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BRCA1/2 Expression Patterns in Different Grades of Oral Squamous Cell Carcinoma

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Abstract

Background: Oral squamous cell carcinoma (OSCC) is the sixth most common cancer worldwide and has a poor prognosis. The breast cancer 1 (*BRCA1*) and breast cancer 2 (*BRCA2*) genes are the key tumor suppressor genes responding in the cases of DNA damage. They repair double-strand DNA breaks to maintain gene stability. Mutations in *BRCA1* and *BRCA2* lead to genetic instability and develop different cancers, mainly familial breast and ovarian cancers. This study aimed to investigate the expression profiles of *BRCA1* and *BRCA2* genes in OSCC through the use of immunohistochemistry (IHC) technique.

Method: In this retrospective study, a total of 60 samples (20 samples of each grade) were collected from the archive of pathology department of Taleghani educational hospital, Tehran, Iran, from 2000-2017. IHC staining was performed for all tissue samples.

Results: BRCA1 immunoreactivity was positive in the cytoplasm and nuclear of 56 and 24 samples, respectively. None of the cancer cells showed nuclear BRCA2 expression; however, BRCA2 cytoplasmic staining existed in 17 cases. Chi-square test showed statistically significant differences between BRCA1 staining (P=0.001) and histological grade, and between BRCA2 expression (P=0.001) and histological grade in the research groups.

Conclusion: Altered subcellular localization of BRCA1/2 and immunostaining of the cancer cells at the invasive front may indicate the critical role of *BRCA1*/2 in the development of OSCC. Early detection of *BRCA* mutation carriers by IHC has a significant impact on successful treatment.

Keywords: *BRCA1*, *BRCA2*, Immunohistochemistry, Mouth, Neoplasm

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Introduction

Head and neck cancers are highly heterogeneous cancers, 90% of which are squamous cell carcinoma developing in the oral cavity.[1] With a poor prognosis, oral squamous cell

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carcinoma (OSCC) is the sixth most prevalent cancer worldwide.[2] Many factors contribute to the development of oral cancer such as age, smoking, HPV infection, genetic factors, and a variety of environmental carcinogens.[3] Breast cancer 1 (*BRCA1*) and breast cancer 2 (*BRCA2*) genes are the key tumor suppressor genes that respond in the cases of DNA damage. They repair double-strand DNA breaks to maintain gene stability.[4] Mutations in *BRCA1*/2 lead to genetic instability and development of different cancers, familial breast and ovarian cancers in particular.[5] Aberrant BRCA1 expression indicates the impairment of DNA repair mechanisms.[6] Therefore, *BRCA1*/2 genes have essential roles in maintaining genomic stability and preserving chromosomal structures.[7] Mutations in *BRCA1*/2 genes are associated with a higher risk for breast, ovary, prostate, and pancreas cancers.[8] In prostate cancer, *BRCA1*/2 mutations are associated with more aggressive types and higher rates of nodal metastasis and distant metastasis.[9] Pancreatic, prostate, and stomach cancers are also related to *BRCA1*/2 mutations. Regarding both prostate and stomach cancers, the risk of developing cancer is higher in patients with *BRCA1* mutation compared to those with *BRCA2* mutation.[10] *BRCA* mutation has been reported in 45%-80% of breast cancers and 18%-40% of ovarian cancers.[11] BRCA1/2 proteins interact with several nuclear proteins; therefore, they play myriad essential roles in cells.[12] Besides, it has been demonstrated that germline mutations in DNA repair genes are related to congenital defects.[5] A very recent study on craniofacial bone development showed that disrupted *BRCA1*/2 in a neural crest cells developed craniofacial abnormalities in mice. Among the craniofacial bones, frontal-nasal and maxilla-mandibular bones were severely compromised. Interestingly, this study indicated that the inactivation of *p53* reduced DNA damage-induced cell death, thereby reducing the craniofacial bone defects due to *BRCA1* deficiency.[9] Immunohistochemistry (IHC) is an easy and inexpensive test that determines the immunolocalization of BRCA proteins in several cancers such as salivary gland tumors, breast cancer, epithelial ovarian carcinoma, and prostate cancer. This test can be further employed as a prognostic biomarker for the mentioned conditions.[4] Accurate techniques are required for early diagnosis. One of these techniques is IHC.[4] This study aimed to investigate the expression profile of *BRCA1*/2 genes in OSCC by using IHC. Understanding the mechanisms and pathways involved in OSCC development helps to find new therapeutic strategies. With appropriate therapy, OSCC patients have a greater chance for prolonged survival and low morbidity.

