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State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine, Ministry of Education, School and Hospital of Stomatology, Wuhan University, Wuhan, China

Mar. 25, 2019

Dear Editor:

Thank you for your kindly consideration on our manuscript. The content has been revised according to comments and requests. The changes were highlighted with yellow background in revised manuscript. Reviewer comments and corresponding reply are shown in the below:

Editor comments:

1. Please check the department of the authors.

Response: We have replaced the former one by the following one:

Ruo-Yi Wu[1,2], Zhe Shao[1,2], Tianfu Wu[1,2,*]

[1]The State key Laboratory Breeding Base of Basic Science of Stomatology (Hubei-MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University.

[2]Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, Wuhan University, Wuhan430079, People's Republic of China

2. Please provide the author contributions.

Response: The author contributions are:

Ruoyi Wu and Zhe Shao contributed equally to this work; Ruoyi Wu and Zhe Shao collected the information and reviewed the literature , Tianfu Wu and Ruoyi Wu wrote the paper.

3. Please provide and upload the approved grant application form(s).

Response: The application forms are uploaded.

4. Informed consent statement: Please add this statement.

Response: We add that" The patient involved has been informed before we submit this case report and signed the consent."

5. CARE Checklist (2016) statement: Please add this statement.

Response: The statement has been uploaded.

6. Please add a new abstract.

Response: New abstract has been added.

Abstract

BACKGROUND

Orthokeratinized odontogenic cyst (OOC) is a benign odontogenic cyst that is not believed to be cancerous by most surgeons. It is a variant of the common odontogenic keratocyst (OKC). This case report describes rare malignant transformation of OOC, with the aim of raising awareness of the malignant potential of OOC, and distinguishing it from OKC.

CASE SUMMARY

In August 2018, a 52-year-old man was referred to the Department of Oral Maxillofacial and Head–Neck Oncology of Wuhan University. The patient presented with severe pain in the left mandible for 2 mo, and had a 5-year history of osteomyelitis and mandibular cyst, with three recurrences. His latest diagnosis by pathological examination was OOC of the left mandible with mild-to-moderate local proliferation. However, this time, the cyst showed malignant potential by radiographic examination. We performed partial mandibulectomy and sent the lesion tissue for pathological examination. As expected the cyst deteriorated to moderately differentiated squamous cell carcinoma. During postoperative follow-up, the patient went for chemotherapy in September 2018 and successfully completed four cycles.

CONCLUSION

Surgeons should be more aware of OOC, which is usually benign but can become malignant.

7. Under the heading of Case Presentation, the following seven aspects must be presented in this order:

   1) Chief complaints;

   2) History of present illness;

   3) History of past illness;

   4) Personal and family history;

   5) Physical examination upon admission;

   6) Laboratory examinations—e.g., routine blood tests, routine urine tests and urinary sediment examination, routine fecal tests and occult blood test, blood biochemistry, immune indexes, and infection indexes;

   7) Imaging examinations—e.g., ultrasound, plain abdominal and pelvic CT scan, high-resolution chest CT scan, and head MRI. The patient case presentation should be descriptive, organized chronologically, accurate, salient, and presented in a narrative form.

Response: Case presentation has been revised as following:

CASE PRESENTATION

In August 2018, a 52-year-old man was referred to the Department of Oral Maxillofacial and Head–Neck Oncology at our hospital. The patient presented with severe pain in the left mandible for 2 mo. His left mandible gradually became swollen with severe pain. He denied a family history. Oral examination revealed a 6 cm × 3 cm firm and ill-defined mass on the left mandible angle and ramus. Other physical examination results were normal. Laboratory examinations such as routine blood tests, routine urine tests and urinary sediment examination, routine fecal tests, serum tumor marker measurement, and blood biochemistry revealed no obvious anomalies. There was a cystic lesion in cone beam computed tomography (CBCT); the left mandibular angle and ramus bone density decreased irregularly; and the lesion boundaries were unclear (Figures 1–4). Combined with history and CBCT images, we considered

that the OOC could be cancerous. Therefore, we performed partial mandibulectomy. The diseased tissue was obtained for pathological examination and showed pleomorphic nests of squamous cells infiltrating muscles and nerves. The results revealed moderately differentiated squamous cell carcinoma (SCC) (Figures 5 and 6). We recommended the patient for subsequent chemotherapy. Sometimes central type oral SCC can be mistaken for odontogenic SCC. However, we believe that the SCC came from OOC for the following reasons: (1) the SCC arose from the primary lesion site; (2) the central type oral SCC keratinized rarely; and (3) combined with clinical and radiographic features, this case conformed more to odontogenic SCC. Therefore, we were sure that the SCC arose from OOC. Besides, the patient had a 5-year history of osteomyelitis and mandibular cyst, with three recurrences.

On the first visit in January 2013, his left tempus and parotid region were painful and swollen for about 1 year, and pus was leaking out of the fistula. CBCT showed that tooth 37 was lost and the root zone of 37 and the left mandible ramus had a lower density, and the mesial tissue of the lesion area formed new bone (Figures 7–10). Abscess drainage and curettage of mandibular osteomyelitis were done, and we diagnosed by pathological examination osteomyelitis and mandibular cyst of the left mandible (Figure 11). After surgery, a fistula appeared in the left cheek with exudation of pale yellow pus, but the patient refused further treatment.

