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Ameloblastic Carcinoma, Primary Type: Case Report, Immunohistochemical Analysis and Literature Review

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Abstract. *Ameloblastic carcinoma (AC) is a rare malignant odontogenic neoplasm with scarce reported cases in the literature and it can be confused with benign ameloblastoma (AM). This study reports a case of AC, and presents a literature review of AC classified into primary type (ACPt) or secondary type (ACSt) by the World Health Organization (WHO). The review addressed 31 cases published in the English literature between the years 2005 and 2011. The majority of cases were ACSt. The mandible was the most common site of occurrence for both AC types. All patients who died of their disease had ACSt. Tumors with plexiform pattern, hyperchromatism, mitosis and necrosis were associated with a higher ratio of histories of recurrence and death by disease, as well as the tumors with clear cells, especially in the ACSt. ACSt appeared to correlate with recurrence and mortality. The histological features may have different prognostic importance depending on the AC type.*

Definition and classification of malignancy in ameloblastoma (AM) has been the subject of controversy, in part because of its rarity, complicated by the confusion in terminology. The term ameloblastic carcinoma (AC) was introduced by Elzay. In the updated World Health Organization (WHO) classification, odontogenic carcinomas include metastasizing AM, AC, primary intraosseous carcinoma, ghost cell odontogenic carcinoma, and clear cell odontogenic carcinoma (2). AC is considered a rare malignant epithelial odontogenic neoplasm which retains the histological features of ameloblastic differentiation with cytological atypia, regardless of whether it has metastasized. Furthermore, AC was classified into primary type (ACPt) not preceded by simple AM (*de novo* carcinoma), and secondary type (ACSt), defined as a malignant transformation of a pre-existing benign AM (carcinoma ex ameloblastoma) (2).

Clinically, AC causes expansion of the jaw, growing rapidly, and frequently causes painful swelling, and can result in perforation of the cortex. AC appears most commonly in the posterior mandible, with about 10% of the reported cases being in the anterior mandible (3). Radiographic findings include poorly defined radiolucency, sometimes with focal radiopacities (3, 4).

The histological features of AC may vary and a single definitive microscopic criterion for AC can be elusive (5). Therefore, in order to ascertain a diagnosis of AC and classify it into ACPt or ACSt, it is necessary to make a careful examination of the submitted tissues. Studies have identified molecular alterations in odontogenic lesions and the use of phenotypic markers has assisted in determining the aggressive and malignant potential of these tumors. The molecular markers studied include Ki-67 proliferative index (6, 7), BCL-2 proto-oncogene protein (5), *p53* mutation (8, 9), alpha-smooth muscle actin (α-SMA) (6, 10) and cytokeratins (CKs) (5, 11).

The treatment of choice for this lesion includes wide local excision with cervical lymph node dissection; radiotherapy and chemotherapy seem to be of limited value (12). Although rare, these lesions may metastasize to the regional lymph nodes or lung (3). Patient prognosis is difficult to assign because of the rarity of well-documented follow-up information. Moreover, most reported cases are either single case reports or small series of cases.

Although the demographic and phenotypic aspects of AC have been investigated, no studies have correlated these features with their classification into ACPt and ACSt. In

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Key Words: Ameloblastic carcinoma, *de novo* carcinoma, demographic data, histological features, clinical course.

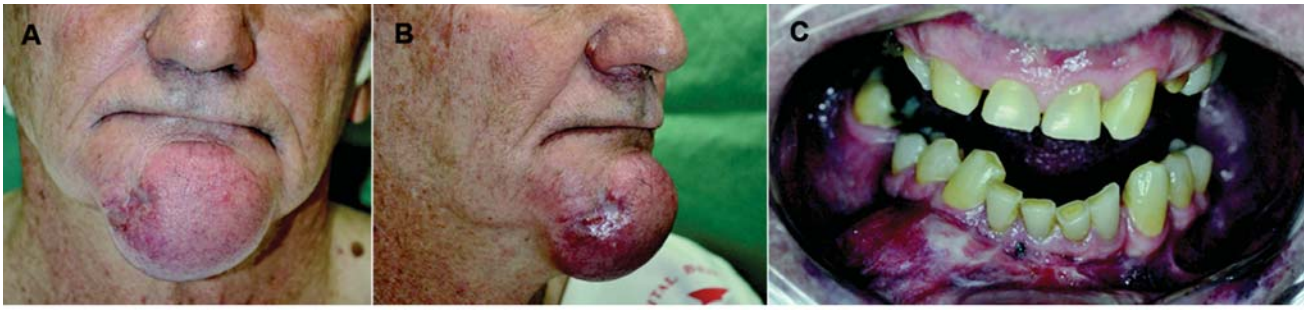

Figure 1. *Clinical photographs of a patient with ameloblastic carcinoma. A and B: Facial features showing a mass in the mandibular symphysis with erythematous surface. C: Intraoral photograph shows a swelling of the lower vestibular area with intact mucosa.*

