Original Article

Expression of Survivin in Oral Potentially Malignant Disorders: An Immunohistochemical Study

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Aim: The aim of this study was to compare the immunohistochemical expression of survivin in oral potentially malignant disorders (OPMDs) and evaluate its prognostic significance among oral leukoplakia (OL), oral submucous fibrosis (OSMF), and oral lichen planus (OLP).

Materials and Methods: The study material consisted of 60 formalin-fixed paraffin-embedded tissue samples: 15 cases each of OL, OSMF, OLP, and normal oral mucosal epithelium as control. Survivin expression was analyzed immunohistochemically, and data analysis was accomplished using Statistical Package for Social Sciences, version 22.0 (SPSS Inc., Chicago, IL). Fisher’s chi-square test was opted to compare the study groups.

Results: Survivin was expressed in all the OPMDs including OL, OSMF, and OLP, but was absent in normal oral tissue samples. Higher immunoreactivity and survivin staining was observed in OLP compared to OL and OSMF whereas OL showed a significant difference in the distribution of survivin immunoexpression against OLP. An increased nuclear expression of survivin along with distribution in the basal and parabasal layers was evident in all OPMDs.

Conclusion: Survivin was expressed more in OLP in comparison to OSMF and OLP, indicating unfavorable prognosis. OL showed increased expression in comparison to OSMF, showing unfavorable prognosis. On the basis of this study, it was concluded that survivin may be used as an important diagnostic and prognostic marker for OPMDs.

Keywords: Immunohistochemistry, oral leukoplakia, oral lichen planus, oral submucous fibrosis, prognostic marker, survivin

INTRODUCTION

Oral cancer is a subgroup of head and neck malignant neoplasms that includes carcinomas arising from the lining mucosa of the lips, the buccal mucosa, the retromolar region, the alveolar ridges, the anterior two-thirds of the tongue, the hard palate, and the floor of the mouth. Oral epithelial dysplasia is a common precursor of oral cancer. The term “oral potentially malignant disorders” (OPMDs) was adopted by the World Health Organization in 2005 to describe oral lesions and conditions associated with a risk of malignant transformation.

Survivin, a unique member of the inhibitor of apoptosis protein (IAP) family, has been demonstrated as an essential protein for mitotic regulation and inhibition of apoptosis. It has been documented that survivin plays an active role in both physiological and pathological conditions such as carcinogenesis in many human cells.

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MATERIALS AND METHODS

Before the commencement of the study, ethical approval was obtained. Appropriate permissions were obtained from another institution to carry out the immunohistochemistry (IHC) procedures.

This retrospective study was conducted by retrieving formalin-fixed paraffin-embedded (FFPE) tissue specimens of oral leukoplakia (OL), oral submucous fibrosis (OSMF), and oral lichen planus (OLP) from the archives and fresh specimens of normal oral epithelium (NOE) of the research institute. The study material comprised 60 FFPE tissue specimens (15 histopathologically confirmed tissue specimens of OL, OSMF, OLP, and NOE as Group I, Group II, Group III, and Group IV, respectively).

Immunohistochemical staining

Upon preparation, tissue sections of 4 μm thickness were mounted on poly-lysine-coated glass slides. The slides were heated and immersed in citrate buffer at a pH of 6 in a microwave oven for antigen retrieval. Primary rabbit monoclonal antibodies against survivin were used. Biotinylated anti-rabbit immunoglobulin G, used as a secondary antibody, was detected using streptavidin-conjugated horseradish peroxidase with 3,3-diaminobenzidine, and Harris’ hematoxylin was used for counterstaining. A strong expression of survivin in high-grade human breast carcinoma specimen was used as positive control. Staining the positive control breast cancer specimen with the secondary antibody alone was used as a negative control.

Criteria for evaluation

The IHC slides were viewed by two observers using an Olympus BX53® (Olympus, Tokyo, Japan) light microscope and compared with their respective hematoxylin and eosin sections. Photomicrography was performed with ProgRes Speed XT Core 3 software (Jena, Thuringia, Germany). Survivin immunopositivity was assessed by the presence of a brown color immunostaining of the nucleus and cytoplasm.

The intensity of staining was estimated based on the criteria followed by Tanaka et al.\(^\text{i}\): no staining (0), mild staining (1), moderate staining (2), and strong staining (4). The percentage of survivin immunopositivity was categorized based on the criteria adopted by Muzio et al.\(^\text{j}\). It was estimated and graded, in five random fields, on a scale of 0–4: <5% immunopositive cells (0); 5%–25% immunopositive cells (1); 26%–50% immunopositive cells (2); 51%–75% immunopositive cells (3); and >75% immunopositive cells (4). The immunoreactivity of survivin was assessed by the immunoreactivity score (IRS; percentage of immunopositive cells × staining intensity). It was scored as negative (0–1), mild (2–3), moderate (4–8), and strongly positive (9–12).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Sciences, version 22.0, for Windows (SPSS Inc., Chicago, IL). Fisher’s chi-square test was opted to compare the variance in distribution, intensity of staining, and percentage of immunopositivity, and to assess pair-wise comparison between the study groups. Analysis of variance was used to compare the IRS of survivin within the four groups. Significant relationship was denoted by a \(P\) value less than 0.05 (\(P > 0.05\) [not significant], \(P < 0.05\) [significant], \(P < 0.01\) [highly significant], and \(P < 0.001\) [very highly significant]).

