Original Article

Immunohistochemical Expression of E-Cadherin and β-Catenin in Oral Squamous Cell Carcinoma

Akindayo Olufunto Akinyamoju, Ahmed Oluwatoyin Lawal, Akinyele Olumuyiwa Adisa, Bukola Folasade Adeyemi, Bamidele Kolude

Department of Oral Pathology, University of Ibadan/University College Hospital, Ibadan, Nigeria

Abstract

Background: The E-cadherin/β-catenin protein complexes are actively involved in the epithelial-to-mesenchymal transition. Alterations in cadherin or catenin expression or function, play important roles in the development of invasive or metastatic phenotypes of cancers. **Objectives:** The aim of this study was to assess the expression of E-cadherin and β-catenin in oral squamous cell carcinoma (OSCC) patients and to compare this with their clinico-pathological parameters. **Materials and Methods:** This was a cross-sectional study to assess the immunohistochemical expression of E-cadherin and β-catenin in 41 cases of OSCC. Data were analyzed using version 26 of SPSS software. Qualitative data were compared using chi-square statistics. Quantitative data were summarized using mean, standard deviation, and confidence interval and compared using a one-way analysis of variance test. The level of significance was set at $P < 0.05$. **Results:** Overall, 95.1% of the cases had positive membrane expression for E-cadherin, while cytoplasmic staining was seen in 90.2% cases. Positive nuclear staining was seen in 46.3% cases. There was a decrease in the percentage of cytoplasmic and nuclear expression of E-cadherin as the OSCC became more poorly differentiated ($\chi^2 = 13.96$, $P = 0.016$). Also, a decrease in the percentage of nuclear expression of β-catenin in poorly differentiated cases was seen. However, no statistically significant difference was seen in the expression of β-catenin between the different histologic grades ($\chi^2 = 4.8$, $P = 0.4$). **Conclusion:** This study shows a reduction in the expression of E-cadherin and β-catenin as OSCC becomes less differentiated.

Keywords: *E-cadherin, oral squamous cell carcinoma, β -catenin*

Introduction

Oral squamous cell carcinoma (OSCC) accounts for over 90% of oral cancers worldwide and it's associated with a high mortality rate.[1] Majority of cancer morbidity and mortality are attributable to metastatic disease and not the primary cancer.[2] Also, cancer metastasis has been reported to be responsible for up to 90% of mortality in some instances.[2] Squamous cells in physiologic conditions consist of polarized, rather immobile cells that adhere to each other and surrounding matrix to form a sheet of cells.[3] Metastasis, on the other hand, involves the detachment of malignant cells from the primary site and their migration/transportation to distant sites. For this to occur, epithelial-to-mesenchymal transition (EMT) is essential.[3]

Furthermore, EMT is a complex, multistep process that includes loss or defective adhesive properties of epithelial cells. Four classic cellular adhesion molecules (CAMs) are known; these are cadherins, selectins, integrins, and immunoglobulins.[3,4] CAMs play important roles in a broad range of physiologic processes, including cell–cell and cell–matrix interactions, cell migration, cell cycle, and signaling, as well as morphogenesis during development and tissue regeneration. CAMs are also important in a variety of pathologies ranging from cancer, inflammation, and pathogenic infections to autoimmune diseases.[4] In cancers and carcinogenesis, CAMs play an important role in EMT.[4,5]

The cadherin family are calcium-dependent glycoproteins that contain an extracellular domain CAM with three to five internal repeats, a single-spanning transmembrane domain, and an intracellular domain.[3,4] Cadherins are known to mediate cell–cell interactions and are important in the maintenance of epithelial cell integrity.[6]

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Address for correspondence:
Dr. Akindayo Olufunto Akinyamoju, Department of Oral Pathology, University of Ibadan/University College Hospital, Ibadan 200285, Nigeria.
E-mail: akindayo2002@yahoo.com

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This function is largely dependent on an interaction between the cytoplasmic domain of the cadherin molecule with α-, β- and γ-catenin.

Consequently, previous studies have suggested that alterations in cadherin or catenin expression or function, play an important role in the development of the invasive or metastatic phenotype of cancers.[7,8] This study aims to describe the immunohistochemical expression of E-cadherin and β-catenin in OSCC patients and to examine the relationship of these expressions with clinicopathological parameters of patients seen in a tertiary health facility.