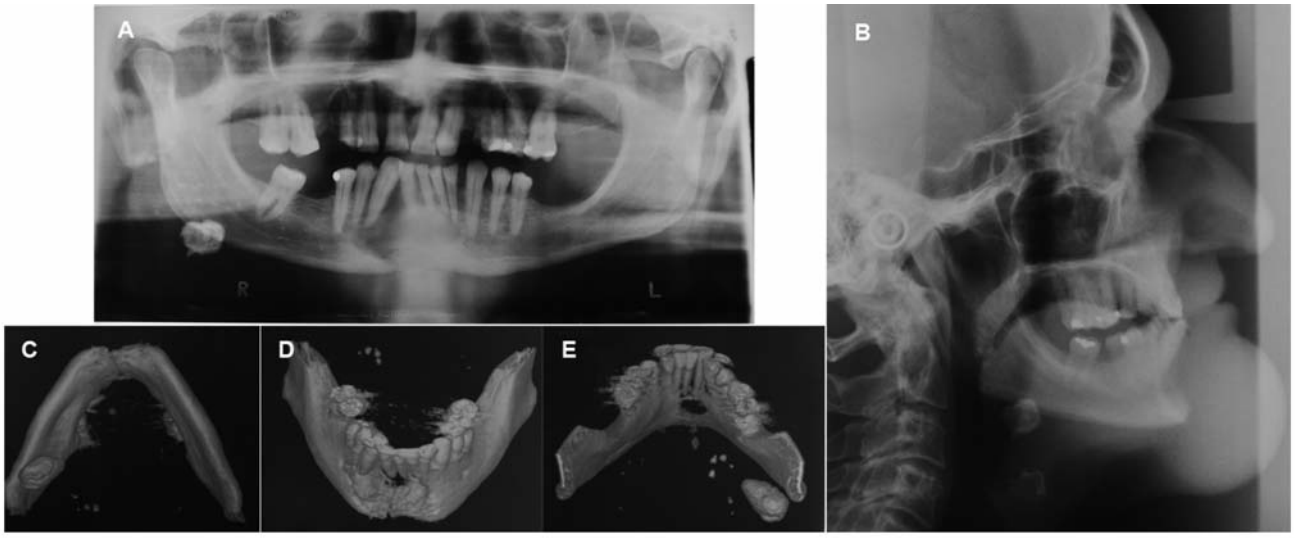

Figure 2. *Radiographs of ameloblastic carcinoma. Panoramic (A) and lateral cephalometric (B) radiographs show a large radiolucent lesion in the anterior mandible causing displacement of the lower incisors. C-E: Computerized tomographic 3D reconstructions show extensive destruction of the mandibular base, and vestibular and lingual cortical bone.*

addition, AC can be confused with benign AM. Therefore, the aims of the present work were: to report the clinical, histological, immunohistochemical and therapeutic details of a case of AC with one-year follow-up; and to review the literature with regard to clinical, follow-up, histopathological and phenotypic information of AC tumors classified as ACPt and ACSt.

Case Report

A 66-year-old man was referred to the Oral Surgery Clinic of the Bauru Institute of Rehabilitation and Maxillofacial Surgery, Bauru, Brazil. His chief complaint was a swelling of the anterior mandible, which had been present for 4 months and exhibited progressive growth; he had noticed the swelling coincidentally after local trauma. The patient's medical history included high blood pressure.

On physical examination, a 60×20 mm painless mass in the symphysis portion, resulting in distortion of the patient's facial features, was observed. This lesion was a soft mass with erythematous irregular surface and well-defined limits (Figure 1A and B). No enlarged cervical lymph nodes were palpable. On intraoral examination, a swelling of the lower vestibular area extending from the right pre-molar region to the incisors was observed. The mucosa was intact and there was no evidence of any surface mucosal disease (Figure 1C).

Radiographic imaging revealed a well-defined large radiolucent lesion in the anterior mandible causing displacement of the lower incisors but without apparent root resorption (Figure 2A and B). Moreover, computerized tomographic (CT) 3D reconstruction demonstrated extensive mandibular base, and vestibular and lingual cortical bone destructions (Figure 2C, D and E). An incisional biopsy was

performed under a provisional diagnosis of central giant cell granuloma and was submitted to histopathological examination.

The microscopic analysis showed islands, nests, and strands of tumor odontogenic cells, similar to ameloblasts, within a collagenous stroma. These nests and strands were arranged in a predominantly solid growth pattern. The epithelial component exhibited cytological malignancy characterized by peripheral cells with loss of reverse polarized nuclei, absence of stellate reticulum-like structures, nuclear pleomorphism, hyperchromatic nuclei, increased nucleous-to-cytoplasm ratio and atypical mitotic figures. Furthermore, focal areas of keratinizing metaplasia and necrosis were observed (Figure 3). The histopathological features were consistent with AC. Extensive surgical resection was carried out that included a left partial mandibulectomy from the left angle of the mandible to the midline area and supraomohyoid neck dissection. Immediate reconstruction was accomplished using a titanium reconstruction 2.4-unilock plate together with a forearm free flap.

The resection specimen with 70×65×60 mm was subjected to multiple sections that represented as many areas of the specimen as possible in order to meticulously examine the possibility of a classic ameloblastoma being found in tumor areas. The histopathological features exhibited no benign areas in any of the analyzed samples, leading to a diagnosis of AC *de novo* carcinoma, ACPt. High proliferation was confirmed by immunohistochemistry as shown by staining for Ki-67 antigen (42.45%) (Figure 4A). In addition, immunohistochemical analysis demonstrated diffuse and strong positivity for BCL-2 and p53 (Figure 4B and C), unlike that for CKs 8 and 18, which were expressed more focally and to a slighter degree, as well as a lack of expression for CK19 (Figure 5). α-SMA was expressed diffusely and strongly in the stroma in close approximation to the epithelial islands (Figure 4D).

Based on the localized nature of the tumor and the fact that all surgical margins and excised lymph nodes were tumor free, it was decided not to treat the patient with postoperative radiation. The patient is currently under close follow-up care. There is no sign of recurrent or metastatic tumor at one year postoperatively. The patient seeks oral reconstruction with osseointegrated dental implants and he is reintegrated into society.

Materials and Methods

All cases of AC classified as ACPt or ACSt published in the English language literature between 2005 and 2011 were reviewed, including a new case analyzed immunohistochemically in this work. The literature review was conducted using the Medline and Lilacs databases using the term *ameloblastic carcinoma* and *case report*. Data analysis included WHO classification, patient age, gender, location, follow-up, status at the last examination, histopathological features and immunohistochemical staining of tumors recorded. The

