

RESEARCH ARTICLE

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## Amelogenin is a Potential Biomarker for the Aggressiveness in Odontogenic Tumors

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### Abstract

Amelogenin (AMEL), the major structural protein of the enamel organic matrix, constitutes more than 90% of the enamel's protein content, Aberrations of amelogenin are thought to be involved in the oncogenesis of odontogenic epithelium. The expression of amelogenin is possibly an indicator of differentiation of epithelial cells in the odontogenic tumors. **Aim of the study:** Investigating the expression of amelogenin in some odontogenic tumors, using an anti-amelogenin polyclonal antibody, and then compare it with AMEL expression in tooth buds as control. **Materials and Methods:** study sample consisted of 10 formalin-fixed, paraffin- embedded specimens of ameloblastoma, 10 Keratocystic odontogenic tumors, and 10 tooth buds were conventionally stained with hematoxylin-eosin and immunohistochemically with AMEL polyclonal antibody. **Results:** All of the odontogenic tumors expressed AMEL in the epithelial component, Intensity of expression in ameloblastoma and Keratocystic odontogenic tumor was lower, compared with tooth buds, Statistical analysis indicated a significant differences between the tumors and tooth buds. **Conclusion:** Amelogenin can be used as a marker for odontogenic epithelium, and the expression of amelogenin is possibly an indicator of epithelial cells differentiation in the odontogenic tumors, and therefore in prediction of the histological behavior of odontogenic tumors.

**Keywords:** Ameloblastoma- Keratocystic odontogenic tumor- Amelogenin- Oncogenesis

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### Introduction

Odontogenic tumors are a group of lesions that arise from the tissues derived from the tooth-forming apparatus. They are thus exclusive to the jaws and represent the only situation in pathology where a primary epithelial tumor may be found within bone. Odontogenic tumors are rare and lack of familiarity with these lesions and their variable appearance may lead to difficulties in diagnosis with occasional serious confusion with more sinister lesions. (Jordan and Speight, 2009).

Tumors arising from the epithelium of the odontogenic apparatus or from its derivatives or remnants exhibit considerable histological variation and are classified into several benign and malignant entities. (Kramer et al., 1992).

Ameloblastoma is a benign odontogenic tumor which possess an aggressive behavior as evidenced by its rapid growth and significance recurrence rates following initial surgical resection (Jhamb and Kramer, 2014).

The clinical presentation of the ameloblastomas is variable, but they are commonly associated with non-painful mandibular displacement due to their slow

growth. Pain and paresthesia are rare symptoms and in general the dental elements associated with these tumors can be impacted or displaced (do Canto et al., 2016).

According to the clinical and radiographic aspects, these tumors can be classified into three main types: solid/multicystic, unicystic and peripheral. Usually, the solid/multicystic type is characterized by a locally aggressive behavior with a high risk of recurrence if not removed adequately This variant can be subdivided histologically as follicular, plexiform, acanthomatous, desmoplastic, granular cell, and basal cell subtypes, with the first two being the most common (Zhong et al., 2011).

Keratocystic Odontogenic Tumor (KCOT) is a locally aggressive developmental cystic neoplasm thought to arise from the odontogenic epithelium. A high recurrence rate of up to 30% has been found following conservative treatment (Hu et al., 2016).

Amelogenin, isolated by Termine et al., (1980), is a representative enamel matrix protein produced by secretory ameloblasts, and plays a major role in organization and mineralization of developing enamel.

Amelogenin proteins are critical to the formation of enamel in teeth and may have roles in controlling growth

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and regulating microstructures of the intricately woven hydroxyapatite (HAP) (Tarasevich et al., 2015).

In humans, the amelogenin proteins are primarily encoded by the AMELX gene on the X chromosome. The AMELY gene on the Y chromosome in males is estimated to be only about 10 as active as AMELX in producing amelogenin proteins. (Salido et al., 1992).

Gene Mutations of amelogenin Lead to Amelogenesis Imperfecta AI in Humans, The basic categories of AI have been divided into hypoplasia enamel that is too thin because of a defect in secretion, hypocalcification a defect in the mineral crystals or hypomaturation protein processing defect with reduced removal of the organic material with autosomal or X linked inheritance patterns (Gibson, 2011).

