Case Series

Proliferative potential of impacted tooth lesions using Ki67 labelling index-A clinicopathological insight

ABSTRACT

Tooth impaction is a frequent phenomenon, and the prevalence and distribution of this entity in different regions of the jaws may vary considerably. The third molars, maxillary canines, maxillary and mandibular premolars, and maxillary central incisors are the most commonly affected teeth. Impacted teeth in children and adolescents are rarely associated with pathological changes, but the prevalence of problems has been found to increase in later decades. Impacted teeth are commonly asymptomatic and not associated with any pathologic lesions for years. Proliferative potential of various odontogenic lesions were calculated using Ki-67 labeling index calculation, with the highest index of Unicystic Ameloblastoma followed by Adenomatoid odontogenic tumor, Unicystic Ameloblastoma, followed by the dental follicle.Ki-67 is a marker of cell proliferation, used as an important diagnostic marker in the pathologic differentiation of various lesions. It is always better to orthodontically treat or extract asymptomatic impacted teeth to avoid or to restrict the proliferative capacity of the dental follicle. Treatment decisions about the third molar have important clinical and cost implications.

Keywords: Impacted tooth lesions, Ki-67 proliferative potential, metaplasia, unerupted tooth

INTRODUCTION

An unerupted tooth is the one that fails to erupt in the oral cavity either due to a lack of eruptive forces or lack of resorption of bone present above it.[1] A tooth that is not erupted in the oral cavity can be either embedded or impacted. An impacted tooth is referred to as impacted if it is completely or partially unerupted many years after normal eruption time or if it is positioned against another tooth, bone, or soft tissue so that its further eruption is unlikely.[2] It is important for one to understand the difference between these two terminologies. An impacted tooth is present in the oral cavity due to two main causes: primary causes and secondary causes. Amongst the various causes of impaction, few of the common ones are retained primary teeth, lack of space and increased density of bone, presence of soft tissues or bony lesions, presence of supernumerary teeth, scar tissue, ectopic position of tooth bud, etc., during eruption, systemic factors-diseases such as neurosis, or any other infections, anemias, rickets, endocrine dysfunction or malnutrition can also trigger impaction.[1]

To understand the abnormality behind the tooth impaction, it should be well understood what happen in a normal tooth eruptive movement. Eruptive tooth movement is a mechanism of eruption that results in axial and occlusal movement of the tooth from its developmental position in the jaw to its final occlusal and functional position. It is comprised of two phases the intraosseous phase and extra-osseous phase. To move in the axial direction towards the occlusal

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plane the tooth pierces the connective tissue domain and active bone resorption occurs at the bony crypt end. Once the tooth erupts, i.e., extra osseous phase begins the muscle movement of the lips, cheeks accommodate the tooth to its final functional position with respect to occlusal plane.[3]

The tooth before erupting is enclosed in a follicle-like bag attached to its cemento-enamel junction. The molecular interplay plays a vital role in the resorption of bone for the eruption of the tooth, colony stimulating factor-1 (CSF-1) secreted from the dental follicle stimulates the monocytes, macrophages, and osteoclast to actively initiate the process of osteoclastogenesis, on the other hand, enamel organ secretes epidermal growth factor (EGF) to further secrete interleukin-1 alpha (IL-1α) to stimulate the follicular cells, these follicular cells further contribute to the resorbing front.[4] A dental follicle is an ectomesenchyme tissue that forms three supporting structures of tooth and signals the reduced enamel epithelium and alveolar bone to signal the process of eruption. The potential transformation of impacted teeth into cystic or neoplastic ones is related to the constituent structures of the follicle, namely: reduced enamel epithelium (REE) and cell rests of serres. The REE also actively secretes proteases which assist in the breakdown of connective tissue domain to produce a path of least resistance. When these remnants are stimulated it creates a proliferative potential leading to the formation of cysts and tumors.[5,6]

