Original Article

Immunohistochemical expression of survivin in oral epithelial dysplasia and different grades of oral squamous cell carcinoma

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Abstract

Background: Survivin, a member of the inhibitor of apoptosis proteins family, is not detectable in most differentiated normal adult tissues but is expressed in a wide range of cancer tissues. Survivin expression in cancer has been associated with poor prognosis, cancer progression, and drug resistance, and the expression levels correlate with more aggressive disease and a poor clinical outcome.

Objective: To evaluate and compare the immunoexpression of survivin in the normal oral epithelium (NOE), oral epithelial dysplasia (OED), and different grades of oral squamous cell carcinoma (OSCC).

Methodology: The patterns of survivin immunoexpression and immunoreactivity were assessed in previously diagnosed, paraffin-embedded sections of 10 tissues of NOE and 15 tissues each of OED and the three grades of OSCC (well-, moderately-, and poorly-differentiated). The pattern of survivin expression was recorded as cytoplasmic, nuclear, or both. Survivin immunoreactivity was assessed semi-quantitatively as the immunoreactive score (IRS). Analysis of variance and Tukey-HSD tests were employed for statistical analysis.

Results: No immunoreactivity for survivin was evident in the NOE tissues. In the OED tissues, the immunoexpression pattern of survivin was predominantly nuclear in the basal cells, and in the OSCC tissues, cytoplasmic and nuclear. IRS was highest among the moderately- differentiated OSCC, followed by poorly- and well-differentiated OSCC and OED, with a statistically significant difference in the IRS scores between the normal and the study groups.

Conclusion: Survivin protein expression may be an important early event in oral carcinogenesis and may predict unfavorable prognosis in OSCC.

Keywords: Inhibitor of apoptosis proteins, oral epithelial dysplasia, oral squamous cell carcinoma, survivin

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common head and neck cancer, mostly with a poor prognosis, the 5-year survival rate being 35–50%, despite recent advances in radiation therapy, improvement in surgical

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techniques, and the advent of aggressive chemotherapy protocols.[1] Improved prognostication would be clinically valuable, particularly in cases where initial therapy might be tailored to tumor aggressiveness. One of the primary reasons for the poor prognosis in OSCC is the lack of significant and unique molecular tumor markers to assess risk and prognosis. Therefore, identification of better prognostic tumor markers is necessary to assist clinicians in more accurate staging and grading of lesions and prediction of prognosis.[2]

Survivin is a member of the inhibitor of apoptosis proteins (IAP) family, whose members have been shown to inhibit activated caspases.[3] Contrary to most IAP family members, survivin mRNA is expressed diffusely during fetal development but is not generally found in normal adult tissues. Moreover, survivin expression has been detected in various human cancers including bladder, colon, liver, brain, lung, and prostate. In the majority of cancers studied to date, survivin expression is associated with poor prognosis.[4]

Recently, the focus of relevant research has shifted towards survivin being exploited as a target in cancer therapy, especially because studies have revealed that the role of survivin in cancer cells is not just limited to inhibition of apoptosis, but may also be associated with aggressive characteristics of cancer, such as angiogenesis and invasiveness.[4] However, there are relatively few investigations on the role of survivin in the pathogenesis of OSCC and the correlation of survivin expression with the degree of differentiation of OSCC.

In the present study, we assessed and compared the immunoexpression patterns and immunoreactivity of surviving in tissue samples of the normal oral epithelium (NOE), oral epithelial dysplasia (OED), and the three grades of OSCC.

MATERIALS AND METHODS

After obtaining approval from the institutional ethical committee, the present study was undertaken by retrieving previous records and paraffin-embedded tissue blocks of diagnosed cases of OSCC. Ten tissue specimens of NOE (Group I) were obtained from patients undergoing routine oral surgical procedures, with patients with a history of tobacco usage or with clinically or histopathologically diagnosed other pathologies being excluded. Fifteen tissue specimens were obtained from patients with histologically diagnosed OED (Group II), and only patients with a history of tobacco usage (smoking/chewing) were included. Of the 45 cases of OSCC, 15 cases were grouped as well-differentiated OSCC (WDSCC) (Group III), 15 cases as moderately differentiated OSCC (MDSCC) (Group IV), and 15 cases as poorly differentiated OSCC (PDSCC) (Group V).