The second visit (first recurrence) was in January 2015, osteomyelitis of the left mandible and fistula of the parotideomasseterica region was diagnosed by clinical examination. Further treatment was recommended but the patient refused. After this recurrence, the disease deteriorated. In February 2017, the patient was admitted to our hospital again (second recurrence), but shortly afterwards, he was sent to Wuhan General Hospital of Guangzhou Military Region because of sudden precordial pain with pus leaking into the thorax. After the pain alleviated and other complications were cured completely, he

was admitted to our hospital in June 2017. CBCT showed that left mandibular angle and ramus density decreased and the cyst had clear boundaries (Figures 12–15). Cyst curettage was done and the pathological diagnosis was OOC of the left mandible. The cyst component had an orthokeratinized lining (Figure 16) and the cystic epithelium showed local mild-to-moderate proliferation (Figure 17).

8. final diagnosis 、treatment、outcome and follow-up：Please add or move those related information under the subtitles.

Response: These sections have been added:

FINAL DIAGNOSIS

The final diagnosis was moderately differentiated SCC.

TREATMENT

The patient was treated twice with curettage, partial mandibulectomy, and chemotherapy.

OUTCOME AND FOLLOW-UP

After surgery, the patient experienced pain in the surgical area. Therefore, he attended another hospital for chemotherapy in September 2018. Positron emission tomography–CT showed recurrence of the left mandibular SCC. The patient underwent four cycles of chemotherapy successfully.

9. Please add a conclusion. The Conclusion section must provide a brief conclusion with evidence-based recommendations. The key points for writing the Conclusion are as follows:
    - • Provide a justified conclusion.
    - • Provide evidence-based recommendations.
    - • Describe how the information learned from this case report will apply to one's own practice.

- • List opportunities for research.
- • Ensure that this section is brief and does not exceed one paragraph.

Response: The conclusion has been added:

CONCLUSION

This case report was about chronic progression of recurrent OOC into SCC. It revealed that OOC can also be cancerous. Surgeons could collect OOC cases for long-term follow-up and examination.

10. Please add PubMed citation numbers to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID.

Response: The PMID number and author list has been uploaded . A paper without PMID has been uploaded too.

Reviewer 1:

1. The language needs polishing to make it easier to read

Response: the manuscript has been polished by MedE Editing Service. The certification has been uploaded.

2. Title: Could be reworded to exclude abbreviations

Response: the title has been replaced by Chronic progression of recurrent orthokeratinized odontogenic cyst into squamous cell carcinoma: a case report and literature review

3. WHO Classification: OKC have been reinstated in the most current WHO classification, but there is debate about its "aggressiveness". There are studies describing PTCH1 gene mutation or inactivation that lead to this cystic lesion being neoplastic. These should be discussed in the manuscript. In addition, OKC can be solid, and a brief note on the term "keratocystic odontogenic tumor" for this variant should also be discussed.

Response: thank you for the advice, we have discussed it in discussion as following: The naming and classification of OKC and OOC have been controversial. In the 1950s, odontogenic cyst with keratin formation was designated as OKC for the first time. Afterwards, researchers found that many other cysts also form keratin; therefore, they were called keratocysts. OKC was used to describe a specific cyst in the 1992 classification[6]. In 2005, there was a controversy between cyst and neoplasm, which involved OKC. The WHO working group recommended that OKC should be replaced by keratocystic odontogenic tumor (KCOT) for the following reasons: (1) aggressive behavior; (2) occurrence of a solid variant; (3) possibility of recurrence; and (4) mutations of the *PTCH* gene[10]. However, some researchers debated whether gene mutations could be found in other non-neoplastic diseases such as fibrous dysplasia. In other words, the genetic variation that influences OKC can also influence other types of cysts that are not defined as neoplasms[10]. There is also controversy about aggressive behavior of OKC. Therefore, in 2017, the WHO working group renamed KCOT as OKC[6]. As for OOC, it was found and described as a type of OKC in 1981[11]. However, because of its different clinical and histological behavior, OOC was accepted as a separate entity in the 2017 classification.

4. Histology description: This could be enhanced, along with use of descriptive terms such as "onion skin-like" keratinization to help non-pathologists understand histologic features better.

Response: the histology description has been enhanced as following:
OOC has a thick “onion skin-like” uniform orthokeratinized squamous epithelial lining of 4–8 cell layers, while OKC has a thin parakeratotic epithelial lining. The basal cells of OOC lack polarity and nuclear hyperchromatism, while prominent palisaded and hyperchromatic basal cells with polarity are common in OKC. Besides, OKC often has a corrugated surface layer of parakeratin[6]. Light microscopy shows small daughter cysts

in OKC but not in OOC, which might be related to the high recurrence rate of OKC.

5. Immunohistochemistry: Images of Ki-67 and p63 stains of these two entities would enhance the value of this manuscript, as they are likely to be used in clinical practice.

