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Immunohistochemical expression of DNA repair proteins in oral tongue and lower lip squamous cell carcinoma

Deborah Gondim Lambert MOREIRA[a]
Everton Freitas de MORAIS[a]
Hellen Bandeira de Pontes SANTOS[a]
Roseana de Almeida FREITAS[a]

[a]Universidade Federal do Rio Grande do Norte – UFRN, Centro de Ciênicas da Saúde, Department of Oral Pathology, Natal, RN, Brazil.

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Corresponding Author:
Roseana de Almeida Freitas
E-mail: roseanafreitas@hotmail.com

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Abstract: The DNA repair system involves genes and proteins that are essential for the maintenance of genome integrity and the consequent control of various cellular processes. Alterations in these genes and proteins play a role in tumor development and progression and might be associated with prognosis. The aims of this study were to analyze the immunoexpression of two DNA repair proteins, XPF and XRCC1, in lower lip squamous cell carcinoma (LLSCC) and oral tongue squamous cell carcinoma (OTSCC), and to investigate possible associations with clinical and histopathological parameters. The immunohistochemical expression of XPF and XRCC1 was analyzed semi-quantitatively in 40 cases each of LLSCC and OTSCC. The chi-squared test or Fisher's exact test, when appropriate, was used to investigate the association between expression of the proteins and clinicopathological characteristics. The cytoplasmic immunoexpression of XPF was high in OTSCC (95% of the cases analyzed) but low in LLSCC (52.5%). Among the clinicopathological parameters evaluated, a statistically significant association was observed between high nuclear expression of XRCC1 and the absence of regional lymph node metastasis in patients diagnosed with OTSCC (p=0.006). The high protein expression of XPF and XRCC1 in OTSCC and LLSCC suggests an important role in the development and progression of these tumors. Our study found an association between high nuclear expression of XRCC1 and the absence of loco-regional metastasis in cases diagnosed as OTSCC, suggesting a role of this protein in tumor progression.

Keywords: Immunohistochemistry; Pathology; Mouth Neoplasms.

Introduction

Squamous cell carcinoma (SCC) is the most common oral malignant neoplasm that accounts for more than 90% of cases in the oral cavity[1] where it can occur at any site. The oral tongue is the most common intraoral site, which is affected in more than 50% of cases.[2]

Oral tongue squamous cell carcinoma (OTSCC) is the most prevalent oral cancer; a higher incidence is observed between the sixth and seventh decade of life. Smoking is the main etiological factor associated with its development.[3] Although the epidemiological profile has changed over time, with considerable regional variations, there is still higher

prevalence of OTSCC among males, as they have greater exposure to risk factors.[1,3] OTSCC exhibits an aggressive biological behavior and has usually a poorer prognosis when compared to tumors at other sites.[4,5,6,7] Lower lip squamous cell carcinoma (LLSCC) is more common among male patients above the age of 50 years and solar radiation is the main etiological factor.[7] LLSCC is usually associated with a good prognosis and low mortality rate.[6,7]

Despite advances in cancer treatment, the survival of patients with oral cancer has remained unchanged over the last three decades.[8,9] Patient survival depends on conventional prognostic factors used in clinical practice. The most common are clinical staging of the tumor and different histological grading systems. Although efficient, these factors require complementary methods for analysis of the development, progression, and prognosis of SCC.[10] Within this context, prognostic biological markers are needed that better reflect the diversity of SCC and that more accurately predict clinical outcomes and responses to certain types of adjuvant therapy.[8]

Carcinogenic agents can trigger the accumulation of genetic alterations, including changes in the normal functions of proto-oncogenes and tumor suppressor genes that affect the cell cycle, immunity and cell differentiation, proliferation and death, as well as modifications in DNA repair.[11,12,13] The DNA repair system plays a fundamental role in the protection of the genome against endogenous and exogenous agents, preventing cells altered by carcinogens from continuing the cell cycle and their inappropriate proliferation.[13,14]