Two fresh sections of 3-μm-thickness were cut from each formalin-fixed and paraffin-embedded tissue block. One set of sections was stained with haematoxylin and eosin. The histological grading of malignancy was analysed under light microscopy according to Brynes's grading system. Another set of sections was taken onto polylysine-coated micro-slides for immunohistochemical staining.

Immunohistochemical procedure

The sections were deparaffinised and rehydrated through xylene and descending grades of alcohol. Antigen retrieval was carried out in a microwave in 10 mM citrate buffer (pH 6.0) at high power for 15 min and at low power for 10 min, followed by washing in Tris-buffer saline. The sections were then incubated after covering with 4% hydrogen peroxide for 30 min to block any endogenous peroxidase activity, and the slides were incubated with primary anti-survivin monoclonal antibody (Biogenex Life Sciences Private Limited CA, USA, 6 ml, ready-to-use) for 60 min at 37°C in a humid chamber. The sections were then incubated with secondary linking antibody (biotinylated anti-immunoglobulins/super enhancer) at room temperature, in a humid chamber for 30 min to enhance the effect of the subsequent polymer step. The sections were incubated with pre-diluted secondary antibody, that is, the conjugate (enzyme-conjugated streptavidin) at room temperature for 30 min. This was followed by incubation with DAB and counter staining with Mayer's hematoxylin. For negative control, tissue sections were treated with all the reagents except the primary antibody. Positive control tissue sections were used to ensure homogenous, accurate, and reproducible staining and this included the NOE tissues.

Immunohistochemical analysis

All the immunohistochemically stained slides from the study groups I, II, III, IV, and V were evaluated for the expression of survivin. Survivin immunopositivity was defined as the presence of a brown-coloured immunostaining of the nucleus and cytoplasm. The pattern of survivin expression in all the groups was recorded based on their localization as cytoplasmic, nuclear, and both cytoplasmic and nuclear expressions. The immunoreactivity of survivin in all the groups was assessed semi-quantitatively by calculating the immunoreactive score (IRS) as follows:

IRS = Percentage of immunopositive cells (A) × Intensity of immunostaining (B) [Table 1].

Table 1: Calculation of immunoreactive score (IRS)

A - Percentage of survivin immunopositive cells	
Points	**Percentage of immunopositive cells**
0	0%
1	<10%
2	10-29%
3	30-59%
4	60-100%
B - Intensity of survivin immunostaining	
Points	**Staining intensity**
0	No staining
1	Mild
2	Moderate
3	Strong
IRS=A × B	
0-1	Negative
2-3	Mild
4-8	Moderate
9-12	Strong

The survivin immunoexpression patterns and the immunoreactivity assessed from the calculated IRS were statistically compared among all the groups using the Statistical Package for Social Sciences (SPSS, version 20.0) statistical analysis software. The values were represented in numbers, percentages, and means ± standard deviation. Analysis of variance (ANOVA) was used to compare the variance in the IRS of survivin within the five groups, and the Tukey-HSD test was used to assess pair-wise comparison between the groups.

RESULTS

In the present study, the immunoexpression patterns and IRS of survivin were recorded and compared among NOE, OED, and the three grades of OSCC (WDSCC, MDSCC, and PDSCC). In NOE, no survivin expression was found [Figure 1a, 1b]. Nuclear expression was seen in maximum samples of OED [14 samples (93.3%)] [Figure 2a, 2b], and both cytoplasmic and nuclear expressions were found more in MDSCC [eight samples (53.3%)] [Figure 3a, 3b] and PDSCC [(nine samples (60.0%)] [Figure 4a, 4b]. Collectively, among all the samples, cytoplasmic expression was found in the least number of samples [10 samples (14.3%)]. Statistically highly significant differences were found in the immunoexpression patterns of survivin among all the five groups ($P = 0.001$) [Table 2 and Graph 1].

