Hyalinizing Clear Cell Carcinoma of the Salivary Gland in an Elderly Female: A Case Report Supported by EWSR1 Molecular Studies

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ABSTRACT

Hyalinizing clear cell carcinoma of the salivary gland is a rare neoplasm, accounting for only less than 1% of malignancies arising from the salivary gland. It is molecularly defined by the expression of the EWSR-ATF1 fusion oncogene. To date, there has been no previous studies published yet in the Philippines regarding the existence of this tumor. In this paper, we present a case of a 70-year-old elderly female who had a 10-year history of a gradually enlarging left lateral neck mass. Histopathologic examination showed a tumor arranged of cords, nests, and trabeculae of monomorphic round cells with abundant clear to lightly eosinophilic cytoplasm surrounded by thick hyalinized collagen bundles. Immunohistochemistry and molecular studies were done which revealed a positive p63 staining, negative SMA and S100, and an EWSR1 rearrangement in Fluorescence in situ hybridization (FISH), thus, confirming the diagnosis.

Keywords: clear cell carcinoma, EWSR1, fluorescence in situ hybridization (FISH)

INTRODUCTION

Hyalinizing clear cell carcinoma of the salivary gland is a relatively rare neoplasm. First reported in 1994, it was first described as monomorphic clear cells that were glycogen rich and mucin negative surrounded by hyalinized bands of foci of myxohyaline stroma, and arranged in trabeculae, cords, islands, and nests.[1] This neoplasm has a low mitotic index and has infiltrative borders. In rare cases, high grade transformation may occur which is identified by the presence of poorly differentiated cells with scant cytoplasm and hyperchromatic nuclei accompanied by atypical mitotic figures and focal necrosis. A rapid progression of disease is usually observed in these cases.[2] It expresses cytokeratin but are negative for S-100 protein and smooth muscle markers like smooth muscle actin (SMA). Majority of cases are female, more than 30 years of age, and most tumors arise from minor salivary glands.[3]

The advent of molecular studies enabled us to reclassify tumors based on their genetic profile, and so far, this tumor showed a consistent EWSR-ATF1 gene fusion. Histologically, there is a difficulty in identifying this tumor due to its rarity and morphologic mimics. Knowledge about its existence thru case reports and case series will greatly help in including this in the differentials once a tumor with similar morphology located in the head and neck region is seen.

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CASE PRESENTATION

This is a case of a 70-year-old female presenting with a ten-year history of gradually enlarging left lateral neck mass. No consult was done until one year prior, there was noticeable increase in the size of the mass, which was accompanied by dysphagia and aspiration episodes. Masses were also noted at the base of the tongue and infra-auricular area. CT scan of the neck area showed a heterogeneously enhancing mass at the left parotid region and a focus at the tongue base area, likely metastatic in nature. Cervical lymphadenopathies, some with necrotic center, were also seen. Imaging studies of the chest and abdomen showed multiple noncalcified pulmonary nodules, likely metastatic in nature, hypodense foci in the liver and spleen that were too small to characterize, and paraaortic lymphadenopathy. There were no masses seen in the kidneys. Excision biopsy of the left infra-auricular area, base of tongue, and cervical lymph nodes was done.

Microscopic examination showed solid sheets of monomorphic tumor cells arranged in cords, nests, and trabeculae surrounded by thick hyalinized eosinophilic collagenous stroma (Figure 1). The tumor cells are mostly round to polygonal, with clear to lightly eosinophilic abundant cytoplasm, single round to oval nucleus with conspicuous nucleoli (Figure 2). There was no morphologic features compatible with a high grade transformation seen in the specimen submitted. Periodic acid Schiff (PAS) and Periodic acid Schiff with diastase (PAS-D) were done showing intracytoplasmic diastase-sensitive material in neoplastic cells that is likely glycogen. Immunohistochemistry studies with p63 showed strong, diffuse, nuclear positivity in tumor cells, while S100 and Smooth muscle antigen (SMA) were both negative (Figure 3). Ewing sarcoma breakpoint region (EWSR1) FISH was done using Vysis LSI EWSR1(22q12) Dual Color Break Apart Rearrangement FISH Probe Kit which showed a positive EWSR1 rearrangement (Figure 4). With the above immunohistomorphologic and molecular features, the case was signed out as hyalinizing clear cell carcinoma of the salivary gland.