RESULTS

Survivin expression was predominantly absent in NOE, but mild nuclear staining was evident in the basal layer in two samples [Figure 1A and B]. However, survivin immunoreactivity was found predominantly in the nuclear region of dysplastic epithelial cells in most cases of OL (73.3%) [Figure 2A and B], OSMF (86.7%) [Figure 3A and B], and OLP cases (66.7%) [Figure 4A.
and B) as as represented in Table 1. Both cytoplasmic and nuclear expression of survivin was found in 20% of OLP, 13.3% of OSMF, and 6.7% of OL cases, whereas cytoplasmic expression of this marker was observed only in few cases of OL (20%) and OLP (13.3%). The intensity of survivin reaction was found to be severe in 46.7% of OL cases. Nevertheless, majority of cases of OL (86.7%), OSMF (80%), and OLP (53.3%) showed moderate staining intensity, which was represented in Table 2. The number of immunopositive cells was higher for OLP with 80% samples exhibiting reaction in 10%–29% cells. OL had immunopositivity in the range of <10% (46.7%) and 10%–29% (46.7%), but a single case (6.7%) demonstrated greater positivity 30%–59%. Most cases (53.3%) of OSMF had expression in only <10% cells and the rest 46.7% cases had survivin expression in 10-29% of epithelial cells as represented in Table 1. Group wise comparison is represented in Table 3 and Graph 1.

**Discussion**
OPMD forms a family of clinical or histological alterations of oral epithelium that indicates a risk

Figure 2: Oral leukoplakia showing survivin immunopositivity in the basal and suprabasal areas (A [100×]; B [200×])

Figure 3: Oral submucous fibrosis demonstrating positive survivin immunoreactivity in the basal and suprabasal areas (A [100×]; B [200×])

Figure 4: Oral lichen planus showing positive survivin immunoreactivity in the basal and suprabasal areas (A [100×]; B [200×])
Rajanna, et al.: Survivin expression in OPMDS

Overexpression of IAPs is thought to be a primary mechanism through which tumor cells acquire resistance to apoptosis. Survivin appears to have an active role in maintaining the tumor cell vitality and has been shown to bind specifically to caspases 3 and 7 resulting in inhibition of apoptosis. In OSCC, a high incidence of survivin overexpression has been reported and correlates with poor survival rates, thus making it a better prognostic marker.

This study demonstrated negative immunoreactivity in majority of NOE samples, but mild nuclear staining was observed in less than 10% of cells predominantly in the basal layer in two samples. The outcome was towards transformation into squamous cell carcinoma. Microscopic examination to identify the existence of epithelial dysplasia is currently the reference standard in the assessment of OPMD to assess the risk of developing oral squamous cell carcinoma. OL, OSMF, and OLP are suggested to have a premalignant potential with varying consistencies. A study by Warnakulasuriya[6] stated that, despite the availability of many molecular markers for the diagnosis of OPMD, an accurate predictive assessment of the clinical behavior of OPMDs will depend on development of newer markers.[6]
also consistent with earlier studies, which stated that the scanty expression could be due to active mitotic figures.8-11 However, a study by Chaiyarit et al.12 demonstrated noticeable expression of survivin in NOE and attributed to processing errors in choice of fixatives, antigen-retrieval methods, usage of primary antibodies, and detecting systems.

Survivin immunoreactivity was demonstrated in most cases of OL (87%) with two samples (one case each of mild and moderate dysplasia) showing negative IRS. This was comparatively higher, as earlier reports had survivin immunopositivity in the range of 33%–65% for OL.13-16 The immunoreactivity varied from mild to moderate with a single case of histopathologically confirmed carcinoma in situ (CIS) demonstrating stronger expression. Gayathri and Rao17 demonstrated a progressive increase in survivin expression based on predominant staining pattern and histological grading. Although our study established stronger survivin expression in one case of CIS, a correlation between the histological grading and survivin expression could not be established.