On comparison of the IRS among all the groups, moderate immunoreactivity was seen in maximum number of samples [35 samples (50%)]. All samples of NOE were negative for survivin immunoreactivity [Figure 1a, 1b]. Moderate immunoreactivity was seen among the maximum

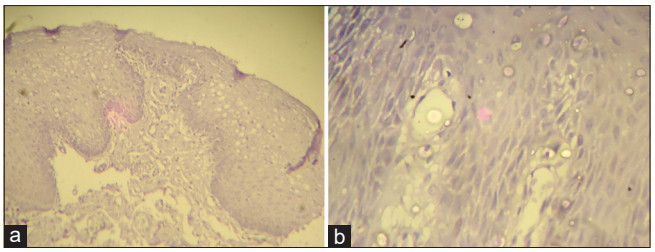


Figure 1: Survivin immunopositivity not observed in the normal oral epithelium (a×100, bx400)

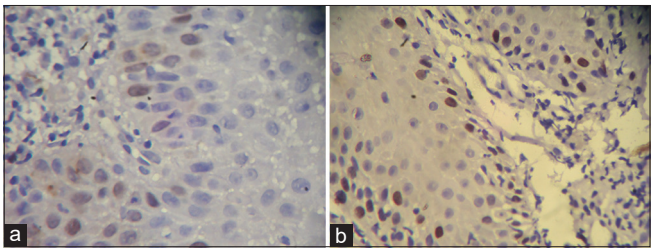


Figure 2: (a and b) Survivin immunopositivity observed in oral epithelial dysplasia (×400)

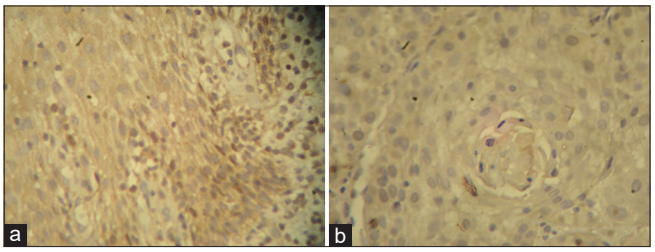


Figure 3: (a and b) Survivin immunopositivity observed in moderately-differentiated squamous cell carcinoma (×400)

samples of OED [11 samples (73.3%)] [Figure 2a, 2b], WDSCC [nine samples (60.0%)] [Figure 5a, 5b], and MDSCC [10 samples (66.6%)] [Figure 3a, 3b], and an equal number of samples of PDSCC [five samples each (33.3%)] [Figure 4a, 4b] showed moderate and strong immunoreactivity. A highly statistically significant difference in the IRS of survivin was found among all the five groups ($P = 0.001$) [Table 3 and Graph 2].

On comparison of the immunoexpression patterns and IRS of survivin in OED with the other groups, it was found that a statistically significant difference was found in the expression pattern of OED and other groups, with a nuclear expression in 14 samples (93.3%) of OED and in seven samples (46.7%) of WDSCC, three samples (20.0%) of MDSCC, and three samples (20.0%) of PDSCC [Figures 2-5]. However, no statistically significant difference was found in the IRS of OED when compared with other groups, with moderate immunoreactivity in 11 samples (73.3%) of OED and in nine samples (60.0%) of WDSCC, 10 samples (66.6%) of MDSCC, and five samples (33.3%) of PDSCC [Tables 4 and 5].