Due to the age of the patient and high surgical risk, the family opted for palliation. The patient eventually died of the disease with clinical lung metastasis, one year after diagnosis.

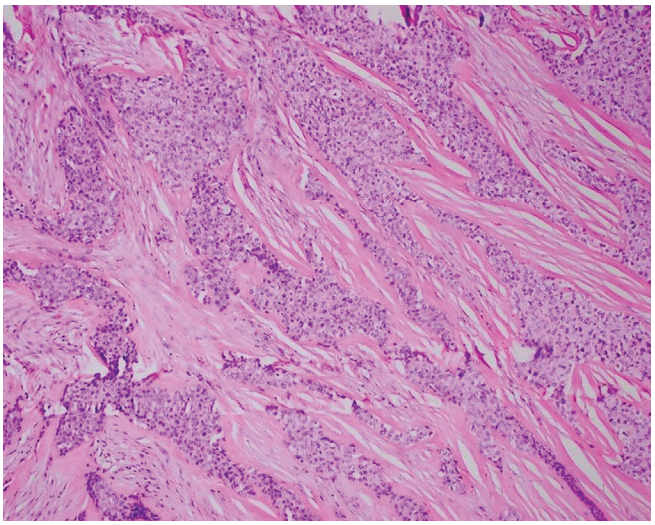

Figure 1. Diffuse sheets of monomorphic tumor cells surrounded by thick bands of hyalinized collagen bundles. (Hematoxylin-eosin, 100X magnification)

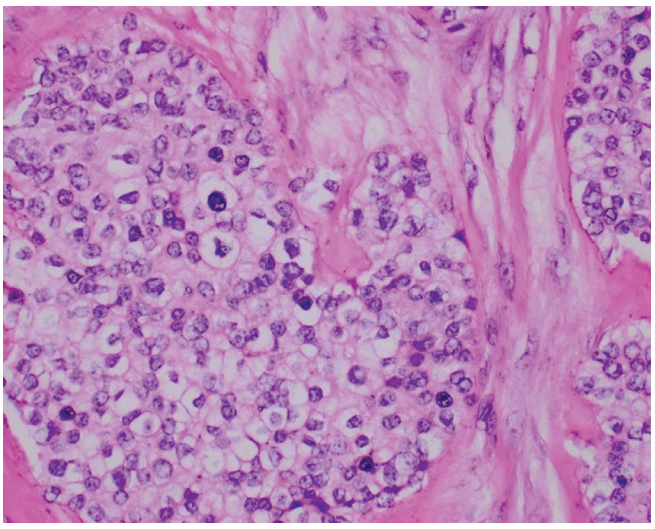

Figure 2. Tumor is composed of monomorphic round cells with distinctly clear abundant cytoplasm. (Hematoxylin-eosin, 400x magnification)

DISCUSSION

Hyalinizing clear cell carcinoma (HCCC) of the salivary gland is a distinct entity with the morphologic characteristics as described. A diagnostic challenge to this tumor is that the presence of clear cells warrants further work-up because this finding is also seen in other primary salivary clear cell tumors such as clear cell myoepithelial carcinoma (CCMEC), epithelial-myoepithelial carcinoma (EMEC), and in clear cell variants of other salivary gland tumors such as acinic cell carcinoma and mucoepidermoid carcinoma.[4,5] Clear cells are also seen in metastatic tumors from the kidney.[6] CCMEC and EMEC can be distinguished from HCCC by the presence of myoepithelial differentiation and are usually S100, SMA and EMA positive. The use of p63 in HCCC is to highlight the squamous differentiation and not as a myoepithelial marker. Clear cell variants of acinic cell carcinoma have periodic Schiff positive and diastase-resistant cytoplasmic zymogen granules, while clear cell mucoepidermoid carcinomas also show PAS-positive diastase-resistant cytoplasmic positivity for mucin. For challenging cases, CRTC1-MAML2 fusion studies may be done, wherein the absence of this gene rules out the latter. Clear cell renal carcinoma is positive for CD10 and vimentin. In our case, since the patient has no masses