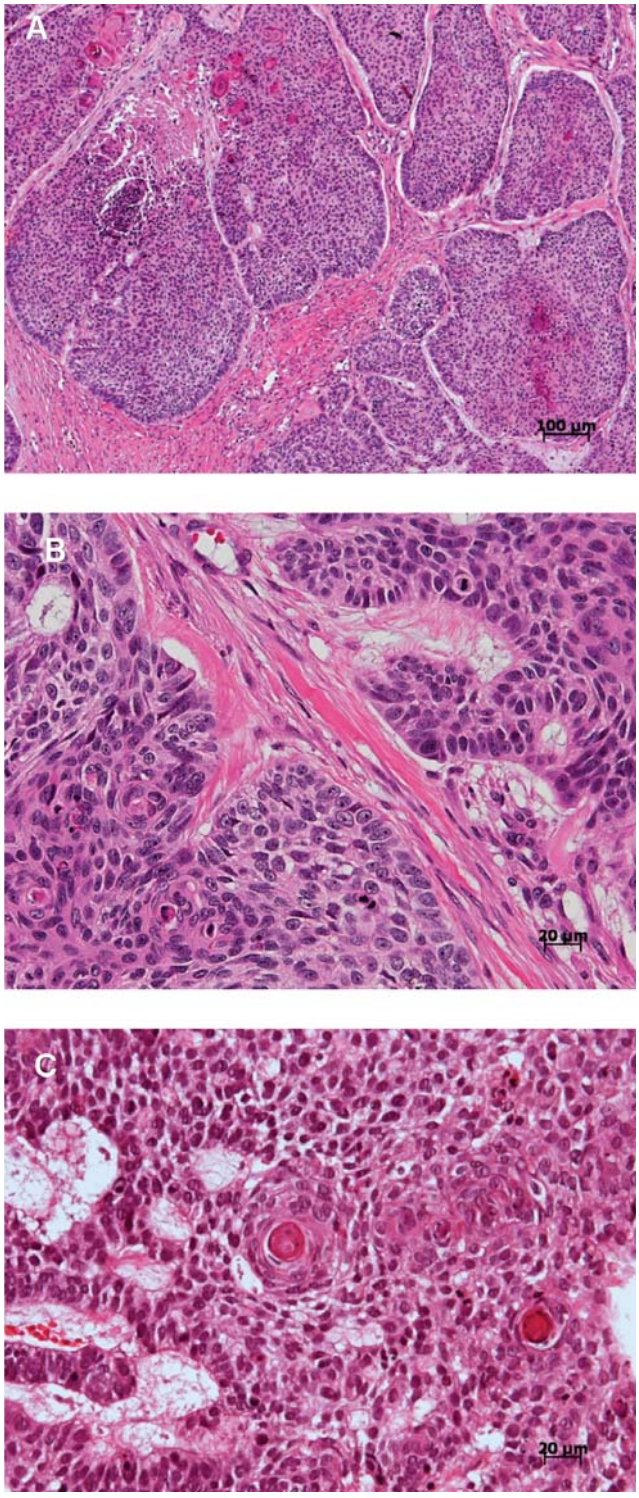

Figure 3. *Histopathological features of ameloblastic carcinoma. A: Ameloblastic carcinoma exhibiting nests and strands in a solid growth pattern with central necrosis (hematoxylin and eosin, ×10). B: Tumor islands demonstrating loss of peripheral palisading and nuclear polarity. Tumor cells exhibit hyperchromatism, nuclear pleomorphism and mitotic figures (hematoxylin and eosin, ×40). C: Keratin pearls can be seen (hematoxylin and eosin, ×40).*