Response: it is a pity that examination of known factors like expression of p63 and Ki67 has not be done. But in this case, the image examination and the clinical features are significant for final diagnosis.

6. Discussion: This section needs to be expanded.

Response: The discussion has been expanded as following.

DISCUSSION

This case showed low patient compliance, which might lead to missed diagnosis of cancerization. Multiple molecular factors and inflammation-cancer chain involved mechanism, and more unknown factors might play crucial roles in the malignant transformation. Examination of known factors like expression of p63 and Ki67, although not performed in this case, might provide meaningful indications for early intervention.

Surgeons should be alert to recurrent OOC, which might change with time. Furthermore, this patient was diagnosed by pathological examination in 2017 with OOC of the left mandible with mild-to-moderate local proliferation, which was a sign of risk. On the whole, we should be more vigilant about OOC, especially recurrent and proliferative OOC.

The naming and classification of OKC and OOC have been controversial. In the 1950s, odontogenic cyst with keratin formation was designated as OKC for the first time. Afterwards, researchers found that many other cysts also form keratin; therefore, they were called keratocysts. OKC was used to describe a specific cyst in the 1992 classification[6]. In 2005, there was a controversy between cyst and neoplasm, which involved OKC. The WHO working group recommended that OKC should be replaced by keratocystic odontogenic tumor (KCOT) for the following reasons: (1) aggressive behavior; (2) occurrence of a solid variant; (3) possibility of recurrence; and (4) mutations of

the PTCH gene[10]. However, some researchers debated whether gene mutations could be found in other non-neoplastic diseases such as fibrous dysplasia. In other words, the genetic variation that influences OKC can also influence other types of cysts that are not defined as neoplasms[10]. There is also controversy about aggressive behavior of OKC. Therefore, in 2017, the WHO working group renamed KCOT as OKC[6]. As for OOC, it was found and described as a type of OKC in 1981[11]. However, because of its different clinical and histological behavior, OOC was accepted as a separate entity in the 2017 classification.

OOC used to be considered as a variant of OKC and unlikely to recur, there are rare reports about its malignant transformation[12]. The systematic review by MacDonald-Jankowski in 2010 showed that only 4% of OCC recurred and the average age at first presentation was 35 years[13]. Although OOC was generally believed to have benign clinical behavior, we have found two case reports of malignant OOCs. The first one was a report of OOC with 8 months chronic inflammation, which turned into SCC[14] Kamarthi reported a case of OOC that turned into verrucous carcinoma after 1 year of painful swelling of the left maxillary alveolus[15]. Beyond that, OOC does not associated with NBCCS, and differs in many aspects from other odontogenic cysts, especially dentigerous cyst and OKC. Therefore, oral and maxillofacial surgeons should distinguish OOC from other types of odontogenic cysts and be more alert to the malignant potential of OCC with a history of recurrence and inflammation, and encourage patients to have frequent visits after treatment.

7. References: This currently includes many case reports and only a few review articles; I would suggest adding some more relevant references, including case series/review articles on the new WHO classification of head and neck tumors.

Response: Thank you for your advice. We read many articles about new WHO classification of head and neck tumors and choose the following as references.

6. M. Soluk-Tekkesin, J. M. Wright, The World Health Organization Classification of Odontogenic Lesions: A Summary of the Changes of the 2017

(4th) Edition. *Turkish Journal of Pathology* **34**, 1-18 (2018); published online EpubJan (10.5146/tjpath.2017.01410).

11. J. M. Wright, THE ODONTOGENIC KERATOCYST - ORTHOKERATINIZED VARIANT. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* **51**, 609-618 (1981); published online Epub1981 (10.1016/s0030-4220(81)80011-4).

Reviewer 2:

1. The provided case history needs to be rewritten more clearly. To my opinion, the time frame of the case would be as follow:

1) The first occurrence: in January 2013, diagnosed as osteomyelitis of the left mandible and mandibular cyst by pathological examination. Suggestion: It needs to describe the case history with radiography; and show the pathological pictures. It needs to explain why it is a case of osteomyelitis.

Response: the first occurrence has been rewrote, and the image figures have been added.

On the first visit in January 2013, his left tempus and parotid region were painful and swollen for about 1 year, and pus was leaking out of the fistula. CBCT showed that tooth 37 was lost and the root zone of 37 and the left mandible ramus had a lower density, and the mesial tissue of the lesion area formed new bone (Figures 7–10). Abscess drainage and curettage of mandibular osteomyelitis were done, and we diagnosed by pathological examination osteomyelitis and mandibular cyst of the left mandible (Figure 11). After surgery, a fistula appeared in the left cheek with exudation of pale yellow pus, but the patient refused further treatment.

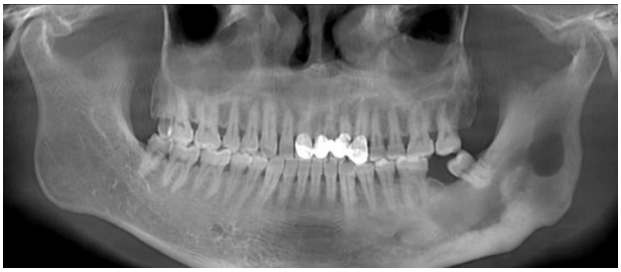

Figure 7 Orthopantomogram from CBCT of the left mandibular angle and ramus: the cyst boundary was clear and tooth 37 was lost (January 2013, first visit).

