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ORIGINAL ARTICLE

SECRETORY CARCINOMA OF SALIVARY GLAND: A CLINICOPATHOLOGICAL ANALYSIS

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Background: Secretory carcinoma of the salivary gland (SC) is a new entity that harbours a specific ETV6 gene rearrangement. The clinical behaviour of this tumour is not well-known as it is a relatively new entity but it is generally considered as a tumour of low malignant potential. The objective of the study was to find out the frequency of ETV6 translocation in cases diagnosed based on histology and immunohistochemistry, to study morphological features and immunohistochemical findings of our cases and to determine the survival and disease-free status of our patients. **Methods:** Twenty-five diagnosed cases of SC were retrieved from the archives of SKMCH and RC. Diagnosis was made primarily based on morphology and immunohistochemistry. Immunohistochemistry includes S100, p63, mammaglobin, DOG 1, GCDFP-15, TTF-1, GATA3, SMA, AMA, and AR. The diagnosis was further confirmed by molecular testing, i.e., Fluorescence in situ hybridization (FISH) studies to observe specific ETV6 gene break. Follow up of the patients was done by developing a questionnaire. Statistical analysis of the data was done using SPSS-23.0. **Results:** The mean age of diagnosis was 41 ± 17.4 and the male to female ratio was 1.5:15. The mean size of the tumour was 45.48 ± 27.35 . The most common site of the tumour was parotid gland (60%). On morphology, SC showed a wide range of morphological patterns, most common being the tubular, microcystic, intraductal, and papillary. Immunohistochemical stains mammaglobin (22/22), GCDFP-15 (15/15) and GATA3 (10/10) showed 100% positive result. However, all cases were negative for p63 (0/18) and DOG 1 (0/11). ETV6 break was seen in 17/17 cases (100%). The mean disease-free survival was 75 months and the overall survival was 51.90 ± 2.80 months. **Conclusion:** This study highlights the presence of specific molecular alteration in all cases, which were diagnosed based on morphology and immunohistochemistry.

Keywords: Secretory carcinoma of Salivary gland; ETV6

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INTRODUCTION

Secretory carcinoma of salivary gland, originally known as mammary analogue secretory carcinoma (MASC), was first described by Skalova *et al*¹ in 2010 in a series of 16 cases that resembled secretory carcinoma of breast morphologically and immunohistochemically but harboured a specific genetic alteration ETV6-NTRK3 gene fusion with translocation t (12;15) (p13; q25).^{2,3} Though, now other fusion partners of ETV6 rearrangement have also been reported.⁴

It was established as a separate entity in the 4th edition of head and neck WHO classification. Before its identification, it was commonly misdiagnosed as acinic cell carcinoma, mucoepidermoid carcinoma or adenocarcinoma, not otherwise specified.⁵ However, it shows a great difference in behaviour from these entities as it is generally

considered as an entity of low malignant behaviour.

There is no much data available about the behaviour of secretory carcinoma in our population. Here, we present the largest study of 25 cases of secretory carcinoma in our population at SKMCH & RC including detailed discussion of demographic details, morphological features, and clinical follow-up.

MATERIAL AND METHODS

This was a descriptive, analytical study. All cases of SC were retrieved from the archives of the surgical pathology department of Shaukat Khanum Memorial Cancer Hospital and Research Center after obtaining ethical approval from the institutional review board of the hospital ethical committee. All cases were diagnosed cases of SC at SKMCH & RC, includes both genders, age ranges from 10 to 90 years and specimen nature consists of excision

specimen only. The diagnosis was made primarily on a morphological basis, further confirmed by immunohistochemical stains. These cases were further blindly reviewed by three pathologists with a special interest in head and neck pathology Referral cases and cases diagnosed as adenocarcinoma, NOS were not included.

For conventional microscopy, formalin-fixed, paraffin-embedded tissues were used that were stained with haematoxylin and eosin. Additional histochemical stains, PAS with and without diastase and mucicarmine, were also used in some cases.

