European Archives of Oto-Rhino-Laryngology (2019) 276:3461–3466
https://doi.org/10.1007/s00405-019-05604-4

HEAD AND NECK

Clinical characteristics of acinic cell carcinoma and secretory carcinoma of the parotid gland

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Received: 21 June 2019 / Accepted: 14 August 2019 / Published online: 22 August 2019

Abstract

Purpose Mammary analogue secretory carcinoma (SC) of the parotid gland is a relatively uncommon cancer associated with the ETV6–NTRK3 fusion product similar to breast cancer. The clinical characteristics and outcome of treatment were reviewed for patients with this tumor at our hospital.

Methods In this retrospective case series, 24 patients with a diagnosis of acinic cell carcinoma (AcCC) of the parotid gland were classified as having either SC or AcCC based on analysis of the ETV6–NTRK3 fusion gene. These two groups were compared with respect to their clinical and imaging characteristics (MRI/US), cytologic findings, accuracy of fine-needle aspiration cytology and frozen section, treatment outcomes, and immunohistochemical findings.

Results Based on re-classification by ETV6–NTRK3 fusion gene analysis, the diagnosis was SC in 14 patients and AcCC in 10 patients. The SC group had a significantly higher proportion of male patients and was also significantly younger than the AcCC group. Imaging studies revealed that SC was significantly more likely to show internal heterogeneity. Correct grading of both tumors was comparable by fine needle aspiration, with the rate being 60% for AcCC and 50% for SC. Diagnosis by frozen section biopsy diagnosis obtained the correct grade in 90% of the AcCC group and 93% of the SC group.

Conclusions In 24 patients previously diagnosed with AcCC, re-analysis of the ETV6–NTRK3 fusion product indicated that 14 patients actually had SC. Although AcCC and SC show similarities of their biological aggressiveness and prognosis, patients with SC were significantly more likely to be male and younger.

Keywords Parotid carcinoma · Acinic cell carcinoma · Secretory carcinoma · ETV6

Introduction

A wide variety of tumors can develop in the parotid gland, with 23 histopathological types of parotid carcinoma being listed in the 2017 World Health Organization classification [1].

Mammary analogue secretory carcinoma (SC) is a rare tumor of the salivary glands that shares histologic and genetic features with a rare malignancy known as SC of the breast [2]. Along with similar histologic findings, both breast and salivary SCs show ETV6 gene (12p13) rearrangement. Before SC was recognized in 2010, these parotid tumors were often diagnosed as acinic cell carcinoma (AcCC) or as adenocarcinoma, not otherwise specified. According to Boon et al. [3], the majority of SCs were previously misclassified as AcCC, with the latter tumor being the chief differential diagnosis.

While the histology of SC has been documented in the literature, and Khalele et al. [4] have subsequently reviewed its clinical features, little is known about the clinical characteristics of patients with this tumor at a single center.

Therefore, we reviewed 24 patients who were previously diagnosed as having AcCC at our hospital from 2000 to 2018 and reclassified them as SC or AcCC based on genetic analysis. We then compared the histological, immunohistochemical, and clinical features of these two groups in the present study.

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Patients and methods

Patients

The protocol of this study was approved by the Ethics Committee of Osaka Medical College. During the 19-year period from 2000 to 2018, 194 new parotid cancer patients were managed at our hospital (Osaka Medical College Hospital, Osaka, Japan). The main histopathological types were mucoepidermoid carcinoma (MEC, $n = 49$), carcinoma ex pleomorphic adenoma ($n = 29$), adenoid cystic carcinoma ($n = 23$), AcCC ($n = 24$), and salivary duct carcinoma ($n = 17$). Retrospective ETV6–NTRK3 gene fusion analysis of the 24 AcCCs was performed, thus leading to their reclassification as 14 SCs and 10 AcCCs. We then reviewed the symptoms, pre- and intra-operative diagnoses, imaging findings from magnetic resonance imaging (MRI) and ultrasound (US), outcome of treatment, histological findings, and results of immunohistochemical analysis in the SC and AcCC groups.

ETV6–NTRK3 fusion gene analysis

After total RNA was extracted from paraffin-embedded blocks, amplification was done as described previously [5]. Then the cDNA thus obtained was subjected to PCR using a pair of primers [sense: TEL971 (complementary to ETV6: 5′-ACCACATCATGGTCTCTGTCTCCC-3′) and antisense: TRKC1059 (complementary to NTRK3: 5′-CAGTTCTCGCTTCAGCACGATG-3′)]. Subsequently, the ETV6–NTRK3 fusion transcript (110-bp product) was amplified by the method of Bourgeois et al. [6]. If the result was positive, direct sequencing of the PCR fragments was done with a Big Dye Terminator Sequence kit (Applied Biosystems, Foster City, CA).