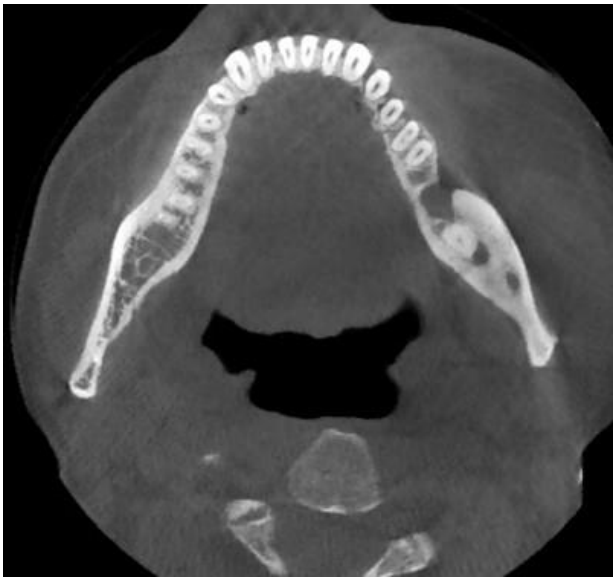

Figure 8 Axial plane of CBCT showing the cyst lesion limited in the region of lost tooth 37 with clear margin (January 2013, first visit).

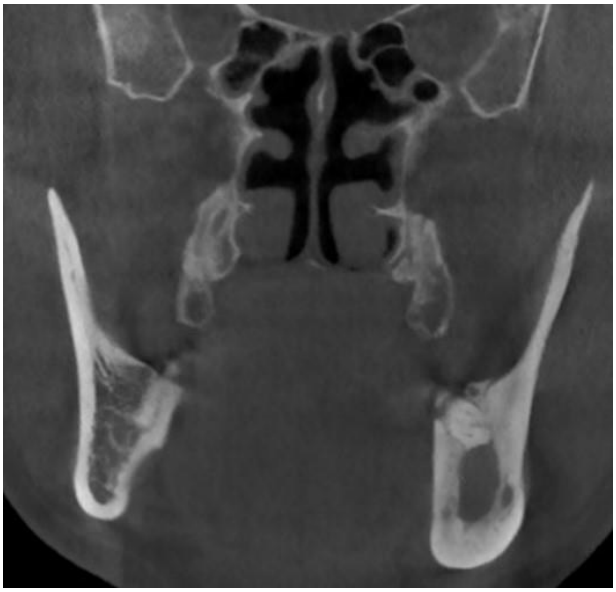

Figure 9 Coronal plane of CBCT showing the cortical bone around the cyst incrassates but not damaged (January 2013, first visit).

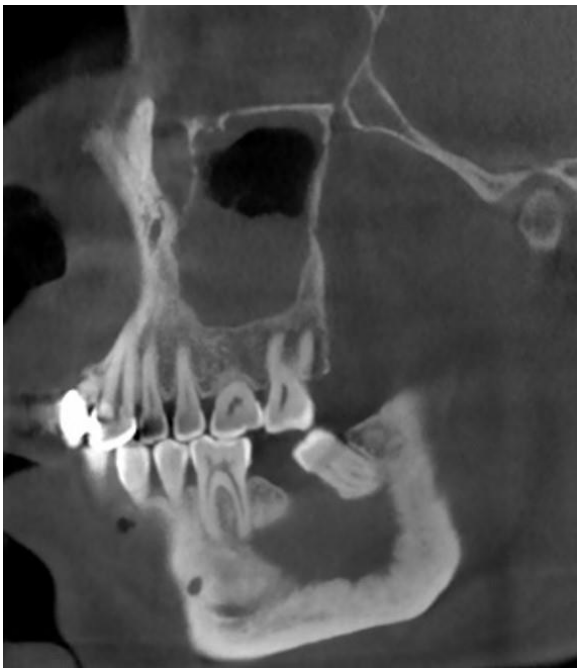

Figure 10 Sagittal plane of CBCT showing a cystic lesion with clear boundaries (January 2013, first visit).