For immunohistochemical studies, 4- μ m-thick sections were used. These sections after being cut from paraffin blocks mounted on slides, deparaffinized in xylene and rehydrated using decreasing concentration (100–70%) of ethanol. Buffer used for antigen retrieval is EDTA; pH 8.0. The incubation time used for primary antibodies was 32 minutes. Staining was done on automated stainers (Ventana benchmark XT and bond III). Appropriate positive and negative controls were used for each antibody. Antibodies against S100 (S100; polyclonal, RTU, Ventana), p63 (p63; 4A4, RTU, Ventana), mammaglobin (31A5, RTU, Ventana), DOG1 (SP31, RTU, Ventana), GCDFP-15 (23A3, RTU, Leica), TTF-1(8G7G3/1, RTU, Ventana), SMA (alpha Sm-1, RTU, Leica), GATA 3 (L50-823, RTU, Cell Marque), AMA (SPM198, RTU, Abcam) and androgens receptor (AR; AR441, 1:50, Dako) were used. Selective immunohistochemical stains were applied to all cases.

For fluorescence in situ hybridization, 4- μ m formalin-fixed paraffin-embedded sections were placed on positively charged slides (Super Frost). FISH analysis was done according to the instructions of FISH probe literature. The FISH probe used in this study was ETV6 Dual Color Breakapart (Vysis LSI ETV6). Hybridized slides were analysed with an Olympus BX61 microscope using DAPI/Green/Red triple-band filter set at 100x magnification. The cut off value set for the ETV6 gene break was 10%.

SPSS software version 23.0 was used for statistical analysis of the data. Mean $\pm$ standard deviation used for continuous variables while frequencies and percentages used for categorical variables. The Kaplan-Meier method was used to estimate survival as a function of time.

A questionnaire was developed including details of surgical procedure, i.e., radical or superficial parotidectomy, neck

dissection, adjuvant treatment (radiotherapy), history of nodal metastasis or distant metastasis, no of recurrences and death. The follow-up period was at least one year. Follow up was done via telephonic contact with the patient and in case of death of the patient, with the next of kin.

RESULTS

The main clinical data and salient features of all 25 cases of SC are summarized in table-2. Fifteen patients were male (60%) and the other 10 patients (40%) were female. The mean age at the time of diagnosis was 41 years (ranging from 14–80 years). Fifteen cases (60%) occurred in parotid gland, three case (12%) in submandibular gland, two cases (8%) on lip, one case (4%) in left buccal sulcus, one case (4%) in right lower alveolus region, one case (4%) on the left side of cheek, one case (4%) was a postauricular mass and one is retriever cyst (as per patient site of biopsy is from face).

The size of the tumour ranges from 7mm to 110mm (mean 45.48 $\pm$ 27.376). The primary stage at the time of the diagnosis was T1 in eight patients (32%), T2 in five patients (20%), T3 in eleven patients (44%) and T4 in one patient (4%). None of the patients presented with local lymph node or distant metastasis at the time of the diagnosis. The gross picture varied from solitary ill-defined lesion to cystic masses. Cut surface was usually grey or brown in colour and firm to rubbery in consistency.

Histologically, SC showed diverse morphological patterns. Low power view showed multilobulated masses with various growth patterns, most common being the tubular, microcystic, intraductal, and papillary. Uncommon pattern, i.e., thyroid like macrocystic growth pattern with abundant secretions (figure 3b) was seen in two cases. Secretions were usually eosinophilic, but basophilic secretions were also seen. They were usually not encapsulated but relatively circumscribed but invasion of the adjacent salivary gland tissue was generally seen. Extra parenchymal extension, i.e., extension into the surrounding adipose tissue was also seen in two cases.

The tumour cells generally had vesicular nuclei with open chromatin with other conspicuous or inconspicuous nucleoli. The nucleus is surrounded by pale to eosinophilic cytoplasm with or without vacuolations. Cellular atypia was ranging from mild to moderate. Mitoses were seen in seven cases whereas no definite mitosis was seen in remaining cases. Maximum 5 mitoses/10 HPF was seen in one

case without any other high-grade features. Perineural invasion was seen in two cases. Lympho-vascular invasion was not identified in any case. The detailed immunohistochemical findings were discussed in table 1. All 25 cases of MASC were positive for S100 (100%). Other cases on which mammaglobin (22/22), GCDFP-15(15/15) and GATA 3(10/10) were applied showed a 100% result. All cases were negative for p63 (0/18), DOG 1 (0/11), SMA (0/2) and TTF-1(0/2). AMA was performed on only one case and it showed positive results. Androgen Receptors showed positive expression in one out of five cases.