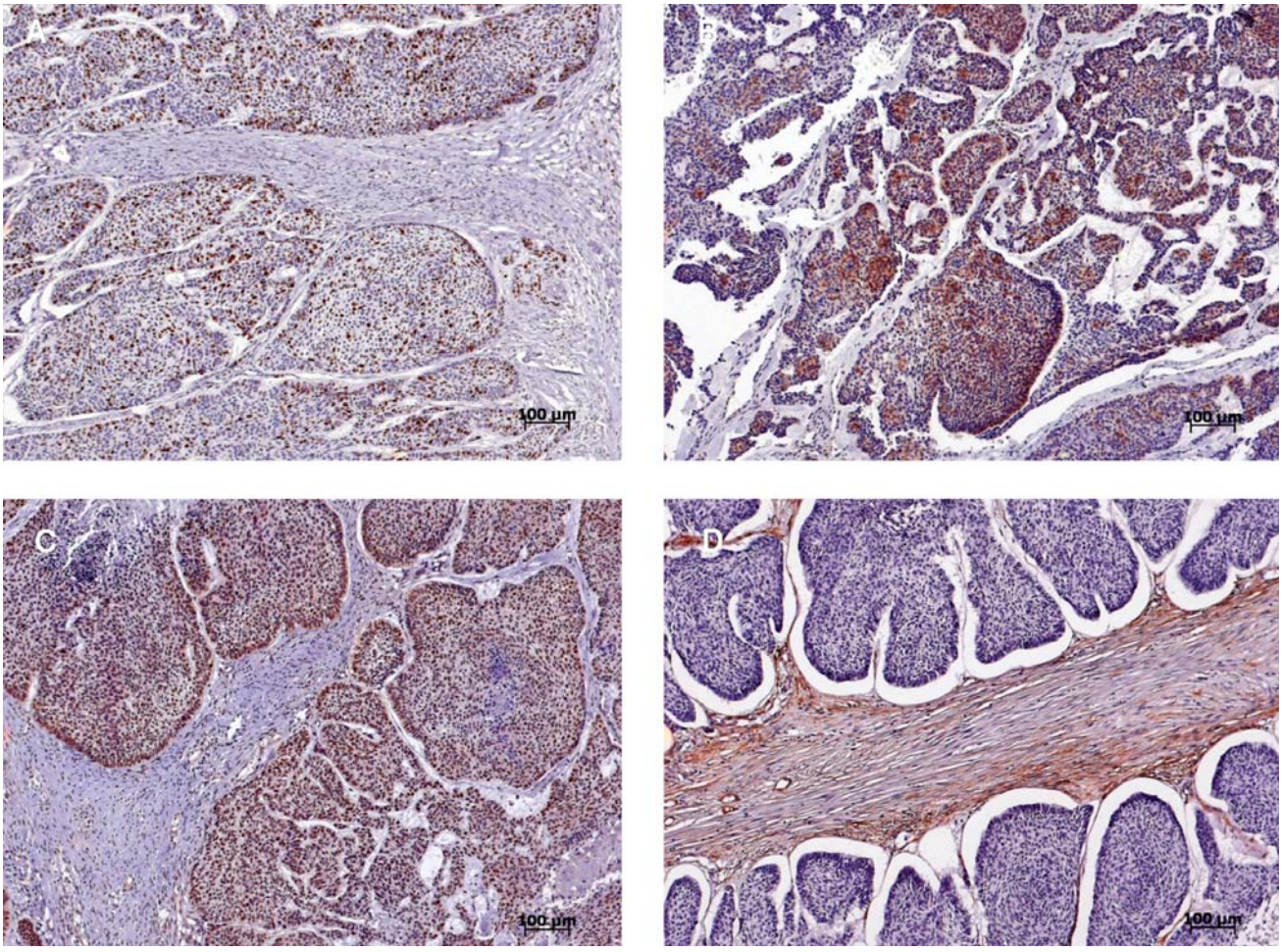

Figure 4. *Immunostaining in ameloblastic carcinoma. A: Strong staining for Ki-67 in the tumor epithelial islands. Diffuse and strong positivity for BCL-2 (B) and p53 (C) in the tumor epithelial islands. D: Diffuse and strong positivity for alpha-smooth muscle actin (α-SMA) in the stroma in close approximation to the tumor epithelial islands.*

articles derived from this search were independently screened by two authors based on the inclusion criteria. These were articles focusing on the case report that included at least clinical information, histopathologic features and WHO classification into ACPt and ACSt.

Results

AC literature review including a new case. A review of the English language literature from 2005 to 2011 revealed 31 reported cases of AC classified as ACPt or ACSt (Tables I, II and III), in addition to our new case, thus totaling 32 cases.

With regard to WHO classification as ACPt and ACSt, the majority of cases arose from a pre-existing AM (24 cases ACSt, 75%), and only eight cases, including our case, developed *de novo* (ACPt, 25%). Of the 24 ACSt, there were 19 males (79.2%) and five females (20.8%), with a male-to-female ratio of almost 3.8:1. Of the eight ACPt cases, six occurred in males (75%) and two in females (25%), resulting in a male-to-female ratio of 3:1. The mandible was involved in 15 ACSt cases (62.5%) and the maxilla in nine (37.5%), with a mandible-to-maxilla ratio of 1.7:1, while for the ACPt, the mandible and maxilla were involved two (25%) and six (75%), respectively, with a mandible-to-maxilla ratio of 1:3. Age at diagnosis of ACSt ranged from 7 to 90 years, with an average age of 48.4 years. For ACPt, the age ranged from 58 to 73 years, with a mean age at diagnosis of 65.4 years.

Of the 24 ACSt and eight ACPt cases, follow-up, recurrence, metastasis and survive information were available for 22 cases carcinoma ex ameloblastoma and seven cases developed *de novo*. Fourteen out of the 22 follow-up cases of ACSt (63.6%) had history of a recurrent tumor, with at higher prevalence in males (10 cases, 71.4%) and in a mandibular site (9 cases, 64.3%). Of the seven follow-up cases of ACPt, only one male patient with initial tumor in the maxilla had recurrence. All patients who died of disease had ACSt (nine out of 22 ACSt, 40.9%) of which seven cases had initial tumor located in the mandible.

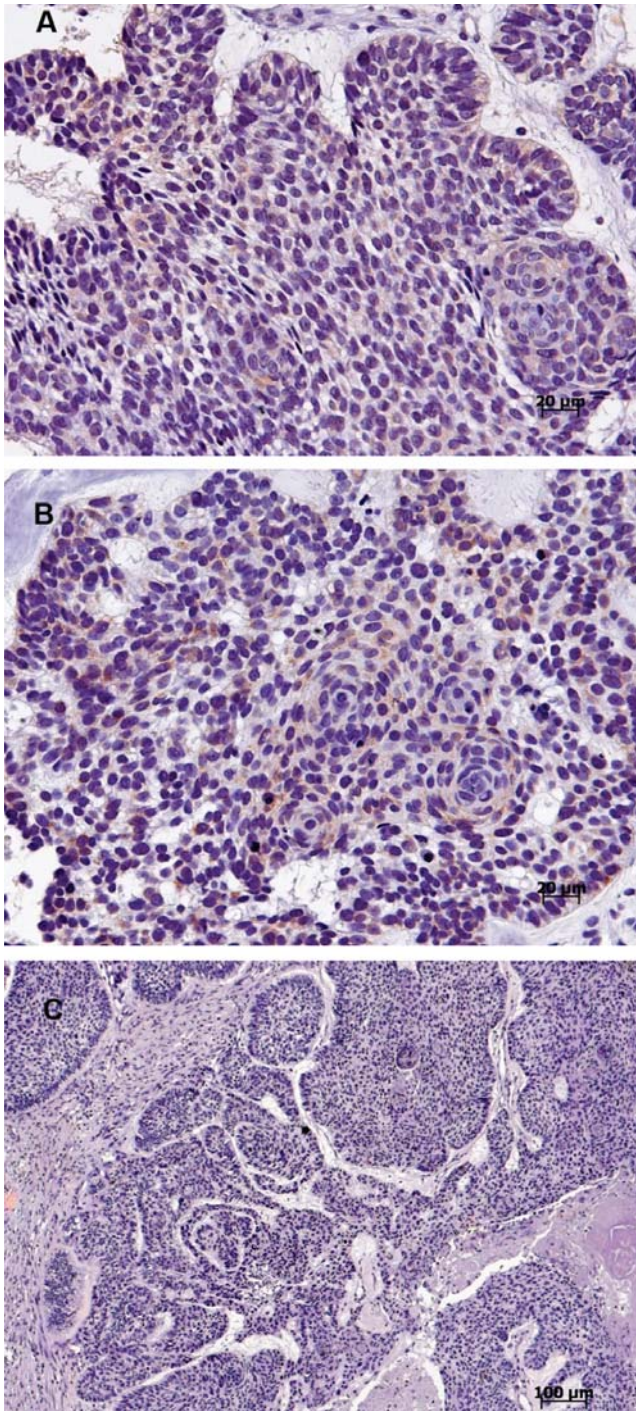

Figure 5. *Immunostaining in ameloblastic carcinoma. Focal and slight positivity for CKs 8 (A) and 18 (B), and negative expression for CK 19 (C) in the tumor epithelial islands.*

Histopathological features. The microscopic growth pattern was assessed for 31 cases, including our new case. The follicular growth pattern was most prominent (20 cases, 64.5%), followed by plexiform (11 cases, 35.5%).

