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MITOTIC INDEX IN CLEAR CELL ODONTOGENIC TUMORS

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Abstract. Clear cell (CC) variants of odontogenic tumors have bland morphology and may be difficult to diagnose especially in small biopsies. We address our study to mitotic index in benign and malignant odontogenic tumors as criterion for differential diagnosis. We reviewed 14 cases of odontogenic carcinomas ("malignant group" including 6 CC odontogenic carcinomas (CCOC), 6 ameloblastic carcinomas (AC) and 2 primary intraosseous squamous cell carcinoma (PISCC)) and 30 cases of ameloblastoma with clear cell component ("benign group"). We compared the mitotic index of the benign group versus the malignant group; the mitoses were counted in consecutive high power fields (hpfs) without necrosis or stromal deposition and the mitotic index was reported as number of mitoses per 10 hpfs. The statistical calculations were performed using EXCEL and EPIINFO programs. The mitotic index was lower in benign tumors (most benign cases had less than 20 mitoses / 10 hpfs) but almost half of the odontogenic carcinomas had similar mitotic count. There is no statistical significance of higher mitotic index in malignant tumors compared to benign ones (P = 0,057). There was also a tendency towards higher number of mitoses in solid / multicystic ameloblastoma than in desmoplastic variant. Frank malignant tumors present strikingly numerous mitoses; otherwise, ordinary ameloblastomas may reveal an impressive number of mitoses, sometimes in such a proportion that the examiner could be induced to consider that certain tumor as being malignant. Unfortunately, in daily practice, the main problem is not represented by high mitotic index in ameloblastoma but by low mitotic index in CCOC. An odontogenic tumor with clear cells may present a reduced number of mitoses but the overall behavior (destruction of the cortical bone, invasion in the soft tissue or even metastases) may allow its classification as malignant. When dealing with an individual case, mitotic index is not a reliable parameter for differentiating benign ameloblastomas from odontogenic carcinomas since many ameloblastomas may have an increased number of mitoses while CCOC may show a not very prominent mitotic activity.
Keywords: clear cell ameloblastoma, clear cell odontogenic carcinoma, odontogenic tumors, mitotic index

Introduction

In every field of pathology, clear cell (CC) tumors represent a challenge for the pathologist due to their intriguing biology. No matter how bland their morphology is, they might show malignant behavior (classic examples being those of the CC renal cell carcinoma [Grawitz tumor] or CC sarcoma [malignant melanoma of the soft tissues]). They are tumors with the most unremarkable microscopic appearance – monomorphic CCs with abundant clear cytoplasm, small round centrally placed nuclei, nonidentifiable nucleoli, no mitoses but with enormous propensity for hematogenic embolisation and subsequent blood-borne metastases.

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CC variants of odontogenic tumors do not represent an exception. Exceedingly rare, they may be difficult to diagnose, especially when dealing with small amounts of tissue (biopsies). In these cases, since invasion in adjacent tissues cannot be evaluated, the presence of cytonuclear atypia should establish the malignant character of the lesion. Unfortunately, tumors with CC are characterized by mild pleomorfism; the presence of numerous mitoses might be useful for differentiation. We address this study to mitotic index in benign and malignant odontogenic tumors as criterion for differential diagnosis.

Material and methods

We reviewed 14 cases of odontogenic carcinomas and 30 cases of ameloblastoma with clear cell component; the cases were retrieved from the personal files of one of the authors (PJS), consisting of consultation cases and cases from several hospitals in the Netherlands. The selection of the cases was done based on H&E microscopic appearance, using the diagnostic criteria of the latest WHO classification [5]. The tumors were included if the CCs structures represented at least 5% of the solid tumoral mass. 5% is an arbitrarily selected margin offering the best compromise between the necessity of including both a high number of cases and a sufficient quantity of CC component for evaluation (1% margin offers the possibility of inclusion of more numerous cases but there is not enough CC component to be analyzed; 10% offers cases with more areas of CC but the overall number of cases decreases).

