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p53 is not expressed in Indian salivary gland neoplasm: A light microscopic and immunohistiochemical retrospective review of 140 salivary gland neoplasms.

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Introduction: Salivary gland neoplasms are a heterogeneous group of tumours with different biological behaviour.

Patients and Methods: A retrospective analysis of gross and light microscopic features of 140 salivary gland neoplasms treated at Government Medical College, Trivandrum was carried out. Immunohistiochemistry for p53 was performed for 15 benign and 15 malignant salivary neoplasms.

Results: A total of 140 cases were studied during this period. Mean age of the patient was 43 year (SD ±16.5) year; ranging from 7 to 87 years. There were 66 (47.1%) females and 74 (52.9%) males. Pleomorphic adenoma was commonest in 54.3%, followed by Warthin's tumour in 15%, pleomorphic adenoma with myoepithelial predominance in 7.9% of the cases. Among the malignant neoplasm mucopidermoid carcinoma was commonest in 7.9%. p53 immunostaining was not observed in any of the cases.

Conclusion: p53 expression is not seen in Indian salivary cancers, this could be due to selection bias or a true negativity.

Introduction

Recent studies of the molecular biology of cancer have demonstrated that p53 tumour suppressor gene aberration is associated with the development and progression of several cancer types [1]. p53 and c-erbB-2 as well as karyotyping, are of disputable benefit for clinical use, but the biologic information they provide give a better understanding on the molecular mechanisms involved in the development and progression of tumours [2]. Studies have also shown that inactivation of tumour suppressor p53 gene is a key point in the development of human carcinomas and that normal p53 protein acts as a "molecular policeman" monitoring the integrity of the genome [3].

Pleomorphic adenoma (PA) is the most common benign tumour of salivary glands. Carcinomas in pleomorphic adenomas (CPAs) may arise by malignant transformation of the epithelial components. Occasionally, transitional zones containing cells with histological features intermediate between those of the benign and carcinomatous components are identified.

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Yamamoto *et al*., [4] studied 12 cases of CPAs and showed a relatively high rate (58%) of mutations (loss of heterozygosity [LOH] and microsatellite alterations) at the p53 gene. In another study, nuclear p53 accumulation by immunohistochemistry was investigated in 109 surgically resected salivary gland tumours, of these 46 were adenomas and 63 carcinomas. Nuclear p53 accumulation was observed in 13.7% tumours, of which 9% showed DNA aneuploidy [5].

The development and progression of cancer are known to be regulated by various oncogenes and tumour suppressor genes. Kamio analyzed 63 primary malignant salivary gland tumours for the expression of p53 proteins. Immunohistochemically, 11% showed diffuse nuclear staining for p53 protein. Overexpression of both p53 and c-erbB-2 proteins (coexpression) was found in certain histological types, such as adenocarcinoma, carcinoma in pleomorphic adenoma, and salivary duct carcinoma [6]. These results suggest that the rate of p53 expression varies for each histological type of tumour and for various geographical regions. We report our results on light microscopy and immunohistochemical p53 expression in salivary gland tumours in Trivandrum region of Kerala, India.

Material and methods

All patients presenting to the medical college hospital between 1997-2001 were retrospectively studied. Gross features included the type of surgery, size of the tumour, location of the tumour, distance from closest margin, surface of tumour, colour, solitary or multiple, cystic or solid, lining and content of cyst, encapsulation, circumscription, haemorrhage, necrosis and extra glandular extension were recorded.

Formalin fixed paraffin embedded tissue was sectioned at 5 µm and was stained with haematoxylin and eosin. Microscopic examination included study of cell type, growth pattern, stromal changes, collagen, fibrosis, hyalinisation, calcification, lymphocytic infiltrate, histocyte, foreign body giant cells, metaplasia, Rosette formation, perineural invasion, intra vascular invasion, invasion of bone and cartilage, muscle and adipose tissue chondromyxoid component, mucus production, cytological uniformity and necrosis.

p 53 immunostaining

Paraffin embedded section from 15 benign and 15 malignant salivary gland tumours were taken on poly – L- Lysine coated slides and were baked at 45^{o}C overnight in incubator or at between 60-70^{o}C for an hour. The slides were given 3 changes of xylene (5-10 minute each), 2 changes of 100% ethanol (2 minute each), 2 changes of 95% ethanol (2 minute each), 2 changes of 70% ethanol (2 minute each), slides were rinsed in tap or distilled water for 5 minutes endogenous peroxidase were blocked by quenching in 15% H_2O_2 for 10 minutes. Slides were again rinsed with tap water for 5 minutes.

