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ORIGINAL ARTICLE

Oral Squamous Cell Carcinomas and Expression of Inflammatory Markers

Carcinoma oral de células escamosas y expresión de marcadores inflamatorios

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Abstract

Objectives: To describe the clinical and histopathological information of oral squamous cell carcinomas an oral pathology clinic of a private university in Rio de Janeiro state, Brazil (1998–2017) and to evaluate the immunoexpression of some inflammatory markers (nterferon-gamma, cluster of differentiation 57, and cluster of differentiation 68).
Methods: Clinical data were collected and histopathological evaluations of 183 oral squamous cell carcinomas were performed at an oral pathology clinic of a private university in Rio de Janeiro state, Brazil (1998–2017). Twenty-two paraffin blocks underwent immunohistochemistry to measure the Interferon-gamma, cluster of differentiation 57, and cluster of differentiation 68 expressions and positivity, and were classified as negative/focal, weak/moderate, or strong.
Results: Thereabout 81% of the sample were male and 57% Caucasian. The average age was 58.6 years. Tongue cancers were the most prevalent (36.6%) and 48.1% had a moderately differentiated oral squamous cell carcinomas. Interferon-gamma was expressed in all cases, and 91% had the maximum degree of marking. The cluster of differentiation 68 expression had a maximum degree in 41% of the tumours, and surprisingly all of them in concomitance with the maximum Interferon-gamma-markings. cluster of differentiation 57 was strongly expressed in 45.5% of the sample.
Conclusion: Oral squamous cell carcinomas was more frequent in Caucasians, in their fifth decade of life, and located on the tongue. The Interferon-gamma expression was observed in all cases, its role in the development of tumours and the concomitant expression of cluster of differentiation 68 suggests a possible differentiation for the M2 phenotype of tumours and a consequently a poor prognosis.

Keywords: Oral squamous cell carcinoma; immunohistochemistry; Interferon gama; macrophages

Resumen

Objetivo: Describir la información clínica e histopatológica de los carcinomas orales de células escamosas de una clínica de patología oral de una universidad privada del estado de Río de Janeiro, Brasil (1998-2017) y evaluar la inmunoexpresión de algunos marcadores inflamatorios (interferón-gamma, clúster de diferenciación 57 y clúster de diferenciación 68).
Métodos: Se recopilaron datos clínicos y se realizaron evaluaciones histopatológicas de 183 carcinomas de células escamosas orales en una clínica de patología oral de una universidad privada en el estado de Río de Janeiro, Brasil (1998-2017). Veintidós bloques de parafina se sometieron a inmunohistoquímica para medir las expresiones y la positividad de Interferón-gamma, clúster de diferenciación 57 y clúster de diferenciación 68, y se clasificaron como negativas/focales, débiles/moderadas o fuertes.
Resultados: Aproximadamente el 81% de la muestra eran varones y el 57% caucásicos. La edad media era de 58,6 años. Los cánceres de lengua fueron los más predominantes (36,6%) y el 48,1% presentaban un carcinoma oral de células escamosas moderadamente diferenciado. El interferón-gamma se expresaba en todos los casos, y el 91% presentaba el máximo grado de marcaje. La expresión del clúster de diferenciación 68 tenía un grado máximo en el 41% de los tumores, y sorprendentemente todos ellos en concomitancia con el máximo marcaje de Interferón-gamma. El clúster de diferenciación 57 se expresaba fuertemente en el 45,5% de la muestra.
Conclusiones: Los carcinomas escamosos orales fueron más frecuentes en caucásicos, en la quinta década de la vida y localizados en la lengua. La expresión de interferón-gamma se observó en todos los casos, su papel en el desarrollo de los tumores y la expresión concomitante del clúster de diferenciación 68 sugiere una posible diferenciación para el fenotipo M2 de los tumores y por consiguiente un pronóstico inconcluso.

Palabras clave: Carcinoma oral de células escamosas; inmunohistoquímica; Interferón gama; macrófagos.

