β-catenin expression in perilesional area of different grades of oral squamous cell carcinoma

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Abstract

Background: Multistep carcinogenesis discusses a stepwise accumulation of alterations, both genotypic and phenotypic. Arresting one or several of the steps may disturb or delay the development of cancer. Current guidelines describe histopathologic margin of >5 mm as "clear margin" and 1-5 mm as "close margin". β-catenin plays a critical structural role in mediating cadherin junctions and is also an essential transcriptional co-activator in the canonical Wnt pathway. A predictor marker is needed to confirm the clearance of margins surrounding a resected tumor. The current study aim is to assess β-catenin expression at the perilesional area of OSCC and relate such expression to OSCC histopathological grading.

Materials and Methods: Immunohistochemical evaluation of β-catenin expression at the perilesional area of 25 OSCC and ten normal oral mucosae from archival paraffin blocks was done. The sections were assessed according to the ability of surface epithelium at the perilesional area of OSCC in showing normal expression pattern of β-catenin in the oral mucosa.

Results: Normal oral epithelium showed strong β-catenin expression at the cell membrane, but no cytoplasmic or nuclear expression. There was no significant difference between the immunoreactivity for β-catenin in the perilesional area of the different histological grade of OSCCs.

Conclusion: β-catenin expression does not represent a valuable tool to predict the free margin.

Keywords: *β-catenin, perilesional area, immunohistochemistry.*

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Introduction:

Oral squamous cell carcinomas illustrate one of the major health affairs, with over 200,000 new cases reported worldwide yearly. Despite significant improvements in the early screening and diagnosis of these cancers, the 5-year disease free survival of those patients remains unacceptably poor. For many years, traditional grading, staging, and site of the tumor are the main prognostic factors of OSCC[1-3]. Identification of biomarkers in normal tissues adjacent to tumors (peri-tumoral cancer fields), probably is helpful for primary chemoprevention studies[4].

Surgery is the primary treatment modality and the best choice in oral cancers owing to anatomical in respect of complex bone and soft tissues in head and neck area[5]. Prognostic risk factors of oral cancer include tumor staging and grading, marginal status, lymphovascular invasion, perineural spread, and perinodal spread of regional disease, of which the only factor under clinician's control is marginal status[6]. Although surgeons always aim at a resection with a clear margin, close margins are inevitable; the oral anatomy complexity demonstrates the fact that positive margins are most frequent in oral cancer resection in comparison to the upper aerodigestive tract cancers[7]. Margins were regarded as clear when histological margin from invasive carcinoma was more than 5 mm, 1–5 mm distance was regarded as close, and less than 1 mm was regarded as involved[8].

On the other hand, individuals with one carcinoma of head and neck region have an increased possibility of getting a second malignancy. The frequency of that event varies from 16% to 36%. When a second malignancy occurs in synchronize with the initial lesion, it is called a synchronous carcinoma[9]. "Lateral cancerization" was subsequently used to indicate the lateral spread of tumors which was due to the progressive transformation of cells adjacent to a tumor, rather than the spread and destruction of the adjacent epithelium by pre-existing cancer cells[10]. It was observed that normal looking cells near malignant cells were abnormal histologically and therefore were

contemplated as a part of the transformed cells in a particular tumor field, and subsequently were responsible for the occurrence of local tumor recurrences[11]. The tendency to develop multiple carcinomas in the upper aero-digestive region is known as "field cancerization" [10-12].

β-catenin is a transcription factor that when over-activated promotes cell migration and invasion [13]. β-catenin plays a dual role in cells as a major structural component of cell-cell adherence junctions and as a signaling molecule in the Wnt pathway [14]. β-catenin of the normal oral epithelial cell was detected strongly at the cell membrane of basal, parabasal and prickle cell layers [15-17]. In OSCC, β-catenin localizes at cytoplasm and nucleus in parallel with Wnt expression at the invasive front [18].

The present study evaluation β-catenin expression distribution and cellular localization in the perilesional area (close and free margins) of OSCC, and relating such expression to the histopathological grading.

Materials and methods:

This retrospective study is concerned with the evaluation of 25 archival paraffin blocks which were previously diagnosed as OSCC with the perilesional area. They were collected from Sulaimani histopathological centers. Ten blocks of normal oral mucosa were obtained from Oral and Maxillofacial Pathology Department at School of Dentistry Sulaimani University and used as a control. The study was approved by the scientific and ethical committee of the School.

