Expression of E-cadherin and B-cell Lymphoma 2 in Oral Cancer: A Ratio-based Planning for Targeted Therapy

Abstract

Background: Oral cancer is known to be governed by the antiapoptotic and loss of cell adhesion properties which dictate its progression. Aim: To study the immunexpression of E-cadherin and Bcl-2 in varying TNM stages and histopathological grades of OSCC. Materials and Methods: 11 cases of well differentiated, 10 cases of poorly differentiated and 11 cases of poorly differentiated OSCC were studied immunohistochemically using archival paraffin embedded tissue specimens. Statistical Analysis: Differences between the different variables were analyzed using ANOVA test, Kruskal–Wallis test and post hoc test followed by Bonferroni test. The resulting data was analyzed using SPSS software version 19. Results: The expression of Bcl-2 and E cadherin immunopositivity was associated positively with tumor grade, high T category and Histopathological grades. Conclusions: The results of this study points to the significance of cell proliferation and invasion as a major determinant of prognosis in OSCC.

Keywords: B-cell lymphoma 2, E-cadherin, oral squamous cell carcinoma, prognosis

Introduction

Oral cancer constitutes a significant part of the global burden of cancer. India contributes up to 7.8% of the global cancer burden and 8.33% of global cancer deaths. International Agency for Research on Cancer, an arm of the World Health Organization (WHO), has ranked oral cancer as sixth most common malignancies. The alarmingly high incidence of oral squamous cell carcinoma (OSCC) is indeed a major health issue for developing nations where it accounts for 20 per 1 lakh new cases every year[1] and the most promising strategy for the treatment of OSCC remains the breakthroughs in the fields of molecular targeted therapy.

E-cadherin acts as a growth suppressor by cyclin-dependent kinase inhibitor p27, dephosphorylates Rb protein, leads to late reduction in cyclin D1 protein and Wnts, upregulates E-cadherin suppressors, i.e., Snail and Twist, and downregulates miR-20014.[2] B-cell lymphoma 2 (Bcl-2) protein is an antiapoptotic gene which is an promoter of invasion, death antagonist which facilitates the permanent acquisition of mutations and malignant transformation.

In vitro experiments have shown that intercellular communication may modulate apoptosis both in normal and tumorous tissue through Wnt signaling pathways.[3]

E-cadherin and Bcl-2 have antagonistic role in progression of oral cancer. Thus, the present study is designed to assess and correlate the immunoexpression of E-cadherin and Bcl-2 in various histopathological grades of OSCC, hypothesized E-cadherin and Bcl-2 antagonistic interaction in pathogenesis of OSCC and to deduce a probable E-cadherin/ Bcl-2 ratio targeted molecular therapy.

Materials and Methods

Patients and tumor samples

The biopsy specimens of cases obtained were fixed with 10% neutral buffered formalin processed and embedded in paraffin wax. Three microns section was obtained for hematoxylin and eosin staining and immunohistochemistry (IHC) procedure. The samples comprised of 32 cases of OSCC from the archives of the department. Clinical data of each case such as and tumor, node, metastasis (TNM) staging were...
collected. Archival specimens were grouped based on the WHO (2005) histopathological grading criteria as follows: Group 1 - Well-differentiated OSCC \((n = 11)\), Group 2 – Moderately differentiated OSCC \((n = 10)\), and Group 3 – Poorly differentiated OSCC \((n = 11)\). Different aspects of the same study were undertaken simultaneously such as clinical parameters, Histopathological grades and pattern of invasion. This study highlights the correlation of expression of markers with TNM stage and histopathological grades of study cases.

**Immunohistochemistry with E-cadherin and Bcl-2**

Immunohistochemistry procedure was done using standard heat induced epitope retrieval methods. Archival sections on glass slides coated with Poly-L-lysine were used to carry out the immunohistochemistry procedure using heat induced epitope retrieval method. Diaminobenzidine (DAB) was used as chromogen to detect Bcl-2 and E-cadherin positivity.

