Original Article

Elucidating the role of excision repair cross-complement group 1 in oral epithelial dysplasia and early invasive squamous cell carcinoma: An immunohistochemical study

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Abstract

Objectives: Oral epithelial dysplasia (OED) is characterized by cellular alterations which have the proclivity of progressing to squamous cell carcinoma. Excision repair cross-complement group 1 (ERCC1) is one of the key proteins involved in nucleotide excision repair (NER) pathway. The expression of ERCC1 has been studied in colorectal, esophageal, ovarian and oral squamous cell carcinoma; but, very few studies have been done to apprehend the expression of ERCC1 in OED and early invasive squamous cell carcinoma (EISCC). The goal of this study is to evaluate the role of ERCC1 in OED and EISCC.

Materials and Methods: Histopathologically diagnosed cases of moderate dysplasia ($n = 10$), severe dysplasia ($n = 10$) and EISCC ($n = 10$) were retrieved. 4 μ thick sections were cut from the formalin-fixed paraffin-embedded tissue blocks. The sections were immunohistochemically stained for ERCC1 following standard protocols. The expression of ERCC1 was evaluated semiquantitatively. Statistical analysis was carried out using Fischer's exact *t*-test.

Results: The expression of ERCC1 was found to be strong (+3) in EISCC, moderate (+2) in severe dysplasia and mild (+1) in moderate dysplasia. Thus, the results were statistically significant between the three groups ($P < 0.001$).

Conclusion: Disruption in the mechanisms that regulate cell cycle checkpoints and DNA repair mechanism results in genomic instability; these alterations might contribute to carcinoma. ERCC1 is essential to repair the DNA damage induced by various carcinogens. The present study shows significant difference in the expression of ERCC1 between EISCC and OED, which suggests ERCC1 could be used as one of the predictive markers.

Keywords: DNA repair proteins, dysplasia, early invasive squamous cell carcinoma, excision repair cross-complement group 1, immunohistochemistry, pathogenesis

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INTRODUCTION

Dysplasia is a premalignant change characterized by cellular atypia and loss of normal maturation. The term precancerous lesions and conditions have been used in the literature to describe the clinical presentation of the

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lesions that have the potential to turn into cancer. In 2005, the term "potentially malignant lesions" has been given to these lesions by WHO, as it conveys, not all the disorders may transform into cancer. Although it is established that oral epithelial dysplastic lesions are statistically more likely to progress to cancer, the actual mechanisms are poorly understood.[1]

The exposure to various endogenous and exogenous agents such as chewing of betel nut or areca nut and consumption of alcohol can lead to oral epithelial dysplasia (OED). Nonetheless, in addition, the genetic instability also plays an important role. These factors lead to disruption of the cell cycle and damage to DNA repair systems; the physiological activities of which are essential in maintaining normal cellular function leading to carcinoma.[2]

The genetic information contained in DNA which is essential for proper cell function is ensured by multiple DNA repair pathways.[3] One of the major DNA repair pathways is NER, which primarily manages the damage caused by the ultra-violet (UV) radiations and responsible for the removal of bulky DNA adducts induced by tobacco carcinogens. The genetic variation in the population of genes involved in NER pathway may cause variations in DNA repair capacity leading to extreme photosensitivity and increased susceptibility to both oral epithelial dysplastic lesions and cancer.[4,5]

Excision repair cross-complement group 1 (ERCC1) is a 32 kDa protein (~300 aminoacid) located on chromosome 19q13.32. It is an important component of cellular machinery and one of the key proteins involved in nucleotide excision repair (NER) pathway.[6,7] ERCC1 forms a complex with ERCC4 (xeroderma pigmentosum – F). This ERCC1-XPF complex is essential to repair various DNA lesions such as intra- and interstrand crosslinks, homologous recombination and DNA double-stranded break.[7]

Expression of ERCC1 has been studied in lung,[8] colorectal,[9] esophageal,[10] ovarian,[11] uterine cervix[12] and oral squamous cell carcinoma.[13] Review of literature shows very few studies done to evaluate the expression of this marker in early invasive squamous cell carcinoma (EISCC) and oral epithelial dysplastic lesions. The goal of the present paper is to study the expression of ERCC1 in moderate, severe dysplasia and EISCC as well as to correlate the expression of ERCC1 with the clinicopathologic parameters.