Two serial, 4μm sections were cut, one section was stained with hematoxylin and eosin to identify the perilesional area of the lesion and histopathological grading of OSCC, the other section was mounted on a positively charged slide and stained immunohistochemically with β-catenin. Sections were deparaffinized, rehydrated, and then retrieved by boiling tissue sections in citrate buffer for 15 min at 95^0C. Endogenous peroxidase activity was blocked by incubating the sections with enough drops of H2O2 for10 min. In order to prevent non-specific binding, sections were incubated with blocking serum for10 min. Then sections were incubated with the primary rabbit polyclonal antibody of β-catenin (**abcam®**; dilution 1:100) for 45 min at 37°C in a humid chamber. Complement was added and incubated for 10 min. Then detection was performed by using goat anti-rabbit HRP conjugate, and it was incubated for 15 min. The reaction was visualized by incubation with DAB for 5 min, and then sections were counterstained with hematoxylin. Negative control slides were obtained by omitting the primary antibody. Washing with phosphate buffer saline three times for 3 min each was done between all steps. Then excess buffer was tapped off gently. All system reagents were equilibrated to room temperature (20-25°C) prior to performing the procedure. Likewise, all incubations were performed at 37°C in an incubator. The sections were not allowed to dry during the staining procedure, by placing the slides in a humid environment (humidified chamber).

Slides were assessed blindly by the author. The sections were evaluated according to the ability of surface epithelium in perilesional area adjacent to OSCC in showing normal pattern of membranous strong staining in basal, parabasal and polyhedral cell layers [15-17], and the location of β-catenin expression was determined (whether membranous, cytoplasmic, or mixed), and accordingly the surface epithelia in perilesional area, was evaluated by using the following score:

1. Unchanged expression, sections revealed strong immunoreactivity that was confined to the cell membrane of basal, parabasal, and polyhedral cell layers.
2. Decreased expression, sections showed faint membranous staining.

SPSS statistical software was used to estimate Chi-square and Fisher's exact tests. Probabilities of less than 0.05 were accepted as significant.

Results:

In normal oral epithelial β-catenin expression is membranous of basal, parabasal and prickle cell layers (Figure 1-A). Among a total of 25tissue samples, 84% were an unchanged β-catenin expression and 16% were decreased β-catenin expression (Table 1, Figure 1-B).

The perilesional area was grouped according to OSCC histopathological grades into three groups: 14 with WDSCC, 7 with MDSCC, and 4 with PDSCC (Table 2). β-catenin expression was predominantly membranous (unchanged) in the perilesional area of WDSCC. Only 3 of them show cytoplasmic expression: 2 of them were mixed membranous and cytoplasmic, and one pure cytoplasmic expression (Figure 1-C). In the perilesional area of MDSCC β-catenin expression was unchanged in 5 cases, only one of them was mixed membranous and cytoplasmic expression. Lastly, all perilesional area of PDSCC was membranous. Nevertheless, they did not reach the significant level ($P>0.05$).

Discussion:

Margin adequacy is an important prognostic factor. Understanding of the molecular alteration of the perilesional area could not only assist the diagnosis and prognosis of oral cancer but might also open up novel therapeutic approaches [19]. Some researchers have recommended up to 2 cm clinical margin, whereas others considered 3 mm as sufficient [20, 21]. Currently, maximum uniformity is "1 cm three-dimensional margin" [22]. This should be reflected into >5 mm of pathological margin [23]. β-catenin function depends on its localization. It is synthesized in the cytoplasm and must be transported into the nucleus to exert its transcriptional effect. In addition to that, in Wnt pathway, it is an essential binding partner for the cytoplasmic tail of various cadherins, such as E-cadherin in adhesion junctions. Though half-life of the signaling pool of β-catenin is in the order of minutes, the adherence junction pool is highly stable. The adhesive

and signaling properties of β-catenin are most likely independent [24].