**Immunoscoring criteria**

Five randomly selected fields with minimum 200 cells per field at 40× magnification were selected and the percentage of positive tumor cells out of total number of neoplastic cells present in the same field were scored as mentioned below: Semiquantitative scoring for E-cadherin and Bcl-2 was done as Low, intermediate, and high \(<10\%\), \(10\%–50\%\), \(>50\%\), respectively.\(^{4,5}\) Similarly, qualitative scoring was done as (+, ++, and +++). The images of five high-power fields \((40×)\) were obtained with a digital camera (Olympus EPL3) Fixed to the research microscope and was transferred to computer system for analysis which was done using a grid. Immunoreactive score was then evaluated by number of positive cells (cytoplasm and nucleus of epithelial cells for Bcl-2 and membranous and cytoplasmic for E-cadherin) per 1000 total tumor cells of all grades of OSCC. Combined scoring was also done and cases divided as low and high grades \((\text{Low as 0-2 , high as 3-4})\).\(^{6}\)

**Statistical analysis**

The resulting data were analyzed using SPSS statistics for Windows, Version 19.0(Armonk, NY; IBM Corp). Data have been expressed as mean and standard deviation. Differences between the different variables were analyzed using ANOVA test, Kruskal–Wallis test and post hoc test followed by Bonferroni test. Besides this, area under the curve values was calculated by applying the receiver operating characteristic (ROC) curve analysis for both the molecules. Pearson’s Chi-square test was carried out to determine the level of correlation or association between the groups under study. \(P < 0.05\) was considered statistically significant.

**Results**

**The expression of Bcl-2 and E-cadherin**

The qualitative scoring of Bcl-2 immunoexpression increased gradually from well to poorly differentiated OSCC with moderate \((++)\) staining intensity present in 72.7% cases of well-differentiated OSCC in comparison to intense \((+++\)) staining intensity present in 100% cases of poorly differentiated OSCC. Semiquantitative scoring of Bcl-2 immunoexpression increased from well to poorly differentiated OSCC with 81.8% well-differentiated OSCC showing 5%-50% positive cells whereas 90.9% poorly differentiated OSCC with >50% positive cells. The qualitative scoring of E-cadherin immunoexpression decreased in a gradual, incremental manner from well to poorly differentiated OSCC with +++ scoring present in 63.6% cases of well-differentiated OSCC in comparison to + scoring present in 72.7% cases of poorly differentiated OSCC. Semiquantitative scoring of E-cadherin immunoexpression decreased from well to poorly differentiated OSCC with 72.7% well-differentiated OSCC showing >50% positive cells whereas 54.5% poorly differentiated OSCC with <10% positive cells. Results for both the parameters were statistically significant, i.e., \(P < 0.05\) [Tables 1 and 2].

The mean score of Bcl-2 immunoexpression quantitatively increased in a gradual gradational manner from well to poorly differentiated OSCC, i.e., well differentiated with 437.55 to poorly differentiated with 926.18 whereas a mean score of E-cadherin immunoexpression quantitatively decreased from well to poorly differentiated OSCC, i.e., well differentiated with 867.45 to poorly differentiated with 301.09. These results were statistically significant when correlated to histopathological grades but not when compared to TNM staging \((P < 0.05)\) [Graphs 1 and 2].

The ratio decreased gradually from well-differentiated OSCC \((1.5454)\) to poorly differentiated OSCC \((0.5454)\), from stage 1 \((1.4)\) to stage 4 \((0.72)\).

**Receiver Operating Characteristic (ROC) curve**

The ROC curve shows that area under the graph for Bcl-2 immunoexpression was 20.8% (with standard error as 0.092), and for E-cadherin immunoexpression was 71.9% (with standard error as 0.095), predicting Bcl-2 to be a less sensitive and specific marker as compared to E-cadherin. However, E-cadherin/Bcl-2 ratio had an area under the graph as 78.6% (with standard error as 0.079), indicating it to be the most sensitive and specific parameter to analyze the progression of OSCC cases. The result was statistically significant \((P < 0.05)\) [Graph 3].

