



# Caveolin-1 Expression in Odontogenic Cysts and Ameloblastomas

## Ameloblastom ve Odontojenik Kistlerde Caveolin-1 Ekspresyonu

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### ABSTRACT

**Objective:** The aim of this study is to evaluate the caveolin-1 expression in a group of odontogenic cysts and tumors.

**Material and Method:** In this cross-sectional study, the expression of caveolin-1 was evaluated immunohistochemically in 75 samples including 18 cases of dentigerous cyst, 18 odontogenic keratocysts, 3 orthokeratinized odontogenic cysts, 2 calcifying odontogenic cysts and 34 ameloblastomas (solid and unicystic).

**Results:** Positive immunohistochemical reaction was found in 100% of odontogenic cysts and this was significantly more than both unicystic (65%) and solid (55%) ameloblastomas.

**Conclusion:** The present study showed the expression of caveolin-1 in all odontogenic cysts and more than ameloblastomas. The results suggested that absence of caveolin-1 might enhance aggressiveness of odontogenic lesions and could be a useful marker for distinguishing ameloblastomas from other odontogenic lesions.

**Key Words:** Ameloblastoma, Odontogenic cyst, Caveolin-1, Immunohistochemistry

### ÖZ

**Amaç:** Bu çalışmanın amacı, bir grup odontojenik kist ve tümörde caveolin-1 ekspresyonunu değerlendirmektir.

**Gereç ve Yöntem:** Bu çalışmada, caveolin-1 ekspresyonu, 18 dentigeröz kist, 18 odontojenik keratosit, 3 ortokeratinize odontojenik kist, 2 kalsifiye odontojenik kist ve 34 ameloblastomu (solid ve unikistik) içeren 75 olguda immünohistokimyasal olarak değerlendirilmiştir.

**Bulgular:** Odontojenik kistlerin % 100'ünde pozitif immünohistokimyasal reaksiyon saptanmıştır. Bu sonuç hem unikistik (% 65) hem de solid (% 55) ameloblastomada saptanandan anlamlı ölçüde yüksektir.

**Sonuç:** Bu çalışma caveolin-1 ekspresyonunun, bütün odontojenik kistlerde ameloblastomalardan daha fazla olduğunu göstermiştir. Bulgular caveolin-1 yokluğunun odontojenik lezyonların agresivitesini arttırabileceğini ve ameloblastomaların diğer odontojenik lezyonlardan ayırt edilebilmesi için yararlı bir gösterge olabileceğini ortaya koymuştur.

**Anahtar Sözcükler:** Ameloblastoma, Odontojenik kist, Caveolin-1, İmmünohistokimya

### INTRODUCTION

Odontogenic cysts and tumors originate from remnants of odontogenic epithelium and have a wide spectrum of histopathologic and clinical characteristics, ranging from benign non-invasive to locally invasive or malignant lesions. Histopathologic similarities between these lesions may challenge the correct diagnosis, especially in small size specimens, and may also lead to unnecessary broad surgery (1- 5). Recently, immunohistochemistry (IHC) has been employed to differentiate various lesions or predict their biologic behavior by using proper markers (6). For example, some cytokeratins have been investigated and found to be suitable for differentiating odontogenic keratocysts from other odontogenic cysts (7). It is also stated that calretinin is

a marker for stellate reticulum-like cells of ameloblastoma and can be used to distinguish this tumor from other odontogenic tumors (8).

Caveolin-1 (cav-1) is the main protein that participates in formation of caveolae that are 50-100 nm omega-shaped invaginations of the plasma membrane (9). Caveolae are primarily formed by caveolin proteins including cav-1, 2, and 3 that are expressed in various mammalian cells. They have a loop structure with common topology: Cytoplasmic N-terminus with scaffolding domain, cytoplasmic C-terminus, and a long transmembrane domain (10). They can move into the cytoplasm and return to the cell surface (11). In 1992, the first protein marker from this family was produced and named caveolin/vip21 which is now

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recognized as cav-1(12). Among caveolin proteins, cav-1 is highly expressed in epithelial cells including keratinocytes, salivary glands and respiratory tract epithelium, as well as, osteoblasts, endothelial cells and skeletal muscles (13,14). It is explained that some signaling molecules are related to the caveolin-scaffolding domain (CSD) (15) and some roles are determined for cav-1 including prevention of cells cycle progression via saving the cell cycle in G0/G1 stage, (7) cell transformation, programming cell death by activating protein kinase B (PKB) pathway (16) and acting as an oncogene or tumor suppressor gene based on the tumor type and stage (15). Also, it is shown that cav-1 can delay tumor metastasis by decreasing of matrix metalloproteinases (MMP) secretion (15).