DNA repair proteins play an important role in different pathways involved in the maintenance of DNA structure and function and might be associated with the clinical prognosis of patients.[13,15] Human X-ray repair cross-complementing 1 (XRCC1) is the first mammalian DNA repair gene isolated that affects cell sensitivity to ionizing radiation. XRCC1 participates in base excision repair (BER) and single-strand break repair (SSBR) to eliminate the DNA damage induced by chemical mutagens and ionizing radiation.[16] XPF partners with ERCC1 to form a bi-partite nuclease that is essential for nucleotide excision repair (NER).[17,18] XPF–ERCC1 heterodimer is directed to the damaged strand by RPA (replication protein A) to create an incision 5′ to the lesion and remove the single-stranded nucleotide through XPG activation.[18,19]

Studies indicate that the expression of these proteins is deregulated in various neoplastic processes, including SCC; however, the role of these proteins in the biological behavior of OTSCC and LLSCC remains controversial.[3,20] In this context, studies analyzing the role of the DNA repair proteins XRCC1 and XPF in OTSCC are scarce and, so far, no study has evaluated their immunoexpression in LLSCC.[3,21,22]

The objective of the present study was to investigate possible associations of the two DNA repair proteins XPF and XRCC1 with clinical and histopathological parameters in OTSCC and LLSCC, analyzing the role of these proteins in the two neoplastic processes that differ in terms of etiological factors, biological behavior, and prognosis.

Methodology

This was an observational, analytical, cross-sectional study that compared clinicopathological and immunohistochemical characteristics of a sample of cases diagnosed as OTSCC and LLSCC. The study was approved by the Research Ethics Committee of the Federal University of Rio Grande do Norte (UFRN) and was conducted in accordance with Resolution 466/12 of the National Health Council (protocol no. 2.332.567). The informed consent was explained, and the signed approval was obtained from all the participants.

Sample

Among the OTSCC and LLSCC cases diagnosed between 2000 and 2012 at the Pathological Anatomy Service of the Department of Dentistry, UFRN, cases whose clinical records contained information about clinical stage, treatment, and follow-up were selected by convenience sampling to evaluate the recurrence and death rates of the patients. Only cases whose paraffin-embedded material was sufficient for immunohistochemical analysis were selected. Patients submitted to radiotherapy,

chemotherapy, or any other treatment before surgery were excluded. Patients with incomplete data regarding patient gender/age, tumor size/extent, presence of regional lymph node metastasis and distant metastasis, and clinical stage were also excluded. In addition, cases of recurrence and cases of squamous cell carcinoma of the tongue base (posterior one-third), a site belonging to the oropharynx, were not included.

Thus, 40 cases of LLSCC and 40 cases of OTSCC were selected and clinical data including patient sex and age, sun exposure, smoking, alcohol drinking, tumor size, metastasis, recurrence, and outcome were collected. Surgical specimens of the selected cases fixed in formaldehyde 10%, included in paraffin were evaluated by histopathological and immunohistochemical analyses.

Histopathological analysis

For this analysis, histological sections stained with hematoxylin and eosin were evaluated using the histological grading systems of malignancy proposed by Brandwein-Gensler et al.[23] and Almangush et al.[24] Three previously calibrated examiners performed these assessments. Cases where examiners disagreed were resolved by consensus.

For the Brandwein-Gensler grading system,[23] the worst pattern of invasion, lymphocytic infiltrate, and perineural invasion were evaluated. The scores obtained for each parameter were summed to give a final score of malignancy for each case. Tumors with a final score of 0 were classified as low-risk, those with a final score of 1 or 2 as intermediate-risk, and those with a final score 3 as high-risk.

The cut-off point for tumor budding was set at five buds (low < 5; high ≥ 5), and the cut-off point for depth of invasion was set at 4 mm (low < 4 mm; high ≥ 4 mm), as preconized by Almangush et al.[24] After grading, tumors were divided as follows: low risk (tumor with < 4 mm depth of invasion and < 5 buds at the invasive front); intermediate risk (tumor with ≥ 4 mm depth of invasion and < 5 buds at the invasive front or superficial tumor < 4 mm, but with ≥ 5 buds), and high risk (tumor with ≥ 4 mm depth of invasion and ≥ 5 buds at the invasive front).

