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CASE REPORT

Calcifying Epithelial Odontogenic Tumour with Clear Langerhans Cells: A Novel Variant, Report of a Case and Review of the Literature

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Abstract Clear cell calcifying epithelial odontogenic tumour (CCEOT) is a rare variant of calcifying epithelial odontogenic tumor (CEOT). While it is not surprising to find clear cells in odontogenic lesions, the exact nature of the clear cells in CCEOT has not been elucidated. Herein, we report a case of peripheral CCEOT of anterior mandible in a 37 year old black female. Histologically, the tumour consisted of cords and small nests of clear cells surrounded by dense deposits of amyloid and basophilic calcifications. The cells possessed abundant clear cytoplasm and eccentrically located indented nuclei. Admixed with the clear cells were eosinophilic cuboidal to polyhedral cells. The clear cells were PAS negative and immunoreactive for S100 protein, CD1a and Langerin. The clear cells were negative for MNF-116, SMA, Desmin and CK-19. It is therefore recommended to recognize two variants of CCEOT, namely, CEOT with clear cell change and CEOT with clear Langerhans cells (LC). We further suggest that the contradictory term "*non-calcifying* variant of *calcifying* epithelial odontogenic tumour with LC" to be abandoned, as the current case clearly indicates that LC could be seen in CEOT irrespective of the presence or absence of calcifications.

Keywords CEOT · Langerhans cells · Immunohistochemistry · CD1a · Clear cells · S100 · Non calcifying CEOT · Langerin

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Introduction

The presence of LC in CEOT has traditionally been associated with paucity or absence of calcifications. This association which has been described in a few isolated case reports, has culminated in the adoption of the contradictory term "*non-calcifying* variant of *calcifying* epithelial odontogenic tumour with LC" [1–4]. LC rich non-calcifying CEOT has further been suggested to be a distinct entity common in the Asian population with a predilection for the anterior maxillary region [3].

While the majority of the case reports [3, 4], with the exception of one case [1], have focused on the presence of dendritic LC in CEOT, the exact nature of clear LC in CEOT has not been clarified. In this paper, we report a case of peripheral CEOT with clear LC in a 37 year old black female, showcase its unique clinical, histomorphological and immunohistological features and address the past scientific efforts in characterizing LC rich CEOT. In addition, the possible role of clear LC in this novel variant of CCEOT is discussed together with its differential diagnosis.

Case Report

A 37 year old black female presented at the oral and maxillofacial surgery unit, Livingstone hospital, Port Elizabeth, South Africa in February 2011 with a chief complaint of a painless slow growing soft tissue swelling of 6 months duration involving the anterior mandible. Her medical history was unremarkable. Intraoral examination revealed a firm fibrous nodule of the anterior edentulous lower alveolar ridge, 1×2 cm in size. A panoramic radiograph showed erosion of the underlying alveolar bone

by the soft tissue growth (Fig. 1). At close radiological examination small calcifications were clearly seen at the site of the lesion. The lesion was excised under local anaesthesia. The post-surgical period was uneventful.

Histological examination of the excised lesion disclosed a polyp surfaced by oral epithelium. The connective tissue core of the lesion contained cords and small nests of clear cells surrounded by dense deposits of a homogeneous eosinophilic material and basophilic calcifications (Fig. 2a). The individual tumour cells were round in appearance and possessed abundant clear cytoplasm and eccentrically located indented nuclei (Fig. 2b). Admixed with the clear cells were a second population of cuboidal to polyhedral cells with modest amounts of eosinophilic cytoplasm and large, round, centrally located hyperchromatic nuclei. Marked pleomorphism was not a feature. Mitoses, necrosis, lymphovascular and perineural invasion were not detectable. Focally, the tumour was continuous with the overlying surface epithelium (Fig. 2c). There were also cellular areas with small rounded cementum-like deposits (Fig. 2d).

The special stain, PAS, was negative for intracytoplasmic glycogen particles and mucin in clear cells. The homogeneous eosinophilic material stained orange-red with Congo-Red and produced an apple-green birefringence on polarized light microscopy (Fig. 3a). A positive control for amyloid was used.

The eosinophilic cells were diffusely immunoreactive for the pancytokeratin, MNF-116 (Fig. 3b) and CK19, while the clear cells were negative for these epithelial markers. The clear cells showed marked nuclear and cytoplasmic staining for S100 protein (Fig. 3c), intense membranous staining for CD1a (Fig. 3d) and strong granular cytoplasmic positivity for Langerin (Fig. 3e, f). Both cell types were negative for SMA and Desmin.