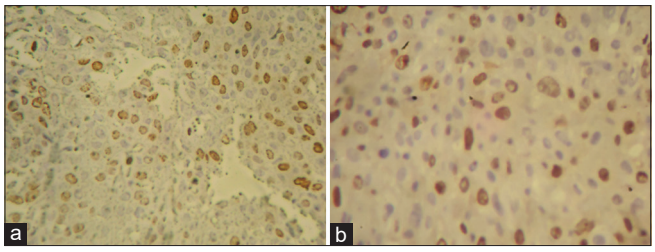


Figure 4: (a and b). Survivin immunopositivity observed in poorly-differentiated squamous cell carcinoma (×400)

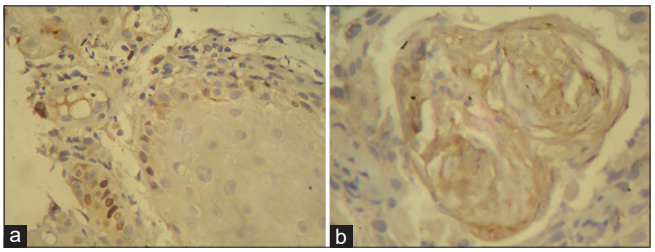
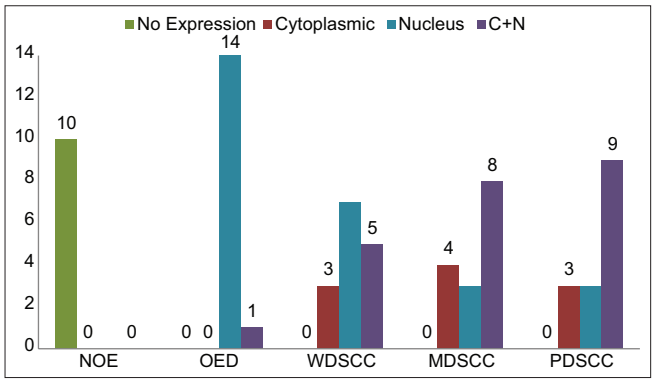
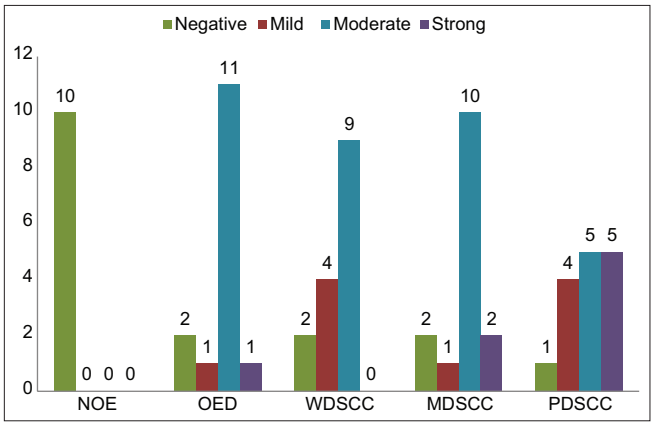


Figure 5: (a and b) Survivin immunopositivity observed in well-differentiated squamous cell carcinoma (×400)



Graph 1: Comparison of immunoexpression patterns among the study groups



Graph 2: Comparison of the IRS among the study groups

Table 2: Comparison of immunoexpression patterns among the study groups

Groups	*n*	Immunoexpression Patterns			
		No expression	Cytoplasmic	Nucleus	C + N
NOE	10	10 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
OED	15	0 (0.0%)	0 (0.0%)	14 (93.3%)	1 (6.7%)
WDSCC	15	0 (0.0%)	3 (20.0%)	7 (46.7%)	5 (33.3%)
MDSCC	15	0 (0.0%)	4 (26.7%)	3 (20.0%)	8 (53.3%)
PDSCC	15	0 (0.0%)	3 (20.0%)	3 (20.0%)	9 (60.0%)
Total	70	10 (14.3%)	10 (14.3%)	27 (38.6%)	23 (32.9%)
χ^2			96.019		
Significance *P*			0.001 (HS)		