Schwab et al. (17) reported cav-1 expression in epithelial and mesenchymal cells of tooth germ in various stages of development. That study determined that cav-1 could participate in odontogenesis (17). Since this protein is a target of treatment in some tumors, evaluation of its function in any specific pathologic lesion is suggested (15). To the best of our knowledge, there is no research about cav-1 expression in odontogenic cysts and ameloblastomas and also regarding its importance as a diagnostic or prognostic marker in lesions originating from odontogenic epithelium. This study aimed to evaluate cav-1 expression in a group of odontogenic cysts and tumors with higher prevalence and with rather similar histopathological features, and hence to investigate its possible role as a diagnostic or prognostic marker.

### MATERIALS and METHODS

In this cross-sectional and analytical study, we analyzed 34 cases of ameloblastomas (14 solid and 20 unicystic types), 18 dentigerous cysts, 18 odontogenic keratocysts (also known as parakeratotic type odontogenic keratocyst and keratocystic odontogenic tumor), 3 orthokeratinized odontogenic cysts and 2 calcifying odontogenic cysts (COC). The subjects were provided from the archive of the oral and maxillofacial pathology department from 1995 to 2012. All samples had definitive diagnosis and adequate epithelial component.

Formalin-fixed and paraffin-embedded blocks were cut with 4µm thickness for immunohistochemical staining, using the Envision Labeled Peroxidase System (DAKO, Carpinteria, CA, USA). Sections were deparaffinized and rehydrated, and then washed with distilled water. Antigen retrieval was performed for 20 minutes. Then, the sections were incubated with anti-caveolin-1 polyclonal rabbit antibody (1:200, Novocastra, Newcastle, UK) for 60 minutes. The chromogen solution was 3, 3 di- aminobenzidine (DAB

liquid). Positive control was provided from colon tissue (18). Endothelial cells were used as internal positive control (19). Negative control sections were provided by replacing the primary antibody with PBS solution. Brown staining of the cell membrane, cytoplasm or both, was considered as positive. All the samples were classified into three groups: negative (staining  $\leq 5\%$ ), focally positive ( $6\% < \text{staining} \leq 50\%$ ) and extensive positive (staining  $>51\%$ ). The staining was estimated in the basal cell layer of cystic lesions and peripheral cells of ameloblastic nests.

Data were analyzed using SPSS version 11. We used the Chi-square test and Monte-Carlo method for statistical analysis. Study groups with less than 10 cases were not evaluated in the statistical analysis. P values of less than 0.05 were considered as significant.

### RESULTS

Expression of cav-1 was found in 88.7% of all selected samples. Table I shows the cav-1 expression in all groups with details. All the cystic lesions were positive for cav-1. Membranous and cytoplasmic immunoreactivity was observed throughout the basal cell layer of all cysts (Figures 1A,B, 2A,B,C). This pattern was similar in all odontogenic cysts. A limited number of odontogenic cysts in each group showed slight staining in superficial cells. Also, one out of two COCs showed cav-1 expression in superficial stellate reticulum-like cells.