Antigen Retrieval and staining

Slides were placed in plastic couplin jar with 0-01 M citrate buffer. Couplin jar was placed is pressure cooker and cooked for 10 minutes and kept at full pressure for 10 minutes. Pressure was released and couplin jar was taken out when it cools. Slides were washed in distilled water, allowed to dry and tissue was circled using DAKO pen. Blocking of non-specific binding was carried out by covering the section with 3% BSA for half an hour. Primary antibody of required dilution in 1% BSA is added after removing the BSA with tissue paper. Slides were incubated overnight at room temperature. Next morning slides were rinsed in 3 changes of 1 X phosphate buffered saline and secondary antibody was added in 200 dilution in phosphate buffered saline. Slides were incubated for 30 minutes. After rinsing thrice in 1 X phosphate buffered saline for 5 minutes each. Streptavidin horse raddish peroxidase is added for 30min slides were placed at room temperature, again after rinsing in 3

changes of phosphate buffered saline of 5 minute duration the slides were incubated with diamino benzidine for 5-10 minutes. Thereafter, the slides were washed in tap water, counter stained with haematoxylin bluing dehydrated in alcohol, clear in xylene and were mounted with DPX observed light microscopic.

Grading of p53 staining

The p53 staining intensity was proposed to be graded semi quantitatively into +1, 2+, and 3+. It was reported negative if there was no staining. Type of staining i.e. nuclear or cytoplasmic if present, was also recorded.

Statistical Analysis

The data is presented as frequency table, percentages were calculated for all.

Results

A total of 140 cases were studied during this period. Mean age of the patient was 43 year (SD ±16.5) year; ranging from 7 to 87 years. There were 66 (47.1%) females and 74 (52.9%) males. The male female ratio was nearly 1:1. Mean length of tumours was 3.5 ± 1.7cm (range 1-11cm) mean breadth of tumours was 2.6cm ± 1.3cm (range 1-9cm) and mean depth of tumours was 1.79 ± 0.9cm (range 0.5-7cm). Parotidectomy was the most common procedure performed. In 39.9% of these the superficial parotidectomy was performed in 19.3% and the type of parotidectomy was not mentioned in 17.9% other procedures performed are shown in (Table 1).

Parotid gland was most frequently involved in 106 (75.7%) patients followed by submandibular gland in 15 (10.7%). Sublingual gland was the least involved. In minor salivary glands palate, cheek, parapharyngeal space and nasal mucosa was equally involved in 2 cases each (1.4%). Nasopharynx was involved in 1 case (0.7%).

Table 1: Procedures performed

Procedure performed	Number	%
Rhinotomy	2	1.4
Excision	33	23.6
Biopsy	14	10
Parotidectomy	25	17.9
Superficial Parotidectomy	27	19.3
Enucleation	2	1.4
Total Parotidectomy	2	1.4
Total Radical Parotidectomy	1	0.7
Total conservative Parotidectomy	1	0.7

Margin was positive in 39 (27.9%) cases. It was more than 1cm in 13 (9.3%), and less than 1cm in 8 (5.7%) cases.

Gross examination

Surface of the tumour was irregular in 60 (42.9%) cases followed by nodular appearance in 28 (20%) cases, lobulated or bosselated surface was seen in 2 cases (1.4%). The colour of tumour varied and was gray white in 69 (49.3%), brownish in 9 (6.4%), gray white brownish in 5 (3.6%), gray in 2 (1.4%) and yellowish white in 2 (1.4%) cases. Tumour was solitary in 83 cases (59.3%) whereas it was multiple in 4 (2.9). In 2 (1.4%) cases specimen was in fragments. Consistency of the tumour varied from solid to cystic, solid tumours were commonest in 49

Table 2: Cell types seen in salivary neoplasm (figures in parenthesis show percentages)

Cell type	n	%	Scanty	Moderate	Abundant	Predominant
Epithelial cell	104	74.3	5(3.6%)	64(45.7%)	32(22.9%)	16(11.4%)
Myoepithelial cell	95	67.9	14(10%)	61(43.6%)	20(14.3%)	14(10%)
Mucous cell	13	9.3	-	4(2.9%)	7(5%)	3(2.1%)
Oncocytic cell	27	19.3	-	-	17(12.1%)	16(11.4%)
Intermediate cell	5	3.6	1(0.7%)	1.4(2%)	-	1(0.7%)
Epidermoid cell	8	5.7	-	4(2.9%)	3(2.1%)	-
Acinic cell	2	1.4	-	-	2(1.4%)	1(0.7%)
Clear cell	1	0.7	-	1(0.7%)	-	-
Ductal cell	1	0.7	-	-	-	-
Stetlate cell	3	2.1	2(1.4%)	1(0.7%)	-	-
Lymphoid cell	1	0.7	-	-	1(0.7%)	1(0.7%)

(35%) followed by cystic 25 (17.9%) and variegated in 5 (3.6%).