The 14 cases of odontogenic carcinomas with CCs ("malignant group") included 6 CC odontogenic carcinomas (CCOC), 6 ameloblastic carcinomas (AC) and 2 primary intraosseous squamous cell carcinomas (PISCC); they were selected from a total of 24 cases of odontogenic carcinomas. All the odontogenic carcinoma cases had cortical perforation and subsequent growth into adjacent soft tissue as unequivocal signs of malignancy [26]; vascular emboli were noticed in one of the cases and in one case distant bone metastases occurred during follow-up.

The 30 cases of ameloblastomas with CCs - "ameloblastomas with CC differentiation" ("benign group") were selected from a total of 240 cases. Histopathologically, they presented tumoral structures composed of clear cells, either replacing the stellate reticulum in solid/multicystic ameloblastomas or forming tumoral structures in desmoplastic ameloblastomas.

We compared the mitotic index of the benign group versus the malignant group. The mitoses were counted in consecutive high power fields (hpfs) without necrosis or stromal deposition (solid nonnecrotic areas of tumor were evaluated whenever possible; in desmoplastic tumors, several consecutive hpfs – up to 4 fields – were summed to obtain a "solid" tumoral field, according to the quantity of intervening collagen in each particular case). Whenever possible, 50 consecutive hpfs were evaluated and then the mitotic index was reported as number of mitoses per 10 hpfs; only integer numbers were used (the integer part of the number or the integer part of the number plus one, according the value of the decimals, less or more than 5 – i.e. 3,4 = 3 and 3,6 = 4). When the number of hpfs to evaluate was lower than 10, the extrapolation to 10hpfs was performed.

Whenever it was necessary Fisher distribution was used to ensure an extrapolation to a more homogeneous distribution of the cases. The statistical calculations were performed using EXCEL and EPIINFO programs.

Results

The 30 cases of ameloblastomas with CC differentiation (benign group) were represented only by solid / multicystic and desmoplastic type ameloblastomas; there were no unicystic ameloblastomas with CC differentiation and the proportion of the desmoplastic ameloblastoma was quite high (9 cases of 30 - 30%) (Table I).

Type of ameloblastoma	No. of cases	%
Solid / multicystic	21	70,00%
Desmoplastic	9	30,00%
Total	30	100.00%

Table I. Type of ameloblastoma with clear cell differentiation

The most frequent histopathologic types of odontogenic carcinomas with a CC component were CCOC and AC (42,85% each) (Table II).

No significant difference was noticed in the proportion of the CC component between benign

Odontogenic carcinomas with CC component	No. of cases	%
CCOC	6	42,85%
AC	6	42,85%
PISCC	2	14,28%
Total	14	100,00%

Table II. Types of odontogenic carcinomas with clear cell component

and malignant tumors; the number of cases composed predominantly of clear cells (over 75% clear cell component in the tumoral mass) was higher for malignant tumors but the difference is not statistically significant (P = 0,99) (Fig 1). Most of the CCOC were composed predominantly of CC but had similar mitotic count (Table IV). There is a tendency to count more numerous mitoses in the malignant group but no statistical significance of higher mitotic index in malignant tumors compared to benign ones (P = 0,057) (Fig 2). There was also a tendency towards higher number of mitoses in solid

