Original Article

SOX10 EXPRESSION IN SELECTIVE MALIGNANT SALIVARY GLAND TUMORS OF INTERCALATED AND EXCRETORY DUCTAL ORIGIN

Madiha Insha Awan[1], Arooj Khurshid[1], Obaid Akhtar[2], Sabeen Nasir[1], Sara Ziaullah[1], Nasiha Bashir[1]

[1]Peshawar Medical and Dental College, Peshawar Pakistan
[2]HBS Medical and Dental College, Islamabad Pakistan

ABSTRACT

Objective: To determine the level of immunohistochemical expression of SOX10 in malignant tumors of salivary gland of intercalated ductal and excretory ductal origin.

Material and Methods: An analytical cross-sectional study was designed. Sample size was 90 and categorised into two groups i.e., intercalated ductal (adenoid cystic carcinoma AdCC and polymorphous low-grade adenocarcinoma PLGA) and excretory ductal (mucoepidermoid carcinoma MEC). Blocks were retrieved from the histopathology lab of Pakistan Institute of Medical Sciences (PIMS), Peshawar Medical College (PMC) and Islamabad Diagnostic Center (IDC) from 2015 – 2020. Hematoxylin & Eosin (H&E) and Immunohistochemistry (IHC) slides were prepared and examined under microscope. Statistical analysis was done using SPSS version 25.

Results: The intensity was found weak in 24 (85.7%) and moderate 4 (14.3%) in excretory ductal tumors. Similarly, the intensity was strong and intense in most of the intercalated ductal tumors compared to none in the excretory ductal tumors. The difference was statistically highly significant (p-value,<0.001). Similarly, the proportion of SOX10 was found with no positive cells in 25 (89.3%) and <10% cells in 3 (10.7%) cases of excretory ductal tumors whereas in the intercalated ductal tumor cases almost two third (62.8%) patients had cells between 51- 80% and above 80%. The difference in proportion of SOX10 was found statistically significant (p-value, <0.001).

Conclusion: The conclusion of this study is that immunohistochemical expression of SOX10 can help in differentiating the origin of tumors of intercalated ductal and excretory ductal origin.

Key Words: Adenoid cystic carcinoma, Polymorphous low-grade adenocarcinoma, Mucoepidermoid carcinoma, SOX1O, Immunohistochemistry, Hematoxylin & eosin.

This article can be cited as: Awan MI, Khurshid A, Akhtar O, Nasir S, Ziaullah S, Bashir N. SOX10 expression in selective malignant tumors of intercalated and excretory ductal origin. Pak J Pathol. 2022; 33(2): 48-52.

DOI: 10.55629/pakjpathol.v33i2.709.

INTRODUCTION

Salivary gland tumors occur 6.3% of almost all head and neck tumors [1]. Salivary gland tumors incidence is 0.5 to 2 per 100,000 people in many parts of the world [2]. In Pakistan, 14% to 22% of tumors arising from salivary gland are carcinomas [3]. The most common benign salivary gland tumor is pleomorphic adenoma whereas mucoepidermoid carcinoma is the most common malignant salivary gland tumor [3].

The cause of salivary gland tumors is not clear , whereas majority of head and neck carcinomas are associated to smoking and drinking [4,5]. Certain studies shows that vitamin C rich diet and diet with low cholesterol might be helpful in prevention of salivary gland tumors [6]. Radiations for other types of head and neck tumors and occupational exposures (metal and nickel compounds, asbestos mining) are the possible risk factors for the tumors of salivary glands [7]. Previous history of neoplasm related to Epstein-Barr virus, immunosuppression, and radiation are the factors that may be helpful in increasing the risk of salivary gland tumors [8]. Tumor can occur in both minor and major salivary glands. Generally malignant tumors arise more frequently in minor salivary glands than in major ones [5]. Majority of tumors of major salivary glands are benign while in minor salivary glands majority of tumors are malignant [9]. Histologically, salivary gland carcinomas are heterogeneous in nature with maximum morphological variations that make their diagnosis difficult [10]. SOX10 gene (SRY- sex determining region Y related HMG box protein 10) is a transcription factor. It regulates embryonic development and also involved in the determination of cell fate [10]. SOX 10 protein plays important role in the development of peripheral nervous system (PNS), neural crest cell and melanocyte development [11,12]. It is also important marker for tumors like gliomas, melanomas and breast carcinomas. It was also revealed in soft tissue and salivary gland tumors , while in normal human salivary gland tissue, SOX10 expression was specific

Correspondence: Dr Madiha Insha Awan, Department of Oral Pathology, Peshawar Medical and Dental College Peshawar Pakistan.