Lining of the cyst was smooth in 20 (14.3%) cases while it was papillary in 5 (3.6%). Cyst contained clear fluid in 12 (8.6%), gelatinous material was seen 4 (2.9%) and blood in 2 (1.4%) cases. Twenty eight (20%) tumours were encapsulated whereas 6 (4.3%) were non-encapsulated and 1 (0.7%) tumour was partially encapsulated. Presence of capsule was not defined in rest.

Microscopy

Majority of patients showed epithelial cells (104; 74.3%) followed by myoepithelial cells (95; 67.9%) and oncocytic (27; 19.3%) other cell types and their quantity is described in detail in (Table 4). Intercalated, ductal like cell, spindle cell, non-specific glandular cell, Schumann cell, endothelial cell, and fibroblasts were not observed in any case (Table 2).

Growth pattern was predominantly in form of cell nest in 93(66.4%) cases followed by ducts in 76(54.3), solid sheets in 68(48.6) and anastomosing cords in 61(43.6%) most of the patients had a combination of growth pattern. (Table 3) describes the growth pattern in detail. Membranous, Follicular and Intercalated nests were not observed in any of the cases.

Stromal changes

Presence of histiocytes (69, 49.3%), foreign body giant cells (70,50%), calcification (64,45.7%) were common stromal changes besides fibrosis and hyalinization (Table 4, 5).

The diagnosis of all the cases is detailed in (Table 6). Pleomorphic adenoma was commonest in 54.3%, followed by Warthin's tumour in 15%, pleomorphic adenoma with myoepithelial predominance in 7.9% of the cases. Among the malignant neoplasm mucopidermoid carcinoma was commonest in 7.9%.

Table 3: Growth pattern seen in salivary gland neoplasms

Growth pattern	Number	Percentage
Duct like or ductular	76	54.3%
Cell nest	96	66.4%
Solid sheets	68	48.6%
Anastomosing cords	61	43.6%
Cribriform	5	3.6%
Tubular	24	17.3%
Papillae	21	15.0%
Trabeculae	11	7.9%
Adenomatous	16	11.4%
Adenoid structures	3	2.1%
Papillary cystic	22	15.7%
Mucocystic pattern	13	9.3%
Solid aggregates	1	0.7%
Others	6	4.3%

After the review the diagnosis was changed only in 3 cases of pleomorphic adenoma to pleomorphic adenoma with myoepithelial predominance in two and cellular pleomorphic adenoma in one.

p 53 in salivary gland neoplasms

Immunostaining for p 53 was carried out in 30 patients (15 pleomorphic adenoma and 15 mucoepidermoid carcinoma), however p 53 was found to be negative in all the cases.

Discussion

Salivary gland neoplasms are a diverse group of tumours, characterized morphologically by cell type, stroma, and growth pattern. Here we have reported a retrospective review of 140 cases of salivary gland neoplasm treated at our centre. Pleomorphic adenoma was the commonest neoplasm identified in 54.3%, followed by Warthin's tumour in 15%, pleomorphic adenoma with myoepithelial predominance in 7.9% of the cases. Among the malignant neoplasm mucopidermoid carcinoma was commonest in 7.9%.

The p53 immunostaining was carried out in 30 cases and none of our case demonstrated p

Table 4 describes the stromal change in salivary neoplasm

Stromal changes	Absent	scanty	moderate	Abundant
Collagen	5(3.6%)	18(12.9%)	-	-
Fibrosis	36(25.7%)	-	-	-
Hyalinisation	23(16.4%)	-	-	-
Calcification	64(45.7%)	-	-	-
Lymphocytic infiltration	36(25.7%)	48(34.3%)	23(16.4%)	2(1.4%)
Histiocytes	69(49.3%)	10(7.1%)	-	-
Foreign body giant cell	70(50%)	5(3.6%)	1(0.7%)	-
Metaplasia	6 (4.3%)			
Adipose	1(0.7%)			
Cartilaginous	3(2.1%)			
Squamous, Osseous	1 (0.7%)			
Sq. cartilaginous	1 (0.7%)			
Chondromyxoid component	41(29.3%)	15(10.7%)	51(36.4%)	19(13.6%)
Production of mucin	90(64.3%)	4(2.9%)	3(2.1%)	1(0.7%)

Table 5. Other findings in Salivary gland neoplasms

Other findings	Absent	Scanty	Moderate
Rosette formation	97(69.3%)	2(1.4%)	
Perineural invasion	94(67.1%)	3(2.1%)	
Intravascular invasion	99(70.7%)	1(0.7%)	
Invasion of bone	98(70%)		
Invasion of cartilage	99(70.7%)		
Invasion of muscle	97(69.3%)		
Invasion of adipose tissue	96(68.6%)		
Cytological uniformity	105(75%)	5(3.6%)	1(0.7%)
Hyperchromatic nuclei	66(97.1%)	41(29.3%)	2(1.4%)
Mitotic activity	96(68.6%)	5(3.6%)	
Necrosis	75(53.6%)	37(26.4%)	

53 staining. These results are in contrast to the literature where the p53 expression has been identified in 10-54% of the cases [7,8,9,10,11,12,13,14,15,16,17,18,19,20,21]. The p53 expression has also been found to be associated with malignant transformation in pleomorphic adenoma and has been correlated with the outcome in malignant tumours.

