ORIGINAL ARTICLE

EXPRESSION OF SURVIVIN IN ORAL MALIGNANCY AND PREMALIGNANCY

Hoor Maryam[1], Sabeen Nasir[2], Mohammad Mumtaaz Khan[2], Abbas Saleem[1], Nasiha Basher[1], Fatima Iqbal[1], Tehmina Naushen[1]

[1]Department of Oral Pathology, Peshawar Dental College, Peshawar - Pakistan

[2]Department of Pathology, Peshawar Medical College, Peshawar - Pakistan

ABSTRACT

Objectives: To evaluate the immunohistochemical expression of survivin in oral squamous cell carcinoma (OSCC), oral potentially malignant disorders (OPMDs) and normal oral mucosa.

Materials and methods: This descriptive cross sectional study comprised of 25 already diagnosed cases of OPMDs, 25 cases of OSCC and 25 normal oral mucosa as a control. Immunohistochemical expression of survivin was analyzed.

Results: Immunoreactivity for survivin was found positive in 60% cases of OPMDs and 100% in OSCC but was negative in normal oral mucosa only one case showed mild staining intensity.

Conclusion: A statistically significant expression of survivin was observed in OPMDs which may indicate the probability of their transformation to OSCC.

Keywords: Survivin, Oral Squamous Cell Carcinoma, Immunohistochemisrty, Oral Potentially Malignant Disorders

This article may be cited as: Maryam H, Nasir S, Khan MM, Saleem A, Basher N, Iqbal F et al. Expression of Survivin in Oral Malignancy and Premalignancy. J Med Sci 2022 January;30(1):14-17

INTRODUCTION

Oral cancer is among the 18th most common malignancies in the world, about 90% of the oral cancers are squamous cell carcinomas [1]. Worldwide, there is a large variation in geographical distribution and incidence of oral cancer. The geographic incidence of Oral squamous cell carcinona (OSCC) is reported 30% in Eastern and 3-6% in Western countries [2]. South East Asia regions including Pakistan, Bangladesh, India, Sri Lanka, and Taiwan are reported with high incidence rates [3,4]. According to the SKMCH&RC, Pakistan's collective cancer registry data (from December 1994 to December 2019), it is the third most prevalent malignancy in Pakistan[5]. Tongue is commonly affected (40-50%) site in oral cancer [6]. Tobacco (smoked and smokeless), alcohol and HPV are the most common risk factors for OSCC. Other causes like poor oral hygiene, low socioeconomic status, ill-fitting dentures, nutritional deficiencies, chronic irritation from fractured or rough teeth, and immunological alterations may also play a role [7,8].

Correspondence
Dr. Hoor Maryam
Department of Oral Pathology, Peshawar Dental College, Peshawar - Pakistan
Email: hoormaryamyounas@gmail.com
Cell: +92 333-0429494
Date Received: 21-12-2021
Date Revised: 03-03-2022
Date Accepted: 07-03-2022

Oral potentially malginant disorders (OPMDs) are clinical manifestations in the oral cavity, whether in a clinically identifiable precursor lesion or clinically healthy mucosa that carry a risk of transformation to carcinoma [9]. World wide the prevalence of OPMDs varies between 1 and 5% [10]. The OPMDs comprises; oral submucous fibrosis, oral lichen planus, leukoplakia and erythroplakia [9]. Buccal mucosa, lateral edge of tongue and floor of mouth are common sites for these lesions [11]. It is important to identify the OPMDs which are at a greater risk of conversion into oral malignancy [12]. Therefore new molecular markers are required to identify high risk OPMDs [13].

Survivin is the recently identified inhibitor of apoptosis protein (IAP) [14]. It has two functions that promotes cell cycle progression and also inhibits apoptosis. In G2M phase of cell cycle survivin is expressed, to support rapidly dividing cells and helps in chromosomes separation during cell division [15]. Survivin inhibits apoptosis by interfering with the action of caspases both directly and indirectly. [15]. The up-regulated expression of apoptosis inhibitors family prevents cancer cells from undergoing apoptosis [16].

Early diagnosis of OPMDs and identification of their possible risk of conversion into malignancy is important to improve the survival rate. Regardless of the availability of several molecular markers for the diagnosis of OPMDs, an accurate predictive evaluation of OPMDs will depend on the development of newer makers with predictive potential. Survivin detection might be helpful as a candidate biomarker to identify the group with high-risk for malignant

transformation in our region. Hence the present study is designed to evaluate the immunohistochemical expression of survivin in OPMDs, OSCC, and in normal oral mucosa.