MATERIALS AND METHODS

This study was approved by the Institution Ethical Committee (IEC - 356/2018). Formalin-fixed paraffin-embedded tumor blocks ($n = 30$) of histopathologically diagnosed cases of moderate dysplasia, severe dysplasia and early invasive oral squamous cell carcinomas were retrieved from the archives of the department. The patient's medical records were accessed to obtain the details pertaining to the clinicopathological features of the cases. 4 microns thick sections were cut from formalin-fixed paraffin-embedded blocks and taken onto poly-L-lysine-coated slides. The sections were deparaffinized with xylene and hydrated with descending grades of alcohol, this was followed by antigen retrieval using pressure cooker for 15 minutes using citrate buffer. Incubation of the sections with primary antibody – mouse monoclonal anti-ERCC1 (Product No MA5-13912 Thermo scientific, USA) was done. The sections were then treated with prediluted primary target binder (PolyExcel Target Binder, PathnSitu) at room temperature for 10 min, followed by secondary antibody (PolyExcel Poly HRP, PathnSitu). The peroxidase activity was developed with diaminobenzidine tetrahydrochloride (DAB), counterstained with Mayer's hematoxylin, dehydrated, cleared and mounted using dibutyl phthalate xylene.

Immunohistochemical evaluation

The slides were evaluated under a bright field microscope (Olympus BX2). A prominent brown nuclear staining was considered as positive for ERCC1 protein expression. Under high-power field, the percentage of positively stained cells in 100 epithelial cells were counted for each case. The percentage of positive tumor cells were evaluated in a semiquantitative manner by two observers independently. Three high-power (×40) fields were identified, and the total number of positive cells was counted. The proportion score of ERCC1 was defined as percentage of positive tumor cells and graded on scale from 1 to 3 [Table 1] as given by Hayes *et al*.[14] Lymph node was taken as a positive control, and it was found to be the positive for biomarker expression. For negative control, the tumor sections were subjected to the standard immunohistochemical procedure, but the primary antibody was omitted [Figure 1a and b].

Statistical analysis

Statistical evaluation was done using Statistical Package for Social Services (SPSS) version 20.0 (Armonk, NY: IBM

Table 1: Scoring criteria

Scoring criteria	Percentage of positive cells
1+	<50
2+	50-75
3+	75-100

Corp., USA). The expression of ERCC1 was compared between the cases of EISCC, severe dysplasia and moderate dysplasia using Fischer's exact *t*-test; the scores of 2 observers were analyzed using kappa (k) measure of agreement. $P < 0.05$ was considered to be statistically significant.

RESULTS

The expression of ERCC1 was semi-quantitatively evaluated in 30 histologically diagnosed cases of EISCC ($n = 10$), moderate dysplasia ($n = 10$) and severe dysplasia ($n = 10$). Among 30 cases, the expression of ERCC1 was found to be high in EISCC [Figure 2] when compared to severe dysplasia [Figure 3] and moderate dysplasia [Figure 4]. 7/10 cases (70%) presented with severe (score 3) expression of ERCC1, 3/10 cases (30%) displayed moderate expression (score2) of ERCC1 in EISCC. In cases of severe dysplasia, 6/10 cases (60%) showed moderate (Score 2) expression of ERCC1, 3/10 cases revealed mild expression (30%) and 1/10 case showed severe (Score 3) expression (10%). Moderate dysplasia cases showed mild (Score 1) expression of ERCC1 in 7/10 cases (70%), and 3/10 cases (30%) revealed moderate expression. Comparison of expression of ERCC1 between the three groups (moderate dysplasia, severe dysplasia and EISCC) is given in Table 2. The association of the expression of ERCC1 between moderate dysplasia and severe dysplasia was done, which was not significant [Table 3]; the association was also not significant between severe dysplasia and EISCC [Table 4], while the association between the expression of ERCC1 between dysplasia and EISCC was found to be statistically significant [Table 5].

The expression of ERCC1 was also correlated with the clinicopathologic parameters [Table 6].

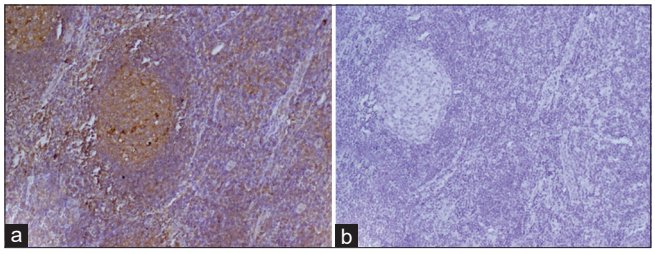


Figure 1: (40×) Positive control (a), Negative control (b)