Comparison of clinical features, treatment and outcome

We investigated the following clinical characteristics of the SC and AcCC groups: sex, age, past medical history, tumor size, tumor stage, and tumor location.

Presenting symptoms/signs and imaging findings

The common symptoms/signs of malignancy associated with parotid carcinoma (spontaneous pain/tenderness, adhesion to the surrounding tissues, and facial palsy) were investigated in both groups. Imaging findings obtained by MRI and US were also investigated, since these are the most important diagnostic modalities.

Fine-needle aspiration cytology (FNA) and frozen section biopsy (FSB)

The results of FNA and FSB were investigated in all 24 patients. FNA was performed once under US guidance. FNA and FSB diagnoses were classified into the following five categories: correct histology and grade, correct grade (histology unknown), malignancy only, benign, and inadequate or indeterminate.

Immunohistochemical analysis

All 24 tumors were investigated immunohistochemically for human epidermoid growth factor receptor type 2 (HER2), androgen receptor (AR), epidermal growth factor receptor (EGFR), and programmed death-ligand 1 (PD-L1) expression, as described previously [7]. Appropriate positive and negative controls were employed for each immunostaining procedure.

Statistical analysis

Fisher's exact test for independence was used to compare the gender, tumor location, T classification, N classification, stage, symptoms/signs and MRI/US findings. Student's *t* test was employed to compare the age at the initial treatment, maximum tumor diameter, duration of symptoms. Statistical analysis was performed using JMP Pro software (version 14), and $p < 0.05$ was considered significant for all analysis.

Results

Clinical characteristics (Table 1)

When the AcCC group ($n = 10$) was compared with the SC group ($n = 14$), significant differences were noted with respect to sex, age, and T classification. The female:male ratio was 9:1 in the AcCC group and 3:11 in the SC group, with a significant difference of this ratio being noted between the two groups ($p < 0.001$). The mean age at initial diagnosis was 56.9 years in the AcCC group and 34.5 years in the SC group, and the SC group was significantly younger ($p < 0.001$). There was no significant difference of the maximum tumor diameter nor of the tumor location (superficial lobe, deep lobe, or lower pole). The interval from initial symptoms to confirmed diagnosis was 26.5 months in the AcCC group and 42.5 months in the SC group, but the difference was not statistically significant. While there was a difference of the T classification with more T2 tumors in the

Table 1 Characteristics of patients with acinic cell carcinoma and secretory carcinoma

	Acinic cell carcinoma ($n=10$)	Secretory carcinoma ($n=14$)	p value
Sex (female:male)	9:1	3:11	<0.001
Age (median + SD)	56.9	34.5	<0.001
Maximum diameter (median + SD; mm)	28.9	25.7	NS
Tumor location (superficial:deep:lower pole)	7:2:1	11:1:2	NS
Duration of symptoms (mean, months)	26.5	42.5	NS
T classification (1:2:3:4)	4:2:3:1	3:10:1:0	0.04
N classification ($N+$:N0)	0:10	1:13	NS
Stage (I:II:III:IV)	4:2:3:1	3:9:1:1	NS

Table 2 Symptoms/signs and MRI/US findings of acinic cell carcinoma and secretory carcinoma

	Acinic cell carcinoma ($n=10$)	Secretory carcinoma ($n=14$)	p value
Pain/tenderness	5 (50%)	4 (29%)	NS
Adhesion	4 (40%)	4 (29%)	NS
Facial palsy	1 (10%)	0 (0.1%)	NS
MRI/US			
Margin (clear:unclear)	9:1	13:1	NS
Tumor architecture (homogeneous:heterogeneous)	6:4	1:13	<0.01

SC group, there was no significant difference of the N classification or tumor staging. Lymph node metastasis was only found in one patient who was from the SC group.