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Introduction

Oral squamous cell carcinoma (OSCC) is the most common head and neck malignancy and represents 2% of all cancers worldwide.[1,2] Survival rates for tumours in advanced stages do not usually exceed five years.[3] Various malignant neoplasms have already been associated with different aspects of immune responses, as well as with the presence of infectious microorganisms with oncogenic potential, such as human papillomavirus (HPV) and Epstein-Barr virus (EBV).[4] These associations, and the low survival rates in OSCCs, have brought a greater understanding of the immune response to these tumors, in the attempt to characterize them beyond the TNM classification.[5]

The infiltration of immunological and inflammatory cells seems to have specific roles that may influence the prognosis in human malignant neoplasms. Among them, natural killer (NK) cells and macrophages have stood out due to their protagonist in immunological response.[6] In fact, NK cells that are characterized by the surface marker CD57, can destroy tumor cells and improve anti-tumor immunity by recognising altered major histocompatibility complex (MHC) class 1 surface molecules.(7) In addition, NK cells play a potential role in the development of oral cavity tumors through mediator molecules such as interferon gamma (IFN-γ).[8]

Most malignant tumors contain macrophages that represent the main stromal cells in their tumour microenvironment. In OSCC, the presence of macrophages is associated with an increase in angiogenesis, tumor invasion, and consequently poor prognosis. This scenario happens because, unlike macrophages in normal tissue, tumor-associated macrophages (TAMs) lose their phagocytosis abilities and antigen presentation. Furthermore, TAMs can differentiate themselves into two distinct phenotypes named M1 and M2.[9] The M1 phenotype is especially induced by IFN-γ and promotes a pro-inflammatory tumor environment secreting cytokines, such as IL-1 and recruiting CD68+ T cells; on the other hand, M2 phenotype secretes anti-inflammatory cytokines, such as IL-10, promoting an immunosuppressive tumor microenvironment.[10]

Cluster of differentiation 68 (CD68) is a recognized marker for both M1 and M2 phenotypes.[9] However, the expression of CD68 is associated with a lower survival rate(7,11) and with the presence of metastatic lymph nodes. On the other hand, the expression of Cluster of differentiation 57 (CD57) in the tumor stroma is associated with an absence of nodal metastasis[1] and better survival rates.[7]

Despite the availability of multiple therapies, the prognosis of OSCC remains poor.[3] Many studies have shown that the tumor microenvironment plays a role in cancer progression. There is a great effort in the search for a marker that allows us to improve the characterization of the risk group, stratify treatments and develop targeted therapies. The identification of changes in the tumor microenvironment will increase the understanding of the malignant progression of OSCC and, in the future, improve the management of these patients.

Thus, the objective of this study was to describe the clinical and histopathological information of OSCC an oral pathology clinic of a private university in Rio de Janeiro state, Brazil (1998-2017), and to evaluated the immunoexpressions of some inflammatory markers (IFN-γ, CD57, and CD68).

Methods

This is a retrospective, observational and descriptive study with laboratory data. The first stage was to form a database from the records of patients who attended at the oral pathology service of a private university in Rio de Janeiro state, Brazil, from 1998 to 2017. Information such as sex, age, race/ethnicity, histopathological diagnosis, location of the lesion, and histopathological characteristics were collected from pathology records. The inclusion criteria were records of patients diagnosed with OSCC showing complete clinical and histopathological information, along with accessible paraffin-embedded tissue samples for histopathological and immunohistochemical analysis. 190 specimens from oral cavity biopsies were histologically classified as squamous cell carcinoma. Cases with inconclusive diagnosis and non-specific histopathological findings were excluded, as were clinical records with incomplete information, thus, 7 specimens were removed from the final sample.

Were allocated for convenience a total of 24 specimens from oral cavity biopsies were selected with the histopathological diagnosis of OSCC through immunohistochemical analysis. Of these, two specimens were excluded due to insufficient epidemiological and tumor data.

This study was approved by the Research Ethics Committee (no: 2502690) of the Estácio de Sá University.

Immunohistochemical reactions

Salinized slides were made up of 3 µm thick histopathological sections to perform the immunohistochemistry reactions, according to protocols described in previous studies.[12,13] The slides were deparaffinized and the sections hydrated. Citrate-citric acid buffer (Citrate Buffer pH 6.0, Diagnostic BioSystems, Pleasanton, California, United States of America) was added to the slides and microwaved (BMX 35ARHNA - Maxi Gratine Inox, Brastemp, Santa Catarina, Brazil) to recover the antigen.