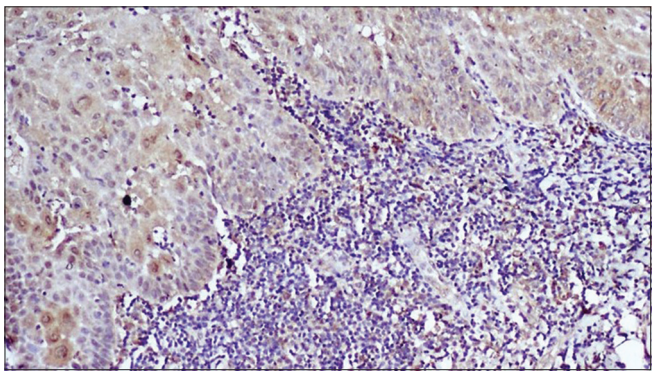


Figure 3: Histopathological image shows the expression of excision repair cross-complement group 1 in severe dysplasia (Moderate = 2+, ×40)

DISCUSSION

DNA damage can be caused by various exogenous and endogenous factors; in addition to these factors, genomic

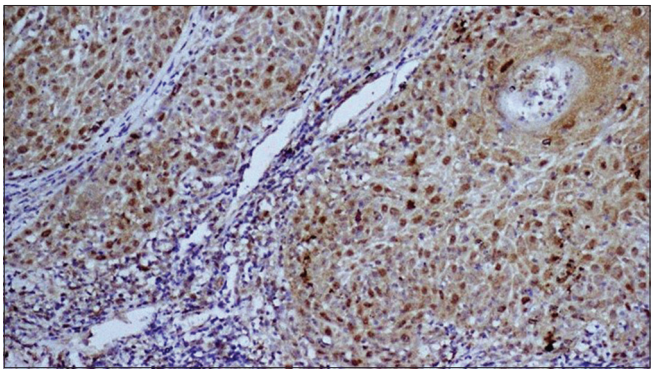


Figure 2: Histopathological image shows the expression of excision repair cross-complement group 1 in early invasive squamous cell carcinoma (Severe = 3+, ×40)

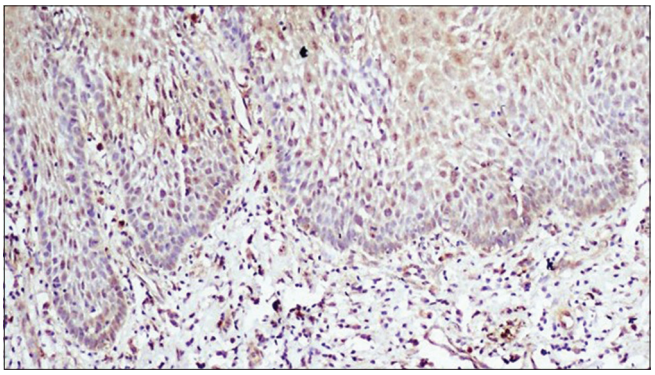


Figure 4: Histopathological image shows the expression of excision repair cross-complement group 1 in moderate dysplasia (Mild = 1+, ×40)

Table 2: Comparison of expression of excision repair cross-complement group 1 between moderate dysplasia, severe dysplasia and early invasive squamous cell carcinoma

Lesion	Mild (+1)	Moderate (+2)	Severe (+3)	Fisher's exact *t*-test	*P*
Moderate dysplasia (%)	7 (70)	3 (30)		17.616	<0.001*
Severe dysplasia (%)	3 (30)	6 (60)	1 (10)		
EISCC (%)		3 (30)	7 (70)		

*$P < 0.05$ highly significant. EISCC: Early invasive squamous cell carcinoma

stability also plays an important role. There are various genes involved in maintaining the genomic stability and necessary for upholding the cellular machinery, which is sustained by various DNA repair systems such as mismatch repair system, base excision repair and NER. NER is the major pathway that helps in the repair of tobacco carcinogen-induced adducts present on the strands of DNA.[15] There are 2 major NER pathways that is global genome NER which recognizes lesions anywhere in the genome and transcription-coupled NER that preferentially repairs lesions that block transcription. The germline mutations in several genes of the NER are associated with cerebro-oculofacial syndrome and trichothiodystrophy and can cause Xeroderma pigmentosum, a disorder characterized by extreme sensitivity to sunlight with propensity to develop skin cancer and other malignancies. The steps involved in the DNA repair mechanism through NER pathway is explained in the flow chart [Table 7].[4]

Table 3: Association of the expression of excision repair cross-complement group 1 moderate dysplasia and severe dysplasia