With regard to WHO classification, the growth pattern was assessed for eight cases of ACPt and 23 of ACSt. Of the eight ACPt, there were seven cases with a follicular growth pattern and only one tumor exhibited a prominent plexiform pattern. In 23 ACSt, the follicular growth pattern was also the most commonly found (13 cases, 56.5%), followed by plexiform (10 cases, 43.5%). Other histopathological features were available for assessment in eight ACPt and 24 ACSt cases. Peripheral palisading was found in all ACPt and 22 out of 24 ACSt cases (91.7%); and reverse polarization was detected in five (62.5%) and 19 (79.2%) of ACPt and ACSt, respectively. However, stellate reticulum-like aspects were seen in only one ACPt case (out of eight tumors), unlike in ACSt (11 out of 24 cases, 45.8%). Some features of malignancy were seen frequently in both tumors; all ACPt and 20 out of 24 ACSt cases (83.3%) exhibited hyperchromatism; mitoses were present in seven (87.5%) and 22 (91.7%) ACPt and ACSt, respectively. Of the eight ACPt, three cases (37.5%) exhibited clear cells and three (37.5%) keratin production. Ghost cells were not found in these ACPt cases. Of the 24 ACSt cases, 10 (41.7%) had areas with clear cells, six cases (25%) presented keratinization, and only two tumors (8.3%) had sites of ghost cells. Areas of dentinoid/osteoid formations and rosette-like structures were found in one case of ACPt. Three ACSt cases (12.5%) exhibited calcification and three (12.5%) had rosette-like structures. Tumor necrosis indicative of malignancy was recognized in five ACPt cases (out of eight cases), while for ACSt, evidence of necrosis was seen only in three tumors (12.5% out of 24 cases).

Immunohistochemical study for Ki-67 labeling index (LI) was performed for 11 cases. Ki-67 LI ranged from 2% to 80%, with an average of 21.6%. Of the eight ACPt and 24 ACSt cases, Ki-67 LI was available for six ACPt and five ACSt cases. The mean Ki-67 LI in ACPt was 18.3%. For ACSt, Ki-67 LI ranged from 2% to 80%, with an average of 25.6%. BCL-2 and α-SMA markers were assessed for two tumors, with one ACPt and one ACSt, both being positive for these molecules. CKs 5, 14 and 18 were positive in all cases in which these molecules were studied (seven cases CKs 5 and 18 and eight cases CK 14), whereas the CK 7 and S-100 protein were negative (seven cases CK 7 and six cases S-100). CK 6 and argyrophilic nucleolar organizing regions (AgNORs) were studied in only one case, with positive immunoreactivity for both molecules. Five out of 10 cases with p53 data available (seven ACPt and three ACSt) showed positive reactivity for this marker, with four cases of ACPt (out of seven) and one case of ACSt (out of three).

Histopathological features and clinical course. With regard to ACPt tumors, six follicular cases had follow-up information of which one had recurrent tumor and another metastasized. The only ACPt case with plexiform pattern no

Table I. *Clinical and follow-up information of 32 cases of ameloblastic carcinoma classified as primary or secondary type.*

WHO classification	Case no.	Authors (ref)	Year	Location	Gender	Age (years)	Follow-up (years)	Recurrence	Metastasis	Outcome
Primary type (n=8)	1	Ward *et al.* (20)	2007	Mx (A)	M	64	3½	No	No	Alive
	2	Yoon *et al.* (9)	2009	Mx (P)	M	63	1¼	Yes	No	Alive
	3			Mx (P-S)	F	73	2½	No	No	Alive
	4			Mx (P-S)	M	61	–	–	–	–
	5			Mx (P)	M	58	1	No	No	Alive
	6			Mnd (P)	M	65	1¼	No	LN	Alive
	7	Matsuzaki *et al.* (15)	2011	Mx (A)	F	73	1	No	No	Alive
	8	*Casaroto *et al.*	2011	Mnd (A)	M	66	1	No	No	Alive
Secondary type (n=24)	9	Abiko *et al.* (8)	2007	Mnd (P)	M	72	–	–	–	–
	10	Akrish *et al.* (3)	2007	Mnd (A-P)	M	80	1	No	No	Alive
	11	Benlyazid *et al.* (12)	2007	Mx (P)	M	90	2	No	No	DOD
	12	Hall *et al.* (16)	2007	Mnd (P)	M	27	9½	Yes	No	DD
	13			Mnd (R)	M	31	41	Yes	No	Alive
	14			Mnd (P)	F	43	5	Yes	LN	DD
	15			Mnd (R)	M	50	13	Yes	No	DD
	16			Mnd (P-R)	M	49	4¾	Yes	No	DD
	17			Mnd (P)	F	53	30¾	No	No	DD
	18			Mnd (A-P-R)	M	59	11¾	Yes	No	DD
	19			Mx (A)	M	15	16¼	Yes	No	Alive
	20			Mx (P-S)	M	16	24	No	No	Alive
	21			Mx (P-S)	M	75	12¾	Yes	No	DD
	22			Mx (P-S)	F	7	9¾	Yes	No	Alive
	23			Mnd (P)	F	17	10¼	Yes	No	DD
	24			Mx (P-S)	M	63	19	Yes	No	DD
	25			Mx (P-S)	M	52	4¼	Yes	Lung Liver	Alive
	26	Angiero *et al.* (5)	2008	Mx (S)	M	68	½	No	No	Alive
	27	Yazici *et al.* (19)	2008	Mx (S)	M	10	½	No	No	Alive
	28	Yoon *et al.* (9)	2009	Mnd (P)	M	46	1½	Yes	LN	Alive
	29	Jindal *et al.* (30)	2010	Mnd (A-P)	F	60	19	Yes	LN	Alive
	30	Kamath *et al.* (10)	2010	Mnd (P)	M	64	–	–	–	–
	31	Karakida *et al.* (17)	2010	Mnd (A-P)	M	43	3¾	No	No	Alive
	32	Fujita *et al.* (23)	2011	Mnd (P, Per)	M	71	¾	No	No	Alive