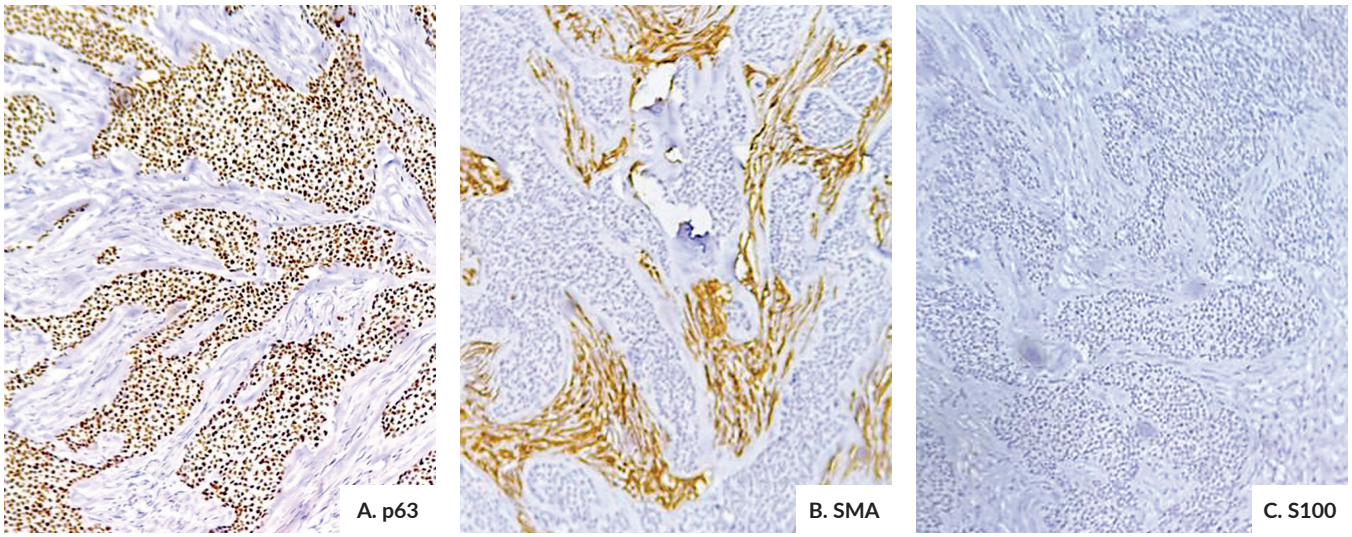

Figure 3. p63 showed a strong, diffuse nuclear staining, while SMA and S100 are both negative. The SMA highlighted the myofibroblasts among the thick hyalinized collagen that is intimately associated with the tumor. (100X magnification)