Materials and Methods

This was a cross-sectional study to assess the immunohistochemical expression of E-cadherin and β-catenin in OSCC cases. The formalin-fixed, paraffin-embedded samples of OSCC cases diagnosed at the Department of Oral Pathology University College Hospital, Ibadan, between the years 2018 and 2020 were retrieved. Freshly prepared sections were stained with hematoxylin–eosin, and the diagnoses were verified by one of the investigators (AOL). Following the specifications of the manufacturer (Dako Cytomation, USA), the sections for immunohistochemistry were deparaffinized, hydrated, and then rinsed in phosphate-buffered solution (PBS). They were immersed in heat-induced epitope retrieval citrate buffer diluted 1:10 with distilled water and incubated at 90°C for 60 minutes. They were then placed in fresh citrate, cooled in water for 20 minutes, and then rinsed in PBS. Positive and negative controls were employed for the antibodies.

Also, hydrogen peroxide (3%) was added to each section for 10 minutes, and the sections were rinsed in 0.1% PBS. The specimens were incubated for 60 minutes with 1:20 dilution of Abcam mouse monoclonal antibody to E-cadherin and β-catenin followed by incubation with undiluted labeled polymer horseradish peroxidase-conjugated with anti-mouse secondary antibody for 30 minutes. Also, 1 ml of diaminobenzidine solution was added to cover the specimen, followed by incubation in a humidity chamber for 15 minutes. The sections were then immersed in aqueous hematoxylin and rinsed in distilled water. The tissue was dehydrated and subsequently rinsed with xylene. Distyrene plasticizer in xylene mounting fluid was then applied, and a cover slip was placed.

Two investigators (AOA and BK) reviewed the slides scoring the pattern and intensity of staining as follows: negative (<10%) (0), weakly positive (10%–25%) (+1), moderately positive (25%–50%) (+2), and strongly positive (>50%) (+3)[8]. The data were analyzed using the IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA). Qualitative data were compared using chi-square statistics. Quantitative data were summarized using mean, standard deviation, and confidence intervals. The level of significance was set at $P < 0.05$.

Results

A total of 41 cases of OSCC were seen over the study period. The age of occurrence ranged from 29–100 years, with mean age of 58.0 (±19.1) years. Table 1 shows the gender and site distribution of lesions. The cases consisted of 27 males and 14 females, with a 1.9:1 male-to-female ratio. The maxilla, with 16 (39%) cases, was the most frequently affected site, followed by the palate and mandible, both of which had eight (19.5%) cases each. Only six (14.6%) cases occurred in the tongue. The moderately differentiated OSCC was the most frequently diagnosed histologic grade (24/58.5%), while 13 (31.7%) and 4 (9.8%) well and poorly differentiated cases, respectively, were also seen.

A total of 39 (95.1%) of the OSCC cases had membranes that positively expressed E-cadherin, while cytoplasmic staining was recorded for 37 (90.2%) cases. Positive nuclear staining for E-cadherin was seen in 19 (46.3) cases [Table 2].

Concerning E-cadherin, all the well-differentiated and poorly differentiated OSCC cases showed membrane positivity, while 91.7% of the moderately differentiated cases demonstrated membrane positivity.

Similarly, positive cytoplasmic expression for E-cadherin was seen in 13 (35.1%) cases of well-differentiated and 2 (5.4%) cases of poorly differentiated OSCC, both constituting 100% and 50% of cases, respectively, while moderately differentiated OSCC recorded 22 (59.5%) cases representing 91.7% of E-cadherin cytoplasmic positive expression.

Regarding the nuclear expression of E-cadherin, eight (42.1%) well-differentiated OSCC recorded positive expression, constituting 61.5% of well-differentiated OSCC. Also, the moderately differentiated OSCC had 10 (52.6%) cases indicating 41.7% E-cadherin positive nuclear expression. The poorly differentiated OSCC consistently had the lowest percentage expression for E-cadherin, with one (5.3%) case having positive nuclear expression, representing 25% of poorly differentiated OSCC [Table 2]. There was a statistically significant difference in the expression of E-cadherin between the different histologic grades ($\chi^2 = 13.96$, $P = 0.016$).

Table 1: Gender and site distribution of OSCC

Site	Male	Female	Total	Percent
Maxilla	14	2	16	39.0
Palate	5	3	8	19.5
Mandible	5	3	8	19.5
Tongue	3	3	6	14.6
Lower lip	0	2	2	4.9
Cheek	0	1	1	2.5
Total	27 (65.9%)	14 (34.1%)	41	100.0

Additionally, 29 (70.7%) cases of OSCC recorded a positive membrane expression for β-catenin, while cytoplasmic and nuclear staining were positively expressed in 31 (75.6%) and 13 (31.7%) OSCC cases, respectively [Table 3]. On the assessment of the different grades of OSCC, β-catenin membrane expression was seen in 10 (34.5%) cases of well-differentiated OSCC, representing 76.9%. Also, 16 (55.2%) and 3 (10.3%) cases of moderately and poorly differentiated OSCC, representing 66.7% and 75% of moderately and poorly differentiated OSCC, respectively, had membrane positively for β-catenin expression.