MATERIALS AND METHODS

This descriptive cross-sectional study comprised of 25 cases of OPMDs and 25 cases of OSCC that has been identified histologically. Non probability convenient sampling technique was adopted. Formalin-fixed paraffin embedded (FFPE) blocks were recruited from the archives of histopathology department of Pakistan Institute of Medical Sciences (PIMS). Recurrent cases and those who were receiving chemotherapy or radiotherapy were excluded from the study. After taking consent, 25 cases of non-inflamed normal oral mucosa were obtained from the patient's alveolar mucosa attached to extracted tooth and other dental procedures in Peshawar Dental College and were used as controls.

Before starting the study, ethical approval was taken under the approval No. Prime /IRB/2019-174 of Prime foundation from the institutional review board. After histopathological evaluation of various types of OPMDs and OSCC, Survivin immunohistochemistry was performed on sections with the maximum epithelial content. Slides kept for immunohistochemistry (IHC) were divided into 5 batches. One slide of colon carcinoma was added as positive control to each batch followed by immunohistochemical procedure.

During procedure antigen retrieval was carried out using microwave oven. Primary antibody (monoclonal mouse antihuman survivin) and secondary antibody (Dako EnVision™ detection system) were used for immunohistochemical staining of selected sections. The existence of brown colour immunostaining of the nucleus confirmed survivin immunopositivity. The survivin immunopositivity percentages was categorized according to the criteria adopted by Celina F.Lima et al and Rajanna et al [17,18]. The percentage of positive cells was examined in at least five areas at 400X magnification and were scored as (0) <5%, (1) 5-25%, (2) 26-50%, (3) 51-75%, (4) >75%. Cases with zero score were considered negative, and with score 1-4 as positive. The intensity of survivin immunostaining was scored as Negative stain (0), Mild staining (1), Moderate (2), Strong (3). Survivin Immunoreactivity was evaluated by the immunoreactivity score (IRS; staining intensity x percentage of immunopositive cells). Statistical package for social sciences (SPSS) version 20 was used to perform statistical analysis. P-value ≤ 0.05 was considered statistically significant.

RESULTS

The majority of patients with OSCC and OPMDs were over 60 years old, with mean ages of 56.76 and 60.9 years, respectively. In OSCC the ratio of male to female was 2:3 and in OPMDs it was 1:1.1. All the layers of epithelium was found positive for survivin expression in OSCC while in OPMDs staining was limited to the basal layer of (84%) cases and only (16 %) cases showed staining in both basal and in suprabasal layers. Table 1 shows the comparison of survivin immunoreactivity status among OSCC and OPDM. The statistical comparison made among the study participants (OSCC and OPMDs) for survivin immunoreactivity showed significant results p value (< 0.05).

Table 2 Shows the IHC staining percentage of survivin-positive cells in the cases of OSCC. The histological grades of OSCC were compared, and significant findings were revealed p value < 0.05.

Table 3 shows the staining percentage of survivin-positive cells in OPMDs. Comparison was made among the types of OPMDs which was statistically significant p value < 0.05. Out of 25 cases of normal oral mucosa only 1 case showed mild Immunopositivity and 24 cases were negative for survivin expression.

Table 1: Comparison of survivin immunoreactivity status among OSCC and OPMDs

Survivin immunoreactivity	Positive	Negative	Total	P-Value
OPMDs n(%)	15(60%)	10(40%)	25(50%)	< 0.05
OSCC n(%)	25(100%)	0	25(50%)	
Total	40(80%)	10(20%)	50	

Table 2: Expression of survivin between different grades of OSCC

	OSCC			Total n (%)	P value
	WDSCC	MDSCC	PDSCC		
0 = <5%	-	-	-		
1 = 5%-25%	1(11.11%)	3(37.5%)	2(25%)	6 (24%)	**< 0.05**
2 = 26% - 50%	3(33.3%)	4(50%)	3(37.5%)	10 (40%)	
3 = 51%-75%	4(44.4%)	1(12.5%)	3(37.5%)	8 (32%)	
4 = > 75%	1(11.11%)	-	-	1 (4%)	
Total	9(36%)	8 (32%)	8 (32%)	25	

Table 3: Expression of survivin among the cases of OPMDs

Staining Percentage of Positive cell	OPMDs			Total n (%)	P value
	Oral Leukoplakia	Oral Lichen planus	Oral Erythroplakia		
0 = <5%	3(17.6%)	4(100%)	1(25%)	8 (32%)	**< 0.05**
1 = 5%-25%	3(17.6%)	-	1(25%)	4 (16%)	
2 = 26% - 50%	2(11.7%)	-	1(25%)	3 (12%)	
3 = 51%-75%	8(47%)	-	1(25%)	9 (36%)	
4 = > 75%	1(5.8%)	-	-	1 (4%)	
Total	17 (68%)	4 (16%)	4 (16%)	25	

DISCUSSION

In present study the common age group for OSCC and OPMDs was above 60 years with a mean age of 56.76 years and 60.9 years, which is consistent with the studies done in Pakistan and Saudia Arabia [19,20]. OSCC and OPMDs are more likely to develop in males than in females but in this study the male to female ratio in OSCC was 2:3, However, a study published from, Singapore, Sweden and Lebanon found that OSCC is more common in females than males in Austria, Ireland, Denmark, Bulgaria and England (the ratio was not mentioned) [21], and in OPMDs it was 1:1.1, it is consistent with the studies done in Iran which shows a slight female predominance [22]. The discrepancy in male to female ratio is might be due to the small size of sample.

