Int.j.pathol.2021;19(3): 151-154

Original Article

Role of Survivin as a Diagnostic Tool for Oral Squamous Cell Carcinoma

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ABSTRACT

Introduction: Oral cancer is among the 10 most common malignancies in the world, about 90% of the oral malignant cancers are oral squamous cell carcinomas (OSCC). Survivin is the recently identified inhibitor of apoptosis protein (IAP). It has a dual function both in cell cycle promotion and apoptosis inhibition.

Objectives: To assess the immuno-histochemical expression of Survivin in oral squamous cell carcinoma and normal oral mucosa.

Material and methods: The study consisted of 30 diagnosed cases of OSCC, and 30 cases of normal healthy oral mucosa as a control. Immuno-histochemical expression of Survivin was analyzed.

Results: Survivin showed positive immunoreactivity in all cases of OSCC (100%) but was absent in normal oral mucosa.

Conclusion: Survivin expression was higher in oral squamous cell carcinoma, indicating that it could be used as a diagnostic marker for OSCC.

Keywords: Oral Squamous Cell Carcinoma, Survivin, Immunohistochemistry.

Introduction

Oral cancer is among the most communal malignancies in the world, about 90% of oral malignant cancers are OSCC [1]. Geographical distribution and incidence of oral cancer vary greatly throughout the world. The geographic incidence of OSCC is reported as 30% in Eastern and 3-6% in Western countries [2]. High incidence rates are reported from the South East Asia region e.g Pakistan, India, Bangladesh, and Taiwan [1,3]. It is the third most common malignancy in Pakistan, according to the collective cancer registry report of SKMCH&RC, Pakistan (from Dec 1994 – Dec 2019) [4]. In the oral cavity, 40–50% of cancers commonly affect the tongue [5]. Etiological factors of OSCC are tobacco smoking, tobacco chewing in the form of betel quid, reverse smoking, infection by human papillomavirus (HPV), and other factors like low socioeconomic status, alcohol consumption, poor hygiene, poor diet, ill-fitting dentures and chronic irritation from rough or fractured teeth and nutritional deficiencies [6, 7].

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Cancer is a heterogeneous group of diseases that results not just from aberrant cellular proliferation but also from a lack of well-regulated cell death. Resistance to apoptosis is one of the important mechanisms by which tumor cells may present chemo-resistance and thus contribute to cancer progression. Consequently, molecules involved in the regulation of apoptosis are considered potential targets for cancer therapy. In this regard, Survivin, the smallest member of the inhibitor of apoptosis protein (IAP), has recently emerged as an attractive drug target due to its dual role in cancer, both in cell cycle progression and apoptosis inhibition [8]. Survivin has become the attention of several cancer research studies, due to its highly specific expression in tumors [9]. Survivin is expressed during the G_2M phase of the cell cycle to assist rapidly dividing cells and aid in chromosomal separation during cell division [10, 11]. Survivin, directly and indirectly, inhibits apoptosis, by impeding the caspases' function [10, 12]. Survivin is indicated as an important candidate in cancer therapy due to many reasons including high expression of Survivin in tumor cells which has prognostic value, its involvement in multiple cellular signaling pathways (i.e., promotes cell proliferation that causes cell death resistance) which contributes to the cancer growth and progression [13]. Therefore, targeting Survivin has been

attracted as a possible point of therapeutic in halting cancer progression and cancer therapy [13]. Expression of Survivin is minimal in normal tissues, therefore it has become the main target for both tumor diagnostic, prognostic as well as anticancer therapies [11].

The purpose of the study is to assess the immuno-histochemical expression of Survivin in both OSCC and normal oral mucosa, to determine its potential role as a diagnostic marker for OSCC.

Materials and Methods

This descriptive cross-sectional study comprised of 30 cases of OSCC and 30 cases of normal oral mucosa. Non probability convenient sampling technique was adopted. In our study, there were 10 well-differentiated, 10 moderately differentiated, and 10 poorly differentiated cases of OSCC. After taking consent, tissue of normal oral mucosa was obtained from the alveolar ridge of the patients undergoing tooth extraction or any other dental procedures. Before starting the study, ethical approval was obtained from the institutional review board under the approval No. Prime /IRB/2019-174 of Prime foundation. This study was done in the Pathology department of Peshawar Medical and Dental College, Pakistan. Formalin-fixed paraffin embedded tissue (FFPE) blocks were acquired from the archives and those fulfilling the inclusion and exclusion criteria were chosen for the study. Already diagnosed cases of OSCC were included. Patient with recurrent lesions of OSCC was excluded. Patients taking chemotherapy and radiotherapy were excluded due to morphological changes in oral mucosal tissue. Severe inflamed mucosa was also excluded due to destruction of normal morphological architecture of tissue by inflammatory cells.

Nuclear staining for Survivin immuno-histochemistry was assessed. Cytoplasmic staining was disregarded. The percentage of Survivin immuno-positivity was categorized based on the criteria adopted by previous researchers [14, 15]. The percentage of positive cells was observed 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% and Cases with score 1-4 were considered as positive and with score zero as negative. The Survivin intensity (immuno-staining) was scored as Negative stain (0), Mild staining (1), Moderate (2), Strong (3).

Statistical Analysis

The statistical package for social sciences (SPSS) version 20 was used for the statistical analysis. P-value ≤ 0.05 was considered statistically significant.

Results

In present study, age of most of the patients in cases of OSCC was above 60 years with the mean age of 56.76 years and female predominance with a Male: Female ratio of 2:3, and tongue was the common site of the lesion. Out of 30 cases of OSCC, 7 cases showed score 1 (5-25%) of Survivin expression, 10 cases showed score 2 (26-50%) of Survivin expression, 9 cases showed score 3 (51-75%) Survivin expression and 4 case showed score 4 more than 75% expression which is shown in (table 1). The comparison among the immuno-positivity of histological grades of OSCC is made which revealed statistically significant results. Expression of Survivin was absent in normal oral mucosa.