**Discussion**

Deregulation of oncogenes and tumor suppressor genes involved in apoptosis has been associated with tumor pathogenesis and progression.\(^{7}\) Bcl-2 protein leads to cellular...
immortalization, contributing to the formation of the tumor and facilitating the permanent acquisition of mutations. On the contrary, loss or reduction of E-cadherin expression can be caused by somatic mutations of CDH1 promoter.

Bcl-2 expression was seen in the form of granular cytoplasmic to nuclear staining. All well and moderately differentiated OSCC tumor islands showed positive expression except the islands with central keratinization where only peripheral cells were positive and central core with less or no expression suggesting that cells expressing antiapoptotic activity have a stem cell property representing permanent self-renewal which was in contrast to Bcl-2 staining restricted to tumor cells within the centre of tumor islands. Diffuse immunopositivity was seen throughout the tumor cell population in PDSCC suggesting overexpression possibly reflecting the resistance of these tumor cells to apoptosis and increased cell survival and the loss of ability of malignant keratinocytes to differentiate terminally.

The Bcl-2 mean score was statistically significant \( (P < 0.05) \) with \( 437.55 \pm 29.737 \) in well-differentiated squamous cell carcinoma, \( 576.10 \pm 10.22 \) in moderately differentiated OSCC, and \( 926.18 \pm 11.28 \) in poorly differentiated OSCC. Semi-quantitative scoring of Bcl-2 immunoexpression also increased from 81.8% WDSCC having 5%–50% positive cells whereas 90.9% PDSCC with >50% positive cells.

It has also been indicated that this oncoprotein may play a role in relatively early events of OSCC but a decrease in number of Bcl-2 positive cells with a decrease in differentiation has also been reported.

### Table 1: Immunoscoring of Bcl-2 expression in study cases

| H/P Grade                  | Qualitative Score | Semi-quantitative Score |
|----------------------------|-------------------|-------------------------|
|                            | Mild (+)          | Moderate (++)           | Intense (+++)          | Low (<5%)     | Intermediate (5-50%) | High (>50%) |
| Well differentiated OSCC (n1) | 9.1% (1/11)       | 72.7% (8/11)            | 18.2% (2/11)           | 0%            | 81.8% (9/11)          | 18.2% (2/11) |
| Moderately differentiated OSCC (n2) | 0%                | 50% (5/10)             | 50% (5/10)             | 0%            | 50% (5/10)           | 50% (5/10)   |
| Poorly differentiated OSCC (n3) | 0%                | 0%                     | 100% (11/11)          | 0%            | 9.1% (1/11)          | 90.9% (10/11) |
| Total (n)                  | 3.1% (1/32)       | 40.6% (13/32)          | 56.3% (18/32)         | 0%            | 46.9% (15/32)        | 53.1% (17/32) |
| P                          | 0.00              | 0.00                   | 0.00                   | 0.00          | 0.00                 | 0.00         |

### Table 2: Immunoscoring of E-cadherin expression in study cases

| H/P grade                  | Qualitative Score | Semi-quantitative Score |
|----------------------------|-------------------|-------------------------|
|                            | Mild              | Moderate                 | Intense                | Low          | Intermediate (5-50%) | High (>50%) |
| Well differentiated OSCC (n1) | 0%                | 36.4% (4/11)           | 63.6% (7/11)          | 0%           | 27.3% (3/11)         | 72.7% (8/11) |
| Moderately differentiated OSCC (n2) | 0%                | 60% (6/10)             | 40% (4/10)            | 0%           | 60% (6/10)           | 40% (4/10)   |
| Poorly differentiated OSCC (n3) | 72% (8/11)       | 18.2% (2/11)           | 9.1% (1/11)           | 54.5% (6/11) | 27.3% (3/11)        | 18.2% (2/11) |
| Total (n)                  | 25% (8/32)        | 37.5% (12/32)          | 37.5% (12/32)         | 18.8% (6/32) | 37.5% (12/32)       | 43.8% (14/32) |
| P                          | 0.00              | 0.00                   | 0.00                   | 0.00         | 0.00                 | 0.00         |
Table 3: Correlation of histopathological grades with mean scores of qualitative, semi-quantitative and combined assessment of Bcl-2 and E-cadherin in study cases