Several studies demonstrated the altered expression of β-catenin in OSCC. This work for the first time evaluates the β-catenin expression at the perilesional area of OSCC. In this work, β-catenin of the normal oral epithelial cell was detected at the cell membrane of basal, parabasal and prickle cell layers. Similarly, other studies found identical expressions [15-17, 25,26]. All the perilesional cases were positive for β-catenin and only 16% of the showed expression. This reduction in β-catenin may be associated with other molecular findings of genetically altered cells. Possibly, these cells could share some or even all genetic markers with the tumor, indicating that both have arisen from a common cell clone [27]. There have been no previous studies available to compare our results with. Thus, expression of β-catenin at the perilesional area of OSCC might give an idea about peri-tumoral cancer field, which might be useful for preventive therapy.

Just 2/14 perilesional area of WDSCC and 2/7 perilesional area of MDSCC showed decreased expression. An aberrant cytoplasmic localization of β-catenin was found in 3/14 perilesional area of WDSCC and 1/7 perilesional area of MDSCC. The loss of membranous β-catenin expression probably comes after cytoplasmic relocalization and considered as a late event [17]. However, statistical findings did not show any significant difference with histopathological grading as $p>0.05$. Thus, histopathological grading is unrelated to β-catenin expression.

Conclusion:

Positive β-catenin expression presents in all perilesional margins of OSCC, with a small percentage of reduced and altered expression. Thus, its expression does not represent a valuable tool to predict the marginal status of OSCC. β-catenin expression is unrelated to histopathological grading.

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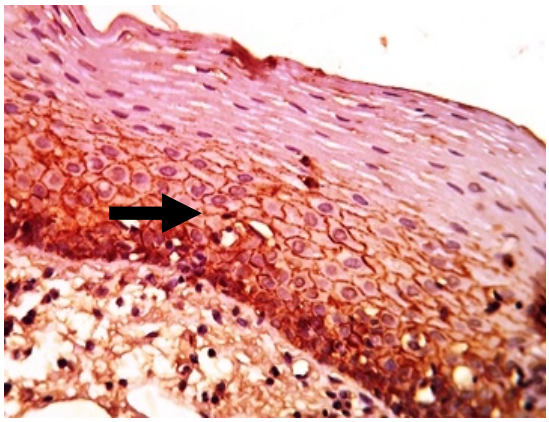
Table1: β-catenin expression in perilesional area of 25 OSCC

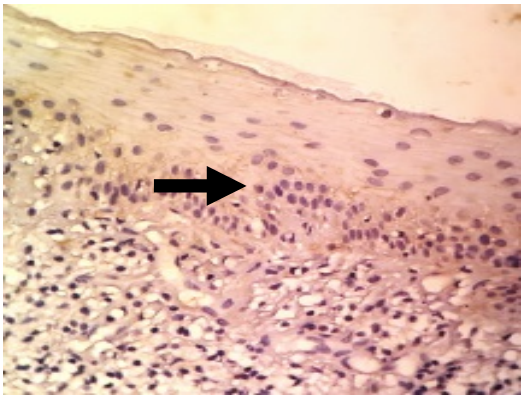
Unchanged expression		**Decreased expression**	
No.	%	No.	%
21	84%	4	16%

Table2 β-catenin expression in relation to histopathological grades at the perilesional area of OSCC

Histopathological grading	**Unchanged expression (21)**	**Decreased expression (4)**
Well (14)	12	2
Moderate (7)	5	2
Poor (4)	4	0
Total	21	4

No significant difference was found as P > 0.05.





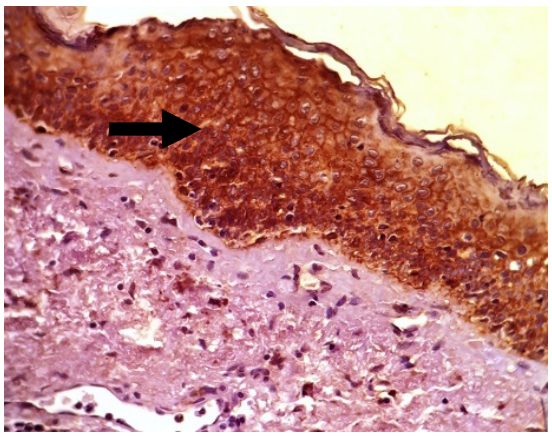


Figure 1: (A) β-catenin membranous expression (arrow) in normal oral mucosa.

β-catenin expressions (arrow) in the perilesional area:

(B) Decreased expression.

(C) Membranous and cytoplasmic staining (IHC, 400X).

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