Various factors may act on dental follicle leading to their proliferation such as chronic inflammation, squamous metaplasia, blockade in signaling pathway, mucous prosoplasia, cell rests of serres, mallazes, and genetic and epigenetic factors. The chronic inflammation stimulates proliferation of oral epithelial cells. In several pathologic conditions, inflammation results in epithelial hyperplasia and metaplasia. Growth factors and cytokines released by the inflammatory infiltrates in various cysts might be responsible for the greater proliferative activity.[7] The cells of the reduced enamel epithelium vary from low columnar to cuboidal, often these cells differentiate into squamous in appearance which is essential for the process of cystification hence leading to squamous metaplasia.[8] Further stimulation to these squamous cells may lead to the formation of mucous cells, ciliated cells, and glandular type. A well-known example of this prosoplastic switch is mucous cell prosoplasia, often seen is origin of central Mucoepidermoid carcinoma from the neoplastic transformation of the cystic lining of pre-existing dentigerous cyst and tumor.[9]

DNA hydroxymethylation and methylation in dental follicle is a common example of genetic and epigenetic stimulus which changes during aging and is gene-specific hypermethylation it leads to silencing of several genes including those responsible for cell cycle control.[10] DL is programmed for apoptosis after tooth bud formation. There may be certain remnants of the DL present in the dental arch, called "cell rests of Serres." These remnants detach from the stalk-like extension provided for the tooth bud by replacing the tissue with the fibrous condensation known as the Gabernacular cord, which aids tooth eruption.[11]

Therefore, we presented a case series with histopathological co-relation and Ki-67 labeling index to observe the proliferative nature of the dental epithelium and also to observe the aid of histopathological analysis in determining the actual pathosis related to an otherwise asymptomatic impacted tooth.

CASE SERIES

Case 1

A female patient of age 14 years complained of pain in the upper right front tooth region since 1–2 years. On radio graphical analysis which included the intra oral periapical and cone beam computed tomography (CBCT), it was observed that there was a presence of an impacted canine in the right maxillary anterior region with a follicle attached to the tooth. There was also a retained deciduous tooth beneath the impacted canine. A provisional diagnosis of adenomatoid odontogenic tumor was considered. Under differential diagnosis, dentigerous cyst and dental follicle were considered [Figure 1].

The grossing image was not indicating or supporting the provisional diagnosis that is adenomatoid odontogenic tumor (AOT) as there was no characteristic feature of AOT which includes nodular proliferation within the tumor area. When histopathological analysis there were odontogenic rests and a hyperplastic epithelium having 2–3 layer thickness comprising flat cells resembling reduced enamel epithelium were observed. Based on the histopathological features, a final diagnosis of inflamed dental follicle was given.

To check the proliferative capability of dental epithelium, a Ki-67 labeling index was performed and it was observed that this case presented with a low proliferative index, i.e.,1.6, suggesting that the proliferative capability was low and hence the dental epithelium did not have the capability to proceed into a more aggravated lesion. If the tooth would not have been treated till then the proliferative capacity of the epithelium would have been increased and it would have progressed into a much more aggravated lesion.

The proliferative capability of dental epithelium has been mentioned by the results of Kucukkolbasi *et al*.[12] who found out

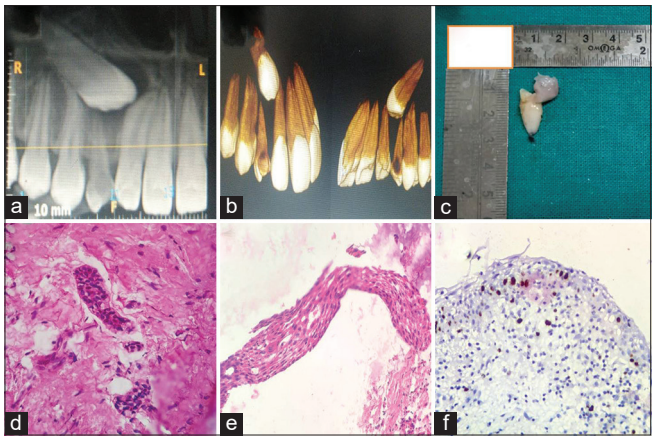

Figure 1: (a) Radiolucency around crown of permanent right maxillary impacted canine. (b) CBCT image of bilateral impacted canine. (c) Gross image of tooth specimen along with soft tissue attachment at CEJ. (d) H and E stained section at 40x showing cell rests of Serres. (e) Reduced Enamel Epithelium at 10×. (f) IHC showing focal positivity of Ki 67 in the basal and suprabasal layer (10×)

