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A Clinicopathological Study of Salivary Gland Lesions and Immunohistochemical Expression of Bcl2 in Benign and Malignant Salivary Gland Neoplasms

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Introduction

Salivary glands neoplasms (SGNs) are rare tumors and comprise approximately 3-10% of the neoplasms of the head and neck region and 1% of the neoplasms of the whole body[(1)]. Annual global incidence of SGNs is between 0.4 to 13.5 cases per 1, 00,000 individuals[(2)]. Among these, malignant salivary gland neoplasms (MSGNs) are 0.40 to 0.65 per 1, 00,000 cases[(3)]. MSGNs are 0.3% of all malignancies[(4)]. Early diagnosis of MSGNs play an important role in better management of these tumors[(5)]. Fine-needle aspiration cytology (FNAC) is used as a screening and early diagnostic tool in SGNs however its role is limited[(6)].

Accurate diagnosis depends upon the histological evaluation of SGNs.

Although haematoxylin-eosin (H&E) staining is still the gold standard for diagnosing the SGNs yet its role is limited in certain cases. Immunohistochemistry (IHC) can enhance the accuracy and may be a helpful tool when there is a need to investigate cell type and differentiation status, cell proliferation, and tumor protein expression that cannot be assessed by routine histological examination alone. There is also a need to investigate new biomarkers that can be useful in the diagnosis and grading of malignant salivary gland neoplasm[(7)]. Bcl-2 (B-cell lymphoma) oncoprotein is a useful marker for investigation in SGNs. Bcl-2 gene family consists of different regulators involved in apoptosis. It is considered as anti-apoptotic protein and plays a key role in preventing programmed cell death by favouring prolonged survival in normal and neoplastic cells. Increased expression has been seen in a number of tumors and is related to resistance to conventional cancer management[(8)]. It's expression has been studied in oral epithelial dysplasia, oral sub mucous fibrosis and oral squamous cell carcinoma and its expression showed increasing with the severity of dysplasia and decreased expression in differentiation of oral carcinoma. The present study was planned to determine the expression of Bcl-2 proteins in benign and malignant salivary gland neoplasms and to determine association of Bcl-2 expression with different grades of malignant salivary gland neoplasms.

Aim and Objectives

1. To document the morphological spectrum of various salivary gland lesions on histopathology at L.L.R.M. Medical College, Meerut.

2. To do the cytological evaluation of salivary gland lesions according to Milan System and to correlate with histopathology.
3. To study the immunohistochemical expression of Bcl2 in benign and malignant salivary gland neoplasms.

Material and Methods

1. **Study Design:** Prospective study
2. **Sample Size :** 80
3. **Methodology:** The study was carried out on 80 cases of salivary gland swellings .Patients with salivary gland swellings and suspected salivary gland swellings were included in the study.

   After informed consent, noting down the demographic data and collection of some important previous reports if available, FNA was performed with 22 or 23 gauze needle attached to a 20ml disposable syringe. The aspirated material was expressed onto the slides and smears were prepared, dried and stained with May-Grunwald-Giemsa stain (MGG) and PAP stain and reported using MILAN system. Confirmed salivary gland lesions were followed up and biopsies were obtained for histopathological examination. Tissue samples in 10% buffered formalin received in histopathology lab, were processed routinely.3–5 µ thick H and E stained sections were analysed for histopathological diagnosis.
4. The MSRSGC (Milan system for Reporting Salivary Gland Cytopathology) consists of 6 diagnostic tiers:

   1 Non-diagnostic

   2 Non-neoplastic

   3 AUS (atypia of undetermined significance)

   4 Neoplasm (subdivided into - 4a benign and - 4b salivary gland neoplasm of uncertain malignant potential or (SUMP)

   5 Suspicious for malignancy

   6 Malignant

Immunohistochemical staining procedure for Bcl2:

For immunohistochemistry, extra 2-4 µ thick sections were taken on Poly-L lysine coated slides and subjected to antigen retrieval by pressure cooker method. Immunohistochemical staining for Bcl2 was performed using primary antibodies. Sections were incubated with the secondary biotinylated antibody and avidin-biotin peroxidase complexes for 30 min. Reaction products were revealed with diaminobenzidine as the chromogen and sections were counterstained with Harris's hematoxylin to enhance nuclear detection .Sections of tonsil were used as positive control. For negative control, primary antibody was substituted with phosphate buffer saline in duplicate sections.