Material and Methods

In this retrospective study, we collected a total of 60 samples (20 samples of each grade) from the archive of pathology department of Taleghani educational hospital, Tehran, Iran, from 2000-2017. The previous diagnosis was confirmed by hematoxylin and eosin (H&E) staining.

IHC staining

We performed IHC staining for all tissue samples. Briefly, the paraffin blocks were cut into 4 µm thick sections. Next, the sections were deparaffinized and dehydrated with graded alcohol. We performed the antigen retrieval in citrate buffer (pH=6). Endogenous peroxidase activity was blocked by using Leica detection kit. Later, the slides were incubated with primary mouse monoclonal anti-BRCA1 antibody (Abcam, ab16780) at 1:90 dilution and primary rabbit polyclonal anti-BRCA2 antibody (Abcam, ab 27976) at 1:80 for 1 hour at room temperature, followed by incubation with secondary antibody for 30 min and immunostaining with DAB (3, 3' diaminobenzidine) for 5 min as a chromogen and hematoxylin as counterstain. We utilized breast carcinoma tissue as a positive control. Eliminating of the primary antibody was used as negative control.

Detection and scoring

Two independent pathologists familiar with IHC scored all the sections. Brown cytoplasmic/nuclear staining and brown cytoplasmic staining were considered as a positive

immunoreactivity for BRCA1 and BRCA2, respectively. Semi-quantitative scoring system was employed for the percentage of positively stained cancer cells as follows: occasional staining <10% was considered negative, 10%-40% was low, 40%-70% was moderate, and >70% was considered as strong staining.[4]

Statistical analysis

The statistical analysis was performed by the Statistical Package for Social Sciences software version 22.0 (Chicago, IL, USA). We used chi-square and one-way ANOVA tests to examine the differences between the variables. Significance level was set at 0.05.

Results

We considered a total of 60 samples for immunohistochemical analysis. BRCA1 immunoreactivity was positive in the cytoplasm and nuclear of 56 and 24 samples, respectively. None of the cancer cells showed nuclear BRCA2 expression; however, BRCA2 cytoplasmic staining existed in 17 cases. According to chi-square test, the difference was statistically significant for both cytoplasmic and nuclear BRCA1 staining (P=0.001 and P=0.001, respectively) and cytoplasmic BRCA2 expression (P=0.001) in the research groups. One-way ANOVA test also showed significant differences between the lesion type and cytoplasmic BRCA1 expression (P=0.001), nuclear BRCA1 expression (P=0.001), and cytoplasmic BRCA2 expression (P=0.001). Table 1 presents the results of chi-square test.

Discussion

In the current study, we studied the expression levels of BRCA1/2 in different grades of OSCC. Immunoreactivity showed that the cytoplasmic/nuclear expression levels of BRCA1 significantly increased in OSCC tissue samples. 38 cases (63.3%) of intermediate grade and high grade tissue samples showed a moderate to strong cytoplasmic immunoreactivity of BRCA1 (Figures 1 and 2). However, moderate to strong nuclear BRCA1 expression was only present in 17 cases (28.3%) of intermediate grade and high grade samples (Figures 3 and 4). Moreover, 33 cases (55.01%) of intermediate grade and high grade samples had moderate to strong cytoplasmic BRCA2 immunoreactivity (Figures 5 and 6). Totally, four cases (two cases for each protein) of low-grade tissue samples exhibited strong cytoplasmic immunolabeling of BRCA1/2 (Figures 7 and 8). In a previously published paper, the expression of BRCA1 was investigated in oral leukoplakia and tongue cancer by immunostaining. A strong BRCA1 expression was found in 33% of leukoplakia with mild to moderate dysplasia. However, only 1% of tongue cancer tissues showed a strong positivity.[13] In the current study, a different manufacturer provided the primary