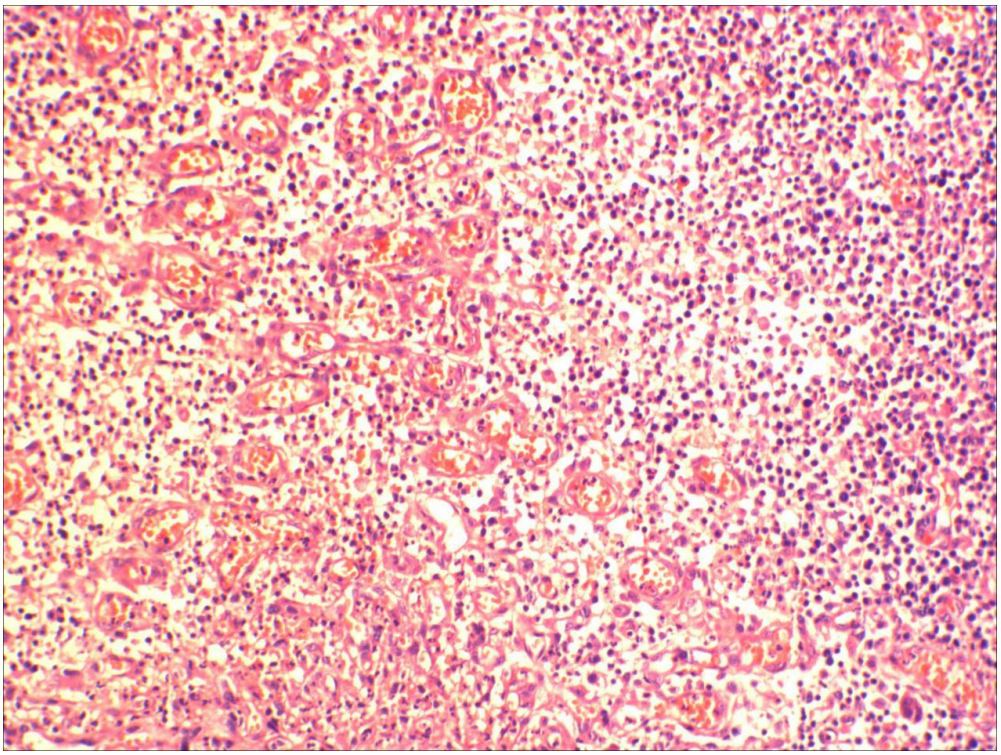

Figure 11 Pathological examination of the lesion tissue consisting of granulation and inflammatory cells (January 2013, first visit).

2) The second time (the first recurrence): in January 2015, diagnosed by pathological examination was still the same as before. Suggestion: It also needs to show the case history with radiography again and also the pathological pictures and needs to explain why it is recurred. It is important to aware that for a benign case, it is not easily to be recurred.

Response: we are sorry that some previous details are wrong, we replaced by a correct and detailed one:

The second visit (first recurrence) was in January 2015, osteomyelitis of the left mandible and fistula of the parotideomasseterica region was diagnosed by clinical examination. Further treatment was recommended but the patient refused.

3) The third time (the second recurrence): in June 2017, pathological diagnosis was the OOC of the left mandible (Figure 2). Suggestion: The case description is too brief. It needs to show the case history in more detail. For instance, what is the treatment modality, etc. Figure 1 is a panoramic view derived from CBCT. Other views such as axial, coronal, sagittal view need to be shown. No cystic stroma (capsule) is shown in figure 2, it would not be sure of cystic lining or detached dysplastic epithelium!?

Response: the case description has been expanded. Other views of CBCT has been added as following. The cystic stroma is clear in other views of CBCT images.

In February 2017, the patient was admitted to our hospital again (second recurrence), but shortly afterwards, he was sent to Wuhan General Hospital of Guangzhou Military Region because of sudden precordial pain with pus leaking into the thorax. After the pain alleviated and other complications were cured completely, he was admitted to our hospital in June 2017. CBCT showed that left mandibular angle and ramus density decreased and the cyst had clear boundaries (Figures 12–15). Cyst curettage was done and the pathological diagnosis was OOC of the left mandible. The cyst component had an orthokeratinized lining (Figure 16) and the cystic epithelium showed local mild-to-moderate proliferation (Figure 17).

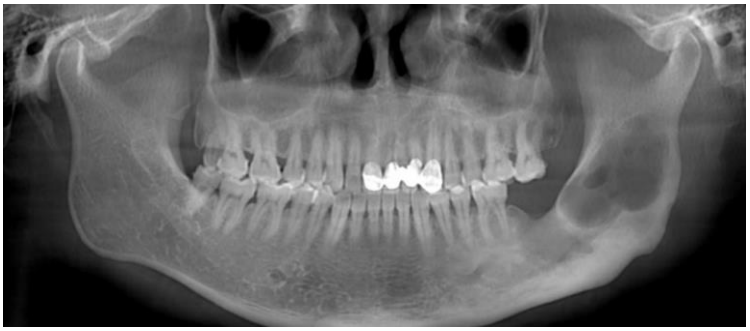

Figure 12 Orthopantomogram showing the cystic lesion in the left mandible angle and ramus, with area of decreased density measuring 15 mm × 25 mm × 41 mm (anterior/posterior × left/right × cranial/caudal). (February 2017, second recurrence).