Table 1: Comparison of expression of Survivin between OSCC groups (P value by fisher's exact test)

Staining Percentage of Positive cell	OSCC			Total n (%)	P value
	WDSCC	MDSCC	PDSCC		
0 = <5%	-	-	-		<0.05
1 = 5%-25%	2(20%)	3(30%)	2(20%)	7 (23%)	
2 = 26% - 50%	3(30%)	4(40%)	3(30%)	10 (33%)	
3 = 51%-75%	4(40%)	2(20%)	3(30%)	9 (30%)	
4 = > 75%	1(10%)	1(10%)	2 (20%)	4 (13%)	
Total	**10(33%)**	**10 (33%)**	**10 (33%)**	**30**	

In cases of OSCC, only 3 cases showed weak, 23 cases showed moderate, and 4 cases showed strong staining intensity which is shown in table 2. Comparison among the intensity of histological grades of OSCC revealed statistically significant results. The intensity of Survivin was absent in normal oral mucosa epithelium.

Table 2: Survivin staining intensity among the cases of OSCC (P value by fisher's exact test)

Intensity	OSCC			Total n (%)	P value
	WDSCC	MDSCC	PDSCC		
Negative (0)	-	-	-		<0.05
Weak (1)	2(20%)	1 (10%)	-	3 (10%)	
Moderate (2)	6(60%)	9(90%)	8(80%)	23 (76%)	
Strong (3)	2(20%)	-	2(20%)	4 (13%)	
Total	**10 (33%)**	**10 (33%)**	**10 (33%)**	**30**	

Table 3: Comparison of immunoreactivity status among OSCC and normal oral mucosa (P value by fisher's exact test)

Survivin Immunoreactivity	OSCC n (%)	Normal oral mucosa n (%)	Total	P value
Positive	30(100%)	0	30 (50%)	<0.05
Negative	0	30(100%)	30 (50%)	
Total	**30 (50%)**	**30 (50%)**	**60**	

Discussion

In present study, the mean age in cases of OSCC was 56.76 years. The results were comparable to reported studies in Pakistan which shows the mean age of 53.13 years in patients of OSCC [2]. In contrast to our findings, a study in India showed patients' age ranging from 20-85 years with a mean of 48.35 years [(16)]. OSCC is generally common in males than in females [17] but in our study male to female ratio in OSCC was 2:3. However one of the published work from Lebanon and Singapore and Sweden showed that OSCC in Austria, Bulgaria, Ireland, Denmark, and England was more common in females than males (the ratio was not mentioned) [18, 19]. In this study tongue was the commonest site of OSCC 52%, the results are comparable with the other locally published study in Pakistan (Peshawar) which showed that the tongue is the commonest site in OSCC and the posterior-lateral surface of the tongue is the most affected area (51.6%) [20]. A study carried out in Switzerland is similar to our study which showed that the tongue is a common site in 40-50% of cases (4) and Poland 40% [21]. The comparison of immunoreactivity score between OSCC and normal oral mucosa was made, which showed statistically significant results (Table 3). The expression of Survivin was positive in 100% OSCC cases, and all the samples of normal oral mucosa were negative for the expression of Survivin, which was in accordance to the study done in India, showed the same results as our study [22]. There was an increased expression of Survivin in OSCC compared to normal oral mucosa which is comparable to other studies [22, 23]. In our study, the expression of Survivin-positive cells was found to be strong in PDSCC followed by MDSCC and WDSCC and the results were statistically significant (Table 1), which was consistent with other studies reported in India. It showed that Survivin expression was strong in PDSCC and WDSCC than in WDSCC [22, 24]. Survivin expression increases with the increase in the grade of OSCC. However, a study with larger sample size is required to find out the immuno-positivity and intensity of Survivin between different histological grades of OSCC to predict the biological behavior of OSCC. This study reported a predominant nuclear positivity for Survivin in all grades of oral squamous cell carcinoma which suggested that nuclear Survivin expression was involved in promoting cell proliferation [22]. The moderate intensity of Survivin expression was found in majority of the cases of MDSCC (90%), PDSCC (80%), and WDSCC (60%). Some of the cases of WDSCC (20%) and PDSCC (20%) showed strong intensity and the results were statistically significant (Table 2). These results are comparable to other studies in India that showed the same results as our study [22]. In this study, normal oral mucosa showed an absence of Survivin expression. The results were comparable with other studies done in India and China showing negative expression in normal oral mucosa [25, 26]. In contrast, some other studies reported from China and India showed expression of Survivin in few cases of normal oral mucosa [15, 23]. This expression of Survivin in normal oral mucosa may be due to mitotic activities or errors in processing [15, 22]. To further support Survivin's role as a prognostic marker, a large sample size study is required to assess the relation of Survivin with different histological grades of OSCC along with the clinical follow-up data to confirm its role as a predictive marker for OSCC. Also comparison between OPMDs and OSCC is required to confirm its role as a prognostic marker.

Conclusion

Survivin expression was higher in oral squamous cell carcinoma, indicating that it could be used as a diagnostic marker for OSCC.

Conflict of interest: None

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HISTORY	
Date received:	21-09-21
Date sent for review:	27-09-2021
Date received reviewers comments:	25-10-2021
Date received revised manuscript:	26-10-2021
Date accepted:	09-01-2022

KEY FOR CONTRIBUTION OF AUTHORS:

A. Conception/Study/Designing/Planning
B. Active Participation in Active Methodology
C. Interpretation/ Analysis and Discussion

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