in the kidneys on imaging studies, the possibility of a renal metastatic tumor is unlikely hence CD10 and vimentin were not requested anymore. Also, the thick hyalinization is not a morphologic feature of clear cell renal cell carcinoma. Though immunohistochemistry stains and special stains help rule out the differential diagnoses, there are still clearly overlapping features between these tumors. The advent of molecular pathology enabled us to further characterize the genetic aspect of its tumorigenesis. Recently, hyalinizing clear cell carcinoma has been classified as a translocation-associated tumor with an EWSR1 rearrangement.[7] Genetically, HCCC shows a consistent EWSR-ATF1 gene fusion.[8] EWSR1 or Ewing sarcoma breakpoint region is a gene located on chromosome 22q12.2 that is first identified in Ewing sarcoma, hence the name. This gene is ubiquitously expressed in various cells proving its multiple roles in various cellular mechanisms and organ development.[9] EWSR rearrangements detected by Fluorescence in situ hybridization (FISH) has been found in a variety of tumors; though in cases of clear cell tumors of the salivary gland, EWSR rearrangement, is almost unique to clear cell carcinomas in that 80% of cases have EWSR rearrangement. It can also be detected in a percentage of clear cell myoepithelial carcinomas[10], albeit less than half of cases. Nevertheless, the presence of EWSR-ATF1 fusion helps separates this entity from its other histologic mimics. The currently available FISH kits in our country can detect EWSR rearrangement but not the partner gene, but since the histologic mimics of HCCC do not usually exhibit EWSR rearrangements, this is enough to commit to the diagnosis. Although, molecular testing by genetic sequencing remains to be the most ideal confirmatory test.

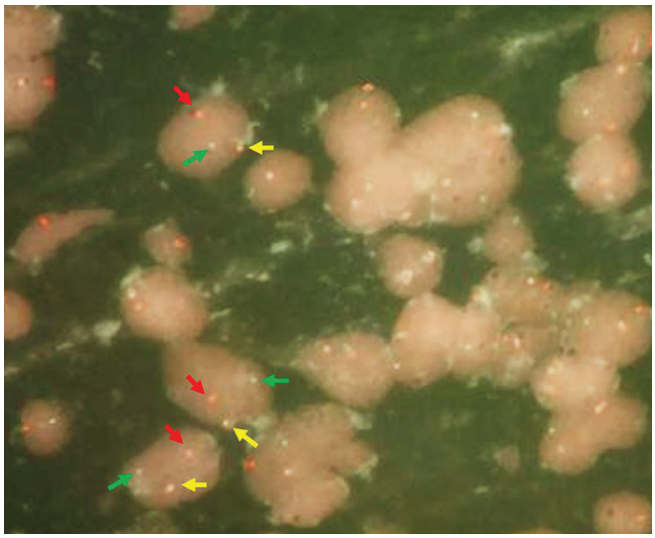

Figure 4. Molecular studies with EWSR1 showed positive EWSR1 rearrangement as demonstrated by the 78% percentage of nuclei with break apart signals. The normal fused signals *(yellow arrows)* are seen with break apart signals *(red and green arrows)*. (This was done using Vysis LSI EWSR1(22q12) Dual Color Break Apart Rearrangement FISH Probe Kit)

Clinically, primary surgical resection with negative margins is the treatment of choice for this tumor, however adjunct radiotherapy has been added in some cases.[11] Prognosis is usually good except for cases with high grade transformation, which requires more frequent follow-up due to possibility of recurrence and continued treatment.[12] One of the largest review of salivary clear cell carcinomas to date showed a relatively good prognosis of 81.3% 5-year disease-specific survival (DSS) and 69.9% 10-year DSS.[13] Factors for recurrence include positive margin status, lymph node status, and presence of tumor necrosis on histologic examination.[14]

Diagnostic accuracy is needed in this type of tumor, especially in differentiating it from its mimics that are metastatic (i.e., renal), because the management of each are different from one another.

CONCLUSION AND RECOMMENDATIONS

In summary, this paper presents a case of a 70-year-old female diagnosed with hyalinizing clear cell carcinoma histologically confirmed and supported by immunohistochemical and molecular studies. The molecular study for this case allowed us to detect EWSR rearrangement but not the partner gene involved in a translocation or fusion. Other FISH rearrangement studies and genetic sequence analyses may be done to identify the specific gene partner involved. To the best of our knowledge, there is no published data yet on the incidence of this type of malignancy in the Philippines. This may be due to the lack of awareness on the existence of this tumor and limitations of other areas when it comes to molecular testing. Reporting similar case will help increase awareness and knowledge regarding the tumor, its characteristics, and natural history.

Statement of Authorship

All authors certified fulfillment of ICMJE authorship criteria.

Author Disclosure

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