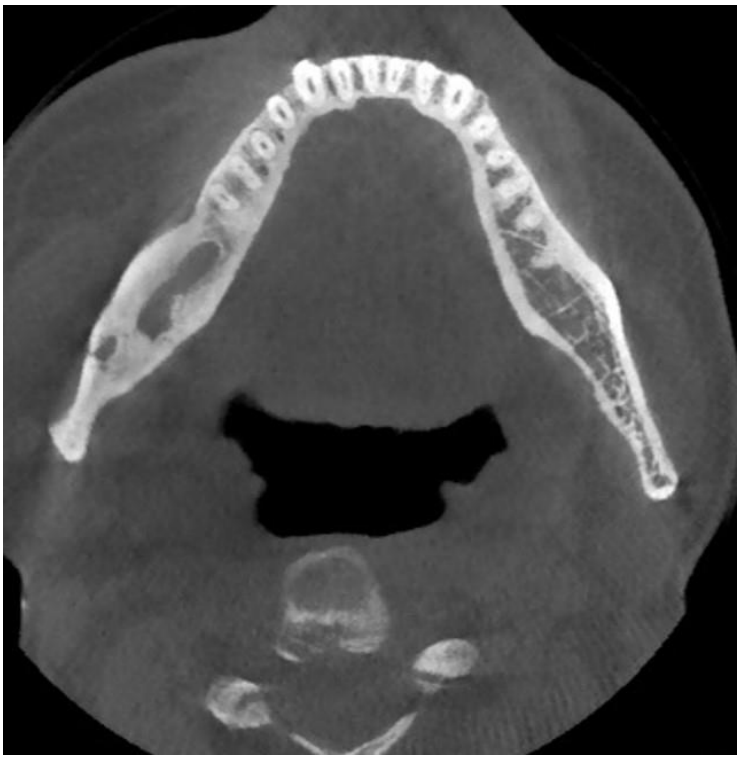

Figure 13 Axial plane view of CBCT showing that the cystic lesion was larger than in the previous image (February 2017, second recurrence).

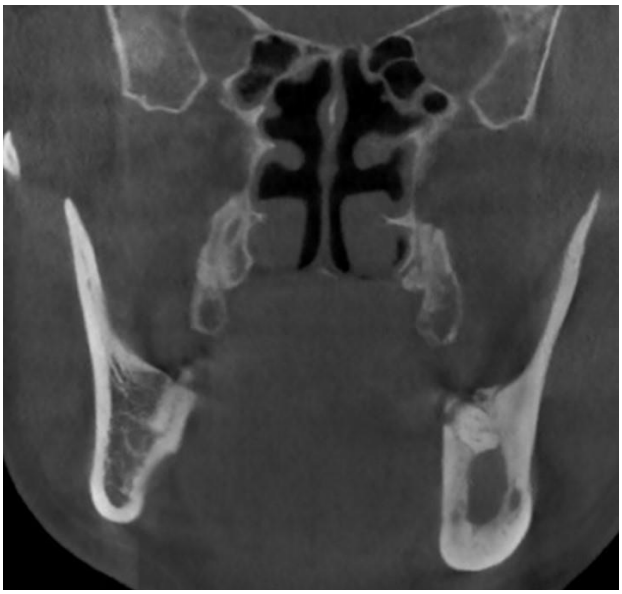

Figure 14 Coronal plane view of CBCT showing a regular-shaped cyst with clear boundaries (February 2017, second recurrence).

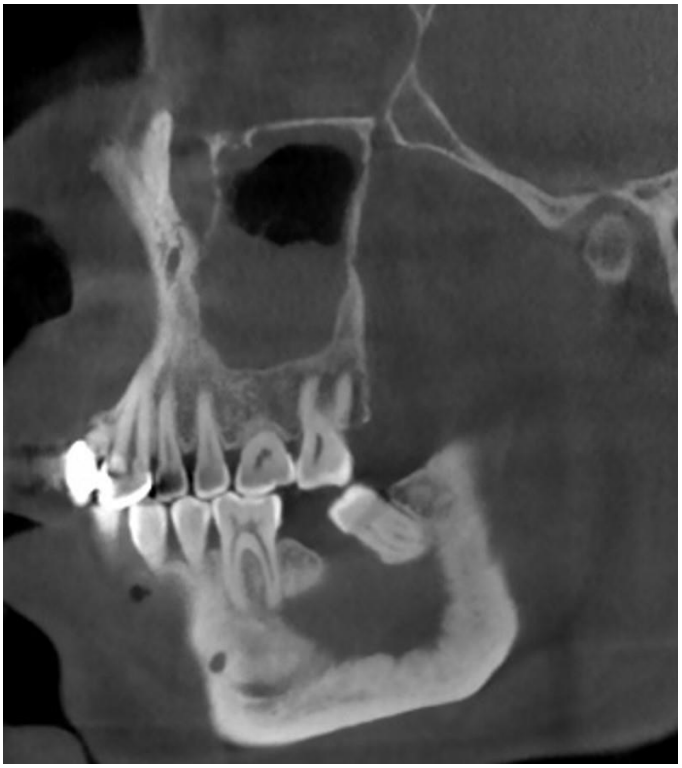

Figure 15 Sagittal plane of CBCT showing a cyst involving the previous positions of teeth 37 and 38 (February 2017, second recurrence).