After this latter step, the slides were submitted to five consecutive 6% hydrogen peroxide (Proquímios, Rio

de Janeiro, Brazil) 5-minute baths to inactivate the endogenous peroxidase and then placed in phosphate-buffered saline (PBS) 1x, pH 7.4 (Laborclin, PR, Brazil).

A PBS/Bovine Serum Albumin (BSA) (1%) solution was used to dilute primary antibodies CD57 (1:100, mouse, Biocare-CM007C, Biocare Medical, California, United States of America), CD68 (1:500, mouse, Dako-M0876, DAKO, California, United States of America), and INF-γ (1:200, rabbit, SC-8308, Santa Cruz Biotechnology, Texas, United States of America). The primary antibodies were applied to the sections and then the slides were placed in a refrigerator for 16 hours. Afterward, the slides received three washes in 1x PBS for 1 minute each.

The secondary biotinylated antibody (labeled streptavidin biotin + system Horseradish Peroxidase − Dako K0690, DAKO, California, United States of America) was applied over the slides and heated at 37° C for 30 minutes. Afterward, the slides were washed in 1x PBS, and the streptavidin-biotin complex was applied. Then the slides were again heated for 30 minutes at 37° C. After this, the slides were washed three times for 1 minute each with 1x PBS.

Liquid 3,3′-Diaminobenzidine (Liquid 3,3′-Diaminobenzidine + Substrate Chromogen System − Dako K3468, DAKO, California, United States of America) was applied on the slides for 5 minutes at room temperature, then the slides were washed under running water for 5 minutes. Subsequently, the slides were counterstained with Carazzi hematoxylin for 2 minutes and then washed under running water for 8 minutes. Finally, the slides were dehydrated and then treated with diaphanization xylol and the mounting xylol, and subsequently mounted on glass slides with Entellan (Meck, Darmstadt, Germany). Positive and negative controls were used for each antibody, according to the manufacturers' instructions.

Image Analysis

Two previously trained evaluators performed the image analyses with an optical microscope (Leica DM500, Heerbrugg, Sweden) following a protocol, established by Ajuz and collaborators (2014),(12) in which each slide was subdivided into five fields (40x magnification).

The independent evaluators assigned values from 0 to 2.0 according to the number of positive markings for the antibody in each field. The observed fields were considered negative when there were no positive cells or less than 5% of the cells were positively stained (Score: 0); weak to moderate when 5% to 50% of the cells were positively stained (Score: 1.0); and intense when more than 50% of the cells were positively stained (Score: 2.0). Each field of the slides evaluated received five independent degrees, representing values from 0 (when all the high-power fields analyzed were negative) to 10 (when all high-power fields analyzed were strongly positive). As this final number summarizes the total value of the five fields, an average for the immunoexpression classification was obtained: negative/focal (final average from 0 to 0.5), weak to moderate (ranging from 0.6 to 1.2), and strong (ranging from 1.3 to 2.0).

Statistical Analysis

Statistical software (Statistical Package for the Social Sciences 21.0; IBM, Armonk, NY) was used for all statistical analyses. The normality of the quantitative variables (age) was checked using the non-parametric Kolmogorov–Smirnov and Shapiro-Wilk tests, as well as graphic analysis. The quantitative variables were described as mean [standard deviation (SD)], and qualitative variable (sex; color; location; degree of differentiation; expression) as frequency [n (%)]. The difference between groups was compared using the non-parametric Kruskall-Wallis test, adjustment for multiple comparisons by post hoc Dunn's test, for quantitative variables. The Kruskal-Wallis test was used because it's indicate to compare more than two independent groups, of the same or different sizes, with a quantitative response variable. This test showed $p < 0.05$. Therefore, the post hoc Dunn's test was performed to identify groups that showed a statistically significant difference. The analysis of agreement among the expressions of IFN-γ, CD57, and CD68 was calculated with the non-parametric correlation of the Kendall posts (tau-b). The correlation coefficients were stratified between 0 and 0.3 (negligible correlation), 0.31 and 0.5 (weak), 0.51 and 0.7 (moderate), 0.71 and 0.9 (strong) and > 0.91 (very strong).[14] The level of significance established in all analyses was 5% ($p < 0.05$).