Immunohistochemical analysis

For immunohistochemistry, 3-µm histological sections were mounted on slides prepared with organosilane adhesive (3-aminopropyltriethoxysilane, Sigma Chemical Co., St. Louis, USA) and submitted to immunoperoxidase staining using primary anti-XPF (Clone 219; dilution: 1:800; Lab Vision Corporation, Freemont, USA) and anti-XRCC1 (Clone 33-2-5; dilution: 1:2,000; Lab Vision Corporation, Freemont, USA) antibodies. The sections were incubated overnight with the primary antibodies diluted in Diamond solution (Cell Marque, Rocklin, USA). Positive and negative controls were established according to manufacturer specifications.

Cells exhibiting brown staining in the nucleus and/or cytoplasm regardless of staining intensity were defined as positive. The immunohistochemical staining of the proteins was analyzed semi-quantitatively at the tumor invasion front by a single previously calibrated examiner who was unaware of the clinical data. The criteria established by Huang et al.[25] were followed and the cases were classified as: 0 (≤ 10% immunostained cells), 1 (11% to 50% immunostained cells), and 2 (> 50% immunostained cells). For statistical analysis, the cases were stratified into low (≤ 50% immunostained cells) and high (> 50% immunostained cells) expression as proposed by Seiwert et al.[17]

Statistical analysis

The results were analyzed statistically using the Statistical Package for the Social Sciences (version 25.0; SPSS, Inc., Chicago, USA). First, descriptive analysis of the data was performed. Statistical tests were then applied to investigate possible associations of the immunoexpression of XPF and XRCC1 with the clinical parameters and histological grade of the OTSCC and LLSCC cases using the chi-squared test or Fisher's exact test, when appropriate.

The immunohistochemical data were analyzed by the Kolmogorov-Smirnov test for normality, which revealed the absence of a normal distribution. Thus, Spearman's correlation test was applied to determine possible correlations between the immunoexpression scores of the proteins. A level

of significance of 5% ($p < 0.05$) was adopted for all tests.

Results

Clinicopathological analysis

The clinicopathological characteristics of the selected cases are shown in Table 1. The age of the patients ranged from 25 to 92 years (mean 60.5 years). Factors associated with carcinogenesis such as use of tobacco and alcohol and sun exposure were evaluated. Among the patients with OTSCC, 72.5% (n = 29) were smokers and 55% (n = 22) alcohol drinkers. Among the patients with LLSCC, 82.5% had a history of chronic sun exposure, 77.5% (n = 31) were smokers, and 30% were alcohol drinkers. With respect to TNM stage, stage III was found in 42% of the OTSCC cases and stage II predominated in the LLSCC cases (55%). A combination of three therapeutic modalities (surgical excision, radiotherapy, and chemotherapy) was the most common treatment in the OTSCC cases, corresponding to 42.5%, followed by surgical resection combined with neck dissection (37.5%). In patients with LLSCC, surgical resection alone was used in 50% of the cases and was combined with neck dissection in 20%.

More than half the patients with OTSCC (65%) exhibited complete remission of the tumor, while remission was observed in 90% of the LLSCC cases. Death due to the tumor was reported for 35% of the patients with OTSCC and for 10% of those with LLSCC.

Morphological analysis using the model proposed by Brandwein-Gensler et al.[23] classified 55% of the LLSCC cases and 55% of the OTSCC cases as intermediate-risk carcinomas, while 35% of the OTSCC cases and 12.5% of the LLSCC cases were classified as high risk. According to the system proposed by Almangush et al.,[24] a tumor depth > 4 mm was observed in 75% of the OTSCC cases and in 65% of the LLSCC cases. Evaluation of tumor budding identified ≥ 5 buds in 62.5 and 45% of the OTSCC and LLSCC cases, respectively. Most OTSCC cases (52.5%) were classified as high risk and most LLSCC cases (50%) as intermediate risk.