In this study, difference between the immunoreactivity score of OPMDs and OSCC was significant statistically ($p < 0.05$) and the results are similar to study reported from India which showed the same statistically significant results [23]. The findings of this study suggests that the expression of survivin in OPMDs points to an early event in their malignant transformation. The survivin expression was found maximum in well differentiated Squamous cell carcinoma (WDSCC) followed by poorly differentiated Squamous cell carcinoma (PDSCC) and moderately differentiated Squamous cell carcinoma (MDSCC), this is inconsistent with the studies done in India which showed maximum expression in MDSCC and PDSCC as compared to WDSCC [14,24]. The difference in expression was due to the large size of the sample of the study done in India.

In cases of OPMDs, oral leukoplakia showed high expression of survivin 68%, which is consistent with other studies reported from India showing identical results [13,18]. In contrast oral lichen planus showed less survivin positivity 16% which is comparable to the study done in India in which oral lichen planus showed lesser percentage positivity [13].

Similarly, oral erythroplakia showed 16% positive cells. After extensive literature research, no study could be found of survivin expression in oral erythroplakia. In this study, the normal oral mucosa exhibited negative expression for survivin, only 1 case showed mild nuclear staining. This sparse expression can be due to the active mitotic figures and may be due to processing errors [18]. The results are comparable to the other studies done in China and India which showed survivin expression in few cases of normal oral mucosa [13,18,25]. In contrast other studies done in China and India showed negative expression in normal oral mucosa [26,27].

Limitation of study is that a study with larger sample size is recommended. The study should include follow-up to determine the role of survivin in OPMDs progressing to OSCC.

Our study concluded that a statistically significant surviving expression was observed in OPMDs and OSCC which may indicate the probability of their transformation to OSCC.

Survivin expression was higher in OSCC, followed by OPMDs.

ACKNOWLEDGEMENT

I am thankful to all the patients of OPMDs and healthy individuals who consented to be part of this research. I am also thankful to all the doctors and other health care professionals of the participating centers for their help.

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2018;68(6):394–424.
2. Sahaf R, Naseem N, Anjum R, Nagi AH, Path FRC. A study of 89 cases of oral squamous cell carcinoma presenting at Teaching Hospitals of Lahore, Pakistan. JPDA. 2017;26(01):27.
3. Xie S, Xu H, Shan X, Liu B, Wang K, Cai Z. Clinicopathological and prognostic significance of survivin expression in patients with oral squamous cell carcinoma: evidence from a meta-analysis. PloS one. 2015;10(2):e0116517.
4. Rivera C. Essentials of oral cancer. International journal of clinical and experimental pathology. 2015;8(9):11884.
5. Yousaf A, Mahmood S, Faraz R, Quader Q. Annual cancer registry report-2018, of the Shaukat Khanum Memorial Cancer Hospital & Research Center, Pakistan. 2019.
6. Porcheri C, Meisel CT, Mitsiadis T. Multifactorial contribu-

tion of notch signaling in head and neck squamous cell carcinoma. International journal of molecular sciences. 2019;20(6):1520.