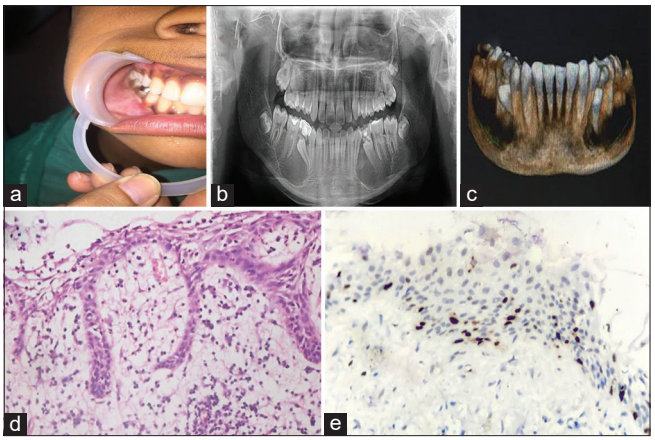

Figure 2: (a) Clinical image of a 7-year-old patient showing diffuse swelling from mesial aspect of right mandibular canine to second molar region. (b) Unilocular radiolucency observed in mandibular right and left region extending from distal aspect of mandibular canine region to second molar region. (c) CBCT image showing impacted bilateral mandibular second premolar. (d) H and E stained section of reduced enamel epithelium showing proliferation under the influence of dense chronic inflammation in the stroma (e) IHC stained section with Ki-67 in focal areas

a weak expression of p53 in dental follicles with reduced and stratified squamous epithelium, and Ki-67 positive cells in basal and suprabasal layers of the same epithelium hence commenting that dental follicles possess proliferative activity. According to Shear M. a weak expression of p53 in dental follicles with reduced and stratified squamous epithelium, and Ki-67 positive cells in basal and suprabasal layers of the same epithelium, dental follicles possess proliferative activity.[13]

Case 2

A 12-year-old male patient reported with the chief complaint of swelling in the lower right back tooth region since 3 weeks. On radiological analysis which included OPG and CBCT it was observed that there was the presence of multilocular lesion involving the molar area bilaterally in the mandible. Also bilaterally there was the presence of impacted premolars with a retrained deciduous tooth [Figure 2].

On the basis of the location, radiographic details and presence of impacted tooth, the provisional diagnosis given for this case was a dentigerous cyst. The differential diagnosis for this case was odontogenic keratocyst, radicular cyst, unicystic ameloblastoma, ameloblastic fibroma, and enlarged follicular space. Whenever multiple radiolucencies are observed in a suspected dentigerous cyst, it is very important to rule out the syndrome associated with multiple dentigerous cyst-Cleidocranial Dysplasia and Maroteaux-Lamy syndrome. The patient was checked for any representation clinically and no symptoms were found ruling out the possibility of association of any of the above-mentioned syndromes.

A very important study was conducted by Shear M *et al*.[13] where he observed in the age groups 10–19 maximum cases were seen to have a prevalence for premolar area for Dentigerous cyst when mandible was evaluated. Both the first and second case was seen to be in the same mentioned age group. When histological analysis was done, it was observed that there was presence of reduced enamel-like lining – 2–3 layers of flat cells with oval to round hyperchromatic nuclei with the presence of odontogenic rests. Hence, based on the histological feature a final diagnosis of dentigerous cyst was given.

As we know inflammation plays an important role in the pathogenesis of dentigerous cyst, it is important to evaluate mast cells in this lesion. With the help of toluidine blue staining the mast cells were detected which were present in both in granulated and degranulated form. It has been postulated that mast cells degranulation promotes collagenolytic activity and releases histamines and prostaglandins which lead to increased contraction of smooth muscles and also activates vasoactive amine chemo attractive gradient leading to subsequent enlargement as well as aggressiveness of the cystic entity.