Immunohistochemical analysis

High nuclear expression of XPF was observed in 72.5% of OTSCC. A similar finding was obtained for LLSCC. However, cytoplasmic immunoexpression of XPF was high in OTSCC (95%), but low in LLSCC (52.5%) (Figure A-B). No significant associations between XPF protein expression and the clinicopathological parameters were observed at either anatomical site ($p > 0.05$).

Immunoexpression of protein XRCC1 was only detected in the nucleus and was high in both OTSCC and LLSCC (Figure C-D). Statistically significant associations were found between high nuclear expression of XRCC1 and the absence of regional lymph node metastasis in patients diagnosed with OTSCC ($p = 0.006$) (Table 2). However, nuclear immunoexpression of XRCC1 was not significantly associated with clinical stage ($p = 0.101$), outcome ($p=0.685$), or recurrence ($p = 0.263$). Regarding tumor size, high nuclear expression was observed in OTSCC categorized as T1 (88.9%) and lower expression levels in tumors categorized as T2-T4 (51.6%).

High nuclear expression of XRCC1 was observed in 71.4% of LLSCC cases classified as advanced clinical stage (III-IV). There was no difference in immunostaining intensity between tumors of different sizes. No association was found between the nuclear expression of XRCC1 and lymph node metastasis. High nuclear expression was observed in patients who died (75%).

No associations were found between XRCC1 expression and the grading systems proposed by Brandwein-Gensler et al.[23] ($p = 1.00$) or Almangush et al.[24] ($p > 0.05$), nor when the morphological parameters were analyzed separately ($p > 0.05$). Tables 2 and 3 show the results of XRCC1 and XPF expression according to clinical parameter and histological grade of malignancy in the cases diagnosed as OTSCC and LLSCC.

Correlation between the immunoexpression of XRCC1 and XPF in OTSCC and LLSCC

No statistically significant correlation was found between the nuclear immunoexpression of XRCC1 and XPF in cases of OTSCC ($r = 0.141$; $p = 0.386$).

Table 1. Descriptive analysis of socio-demographic and clinical variables of the oral tongue squamous cell carcinoma (OTSCC) and lower lip squamous cell carcinoma (LLSCC) patients.

Parameters	Location	
	OTSCC (n = 40)	LLSCC (n = 40)
	n (%)	n (%)
Gender		
Male	27 (67.5)	30 (75.0)
Female	13 (32.5)	10 (25.0)
Age	60.55 ±13.95	
Tobacco use		
Ever	29 (72.5)	31 (77.5)
Never	7 (17.5)	1 (2.5)
NI	4 (10.0)	8 (20.0)
Alcohol drinking use		
Ever	22 (55.0)	12 (30.0)
Never	18 (45.0)	7 (17.5)
NI	-	21 (52.5)
Chronic solar exposure		
Ever	-	33 (82.5)
Never	-	0 (0.0)
NI	-	7 (17.5)
Tumor size		
T1	9 (22.5)	8 (20.0)
T2	16 (40.0)	28 (70.0)
T3	12 (30.0)	2 (5.0)
T4	3 (7.5)	2 (5.0)
Node		
N0	23 (57.5)	30 (75.0)
N1	8 (20.0)	7 (17.5)
N2	9 (22.5)	1 (2.5)
N3	0 (0.0)	2 (5.0)
Metastasis		
M0	40 (100.0)	38 (95.0)
M1	0 (0.0)	2 (5.0)
Clinical stage		
Stage I	7 (17.5)	4 (10.0)
Stage II	8 (20.0)	22 (55.0)
Stage III	17 (42.5)	8 (20.0)
Stage IV	8 (20.0)	6 (15.0)
Treatment		
Surgery	8 (20.0)	20 (50.0)
Surgery + ND	15 (37.5)	8 (20.0)

Continue...