Email: **madihainsha53@gmail.com**

Received: ***28 Feb 2022****; Revised:* ***19 May 2022****; Accepted:* ***22 Jun 2022***

to the nuclei of acini and both luminal and abluminal cells of intercalated ducts but not in other sites [13].

The immunohistochemical expression of SOX 10 clearly differentiate between acinic origin and ductal origin of tumors arising from salivary glands [14]. But it is very difficult to differentiate between the ductal origin salivary gland tumors that are intercalated ductal and excretory ductal origin. The aim of this study is to differentiate between few tumors of ductal origin salivary gland tumors that are intercalated ductal and excretory ductal based on SOX10.

MATERIAL AND METHODS

This is analytical cross-sectional study and was conducted in Peshawar Medical college (department of Pathology). Sampling was done using non-probability consecutive sampling technique. Sample size was measured by using G power software, with an effective size of 0.3, alpha 0.05, power 80% and degree of freedom 2%. The research was conducted following the approval by the Institutional Review Board, Peshawar.

Already diagnosed cases of Salivary gland tumors of intercalated ductal origin i.e., adenoid cystic carcinoma (38), polymorphous low-grade adenocarcinoma (24) and excretory ductal origin i.e. mucoepidermoid carcinoma (28) were included. Cases were collected from pathology department at Pakistan Institute of Medical Sciences (PIMS), Peshawar Medical College (PMC) and Islamabad Diagnostic Center (IDC). Laboratory procedures were done in pathology department, Peshawar Medical and Dental College, Peshawar.

Formalin fixed paraffin embedded salivary gland tumor tissue blocks were taken and thin sections were made (4-5 microns) for both H&E staining and IHC procedure. Microscopic examination of H&E and Immunohistochemical (SOX10) slides were done. SOX10 was observed in nuclei of positive cells. SOX 10 Immunoreactivity was assessed by the immunoreactivity score (IRS; percentage of immune positive cells x staining intensity). It was scored as Strong and diffuse staining (final score ≥ 8), Dim or focal staining (final score from 3-7) and Negative staining (score < 3).

Immunohistochemistry: Formalin fixed paraffin embedded tissue sections were de paraffinized prior to staining. Antigen retrieval was carried out by citrate-buffer and then heating was carried out in microwave oven at 95-100C for 20 min and then slides were allowed to cool for 15-20 mins at room temperature. Slides were then washed with distilled water. Phosphate buffer saline (PBS) a Peroxidase blocking solution was added to the slides for 10min. slides were then washed with PBS for 6 minutes. Primary antibody (Bio SB, diluted in buffered pH 7.5 (predilute 7.0ml), monoclonal) was applied to the slide and incubation was done for 60 minutes at room temperature. Slides were washed again after 1 hour. Secondary antibody (HRP) was then applied to slides and then incubation was done for 30mins at room temperature. Again, rinsing of slides was done for 06 minutes with PBS. Substrate/ Chromogen was applied and then incubated in peroxidase substrate solution to disclose color of the antibody. Color was allowed to develop for less than 5 minutes and slides were washed again. Counterstaining was performed by immersing slides in hematoxylin for 1-2 mins. Slides were again washed in running tap water for about 15 mins. Dehydration was done with alcohol for 5 mins each. Clearing was done through xylene. In the end DPX solution was applied on slide and cover slip was placed over it. Positive control (metastatic melanomas, nodal capsular navus) Negative control (fibroblast and histocytes). The quantitative variables are age. The qualitative/ categorical variables are gender and intensity of SOX10 expression. Statistical analysis was done using Chi square test. The P-value less than or equal to 0.05 is considered as significant.

RESULTS

Total 90 cases of salivary gland carcinomas were enrolled in this study. Overall mean age of patients was 45.1 ± 13.1 years. There were very few younger patients, only 4 (4.4%) cases were below 25 years of age where almost all patients were of older ages. It was witnessed that most of the study cases were between 26 and 50 years of age 58 (64.4%). Male gender was predominant in the study with 51 (56.7%) proportion (Table-I).