The Ki-67 labeling index was calculated and it was observed that the value was approximately 3.2, which is higher in comparison to the previous case. It can be assumed that because in this case there was a high proliferative index in comparison to the previous case, there was an increase proliferative potential of the dental epithelium which lead to the development of cystic lesion.

Case 3

A female patient of age 24 years complained of pain in the

lower right back tooth region since 1 month. Radiologically when the analysis was done, it was observed that there was a unilocular radiolucency involving the third molar area with a follicle-like lining [Figure 3].

Based on above findings, a provisional diagnosis of dentigerous cyst and a differential diagnosis of inflamed dental follicle was given. Histologically, two areas were seen, there was a cystic lining having 2–3 layers of flat cells with oval to round hyperchromatic nuclei with the presence of odontogenic rests. The proliferation of the cystic lining and presence of Vickers's and Gorlin's criteria which included (Tall columnar cells, Palisading, Loss of basal polarity, Subnuclear vacuolisation, and Sub epithelial Hyalinisation) were observed. On the basis of the features present a final diagnosis of Dentigerous Cyst transforming into Unicystic ameloblastoma was given.

On evaluating it was seen that there was presence of:

- Odontogenic epithelium
- Metaplastic/hyperplastic changes in the epithelium
- Dental papilla-like tissue presence of chronic inflammatory infiltrate
- Nature of connective tissue stroma was loose fibrous followed by dense fibrous areas in ameloblastomatous areas.

The most notable finding was the presence of a highest proliferative index in this case in comparison to other cases. The proliferative index was seen to be 4.3 indicating that because of such proliferative index, the epithelium was capable of inducing such changes leading to the development of a cystic as well as ameloblastomatous changes leading to the development of such a hybrid lesion.

Case 4

A 25-year-old female patient complained of pain in left lower anterior tooth region since 2 months. On clinical examination, a bluish hue, solitary well-defined swelling was seen with respect to right mandibular vestibular region of approximately 2 × 1.3 cm in its greatest dimension, extending antero-posteriorly from midline to the mesial aspect of 44 and supero-inferiorly from approximately 0.5 cm below cervical margin of 31 41 42 83 44 involving attached gingiva, with obliteration of labial vestibule was observed. Radio graphically there was presence of an impacted canine in the mandibular anterior area. On grossing we found the presence of a nodular proliferation in the lumen, a lumen, and an impacted tooth [Figure 4].

Hence a provisional diagnosis of adenomatoid odontogenic tumor was given and based on the location and extent involved differential diagnosis of dentigerous cyst, odontogenic keratocyst, calcifying odontogenic cyst, and calcifying epithelial odontogenic tumor were considered.

On histopathological analysis, as suspected there was presence of pseudoductal areas, rosette formation, cribriform lace like areas, duct-like spaces lined by single row of cuboidal or low columnar epithelial cells, tumor droplets, ovoid nuclei polarized away from luminal surface were observed which are pathognomic for the diagnosis of Adenomatoid odontogenic tumor and hence a final diagnosis of AOT was given.

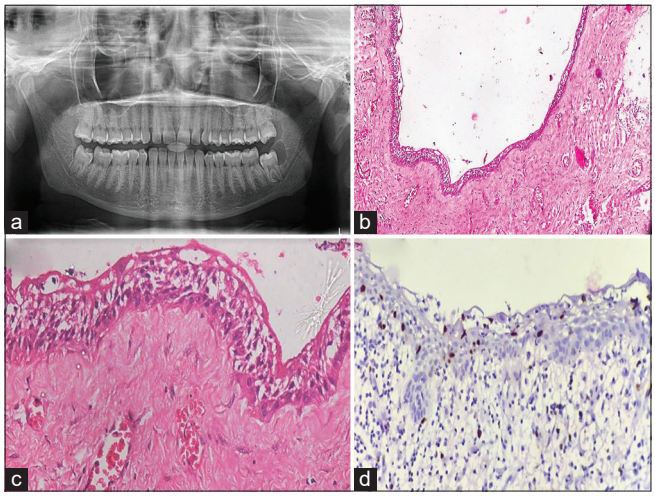

Figure 3: (a) Radiolucency observed distal to the crown of partially impacted mandibular left third molar. (b) H and E stained section at showing at 10× showing cystic lining with underlying capsular stroma. (c) Presence of tall columnar to cuboidal cells showing ameloblast-like cells with stellate reticulum-like cells. (d) Presence of focal positivity of IHC marker Ki-67 in supra-basal layer