Results

Socio-demographic compilation

Between 1998 and 2017, 183 specimens from oral cavity biopsies were histopathologically classified as squamous cell carcinoma. Most of the patients were male (80.9%) and Caucasian (56.8%) and were in their fifth decade of life (31.7%). The average age was 58.6 years (standard deviation = 11.84; range 34–87); 57.5 years for men and 63.1 years for women. Tongue cancers were the most prevalent (36.6%; 27.9% lateral border and 6.0% belly), followed by the floor of the mouth (14.2%) and alveolar ridge (14.2%). Concerning the degree of differentiation, 48.1% had moderately differentiated OSCC and 25.1% had well differentiated (Table 1).

***Table 1**- Distribution of Oral Squamous Cell Carcinoma cases according to the sociodemographic variables and tumor characteristics*

Variables	Cases n (%)
Sex	
Female	35 (19.1)
Male	148 (80.9)
Age (Average) 58.6 (sd 11.84)	
Color	
White	104 (56.8)
Brown skinned	39 (21.3)
Black	26 (14.2)
Not Reported	14 (7.7)
Location	
Tongue	67 (36.6)
Floor of Mouth	26 (14.2)
Alveolar Edge	26 (14.2)
Soft Palate	18 (9.8)
Lip	14 (7.7)
Retromolar	11 (6.0)
Hard Palate	8 (4.4)
Buccal Mucosa	5 (2.7)
Bottom of the Vestibule	4 (2.2)
Tonsillar Pillar	4 (2.2)
Degree of Differentiation	
Well	46 (25.1)
Moderate	88 (48.1)
Poor	5 (2.7)
Microinvasive	15 (8.2)
Basaloid	9 (4.9)
In Situ	3 (1.6)
Absent	17 (9.3)

Note: sd = standard deviation.

Immunohistochemical assays

Twenty-two OSCC samples were examined and positivity for the IFN- γ, CD68, and CD57 expressions was analysed. The group was composed of 15 men (68.2%) and 7 women (31.8%), and the average age was 60.2 years (range 38–85); 59.2 years for males and 62.3 years for females, with no statistical difference between the mean age. OSCC occurred more frequently on the tongue (50%; 36.4% on the lateral border and 13.6% on the belly), followed by the retromolar region (18.2%) and floor of the mouth (13.6%). There was no significant difference among the sociodemographic variables and tumour characteristics with the same biomarker.