Unfortunately, the formalin-fixed tissue was unsuitable for ultrastructural studies. Formalin-fixed paraffin embedded tissue was analysed using Fluorescence in situ hybridization (FISH) and the EWSR1 (22q12) dual colour, break apart rearrangement probe (Vysis, Inc, Abbott Laboratories SA, Downers Grove, IL, USA). Normal fusion signal patterns were observed and no rearrangements of the EWSR1 gene were demonstrable in the sections examined. Based on the microscopic features, histochemical, immunohistochemical and molecular studies, the diagnosis of a CEOT with clear LC was established. Follow up at 6 and 18 months showed no evidence of recurrence.

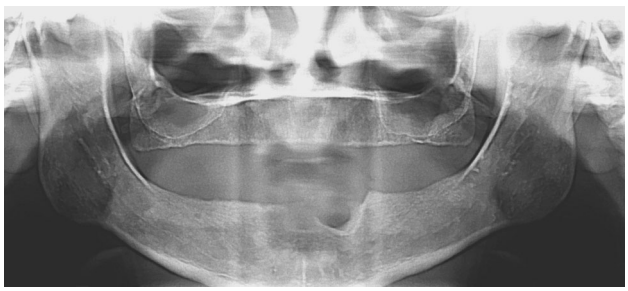

Fig. 1 A panormaic radiograph shows erosion of the anterior lower alveolar ridge by the soft tissue mass

Discussion

CEOT is a slowly growing, benign, but locally invasive epithelial odontogenic neoplasm, initially described by Pindborg in 1955 [5]. CEOT is predominantly an intraosseous (central) neoplasm but it also occurs as a rare less aggressive peripheral (extraosseous) tumour. It consists of sheets and islands of eosinophilic polyhedral epithelial cells associated with homogeneous pink amyloid deposits that have a tendency for calcification. A clear cell variant has been recognized, which was first reported by Abrams and Howell in 1967 [6]. CCEOT is a rare variant of CEOT with less than 30 cases reported in the literature [7–9]. While it is not surprising to find clear cells in odontogenic lesions, as many are speculated to originate from remnants of the dental lamina which exhibit a reasonable proportion of clear cells [10], the exact nature of the clear cells in CCEOT has not been elucidated.

In general, clear cells are seen in several different tumours and could result from fixation artifacts, intracytoplasmic accumulation of various substances such as glycogen, mucin, lipid and even scarcity of organelles. It appears that intracytoplasmic glycogen accumulation accounts for the clear nature of the cells observed in most cases of CCEOT. The majority of the clear cells in CCEOT are reported to be PAS positive, possess centrally located round hyperchromatic nuclei and have been shown to be immunoreactive for EMA and cytokeratins [7, 11, 12]. These findings indicate transformation of the typical polyhedral eosinophilic cells of CEOT to epithelial cells with optically clear cytoplasm through cytoplasmic accumulation and storage of glycogen particles.

In 1990, Asano et al. [1] described a variant of CEOT with LC. The case was reported to be unusual in that no calcifications were identifiable in the histological sections examined. Moreover, in addition to the polyhedral eosinophilic cells, a population of clear cells were observed. The clear cells were negative for glycogen particles when stained with PAS and were immunoreactive for S100 protein. Ultrastructurally they lacked desmosomes and tonofilaments and showed cytoplasmic Birbek granules. The clear cells were interpreted to be LC.

