

CLINICOPATHOLOGICAL CORRELATION OF THE EXPRESSION OF EXCISION REPAIR CROSS COMPLEMENTATION GROUP 1 (ERCC1) IN ORAL CAVITY SQUAMOUS CELL CARCINOMAS: AN IMMUNOHISTOCHEMICAL STUDY

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Background: The nucleotide excision repair pathway is a sophisticated DNA repair mechanism that reduces DNA damage caused by exogenous factors. Excision repair cross complementation group 1 (ERCC1) is a prominent member of this pathway that maintains the genomic stability. The *aim* of this study is to determine the association between the immunohistochemical expression of ERCC1 and the clinical and pathological features of oral cavity squamous cell carcinomas. **Materials and Methods:** The sections of formalin fixed paraffin embedded tissue blocks of oral squamous cell carcinomas (n = 60) were immunohistochemically stained with anti-ERCC1 antibody. The association between the nuclear expression of ERCC1 and the clinicopathological parameters of the tumors and the patient outcomes was evaluated using the chi-square test. **Results:** ERCC1 expression was evident in all studied cases of the oral squamous cell carcinomas. A high ERCC1 expression was associated with smaller tumors, tumors without lymph node involvement and well-differentiated tumors ($p < 0.001$). Better outcomes were associated with higher expression of ERCC1 ($p = 0.028$). **Conclusion:** ERCC1 seems to be an efficient biomarker for prognostication of oral squamous cell carcinomas. High expression of ERCC1 indicates more favorable course of the disease. **Key Words:** DNA damage, ERCC1, nucleotide excision repair, nuclear expression, oral cancer.

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Oral squamous cell carcinoma (OSCC) is the sixth most widespread neoplasm in the world and is the leading cancer in India [1, 2]. Age-adjusted rates of oral cancer in India are high, that is 20 per 100,000 population accounting for over 30% of all cancers in the country [3]. In India, the high incidence of OSCC is predominantly because of the widespread practice of tobacco use in various forms including betel quid chewing and alcohol consumption [4].

OSCC is associated with high mortality and morbidity among patients. In spite of the advances in chemotherapy and radiotherapy, the 5-year survival rate of OSCC patients continues to remain at about 50% [5]. The reason for this dismal survival is that most cases are diagnosed at the late stages. Second, prognostication of these tumors is challenging because tumors that present with a similar stage and grade follow different clinical courses [6, 7].

A plethora of data specifies that OSCC occurs due to stepwise genetic and epigenetic alterations. The genomic DNA is under constant degradation due to multiple factors that are both intrinsic (proto-oncogenes) and extrinsic (ultraviolet (UV)-A, UV-B, cosmic rays etc), thus compromising its function [8]. The DNA repair systems include the nucleotide excision repair (NER) pathway, mismatch repair pathway, base exci-

sion repair pathway, single strand and double strand repair pathways [9].

The NER pathway is an efficient DNA damage removal pathway. This pathway manages mutations that are triggered by UV radiations and DNA complexes formed by chemical adducts [10]. The excision repair cross complementation group 1 (ERCC1) gene locating on chromosome 19q13.32 is a component of NER pathway. ERCC1 is involved in various biological processes such as chromosomal organization, UV protection, post-embryonic hematopoiesis, cellular proliferation and cellular aging. ERCC1 is essential to repair various DNA lesions such as intra- and inter-strand crosslinks and DNA double-strand breaks [11, 12].

The ERCC1 expression was studied in various carcinomas including lung, breast, colorectal, esophageal, ovarian and uterine cervix [12–17]. However, the association between the expression of ERCC1 and its role in OSCC is less explored and is controversial [18, 19]. Here, an attempt is made to understand the association between the expression of this biomarker and clinicopathological parameters in OSCC.

MATERIAL AND METHODS

Patients. The study was designed as a retrospective cohort study on a group of OSCC patients (n = 60). All the patients were treated at Kasturba Medical Hospital, Manipal, Karnataka, India. The Institution ethics committee approved the study. The patients with newly diagnosed OSCC prior to chemotherapy or radiotherapy were included in the study. The patients with systemic diseases or immune-compromised state were not included.