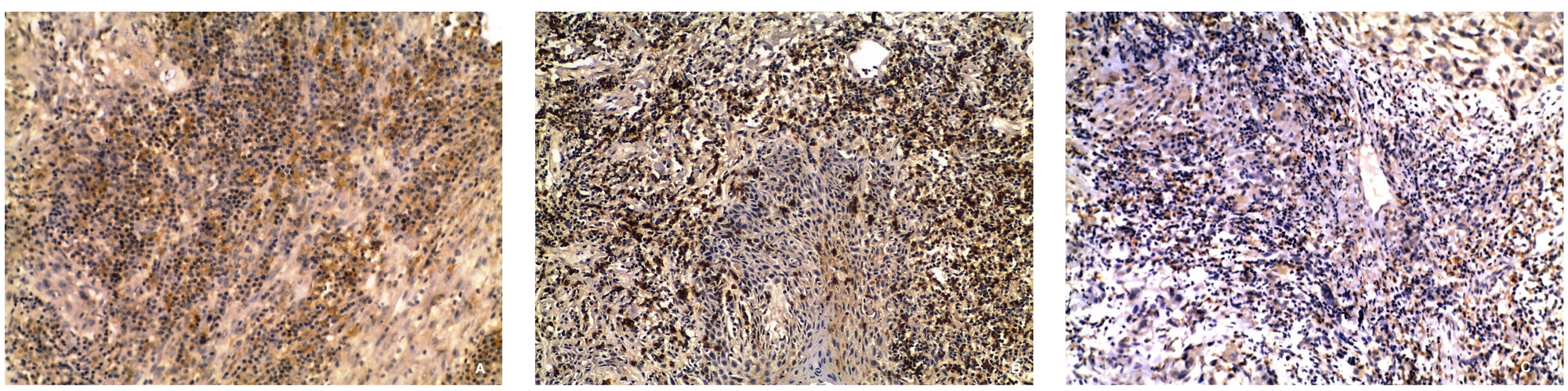

***Fig. 1**- Photomicrographs of the histopathological sections with positive markings for A: IFN-γ, B: CD68, and C: CD57 (Immunoperoxidase; 40x).*

Figure 1 shows the photomicrographs of histopathological sections with positive expressions for IFN- γ, CD68, and CD57. The IFN-γ expression was observed in all cases, with one (4.5%) focal, one (4.5%) weak/moderate, and 20 (91%) strong; consequently, 85% of the cases reached the maximum score (2.0). The CD68 expression was the maximum in nine tumours (41%), weak/moderate in 11 (50%), focal in one (4.5%), and negative in one (4.5%). In the CD57 expression, 10 (45.5%) were strongly expressed, with a maximum score in five specimens,

six (27.3%) with a weak to moderate expression, and six (27.3%) with a focal expression. The average expression of each marker is shown in Table 2. There was a significant difference between the average expressions of the inflammatory markers ($p < 0.001$), except for the average expression of the female, retromolar, and floor of the mouth groups that did not show a significant difference.

The value of tau (τ) (Kendall's tau-b correlation) and the p-value analysed are shown in Table 3. No significant correlation was found among the expressions of all three inflammatory markers, and therefore τ was considered negligible ($\tau < 0.3$).

***Table 2**- Expression of IFN-γ, CD68, and CD57 in 22 specimens of Oral Squamous Cell Carcinoma biopsies*

	IFN- γ	CD68	CD57	P value[3]
		Expression (n)[2]		
Negative/Focal	1 (4.5%)	2 (9%)	6 (27.3%)	
Weak/Moderate	1 (4.5%)	11 (50%)	6 (27.3%)	
Strong	20 (90%)	9 (41%)	10 (45.4%)	
Average[1]	1.86 ± 0.38	1.17 ± 0.55	1.13 ± 0.73	< 0.001
		Age[1]		
< 60 years old	1.83 ± 0.46	1.08 ± 0.45	1.20 ± 0.85	0.0047
≥ 60 years old	1.88 ± 0.27	1.28 ± 0.67	1.04 ± 0.57	0.0027
		Sex[1]		
Female	1.66 ± 0.63	0.94 ± 0.69	1.11 ± 0.736	0.1184
Male	1.95 ± 0.19	1.28 ± 0.46	1.17 ± 0.761	0.0001
		Location[1]		
Tongue	1.96 ± 0.12	1.29 ± 0.48	1.13 ± 0.69	0.0008
Retromolar	1.60 ± 0.80	0.80 ± 0.63	1.20 ± 0.91	0.2739
Hard Palate[4]	2.00	1.40	1.00	
Floor of the Mouth	1.67 ± 0.42	1.27 ± 0.64	1.27 ± 0.95	0.7248
Vermillion Lip[4]	2.00	1.80	2.00	
Alveolar Edge[4]	2.00	0.20	0.20	
Tonsillar Pillar[4]	1.80	1.20	0.6	
		Degree of Differentiation[1]		
Well	1.96 ± 0.09	1.16 ± 0.43	1.48 ± 0.79	0.0464
Moderate	1.88 ± 0.18	1.04 ± 0.73	0.52 ± 0.33	0.0143
Absent[4]	2.00	1.60	1.80	
Microinvasive	1.78 ± 0.52	1.20 ± 0.57	1.18 ± 0.72	0.0167

Note: 1Values are expressed as mean and standard deviation.2Values are expressed as n(%). 3 P value refers to the Kruskal-Wallis test adjustment for multiple comparisons by post hoc Dunn's test. 4There are no poorly differentiated samples (p-value did not calculate because n = 1).