Observation and Results

In present study, out of total 80 cases, maximum number of cases were of benign neoplasms, 46 cases (57%) followed by malignant neoplasms, 26 cases (32%) and non - neoplastic lesions, 8 cases (10%)

Among benign neoplasms, most common was pleomorphic adenoma, 28 cases (60%) followed by Warthin's tumor, 9 cases (19%), myoepithelioma, 3 cases (6%), basal cell adenoma, 2 cases (4%) and single case each (2%) of oncocytoma, duct papilloma, monomorphic adenoma and canalicular adenoma .

Among malignant neoplasms, most common was mucoepidermoid carcinoma, 14 cases (53%), followed by adenoid cystic carcinoma, 4 cases (15%) and polymorphous low grade adenocarcinoma, 2 cases (7%) (Table - 1)

Table: 1 Morphological Spectrum of salivary gland lesions according to final histological diagnosis (n=80)

S.No	Category	Diagnosis	No. of cases	Percentage (%)
1.	Non Neoplastic Lesions (8)	Sialadenosis	3	3.75
		Acute sialadenitis	1	1.25
		Chronic sialadenitis	3	3.75
		Granulomatous sialadenitis	1	1.25
2.	Benign Neoplasms (46)	Pleomorphic adenoma	28	35
		Warthin tumor	9	10
		Myoepithelioma	3	3.75
		Basal Cell Adenoma	2	2.5
		Oncocytoma	1	1.25
		Duct Papilloma	1	1.25
		Monomorphic adenoma	1	1.25
		Canalicular adenoma	1	1.25
3.	Malignant Neoplasms (26)	Mucoepidermoid carcinoma(MEC)	14	17.5
		Adenoid cystic carcinoma (ADCC)	6	7.5
		Acinic cell carcinoma (ACC)	4	5
		Polymorphous low-grade adenocarcinoma (PLGA)	2	2.5
	Total		80	100

Maximum number of cases, 20 cases (20%) were seen in 21-30 years of age group , followed by 16 cases (16%) in 41-50 years of age group , 14 cases (14%) in between 31-40 years , 13 cases (13%) in between 51-60 years . Single case (1%) was seen in between 71-80 years of age group (Table - 2)

Table: 2 Age wise Distribution of total cases (n=80)

Age Distribution	Number Of Cases	Percentage (%)
1-10	7	8.75
11-20	7	8.75
21-30	20	2
31-40	14	17.5
41-50	16	20
51-60	13	16.25
61-70	2	2.5
71-80	1	1.25
Total	80	100

Out of total 80 cases, males, 55 cases (55%) were outnumbered the females, 25 cases (25%) with male:female ratio of 2.2.: 1 (Table -3)

Table 3: Sex Distribution of total cases (n=80)

Sex Distribution	Number Of Cases	Percentage (%)
Male	55	68.75
Female	25	31.25
Total	80	100

Maximum number of cases were seen in Cat.4 (benign), 45 cases, followed by Cat.6 (malignant) 18 cases, Cat.2 (non-neoplastic) 07 cases, Cat.5 (suspicious for malignancy) 05 cases , Cat.4b (SUMP) 03 cases and Cat.3 (atypia of undetermined significance) 02 cases . None of the case fall in Cat. 1 (non- diagnostic)

In our study, we received histopatholoical follow up of all the 80 cases . Risk of malignancy (rom) was zero for Cat.2 and Cat.3. ROM was 2 % for

Cat.4a and 67 % for Cat. 4b while ROM was 100 % for Cat. 5 and Cat. 6. Overall ROM was 32.5 % in our study. (Table 4)