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Abbreviations used: ERCC1 – excision repair cross complementation group 1; FFPE – formalin fixed paraffin embedded; NER – nucleotide excision repair; OSCC – oral squamous cell carcinoma; UV – ultraviolet.

The study group involved patients aged from 20 to 80 years (mean 50 ± 5), 46 males and 14 females. The number of patients with tobacco habit (either smoking or chewing) was 44/60 (73%). Among 60 cases of OSCC, there were 16 early stage tumors and 44 advanced stage tumors. The tumors were located in buccal mucosa (21 cases), the tongue (18 cases), and other sites such as the gingiva, alveolus and the lip (21 cases). The distribution by the stages of the disease was the following: stage I — 7 patients I, stage II — 9 patients, stage III — 5 patients and stage IV — 39 patients. The clinical details were obtained from the medical records. The follow-up period ranged from 1½ years to 5 years. The formalin fixed paraffin embedded (FFPE) blocks of the diagnosed cases were retrieved from the departmental repository. Three FFPE blocks of normal oral mucosa and one of normal lymph node were included to serve as positive controls for the study.

Immunohistochemical staining. 4-micron thick sections were cut from FFPE tissue blocks and taken onto positively charged slides (PathnSitu Biotechnologies, India). Sections were then deparaffinised in xylene and rehydrated through descending grades of alcohol. The prepared sections were incubated with primary antibody — mouse monoclonal anti-ERCC1 (clone 8F1, Thermo Fischer, USA) and then — with secondary antibody (PolyExcel Poly HRP, PathnSitu, India). The peroxidase activity was developed with diaminobenzidine tetrahydrochloride. Finally, the sections were counter stained with Mayer's hematoxylin. For the negative control, the entire immunohistochemical process was followed except that the sections were not treated with the primary antibody.

Evaluation of ERCC1 expression. A dark brown staining in the nucleus of the epithelial cells was considered positive for the ERCC1 expression in tumor sections. A semi-quantitative analysis was carried under an Olympus-BX21 light microscope by two observers. The modified criteria by Hayes et al. [20] were followed to assess the immunohistochemical expression of ERCC1 in the tumor tissues. Four high-power fields of the tissue sections were examined. The percentage of tumor cells with a positive nuclear expression in each field was calculated. The mean of the percentage of positive cells in all the four fields was considered and graded on a scale of 1–3. Score 1 (+) = < 50% of positive cells, score 2 (++) = 50%–75% of positive cells and score 3 (+++) = > 75% of positive cells. Finally, score 1 was considered as low expression and scores 2 and 3 were considered as high expression. The scores of the two observers analyzed using interclass correlation revealed a good reproducibility between the two observers (ICC = 0.925).

Statistical analysis. Statistical analyses were carried out using the Statistical Package for Social Sciences version 23. Descriptive analysis was carried to determine patient's characteristics. The expression in ERCC1 was correlated with the clinicopathological parameters using the Chi Square test. p value of < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

We have evaluated semi-quantitatively the ERCC1 expression in 60 histologically confirmed cases of OSCC. In all 60 cases, positive ERCC1 expression in the nucleus was observed. A score 1(+) expression of ERCC1 was seen in 20/60 (33.3%) cases (Fig. 1), a score 2 (++) ERCC1 expression — in 25/60 (41.6%) cases (Fig. 2) and a score 3 (+++) ERCC1 expression — in 15/60 (25%) cases (Fig. 3). The association of ERCC1 expression with clinicopathological parameters is given in the Table.

There was no significant association of the expression of ERCC1 with the age of the patients, gender of the patients, exposure to tobacco or the site of the tumor. A high ERCC1 expression was associated with smaller tumors ($p = 0.043$) and tumors with no clinical lymph node metastasis ($p = 0.001$). A high expression of ERCC1 was also associated with well-differentiated tumors ($p < 0.001$). The follow-up data of the patients showed that there were 33 patients with either recurrence or death. Patients who were disease-free following treatment had tumors that showed a high expression of ERCC1 ($p = 0.028$).