	Mild (%)	Moderate (%)	Strong (%)	*P*
Moderate dysplasia	7 (70)	3 (30)	0	0.17
Severe dysplasia	3 (30)	6 (60)	1 (10)	

Table 4: Association of the expression of excision repair cross-complement group 1 between severe dysplasia and early invasive squamous cell carcinoma

	Mild (%)	Moderate (%)	Strong	*P*
Severe dysplasia	3 (30)	6 (60)	1 (10)	0.013
EISCC		3 (30)	7 (70)	

EISCC: Early invasive squamous cell carcinoma

Table 5: Association between the expression of excision repair cross-complement group 1 between dysplasia and early invasive squamous cell carcinoma

	Mild (%)	Moderate (%)	Strong (%)	*P*
Dysplasia	10	9	1	0.0002*
EISCC	0	3	7	

*$P < 0.05$ highly significant. EISCC: Early invasive squamous cell carcinoma

ERCC1 which is one of the key proteins involved in NER pathway is necessary for various biological activities such as chromosomal organization, UV protection, cellular multiplication, cellular aging and homogeneity. ERCC1 gene forms a heterodimer with ERCC4 (XPF- Xeroderma pigmentosum complementation Group –F), together they function as nuclease in the NER pathway and incise the harmful DNA adducts.[6] Other DNA repair genes belonging to NER pathway include XPA (Xeroderma pigmentosum complementation Group – A), XPC (Xeroderma pigmentosum complementation Group – C), XPD (Xeroderma pigmentosum complementation Group – D), ERCC2, ERCC3, ERCC4 and ERCC5. The expression of ERCC2 has been proved in premalignant lesions, and the polymorphism associated with this gene has been associated with high risk of oral cancer.[16] High-throughput reverse-phase protein lysate microarray (RPPA) assay done to measure the NER proteins expression levels, revealed reduced expression levels of ERCC2, ERCC4, XPA and XPC which were associated with increased risk of head and neck squamous cell carcinoma.[17] Excluding ERCC1 and ERCC2, the other DNA repair genes belonging to NER pathway are yet to be studied in oral potentially malignant lesions.

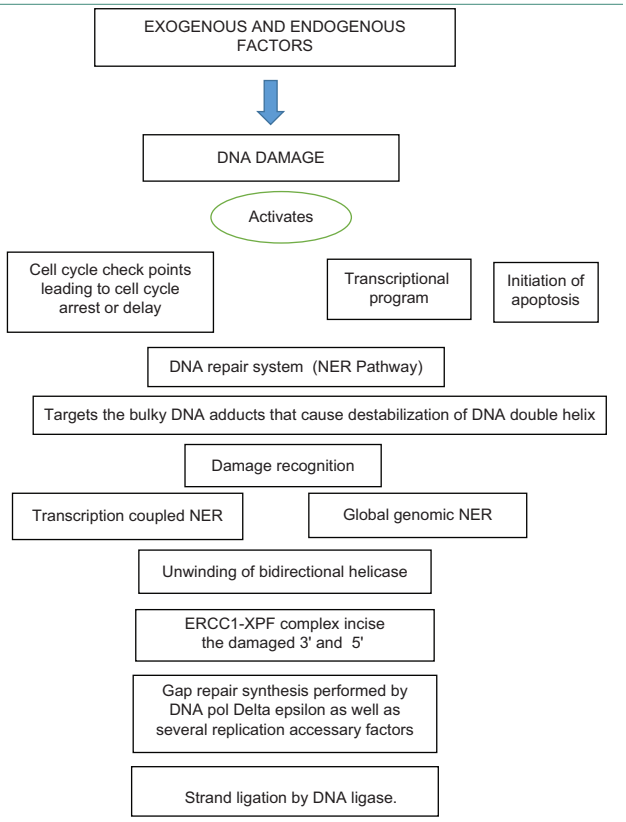
In the present study, we found that the expression of ERCC1 was severe (70% = 3+) in cases of EISCC when compared to OED (severe dysplasia - 60% = 2+, Moderate dysplasia - 70% = 1+), which indicates that the cells are in the state of repair through ERCC1-XPF mechanism. There were few cases which did not take up the stain; the reason could be polymorphism that has occurred in few of the cells, degradation of the proteins by reactive oxygen species or due to epigenetic change. All these molecular changes can lead to deficiency of ERCC1 that results in poor repair capability as well as gradual loss of transcription process which decrease the proportion of cells to express ERCC1. Wang *et al*.[4] conducted study on 144 oral potentially malignant lesions, genomic DNA was