Thereafter, cytoplasmic positive expression for β-catenin was seen in 10 (32.2%) cases of well-differentiated OSCC, representing 76.9%. Likewise, 18 (58.1%) and 3 (9.7%) cases of moderately and poorly differentiated OSCC stained positively for β-catenin, representing 75% of both moderately and poorly differentiated OSCC cases.

In addition, nuclear expression for β-catenin was seen in five (38.4%) cases of well differentiated OSCC, corresponding to 38.4% of well differentiated OSCC. Also, nuclear expression for β-catenin was seen in seven (53.9%) cases of moderately differentiated OSCC, representing 29.1% of moderately differentiated OSCC, and in one (7.7%) case of poorly differentiated OSCC, representing 25%. However, there was no statistically significant difference in the expression of β-catenin between the different histologic grades ($\chi^2 = 4.8$, $P = 0.4$).

Discussion

β-catenin is a member of the Armadillo family of proteins and has several functions that are dependent on cellular localization.[9,10] β-catenin's functions are derived by the interactions of other proteins with components of their membrane, cytoplasm, and nucleus.[10,11] β-catenin and E-cadherin form a complex that promotes cell-to-cell adhesion, which is important in the structural formation

Table 2: E-cadherin expression in histologic grades of OSCC

Tumor grade (differentiation)	-ve (%)	+1 (%)	+2 (%)	+3 (%)	Total +ve *n* (%)
Membrane staining					
Well	–	5 (12.8)	4 (10.3)	4 (10.3)	13 (33.3)
Moderate	2	9 (23.1)	10 (25.6)	3 (7.7)	22 (56.4)
Poorly	–	3 (7.7)	–	1(2.6)	4 (10.3)
Total (%)	2	17 (43.6)	14 (35.9)	8 (20.6)	39 (100)
Cytoplasmic staining					
Well	–	7 (18.9)	5 (13.5)	1 (2.7)	13 (35.1)
Moderate	2	13 (35.1)	9 (24.1)	–	22 (59.5)
Poorly	2	1 (2.7)	1 (2.7)	–	2 (5.4)
Total (%)	4	21 (56.7)	15 (40.5)	1 (2.7)	37 (100)
Nuclear staining					
Well	5	8 (42.1)	–	–	8 (42.1)
Moderate	14	6 (31.6)	4 (21.0)	–	10 (52.6)
Poorly	3	1 (5.3)	–	–	1 (5.3)
Total (%)	22	15 (79.0)	4 (21.0)	–	19 (100)

Table 3: Expression of β-catenin in histologic grades of OSCC

Tumor grade (differentiation)	-ve (%)	+1 (%)	+2 (%)	+3 (%)	Total +ve *n* (%)
Membrane staining					
Well	3	4 (13.8)	2 (6.9)	4 (13.8)	10 (34.5)
Moderate	8	10 (34.5)	2 (6.9)	4 (13.8)	16 (55.2)
Poorly	1	1 (3.4	2 (6.9)	–	3 (10.3)
Total (%)	12	15 (51.7)	6 (20.7)	8 (27.6)	29 (100)
Cytoplasmic staining					
Well	3	5 (16.1)	5 (16.1)	–	10 (32.2)
Moderate	6	12 (38.7)	6 (19.4)	–	18 (58.1)
Poorly	1	2 (6.5)	1 (3.2)	–	3 (9.7)
Total (%)	10	19 (16.3)	12 (38.7)	–	31 (100)
Nuclear staining					
Well	8	3 (23.1)	2 (15.3)	–	5 (38.4)
Moderate	17	6 (46.2)	1 (7.7)	–	7 (53.9)
Poorly	3	1 (7.7)	–	–	1 (7.7)
Total (%)	28	10 (77.0)	3 (23.0)	–	13 (100)

of the stratified squamous epithelium of oral mucosa and is essential in preventing cellular dissociation necessary for cancer invasion and progression.[12,13]