Continuation		
Surgery + RT or ND + ChT	0 (0.0)	2 (5.0)
Surgery + RT + ND	0 (0.0)	4 (10.0)
Surgery + RT + ChT	17 (42.5)	0 (0.0)
NI	0 (0.0)	6 (15.0)
Local recurrence		
No	30 (75.0)	36 (90.0)
Yes	10 (25.0)	4 (10.0)
Survival status		
Alive at the end period of the study	26 (65.0)	36 (90.0)
Death due to tumor	14 (35.0)	4 (10.0)

NI: not informed; ND: neck dissection; RT: radiotherapy; ChT: chemotherapy.

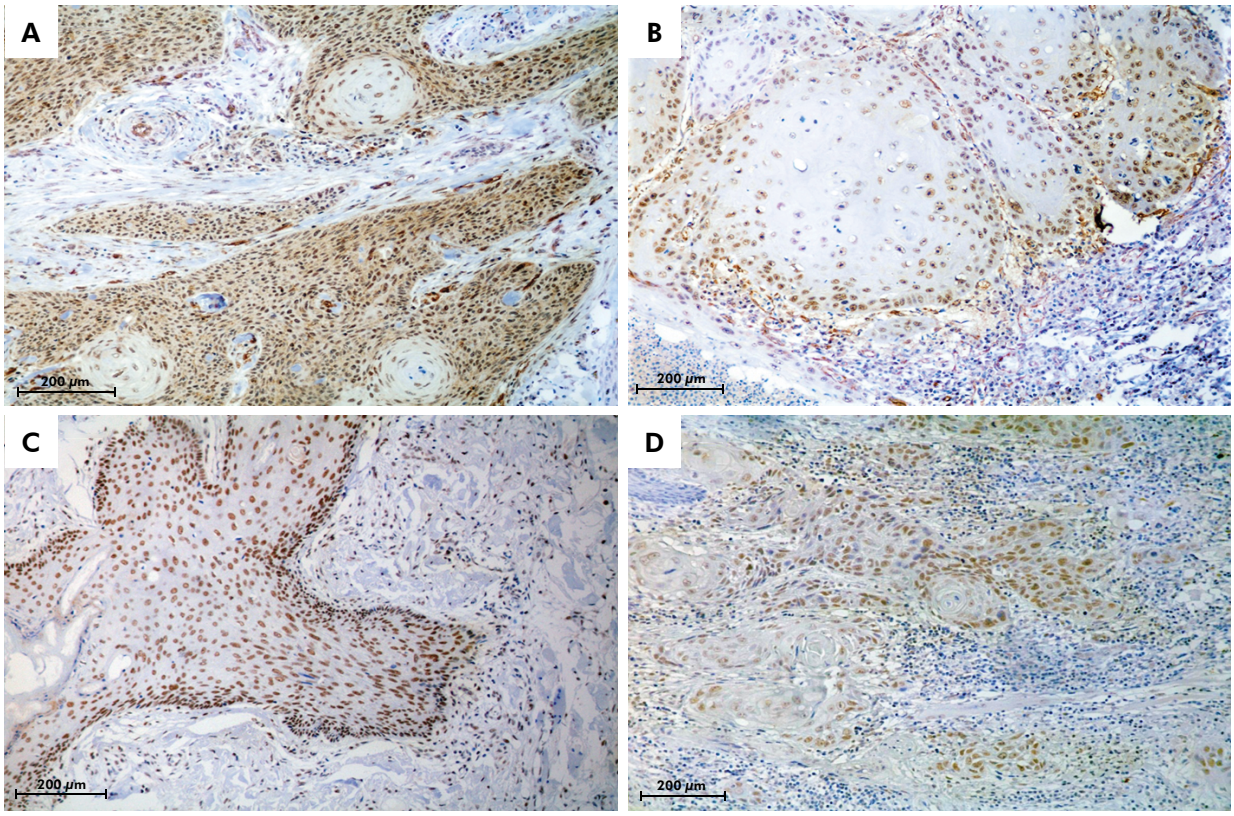

Figure. A. Immunoexpression of XPF protein at the tumor invasion front of lower lip squamous cell carcinoma (LLSCC): positive immunoexpression in the nucleus and cytoplasm of more than 50% of cells. B. Immunoexpression of XPF protein at the tumor invasion front of oral tongue squamous cell carcinoma (OTSCC): positive immunoexpression in 11 to 50% of cells. C. Immunoexpression of XRCC1 protein at the tumor invasion front of LLSCC: positive immunoexpression in 11 to 50% of cells. D. Immunoexpression of XRCC1 protein at the tumor invasion front of OTSCC: positive immunoexpression only in the nucleus of more than 50% of cells.