| H/P Grade       | Bcl-2 Qualitative | Bcl-2 Semi-quantitative | Bcl-2 Combined score | E-cadherin Qualitative | E-cadherin Semi-quantitative | E-cadherin Combined score |
|-----------------|-------------------|-------------------------|----------------------|------------------------|------------------------------|---------------------------|
| Well differentiated OSCC (n1) | 1.09±0.53         | 1.18±0.405              | 1.27±0.46             | 1.63±0.5045            | 1.73±0.467                   | 1.82±0.405                |
| Moderately differentiated OSCC (n2) | 1.5±0.527         | 1.50±0.527              | 1.70±0.483             | 1.4±0.516               | 1.40±0.516                   | 1.60±0.516                |
| Poorly differentiated OSCC (n3) | 2±0               | 1.91±0.302              | 2.00±0.00             | 0.36±0.67              | 0.64±0.809                   | 1.18±0.405                |
| Total (n)       | 1.53±0.56         | 1.53±0.507              | 1.66±0.483             | 1.125±0.79311          | 1.25±0.762                   | 1.53±0.507                |
| P               | 0.001             | 0.000                   | 0.000                 | 0.004                  | 0.000                        | 0.002                     |
Table 4: Correlation of TNM stages with mean scores of qualitative, semi-quantitative and combined assessment of Bcl-2 and E-cadherin in study cases

| Clinical parameters | Bcl-2                          | E-cadherin                       |
|---------------------|--------------------------------|---------------------------------|
|                     | Qualitative                | Semi quantitative | Combined score | Qualitative                | Semi quantitative | Combined score |
| Tumor size          |                              |                                |                |                              |                                |                |
| T1                  | 1±0.6324                    | 1.33±0.516                     | 1.33±0.516     | 1±0.632                     | 1.67±0.516         | 1.67±0.516     |
| T2                  | 1.16±0.408                  | 1.17±0.408                     | 1.33±0.516     | 1.5±0.5477                  | 1.50±0.548         | 1.83±0.408     |
| T3                  | 1.77±0.4277                 | 1.67±0.485                     | 1.83±0.383     | 1±0.8401                    | 1.17±0.786         | 1.44±0.511     |
| T4                  | 2±0                          | 2.00±0.00                      | 2.00±0.00      | 0                            | 0.00±0             | 1.00±0         |
| Total               | 1.53±0.56                   | 1.53±0.507                     | 1.66±0.483     | 1.125±0.79311               | 1.25±0.762         | 1.53±0.507     |
| P                   | 0.008                        | 0.123                          | 0.003          | 0.2076                       | 0.079              | 0.047          |
| Nodal status        |                              |                                |                |                              |                                |                |
| N0                  | 1.07±0.435                  | 1.38±0.506                     | 1.38±1.38      | 1.38±0.506                   | 1.62±0.506         | 1.69±0.480     |
| N1                  | 1.7±0.4830                  | 1.50±0.527                     | 1.70±0.483     | 1.3±0.9486                   | 1.10±0.876         | 1.60±0.516     |
| N2                  | 2±0                          | 1.78±0.441                     | 2.00±0         | 0.55±0.726                   | 0.89±0.782         | 1.22±0.441     |
| N3                  | 0                            | 0                              | 2.00±0         | 0                            | 0                  | 0              |
| Total               | 1.53±0.56                   | 1.53±0.507                     | 1.66±0.483     | 1.125±0.79311               | 1.25±0.762         | 1.53±0.507     |
| P                   | 0.000                        | 0.338                          | 0.002          | 0.06                         | 0.134              | 0.040          |
| TNM                 |                              |                                |                |                              |                                |                |
| Stage 1             | 1±0.63                      | 1.33±0.516                     | 1.33±0.516     | 1.5±0.54                     | 1.67±0.516         | 1.67±0.516     |
| Stage 2             | 1.25±0.5                    | 1.25±0.500                     | 1.25±0.500     | 1.25±0.5                     | 1.50±0.577         | 1.75±0.500     |
| Stage 3             | 1.76±0.438                  | 1.54±0.519                     | 1.69±0.480     | 1.307±0.85                   | 1.23±0.832         | 1.62±0.506     |
| Stage 4             | 1.88±0.33                   | 1.78±0.441                     | 2±0.00         | 0.55±0.726                   | 0.89±0.782         | 1.22±0.441     |
| Total               | 1.53±0.56                   | 1.53±0.507                     | 1.66±0.483     | 1.125±0.79311               | 1.25±0.762         | 1.53±0.507     |
| P                   | 0.012                        | 0.233                          | 0.017          | 0.1327                       | 0.253              | 0.181          |