In a series of 219 salivary gland tumours (103 carcinomas and 116 benign tumours) p53 protein expression was studied using immunohistochemistry, and mutations in p53 gene using non- radioactive single strand conformation polymorphism (SSCP). p53 expression was present in 36% of the benign tumours and in 54% of the carcinomas. The highest prevalence of p53 expression was found in adenoid cystic carcinomas (69%), followed by mucoepidermoid carcinomas (MEC) (67%) [22]. Li *et al*., studied [23] p53 protein in 45 salivary gland lesions and the protein expression was found in 34.4% carcinomas. Nuclear p53 expression was detected in tumour cells but not in non-neoplastic cells, except in one salivary duct carcinoma [23].

Adenoid cystic carcinoma (ACC) of the salivary gland is generally an indolent tumour that pursues a protracted clinical course with recurrences and late metastasis. It is a malignant tumour of salivary gland origin having a propensity for spread by direct extension or perineural invasion and frequent recurrences. Overexpression of p53 protein was demonstrated in the dedifferentiated component in one case [24]. Kiyoshima *et al*, detected p53 alterations in 17.6% of ACC and in 14.8% of MEC, and were only found in carcinomas arising in the minor salivary glands [20]. Polymorphous low-grade adenocarcinoma of minor salivary glands (terminal duct carcinoma, lobular carcinoma) was first defined more than a decade ago. A 17% recurrence rate and a 9% metastasis rate have been reported. Fifteen formalin-fixed, paraffin-embedded archival cases were analyzed for abnormal p53 gene product Qualitative assessment revealed p53 positive staining in 4 of 15 tumours; positive cells comprised 5% to 10% of the tumour [25].

Basal cell adenocarcinoma (BCAC) of the salivary gland is a rare tumour. p53 expression in these tumours was compared with basal cell adenoma (BCA). Considering those cases expressing p53 or EGFR in > 10% of tumour cells as positive, 54% BCAC cases were positive for p53 [26].

Table 6: Final diagnosis in patients with salivary gland neoplasm

Previous diagnosis	N	%
Pleomorphic adenoma (PA)	76	54.3
PA with myoepithelial predominance	11	7.9
PA hyaline type	1	0.7
PA with cystic degeneration	1	0.7
Warthin's tumour	21	15
Basal cell adenoma	3	2.1
PD adenocarcinoma in pleomorphic adenoma	2	1.4
SCC in pleomorphic adenoma.	2	1.4
Mucoepidermoid carcinoma	11	7.9
Syringocystadenoma papilliferum	1	0.7
Non Hodgkin lymphoma	1	0.7
Poorly differentiated carcinoma	1	0.7
Adenoid cystic carcinoma	3	2.1
Sebaceous lymphadenoma with SCC	1	0.7
Acinic cell carcinoma	2	1.4
Adenoid cystic carcinoma ex PA	1	0.7
Adenocarcinoma NOS	1	0.7
Mixed tumour, myoepithelial predominance	1	0.7

Soini *et al*., conducted similar study and showed that the malignant salivary gland tumours expressed p53 in more than 1% of positive nuclei in every case, suggesting that mutations of the p53 gene is infrequent in salivary gland tumours [27]. This is perhaps the only study where the results are somewhat similar to our study. It is suggested that the relatively indolent course of some histological types of malignant salivary gland tumours could thus be associated with the preservation of the non-mutated p53 gene in most of these tumours. The presence of p53 positivity in some pleomorphic adenomas might, on one hand, suggest that p53 gene alterations are also present in these tumours; on the other hand, the accumulation of the p53 protein in these tumours might also be due to some unknown mechanism, not necessarily related to p53 gene mutation [27].

The reason for low positivity in our cases could be due to selective sampling as not all cases were stained with p53 antibodies or use of archival tissue that might have led to antigen masking or loss of antigen. Being a retrospective study, the quality of fixation could not be controlled.

Conclusions

The results of the literature review suggest that p53 overexpression and mutations are present in benign and malignant neoplasms of the salivary gland. Though the frequency of these mutations is low and range from 10-50%.

Authors' Contribution

MS: Carried out the work, prepared the manuscript.
NLR: Conceived and designed the study
MRP: Conducted the IHC work and contributed in writing of manuscript
RD: Literature search and draft manuscript preparation
MP: Design of study, review of manuscript and editing.

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