In LLSCC, Spearman's correlation test revealed a significant and strong positive correlation between the nuclear immunoexpression of XRCC1 and XPF ($r = 0.728$; $p < 0.0001$) (Table 4). Since there was no variation in the cytoplasmic expression of XRCC1 in OTSCC or LLSCC, it was not possible to calculate the correlation between the cytoplasmic expression of XRCC1 and XPF in these tumors (Table 4).

Table 2. XRCC1 and XPF nuclear expression in relation to clinicopathological findings of oral tongue squamous cell carcinoma.

Parameters	XRCC1 (nuclear)				XPF (nuclear)			
	Low	High	Total	p-value	Low	High	Total	p-value
Tumor size								
T1	1 (11.1)	8 (88.9)	9 (100.0)	0.061**	1 (11.1)	8 (88.9)	9 (100.0)	0.399**
T2–T4	15 (48.4)	16 (51.6)	31 (100.0)		10 (32.3)	21 (67.7)	31 (100.0)	
Node status								
N0	5 (21.7)	18 (78.3)	23 (100.0)	0.006*	4 (17.4)	19 (82.6)	23 (100.0)	0.153**
N+	11 (64.7)	6 (35.3)	17 (100.0)		7 (41.2)	10 (58.8)	17 (100.0)	
Clinical stage								
I–II	3 (21.4)	11 (78.6)	14 (100.0)	0.101**	3 (21.4)	11 (78.6)	14 (100.0)	0.715**
III–IV	13 (50.0)	13 (50.0)	26 (100.0)		8 (30.8)	18 (69.2)	26 (100.0)	
Survival status								
Alive at the end period of the study	11 (42.3)	15 (57.7)	26 (100.0)	0.685*	8 (30.8)	18 (69.2)	26 (100.0)	0.715**
Death due to tumor	5 (35.7)	9 (64.3)	14 (100.0)		3 (21.4)	11 (76.8)	14 (100.0)	
Recurrence								
Yes	2 (20.0)	8 (80.0)	10 (100.0)	0.263**	2 (20.0)	8 (80.0)	10 (100.0)	0.696**
No	14 (46.7)	16 (53.3)	30 (100.0)		19 (30.0)	21 (70.0)	30 (100.0)	
Brandwein Gensler et al. (2005)								
LR–IR	10 (38.5)	16 (61.5)	26 (100.0)	0.787*	8 (30.8)	18 (69.2)	26 (100.0)	0.715**
HR	6 (42.9)	8 (57.1)	14 (100.0)		3 (21.4)	11 (78.6)	14 (100.0)	
Almangush et al. (2015)								
Score 0	6 (31.6)	13 (68.4)	19 (100.0)	0,301*	7 (36.8)	12 (63.2)	19 (100.0)	0.293**
Scores 1–2	10 (47.6)	11 (52.4)	21 (100.0)		4 (19.0)	17 (81.0)	21 (100.0)	

LR: low risk; IR: intermediate risk; HR: high risk; *$\chi 2$ test; **Fisher's exact test.

Discussion

The cellular DNA is frequently damaged to different extents by endogenous and exogenous mutagenic agents.[13,26] Once damaged, the DNA can elicit through certain molecules responses ranging from the induction of cell apoptosis to growth deregulation, which can lead to conditions associated with genome instability such as cancer.[27] The genes that encode these molecules involved in the repair of DNA damage have been studied as biological markers of cancer and might be associated with tumor development and patient prognosis.[14,28,29]

OTSCC is frequently diagnosed in an advanced clinical stage and is associated with high rates of morbidity and mortality when compared to LLSCC.[30] Given the heterogeneity of head and neck cancers and their biological behavior and etiological factors, it is important to evaluate markers associated with DNA repair in SCC arising from different anatomical sites.