Table 4: Histological follow up of MILAN system diagnostic categories and evaluation of risk of malignancy (ROM) (n=80)

Milan Category	Cat .1	Cat. 2	Cat. 3	Cat.4a	Cat.4b	Cat. 5	Cat. 6	Total
No. of cases	00	07	02	45	03	05	18	80
No. of cases with hist ological follow up	00	07	02	45	03	05	18	80
Non neoplastic	00	07 (100%)	01 (50%)	-	-	-	-	08 (10%)
Benign	00	-	01 (50%)	44 (98%)	01 (25%)	-	-	46 (57.5%)
Malignant	00	-	-	01 (2%)	02 (67%)	05 (100%)	18 (100%)	26 (32.5%)
Risk of malignancy	00	00	00	01 (2%)	02 (67%)	05 (100%)	18 (100%)	26 (32.5%)

Bcl2 expression was studied in all biopsy proven cases of benign and malignant salivary gland neoplasm

Among benign neoplasms, Bcl2 positivity was seen in 75 % (21/28) cases of pleomorphic adenoma, 55% (5/9) cases of Warthin's tumor , 66 % (2/3) cases of myoepithelioma and 50% (1/2) of cases basal cell adenoma. No Bcl 2 expression was seen in cases of oncocytoma, duct papilloma, monomorphic adenoma and canalicular adenoma.

Among malignant neoplasms, Bcl2 positivity was seen in 71 % (10/14) cases of mucoepidermoid carcinoma , 100 % (6/6) cases of adenoid cystic carcinoma and 50 % (1/2) cases acinic cell carcinoma and polymorphous low-grade adenocarcinoma. Overall Bcl2 positivity was 63 % (29/46) cases in benign and 73 % (19/26) cases in malignant neoplasms. (Table - 5)

Table 5: Bcl-2 expression in different Benign and Malignant Salivary Gland Tumors (n=72)

Diagnosis	Histological Diagnosis	No. of ca ses	Positive cas es	% of Bcl2 posi tivity
Benign Neoplasm (46)	Pleomorphic adenoma	28	21	75
	Warthin tumor	9	5	55
	Myoepithelioma	3	2	66
	Basal Cell Adenoma	2	1	50
	Oncocytoma	1	-	-
	Duct Papilloma	1	-	-
	Monomorphic adenoma	1	-	-
	Canalicular adenoma	1	-	-
Malignant Neoplasm (26)	Mucoepidermoid carcinoma	14	10	71
	Adenoid cystic carcinoma	6	6	100
	Acinic cell carcinoma	4	2	50
	Polymorphous low-grade adenocarcinoma (PLGA)	2	1	50
Total		72	48	100

Discussion

Present study was done on 80 cases of salivary gland lesions. All the cases underwent FNA and were followed by histopathology. Among the 80 cases, non-neoplastic lesions were (10%), benign neoplasms (57.5 %), and malignant neoplasms

(32.5 %). Among the benign category, most common was pleomorphic adenoma (35%) , while among malignant category, most common neoplasm was mucoepidermoid carcinoma (17.5%) .

Out of total 80 cases , males, 55% outnumbered the females, 25% .Maximum number of cases, 20% were seen in 21-30 years of age group , followed by 16% in 41-50 years of age group .

ROM was zero for Cat.2 and Cat.3 while ROM was 02% for Cat 4a and 67% for Cat 4b. ROM was 100% for Cat 5 and Cat 6. Overall ROM was 32.5%.

Previous studies done by Kala C et al.[11] showed similar results as present study. ROM was 5% for Cat.2 , 20% for Cat.3 , 4.4% for Cat 4a and 33.3% for Cat 4b. ROM was 85.7% for Cat 5 and 97.5% for Cat 6. Overall ROM was 31.4%.