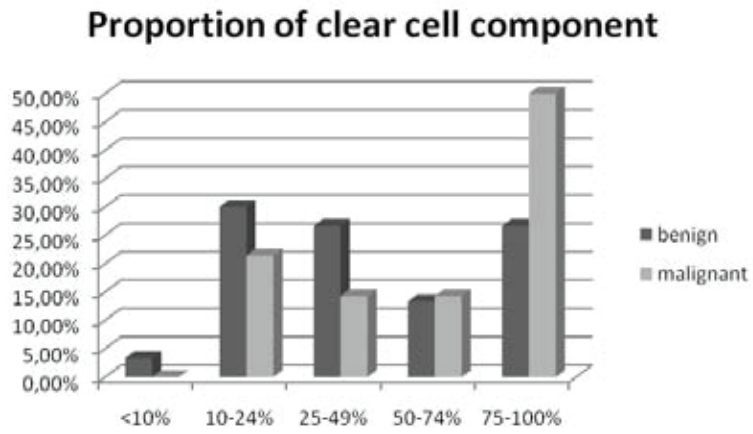

Figure 1. Proportion of clear cell component

% of CC component	Benign tumors		Malignant tumors	
<10%	1	3,33%	0	0,00%
10-24%	9	30,00%	3	21,42%
25-49%	8	26,67%	2	14,28%
50-74%	4	13,33%	2	14,28%
75-100%	8	26,67%	7	50,00%

Table III. Proportion of CC component

there was one case with CC component of only 25%. No AC contained more than 50% CC component; one PISCC had 90% CC while the other PISCC revealed only10% CC component (Table III).

The mitotic index (number of mitoses per 10 hpfs) was lower in benign tumors (most benign cases had less than 20 mitoses / 10 hpfs) but also, more than half of the odontogenic carcinomas / multicystic ameloblastoma than in desmoplastic variant (Table V).

Discussion

The presence of CCs is not uncommon in odontogenic lesions. CCs may be found in the epithelium of several cystic lesions (as dentigerous cyst) and

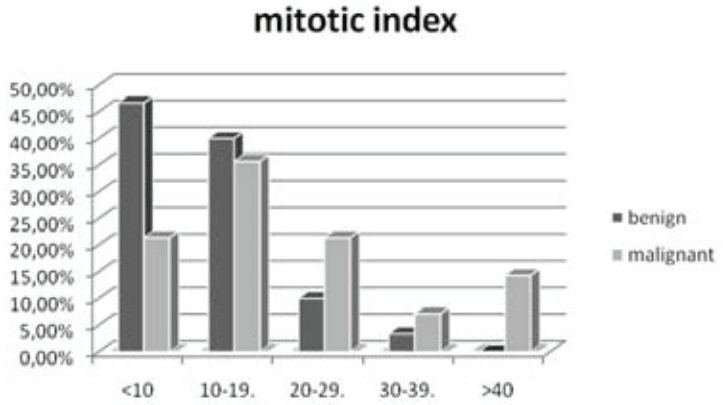

Figure 2. Mitotic index in benign and malignant group

mitotic index	benign		malignant	
<10	14	46,67%	3	21,42%
10-19.	12	40,00%	5	35,71%
20-29.	3	10,00%	3	21,42%
30-39.	1	3,33%	1	7,14%
>40	0	0	2	14,28%
P (χ test)		0,057		

Table IV. Mitotic index in benign and malignant group

Type of ameloblastoma	Mitotic index per case	median	average
Desmoplastic	2, 2, 3, 5, 7, 8, 11, 15, 20	7	8
Solid / multicystic	5, 5, 6, 6, 7, 8, 8, 9, 10, 10, 10, 10, 11, 13, 13, 15, 15, 16, 20, 23, 37	10	12

Table V. Mitotic index according to the type of ameloblastoma

represent a prerequisite for the microscopic diagnosis of the lateral periondontal cyst. CC components were described in benign odontogenic tumors such as ameloblastomas [18, 20-21], calcifying epithelial odontogenic tumor (Pindborg tumor both in intraosseous or peripheral variants) [2, 17, 22] and also, but rarely, in calcifying cystic odontogenic tumor [8,19], ghost cell odontogenic tumor [29] or in peripheral odontogenic fibroma [10, 24]; in some cases of benign tumors, a more aggressive biologic behavior was recorded compared to that of the conventional counterpart [23]. CCOC is the main representative of the malignant odontogenic tumors with CCs; CCs may be present in other malignant odontogenic tumors as ameloblastic carcinoma [5] or ghost cell odontogenic carcinoma[3].