Table 6: Association between the expression of excision repair cross-complement group 1 and different clinical parameters

	Mild (+), *n* (%)	Moderate (++), *n* (%)	Strong (+++), *n* (%)	Fischer's exact *t*-test	*P*
Males (21)	10 (90)	6 (66.7)	5 (62.5)	2.222	0.337
Females (9)	1 (10)	4 (33.3)	4 (37.5)		
	Mild (+), *n* (%)	**Moderate (++), *n* (%)**	**Strong (+++), *n* (%)**	**ANOVA**	***P***
Age (years)					
<50	0.0	30.0	70.0	0.244	0.785
50-75	25.0	50.0	25.0		
>75	87.5	12.5	0.0		
	Mild (+), *n* (%)	**Moderate (++), *n* (%)**	**Strong (+++), *n* (%)**	**Fischer's exact t-test**	***P***
Habits					
With habits	0.00	16.7	25.0	2.568	0.337
Without habits	100	83.3	75.0		

+: <50 % of positive tumor cells; ++: 50-75 % of positive tumor cells; +++: 75-100 % of positive tumor cells

Table 7: Flowchart showing the events in the nucleotide excision repair pathway



isolated from peripheral blood samples using proteinase K digestion and polymerase chain reaction was used for genotyping of these genes. Their study concluded that patients with dysplasia were prone to cancer due to deficiency of NER pathway and polymorphism in NER genes that lead to genetic instability. Other reason that can be linked to is the degree of differentiation, as the degree of differentiation of tumor cells decreases, tumor cells are prone to undergo mutations.[18,19]

The correlation between the expression of ERCC1 and the clinical parameters, that is, age, sex and habits were done, which did not show any statistical significance [Table 5]. Although studies have been done to evaluate the expression of ERCC1 in various carcinomas, very few studies were done to apprehend the expression of ERCC1 in oral squamous cell carcinoma and OED. Souza *et al*.[2] analyzed the expression of p53, APE1, hMSH2, ERCC1 in actinic chelitis (AC) and lip squamous cell carcinoma (LSCC). The results of their study showed that the expression of ERCC1 was higher in LSCC when compared to AC (low-risk AC - the expression of ERCC1 was mild/absent and high-risk AC - moderate/severe expression of ERCC1). Study done by Terayama *et al*.[18] suggested that the expression of p16, RAR-Beta2, TIMP-3, ERCC1 and BRCA1 protein can might occur regardless of promoter methylation and might promote the process of inflammatory carcinogenesis caused by *Candida* albicans infection.

Bajpai *et al*.[12] conducted a study on squamous intraepithelial lesion and invasive squamous cell carcinoma of the cervix which included 50 cervical cancer and 40 squamous intraepithelial lesions using western blot technique. They found that the expression of ERCC1 was reduced in these patients when compared to the controls (disease free). Their study concluded that the reduction in expression of DNA repair genes is associated with an early event in the progression to cervical cancer.

There has been a relationship between p53, epidermal growth factor like receptors (EGFR) and ERCC1. Although p53 is a tumor-suppressor gene at times, it acts as a double-edge sword. The wild type of p53 induces the expression of heparin-binding epidermal-like growth factor which is EGFR ligand, which binds to the EGFR receptor and activates EGFR mechanism. On internalization of EGFR into the nucleus, EGFR combines with DNA- protein kinase/ERCC1, thereby enhancing the kinase activity. The ionizing radiations can also lead to internalization of EGFR into the nucleus. Thus, the combination of EGFR and ERCC1 helps in the repair of DNA double-stranded breaks.[20]

ERCC1 is one the predictive markers for radically resected head and neck squamous cell carcinoma patients treated with surgery and chemoradiation. High expression of ERCC1 has showed cisplatin resistance and poor outcome (cisplatin which is the back bone of chemotherapeutic regimens, used to treat malignancies). Their primary cytotoxic activity is based on formation of mono/bifunctional adducts in the DNA which causes inter/intrastrand cross-linking. Elevated levels of ERCC1 lead to increased rate of NER and decreased sensitivity to cisplatin, whereas cancer cells with lower level of ERCC1 are more sensitive to cisplatin.[21,22]

CONCLUSION

Reduced DNA repair capacity can lead to genomic instability and constitute a risk factor for cancer development. Mutations and inherited polymorphism of the DNA repair genes alter the expression of ERCC1. As per our study results, the expression of ERCC1 was higher in EISCC when compared to the lesions of OED; however, further studies with increased sample size need to be done to confirm ERCC1 as one of the predictive markers.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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