Table-I: Comparison of Age and Gender between Intercalated ductal and Excretory ductal origin of Salivary gland tumor.

	Intercalated ductal Tumors (n=62)	Excretory ductal Tumors (n=28)	Total (n=90)
Age (years)			
Mean ± SD	44.1±11.2	47.0±16.5	45.1±13.1
Age categories			
Up to 25	1 (1.6%)	3 (10.7%)	4 (4.4%)
26 to 50	44 (71.0%)	14 (50.0%)	58 (64.4%)
51 to 60	12 (19.4%)	5 (17.9%)	17 (18.9%)
61 or above	5 (8.1%)	6 (21.4%)	11 (12.2%)
Gender			
Male	37 (59.7%)	14 (50.0%)	51 (56.7%)
Female	25 (40.3%)	14 (50.0%)	39 (43.3%)

Table-II: Comparison of lesion site between Intercalated ductal and excretory ductal origin of Salivary gland tumors.

Site of lesion	Intercalated ductaltumors (n=62)	Excretory ductaltumors (n=28)
Submandibular gland	38 (61.2%)	5 (17.8%)
Hard palate	9 (14.5%)	3 (10.7%)
Parotid Gland	4 (6.4%)	18 (64.2%)
Maxilla	4 (6.4%)	0 (0.0%)
Tongue mass	2 (3.2%)	1 (3.6%)
Others	5 (8.0%)	1 (3.6%)

The lesion site was seen significantly variable between the two groups. The most frequent sites in intercalated ductal tumors were submandibular gland 38 (61.2%), hard palate 9 (14.5%), maxilla 4 (6.4%) and parotid glands 4 (6.4%). On the other hand the most frequent lesion sites in excretory ductal tumors were parotid glands 18 (64.2%) and submandibular glands 5 (17.8%). (Table-II)

Table-III: Comparison of intensity and proportion between Intercalated ductal and Excretory ductal origin of Salivary gland tumors.

	Intercalated ductaltumors (n=62)	Excretory ductal tumors (n=28)	p-value
Intensity			
Weak (1)	0 (0.0%)	24 (85.7%)	<0.001
Moderate (2)	3 (4.8%)	4 (14.3%)	
Strong (3)	49 (79.0%)	0 (0.0%)	
Intense (4)	10 (16.1%)	0 (0.0%)	
Proportion			
No positive cells (0)	0 (0.0%)	25 (89.3%)	<0.001
< 10% (1)	4 (6.4%)	3 (10.7%)	
10-50% (2)	19 (30.6%)	0 (0.0%)	
51-80% (3)	26 (41.9%)	0 (0.0%)	
>80% (4)	13 (20.9%)	0 (0.0%)	

The intensity and proportion of SOX10 interpretations were assessed according to intercalated ductal tumors and excretory ductal tumors. The intensity was found weak in 24 (85.7%) and moderate 4 (14.3%) in excretory ductal tumors. Similarly, the intensity was strong and intense in most of the intercalated ductal tumors compared to none in the excretory ductal tumors and this difference was statistically highly significant (p-value, <0.001) (Table-III).

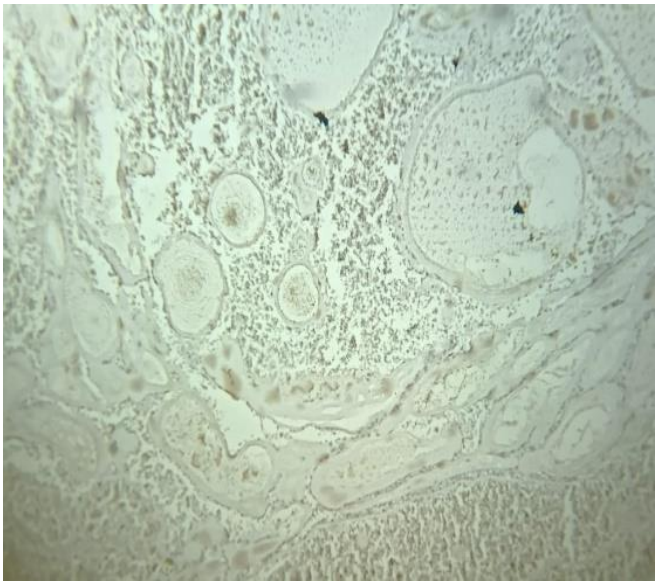

Figure-I: Adenoid cystic carcinoma (SOX10,10x).