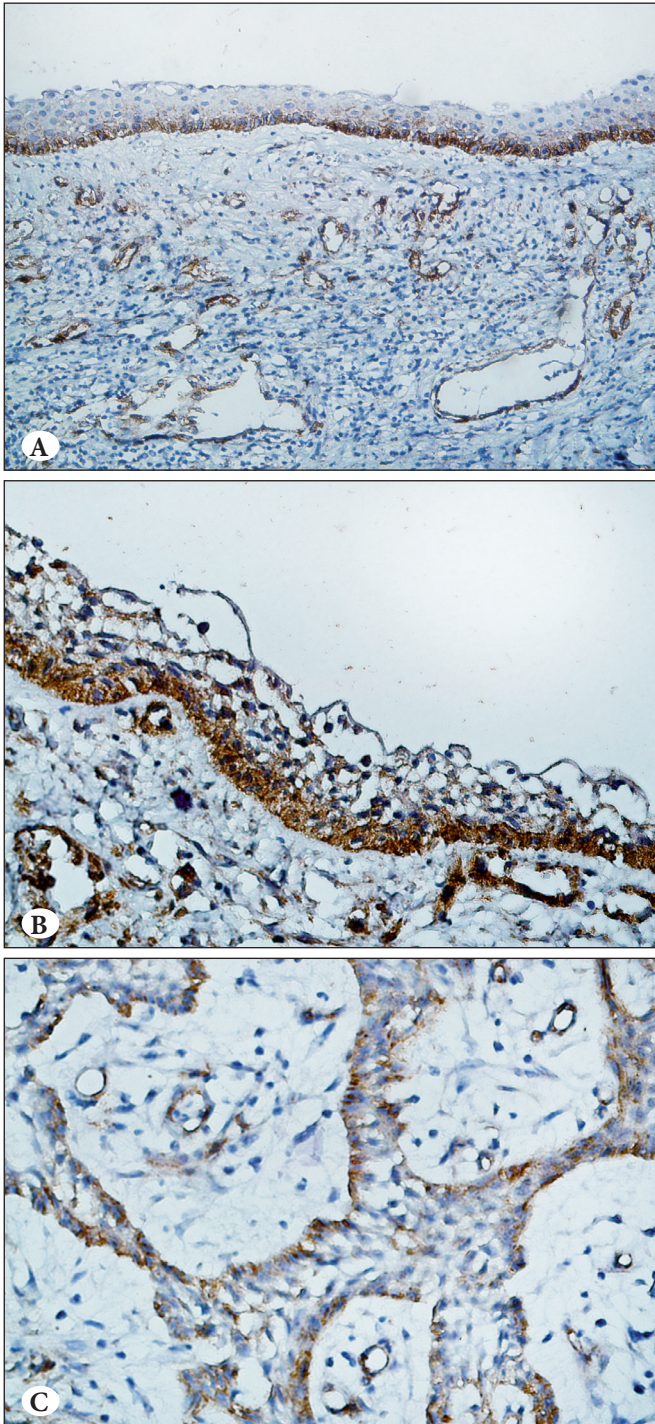
We observed cav-1 expression in 65% of unicystic and 55% of solid ameloblastoma. In the luminal unicystic ameloblastoma, the basal ameloblast-like cells were positive. Superficial epithelial cells were stained in a few cases. Mural and solid ameloblastomas showed immunoreactions in the peripheral ameloblast-like cells of the tumoral nests

**Table I:** Caveolin-1 expression in all study groups

	Negative	Focally positive	Extensive positive
<b>Study groups (n)</b>	0-5%	6-50%	>50%
Ameloblastoma(14)	35%	10%	55%
UA (20)	44.9%	7.1%	48%
Odontogenic keratocyst(18)	0%	0%	100%
Dentigerous cyst(18)	0%	0%	100%
Orthokeratinized OC (3)	0%	0%	100%
COC(2)	0%	0%	100%