Major etiological and predisposing factors for oral SCC include mostly smoking and drinking habits, and ultraviolet radiation (specifically for LLSCC). Cigarette smoking may induce different types of DNA damage including adducts, strand breaks, and cross-links, which are repaired through different DNA repair pathways.[13] In addition, both UVA and UVB radiation is proven to produce DNA damage directly and indirectly through oxidative stress, promoting mutation in key genes associated with cell proliferation, DNA repair, and / or apoptotic cell death.[31]

Table 3. XRCC1 and XPF nuclear expression in relation to clinicopathological findings of lower lip squamous cell carcinoma.

Parameters	XRCC1 (nuclear)				XPF (nuclear)			
	Low	High	Total	p-value	Low	High	Total	p-value
Tumor size								
T1	3 (37.5)	5 (62.5)	8 (100.0)	1.000**	2 (25.0)	6 (75.0)	8 (100.0)	1.000**
T2–T4	10 (31.2)	22 (68.8)	32 (100.0)		10 (31.2)	22 (68.8)	32 (100.0)	
Node status								
N0	9 (30.0)	21 (70.0)	30 (100.0)	0.700**	8 (26.7)	22 (73.3)	30 (100.0)	0.451**
N+	4 (40.0)	6 (60.0)	10 (100.0)		4 (40.0)	6 (60.0)	10 (100.0)	
Clinical stage								
I–II	9 (34.6)	17 (65.4)	26 (100.0)	1.000**	8 (30.8)	18 (69.2)	26 (100.0)	1.000**
III–IV	4 (28.6)	10 (71.4)	14 (100.0)		4 (28.6)	10 (71.4)	14 (100.0)	
Survival status								
Alive at the end period of the study	12 (33.3)	24 (66.7)	36 (100.0)	1.000**	11 (30.6)	25 (69.4)	36 (100.0)	1.000**
Death due to tumor	1 (25.0)	3 (75.0)	4 (100.0)		1 (25.0)	3 (75.0)	4 (100.0)	
Recurrence								
Yes	0 (0.0)	4 (100.0)	4 (100.0)	0.284**	0 (0.0)	4 (100.0)	4 (100.0)	0.297**
No	13 (36.1)	23 (63.9)	36 (100.0)		12 (33.3)	24 (66.7)	36 (100.0)	
Brandwein Gensler et al. (2005)								
LR–IR	12 (34.3)	23 (65.7)	35 (100.0)	1.000**	11 (31.4)	24 (68.6)	35 (100.0)	1.000**
HR	1 (20.0)	4 (80.0)	5 (100.0)		1 (20.0)	4 (80.0)	5 (100.0)	
Almangush et al. (2015)								
Score 0	11 (39.3)	17 (60.7)	28 (100.0)	0.271**	10 (35.7)	18 (64.3)	28 (100.0)	0.285**
Scores 1–2	2 (16.7)	10 (83.3)	12 (100.0)		2 (16.7)	10 (83.3)	12 (100.0)	

LR: low risk; IR: intermediate risk; HR: high risk; *$\chi 2$ test; **Fisher's exact test.

Table 4. Correlation between XRCC1 and XPF immunoexpression.*

XRCC1/ XPF (nuclear)	r	p-value
OTSCC	0.235	0.145
LLSCC	0.728	< 0.0001

*Spearman's correlation test. LLSCC: lower lip squamous cell carcinoma; OTSCC: oral tongue squamous cell carcinoma.

DNA damage is associated with an increased risk of cancer, in which the reduced capacity of DNA repair can accelerate changes and mutations in essential genes, triggering carcinogenesis.[13,20,29] In this study, we observed a significant difference in the cytoplasmic protein expression of XPF between the OTSCC and LLSCC groups, with higher expression in OTSCC cases; however, there was no association with the clinical parameters analyzed. This cytoplasmic expression of XPF may indicate deregulation of protein function in OTSCC, which could contribute to the more aggressive behavior of the tumor at this anatomical site.

