Changes in immunoexpression of E-cadherin and β-catenin in oral squamous cell carcinoma with and without nodal metastasis

Fernanda Ferreira Lopes, Márcia Cristina da Costa Miguel, Antônio Luiz Amaral Pereira, Maria Carmen Fontoura Nogueira da Cruz, Roseana de Almeida Freitas, Leão Pereira Pinto, Lélia Batista de Souza.

Abstract

The aim of this study is to analyze the immunohistochemical expression of E-cadherin and β-catenin in oral squamous cell carcinoma to better understand the biological behavior of this lesion. The sample consisted of 15 cases of the tongue and 15 of the lower lip. The pattern and intensity of the labeling and the analysis of the percentage of tumor cells immunopositive in membrane for E-cadherin and β-catenin were related to the anatomic location of the lesion, the presence or absence of nodal metastasis, and the histological gradation of malignancy in the tumor invasion front. The presence or absence of cytoplasmic and nuclear labeling was also recorded. The membrane expression for E-cadherin and β-catenin predominately displayed a heterogeneous pattern in the carcinomas studied. No significant difference was observed between the expression pattern and the quantity of cells immunopositive for E-cadherin and β-catenin and the anatomic location of the lesion or the presence or absence of nodal metastasis. However, a statistically significant difference was found between the reduced expression of these proteins and the high malignancy score. The reduced immunoexpression of these proteins in the membrane may be related to the high degree of cell differentiation in cases of oral squamous cell carcinoma with high scores.

Keywords: Squamous cell carcinoma; Oral cancer; E-cadherin; β-catenin; Immunohistochemistry

1. Introduction

Cancer represents a global public health problem, which is currently the third cause of death resulting from disease [1]. Among the malignant neoplasias that affect the oral cavity, squamous cell carcinoma is the most frequently reported, accounting for more than 90% of cases [2]. In spite of new and advanced therapeutic strategies, oral squamous cell carcinoma (OSCC) shows poor survival rates [3].

The anatomical areas most affected by OSCC are the tongue and lower lip [2], with tongue tumors showing a high propensity for regional metastasis, poor prognosis, and worse survival rates, whereas lower lip neoplasias show better biological behavior than tumors in other anatomical areas [4,5].

In OSCC, the presence or absence of cervical lymph node involvement and distant metastases are important in patient prognosis. It therefore becomes relevant for the pathologist to search for characteristics in the histopathological analysis to determine the stage of neoplasias invasion. The histological characteristics of this carcinoma may differ from one area to another within the same tumor owing to its heterogeneous nature. Consequently, data collected in the tumor invasion front may be more significant [6,7].

Molecular events important to tumor growth, such as the loss and gain of adhesion molecules, occur in tumor-host interaction, that is, in the front of the invasion front [7,8].
E-cadherin is a transmembrane glycoprotein dependent on the calcium present in most epithelial cells that play an important role in intercellular adhesion and maintenance, cellular polarity, and tissue architecture. It is composed of an extracellular, transmembrane, and intercellular domain, which links to the catenins and which is involved in signal transduction mediated by these catenins. The cytoplasmic domain of E-cadherin is directly linked to β-catenin or γ-catenin, whereas the α-catenin connects the E-cadherin–β-catenin and γ-catenin to the actin cytoskeleton. Recent studies show that β- and γ-catenin act in cell adhesion and in the Wnt signaling pathway, with the stability of both proteins regulated by adenomatous polyposis coli [9].

Consequently, immunohistochemical studies using anti–E-cadherin and anti-β-catenin antibodies, molecules that participate in the cell-cell adhesion process in epithelial tissues, have been performed to understand the significance of the expression of these proteins in human cancers [10-12].

Alterations in E-cadherin and catenin expression are associated with the loss of differentiation, acquisition of an invasive phenotype, and poor clinical course of many types of cancer. The role of these proteins in invasion and neoplastic metastasis has attracted great interest owing to its promise as a possible prognostic marker. This article aimed to analyze the immunohistochemical expression of E-cadherin and β-catenin in squamous cell carcinoma of the lower lip and tongue, correlating this expression to the anatomic location of the lesion, the history of nodal metastasis, and the histological gradation of malignancy proposed by Bryne in 1998 [7] to obtain a better understanding of the biological behavior of this lesion.

2. Materials and methods

The sample is composed of 30 diagnosed cases of OSCC, 15 cases of the lower lip and 15 cases of the tongue, from the files of the Instituto Maranhense de Oncologia Aldenora Bello, São Luís/MA. Cases resulting from surgical treatment, without prior chemotherapy or radiotherapy, were included in the sample, whereas those whose paraffin-embedded material was insufficient were excluded.