The presence of CCs in such a wide variety of odontogenic lesions may represent a source of problems in differential diagnosis, even when differentiating between benign and malignant. In fact, when CCOC was first described by Hansen (3 cases reported as CC odontogenic tumor in 1985 [14]), the lesion was considered benign based on overall unremarkable histopathologic appearance. Since then, 58 similar cases of tumors were reported (a total of 61 cases – based on MEDLINE search)

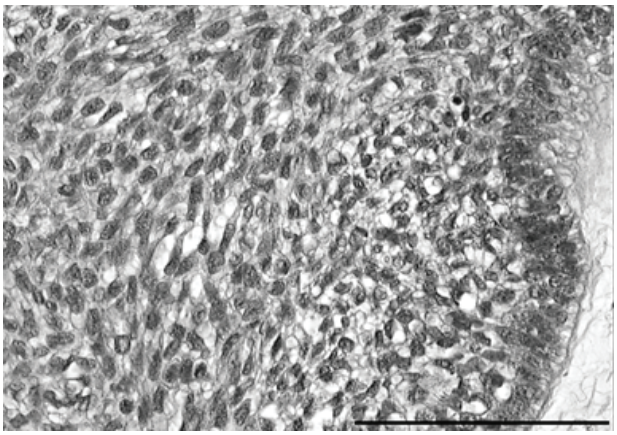

Figure 3. Solid ameloblastoma with follicular pattern; clear cells with oval nuclei with bland morphology (no nuclear pleomorphism, fine granular chromatin, no visible nucleoli, no mitoses). HE, original magnification x 400, scale bar 100 μ

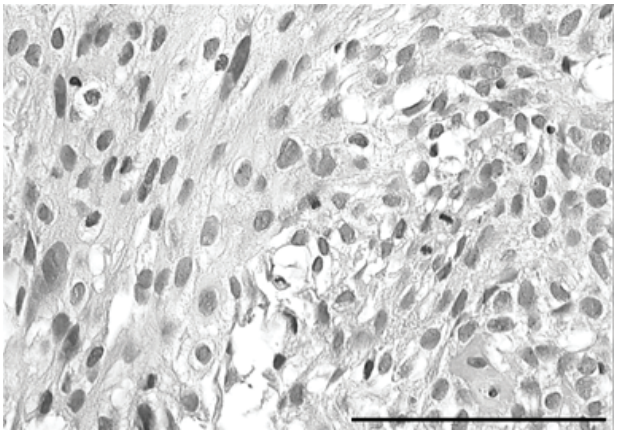

Figure 4. Solid ameloblastoma with follicular pattern; clear cells with oval nuclei with mild pleomorphism, occasional small nucleoli, occasional mitoses. HE, original magnification x 400, scale bar 100 μ

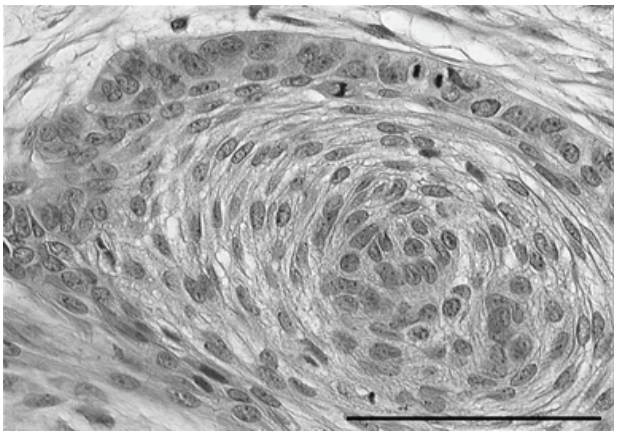

Figure 5. Solid ameloblastoma with follicular pattern; clear cells with oval nuclei with bland morphology (no nuclear pleomorphism, fine granular chromatin, no visible nucleoli) but relatively frequent mitoses. HE, original magnification x 400, scale bar 100 μ