WHO, World Health Organization; Mx, maxilla; Mnd, mandible; P, posterior (distal to canine); A, anterior (incisor-canine); R, involvement of ramus; S, involvement of maxillary sinus; Per, peripheral; DD, dead of disease (AC); DOD, dead of other cause; LN, lymph node; –, not otherwise specified. *New case.

had history of recurrent on metastatic tumors. None of the patients with ACPt, follicular or plexiform, died of their disease. Of the seven ACPt cases with hyperchromatism and follow-up information, one recurred and other metastasized. For the tumors with mitotic figures, out of the six cases, one recurred and another metastasized. The same occurred for the five tumors with necrosis; one tumor was recurrent and one was metastatic. Of the three tumors with clear cells, only one patient had a history of metastasis.

For the ACSt, follow-up information was available for 13 follicular and eight plexiform pattern tumors. Of the follicular cases, history of a recurrence was found in eight out of the 13 patients (61.5%), metastasis in four cases (30.8%), and five patients (38.5%) died of their disease. Of the eight plexiform cases, recurrent tumors were seen in five and death by disease in three; no patient had any history of metastasis. Histopathological features indicative of malignancy were also compared with the clinical course of the ACSt. Thus, 61.1% (11 out of the 18 cases) of the patients with hyperchromatic tumor areas had a history of recurrence, 16.7% (3 cases) had metastasis, and 44.4% (8 cases) died of their disease. For the tumors with mitosis, 20 cases had follow-up, with 60% (12 cases) and 20% (4 cases) showing histories of recurrence and metastasis, respectively; 40% (8 cases) also died. Of all ACSt with clear cells (10 cases), the majority of patients had a history of recurrence (eight cases, 80%), and died of their disease (seven cases, 70%); two (20%) had a history of metastasis. All cases with tumor necrosis (3 cases) expressed recurrence, in addition, two of these had metastatic tumors, and one died from their disease.

Immunohistochemistry for Ki-67, regardless of LI, was performed in 12 cases with follow-up information. Of these, no patient died of disease, but two had a history of recurrence and metastasis (16.7%), one being ACPt and the

Table II. *Histopathologic features of 32 cases of ameloblastic carcinoma classified as primary or secondary type.*

WHO	Case no.	Authors (ref)	Year	Benign microscopic features				Malignant microscopic features							
				GP	P Pal	R Pol	S Ret	Hyp	Mit	C Cells	G Cells	Ker	Den/Os	R-lik	Nec
Primary type	1	Ward *et al*. (20)	2007	Fol	Yes	No	No	Yes	Yes	Yes	No	No	No	No	No
(n=8)	2	Yoon *et al*. (9)	2009	Fol	Yes	Yes	No	Yes	Yes	No	No	Yes	No	No	Yes
	3			Plex	Yes	Yes	No	Yes	No	Yes	No	No	No	No	Yes
	4			Fol	Yes	Yes	No	Yes	Yes	No	No	No	Yes	Yes	No
	5			Fol	Yes	Yes	No	Yes	Yes	No	No	No	No	No	No
	6			Fol	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes
	7	Matsuzaki *et al*. (15)	2011	Fol	Yes	No	No	Yes	Yes	No	No	Yes	No	No	Yes
	8	*Casaroto *et al*.	2011	Fol	Yes	No	No	Yes	Yes	No	No	Yes	No	No	Yes
		Total		7 Fol 1 Plex	8	5	1	8	7	3	0	3	1	1	5
Secondary type	9	Abiko *et al*. (8)	2007	Plex	No	Yes	Yes	Yes	Yes	No	No	No	No	Yes	No
(n=24)	10	Akrish *et al*. (3)	2007	Fol	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No
	11	Benlyazid *et al*. (12)	2007	Plex	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No
	12	Hall *et al*. (16)	2007	-	No	No	No	Yes	Yes	No	No	No	Yes	No	No
	13			Plex	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	No	No
	14			Fol	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No	No
	15			Fol	Yes	No	No	No	No	Yes	No	No	No	No	No
	16			Plex	Yes	No	No	Yes	Yes	Yes	No	No	No	No	No
	17			Fol	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No
	18			Plex	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No	No
	19			Plex	Yes	No	No	Yes	Yes	No	No	No	No	No	No
	20			Plex	Yes	Yes	No	No	Yes	Yes	No	No	No	No	No
	21			Plex	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes
	22			Fol	Yes	Yes	No	Yes	Yes	No	No	No	No	No	No
	23			Fol	Yes	No	No	Yes	Yes	Yes	No	No	No	No	No
	24			Fol	Yes	Yes	No	Yes	Yes	Yes	No	No	No	No	No
	25			Fol	Yes	Yes	No	No	Yes	Yes	No	No	No	No	Yes
	26	Angiero *et al*. (5)	2008	Fol	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	No	No
	27	Yazici *et al*. (19)	2008	Plex	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No
	28	Yoon *et al*. (9)	2009	Fol	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	Yes	Yes
	29	Jindal *et al*. (30)	2010	Fol	Yes	Yes	No	Yes	Yes	No	No	No	No	No	No
	30	Kamath *et al*. (10)	2010	Plex	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	No	No
	31	Karakida *et al*. (17)	2010	Fol	Yes	Yes	Yes	Yes	Yes	No	No	Yes	No	No	No
	32	Fujita *et al*. (23)	2011	Fol	Yes	Yes	No	Yes	Yes	No	No	Yes	No	Yes	No
		Total		13 Fol 10 Plex	22	19	11	20	22	10	2	6	3	3	3

WHO, World Health Organization; GP, growth pattern; Fol, follicular; Plex, plexiform; Tra, trabecular; P Pal, peripheral palisading; R Pol, reverse polarization; S Ret, stellate reticulum; Hyp, hyperchromatic nuclei; Mit, mitoses; C Cells, clear cells; G Cells, ghost cells; Ker, keratin; Den/Os, dentinoid/osteoid; R-lik, rosette-like structure; Nec, necrosis. *New case.

other ACSt. Neither of the two cases positive for BCL-2 and α-SMA had recurrent or metastatic tumors, no did they die. In addition, of the five cases positive for p53, only one had recurrent tumors, while of the five p53-negative cases, one had recurrence and two had metastasis.