The clinical data collected were age and sex of the patients and the presence or absence of nodal metastasis at diagnosis. Histological cuts 5 μm thick were obtained from the paraffin-embedded material and stained using the hematoxilin/eosin technique to evaluate the areas of deepest tumor invasion based on the malignancy criteria recommended [7]. Specimens with a score of 4 to 8 points were classified as having a high degree of malignancy, whereas those with more than 8 points were considered to have a high degree of malignancy, as proposed by Miranda (2002) [13], which is an adaptation of Bryne’s classification (1998) [7].

Histological cuts 3 μm thick were obtained and submitted to the immunohistochemical method, using the streptavidin-biotin technique. Following antigen recovery, using the citric acid (pH 6.0) steamer method, the primary anti–E-cadherin (clone NCH-38, 1:50, DakoCyto-mation, Carpinteria, CA) and anti–β-catenin antibodies were incubated (clone Ab-1, 1:500, Labvision/Neomarkers, Fremont, CA).

The pattern and intensity of the marking in the cytoplasmic membrane for E-cadherin and β-catenin were classified into 4 scores: 1 = absent, 2 = focal heterogeneous, 3= reduced homogeneous, and 4 = strong homogeneous. The semiquantitative analysis of the percentage of tumor cells positive for E-cadherin and β-catenin with membrane expression was classified into 4 scores: 1 = less than 25%, 2 = 26% to 50%, 3 = 51% to 75%, and 4 = greater than 75% of positive cells [14]. For the analysis of β-catenin, the presence of cytoplasmatic and nuclear marking in the tumor cells at the invasion front was also analyzed [15].

As a negative control, the primary antibody was substituted for 1% bovine serum albumin in a buffer solution, and the cuts were subsequently submitted to all the steps described in the technique. As a positive control, normal oral epithelium present in the areas circumjacent to the lesions was used [14].

The Mann-Whitney test, Spearman coefficient correlation, and Fisher exact test were used for the statistical analysis, considering a significance level of 5%. This study

<table>
<thead>
<tr>
<th>Expression pattern</th>
<th>Location</th>
<th>n</th>
<th>Median</th>
<th>Q25-Q75</th>
<th>Mean of ranks</th>
<th>Sum of ranks</th>
<th>U</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin</td>
<td>Lower lip</td>
<td>15</td>
<td>2</td>
<td>1-3</td>
<td>15.27</td>
<td>229.00</td>
<td>109.00</td>
<td>.879</td>
</tr>
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<td></td>
<td>Tongue</td>
<td>15</td>
<td>2</td>
<td>1-3</td>
<td>15.73</td>
<td>236.00</td>
<td></td>
<td></td>
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<tr>
<td>β-catenin</td>
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<td>2-3</td>
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<td>242.50</td>
<td>102.50</td>
<td>.650</td>
</tr>
<tr>
<td></td>
<td>Tongue</td>
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<td>2</td>
<td>2-3</td>
<td>14.83</td>
<td>222.50</td>
<td></td>
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</tr>
</tbody>
</table>

* Mann-Whitney test.
was approved by the Research Ethics Committee of the University Hospital of the Universidade Federal do Maranhão.

3. Results

At diagnosis, the age of the patients varied between 34 and 91 years (mean age, 64.2 years). There were more men than women. Nodal metastasis occurred in 11 (73.33%) of the 15 cases of tongue carcinoma and in 3 (20%) of the 15 lower lip cases, a statistically significant association ($P = .009$), with the tongue more often affected.

Following the morphological analysis, we observed that 11 (73.33%) of the 15 (73.33%) cases of squamous cell carcinoma of tongue and 9 (60%) of the 15 lower lip cases were among the group of high-score malignancy, with no statistically significant association found between lesion location and malignancy scores ($P = .700$).

The presence of nodal metastasis was observed at diagnosis in 14 cases of OSCC and absence in the remaining 16 cases. However, there was also no statistically significant association ($P = .709$) found between nodal metastasis and histological gradation of malignancy.

The cytoplasmic membrane expression for E-cadherin was predominant, with a heterogeneous pattern (score 2) for both lower lip and tongue carcinomas, as well as membrane expression for $\beta$-catenin (Table 1) (Figs. 1-6). No significant difference was found between the means for expression pattern and the amount of immunopositive cells in the antibodies analyzed.