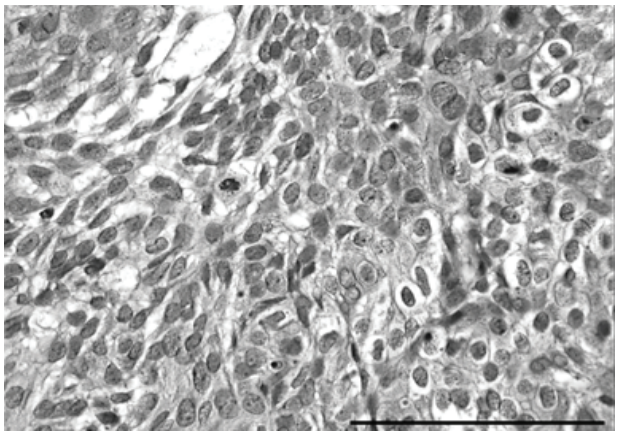

Figure 6. Ameloblastic carcinoma with clear cells in the center of the tumoral islands; clear cells with round to oval nuclei with mild nuclear pleomorphism; fine granular chromatin with occasional small nucleoli, mitoses are present. HE, original magnification x 400, scale bar 100 μ

[1, 4, 6-7, 9, 16, 25, 27-28]. Accumulating data changed the former perception of this entity from a benign locally invasive neoplasm ("CC odontogenic tumor" with an expected biological behavior closer to an ameloblastoma – 1992 WHO classification of odontogenic tumors [15]) to a more aggressive neoplasm, denominated as CCOC – 2005 WHO classification of head and neck tumors [5], as follow-up of the patients revealed a more aggressive biological behavior with frequent recurrences and occasional metastases. However, when analyzing the pictures of the CCOC reported in field literature, none of the lesions have prominent cytological or nuclear pleomorphism, features usually associated with malignancy. Since CCOC may present an ameloblastomatous pattern (clear cells nests with ameloblastoma-like peripheral palisading) [9, 11-12], differentiating between ameloblastoma with CC and CCOC or even AC with CC may occur more frequently than predicted in daily practice.

This is the first study addressed to the issue of mitotic index in ameloblastomatous tumors, at least to our knowledge. Presence of mitotic activity is rarely noted in ameloblastoma [5] and it is tempting to use this criterion to differentiate between ameloblastoma and odontogenic carcinomas.

In our study the mitotic index was, as expected, higher in the malignant group but we have to underline that almost one half of the benign group cases presented a mitotic index higher than 20 mitoses/10HPF.

We question our method of quantification of the mitotic index as a possible cause of obtaining high mitotic indexes in benign tumors. Two biases might occur:

- "compressing" several consecutive hpfs in order to obtain "solid" tumoral fields might artificially increase the number of mitoses, especially in desmoplastic ameloblastomas
- extrapolating the mitotic count obtained in few hpfs to 10 hpfs might also artificially augment the mitotic index by a sort of sampling error effect, especially in small biopsies.