ERCC1 is an enzyme that forms heterodimer with XPF endonuclease to participate in the NER pathway. ERCC1-XPF functions as a nuclease that incises the

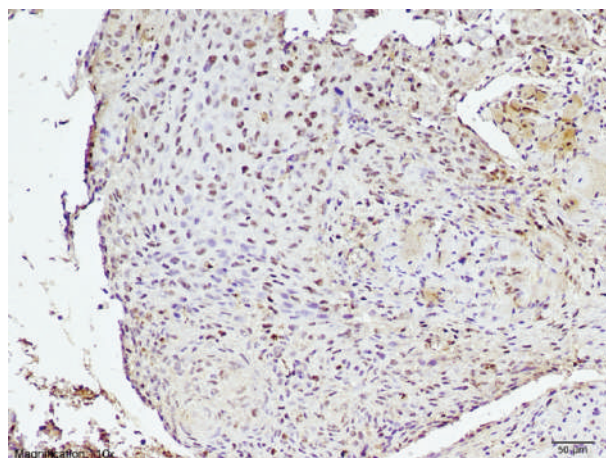


Fig. 1. Photomicrograph of poorly differentiated squamous cell carcinoma showing a score (+1) ERCC1 expression, IHC ($\times 100$)

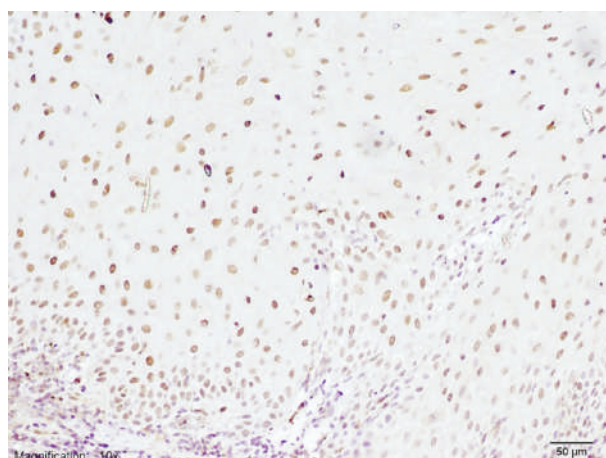


Fig. 2. Photomicrograph of well-differentiated squamous cell carcinoma showing a score (+2) ERCC1 expression, IHC ($\times 100$)

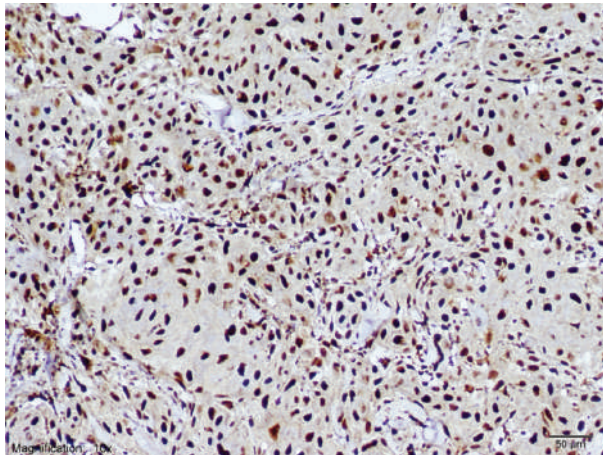


Fig. 3. Photomicrograph of moderately differentiated squamous cell carcinoma showing a score (+3) ERCC1 expression, IHC (× 100)

damaged strand DNA 5' to the adducts. This incision creates 3' end, which is used as a primer by the duplication machinery to replace the excised nucleotide [21]. In this study, a high expression (score 2 and score 3) of ERCC1 was evident in 40/60 (66%) of the OSCC cases. A high ERCC1 expression was reported in 73%, 46% and 43% of OSCC by Jun et al. [18], Chiu et al. [19], and Hayes et al. [20], respectively. Being a major part of the NER pathway, ERCC1 maintains the genomic integrity, both in normal and tumor cells.