Discussion

In the previous WHO classification, both AC and metastasizing AM were categorized as malignant AM. After much debate (1, 4, 13, 14), the classification was revised to describe AC as a malignant change in a pre-existing benign AM (carcinoma *ex* ameloblastoma, ACSt) or as a primary AC not preceded by an ordinary AM (*de novo* carcinoma, ACPt) (2). The case reported here exhibited only malignant features histologically, with no benign areas, leading to a diagnosis of ACPt.

Initially, the anterior mandibular occurrence, normal-appearing mucosal surface without ulceration, and the well-defined margin of the lesion although vestibular and lingual cortical bone resorption observed on CT 3D images led us to think of a benign tumor as diagnostic hypothesis, *i.e.* central giant cell lesion. The final diagnosis of AC was only confirmed after microscopic analysis. Others authors also

Table III. *Immunohistochemical staining of 32 cases of ameloblastic carcinoma classified as primary or secondary type.*

WHO	Case no.	Authors (ref)	Year	Ki-67 LI (%)	Immunohistochemical staining	
					Positive	Negative
Primary type	1	Ward *et al.* (20)	2007	-	-	-
(n=8)	2	Yoon *et al.* (9)	2009	13.9 (SD 6.96;	Ki-67; CKs 5, 14, 18; p53	CK 7, S-100
	3			range 9.3-22.9)**	Ki-67; CKs 5, 14, 18; p53	CK 7, S-100
	4				Ki-67; CKs 5, 14, 18	CK 7, S-100, p53
	5				Ki-67; CKs 5, 14, 18	CK 7, S-100, p53
	6				Ki-67; CKs 5, 14, 18	CK 7, S-100, p53
	7	Matsuzaki *et al.* (15)	2011	-	Ki-67, p53	-
	8	*Casaroto *et al.*	2011	42.45	Ki-67; BCL-2; α-SMA; p53; CKs 14, 18	CKs 7, 19
Secondary type	9	Abiko *et al.* (8)	2007	80	Ki-67, CK 19	p53
(n=24)	10	Akrish *et al.* (3)	2007	20	Ki-67	-
	11	Benlyazid *et al.* (12)	2007	-	Ki-67	-
	12	Hall *et al.* (16)	2007	-	-	-
	13			-	-	-
	14			-	-	-
	15			-	-	-
	16			-	-	-
	17			-	-	-
	18			-	-	-
	19			-	-	-
	20			-	-	-
	21			-	-	-
	22			-	-	-
	23			-	-	-
	24			-	-	-
	25			-	-	-
	26	Angiero *et al.* (5)	2008	-	Ki-67; BCL-2; CKs 5, 6	-
	27	Yazici *et al.* (19)	2008	10	Ki-67, CK 14	-
	28	Yoon *et al.* (9)	2009	13.9 (SD 6.96; range 9.3-22.9)**	Ki-67; CKs 5, 14, 18	CK 7, S-100, p53
	29	Jindal *et al.* (30)	2010	-	-	CKs
	30	Kamath *et al.* (10)	2010	-	AgNORs, α-SMA	-
	31	Karakida *et al.* (17)	2010	2	Ki-67, p53	-
	32	Fujita *et al.* (23)	2011	-	-	-

WHO, World Health Organization; AgNORs, argyrophilic nucleolar organizing regions; LI, labeling index; -, not otherwise specified. *New case. **Mean Ki67 LI of 6 cases.

wrongly diagnosed cases with clinical features similar to our case as being benign tumors (15). However, retrospectively, we recognized the vestibular and lingual cortical bone resorption due to bone invasion by the tumor as a characteristic finding suggestive of malignancy.

A Medline search for cases of AC reported since 2005 identified 31 cases in 13 papers with analysis of WHO classification, clinical information, histopathological and immunohistochemical features of tumors, including the new case addressed in the present work. These cases reported in the English language literature consist mainly of single cases or small series of case reports, and the demographic features of the tumors presented in the literature differ.

In the present analysis of all published AC, the mandible-to-maxilla ratio (1.1:1) was slightly lower than that reported in the literature (3, 9, 16). On review also showed a high incidence of AC in men, of almost four fold that in women, there being a higher proportion of male cases compared studies by other reviewers (3, 12, 15, 16). The mean age (52.7 years) was older than reported by some studies (4, 9, 12), but it is similar to results reported by Akrish *et al.* (3) Based on follow-up information, the majority of the recurrent and metastatic cases presented initial tumors in males and the mandible. The recurrences appear to correlate with the mortality cases (9, 16), since almost all cases of mortality had a previous history of tumor recurrence. On the other hand, most patients who died of their disease had no reports of metastasis. We also highlight that the incidence of metastasis was low, although AC is known to be associated with metastasis (3, 9, 12, 16). Our patient has not yet

presented recurrence or metastasis with one-year follow-up. Continued and long-term follow-up is mandatory to detect late recurrence and metastasis.

Considering the WHO classification, the results of our study revealed that the most of the cases were malignant transformation of an AM, *i.e.* they were ACSt. However, these findings differ from Akrish *et al.* (3) and Karakida *et al.* (17) who show most cases being ACPt. These works reviewed the literature before 2004, prior to the latest WHO classification, during the period that the terminology of these lesions was rather controversial. Moreover, these reports included cases that arose after multiple recurrences and metastasis of a benign AM as AC, but without malignant microscopic features. In fact, AM cases that metastasize in spite of a benign histological appearance are known by the WHO classification as metastasizing (malignant) AM (18). This can explain the difference between the data found in the literature.