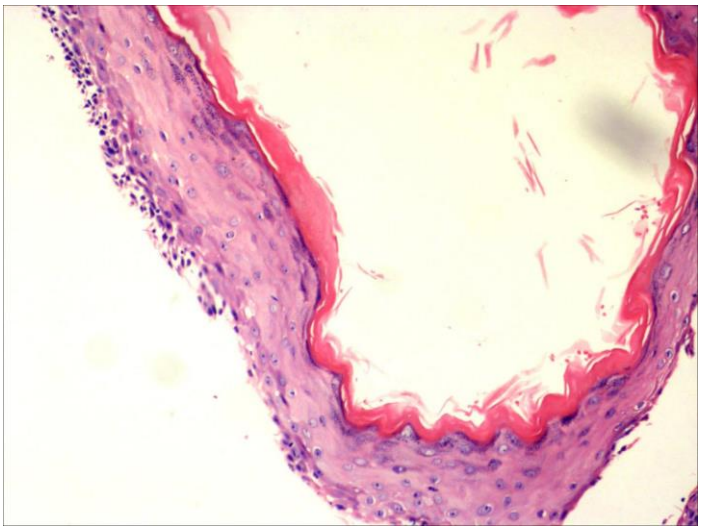

Figure 16 Pathological examination of the disease tissue showing a cyst with thick "onion skin-like" uniform orthokeratinized squamous epithelial lining (February 2017, second recurrence).

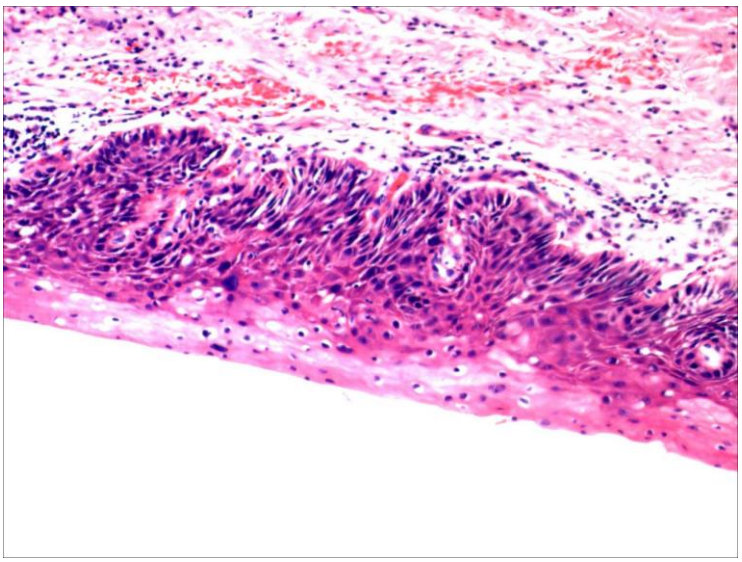

Figure 17 Pathological examination of the disease tissue showing mild-to-moderate epithelial dysplasia of the local lesion (February 2017, second recurrence).

4) The fourth time (the third recurrence): in August 2018, performed partial mandibulectomy, pathological examination revealed moderately differentiated squamous cell carcinoma (Figure 4). Suggestion: It needs to explain why partial mandibulectomy is performed. Up to now, it is diagnosed as OOC, which is a benign lesion! Figure 3 is a panoramic view derived from CBCT. Other views such as axial, coronal, sagittal view need

to be shown. Figure 4 does not have the cystic component; it is again hard to confirm to an original cystic lesion. In summary, the authors would first need to rewrite the case history in more detail and provide more accuate information so as make sure whether it is actually a case of central type oral squamous cell carcinoma!!

Response: other views of CBCT have been added, which show malignant potential. Combined with the latest Pathological examination- mild-to-moderate epithelial dysplasia of the local lesion, we deem the disease may be cancerization. Therefore, partial mandibulectomy is performed. Other views such as axial, coronal, sagittal view have been added.

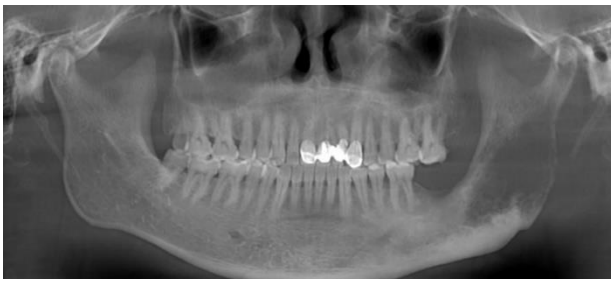

Figure 1 Orthopantomogram showed a lesion in the left mandibular gonial area and ramus with decreased density, that measured 14 mm × 32 mm × 60 mm (anterior/posterior × left/right × cranial/caudal) (August 2018, third recurrence).

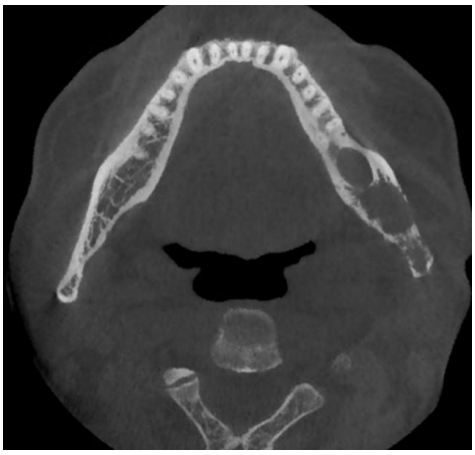

Figure 2 Axial plane view showing cystic lesion with irregular boundaries in the mandible (August 2018, third recurrence).