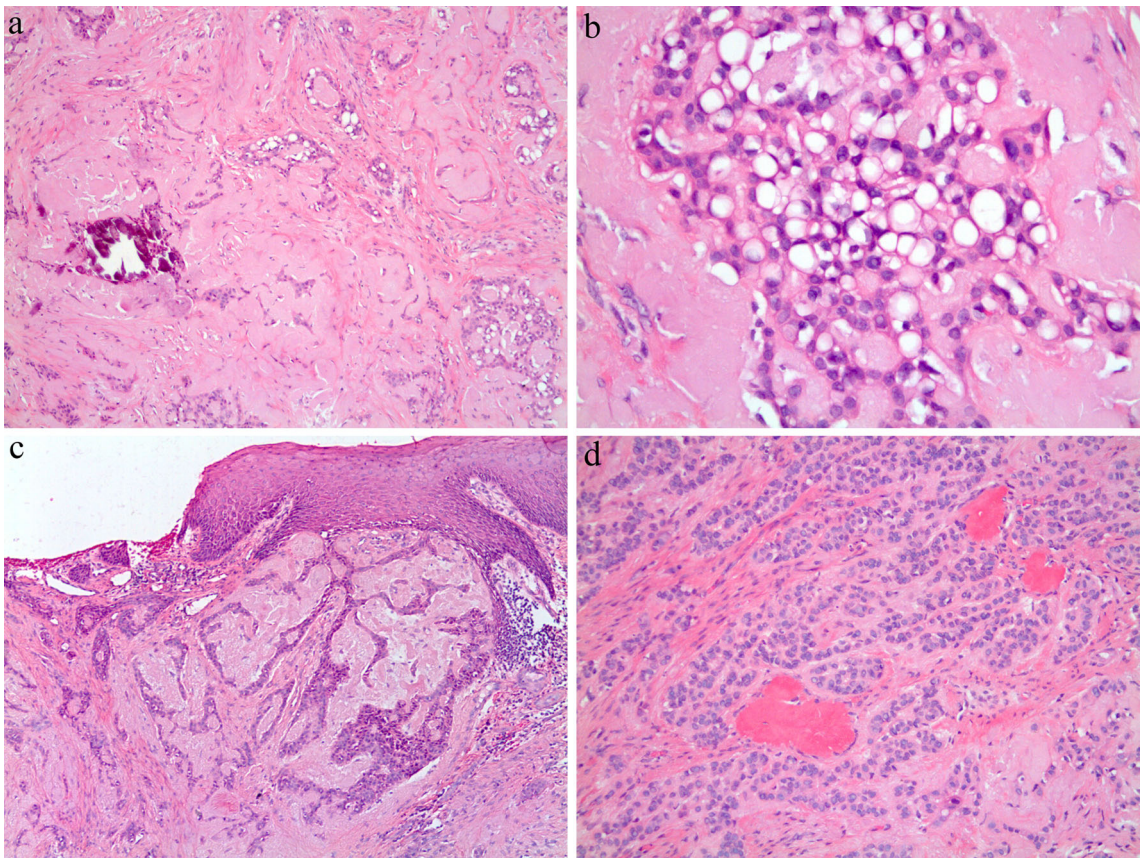

Fig. 2 Light microscopic features of CEOT with clear LC, **a** cords and small nests of clear cells surrounded by dense deposits of amyloid and basophilic calcifications, 100×; **b** the clear cells have eccentrically located indented nuclei, admixed with clear cells are cuboidal to polyhedral eosinophilic cells, 400×; **c** focally, the tumour blends with the overlying surface epithelium, 200×; **d** cellular area with rounded cementum-like deposits, 400×

The finding of PAS negative clear cells in the current case report with eccentrically located indented nuclei, which are immunoreactive for S100 protein, CD1a and the LC marker Langerin, confirms the dendritic nature of the clear cells initially described by Asano et al. [1]. However, in contrast to Asano's observation, calcifications were a frequent finding in the present case.

Langerhans cells are unique granule-containing dendritic cells (DC) that are found in squamous epithelium, the corium, lymph node and thymus. LC are able to internalize antigens and migrate through lymphatic channels into the regional lymph nodes, where they exhibit antigen-presenting and lymphocyte stimulating functions [13]. LC are the prime cells involved in initiating an immune response.

In early 1980s, LC were shown to express S100 antigen and subsequently S100-expressing dendritic cells were detected in several neoplasms [14, 15]. This gave rise to the widespread notion that LC can phagocytose and process tumour antigens and as such may play a crucial role in modulating antitumour immunity.

The assumption that LC may play an integral role in antitumour immunity prompted many scholars to investigate a possible relationship between the levels of tumor-infiltrating DC and the patient's prognosis. For example Ambe et al. [16] demonstrated that, increasing numbers of S100 + DC in specimens of patients with colorectal adenocarcinomas correlated with longer survival, most often in those with no metastases. Nomori et al. [17] found a direct relationship between the degree of density of DC and prognosis in biopsy specimens of nasopharyngeal carcinomas.

While it appears that an increasing number of LC in neoplastic tissues is associated with a more favourable outcome, alterations in shape, maturation and function of LC in the tumour microenvironment have recently been proposed and supported [18–20].

The clear cell appearance of the LC in the present case may reflect an effect of the tumour cells/microenvironment on LC morphology. Gatter et al. [21] in 1984, for the first time described many defective and deformed LC with rounded cell bodies and shortened or missing dendrites in basal cell carcinoma of the skin, an ingenious tumour mechanism for evading the host's immune response.