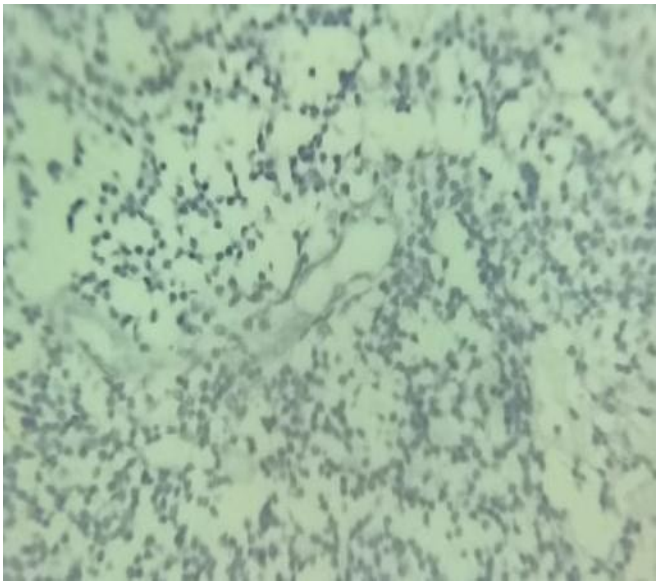

Figure-II: Mucoepidermoid carcinoma (SOX10,10x)

DISCUSSION

Our study is based on two different origin of salivary gland tumors i.e., intercalated ductal (adenoid cystic carcinoma AdCC and polymorphous low-grade adenocarcinoma PLGA) and excretory ductal origin i.e. (Mucoepidermoid carcinoma).

In our study a total of 90 cases (62+28) each group of salivary gland tumors were enrolled. Majority of cases observed were between 26 to 50 years of age. There were very few younger patients; only 4 cases were below 25 years of age where almost all patients were of older ages mentioned in Table-I. A study performed in Armed forces of Pathology Rawalpindi in 2011 on the patients that were enrolled for salivary glands tumors found that their age ranged from 40-50years which is in line with our study [15].

The reason of an early occurrence of salivary gland tumors might be due to the previous radiation therapies that were performed on the patient or might be due to early start of smoking in some population. In older patient the reason is radiation therapies, prolonged smoking and occupational cause. In our study, male gender was predominant (table 1) with male: female 1.2:1 which corresponds to the study performed by Erdem Mengi et al. that male: female ratio was found as 1.3:1 for malignant salivary gland tumors [16]. The changes in the findings of gender can be a result of different reasons such as geography and life style. As the ratio of male smokers is high as compared to females in our region, the study resulted in a higher finding of the tumor in males than in females. The other cause is industrial, such as asbestos mining, exposure to metal and nickel compounds that is the cause of salivary gland tumor.

The next focus of our study of salivary gland tumor is its site. The most common site for the tumor is submandibular followed by hard palate and then parotid and other minor salivary glands (Table-II). Ogle et al. concluded from his study that the most common site for salivary gland tumor is submandibular followed by parotid and then other minor salivary glands [17] which is similar to our study. The reason in some cases where parotid gland is commonly involved is that skin cancer can spread into the parotid gland and cause cancer in it. Submandibular may be commonly involved due to excessive radiations and smoking.

The proportion and intensity of SOX10 interpretations were assessed according to intercalated ductal tumors and excretory ductal tumors. In our study the proportion of SOX10 (Table-III) in intercalated ductal tumor (adenoid cystic carcinoma, polymorphous low-grade adeno-carcinoma) cases was 51-80% in two third cases and 10-50% in one third cases. Similarly, the proportion of SOX10 in excretory ductal tumor (mucoepidermoid carcinoma) was found with no positive cells 0% in two third cases and >10% in one third cases. In the original research of Ohtomo et al. the proportion of SOX10 in intercalated ductal tumors was positive in almost all cases and was negative in all cases of excretory ductal tumor which is correspondent to our study [14].

Intensity of SOX10 in our study (Table-II) was found weak in 24 (85.7%) and moderate 4 (14.3%) in excretory ductal tumors. Similarly, the intensity was strong and intense in most of the intercalated ductal tumors. Ohtomo et al. study shows that the intensity was high in almost all cases of intercalated ductal tumors and low and absent in excretory ductal tumors which corresponds to our study [14].