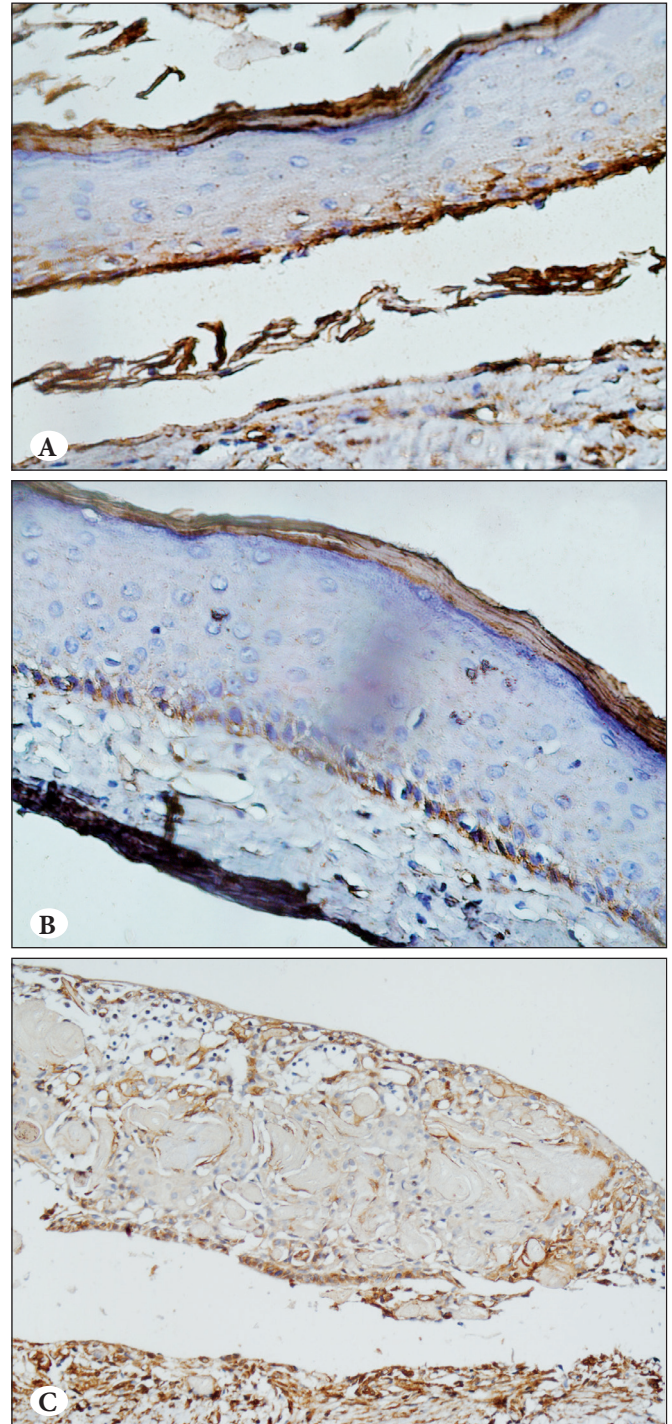
UA: Unicystic ameloblastoma, OC: odontogenic cyst, COC: Calcifying Odontogenic Cyst





**Figure 1:** Immunohistochemical staining of cav-1 in A) Dentigerous cyst (x200), B) Unicystic ameloblastoma (x200). C) Ameloblastoma (x400).

(Figure 1B,C). The central stellate reticulum-like cells were stained in some cases, although most of the cases did not show immunoreaction in the central portion of the tumoral nests. The area of squamous metaplasia also was not stained. Cav-1 expression was also membranous and



**Figure 2:** Immunohistochemical staining of cav-1 in A) Odontogenic keratocyst (x200). B) Orthokeratinized odontogenic cyst (x200). C) Calcifying odontogenic cyst (x200).

cytoplasmic in ameloblastomas. Chi-square test showed significant difference between ameloblastoma (unicystic and solid) and other odontogenic lesions regarding cav-1 expression. However, the other study groups were similar regarding the marker expression (P-value <0.05).



## DISCUSSION

In the present study, we evaluated the expression of cav-1 in a group of odontogenic cysts and tumors for the first time. Our results showed that all odontogenic cystic lesions extensively expressed the marker in the basal cell layer, without significant difference. However, both solid unicystic ameloblastomas revealed immunoreactivity to a smaller degree and more focally. These findings might suggest that cav-1 could be a tumor suppressor in odontogenic tumors. It has been stated that cav-1 plays opposing roles in different tumors (11). Previous studies have shown a tumor suppressor function in sarcomas and ovarian carcinomas (20, 21). Reduced cav-1 expression was also described in mucoepidermoid carcinoma of salivary glands, colon and breast cancers as well as lung carcinoma cell lines (9). It was revealed that hypermethylation of cav-1 gene promoter caused reduced protein expression in these cancers (22).