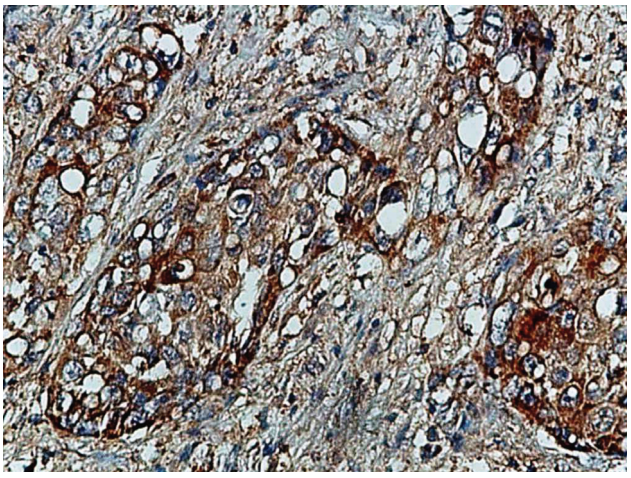

Figure 1. High magnification illustrates strong *BRCA1* positivity in intermediate grade tumor. Cytoplasmic staining is evident in cancer cells at the invasive front (×400).

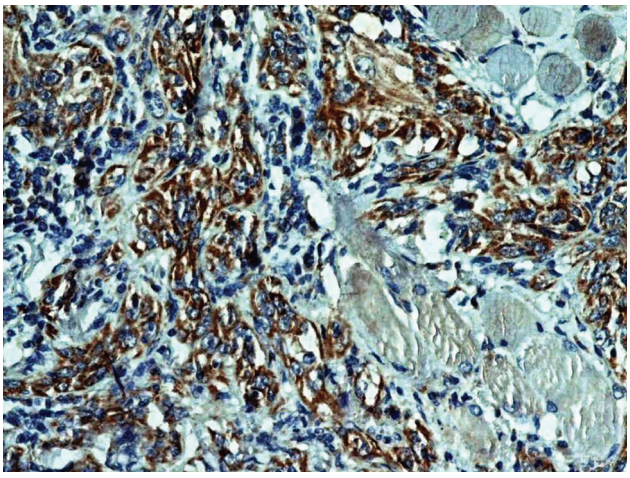

Figure 2. Paraffin section of high grade tumor shows strong cytoplasmic positivity of *BRCA1* in tumor cells at invasive front and detached tumor cells (×400).

Table 1. The expression levels and histopathological variables in different grades of oral squamous cell carcinoma

Histopathological variables	Low grade	Intermediate grade	High grade	*P*
***BRCA 1*(cytoplasmic)**				
Weak	12 (60%)	2 (10%)	0	=0.001*
Moderate	2 (10%)	10 (50%)	4 (20 %)	
Strong	2 (10%)	8 (40%)	16(80 %)	
Negative	4 (20%)	0	0	
***BRCA1* (nuclear)**				
Weak	0	7 (35 %)	0	=0.001*
Moderate	0	5 (25 %)	4 (20 %)	
Strong	0	3 (15 %)	5 (25 %)	
Negative	20 (100 %)	(25 %)	11(55 %)	
***BRCA2* (cytoplasmic)**				
Weak	12(60 %)	5 (25%)	2 (10 %)	=0.001*
Moderate	3 (15 %)	6 (30 %)	6 (30 %)	
Strong	2 (10%)	9 (45 %)	12(60 %)	
Negative	3 (15 %)	0	0	

antibody was provided from a different manufacturer. A published work on carcinoma ex pleomorphic adenoma demonstrated a positive cytoplasmic and nuclear staining for BRCA1 in 93.3% of the samples.[4] Another study on breast cancer reported that BRCA1 mutated cases were associated with higher histological grades and higher nodal infiltration rates.[14] Furthermore, one study on breast cancer tissue samples documented increased expressions of BRCA1 and BRCA2 proteins in 65% and 60% of samples, respectively.[15] A previously published work on breast cancer compared the expression levels of BRCA1 between young and older patients. According to the results of this study, 65.4% of the young group and 35.7% of the older group exhibited cytoplasmic positivity. Moreover, the young patients had higher histopathologic grade and lymph node metastasis rates.[16] In a study on ovarian cancer, BRCA1/2 expression levels were higher in tumor samples compared to the control tissues. Additionally, BRCA2 expression level augmented with tumor histologic grade.[17] A prospective study on women with BRCA1/2 mutations reported a significantly higher risk of colorectal cancer.[18] In a study on soft tissue sarcomas, immunostaining of BRCA1/2 was evaluated. Both cytoplasmic and nuclear BRCA1/2 stainings were noticed and correlated to the histologic subtypes.[19] Utilizing IHC