In relation to nodal metastasis, no significant difference was found between the means of expression pattern and the amount of immunopositive cells for either E-cadherin or $\beta$-catenin.
β-catenin (Table 2). A statistically significant difference was observed in the membrane expression of the E-cadherin and β-catenin proteins in relation to malignancy scores. Cases with a low-malignancy score had significantly higher means in both the expression pattern and amount of cells immunopositive for E-cadherin β-catenin (Table 3).

There was no significant association between the nuclear expression of β-catenin with the anatomic location of the lesion, nodal metastasis, and histological gradation of malignancy. There was also no significant association between β-catenin expression in cytoplasm and the location of the lesion and nodal metastasis. A significant association was found between β-catenin expression in the cytoplasm and histological gradation of malignancy, with β-catenin present in the cytoplasm more often in cases of high malignancy scores (Table 4).

4. Discussion

Among the squamous cell carcinomas that occur in the oral cavity, those of the tongue are the neoplasias that most frequently develop nodal metastasis in initial tumors and are associated with poor prognosis and low survival rates [3]. In this study, we observed a high frequency of nodal metastasis in the tongue cases (more than 70% of the sample), which is in agreement with previous studies [16]; however, less alarming values were presented [10], who detected the presence in 41.18% of his sample.

In the lower lip, the presence of nodal metastasis was significantly less frequent than in the tongue, corroborating others studies, which found 79% of patients with squamous cell carcinoma of lip without nodal metastasis [4], and reported that lip neoplasias have better biological behavior than that of tumors in other anatomical sites, possibly due to the low rate of nodal metastasis and early detection because of their location [5].

The vascular and lymphatic systems vary between distinct anatomical sites, a fact that may influence tumor development [17]. The lower lymphatic vascularization in the lower lip area appears to contribute to the low nodal metastasis rate in cases of squamous cell carcinomas in this region at diagnosis, as observed in this study. However, despite the lower lip carcinoma’s being negative at diagnosis, it may develop nodal metastasis at a later stage, in most cases within a 2-year period [18].

In the morphological analysis of the sample in this study, we observed a predominance of lesions classified as high-score malignancy, both for cases of the tongue (73.33%) and of the lower lip (60%), with no statistically significant association found between anatomical location and malignancy scores.

In relation to the tongue lesions, our results were similar to others reported by the literature [19], which observed a predominance of squamous cell carcinomas of the tongue with high malignancy scores. In other study [13], the 12 cases of tongue carcinoma (100%) had high malignancy scores, whereas among the 12 cases of lower lip carcinoma, only 8.33% were classified with a high score compared to 91.67% with a low malignancy score, differing from our findings for the lower lip.

The distinct biological behavior of OSCC, when the anatomical location of the primary tumor is analyzed, is defended by some authors [4,5]. We found an association between anatomical location and the formation of nodal metastasis, which reflects the differences in biological behavior between neoplasias and distinct anatomical regions [2]. However, no association was found between either the location of the tumor and the histological gradation of malignancy, or location and nodal metastasis. This corroborates the previous findings of Okamoto et al [20] and diverges from those of Odell et al [6], who found a relation between nodal metastasis and the histological gradation of malignancy.
E-cadherin links directly with the β-catenin protein, forming the E-cadherin/β-catenin complex. β-Catenin may also act in the Wnt signaling pathway. In the absence of Wnt signaling, β-catenin has rapid phosphorylation and degradation; however, with the activation of this signaling method, the concentration of β-catenin in the cytoplasm rises, facilitating its translocation to the nucleus. In the nucleus, the β-catenin links with members of the TCF/LEF family of transcription factors and activates the expression of TCF/LEF target genes, which include the protooncogenes c-Myc and cyclin D1 [8,21].

The results of this study, in relation to the immunohistochemical expression of E-cadherin and β-catenin molecules, proved what was described previously [22,23]. These studies revealed that cells positive for E-cadherin and β-catenin had a cuboidal format, whereas the negative cells were fusiforms with greater loss of differentiation. In our research, the tumor cells were predominantly negative when found in small infiltrated or isolated islands, whereas the central cells of the tumor masses were generally immunopositive, albeit with a variable immunomarking pattern [24].

Also correlated in this study was the expression of E-cadherin and β-catenin with the anatomic location of the lesion, the presence of nodal metastasis and the histological gradation of malignancy proposed by Bryne [7]. The results showed that the heterogeneous focal pattern for E-cadherin was predominant in both the lower lip and the tongue. These results corroborate those of previous study [25], who reported that in malignant tumors, the E-cadherin immunoeexpression is heterogeneous, with quantitative and qualitative marking alterations. They also observed that in normal oral mucous adjacent to the tumors, the E-cadherin molecule displayed a strong pericellular immunopositivity in the basal, suprabasal, and spinosum layers, with varying intensity, confirming others’ findings [5], who analyzed 85 epidermoid carcinomas of the tongue.