Table 3: Comparison of IRS among the study groups

Groups	*n*	Immunoreactivity Score (IRS)			
		Negative	Mild	Moderate	Strong
NOE	10	10 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
OED	15	2 (13.3%)	1 (6.7%)	11 (73.3%)	1 (6.7%)
WDSCC	15	2 (13.3%)	4 (26.7%)	9 (60.0%)	0 (0.0%)
MDSCC	15	2 (13.3%)	1 (6.7%)	10 (66.6%)	2 (13.3%)
PDSCC	15	1 (6.7%)	4 (26.7%)	5 (33.3%)	5 (33.3%)
Total	70	17 (24.3%)	10 (14.3%)	35 (50.0%)	8 (11.4%)
χ^2			51.712		
Significance *P*			0.001 (HS)		

On comparison of the immunoexpression patterns and IRS of survivin in WDSCC and the other grades of OSCC, no statistically significant difference was found in the immunoexpression patterns, with nuclear expression although seen more in WDSCC [three samples (46.7%)] as compared to MDSCC [three samples (20%)] and PDSCC [three samples (20%)], while cytoplasmic and nuclear expression seen more in PDSCC [nine samples (60%)] than in WDSCC [five samples (33.3%)] [Figures 3-5]. Likewise, no significant difference was found in the IRS of WDSCC when compared with those of MDSCC and PDSCC, with nine samples (60%) of WDSCC, 10 samples (66.6%) of MDSCC, and five samples (33.3%) of PDSCC showing moderate immunoreactivity [Tables 4 and 5].

Lastly, on comparison of the immunoexpression patterns and IRS of survivin in MDSCC and PDSCC, no statistically significant difference was found in the expression patterns of both the groups, although with cytoplasmic and nuclear expression being slightly more in PDSCC [nine samples (60.0%)] as compared to in MDSCC [eight samples (53.3%)] [Figures 3 and 4]. Likewise, no significant difference was found in the IRS of MDSCC and PDSCC, although with moderate immunoreactivity being seen in maximum samples of MDSCC [10 samples of (66.6%)] and only five samples (33.3%) of PDSCC and strong immunoreactivity being seen in two samples (13.3%) of MDSCC and five samples (33.3%) of PDSCC [Tables 4 and 5].

DISCUSSION

Carcinogenesis is a multistage process involving the activation of oncogenes and the inactivation of tumor suppressor genes. Thus, most human tumors are

Table 4: Pairwise comparison of immunoexpression patterns among the study groups

Groups	*n*	Immunoexpression Patterns				χ^2	*P*
		No Expression	Cytoplasmic	Nucleus	C + N		
OED	15	0 (0.0%)	0 (0.0%)	14 (93.3%)	1 (6.7%)	8.000	0.018 (S)
WDSCC	15	0 (0.0%)	3 (20.0%)	7 (46.7%)	5 (33.3%)		
OED	15	0 (0.0%)	0 (0.0%)	14 (93.3%)	1 (6.7%)	16.562	0.001 (HS)
MDSCC	15	0 (0.0%)	4 (26.7%)	3 (20.0%)	8 (53.3%)		
OED	15	0 (0.0%)	0 (0.0%)	14 (93.3%)	1 (6.7%)	16.518	0.001(HS)
PDSCC	15	0 (0.0%)	3 (20.0%)	3 (20.0%)	9 (60.0%)		
WDSCC	15	0 (0.0%)	3 (20.0%)	7 (46.7%)	5 (33.3%)	2.435	0.296(NS)
MDSCC	15	0 (0.0%)	4 (26.7%)	3 (20.0%)	8 (53.3%)		
MDSCC	15	0 (0.0%)	4 (26.7%)	3 (20.0%)	8 (53.3%)	0.202	0.904(NS)
PDSCC	15	0 0.0%)	3 (20.0%)	3 (20.0%)	9 (60.0%)		
WDSCC	15	0 (0.0%)	3 (20.0%)	7 (46.7%)	5 (33.3%)	2.743	0.254(NS)
PDSCC	15	0 (0.0%)	3 (20.0%)	3 (20.0%)	9 (60.0%)		