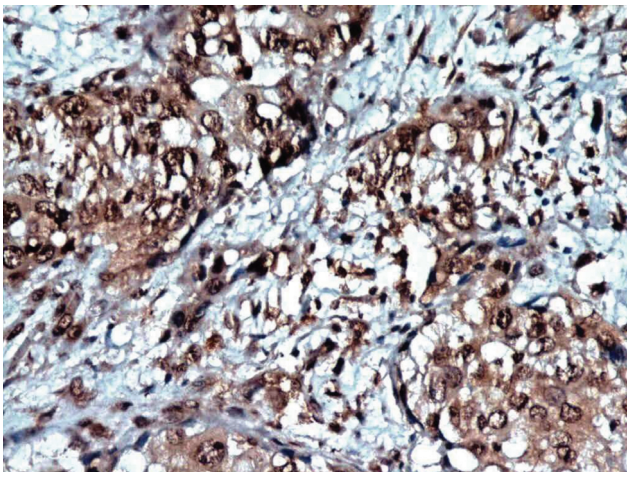

Figure 3. High power section of intermediate grade tumor demonstrates strong positive products of *BRCA1* localized in the cytoplasm and nucleus of tumor cells (×400).

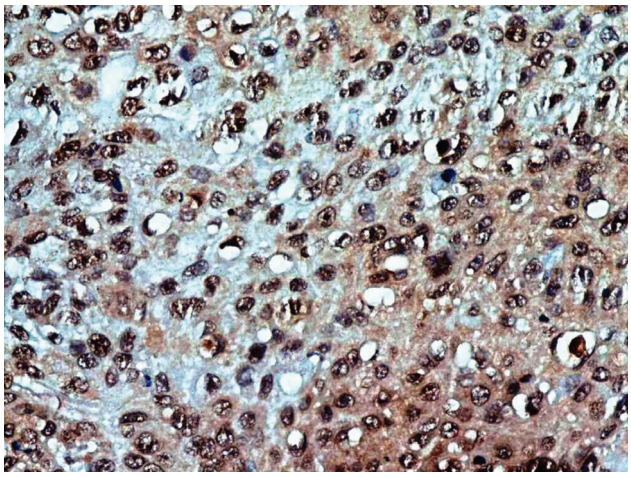

Figure 4. Histologic section of high grade tumor shows clear and strong cytoplasmic and nuclear *BRCA1* labeling in cancer cells (×400).

technique, the protein expression of BRCA1/2 was studied in gastric cancer, colorectal cancer, hepatocellular carcinoma, and pancreatic cancer. In this study, a positive cytoplasmic and nuclear BRCA1 immunostaining was visualized in the cancer cells of all tissue samples; however, BRCA2 was only detected in the cytoplasm of normal and cancerous cells in all samples except one pancreatic cancer tissue. Interestingly, low cytoplasmic BRCA1/2 expression was associated with poor histologic cancer cell differentiation in gastric and colorectal cancers. The authors suggested that the cytoplasmic localization of BRCA1/2 was an independent predictor of good prognosis in gastric and colorectal cancers and BRCA1/2 could be used as biomarkers in these cancers.[20] In a study on pancreatic ductal adenocarcinoma, reduced BRCA1 intensity was associated with a higher pathologic stage. The authors suggested a possible association between BRCA1 expression pattern and pathologic stage, indicating the potential role of *BRCA1* in the development and growth of pancreatic ductal adenocarcinoma.[21]

A published study revealed that the loss of *BRCA1* was not sufficient to develop a cancer, and certain other mutations and signaling pathways were further required for tumorigenesis.[22] Interactions between *BRCA1*/2 and *p53*, a tumor suppressor gene and *c-myc*, an oncogene, might suggest the role of *BRCA1*/2 in the repair of damaged DNA.[23] *BRCA1* and *p53* contribute to some cellular processes such as DNA repair, cell-cycle arrest, and apoptosis. For instance, in breast cancer, BRCA1 positive tumors showed elevated p53 expression levels.[24, 25] Besides, *p53* mutation was present in 20%-40% of breast cancer patients and was correlated with the histologic grade.[26] Mutant p53 protein existed in thyroid cancer and OSCC.[27,28] In a study worked on OSCC, p53 mutation was observed in 63.3% of the tissue samples.[29] A previously published work on oral leukoplakia and OSCC showed that the expression of p53 increased by the progression of the lesion towards the malignancy. In addition, less histologic differentiation of OSCC was associated with higher p53 expression levels.[30] P53 immunoreactivity in thyroid cancers was further associated with less differentiated and more aggressive tumors.[31] According to a previous study, women smoking cigarettes were more *BRCA1* mutation carriers (78%) and ran a higher risk of breast and ovarian cancers.[32] A published investigation found that 83.1% of patients with OSCC and Shisha smoking history showed a positive staining for p53 expression. The authors strongly proposed that p53 mutation was related to Shisha smoking in OSCC.[33] Accumulating evidence indicates the possibility of an increased risk of breast cancer among *BRCA1* carriers associated with previous, not current, smoking. This finding suggests that the effect of carcinogens