OSCCs are considered as associated with tobacco-related DNA damages. The primary carcinogens in tobacco are aromatic hydrocarbons, tobacco specific nitrosamines can attack many electron rich sites

in proteins and nucleic acids to form covalent addition products. The addition products at the hydrogen binding sites of base pairs can cause miscoding [22]. The ERCC1–XPF structure protein incises the damaged strand of DNA. This protein can also repair double strand breaks in DNA and can repair cross-link damages that harmfully links two DNA strands [11]. In the present study, tumors from 28/44 (63.6%) patients who had a history of tobacco exposure had a high expression of ERCC1. The high expression of ERCC1 in these tumors could suggest the activation of repair system induced by tobacco carcinogens. However, a reduced DNA repair capacity is linked with smoking-related cancers such as lung cancer [23]. There is a chance that carcinogenic exposure leads to failure of cells to activate damage-sensor proteins, thus leading to reduced levels of expression of ERCC1 [17].

High ERCC1 expression was found in 19/34 (55%) T3/T4 tumors and in 21/26 (80%) T1/T2 tumors. However, Jun et al. [18] and Chiu et al. [19] found high ERCC1 expression in T3/T4 tumors compared to T1/T2. As the NER pathway comprises several proteins other than ERCC1, which are involved in DNA repair, its expression need not necessarily indicate the degree of repair activity. It may be assumed that as the mass of the tumor rises, the capacity of the NER pathway to repair mutation declines due to the much altered genetic framework that takes place due to uncontrolled cell division [24]. Alternatively, in a study on breast carcinomas, smaller size tumors had higher expression of ERCC1. The wide discrepancies in the expres-

Table. Association of the expression of ERCC1 with clinicopathological parameters and treatment outcomes

Parameters	ERCC1 expression		X ² -value (df)	p-value
	Low	High		
Age				
< 40 years (6 cases)	3 (50%)	3 (50%)	0.833 (1)	0.314
> 40 years (54 cases)	17 (32%)	37 (68%)		
Gender				
Male (46 cases)	17 (37%)	29 (63%)	1.165 (1)	0.228
Female (14 cases)	3 (21%)	11 (79%)		
Habits				
Chewing tobacco (35 Cases)	12 (34%)	23 (66%)	1.014 (2)	0.602
Smoking tobacco (9 cases)	4 (44%)	5 (56%)		
No habits (16 cases)	4 (25%)	12 (75%)		
Site				
Buccal mucosa (21cases)	7 (33%)	14 (67%)	0.15 (2)	0.990
Tongue (18 cases)	6 (33%)	12 (67%)		
Gingiva/ lip (21 cases)	7 (33%)	15 (67%)		
Size of the tumor				
T1–T2 (26 cases)	5 (19%)	21 (81%)	4.106 (1)	0.043*
T3–T4 (34 cases)	15 (44%)	19 (56%)		
Lymph node status				
N0 (23 cases)	2 (9%)	21 (91%)	10.188 (1)	0.001*
N1–N3 (37 cases)	18 (49%)	19 (51%)		
Distant metastasis				
M0–Mx (59 cases)	19 (32%)	40 (68%)	2.034 (1)	0.154
M1 (1 case)	1 (100%)	0 (0%)		
Stage				
Early stage (16 cases)	2 (12%)	14 (88%)	4.261 (1)	0.035*
Advanced stage (44 cases)	18 (41%)	26 (59%)		
Grade				
Well (20 cases)	0 (0%)	20 (100%)	51.450 (2)	<.001**
Moderately (20 cases)	1 (5%)	19 (95%)		
Poorly (20 cases)	19 (95%)	1 (5%)		
Patient outcome				
Disease free survival (27 cases)	5 (18%)	22 (82%)	4.848 (1)	0.028*
Recurrence/Death (33 cases)	15 (46%)	18 (54%)		

Note: *The difference is significant at $p < 0.05$; **the difference is significant at $p < 0.001$.

sion levels of DNA repair genes reflect the existence of genetically determined factors that may contribute to inter-individual differences in cancer risk susceptibility [17].