The biological significance of LC in neoplastic tissues for now remains a matter of conjecture and its thorough

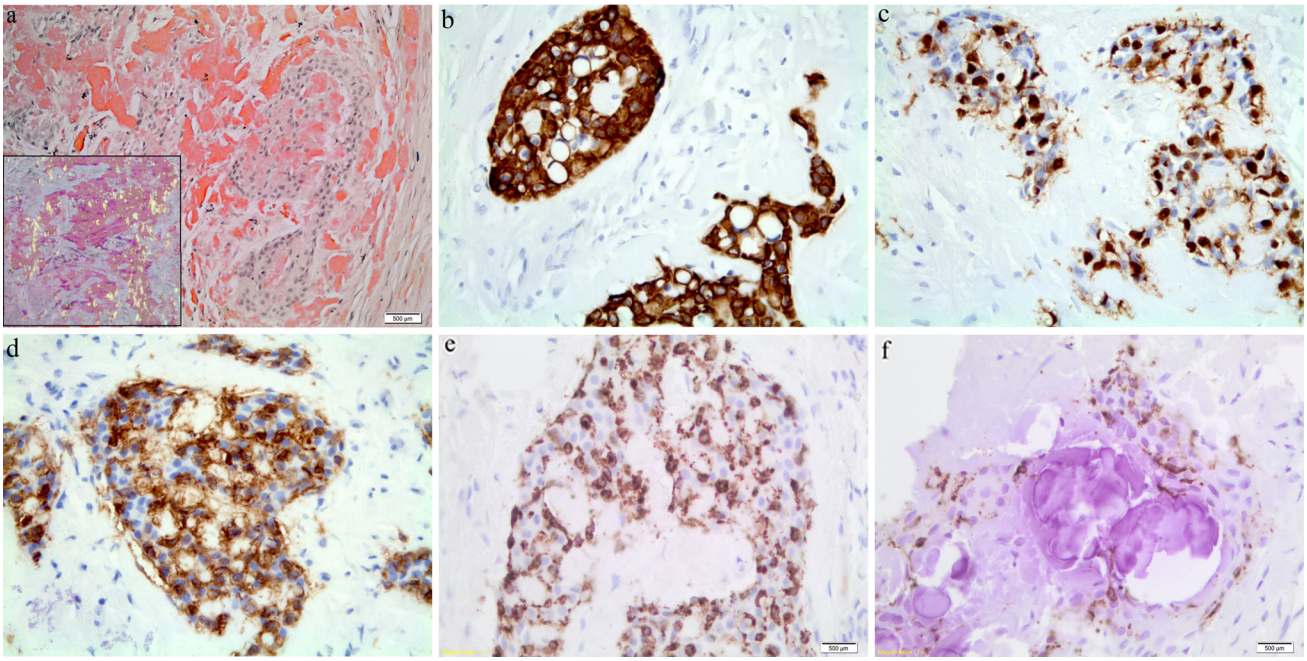

Fig. 3 Histochemical and immunohistochemical features, **a** the amyloid deposits show marked "congophilia" on Congo-Red staining, and on polarization exhibit an apple green birefringence (*inset lower left*), 200×; **b** clear cells are negative for MNF-116, 400×; **c** clear cells show strong nuclear and cytoplasmic staining for S100 protein, 400×; **d** clear cells are positive for CD1a, 400×; **e** clear cells show intense cytoplasmic positivity for the antibody Langerin, 400×; **f** foci of basophilic calcifications surrounded by Langerin positive cells, 400×

Table 1 Reported cases of CEOT with LC

Authors	Age & sex	Location	Clear cells	PAS	Calcification	Treatment	Follow up
Asano et al. [1]	44 F	Anterior Maxilla	Yes	Negative	No	Partial maxillectomy	No follow up
Takata et al. [2]	58 M	Anterior Maxilla	No	–	No	Enucleation	10 Years with no recurrence
Wang et al. [3]	38 M	Posterior Mandible	Few	Positive in most clear cells	No	Partial mandibulectomy	2.5 Years with no recurrence
Wang et al. [3]	39 F	Posterior Maxillary gingiva	Few	Positive in most clear cells	No	Excision	2 Years with no recurrence
Wang et al. [4]	52 F	Anterior Maxilla	No	–	No	Partial maxillectomy with (R) Supra-omohyoid neck dissection	No follow up

understanding requires a more mature perception of the tumour-host microenvironment in which the LC reside.

Following the first report of a non-calcifying CEOT with LC, an additional four cases have been reported in the literature, all of which predominantly lack clear LC [2–4] (Table 1). In 1993, Takata et al. [2] suggested a correlation between paucity/absence of calcifications in CEOT and the presence of LC. In 2005, Wang et al. [3] based on the observations of Asano and Takata et al. [1, 2], concluded that LC rich non-calcifying CEOT is a distinct entity common in the Asian population with a predilection for the anterior maxillary region.