Our results disagree with Vaezi et al.[22] and Seiwert et al.[17] who demonstrated a significant association between high XPF expression and poor clinical outcome in patients with head and neck SCC. On the other hand, consistent with our results, the recent study by Prochnow et al.[32] found no association between the expression of XPF and clinical stage or prognosis in the cases analyzed.

Several studies suggest that the expression of XPF and XCRR1 can influence the prognosis in cases treated with DNA-damaging agents because of the

association of these drugs with the chemoresistance of neoplastic cells.[25,33,34] Like XRCC1, XPF plays an important role in the DNA repair pathways, particularly nucleotide excision repair. This pathway is used by the neoplastic process to correct the damage caused by the genotoxicity of platinum compounds and radiation. Studies using cells from XPF-deficient patients and animal models have shown that low expression of XPF is associated with higher sensitivity to DNA-damaging agents.[21,29]

Ang et al.[21] observed high nuclear expression of protein XRCC1 in head and neck carcinomas, which was related to poor patient survival, especially among those undergoing chemotherapy. In the study of Mahjabeen et al.,[14] expression of XRCC1 was associated with poor survival in a series of head and neck carcinomas and immunostaining for the protein was observed in both cytoplasm and nucleus. The authors reported cytoplasmic staining to be associated with a mutation in the gene and suggested that the deregulation of DNA repair genes such as XRCC1, APEX1, and OGG1, combined with high Ki67 expression, contributes to the progression of head and neck carcinomas. In our study, there was no cytoplasmic expression of XRCC1 in OTSCC, but high nuclear expression was associated with the absence of loco-regional metastasis, suggesting the use of high nuclear staining and absent cytoplasmic expression as a favorable prognostic marker in patients with OTSCC. Further studies are necessary to confirm these findings.

In previous studies investigating the expression of XRCC1 in tumors diagnosed at different anatomical sites such as those of Sultana et al.[35] in breast cancer and Liu et al.[36] in clear cell renal cell carcinoma, low expression of this protein was associated with more aggressive tumors, more advanced stages, higher rates of lymph node metastases, and poor survival. The authors concluded that deficient XRCC1 expression can be used as a prognostic biomarker for these carcinomas.

Regarding the immunoexpression of XPF, Vaezi et al.[22] showed that high expression of this protein is associated with tumor progression. The authors found a better clinical outcome for tumors that expressed low levels of XPF. Seiwert et al.[17] analyzed the expression of XPF in head and neck carcinoma and concluded that expression of this protein varies among carcinomas at different sites. We observed higher expression of XPF in cases diagnosed as OTSCC compared to the sample of LLSCC. This is the first study that evaluated immunostaining of the DNA repair proteins XPF and XRCC1 in LLSCC and it is possible that the two proteins act differently in LLSCC due to the peculiarities of this tumor when compared to OTSCC, especially in terms of the etiological factor. Applebaum et al.[37] observed a higher frequency of the XRCC1 gene polymorphism in smoking patients and suggested that this polymorphism may confer susceptibility to head and neck SCC in smokers. However, there are no studies evaluating the effect of solar radiation on polymorphisms in DNA repair proteins.

In conclusion, by affecting genomic stability, the deregulation of DNA repair proteins may be associated with the development and progression of SCC. Therefore, studies using different techniques are needed to identify the association between the proteins analyzed and the development, progression, and prognosis of SCC at different anatomical sites according to their peculiarities. Our study indicated an association between high nuclear expression of XRCC1 and the absence of loco-regional metastasis in OTSCC cases, suggesting that this protein is an important prognostic marker of this tumor. We also observed differences in the expression pattern of XPF between the anatomical sites analyzed; however, the expression of this protein was not significantly associated with the clinical course of LLSCC or OTSCC. Finally, immunohistochemical analysis of these proteins can be auxiliary to the methods of evaluation of staging and histological classification of the tumor to analyze the behavior of the tumor.

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