In this study, 70.7% of OSCC cases recorded positive expression of β-catenin, while 95.1% of cases had positive expression of E-cadherin. This high expression of E-cadherin was similar to what was obtained in the study by Shakil *et al*.[14], who recorded a positive expression of 91.4%. However, this was in contrast with the findings by Laxmidevi *et al*.,[15] who reported 56.6% of β-catenin positivity in OSCC but reported 83.3% positivity in verrucous carcinoma. Similarly, the findings in this study also differed from findings obtained from Zaid,[16] who reported 61.2% of E-cadherin positive expression and 67.1% positive expression of β-catenin in OSCC. These findings in the above-mentioned previous studies have been supported by suggestions of a decreased expression of E-cadherin and β-catenin in OSCC tumor cells when compared with control groups that had strong expressions of E-cadherin and β-catenin.[17] In addition, studies by Diniz-Freitas *et al*.[18] and Andrews *et al*.[19] showed that reduced expression of E-cadherin was associated with higher histological tumor grades and metastasis, while a high E-cadherin expression was seen in well differentiated OSCC.[20,21] These findings agreed with results obtained in the present study, where reduced expression of E-cadherin and β-catenin were seen in poorly differentiated OSCC. However, this finding contrasted from those of Kaur *et al*.[22] who reported a reduced expression of E-cadherin, more in the well and moderately differentiated OSCC, as well as Sharma *et al*.[23], who reported more staining in poorly differentiated OSCC.

In this study, poorly differentiated OSCC had a relatively lower expression rate for β-catenin compared with the well and moderately differentiated OSCC at the membrane, cytoplasmic and nuclear levels; however, there was no statistically significant difference. This finding differed from those of Zaid,[16] who reported cytoplasmic expression of β-catenin was least in well differentiated OSCC. Also, Cai *et al*.[24] reported 3 cases in their study had nuclear β-catenin expression and 17 cases had cytoplasmic β-catenin expression, and there was no statistically significant difference between the patients with nuclear or cytoplasm β-catenin expression and patients without cytoplasm β-catenin expression. This is comparable to the results in the present study, which recorded 1 case and 31 cases with positive nuclear and cytoplasmic β-catenin expression, respectively.

Additionally, β-catenin is a multifunctional protein that is involved in two independent processes, that is, cell–cell adhesion and signal transduction. Aside from its role in regulating E-cadherin-mediated cell adhesion, β-catenin is also involved as a transcription cofactor in the wingless (Wnt) signaling pathway and a target of the adenomatosis polyposis coli (APC) gene product. APC gene mutations can lead to the accumulation of cytoplasmic and nuclear β-catenin.[25,26] Furthermore, mechanisms such as deregulated expression of β-catenin, which may result from APC defects, activating mutations in the β-catenin gene itself, or other alterations in the Wnt pathway, have been implicated as important steps in carcinogenesis.[26] Subsequently, aberrant expression of β-catenin immunostaining has been suggested for inclusion as an ancillary and complementary standard prognostic biomarker for the evaluation of patients with OSCC.[27]

Also, a report by Pirinen *et al*.[28] showed that a reduction in the nuclear expression of β-catenin was an indication of more aggressive tumor behavior, while Pukkila *et al.*, in their study of 161 patients with primary oropharyngeal and hypopharyngeal squamous cell carcinoma, observed patients without nuclear β-catenin had shorter overall survival.[29] They also reported positive nuclear β-catenin expression in 23% of cases, which is slightly lower than the finding in the present study.

In essence, this study describes E-cadherin and β-catenin immunostaining of OSCC cases. However, an in-depth comparison of results from this study with those of other studies was a challenge due to the dearth in literature. Notwithstanding, the variation in results obtained may be partly due to differences in methodology employed in the different studies. More so, immunostaining methods, criteria, and definition for expression, as well as under-expression and intensity of staining, may differ in these studies. Also, the racial and genetic/molecular basis of each cancer may play an important role in determining the level of expression of the molecules of interest in each cancer. Thus, further studies would be necessary to substantiate the role of E-cadherin and β-catenin in carcinogenesis.

In conclusion, this study showed a reduced E-cadherin and β-catenin expression in the poorly differentiated OSCC. Also, there was a statistically significant lower expression of E-cadherin in the higher histological grades of OSCC, but there was no statistically significant difference in the expression of β-catenin among the histologic grades of OSCC. Although conclusive deductions may not be made from this study owing to the relatively small sample size, findings from this study suggest low expression of E-cadherin and β-catenin may be synonymous with a higher tumor grade.

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Conflicts of interest

There are no conflicts of interest.

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