Immunohistochemically, this protein has been examined in several odontogenic tumors (Mori et al., 1991; Saku et al., 1992; Abiko et al., 2001; Kumamoto et al., 2001; Papagerakis et al., 1999; Takata et al., 2000).

The expression of amelogenin is possibly an indicator of di-erentiation of epithelial cells in the odontogenic lesions. The detection of amelogenin expression may thus help in the understanding of not only the pathogenesis of the lesions, but also play a part in the prediction of the histological behavior and by extension the clinical nature of the lesion (Anigol et al., 2014)

The current study aimed to assess the positive intensity of AMEL expression in some odontogenic epithelium tumors.

## Materials and Methods

The sample consisted of 10 formalin-fixed, paraffin- embedded blocks of ameloblastoma, 10 other of Keratocystic odontogenic tumor, and 10 tooth buds were collected from archival files of The Damascus University.

Paraffin sections of formalin fixed tissues were used for both histological and immunohistochemical evaluation. Hematoxylin and eosin stained sections were used for routine histological examination. For immunohistochemical examination, 4 μ sections were made and loaded on slides. Sections were deparaffinized, washed in deionized water and subjected to antigen retrieval. Nearly 3% hydrogen peroxide was used to block endogenous peroxidase.

After pre-treatment, sections were incubated with

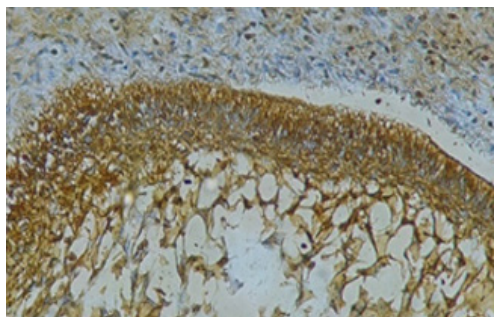


Figure 1. Intense Positivity of Amelogenin in the Inner Enamel Epithelium Layer, Stratum Intermedium and Stellate Reticulum.

primary antibody rabbit polyclonal to AMELX antibody (ab129418) (Abcam , US) , in a humid chamber at 4°C overnight with a dilution of 1/10 - 1/50 , Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. The primary antibody was diluted in antibody diluent with background reducing components (S3022, Dako, Denmark).

The standard streptavidin-biotin-peroxidase complex method was performed to bind the primary antibodies (BioGenex Life Sciences Ltd., CA, USA). The reaction products were visualized by treating with diaminobenzidine solution diluted according to the manufacturer’s instructions. For control studies of the antibodies, the serial sections were treated with all the mentioned reagents but omitting the primary antibody and were confirmed to be unstained.

## Results

### Human tooth buds (n = 10)

The inner enamel epithelium, stratum intermedium and stellate reticulum was intensely positive in early bell stage.

### Follicular Ameloblastoma (n=6)

Four cases showed moderately positive in ameloblast like cells and stellate reticulum like cells, two cases expressed mild positive in ameloblast like cells and stellate reticulum like cells.

### Plexiform Ameloblastoma (n=4)

Three cases showed moderately positive in ameloblast like cells and stellate reticulum like cells, other one mild positive in ameloblast like cells and stellate reticulum like cells

### Keratocystic odontogenic tumor (n=10)

Eight cases moderately intense positive of amelogenin was seen throughout the epithelium and two cases showed mild staining.

### Statistical analysis

The results were computed and subjected to statistical analysis using Kruskal-Wallis test and Mann-Whitney test.

Intensity of expression AMEL in ameloblastoma and keratocystic odontogenic tumour was lower compared with AMEL expression in tooth buds as control, Statistical

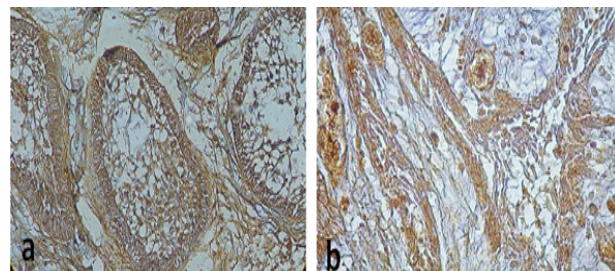


Figure 2. a, (Follicular Type) moderately intense positivity of amelogenin of ameloblast - like cells and stellate reticulum - like cells; b, (Plexiform Type) moderately intense positivity of amelogenin of ameloblast - like cells