In the present study, it was observed that 18/37 (48.6%) tumors with nodal involvement show a strong expression of ERCC1. Earlier, Yuanming et al. [14] demonstrated that in patients with colorectal cancer with nodal metastasis, ERCC1 expression was significantly lower than in patients without lymph node metastasis. In the current study we found that 21/23 (91%) tumors without lymph node metastasis had a high expression of ERCC1.

In the present study, a high expression of ERCC1 was associated ($p = 0.035$) with early stage of the disease. Among the tumors at the early stage, 14/16 (88%) cases had a high expression of ERCC1, whereas only 26/47 (59%) cases of advanced stage showed such expression. The study involving patients with non-small cell lung cancer unveiled that the expression of ERCC1 declined as the clinical stage of the disease increased from stage I to stage III [23]. In another study by Hayes et al. [20], in OSCC 72% of stage IV tumors and 28% of stage II/III tumors showed a high ERCC1 expression. A higher expression of ERCC1 in stage IVA tumors compared to stage III tumors was also seen by Jun et al. [18]. The function of DNA repair proteins and enzymes may be influenced by the level of transcription, splicing, stability of mRNA, post-translational modification and action of inhibitors or stimulators [17].

The current study shows a significant association between the tumor grade and the expression of ERCC1. All 20 cases of well-differentiated OSCC showed high expression for ERCC1, while only 1/20 poorly differentiated tumor showed a high expression for ERCC1. Likewise, in a study by Hayes et al. [20], 23/44 (72%) cases of moderately differentiated tumor showed high ERCC1 expression in contrast to only 3/11 (9%) of the poorly differentiated tumors. As the degree of differentiation decreases, tumor cells are prone to undergo further mutations. Mutation in the 19q13 gene affects the polymorphism in the gene, which alters the expression of ERCC1 protein [25]. This polymorphism is related to the reduced rates of transcription of the *ERCC1* gene, which causes low levels of the protein in nucleus [18]. Moreover, some amino acid substitutions in ERCC1 proteins affect its DNA repair capacity [24]. Bilen et al. [26] did not observe notable association between the ERCC1 expression and tumor grading in esophageal cancer. However, An et al. [27] demonstrated the association between expression of ERCC1 and the degree of differentiation in oropharynx and oral cavity cancer.

While assessing the association between ERCC1 expression and patient's outcomes following treatment, among 27 patients without signs of tumor growth, 22/27 (81%) cases showed a high expression of this protein. Among 33 patients who presented with recurrence or who did not survive, we found high expression in 18/33 (54%) cases. A high ERCC1 ex-

pression was associated ($p = 0.028$) with disease-free survival. In contrast, Jun et al. [18] found that a low ERCC1 expression was a predictor of longer survival.

In addition, the elevated level of ERCC1 is related to increased rate of NER and decreased sensitivity to cisplatin, whereas cancer cells with a lower level of ERCC1 are more sensitive to platinum [20]. In the present study, three patients were rendered with surgery and cisplatin chemotherapy. In these three patients, score of 2 for the expression of ERCC1 was demonstrated. Two patients had survived. The question of the association between cisplatin sensitivity and ERCC1 expression has not yet been solved. Further studies designed to correlate the expression of ERCC1 in OSCC of patients treated with cisplatin based therapeutic regimes will aid in employing ERCC1 expression as a prognostic indicator.

To sum up, we investigated the association between the clinicopathological features of OSCC and the expression of ERCC1 that might be an efficient biomarker for prognostication of this disease. High expression of ERCC1 seems to be associated with more favorable outcomes. Based on the expression of ERCC1 personalized treatment protocols can be developed for oral cancer patients.

DISCLOSURE OF INTEREST

The authors declare that there is no conflict of interest.

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