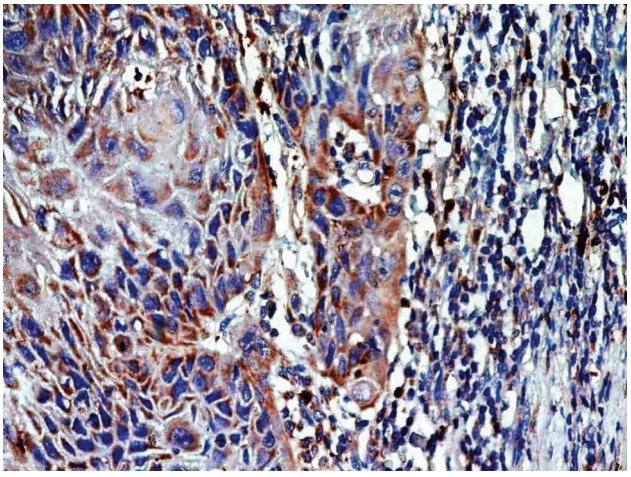

Figure 5. High magnification of intermediate grade tumor indicates strong BRCA2 immunoreactivity in the cytoplasm of cancer cells. (×400)

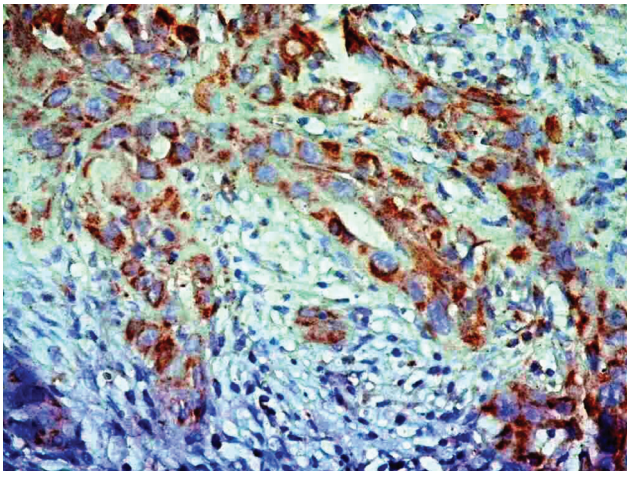

Figure 6. The high power magnification view of a high grade tumor shows strong immunolocalization of BRCA2 in the cytoplasm of tumor cells. (×400)

on *BRCA* mutations depends on the exposure time.[34] Inversely, alcohol consumption does not have any effect on the risk of breast cancer in women carrying a *BRCA* gene mutation.[35,36] Moreover, a relationship between smoking and aggressive prostate cancer has been reported.[37] Women with more than 10 years of smoking history run a higher risk of mucinous ovarian cancer.[38] Because BRCA proteins are involved in the repair of double stranded DNA breaks and damage due to oxidative stress, and due to the incomplete penetrance of *BRCA* mutation, it is suggested that exogenous factors such as cigarette smoking may be involved in the development of OSCC via the mutation of *BRCA1/2* genes.

Additionally, *BRCA1* controls the expression of some cell-surface molecules such as E-cadherin and vimentin; these are the most important molecules involved in epithelial-mesenchymal transition (EMT) phenomenon.[1,34] A previous study reported that *BRCA1* bound to miR-205 to involve in EMT and tumor invasion.[39] A recently published paper on breast cancer indicated the role of *BRCA1* in the cancer cell migration and EMT phenomenon.[34] Cancer cell migration significantly influences invasion and metastasis. In the present study, BRCA1 was present at invasive front and in the detached cells (Figures 1and 2).