***Table 3**. Correlation analysis of the IFN-γ, CD68, and CD57 expressions*

	IFN- γ	CD68	CD57
IFN- γ	1	0 ($p = 1$)	-0.13 ($p = 0.48$)
CD68	0 ($p = 1$)	1	0.14 ($p = 0.40$)
CD57	-0.13 ($p = 0.48$)	0.14 ($p = 0.40$)	1

Note: Values are expressed ▫ (Kendall's tau-b correlation) and p (significance level).

Discussion

The global epidemiological profile of OSCC is characterized by its higher prevalence in males and after the fifth decade of life, [2,16,17] which was also shown in the present study. The most common anatomical site was the tongue, in agreement with other studies,[16,17] and the most frequent histological grade (moderately differentiated) corroborates with other research.[17]

The present study showed a specific tumor's inflammatory activity in the specimens analyzed, however, confirmation of the aggressiveness of these tumors could not be endorsed, even though the immunological markers analyzed are associated with poor prognosis, especially the high expression of IFN-γ. The IFN-γ is a

pro-inflammatory cytokine with anti-tumor activity, which is secreted by activated T cells and NK cells.[9] Previous reports identified the expression of IFN-γ both in tumor cells and tumor infiltrated lymphocytes (TILs).[10] On the one hand, this result also shows that the immune stratification of the OSCCs in non-T cells is characterised by a low density of inflammatory infiltrates, a decreased human leucocyte antigen (HLA) expression, and low IFN-γ expression in the TILs. On the other hand, T cell inflamed tumours have a high density of immune infiltrates, a high level of HLA and a high expression of IFN-γ. This suggests that the general principle of non-T cell inflamed tumours also applies to OSCC.

Macrophages have functional types that can be activated in different ways. Macrophages M1 are activated by patterns associated with pathogens, whereas macrophages M2 are stimulated by immune and interleukin complexes (IL-4, IL-13, IL-21, and IL-33). Each phenotype expresses pro-inflammatory factors. M1 expresses IFN-γ, IL-1, IL-6, IL-12, IL-18, IL-23, tumor necrosis factor (TNFα), reactive oxygen specimens (ROS), and is associated with higher levels of MHC I and II, thus participating in the response to tumors.[18]

One study[18] has already reported the importance of chronic inflammation in the development of prostate tumors, where the presence of inflammatory cytokines perpetuates the condition. In prostate tumors, macrophages change from an M1 phenotype, common in areas of chronic inflammation, in tumor development, in atrophy, and in intraepithelial neoplasia with a high degree of malignancy, to an M2 phenotype, present in established tumor areas. Among these tumors, 20% are associated with M1 and 80% with M2 macrophages, which have high concentrations of IFN- γ and other inflammatory cytokines when compared to normal tissues.

The greater number of TAMs, especially in the M2 phenotype, may be associated with the more aggressive biological behavior of OSCC and, consequently, with a decrease in patient survival. [19] The influence of macrophage polarization in OSCC has not been definitively proven and in carcinomas treated with radiotherapy, an association of CD163—an M2 phenotype TAM marker—was associated with longer survival. The macrophages present in tumors generally have an M2 phenotype and express CD68 and CD163. A high density of TAMs represents a poor prognosis for several tumors and may influence tumor initiation and progression. In the case of OSCCs, the mechanisms involving macrophages are not yet clearly defined.[3] In the samples analyzed in the present study, the highest expression of IFN-γ was found in all specimens with high CD68 expression, suggesting a differentiation for the M2 phenotype.

Some studies[9,11,19,20] have reported that CD68+ macrophages are associated with tumors with a poor prognosis, however, other authors[1,5] have not corroborated this connection. In a meta-analysis,[7] the high infiltration of CD68 in OSCC and their stroma was associated with low survival rates and impacts disease-free survival; however, the authors did not detect any significance between the expression of CD68 in the tumor environment and survival rates. These findings indicated that macrophages of different regions of the tumor could differentiate into polarised macrophages in different ways. Nevertheless, further studies are needed to validate the correlation of macrophage polarization with their locations in the tumor microenvironment.