Bcl-2 is a proto-oncogene, which endows B cells with a selective survival advantage that promotes their neoplastic expansion .Bcl-2 proteins play a key role in preventing programmed cell death by favoring prolonged survival in normal and neoplastic cells .Expression of Bcl-2 is highly variable in epithelial malignancies.

Bcl-2 has been studied in benign salivary tumors such as pleomorphic adenoma, myoepithelioma, Warthin's tumor and oncocytoma and in malignant salivary gland tumorsThe increased expression of Bcl-2 in epidermoid cells of Mucoepidermoid carcinoma (MEC) can be correlated with increased survival rate of these cells and tumors with predominance of these cells.

In present study , overall Bcl2 positivity was 63% in benign neoplasm and 73 % in malignant neoplasms. Among benign neoplasms, Bcl2 positivity was seen in 75 % cases of pleomorphic adenoma , 55% cases of Warthin's tumor , 66 % cases of myoepithelioma and 50% cases of basal cell adenoma .Among malignant neoplasms, Bcl2 positivity was seen in 71 % cases of mucoepidermoid carcinoma , 100 % cases of adenoid cystic carcinoma and 50 % cases each of acinic cell carcinoma and polymorphous low-grade adenocarcinoma.

Previous studies done by Soini et al[10]. showed similar results as present study .

Soini et al.[10] compared Bcl-2 expression in pleomorphic adenomas and in malignant salivary gland tumors comprising of mainly ACC and MEC, found 100% positivity in pleomorphic adenoma and 64% positivity in malignant tumors. In contrast, our study showed a similar but somewhat higher expression in malignant salivary gland tumors and lesser expression in pleomorphic adenoma. Thus the cell survival rate is generally higher in malignant neoplasms than benign neoplasms.

Manjunatha et al.[12] reported Bcl-2 expression was seen in all benign salivary gland tumors except canalicular adenoma.A higher degree of Bcl-2 expression was seen in malignant salivary gland tumors (78%) than in benign tumors (57%). In contrast, our study showed a similar results, with overall Bcl2 expression positivity of 73 % in malignant neoplasms and 63% in benign neoplasm with no expression in canalicular adenoma.

These findings were in contrast with the results of a study by Gordón-Núñez et al.[13] that found most PA cases negative for Bcl-2 protein which made them conclude that this protein was not involved in the pathogenesis of PA.

According to Al-Rawi et al.[14] research 70% of PA samples did not show Bcl-2 expression. They found that all cases of malignant salivary gland tumors were positive for Bcl-2. The highest recorded score was observed in ACC and the lowest in both low grade carcinoma ex-PA and low grade MEC.

Conclusion

In present study, benign neoplasms were 57.6% while malignant were 32.5%. Most common benign neoplasm was pleomorphic adenoma 35% while most common malignant neoplasm was mucoepidermoid carcinoma 17.5% .

Overall Bcl2 expression was 63% in benign neoplasms and 73 % in malignant neoplasms. Among benign neoplasms, Bcl2 positivity was seen in 75 % cases of pleomorphic adenoma , 55% cases of Warthin's tumor, 66 % cases of myoepithelioma and 50% cases of basal cell adenoma.

Among malignant neoplasms, Bcl2 positivity was seen in 71 % cases of mucoepidermoid carcinoma, 100 % cases of adenoid cystic carcinoma and 50 % cases of acinic cell carcinoma and polymorphous low-grade adenocarcinoma.

A higher degree of Bcl-2 expression was seen in malignant salivary gland tumors than in benign tumors .This variable expression may be suggestive of a different susceptibility rate of tumor cells to apoptosis or 'programmed cell death'.It is well established fact that morphology or appearance of cells remains the mainstay in diagnosis of salivary gland tumors. But, the immunohistochemistry is a valuable adjuvant in the diagnosis of malignant tumors of salivary gland origin as these have a very diverse histology.Positive expression of Bcl-2 in malignant salivary gland tumors can help in predicting the behavior of these tumors regarding their potential for aggressiveness.In addition, molecular targeted therapy against BCL-2 can be planned in future for better management of salivary gland tumors.

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