There was no significant difference in the pattern of immunoeexpression and amount of immunopositive cells for the antibodies analyzed in this study, between the anatomical locations of lesions in the lower lip and tongue. This result corroborates with that of Bowie et al [26], who found no association between E-cadherin expression and the anatomical location of tumors in 28 cases of squamous cell carcinoma of the head and neck. There is, however, a scarcity of studies comparing the variables of E-cadherin and β-catenin expression with tumor location. Most studies of these proteins are correlated to histological gradation and the appearance of metastasis.

There was also no significant difference between expression pattern and the amount of immunopositive cells for either E-cadherin or β-catenin and the nodal metastasis variable. Studies with uterine carcinoma also found no relation between the E-cadherin and β-catenin expression and clinical-pathological data, such as recurrence, metastasis, and survival [27]. They were also similar to the results of Chow et al [10], who found no correlation between

<table>
<thead>
<tr>
<th>Expression pattern</th>
<th>Score of malignancy</th>
<th>n</th>
<th>Median</th>
<th>Q25-Q75</th>
<th>Mean of ranks</th>
<th>Sum of ranks</th>
<th>U</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin</td>
<td>Low</td>
<td>10</td>
<td>3</td>
<td>2-3.25</td>
<td>21.50</td>
<td>215.00</td>
<td>40.00</td>
<td>.005</td>
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<td></td>
<td>High</td>
<td>20</td>
<td>2</td>
<td>1-2</td>
<td>12.50</td>
<td>250.00</td>
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<tr>
<td>β-catenin</td>
<td>Low</td>
<td>10</td>
<td>3</td>
<td>2-4</td>
<td>21.05</td>
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<td>.008</td>
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<td></td>
<td>High</td>
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<tr>
<td>Immunopositive cells</td>
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<td>10</td>
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<td>2-4</td>
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<td>20</td>
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<td>1-2</td>
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<tr>
<td></td>
<td>β-catenin</td>
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<td>10</td>
<td>4</td>
<td>2.75-4</td>
<td>22.80</td>
<td>228.00</td>
<td>27.00</td>
</tr>
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<td></td>
<td>High</td>
<td>20</td>
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<td>1-2</td>
<td>11.85</td>
<td>237.00</td>
<td></td>
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</tr>
</tbody>
</table>

Table 3

Expression pattern and amount of cells immunopositive for E-cadherin and β-catenin in relation to the histological gradation of malignancy

<table>
<thead>
<tr>
<th>Expression pattern</th>
<th>Score of malignancy</th>
<th>n</th>
<th>Median</th>
<th>Q25-Q75</th>
<th>Mean of ranks</th>
<th>Sum of ranks</th>
<th>U</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cadherin</td>
<td>Low</td>
<td>10</td>
<td>3</td>
<td>2-3.25</td>
<td>21.50</td>
<td>215.00</td>
<td>40.00</td>
<td>.005</td>
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<tr>
<td></td>
<td>High</td>
<td>20</td>
<td>2</td>
<td>1-2</td>
<td>12.50</td>
<td>250.00</td>
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<tr>
<td>β-catenin</td>
<td>Low</td>
<td>10</td>
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<td></td>
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<td>237.00</td>
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</table>

*a Mann-Whitney test.
E-cadherin and β-catenin expression and nodal metastasis in 85 squamous cell carcinomas of the tongue.

Diverging from our results, studies on oral carcinomas [12] and on tongue carcinomas [19] showed significant association between E-cadherin expression and lymph node metastasis, considering the low expression of this protein as an indicative value for cervical metastasis. However, Takes et al [11], despite finding a significant correlation between the presence of nodal metastasis and low E-cadherin expression in OSCC, did not observe this relationship in cases located in the pharynx.

In relation to β-catenin expression in the membrane, the results of our study confirm those of Kurtz et al [28], who found no significant association between β-catenin expression in the membrane and nodal metastasis in head and neck carcinomas. However, our results diverge those reported by other studies [9,12], which found that low β-catenin expression was significantly related to nodal metastasis in OSCCs and, according to the latter, also related to poor patient prognosis.

This may be justified by the fact that the process of tumor development and metastasis is complex, and it is unlikely that a single molecular parameter could predict the behavior of metastatic tumor cells [11]. The existence of other factors involved in the formation of nodal metastasis is still unclear [28].