Analyzing the number of mitoses according to the type of ameloblastoma (table V) we discovered that, despite our method, only one case of desmoplastic ameloblastoma presented a mitotic index of 20 and in this particular case the whole CC component was 15% of the total tumoral mass in a small biopsy – by "compressing" only 3 fields of tumoral CC we obtained a total of 6 mitoses; the other case of desmoplastic ameloblastoma with a mitotic index of 15 presented 9 mitoses in a total of 6 "compressed" hpfs of CC (the CC component in this case was also small, only 10% of the total tumoral mass). The other 3 cases with high mitotic indexes (over 20) were solid / multicystic ameloblastomas (mitotic indexes of 20, 23 and 37); in these cases no "compression" was necessary; however, the case with mitotic index of 37 presented 11 mitoses from a total of 3 hpfs of CC (the CC component in this case was very small, only 5% of the total tumoral mass), so that an artificial rise of mitotic index could be due to extrapolation of a particular area of the tumor which might not be representative for the whole lesion (it is not uncommon to discover occasional hpfs with even 3 or 4 mitoses in an otherwise ordinary ameloblastoma). In total we had 5 cases with small CC component in which an extrapolation was performed in order to obtain a mitotic index reported to 10 hpfs (the mitoses were counted in 3, 3, 5, 5 and respectively 6 hpfs with number of mitoses of 6/3hpfs, 11/3hpfs, 1/5hpfs, 4/5hpfs, 9/6hpfs), all but one being desmoplastic ameloblastomas. Six other cases presented less than 50 high power fields of CC for analysis, in those cases the mitotic counts being 11/11hpfs, 6/13hpfs, 6/20hpfs, 14/25hpfs, 28/26hpfs and 18/35hpfs. However, even accepting that maybe the mitotic index is not as high in ameloblastomas, we still have 2 cases of solid / multicystic ameloblastomas with mitotic indexes of 20 and 23 (those cases were resection specimens for ameloblastomas with CC component representing 50%, respectively 100% of the tumoral mass, allowing the count of mitoses in 50 hpfs with consecutive conversion of the result into mitotic index per 10 hpfs).

Our study reveals that mitotic index is not a reliable factor for differentiating between benign and malignant odontogenic tumors. Frank malignant tumors present strikingly numerous mitoses; otherwise, ordinary ameloblastomas may reveal an impressive number of mitoses, sometimes in such a proportion that the examiner could be induced to consider that certain tumor as being malignant. Unfortunately, in daily practice, the main problem is not represented by high mitotic index in ameloblastoma but by low mitotic index in CCOC. An odontogenic tumor with clear cells may present a reduced number of mitoses but the overall behavior (destruction of the cortical bone, invasion in the soft tissue or even metastases) may allow its classification as malignant. When dealing with an individual case, mitotic index is not a reliable parameter for differentiating benign ameloblastomas from odontogenic carcinomas since many ameloblastomas may have an increased number of mitoses while CCOC may show a not very prominent mitotic activity.

References:

1. Adamo AK, Boguslaw B, Coomaraswarmy MA, Simos C. (2002) Clear cell odontogenic carcinoma of the mandible: case report. *J Oral Maxillofac Surg.***60:121-126**

2. Anavi Y, Kaplan I, Citir M, Calderon S. (2003) Clear-cell variant of calcifying epithelial odontogenic tumor: clinical and radiographic characteristics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* **95:332-339.**

3. Ariyoshi Y, Shimahara M, Miyauchi M, Nikai H. (2002) Clear cell odontogenic carcinoma with ghost cells and inductive dentin formation - report of a case in the mandible. *J Oral Pathol Med.* **31:181-183.**

4. August M, Faquin W, Troulis M, Kaban L. (2003) Clear cell odontogenic carcinoma: evaluation of reported cases. *J Oral Maxillofac Surg.* **61:580-586.**

5. Barnes L, Eveson JW, Reichart P, Sidransky D. (ed) (2005) Pathology and Genetics of Tumours of Head and Neck Tumours, *WHO Press*

6. Brandwein M, Said-Al-Naief N, Gordon R, Urken M. (2002) Clear cell odontogenic carcinoma: report of a case and analysis of the literature. *Arch Otolaryngol Head Neck Surg.* **128:1089-1095.**

7. Braunshtein E, Vered M, Taicher S, Buchner A. (2003) Clear cell odontogenic carcinoma and clear cell ameloblastoma: a single clinicopathologic entity? A new case and comparative analysis of the literature. *J Oral Maxillofac Surg.* **61:1004-1010.**

8. Colmenero C, Patron M, Colmenero B. Odontogenic ghost cell tumours. (1990) The neoplastic form of calcifying odontogenic cyst. *J Craniomaxillofac Surg.* **18:215-218.**

9. Dahiya S, Kumar R, Sarkar C, Ralte M, Sharma MC. (2002) Clear cell odontogenic carcinoma: a diagnostic dilemma. *Pathol Oncol Res.* **8:283-285.**