Symptoms/signs and imaging findings (Table 2)

When symptoms/signs were compared between the AcCC and SC groups, indicators of malignancy such as pain/tenderness, adhesion to the surrounding tissues, and facial palsy were more common in the AcCC group, but the difference was not significant. Imaging findings obtained by US and MRI were compared with respect to the tumor margins (clear or unclear) and internal architecture (homogenous or heterogeneous). None of the tumors showed different findings between US and MRI. Tumor margins were clear in the majority of patients from both groups, and there was no significant difference. On the other hand, the homogenous:heterogeneous ratio of the internal tumor structure was 6:4 in the AcCC group versus 1:13 in the SC group, with significantly more tumors in the SC group being heterogeneous ($p<0.01$).

FNA and FSB diagnosis (Table 3)

When pre- and intra-operative diagnoses by FNA and FSB were evaluated in both groups, it was found that preoperative FNA could correctly identify the histology in 5/10 patients from the AcCC group and 5/14 patients from the SC group.

Table 3 FNA and FSB diagnosis of acinic cell carcinoma and secretory carcinoma

	Acinic cell carcinoma, $n=10$		Secretory carcinoma, $n=14$	
	FNA	FSB	FNA	FSB
Correct histology/grade	5	9	5	11
Correct grade (histology unknown)	1	0	2	2
Malignant	2	0	3	1
Benign	2	0	3	0
Inadequate/indeterminate	0	1	1	0

On the other hand, malignancy could not be diagnosed by FNA in two patients in the AcCC group and four patients in the SC group. The FSB diagnosis was correct in 9/10 patients from the AcCC group and 13/14 patients from the SC group, with malignancy not being diagnosed by FSB in only one SC patient.

Immunohistochemical findings

Immunohistochemical examination revealed that HER2 and AR were negative in all tumors from both groups. EGFR was positive in 2/10 tumors from the AcCC group and 2/14 from the SC group, while PD-L1 was positive in 1/10 and 5/14 tumors, respectively.

Treatment and outcomes (Table 4a, b)

In the AcCC group, 7/10 patients underwent (partial) parotidectomy and 3 patients received subtotal or total parotidectomy. The facial nerve was preserved in six patients, while two patients had partial sacrifice of the facial nerve, and another two patients required complete sacrifice. Selective neck dissection was performed in six patients, and all of them were pN0. Tumor recurrence was noted in three patients. Two of them showed local recurrence after 8 and 12 years, respectively, while the third patient developed AcCC on the opposite side at 7 years after resection of the first tumor. Postoperative local radiation therapy (60 Gy) was given to all two patients. All ten patients in the AcCC group are still alive, including those with recurrence.

In the SC group, 13/14 patients underwent partial parotidectomy or lower pole resection, while one patient had subtotal parotidectomy. Partial sacrifice of the facial nerve was done in 3 patients, while the nerve was preserved in the other 11 patients. Seven patients received selective neck dissection and were found to be pN0. In addition, one patient who was cN2b underwent selective neck dissection and four node metastases were found. Lymph node recurrence and subcutaneous recurrence at a site away from the primary lesion that was considered to be tumor dissemination were noted in one patient each. Postoperative local radiation therapy (60 Gy) was administrated to all three patients. All 14 patients in the SC group are still alive, including those with recurrence.

Discussion

The present study shows the case series of patients with parotid SC obtained from a single center, focusing on the clinical characteristics and treatment outcomes.

There have only been a few reports about case series of patients with parotid SC. According to a review by Khalele [4], very few reports have included more than 15 patients with parotid SC, with Choisea et al. [8] reporting 26 patients, Shah et al. [9] reporting 16 patients, Skalova et al. [10] reporting 16 patients, and Bishop et al. [11] reporting 15 patients. All of these reports were focused on the histopathological features of SC. In 2018, Boon et al. [3] summarized the clinical features of 18 patients with parotid SC, but their data were collected from 4 centers.

Table 4 Clinical findings of 10 patients with acinic cell carcinoma (a) and 14 patients with secretory carcinoma (b)