Table 5: Pairwise comparison of IRS among the study groups

Groups	*n*	Immuno Reactivity Score (IRS)				χ^2	*P*
		Negative	Mild	Moderate	Strong		
OED	15	2 (13.3%)	1 (6.7%)	11 (73.3%)	1 (6.7%)	3.000	0.392 (NS)
WDSCC	15	2 (13.3%)	4 (26.7%)	9 (60.0%)	0 (0.0%)		
OED	15	2 (13.3%)	1 (6.7%)	11 (73.3%)	1 (6.7%)	0.381	0.944 (NS)
MDSCC	15	2 (13.3%)	1 (6.7%)	10 (66.6%)	2 (13.3%)		
OED	15	2 (13.3%)	1 (6.7%)	11 (73.3%)	1 (6.7%)	7.050	0.070(NS)
PDSCC	15	1 (6.7%)	4 (26.7%)	5 (33.3%)	5 (33.3%)		
WDSCC	15	2 (13.3%)	4 (26.7%)	9 (60.0%)	0 (0.0%)	3.853	0.278(NS)
MDSCC	15	2 (13.3%)	1 (6.7%)	10 (66.6%)	2 (13.3%)		
MDSCC	15	2 (13.3%)	1 (6.7%)	10 (66.6%)	2 (13.3%)	5.086	0.166(NS)
PDSCC	15	1 (6.7%)	4 (26.7%)	5 (33.3%)	5 (33.3%)		
WDSCC	15	2 (13.3%)	4 (26.7%)	9 (60.0%)	0 (0.0%)	6.476	0.091(NS)
PDSCC	15	1 (6.7%)	4 (26.7%)	5 (33.3%)	5 (33.3%)		

characterized by an imbalance of regulatory mechanisms controlling cell cycle progression, cell death/viability balance, and apoptosis. Apoptosis has become a basic tool in developing cancer research and establishing new cancer strategies.[5] Regulation of apoptosis plays a crucial role in embryonic development, tissue morphogenesis, and homeostasis, as a physiologic event that regulates cell number and eliminates damaged cells. On the other hand, resistance to apoptotic stimuli is frequently involved in cancer development and progression as well as in autoimmune disorders. One of the mechanisms through which tumor cells are believed to acquire resistance to apoptosis is by overexpression of IAPs, one of the IAPs being the survivin protein. In OSCC, a high incidence of survivin overexpression has recently been reported.[6]

The role of survivin protein is unique, having been shown to specifically bind to caspases-3 and -7 and inhibit apoptosis in vitro.[7] Furthermore, Li *et al*.[8] reported that survivin expresses during the G2/M phase of the cell cycle and the disruption of survivin-microtubule interactions results in an increased caspase-3 activity and accelerated apoptotic cell death. Ito *et al*.[9] reported that hepatocellular carcinoma cell lines transfected with survivin showed a significant decrease in cells in the G0/G1 phase and an increase in cells in the S and G2M phases. These findings indicate that survivin protein expression may correlate not only with reduced apoptotic cell death but also with increased proliferative activity of cancer cells.[10] In particular, survivin expression is often increased in poorly differentiated tumours, even if, the differences have not been statistically significant. Furthermore, a high survivin expression has been shown to be correlated with poor survival rates.[5]

In our study, we analyzed the immunoexpression patterns and IRS of survivin in NOE, OED, and the three grades of OSCC. We found no survivin immunoexpression in the NOE samples, which was in accordance with the results published by Jinbu *et al*.,[11] Khan *et al*.,[12] Lin *et al*.,[6] and Li *et al*.[13] However, in our study, we found a nuclear localization of survivin in 93.3 percent samples of OED, while similar studies by Jinbu *et al*.,[11] Khan *et al*.,[12] Jane *et al*.,[14] and Lin *et al*.[6] have reported a cytoplasmic localization. Likewise, Pannone *et al*.[15] observed survivin immunostaining in all cases of oral pre-malignant lesions, with an intracellular localisation of survivin, prevalently in the cytoplasm with focal nuclear expression. In our study, we found 73.3% of the OED samples exhibiting survivin positivity; however, Negi *et al*.[16] in their study, observed only 53.3% of cases with dysplasia to be survivin positive.