7. Salian V, Dinakar C, Shetty P, Ajila V. Etiological trends in oral squamous cell carcinoma: A retrospective institutional study. Cancer Translational Medicine. 2016;2(2):33.
8. Hellen-Bandeira-de-Pontes Santos T, dos Santos K-G, Paz A-R, Cavalcanti Y-W, Cassiano-Francisco-Weege Nonaka G-P, Godoy P-MA. Clinical findings and risk factors to oral squamous cell carcinoma in young patients: A 12-year retrospective analysis. Medicina oral, patologia oral y cirugia bucal. 2016;21(2):e151.
9. El-Naggar AK, Chan JKC, Takata T, Grandis JR, Slootweg PJ. The fourth edition of the head and neck World Health Organization blue book: editors' perspectives. Hum Pathol. 2017 ;66:10–2.
10. Khan Z, Khan S, Christianson L, Rehman S, Ekwunife O, Samkange-Zeeb F. Smokeless tobacco and oral potentially malignant disorders in South Asia: a protocol for a systematic review. Systematic reviews. 2016;5(1):1–7.
11. Speight PM, Khurram SA, Kujan O. Oral potentially malignant disorders: risk of progression to malignancy. Oral surgery, oral medicine, oral pathology and oral radiology. 2018;125(6):612–27.
12. Sarode GS, Sarode SC, Maniyar N, Sharma N, Yerwadekar S, Patil S. Recent trends in predictive biomarkers for determining malignant potential of oral potentially malignant disorders. Oncology reviews. 2019;13(2).
13. Angelin D, Nair BJ. Comparative evaluation of survivin expression in leukoplakia, lichen planus, and oral squamous cell carcinoma: An immunohistochemical study. Journal of Cancer Research and Therapeutics. 2020;16(3):569.
14. Gayathri C, Rao GV. Immunohistochemical expression of Survivin in oral leukoplakia and oral squamous cell carcinoma. Journal of Dr NTR University of Health Sciences. 2017;6(1):39.
15. Deo PN, Deshmukh R. Expression of survivin in dysplasia and different grades of oral squamous cell carcinoma. Translational Research in Oral Oncology. 2017;2:2057178X17710147.
16. Shamsabadi FT, Eidgahi MRA, Mehrbod P, Daneshvar N, Allaudin ZN, Yamchi A, et al. Survivin, a promising gene for targeted cancer treatment. Asian Pacific Journal of Cancer Prevention. 2016;17(8):3711–9.
17. Lima CF, Crastechini E, Dos Santos NC, Prado Ribeiro AC, Brandão TB, Castro FC, et al. Smoking cessation leads to changes in survivin expression in oral mucosa. Journal of Oral Pathology & Medicine. 2018;47(3):293–8.
18. Rajanna VR, Raveendranath MC, Kathiresan S, Srinivasan S, Ilango J. Expression of survivin in oral potentially malignant disorders: An immunohistochemical study. Journal of Pharmacy & Bioallied Sciences. 2020;12(Suppl 1):S382.
19. Ahmed R, Malik S, Khan MF, Khattak MR. Epidemiological and clinical correlates of oral squamous cell carcinoma in patients from north-west Pakistan. J Pak Med Assoc. 2019;69(8):1074–8.
20. Vohra F, Al-Kheraif AA, Qadri T, Hassan MIA, Ahmed A, Warnakulasuriya S, et al. Efficacy of photodynamic therapy in the management of oral premalignant lesions. A systematic review. Photodiagnosis and photodynamic therapy. 2015;12(1):150–9.
21. El Toum S, Cassia A, Bouchi N, Kassab I. Prevalence and distribution of oral mucosal lesions by sex and age categories: A retrospective study of patients attending lebanese school of dentistry. International journal of dentistry. 2018;2018.
22. Irani S, Esfahani AM, Ghorbani A. Dysplastic change rate in cases of oral lichen planus: A retrospective study of 112 cases in an Iranian population. Journal of oral and maxillofacial pathology: JOMFP. 2016;20(3):395.
23. Negi A, Puri A, Gupta R, Nangia R, Sachdeva A, Mittal M. Comparison of immunohistochemical expression of antiapoptotic protein survivin in normal oral mucosa, oral leukoplakia, and oral squamous cell carcinoma. Pathology research international. 2015;2015.
24. Ajithkumar M, Murali CR, Vani NV. Prognostic Implication of Survivin Expression in Oral Squamous Cell Carcinoma—An Immunohistochemical Study. The journal of contemporary dental practice. 2019;20(5):577–81.
25. Sakthivel R, Ramamoorthy A, Jeddy N, Singaram M. Evaluation and Expression of Survivin in Potentially Malignant Lesions and Squamous Cell Carcinoma: A Comparative Study. Cureus. 2020;12(4).
26. Basheer S, Shameena PM, Sudha S, Varma S, Vidyanath S, Varekar A. Expression of survivin and p53 in oral lichen planus, lichenoid reaction and lichenoid dysplasia: An immunohistochemical study. Journal of oral and maxillofacial pathology: JOMFP. 2017;21(3):456.
27. Rong L, Guo H, Liu K. The role of survivin in oral squamous cell carcinoma. Biomedical Research (0970-938X). 2018;29(4).

Author Declaration: The article is based on M.Phil (Oral Pathology) research thesis of **Dr. Hoor Maryam**, Riphah International Univeristy Rawalpindi.

CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE: NIL

AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Maryam H:	Conceiving and designing the study, analysis, interpretation of data
Nasir S:	Designing of study, Analysis, interpretation of data
Khan MM:	Designing study design, Approval of Final draft
Saleem A:	Data analysis, Critical review of draft
Basher N:	Data analysis
Iqbal F:	Data collection
Naushen T:	Bibliography

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.