ETV6 gene break was seen in 17/17 cases by Fluorescence in situ hybridization (100%). All cases had shown more than 10% break apart signals. The reason for not having molecular genetic studies on all cases was poor fixation, cost-effectiveness, and restriction by patients.

Among fifteen patients with parotid site tumour, four patients were treated with superficial parotidectomy, seven patients with near-total parotidectomy and four patients with radical parotidectomy treatment. Three patients had undergone neck dissection. The patients with SC of other salivary glands like submandibular and other minor salivary glands had undergone wide local excision. Nine patients had received adjuvant radiotherapy (ranging up to 30 cycles).

Clinical follow up was available in 22 cases, whereas three patients lost to follow up. The mean follow up time was one year (range 1–9 years). Two deaths were reported, but in both cases, the age of the patients was 60 and 70 years respectively and they have other associated morbidities as well. Five patients were having a recurrent disease at the time of diagnosis but previous histopathological diagnosis was not available. Only one patient had developed recurrence after 7 years of initial diagnosis. But after resection, there was no evidence of disease from last 1.5 years. Nineteen patients were alive till the date of follow-up without any recurrence and had no evidence of disease. The overall survival was 51.90 ± 2.80 months and projected two and three-year overall survival was calculated to be 96% and 88%, respectively and disease free period was 100% till 75 months (6 years and 3 months).

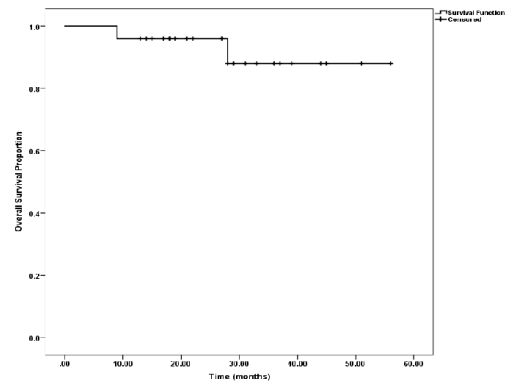


Figure-1: Overall survival of all patients of secretory carcinoma of salivary gland

DISCUSSION

Secretory carcinoma of salivary gland (SC) is a new entity that has been established in the 4th edition of WHO classification of salivary gland tumours (2017). We have presented a detailed clinicopathological study of 25 cases of SC. Before the entity is well known, it was used to be misdiagnosed as acinic cell carcinoma (ACC), mucoepidermoid carcinoma, adenocarcinoma (NOS), low-grade salivary duct carcinoma, and pleomorphic adenoma and used to lead to wrong therapeutic interventions.^{6,7} The main causes of the missed diagnosis are overlapping growth patterns which usually includes tubulocystic and papillary growth patterns and diffuse S100 positivity, which is also seen in many other salivary gland neoplasms.⁸ The first principal consideration in the differential diagnosis of SC is acinic cell carcinoma. Acinic cell carcinoma is characterized by great morphological and cytological diversity, mainly composed of a mixture of vacuolated, clear, hobnail, intercalated duct-like, serous acinar and non-specific glandular structures arranged in papillary, microcystic, solid-lobular and follicular growth patterns.⁹ This architectural diversity shown by both tumours is the main cause of confusion on morphology. However, in contrast, the cells of SC do not show zymogen granules which is present in the majority of the cases of Acinic cell carcinoma. Instead, cells of SC show eosinophilic pink to vacuolated cytoplasm without zymogen granules. Acinic cell carcinoma on the other hand shows a very different immunohistochemical profile from SC. SC typically shows strong expression for DOG1¹⁰, whereas DOG1 was essentially negative in all cases of SC in our study and another study conducted by Shah AA *et al*.¹¹ Other potential differential is pleomorphic adenoma (PA) that can easily be ruled out by staining with mammaglobin and MUC 4, which are reportedly negative in all cases of pleomorphic adenoma.¹² p63 can also be used in differentiating these two entities as p63.