Age	Sex	T	N	Local resection	Tumor location	Neck dissection	Postope radiation	Facial nerve	Recurrence	Outcome	Observation (months)
(a) 10 patients with acinic cell carcinoma											
45	F	1	0	Ex → Lob	Sup	SND	–	Pres	Local 12 years	Alive	168
60	F	1	0	Par	Sup	–	–	Pres	–	Alive	77
46	F	1	0	Par	Deep	SND	–	P. Sacr	–	Alive	41
54	F	1	0	Par → Par	Sup	–	–	Pres	Local 8 years	Alive	3
61	F	2	0	Par	Sup	–	+	Pres	–	Alive	64
53	M	2	0	Par	Sup	SND	–	Pres	–	Alive	25
51	F	3	0	Tot	Deep	SND	–	T. Sacr	Opposite 7 years	Alive	196
64	F	3	0	Sub	Low	SND	–	P. Scar	–	Alive	158
71	F	3	0	Par	Sup	–	–	Pres	–	Alive	14
64	F	4a	0	Sub	Sup	TND	+	T. Sacr	–	Alive	102
(b) 14 patients with secretory carcinoma											
45	F	1	0	Ex → Lob	Sup	SND	–	Pres	Local 12 years	Alive	168
60	F	1	0	Par	Sup	–	–	Pres	–	Alive	77
46	F	1	0	Par	Deep	SND	–	P. Sacr	–	Alive	41
54	F	1	0	Par → Par	Sup	–	–	Pres	Local 8 years	Alive	3
61	F	2	0	Par	Sup	–	+	Pres	–	Alive	64
53	M	2	0	Par	Sup	SND	–	Pres	–	Alive	25
51	F	3	0	Tot	Deep	SND	–	T. Sacr	Opposite 7 years	Alive	196
64	F	3	0	Sub	Low	SND	–	P. Scar	–	Alive	158
71	F	3	0	Par	Sup	–	–	Pres	–	Alive	14
64	F	4a	0	Sub	Sup	TND	+	T. Sacr	–	Alive	102

M male, *F* female, *Ex* extirpation, *Lob* lobectomy, *Par* partial lobectomy, *Sub* subtotal parotidectomy, *Tot* total parotidectomy, *Sup* superficial lobe, *Deep* deep lobe, *Low* lower pole, *SND* selective neck dissection, *TND* total neck dissection, *Pres* preservation, *P. Sacr* partial sacrifice, *T. Sacr* total sacrifice, *Subcut* subcutaneous dissemination

Parotid cancer is relatively rare, and its diagnosis and treatment tend to vary among different centers, so data obtained from a single center may be more reliable.

The reason is not clear, but it is reported in several articles that SC was claimed to be encountered more in men than in women and younger than in AcCC.

According to a review of 279 patients from 57 centers, the mean age at diagnosis of SC was 45.7 years [4]. Boon et al. [3] reviewed 31 patients who had SC and reported that their median age was 49 years, with young patients being relatively uncommon and only 6 patients aged from 18 to 35 years. Sethi et al. [12] reviewed 92 patients with SC and reported that their mean age was 44.2 years. The median age of 2362 patients in an AcCC database was 54 years, but it is unknown how many patients with SC are included [13].

In 279 patients with SC reviewed by Khalele [4], the male:female ratio was 1.5:1 and there was no sex difference. Chiosea et al. [8]. reviewed 36 patients with SC and found a male:female ratio of 21:15, thus favoring males. In the current series, the male:female ratio of the SC group was 11:3 and there was significant male predominance. On the other hand, we found that the male:female ratio was 1:9 in the AcCC group and was different from the SC group. In the 2362 patients from the AcCC database, the male:female ratio was 1:1.6, slightly favoring females [13].

Before parotid SC was recognized as a specific disease entity, these tumors were diagnosed as AcCC, polymorphous adenocarcinoma, adenocarcinoma NOS, or MEC, with the majority considered to be AcCC [11]. Among 194 patients with parotid cancer treated at our hospital over the past two decades, only one patient had polymorphous adenocarcinoma and 6 patients had adenocarcinoma NOS, while there were 49 patients with MEC. However, according to Chiosea et al. [8], only one patient with SC was found by a review of 165 patients with MEC. Based on these reports, the present study focused on 24 patients with a diagnosis of AcCC and analysis of ETV6–NTRK3 fusion product positivity which led to a diagnosis of SC in 14 patients. Therefore, SC was detected in 14/194 (7.2%) patients with parotid cancer, but the frequency of this tumor may have been slightly higher if ETV6–NTRK3 analysis had been conducted in all patients.

Almost all SC cases had cystic lesions with hemorrhage and necrotic tissue, and hemorrhage and related findings (such as hemosiderin deposition and cholesterol clefts) were present in all SC cases. The AcCC showed solid and focal cystic patterns, and histologically they revealed unequivocal serous acinar differentiation. Another important method for distinguishing SC from AcCC is immunohistochemical analysis using the antibody for S-100 protein, mammaglobin and DOG1, as seen in recent our study [5]. An immunohistochemical panel of S-100, mammaglobin and DOG1 distinguish SC from AcCC.