The complexity of molecular mechanisms involved in the formation of nodal metastasis was shown previously in a study about head and neck squamous cell carcinomas [28], which found no correlation between the presence of nodal metastasis and low E-cadherin expression despite their finding a significant association of the latter variable with vascular invasion and lower survival of patients. Perhaps the loss of cellular adhesion is merely an initial event in tumor invasion, with other molecules necessary, such as matrix metalloproteinases and integrins that function in extracellular cell-matrix interaction, to consolidate the invasion and promote the formation of nodal metastasis.

In this study, a statistically significant difference was found in membrane expression of the E-cadherin and β-catenin proteins in relation to malignancy scores. A moderately negative correlation can be observed between the expression pattern, the amount of immunopositive cells for E-cadherin and β-catenin, and the total malignancy score. These results are similar to some studies [19,23,24] that showed E-cadherin expression was inversely proportional to the level of tumor differentiation; however, they differ from the studies [10,26] that did not find this correlation.

The significant correlation between the reduced β-catenin expression in the membrane and the high malignancy score observed in this study differ from those of Castro et al [29], who found no such relation in esophageal carcinomas. However, our results and those of Bánkfalvi et al [8] support the theory that E-cadherin and β-catenin are involved in tissue polarity and architecture and are significantly related to histological gradation of the tumor invasion front in OSCC.

The immunohistochemical expression of β-catenin, not only in the membrane but also in the cytoplasm and the nucleus, showed that this expression in the cytoplasm was more frequent than in the nucleus because, among 30 cases of OSCC in this study, 22 (73.33%) showed cytoplasmatic expression and 6 (20%) showed expression in the nucleus, confirming that nuclear β-catenin appears not to be a common finding in OSCC [30].

With regard to β-catenin expression in the cytoplasm, our results were similar to those of Lo Muzio et al [22], who found this marking in 18 of 30 cases of OSCC. In this study, a significant association was observed between the cytoplasmic marking for β-catenin and the histological gradation of malignancy, with the latter being more frequent in case of high malignancy scores.

On the contrary, a study with ovarian carcinomas [21] found no association between β-catenin expression in the membrane and in cytoplasm with histological gradation of malignancy. This difference in results may be explained by the type of tumor, diversity of histological subtypes of ovarian carcinomas, and differences in data analysis.

It is worth emphasizing that Lyakhovitsky et al [14] observed the presence of β-catenin in the cytoplasm only in cutaneous squamous cell carcinomas and not in precursor lesions, suggesting that cytoplasmic β-catenin may reflect the invasive potential of cells. The location of β-catenin is considered important because the higher frequency of cytoplasmic β-catenin was, the predetermining factor in the
transference of this molecule to the nucleus of colorectal carcinomas [15].

In this study, we found 6 (20%) cases with β-catenin expression in the nucleus, and no association was found between lesion location, nodal metastasis, and histological gradation. In a sample of 24 OSCCs, only 2 (8.33%) cases revealed nuclear expression located only in invasive cells [31], whereas no nuclear marking was observed in 18 cases of metastatic OSCCs [30].

The 20% frequency in our study of cases with of β-catenin nuclear expression is similar to that detected by Pukkila et al [32], who confirmed this expression in 23% (27 cases) of the 138 cases of squamous cell carcinoma of the pharynx. They observed a correlation between β-catenin nuclear expression and patient survival, whereas in our study, no such correlation was found. In addition, patients with squamous cell carcinoma of the esophagus that showed β-catenin nuclear expression had lower mean survival in months than those who did not show this expression [29], confirming the need for further studies on β-catenin in carcinomas.

The clinical significance of β-catenin expression in the cytoplasm and/or nucleus is controversial, where the intense nuclear superexpression of this protein has been considered a significant prognostic parameter for the low survival rate in colorectal carcinoma. On the contrary, at other times, the discovery of this high nuclear expression has been shown to be a significant independent marker for a more favorable prognosis in non–small cell lung carcinomas [9].

When studying β-catenin, we found that its cytoplasmatic and nuclear immunoeexpression findings are omitted in various studies cited in the literature. More research on the immunohistochemical expression of E-cadherin and β-catenin is needed. Studies should investigate the presence or absence of nodal metastasis in addition to patient survival times to reach more significant conclusions.

Finally, we found that alterations in the immunohistochemical expression of E-cadherin and β-catenin in the membrane and the presence of β-catenin in the cytoplasm and nucleus of OSCC cells observed in this study suggest that these proteins may be involved in the aggressive biological behavior of this neoplasia in invasive tumor areas, with a greater loss of differentiation where this association is evident.

Acknowledgments

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