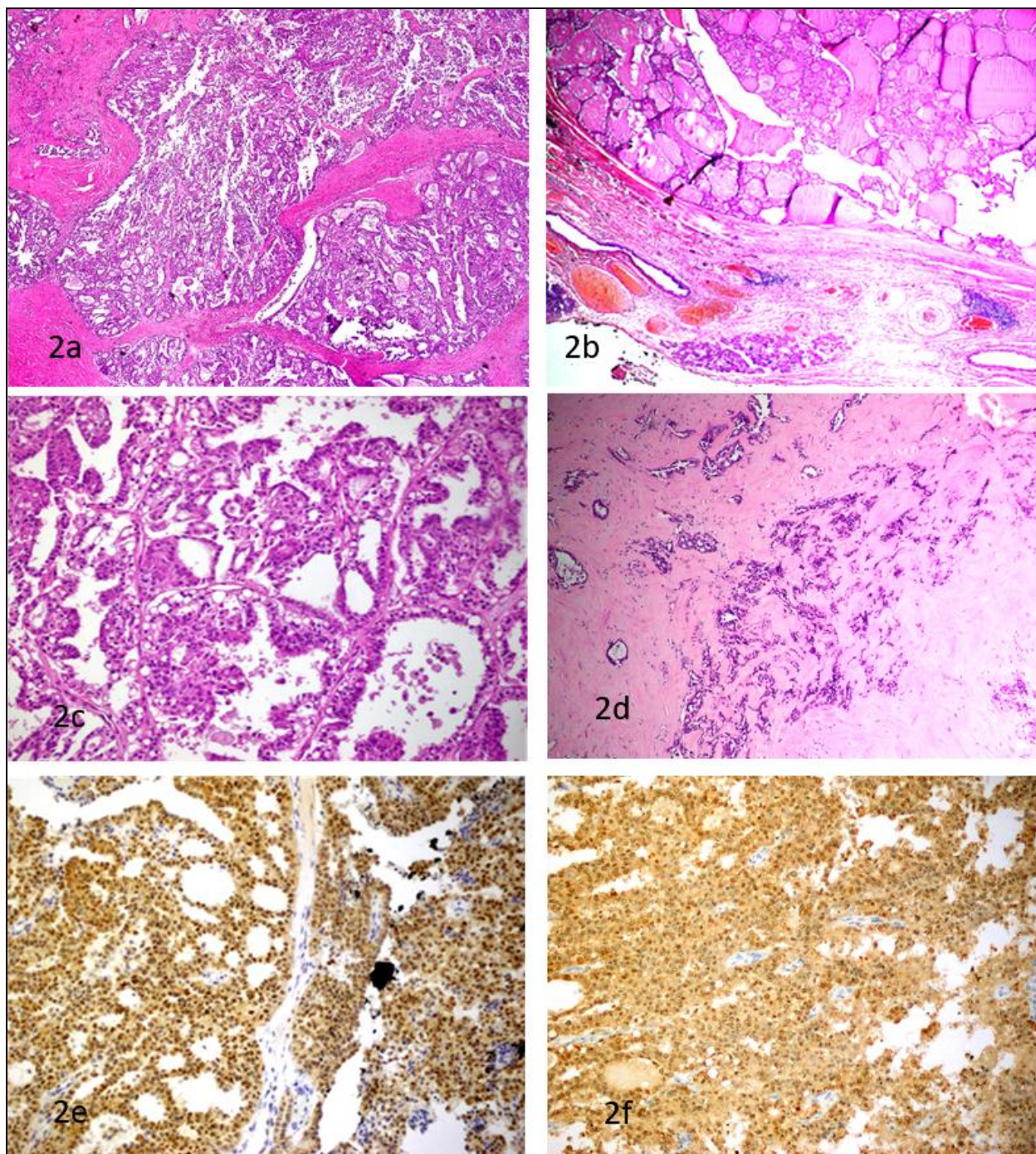


Figure 2a; H&E section of (mammary analogue) secretory carcinoma showing predominantly lobulated growth pattern with thick sclerotic bands. **Figure 2b;** H&E section showing macrocystic growth pattern with abundant colloid like secretions and overall pink appearance (resembling thyroid). **Figure 2c;** H&E section showing high power view of papillary growth pattern with low grade bland and pale nuclei with prominent nucleoli. **Figure 2d;** H&E section showing another less common pattern of MASC with cells arranged in trabecular pattern embedded in dense sclerotic stroma. **Figure 2e;** Cells of MASC showing strong diffuse staining with GATA 3. **Figure 2f;** Cells showing strong staining with S100 immunohistochemical stain.