10. de Villiers Slabbert H, Altini M. (1991) Peripheral

odontogenic fibroma: a clinicopathologic study. *Oral Surg Oral Med Oral Pathol.* **72:86-90.**

11. Ebert CS Jr, Dubin MG, Hart CF, Chalian AA, Shockley WW. (2005) Clear cell odontogenic carcinoma: a comprehensive analysis of treatment strategies. *Head Neck.* **27:536-542.**

12. Eversole LR. Malignant epithelial odontogenic tumors. *Semin Diagn Pathol.* **1999;16:317-24.**

13. Germanier Y, Bornstein MM, Stauffer E, Buser D. Calcifying epithelial odontogenic (pindborg) tumor of the mandible with clear cell component treated by conservative surgery: report of a case. *J Oral Maxillofac Surg.* **2005;63:1377-82.**

14. Hansen LS, Eversole LR, Green TL, Powell NB. Clear cell odontogenic tumor--a new histologic variant with aggressive potential. *Head Neck Surg.* **1985;8:115-23.**

15. Kramer IRH, Pindburg JJ, Shear M: *WHO Histological Typing of Odontogenic tumors (ed2).* **Berlin, Germany, Springer-Verlag, 1992**

16. Li TJ, Yu SF, Gao Y, Wang EB. Clear cell odontogenic carcinoma: a clinicopathologic and immunocytochemical study of 5 cases. *Arch Pathol Lab Med.* **2001;125:1566-71.**

17. Mesquita RA, Lotufo MA, Sugaya NN, De Araujo NS, De Araujo VC. Peripheral clear cell variant of calcifying epithelial odontogenic tumor: Report of a case and immunohistochemical investigation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* **2003;95:198-204.**

18. Muller H, Slootweg P. Clear cell differentiation in an ameloblastoma. *J Maxillofac Surg.* **1986;14:158-60.**

19. Ng KH, Siar CH. Clear cell change in a calcifying odontogenic cyst. *Oral Surg Oral Med Oral Pathol.* **1985;60:417-9.**

20. Ng KH, Siar CH. Peripheral ameloblastoma with clear cell differentiation. *Oral Surg Oral Med Oral Pathol.* **1990;70:210-3.**

21. Odukoya O, Arole O. Clear-cell ameloblastoma of the mandible (a case report). *Int J Oral Maxillofac Surg.* **1992;21:358-9.**

22. Orsini G, Favia G, Piattelli A. Peripheral clear cell calcifying epithelial odontogenic tumor. Report of a case. *J Periodontol.* **2000;71:1177-80.**

23. Philipsen HP, Reichart PA. Calcifying epithelial odontogenic tumour: biological profile based on 181 cases from the literature. *Oral Oncol.* **2000;36:17-26.**

24. Siar CH, NG KH. Peripheral odontogenic fibroma with clear cell odontogenic epithelium. *J N Z Soc Periodontol.* **1995;79:21-4.**

25. Siriwardena BS, Tilakaratne WM, Rajapaksha RM. Clear cell odontogenic carcinoma-a case report and review of literature. *Int J Oral Maxillofac Surg.* **2004;33:512-4.**

26. Slootweg PJ. Malignant odontogenic tumors: an overview. *Mund Kiefer Gesichtschir.* **2002;6:295-302.**

27. Waldron CA, Small IA, Silverman H. Clear cell ameloblastoma--an odontogenic carcinoma. *J Oral Maxillofac Surg.* **1985;43:707-17.**

28. Yamamoto H, Inui M, Mori A, Tagawa T. Clear cell odontogenic carcinoma: A case report and literature review of odontogenic tumors with clear cells. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* **1998;86:86-9.**

29. Yoon JH, Ahn SG, Kim SG, Kim J. Odontogenic ghost cell tumour with clear cell components: clear cell odontogenic ghost cell tumour? *J Oral Pathol Med.* **2004;33:376-9.**