We dispute the ideas put forward by these authors, since the present case clearly indicates that LC could be seen in CEOT irrespective of the presence or absence of calcifications. We further suggest that the contradictory term "*non-calcifying* variant of *calcifying* epithelial odontogenic tumour with LC" to be abandoned and discontinued. Moreover, it is recommended to further recognize two variants of CCEOT, namely, CEOT with clear cell change and CEOT with clear LC.

In general, the differential diagnosis of CEOT with clear LC includes odontogenic, salivary gland and metastatic tumours with a prominent clear cell component. The most difficult differential diagnosis is CCEOT versus clear cell odontogenic carcinoma (CCOC)/(hyalinizing) clear cell carcinoma (CCC). The distinction from CCOC/CCC is of paramount importance, since CCOCs and CCCs can be clinically aggressive with loco-regional recurrences and metastases to regional lymph nodes and distant sites [22, 23]. This is in contrast to CCEOTs which tend to have an excellent prognosis when adequately excised [7].

The presence of amyloid in CCEOT, may closely resemble the hyalinization seen in CCOC and CCC. In addition, CCEOTs may contain variable amounts of calcifications and amyloid, which may be scanty in some cases. In fact, in a fairly recent study which aimed to evaluate EWSR1 gene rearrangements in 12 CCOCs, two cases that were found to be negative for EWSR1 translocation were re-evaluated histologically and classified as CCEOTs [24].

The Congo-Red positive amyloid, even though scanty, aids in distinguishing CCEOT from CCOC and CCC. Moreover, a significant proportion of CCOCs and CCCs have been shown to harbor EWSR1 translocations [24], which may further help in discriminating CCEOT from CCOC and CCC. The expression of S100 protein, CD1a and Langerin immunomarkers is also supportive of the diagnosis of CCEOT with clear LC and electron microscopic evaluation of fresh tissue in gluteraldehyde for cytoplasmic Birbek granules may further strengthen and consolidate the diagnosis.

An ameloblastoma with clear cells will definitely and at least focally demonstrate peripheral tall columnar ameloblast-like cells with reverse nuclear polarization and central stellate-reticulum-like cells. The clear cell variant of mucoepidermoid carcinoma (MEC) may complicate the differential diagnosis, but MEC exhibits at least focally solid nests of tumour cells consisting of a mixture of epidermoid, mucous and intermediate cells. In addition, up to 70 % of cases of MEC demonstrate a specific chromosomal translocation t(11;19)(q21;p13), which creates the MECT1–MAML2 fusion transcript, which could be used to establish the diagnosis [25].

Acinic cell carcinomas may contain clear cells. CCEOT lacks the microcystic or follicular growth pattern of acinic cell carcinoma and its clear cells do not possess the characteristic cytoplasmic diastase-resistant PAS-positive zymogen secretory granules. Metastatic renal cell carcinoma should be excluded by careful clinical (e.g. ultrasound) and histological examination. In metastatic RCC, microscopic examination reveals smaller islands of clear cells and distinct capillary septa with the clear cells being positive for vimentin, CD10 and the marker RCC [23].

Hicks et al. [26]suggested that CEOTs with clear cell change may have a more aggressive behaviour. However, there are only a few reported cases of CCEOT with recurrence, and it appears that recurrent CEOTs with clear cell change result from inadequate surgical treatment (curettage and partial resection) [7]. We fully agree with Anavi et al. [8], in that Hick's notion of CCEOT's aggressive behaviour emanated from the earlier reports of clear cell odontogenic tumour [27], which is now recognized as a low grade malignancy and classified as CCOC.

Conclusion

In conclusion, CEOT with clear LC is a distinct entity and should be distinguished from CEOT with clear cell change. Given the similarities with other clear cell tumours of the head neck, the diagnosis of CEOT with clear LC may be challenging. The distinction from CCOC and CCC is of paramount importance, since CCOCs and CCCs can be clinically aggressive with loco-regional recurrences and metastases to regional lymph nodes and distant sites. The presence of Congo-Red positive amyloid deposits and immunoexpression of S100, CD1a and Langerin can reliably separate CEOT with clear LC from other clear cell lesions in most cases. We further suggest that the contradictory term "*non-calcifying* variant of *calcifying* epithelial odontogenic tumour with LC" to be abandoned, as the current case clearly indicates that LC could be seen in CEOT irrespective of the presence or absence of calcifications.

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Conflict of interest The authors declare that they have no conflict of interest.

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