Hu and collaborators[3] reported the expression of CD68 in 83% of 127 cases of OSCC, 27% with a high marker expression. This high expression of the marker was associated only with tumors with lymph node metastases, low survival, and the invasion of the tumor stroma by CD68 positive macrophages. In the present study, it was not possible to collect data of survival and tumor staging, since the samples came from a histopathology laboratory that provides diagnosis for professionals that route patients to referral hospitals for cancer treatment, and consequentially are unable to provide data on the follow-up of these individuals.

Macrophages can be potential therapeutic targets and used as predictors of malignant transformation. One study showed that the positivity of CD68 in the leukoplakia samples that transformed into OSCC was higher than those that did not undergo malignancy within five years, nor those in the healthy mucosa.[21] Furthermore, the study[21] reported that there was no significant difference in leukoplakia without malignancy and healthy mucosa, and the CD68 expression was higher in the OSCC. The current method of evaluation performed on potentially malignant lesions uses a histopathological grade of dysplasia during or after treatment since leukoplakia with no dysplastic changes can show malignant transformation, while those with a high degree dysplasia can regress spontaneously. Therefore, markers that can predict malignant transformation would be of high clinical value.[21] This is also true for tumors that could develop recurrence, despite having received an early and satisfactory diagnosis and treatment; all of which leads us to the believe that immunological factors are involved in unfavorable prognosis.[22]

TAMs and cancer-associated fibroblasts (CAFs) show a distinct pattern of CD68 expression in the tumor microenvironment of OSCC.[20] The presence of CD68 CAFs in the tumor environment was associated with a higher survival rate, disease-free survival, and recurrence-free survival. The high expression of CD68 CAFs in the "invasive margin" has been seen in patients with longer disease-free survival.[20]

The presence of NK cells seems to be important in the interaction between innate and adaptive responses, in addition to the effectiveness of tumor immune responses. An immunohistochemical study[23] with 1,000 samples of various histopathological grade tumors evaluated NK cells (CD57) as a prognostic factor for OSCC. The CD57 expression observed in mononuclear cells of the tumor had a proportional decrease between the well differentiated and the poorly differentiated carcinomas, likewise, there seems to be an association between

a strong expression of CD57 and a longer survival rate in patients with OSCC.[23]

A high infiltration of CD57 seems to be associated with better survival[7,24] and lower recurrence, which seems to be consistent with the CD57 ability to kill abnormal cells.[24] However, CD57 must be carefully interpreted due to the limited number of studies, the small sample size, and the significant heterogeneity among its subsets.[7] In the present study, most of the samples that have strong CD57 expression also showed a maximum degree of IFN- γ expression, showing a possible anti-tumor reaction, even in cases with the presence of CD68, suggesting that the immune system was attempting to limit the progress of the tumor.

This study was carried out with paraffin-embedded material, which did not allow access to the clinical data of these cases and possibilities for comparison. However, research into these markers is important and promising, and may be used as potential targets for malignant lesions and disorders, bringing advances in treatments with a better prognosis for patients.

Conclusion

In this work, the incidence of OSCC was more frequent in Caucasians, in their fifth decade of life, and located on the tongue. The IFN- γ expression was observed in all cases. The mean expressions of CD57 and CD68 were very close to each other, and both were classified as moderate. Although no significant correlation was found among the expressions of the three inflammatory markers, all tumors with high CD68 expression also showed maximum expression of IFN-γ, suggesting a differentiation for the M2 phenotype and a poor prognosis.

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Conflict of interest

No declared.

Author Contributions

This article represents original material, which has not been previously published and is currently not being evaluated for publication in another journal. All authors reviewed the manuscript, agreed for the authors own contributions, and approved the submitted version. RTR conceived and designed of the study, collected the data, conceived and carried out experiments and performed analysis. KDMF processed the data, performed analysis, and writing the paper. DCF collaborated with the designed of the study, the analysis of data and in the critical review of the manuscript. MSV collected the data. ALRA, TCRBS, AMPS and LSG contributes to review of articles. LA carried out experiments, analyses data, and critical review of the manuscript. MGR conceived and designed of the study, performed a critical review of the manuscript.