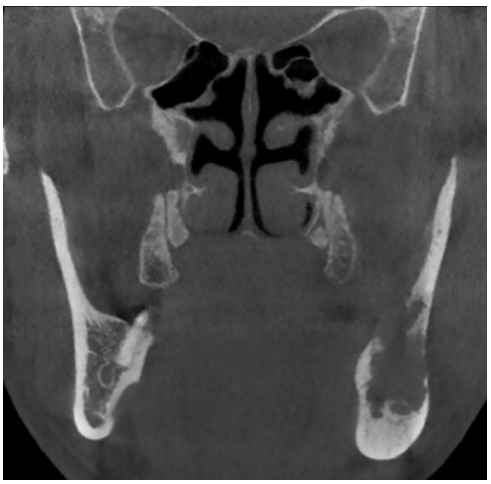

Figure 3 Coronal plane view showing the mandibular cortex was invaded by the cystic lesion (August 2018, third recurrence).

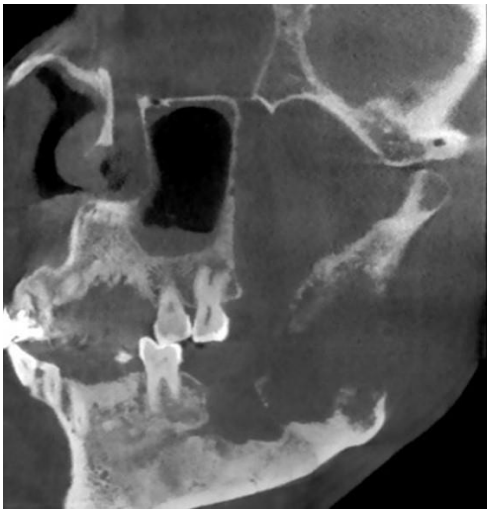

Figure 4 Sagittal plane view showing the left mandible was significantly damaged and teeth 37 and 38 were lost (August 2018, third recurrence).

Sometimes central type oral SCC can be mistaken for odontogenic SCC. However, we believe that the SCC came from OOC for the following reasons: (1) the SCC arose from the primary lesion site; (2) the central type oral SCC keratinized rarely; and (3) combined with clinical and radiographic features, this case conformed more to odontogenic SCC. Therefore, we were sure that the SCC arose from OOC.

2. Usually, no need to have citations in abstract section.

Response: citations in abstract section have been moved.

3. Please make sure whether the keyword “cancerate” is correct.

Response: the keyword “cancerate” has been replaced by “cancerization”.

4. Discussion is too simple. It needs to rewritten to have more information.

Response: Discussion has been expanded.

DISCUSSION

This case showed low patient compliance, which might lead to missed diagnosis of cancerization. Multiple molecular factors and inflammation-cancer chain involved mechanism, and more unknown factors might play crucial roles in the malignant transformation. Examination of known factors like expression of p63 and Ki67, although not performed in this case, might provide meaningful indications for early intervention.

Surgeons should be alert to recurrent OOC, which might change with time. Furthermore, this patient was diagnosed by pathological examination in 2017 with OOC of the left mandible with mild-to-moderate local proliferation, which was a sign of risk. On the whole, we should be more vigilant about OOC, especially recurrent and proliferative OOC.

The naming and classification of OKC and OOC have been controversial. In the 1950s, odontogenic cyst with keratin formation was designated as OKC for the first time. Afterwards, researchers found that many other cysts also form keratin; therefore, they were called keratocysts. OKC was used to describe a specific cyst in the 1992 classification[6]. In 2005, there was a controversy between cyst and neoplasm, which involved OKC. The WHO working group recommended that OKC should be replaced by keratocystic odontogenic tumor (KCOT) for the following reasons: (1) aggressive behavior; (2) occurrence of a solid variant; (3) possibility of recurrence; and (4) mutations of the *PTCH* gene[10]. However, some researchers debated whether gene mutations could be found in other non-neoplastic diseases such as fibrous dysplasia. In other words, the genetic variation that influences OKC can also

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OOC used to be considered as a variant of OKC and unlikely to recur, there are rare reports about its malignant transformation[12]. The systematic review by MacDonald-Jankowski in 2010 showed that only 4% of OCC recurred and the average age at first presentation was 35 years[13]. Although OOC was generally believed to have benign clinical behavior, we have found two case reports of malignant OOCs. The first one was a report of OOC with 8 months chronic inflammation, which turned into SCC[14] Kamarthi reported a case of OOC that turned into verrucous carcinoma after 1 year of painful swelling of the left maxillary alveolus[15]. Beyond that, OOC does not associated with NBCCS, and differs in many aspects from other odontogenic cysts, especially dentigerous cyst and OKC. Therefore, oral and maxillofacial surgeons should distinguish OOC from other types of odontogenic cysts and be more alert to the malignant potential of OCC with a history of recurrence and inflammation, and encourage patients to have frequent visits after treatment.

5. Please pay attention to the grammar and typo errors for the entire manuscript.

Response: the manuscript has been polished by MedE Editing Service. The certification has been uploaded.