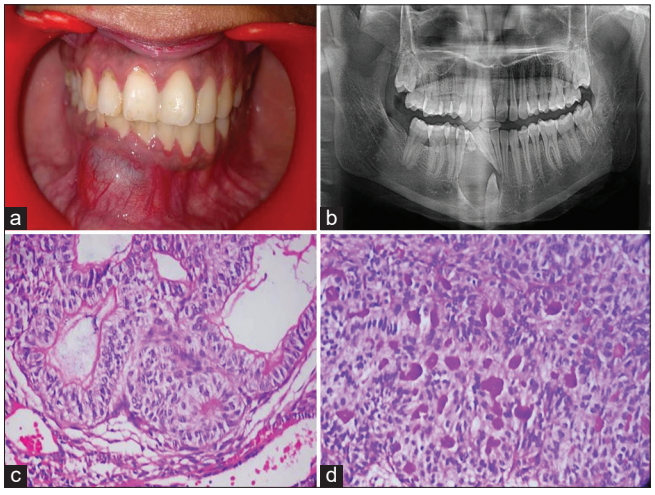

Figure 4: (a) Swelling extending from mandibular right central incisor to mesial aspect on mandibular right premolar. (b) Radiolucency observed extending from mesial aspect of mandibular right central incisor to mesial aspect on mandibular right premolar with impacted crown of mandibular canine. (c) H and E stained section showing rosettes and whorls with secretions showing basement like material. (d) Presence of eosinophilic droplets

Though clinically and radio logically the case was similar to the first case but when histologically analyzed both the cases represented two very different entities. Hence, a Histopathological analysis is very important in identifying the actual pathosis related to imapacted teeth and also it is very important to evaluate the proliferative index of such lesions with impacted tooth. The Ki-67 labeling index was calculated and it was observed that the value was approximately 1.9.

DISCUSSION

Impacted tooth is common in dental practice. Dentists usually remove them if they are associated with any pathologic sign or symptom. The challenge is whether to extract the asymptomatic tooth or not. The universal questions that arise are: Is it necessary to evaluate impacted teeth that are asymptomatic? and Is it necessary to extract impacted teeth for avoiding any presumed pathosis related to impacted teeth?

There have been many studies where comment has been made regarding whether to extract or evaluate asymptomatic third molar or not. In a study done by Urs AB *et al*. in 2010,[14] it was seen that cystic change was recorded when squamous metaplasia was noted in the follicle lining.

Many authors have suggested certain criteria in literature to detect early changes in the dental epithelium leading to the development of different pathosis depending on the proliferative nature of the dental epithelium.

The criteria that have been addressed are:
1. Presence of an odontogenic epithelium
2. Presence of hyperplastic/metaplastic epithelium
3. Changes in the dental papilla like tissue
4. Nature of the connective tissue stroma.

Hence, the absence of radiographic disease is not necessarily reflective of the absence of disease. Hence, it could be said that histological analysis is important to evaluate an otherwise asymptomatic radiographically normal impacted tooth to evaluate for any cystic changes or tumoural changes.

CONCLUSION

Ki-67 is a marker of cell proliferation, used as an important diagnostic marker in the pathologic differentiation of various lesions. It is always better to orthodontically treat or extract asymptomatic impacted teeth to avoid or to restrict the proliferative capacity of the dental follicle. Treatment decisions about the third molar have important clinical and cost implications. No consensus criteria are available with regard to treatment decisions of asymptomatic impacted third molars. Our study suggests that regular radiographic follow-up is necessary so as to be able to surgically intervene when pathology arises. The incisional biopsy conducted along with molecular analysis of Ki-67 can guide us in deciding the further treatment modality.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient has given her consent for her images and other clinical information to be reported in the journal. The patient understands that her name and initials will not be published and due efforts will be made to conceal identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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