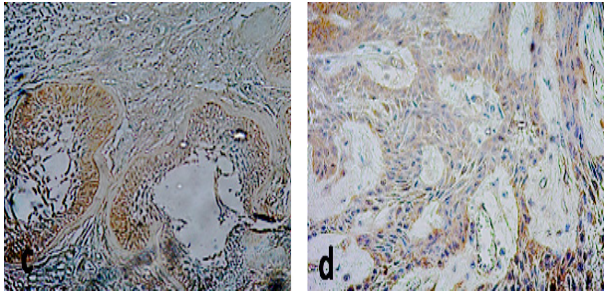


Figure 3. c: (Follicular Type) mild intense positivity of amelogenin of ameloblast-like cells and stellate reticulum - like cells; d, (Plexiform Type) mild intense positivity of amelogenin of ameloblast - like cells

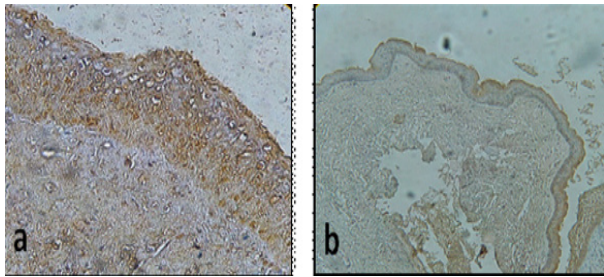


Figure 4. (a) moderately intense positivity of amelogenin of epithelium  $\times 400$  (b) Mild intense positivity of amelogenin of epithelium  $\times 100$

analysis indicate significant differences between the expression in tumors and tooth buds and the expression was found to be statistically not significant between the groups of Ameloblastoma and Keratocystic odontogenic tumour as both groups expressed enough positivity between the samples to the antibody.

## Discussion

Accurate diagnosis of pathological lesions is the ultimate goal of every pathologist and tumor markers

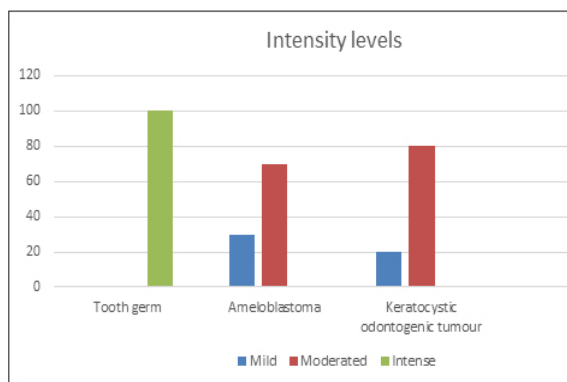


Figure 5. Intensity Levels of the Marke

Table 2. A Binary Comparison of Odontogenic Tumors with Tooth Buds about to Amelogenin Intensity

Sample 1	Sample 2	P value	U value	Differences
Ameloblastoma	Keratocystic odontogenic tumour	0.615	45	No Significant differences
Ameloblastoma	tooth buds (as control)	0	0	Significant differences
Keratocystic odontogenic tumour	tooth buds (as control)	0	0	Significant differences

Table 1. Statistical Comparison of Amelogenin Intensity in Odontogenic Tumors and Tooth Buds Using Kruskal-Wallis Test

Chi-square	Intensity levels	P-value	Differentiation
29,025	3	0	Significant differences

are useful tools for this purpose. There are only a handful of tumor markers that can be used by pathologists for diagnosis of odontogenic tumors. Many other potential markers are constantly under development. Even though histopathology continues to be staple in the diagnosis of odontogenic tumors, tumor markers will play an increasingly important role as adjuvant tools (Premalatha et al., 2013).

The development and progression of odontogenic tumors are affected by alterations of many kinds of genes and molecules. In particular, the characteristics of odontogenic tumors appear to depend on the molecular mechanisms associated with (i) tooth development, (ii) bone metabolism, and (iii) the malignant potential of tumors. Further molecular studies, including genomic and proteomic-based profiling, are required to clarify the etiology and pathogenesis of odontogenic tumors. A better understanding of underlying molecular mechanisms will help to predict the course of odontogenic tumors and lead to the development of new therapeutic applications, such as molecular-targeted treatment and patient-tailored therapy, for odontogenic tumors (Kumamoto, 2006).