In the OSCC samples, we found a predominantly nuclear localisation in samples of WDSCC and a predominant cytoplasmic-nuclear localization in samples of MDSCC and PDSCC. Likewise, Jinbu *et al*.,[11] Lippert *et al*.,[17] Li *et al*.,[13] Lauxen *et al*.,[18] and Pannone *et al*.[15] reported both nuclear and cytoplasmic expression of survivin in the keratinocytes of OSCC. However, Khan *et al*.[12] and Lin *et al*.[6] observed cytoplasmic survivin staining in OSCC cases and Preuss *et al*.[19] observed nuclear survivin expression, moreover, adding a note on poor overall survival rate in these patients compared with those with a non-nuclear expression of survivin. Lo Muzio *et al*.[5] observed cytoplasmic prevalent survivin immunostaining in poorly differentiated cases, with sporadic prominent nuclear staining in well-differentiated areas.

Kim *et al*.[20] reported weak survivin expression 21% of the OSCC biopsy specimens and 79% of his samples showed strong survivin expression. Jane *et al*.[14] reported that WDSCCs had weak survivin expression, predominantly in the keratinocytes, MDSCCs showed weak to moderate cytoplasmic survivin expression, and all PDSCCs showed moderate to strong survivin expression, with two samples showing distinct nuclear expression of survivin. In our study, we observed higher overall immunoreactivity as compared to these studies, with moderate immunoreactivity in 60% samples of WDSCC and 66.6% samples of MDSCC and an almost equal number of samples of PDSCC showing mild, moderate, and severe immunoreactivity. Qi *et al*.[21] reported high expression of nuclear and/or cytoplasmic survivin in 54% of head and neck squamous cell carcinoma (HNSCC) cases and stated that survivin-positive cells were observed predominantly in the periphery of the tumor nests in well-differentiated HNSCCs, while in poorly-differentiated HNSCCs, the cells were present throughout the tumor nests. On the contrary, Pickhard *et al*.[4] reported that the expression of survivin was decreased in both tumor tissue and lymph node tissue with metastasis, as compared with noncancerous tissues.

Comparing survivin expression in OSCC and premalignant lesions to normal tissue, Lin *et al*.,[6] Khan *et al*.,[12] and Pannone *et al*.[15] reported higher levels in OSCC and premalignant lesions than normal oral tissue. De Maria *et al*.[22] and Su *et al*.[23] observed markedly elevated levels of survivin in OSCC compared to normal tissue. Zhou *et al*.[24] found significantly higher survivin expression in OSCC tissues transformed from oral submucous fibrosis compared with normal tissues. Negi *et al*.[16] stated that although the difference in the number of survivin positive cells was statistically insignificant between normal oral mucosa and leukoplakia, it was found to be statistically significant between leukoplakia and OSCC and between normal oral mucosa and OSCC. Lo Muzio *et al*.[5] reported that although the survivin expression was often increased in poorly differentiated tumors, the differences that resulted were not statistically significant. In our study, all the NOE samples were negative for survivin expression, and the immunoexpression pattern of survivin in OED was significantly different than WDSCC and highly significantly different than MDSCC and PDSCC. However, the immunoreactivity of OED was not significantly different from any grade of OSCC. Furthermore, in our study, no statistically significant differences were observed in the immunoexpression patterns and immunoreactivity of survivin among the three grades of OSCC.

CONCLUSION

The survivin protein expression patterns were significantly different in the OED samples and the samples of OSCC; however, the immunoreactivity was not found to be significantly different. All the OSCC samples showed moderate to high survivin immunoreactivity, backing up the potential role of survivin in dysplasia and malignancies. Since none of the NOE samples expressed survivin, the overexpression of the protein in cases of OED and OSCC can help us target it for diagnostic, prognostic, and therapeutic purposes.

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Conflicts of interest
There are no conflicts of interest.

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