Figure 1: Well-differentiated oral squamous cell carcinoma showing immunopositivity of Bcl-2. Periphery cells of tumor islands shows Bcl-2 positivity (×10, inset ×40)

Figure 2: Well-differentiated squamous cell carcinoma showing immunopositivity of E-cadherin. All cells in tumor islands showing positive expression of E-cadherin (×10, inset ×40)

Conclusions

Collectively, the present work has provided new insights on how elevated expression of antiapoptotic Bcl-2 not only increases tumorigenicity but, through induction of an epithelial-mesenchymal transition-like phenotype, triggers an invasion-metastatic profile. It remains unclear exactly the means by which Bcl-2 induce such a diverse set of crucial signaling events culminating in enhanced metastatic potential. Thus, a combined ratio of both molecules is a helpful guide to categorize OSCC cases based on their prognostic implications. Moreover, loss of E-cadherin in invasive tumor cells may lead to increased Bcl-2 expression and resistance to chemotherapeutic drugs.

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Nil.
Table 5: Combined score E-cadherin/Bcl-2 ratio correlation to histopathological grades and TNM stages

| Well differentiated OSCC | TNM          |
|-------------------------|-------------|
|                         | Stage1 | Stage2 | Stage3 | Stage4 |
| n 1                     | 1      | 5      | 2      | 4      | 0      |
| E-cadherin/Bcl-2        | 1.54 ± 0.5222 | 1.4 ± 0.54 | 1.5 ± 0.707 | 1.75 ± 0.5 | 0      |
| Moderately differentiated OSCC | 10 | 1 | 2 | 5 | 2 |
| E-cadherin/Bcl-2        | 1.1 ± 0.6245 | 1 ± 0   | 1.25 ± 1.06 | 1.2 ± 0.75 | 0.75 ± 0.35 |
| Poorly differentiated OSCC | 11 | 0 | 0 | 4 | 7 |
| E-cadherin/Bcl-2        | 0.5454 ± 0.1507 | 0 | 0 | 0.625 ± 0.25 | 0.5 ± 0 |
| Total (n)               | 32     | 6      | 4      | 13     | 9      |
| E-cadherin/Bcl-2        | 1.20 ± 0.75822 | 1.8 ± 0.2647 | 1.64 ± 0.4870 | 1.11 ± 0.8539 | 0.72 ± 0.60914 |
| P                       | 0.000  |        |        | 0.022  |        |

Conflicts of interest

There are no conflicts of interest.

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Figure 3: Poorly differentiated squamous cell carcinoma showing diffuse and intense immunopositivity of Bcl-2 in tumor cells (×10, inset ×40)

Figure 4: Poorly differentiated oral squamous cell carcinoma showing weak immunopositivity of E-cadherin in tumor cells (×10, inset ×40)
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