Existence of survivin in two subcellular pools, cytoplasmic and nuclear, has been documented. Majority of OL samples in our study demonstrated nuclear expression of survivin (73.3%). Conversely, most studies had reported cytoplasmic expression of survivin in OPMDs, especially OL.5,10,11,13 Survivin was predominantly distributed in the basal and parabasal layers of OL. Nevertheless, it was localized in the parakeratin/keratin layers and the prickle cell layers in most studies.14-17 Despite the fact that nuclear expression of survivin is an unfavorable factor for prognosis in various tumors and OPMDs occurring in humans, some authors have proposed survivin nuclear positivity as a favorable prognostic marker.16

Almost 46.7% of OL samples had survivin immunopositivity in less than 10% of cells. In 46.7% samples, survivin was expressed in 10%–29% cells whereas a single sample showed positivity in 30%–59% cells. No significant difference was noted in the immunopositivity patterns between our study and earlier studies.13,18

This study demonstrated strong staining intensity in most samples (46.7%) of OL followed by moderate (33.3%) and mild (20%) staining intensity. Gayathri and Rao17 demonstrated predominantly moderate (37%) staining intensity followed by mild (33%) and strong (17%) staining intensity in OL. This could be attributed to the fact that the measure of staining intensity could not be considered as an endpoint measurement of survivin expression due to variations present during sample collection, fixation, processing, staining, and observation.

A high potential for malignant transformation was demonstrated in OSMF cases by Murti and Bhosle19 and Ekanayaka and Tilakaratne.20 Murti and Bhosle19 stated that the malignant transformation rate rose from 4.5% to 7.6% with a 2-year increase in observation period. OSCC that originated from OSMF tissues demonstrated survivin localization in the nucleus with different brown granules.21 In our study, survivin location in OSMF cases was predominantly noted to be nuclear and distributed in the basal and parabasal layers. However, the distribution pattern varied according to the stages of OSMF in previous studies. The intensity of survivin positivity was in accordance with the observation made by Zhou et al.9,21

This study had survivin expression in 100% samples. The majority of cases showed moderate immunoreactivity in basal and suprabasal layers of epithelium whereas a study by Suganya et al.22 demonstrated mild-to-moderate survivin expression predominantly in the basal layer. The survivin staining intensity was moderate in 60% samples, strong in 27% samples, and mild in 13% of samples.

Studies with intergroup analyses for survivin expression performed earlier include Zhou et al.9 (OPMD, OSCC, and NOE), Oluwadara et al.15 (OL, OLP, OSCC, and NOE), Gayathri and Rao17 (OL, OSCC, and NOE), and Zhou et al.22 (OPMD, OSCC, and NOE). Intergroup comparison using IRS in our study revealed significant difference between OL and OLP and between OSMF and OLP. However, no significant difference was observed between OL and OSMF. Although most of the OL lesions were cases of mild dysplasia, majority of the OLP cases were erosive OLP. It has been documented that the malignant potential among the OLP lesions is comparatively high in erosive OLP.22 The higher IRS in OLP group can be attributed to the fact that the lesions that were selected for IHC were predominantly erosive OLP.

The distribution of survivin expression in different epithelial layers was insignificant between OL and OSMF and between OSMF and OLP, but there was a significance of difference between OL and OLP. Although the expression of survivin in majority of OL cases was noted to be in the basal and suprabasal layers, almost 33% of OL cases showed expression restricted only to the epithelial basal layers. In contrast, all the cases of OLP showed expression in the basal and suprabasal layers. There was one case of histopathologically
confirmed CIS, which showed expression across the full thickness of the epithelium demonstrating that survivin expression indeed correlated with the progression of dysplastic features across the epithelial layers.

The mean immunopositivity showed significance between OSMF and OLP but was insignificant between the other two lesions. No significance could be evaluated between the groups based on staining intensity. Most cases of OLP (80%) showed positive expression in the 10%–29% range whereas 50% cases of the OSMF group showed expression in less than 10% of the cells. This can be again attributed to the fact that the malignant transformation in OLP and OSMF is significantly different. This attains more importance as most OLP cases included in the study were erosive OLP, and the 13% of OLP cases that showed expression in less than 10% of cells were reticular OLP. Increased immunopositivity can be considered to demonstrate an increase in malignant transformation potential, but as the measurement of immunopositivity alone is very subjective, further large-scale studies may be required before arriving at a consensus.\(^{[36]}\)

In our study, significant difference was noted between OL, OSMF, and OLP against the NOE as control. However, this diverse expression of survivin in normal versus malignant tissue is a strong advocate for progression of survivin-based cancer drug research.

**CONCLUSION**

OPMDs provide an excellent model for studying the malignant transformation process and the development of cancer. Survivin was not/minimally expressed in normal oral tissue samples, consistent with many studies that had repeatedly proved that survivin is not expressed in normal tissues that do not show high proliferative activity. Survivin was expressed in all the OPMDs including OL, OSMF, and OLP in comparison with NOE. This study suggests that survivin detection may contribute to a new avenue to identify OPMDs in patients at high risk of antagonistic outcomes. This is particularly relevant in cases of survivin expression in patients with an ambiguous histological outcome, for whom new molecular indicators of dysplasia are needed with priority.

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**Conflicts of interest**

There are no conflicts of interest.

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