However, some studies have demonstrated an oncogenic role for cav-1 in some tumors. Its upregulation was observed in primary and metastatic prostate cancer (9). Also, increasing expression of the marker was found from normal oral epithelium to dysplastic and neoplastic epithelial tissue (squamous cell carcinoma) (9). Bau et al. (23) demonstrated that polymorphism of the cav-1 gene in some genotypex was associated with oral cancer susceptibility. They found the expression of cav-1 in the basal and parabasal layers. The expression of cav-1 in our samples was also observed in the basal cell layer. Despite the fact that the immunoreaction was mainly found in the basal cells, it seems that this marker is not related to proliferation activity in our samples. Various studies have evaluated the expression of proliferation markers (such as Ki-67) in odontogenic cysts and tumors and reported that ameloblastomas had higher proliferative activity in comparison with other odontogenic cysts (24). In our study the tumoral lesions that have higher proliferation rate, revealed similar or lower expression of cav-1, compared to the other lesions. It has also been shown that odontogenic keratocysts had higher expression of proliferation markers in comparison with other cystic lesions, and that the parabasal cell layer of this lesion revealed a higher expression rate than the basal layer (25). However, our study showed different results for cav-1 expression. It was therefore concluded that this protein had no role in proliferative activity in odontogenic cysts and tumors. Other studies suggested a possible negative regulation of proliferation due to high expression of cav-1 in terminally differentiated cells (26- 28). However, we found this marker in less differentiated cells in the cysts and tumors (basal cells, ameloblast-like and some stellate

reticulum- like cells) and not in differentiated superficial cells of odontogenic cysts and squamous change in ameloblastoma.

Our samples revealed both cytoplasmic and membranous immunoexpression. The caveolin proteins have two main domains (scaffolding and membranous) and they are in relation with different molecules such as C- Src and Cirkb-7, resulting in different roles in various pathologic lesions (22).

Expression of cav-1 has been shown previously in enamel organ, especially inner enamel epithelium and ameloblast cells of developing tooth germ (17). It was also demonstrated that cav-1 participated in cell transformation and differentiation (15). So, this study hypothesizes that cav-1 may be a useful marker for differential diagnosis of odontogenic lesions. Odontogenic keratocyst and orthokeratinized odontogenic cyst have histopathologic similarities. Also, differentiating unicystic ameloblastoma from dentigerous cyst and COC may be difficult. According to our results, the similar cystic lesions expressed the marker with the same pattern. All cases of keratinized odontogenic cysts and dentigerous cyst showed extensive immunoreaction in basal cell layer. Although, we found cav-1 expression in the superficial cells of one out of two COCs and a few cases of unicystic ameloblastoma, the limited number of the cases necessitates the further research for evaluation of usefulness of this marker for differentiating of these lesions. Unicystic ameloblastoma and solid ameloblastoma also showed similar expression. Therefore, this marker could not help in distinguishing these lesions from each other. However, due to significant difference between unicystic ameloblastomas and dentigerous cyst, absence of cav-1 expression supports the unicystic ameloblastoma diagnosis. Moreover, this result could be confirmed by future studies with larger sample size. Also, it suggested that other odontogenic tumors should be examined in comparison with ameloblastomas for evaluation of usefulness of this marker for differential diagnosis.

In the present study, we observed diminished expression of cav-1 in ameloblastomas that have a locally aggressive nature when compared to the other study groups. Previous studies revealed the diverse role for cav-1 in different lesions and since cav-1 might be a target of treatment in various tumors, it was suggested that this role should be explained in any pathogenic condition (15). We conclude that this protein may be in association with increased invasiveness of ameloblastoma. A study has shown co-localized expression of cav-1 with extracellular matrix metalloproteinase

inducer (EMMPRIN) in developing tooth germ and has concluded that cav-1 regulates the EMMPRIN-induced expression of MMPs (29). It was suggested that EMMPRIN induces the MMP dependent degradation of extracellular matrix during tumor progression (29). Cav-1 on the cell surface decreases glycosylation of EMMPRIN, resulting in diminished expression of MMPs that participates in tumoral invasion (17). Also, it has been shown that down regulation of cav-1 decreases the expression of E-cadherin and increases the activation of  $\beta$ -catenin, both resulting in enhanced invasiveness of neoplasms (15).

In conclusion, our results showed cav-1 expression in all odontogenic cysts and 58% of ameloblastomas. This protein could function as a tumor suppressor in odontogenic lesions and may decrease their aggressiveness. It is suggested that these results be evaluated in further studies with gene analysis. The absence of its expression might provide a diagnostic aid for distinguishing unicystic ameloblastomas from other odontogenic cysts.

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#### CONFLICT OF INTEREST

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