On immunostaining, SC was not positive for HER2 or AR, while EGFR was positive in 14%. In 132 patients with parotid cancer, the positive rates for HER2, AR, and EGFR were reported to be 14%, 14%, and 70%, respectively [14]. Compared to these results, the positive rates for HER2, AR, and EGFR were all lower in our series. On the other hand, it is interesting that 5/14 tumors (36%) were positive for PD-L1 in SC. Our review showed that the PD-L1 positive rate was 30% for all types of parotid cancer, but only 19% for tumors of low to intermediate malignancy (unpublished observation of submitted data). Thus, the PD-L1 positive rate of parotid SC seems to be relatively high. In cases of recurrence, it may be possible that molecular-targeting therapy is indicated for such SC disease.

A characteristic FNA feature of SC is reported to be cells containing cytoplasmic vacuoles [14], but such cells are also observed in AcCC and in MEC as well. There have also been reports suggesting that FNA cannot be used to make a definitive diagnosis of SC [15].

Once information on staging and grade is obtained, the decision about surgery for parotid cancer can be made. In the diagnoses of FNA and FSB, we have defined a correct diagnosis as having correct grade in this study because of the above reason. Both AcCC and SC are considered to be tumors of low to intermediate malignancy, so diagnosis of either tumor was regarded as the correct grade. Consequently, FNA showed a correct diagnosis rate of 60% for AcCC and 50% for SC, with the correct diagnosis rate being almost comparable. At our department, FNA was done in 163 parotid cancer patients, and the correct grade was obtained in 34%, falling to 25% among patients with tumors of low to intermediate malignancy [16]. Therefore, the correct diagnosis rate of AcCC and SC by FNA was better than that for other histologic types of parotid cancer. On the other hand, the correct grade was determined by FSB in 90% of the AcCC group and 93% of the SC group. In 117 patients with parotid cancer, the correct grade was obtained by FSB in 72%, decreasing slightly to 68% for patients with tumors of low to intermediate malignancy [14]. Thus, the correct diagnosis rate of AcCC and SC by FSB was also higher than that for other histologic types.

Scherl et al. [9] analyzed 2362 patients with parotid AcCC in the National Cancer Database from 2004 through 2012. Although patients with SC were presumably included, a re-classification was not conducted. The 5-year overall survival rate in AcCC was high (88.6%). Factors related to a poor prognosis were high-grade histopathological malignancy, advanced T stage, positive lymph node metastasis, and age $\geq$ 70 years, with high-grade malignancy being the most important factor. The 5-year survival rate was 92.0% for patients with tumors of low to intermediate-grade malignancy, while it decreased to 41.7% for those with high-grade malignancy. However, only 5.1% of all patients

had high-grade tumors. In this series, none of the patients in the AcCC group nor those in the SC group had tumors with high-grade malignancy, and all patients survived after treatment.

Recurrence of AcCC was reported to be local relapses more frequently than lymph node or distant metastasis [16]. When enucleation of the tumor was performed, it was reported that 67% of patients developed recurrence [17]. Therefore, we performed partial parotidectomy for AcCC and SC in principle, resulting in local recurrence in 2/24 patients. Both patients had AcCC, and there was no local relapse of SC. The local recurrence rate is important because it may influence the indication for postoperative radiation therapy. We preserve the facial nerve in removing the tumor as our basic policy for low/intermediate carcinoma of the parotid gland if the tumor is not adhesive or invasive to the nerve. In this case, the surgical margin may not have been enough, so we basically performed PORT in case of demonstrating microscopic residual cancer. AcCC sometimes develops in the contralateral parotid gland, although this was reported to occur in only 3% of patients [16].

Conclusions

In 24 patients with a diagnosis of AcCC, ETV6–NTRK3 fusion product analysis led to re-classification of the tumor as SC in 14 patients and confirmation of AcCC in 10 patients. Thus, there were 14 patients with SC (7.2%) among 194 parotid cancer patients treated at our hospital. When the AcCC and SC groups were compared, the SC group showed significant male predominance and was significantly younger than in the AcCC group. These tumors showed low-grade malignancy, and all patients are still alive after treatment. Local recurrence was observed in two patients with AcCC. While AcCC and SC seem to show clinically similar conditions, further investigation and a long-term follow-up are needed.

Funding The authors have no funding, financial relationships, or conflicts of interest to disclose.

Compliance with ethical standards

Conflict of interest The authors have no funding, financial relationships, or conflicts of interest to disclose.

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