LIMITATION

All the tumors of intercalated ductal and excretory ductal origin of salivary gland tumors could not be included in study due to short time frame.

RECOMMENDATION

In future it can be used with other types of antibodies for the purpose of histopathological diagnosis. Other malignant salivary gland neoplasms of different origin can be accounted in the future for comparative studies. By increasing the sample size and variation in the salivary gland tumors, better results can be achieved.

CONCLUSION

The conclusion of this study is that immunohistochemical expression of SOX10 can help in differentiating the origin of tumors of intercalated ductal and excretory ductal origin.

AUTHOR CONTRIBUTION

Madiha Insha Awan: Article writing
Arooj Khurshid: Data collection
Obaid Akhtar: Data collection
Sabeen Nasir: Statistical analysis
Sara Ziaullah: Staining, literature review
Nasiha Bashir: Article review

REFERENCES

1. Seethala RR, Stenman G. Update from the 4th edition of the World Health Organization classification of head and neck tumours: tumors of the salivary gland. Head Neck Pathol. 2017;11(1):55-67.
2. Ata-Ali J, Zurriaga O, Alberich C. Incidence and survival rates for malignant salivary gland tumors. J Oral Sci. 2016; 58(1): 67-73.
3. Khattak MS, Ahmad S, Noman N. The histopathological pattern of salivary gland tumors. Gomal J Med Sci. 2016;14(4): 203-207.
4. Lin HH, Limesand KH, Ann DK. Current state of knowledge on salivary gland cancers. Crit Rev Oncog. 2018;23(3-4). 139–151..
5. Seethala RR. Salivary gland tumors: current concepts and controversies. Surg Pathol Clin. 2017;10(1):155-76.
6. Skalova A, Michal M, Simpson RH. Newly described salivary gland tumors. Modern Pathol. 2017;30(1):S27-43.
7. da Silva LP, Serpa MS, Viveiros SK, Sena DA, de Carvalho Pinho RF, de Abreu Guimarães LD, et al. Salivary gland tumors in a Brazilian population: A 20-year retrospective and multicentric study of 2292 cases. J Craniomaxillofac Surg. 2018; 46(12): 2227-33.

8. Harada K, Ferdous T, Ueyama Y. PD-L1 expression in malignant salivary gland tumors. BMC Cancer. 2018; 18(1): 1-6.
9. Zakeri K, Wang H, Kang JJ, Lee A, Romesser P, Mohamed N, *et al*. Outcomes and prognostic factors of major salivary gland tumors treated with proton beam radiation therapy. Head Neck. 2021; 43(4): 1056-62.
10. Young A, Okuyemi OT. Malignant Salivary Gland Tumors. StatPearls. 2021 Oct 9.
11. Haldin CE, LaBonne C. SoxE factors as multifunctional neural crest regulatory factors. Int J Biochem Cell Bio. 2010; 42(3): 441-44.
12. Schock EN, LaBonne C. Sorting sox. Diverse roles for sox transcription factors during neural crest and craniofacial development. Frontiers Physiol. 2020; 11: 606889..
13. Lee JH, Kang HJ, Yoo CW, Park WS, Ryu JS, Jung YS, et al. PLAG1, SOX10, and Myb expression in benign and malignant salivary gland neoplasms. J Pathol Translational Med. 2019; 53(1): 23-30.
14. Ohtomo R, Mori T, Shibata S, Tsuta K, Maeshima AM, Akazawa C, et al. SOX10 is a novel marker of acinus and intercalated duct differentiation in salivary gland tumors: A clue to the histogenesis for tumor diagnosis. Modern Pathol. 2013; 26(8): 1041-50.
15. Asif M, Malik S, Khalid A, Anwar M, Din HU, Khadim MT. Salivary gland tumors-a seven years study at Armed Forces Institute of Pathology Rawalpindi, Pakistan. Pak J Pathol. 2020;31(3):64-8.
16. Mengi E, Kara CO, Tumkaya F, Ardic FN, Topuz B, Bir F. Salivary gland tumors: A 15-year experience of a university hospital in Turkey. Northern Clin Istanbul. 2020; 7(4): 366.
17. Ogle OE. Salivary gland diseases. Dental Clin. 2020; 64(1): 87-104.