is positive in pleomorphic adenoma¹³ and negative in all cases of SC. Another major differential diagnosis is mucoepidermoid carcinoma on morphology,

however mucocytes are always a necessary finding for mucoepidermoid carcinoma which were not present in any case of SC. Also, mucoepidermoid

carcinomas harbour mastermind-like 29(MAML2) gene translocation^{14,15}, whereas ETV6 translocation is specific for SC. ETV6 translocation is also seen in our cases and according to literature, ETV6 translocation seems to be specific for the diagnosis of SC.¹⁶⁻¹⁹ Other important differential diagnoses includes adenoid cystic carcinoma and cystadenocarcinoma.^{20,21}

One of the primary considerations of this study was that whether FISH studies are necessary for the diagnosis of this entity or it can be diagnosed based on morphology and immunohistochemical stains only. Because in developing countries like Pakistan, there are not as many resources available to apply on molecular tests in all cases. In our study, FISH studies showed positive results in all cases, which were diagnosed based on morphology and Immunohistochemistry. This fact was in support of the argument that FISH studies are not necessary for the diagnosis of all cases of SC, especially in excision specimen where one has classic morphology and supportive immunohistochemistry. However, molecular studies are always helpful when there is limited biopsy material and uncertain morphology. As, one of the limitations of the study was that all the selected cases showed classic morphology and no such cases were included in the study which were previously diagnosed as adenocarcinoma, NOS.

The main site of the tumour in our study was parotid gland as 60% of the tumours occurred in parotid gland which is in accordance with many other studies by Skalova *et al*²¹, Bissinger *et al*⁸ etc. 20% of the tumour occurred in minor salivary gland which is the second most common site as also described by Boon *et al*.²² The male to female ratio was turned out to be 1.5:1 which is in agreement with a data review of 279 patients by BA *et al*.²³

Secretory carcinoma of salivary gland (SC) is generally regarded as a low-grade malignancy with good prognosis, but recurrences, high-grade transformation and death have also been reported.^{24,25} In our study, one patient developed local recurrence and overall survival is 96% and 88% at two and three years respectively, which is as per another recently published study by Boon *et al*.²² However, in our study five cases are already recurrent cases at the time of diagnosis and unfortunately, previous histopathological diagnoses were not available. Diagnosis, at this time, is confirmed by FISH studies. But the reason for slightly high recurrence in our country can be because people usually did not have margin free surgeries in the past. Distant metastasis is not seen in any of our cases or any case in the literature.²²

A study conducted by Chiosea *et al* explained 17.6% rate for nodal metastasis²⁶ though

no nodal metastasis is seen in any of our cases. Death occurred in only two cases and on detailed review, both cases were diagnosed at a very late age and probably other morbid factors like cardiopulmonary issues, diabetes and no treatment also contributed towards the death of patients. Based on these facts, there is a proposition that cases of SC with low-grade morphology can be labelled as secretory tumour with intermediate behaviour and only those cases of secretory carcinoma in which there is high-grade features should be labelled as secretory carcinoma.

The overall prognosis of SC is generally good when excised with clear margin and there is no role of radiotherapy on the prognosis. This argument is also supported in a recently published series of 31 cases by Boon *et al*.²²

Entrectinib (formerly RXDX-101) which is an inhibitor of kinases encoded by the gene NTRK3 (common in SC). Clinical trials have shown that patients with NTRK1/2/3 gene rearrangements may get benefit from Entrectinib therapy.²⁷

CONCLUSION

This study is the largest series of SC of salivary gland in Pakistan highlighting the presence of specific molecular alteration in all cases, which were diagnosed based on morphology and immunohistochemistry. The mean disease-free survival period of 22 cases of SC with was 75 months and the overall survival was 51.90±2.80 months. However, FISH studies are not always necessary for the diagnosis of cases of SC with classic morphology. Correct diagnosis of this entity is important for proper treatment. More studies with follow-up are required to determine the behaviour of this entity. In this study, we propose that this entity can also be designated as the tumour of intermediate grade behaviour and only those cases with high-grade features should be named as carcinoma.

Conflict of interest: The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

NA: Conceptualization of study concept. AA: Literature search, data collection, write-up. AL: Proof reading, data analysis. UH: Proof reading. MH: Helped in write-up. MAB: Data analysis, data interpretation. SM: Conceptualization of study design, proof reading.

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