The odontogenic epithelium is responsible for tooth development under physiologic conditions but may give rise to tumors in the jaws (Eversole, 1999).

Odontogenic epithelial tumors represent a group of lesions ranging from malignant and benign neoplasms, all arising from the odontogenic epithelium. As tumor markers have become an integral part of modern pathology, this article reviews significance of amelogenin as markers in diagnosis and prognostic assessment of odontogenic tumors.

Ameloblastoma is the second most common odontogenic tumor which is benign and locally aggressive with devastating morbidity if left untreated, due to its unlimited growth potential. Since the treatment is radical surgical intervention and long term follow up, diagnosis and assessment of prognosis is very important.

KCOT was previously grouped under odontogenic cystic lesions with two histological

variants: parakeratinized and orthokeratinized. Considering the biological behavior and genetic abnormalities, WHO working group 2005 grouped parakeratinized OKC as a benign neoplasm and orthokeratinised variant as a separate entity- orthokeratinised odontogenic cyst (OOC).

KCOT is an important neoplasm because of its high recurrence rate and aggressive behaviour (Barnes, 2005).

The AMEL protein was used as marker of odontogenic lesions to segregate other types of epithelial lesions that may develop within the oral and maxillofacial regions (Kumamoto et al., 2001; Taylor, 2008; Mori et al., 1991; Abiko et al., 2001).

The pathogenetic mechanism of odontogenic tumors is closely related to the developmental processes of tooth bud. As a result, the molecular signaling mechanisms for normal enamel organs and odontogenic tumors have been closely compared (Lee and Kim, 2013).

Amelogenin is a low-molecular-weight enamel matrix protein. It has been consistently demonstrated in inner enamel epithelium, stratum intermedium and stellate reticulum of enamel organ and with intense positivity this was in accordance with previous studies (Deutsch et al., 2006).

In a study by Anigol et al, amelogenin expression was positive in ameloblastoma and Keratocystic odontogenic tumour with variable intensity (Anigol et al., 2014).

The present study demonstrated that all the ameloblastomas reacted positively to amelogenin in the peripheral ameloblast-like cells and stellate reticulum like cells but intensity variable, this was in accordance with previous studies (Kumamoto et al., 2001).

The expression of AMEL in Follicular Ameloblastoma was moderate of four cases and mild of two cases, in Plexiform Ameloblastoma showed moderate expression of three cases and one was positive with mild intensity.

This was in contrast with a previous study (Saku et al., 1992) amelogenin was only demonstrated in such lesions with mineralization as adenomatoid odontogenic tumor (AOT), and calcifying epithelial odontogenic tumor (CEOT), positive stainings for the enamel proteins were limited to mineralized foci, hyaline material and the epithelial cells surrounding them, and no positive for enamel proteins in ameloblastoma and explanation is that ameloblastoma cells are too immature to express a detectable amount of enamel proteins.

Also, this was in contrast with Crivelini et al study, ameloblastoma had negative results for antibody anti (amelogenesis-related proteins) due to their undifferentiated nature (Crivelini et al., 2012).

It has been reported that Ameloblastoma expressed amelogenin gene by mRNA phenotyping, northern blot analysis and in situ hybridization (Snead et al., 1992; Tsujigiwa et al., 2005).

The positive expression of AMEL was in the epithelial linings of the Keratocystic odontogenic tumour, eight cases moderately positive of amelogenin was seen throughout the epithelium, this was in accordance with previous studies (Anigol et al., 2014) and two cases showed mild staining.

So, it is obvious that the amelogenin gene expressed in normal developing tooth germ is different from odontogenic tumours.

The low expression in ameloblastoma and Keratocystic odontogenic tumours' cells reflected the fact that cells aren't differentiated and the tumour cells do not attain functional maturation and that agree with this tumors

nature and the aggressive clinical behaviors of both. This could in part explain the lack of expression of this protein as well as the absence of calcified structures in this classic odontogenic tumor.

In conclusion, the AMEL protein can be used as a marker for odontogenic tumors and odontogenic epithelium component.

Intensity of expression of AMEL protein in odontogenic tumors are variable and may explain the differentiation of the tumors' cells.

The AMEL protein predict the behavior of odontogenic tumors and we need of more studies with a large number of tumors to support the observation of the biologic behavior of this protein.

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