Nevertheless, no previous study has correlated the demographic data and histopathological features of AC on the basis of the WHO classification, *i.e.* as ACPt and ACSt. In the present review, both ACPt and ACSt were more common in males. In contrast, ACSt differed from ACPt with regard to the location and average age at diagnosis. ACSt appears to occur more in the mandible, unlike ACPt which was reported more in the maxilla, and ACPt appears to occur in older patients. The majority of the recurrent cases as well as all cases of mortality had a previous history of tumor classified as ACSt. Moreover, ACSt appears to correlate with recurrence and mortality, suggesting it to be more aggressive than ACPt. However, the follow-up time of ACSt ranged between 6 months (5, 19) and 41 years (16), while that for ACPt ranged between 1 (9) and 3.5 years (20). The reported follow-up time of cases developed *de novo* is too short to associate this tumor with more favorable prognosis when compared with ACSt.

The microscopic diagnosis of AC requires familiarity with histological features of AM. Despite the existence of areas or foci that resemble AM, AC shows changes in patterns and cytological features. Gardner considered that because numerous mitotic figures are unusual in AM, cases where they are sufficiently numerous probably justify the diagnosis of AC (21). In contrast, Slater designated that an increased mitotic index because of basilar hyperplasia is insufficient to permit a diagnosis of AC in the absence of other histological evidence of malignancy (22). In our study, most AC tumors reviewed exhibited a follicular growth pattern, with focal benign features, such as peripheral palisaded cells and reverse polarization. However, stellate reticulum-like morphological aspects, commonly found in AM, were not observed in the majority of these cases. Thence, the absence of this aspect may suggest a malignant process (16, 23). Other features of malignancy include hyperchromatism and increased mitotic figures, which were seen in almost all cases. On the other hand, necrosis, also indicative of malignancy, was recognized in only 25% of tumors. AC may not exhibit necrosis, but this should be carefully evaluated before excluding this diagnosis. Occasional clear cells were found in almost half of the tumors. Although ghost cells, keratin production, calcifications and rosette-like structures were rare in AC cases, these features should also be carefully evaluated when present in this tumor.

In cases where AC arises from a recurrent AM, the diagnosis of AC may be simple because most lesions have a malignant transition area coexisting with the benign AM (9). However, in cases where AC arises *de novo*, the microscopic distinction from AM is not always obvious and may be subjective (21, 22). According to our search, follicular growth was the most predominant pattern in both ACPt and ACSt. Focal areas with peripheral palisaded cells and reverse polarization, as well as features of malignancy such as hyperchromatism and increased mitotic figures, were also seen in the majority of cases, with there being no difference between ACPt and ACSt. However, the stellate reticulum-like aspect was the histological feature that differed between the tumors. Almost all ACPt cases were shown lack a stellate reticulum-like aspect, while half of the ACSt cases presented this aspect. Furthermore, the presence of tumor necrosis was detected in 62.5% of ACPt and in only 12.5% of ACSt.

Additionally, with regard to the phenotypic aspects, there were no correlations between the classification of tumors as ACPt and ACSt and immunohistochemical staining for Ki-67, BCL-2, α-SMA, CKs, S-100 and AgNOR markers. Only p53 staining was observed in a greater number of ACPt (9, 15) than in ACSt cases (17). In on presented case, we observed a high positive expression of Ki-67, BCL-2 and p53, which represent major cellular activity and may be involved in the aggressiveness of this malignant lesion (5, 24). The nuclear protein Ki-67 has been used to determine the proliferation rate of many types of tumors and cystic lesions (3, 7). BCL-2 protein is a product of a proto-oncogene and one of the regulators of apoptosis associated with odontogenesis and growth in odontogenic tumors (25, 26). Mutation of p53, a tumor suppressor gene, is the most frequently occurring mutation observed in the process of malignant transformation (27, 28). On the other hand, different data are found in the literature regarding p53 expression in AC. No staining for p53 was observed in benign and malignant areas of the AC case presented by Abiko *et al.* (8). Yoon *et al.* (9) reported that two AC cases exhibited strong positivity for p53 and four cases were p53-negative expression for this molecule. Moreover, we observed diffuse and strong staining of α-SMA in the stroma in close association with the odontogenic epithelial islands. This α-SMA expression suggests a possible mechanism of tissue invasion, probably due to differentiation of fibroblasts

into myofibroblasts. The increase of stromal myofibroblasts is associated with aggressive behavior and poor prognosis (6, 10, 29). Finally, in accordance with Jindal *et al*. (30), we detected reduced or a lack expression of CKs 8, 18 and 19, commonly expressed by odontogenic cells (11), suggesting a different phenotype of odontogenic epithelial cells when under malignant conditions. However, further studies involving larger sample sizes and survival analyses are necessary to better understand the role of these molecules as markers of the biological behavior of epithelial odontogenic neoplasms.

With regard to the correlations of histopathological features with the clinical course of the lesion, we observed a major percentage of cases with histories of recurrence and death by disease in those with the plexiform pattern rather than follicular growth pattern, regardless of the WHO classification. Nevertheless, recurrences are more frequently observed in follicular AM (31), but it is noteworthy that this growth pattern is the most common histological type of AM. Our finding showed that half of the AC cases with hyperchromatism, increased mitotic index and tumor necrosis presented recurrent tumors, and one third of patients with these features died of their tumor. Another important prognostic histological indicator was the presence of clear cells since the majority of cases with these cells had a history of recurrence and death due to their disease. Similarly, Hall *et al*. (16) detected that patients with clear cells AC had almost twice more recurrences than nonclear cell group. We emphasize that all these correlations were more evident in ACSt.

Finally, AC is a rare odontogenic tumor that exhibits malignant histological features in ACPt, as well as in ACSt, but the frequency of malignant features appears to be more pronounced in the former. Although the reported cases are scarce, this review shows that most of these cases arose from malignant transformation of an AM, *i.e*. ACSt, and this type of tumor appeared to correlate with recurrence and mortality. Furthermore, histological features, such as plexiform pattern, hyperchromatism, mitosis and necrosis, may have prognostic importance, as may the presence of clear cells. On the other hand, there was no correlation between the WHO classification and immunohistochemical staining. It is important to keep in mind that although the correlations may be interpreted only as a trend due to the rarity of this neoplasm and the low number of cases published in Medline and Lilacs databases, our findings may be useful to the pathologist, and may alert clinicians to the prognosis and tumor recurrence.

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