A recent work reported the association of *BRCA1*/2 genes with non-syndromic cleft lip/palate.[35] The authors concluded that the involvement of certain genes at the early stages of embryonic development might play a role in cancer development later in life.[35] It is known that *BRCA1*/2 affects the development of craniofacial bones and more specifically, non-syndromic cleft lip and palate.[5,35] Of note, in patients with non-syndromic cleft lip and palate, the expression level of *BRCA1*/2 genes significantly decreased in dental pulp cells compared to controls.[36] Interestingly, previously published works have demonstrated an increased risk of cancers in the families and parents of children born with cleft lip/palate.[37,38] This finding might demonstrate the involvement of the same genes in cancer patients and those with oral clefts.[40-42] Taken together, *BRCA1*/2 are involved in the development of cancers such as breast and oral cancers. *BRCA1* collaborates with other genes such as *p53* to develop cancer. Moreover, *BRCA1* is able to induce craniofacial bone defects partially via inactivation of *p53*.[5] Furthermore, *BRCA1* induces EMT, an important event during embryogenesis such as palatogenesis. EMT has also been reported in cancers.[1,43,44]

In conclusion, the current study proved that BRCA1/2 positive oral cancer samples have a higher histologic grade. These findings suggest that the altered subcellular localization of BRCA1/2 and immunostaining of cancer cells at the invasive front may indicate the critical role

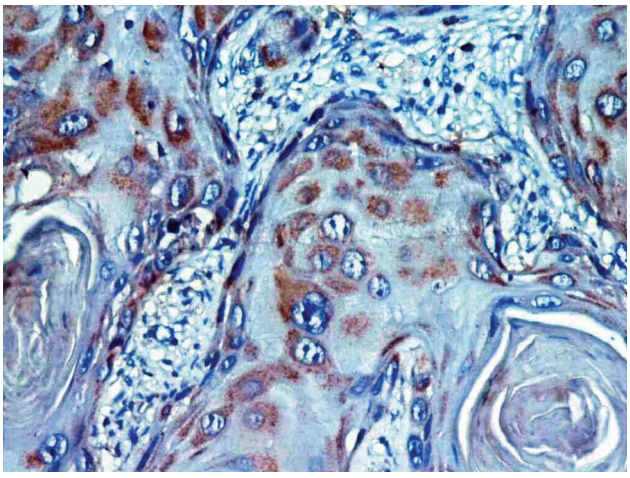

Figure 7. Histologic section of low grade tumor indicates the strong cytoplasmic BRCA1 labeling in cancer cells (×400).

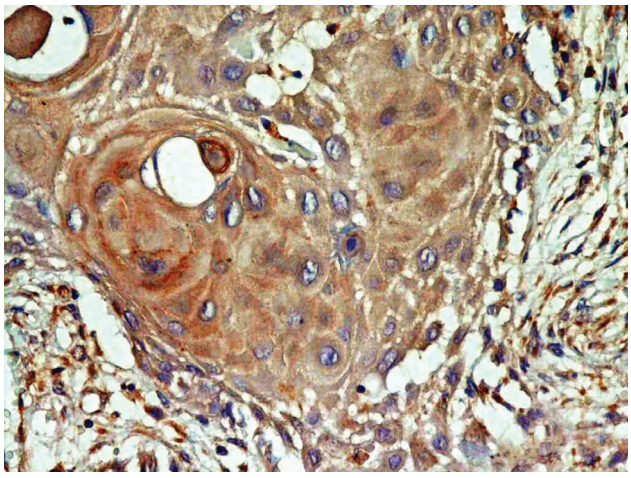

Figure 8. High magnification of low grade cancer illustrates strong BRCA2 localization in the cytoplasm of tumor cells (×400).

of *BRCA1*/2 in the development of OSCC. In addition, the immunohistochemical BRCA expression profile can be employed in the prevention and treatment options of OSCC patients. It is clear that with appropriate management and surveillance, *BRCA* mutation carriers have the chance for prevention or early detection of cancer. This provides a greater chance for successful treatment. Additionally, the current study proposes altered BRCA1/2 expression profiles as biomarkers for evaluating the prognosis in OSCC patients. IHC technique may be conducive to increase our understanding about the mechanisms involved in the development of OSCC. Further investigations are required to find the underlying mechanisms.

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Conflict of Interest

None declared.

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