

Original article

Evaluation of the Biological Behaviour of Odontogenic Keratocysts Compared with Ameloblastoma and Radicular Cysts Using Cyclin-Dependent Kinase 1 Biomarker

Asmaa Algharyani^{1*}, Marwa Maghrabi¹, Yousef Hasen²¹Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Tripoli University, Tripoli, Libya²Department of Pathology, Faculty of Dentistry, University of Zawia, Zawia, LibyaEmail: asm_1981mmm@yahoo.com**Keywords.**

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Abstract

The odontogenic keratocyst (OKC) is classified as a cyst in the 2022 World Health Organisation classification, despite its aggressive behaviour and high recurrence rate. This study aimed to evaluate the expression of cyclin-dependent kinase-1 (CDK1) in odontogenic keratocysts, radicular cysts (RC), and ameloblastoma (AM), and to correlate it with the established behaviour of these lesions. In this retrospective study, we analysed eighty-eight archived formalin-fixed, paraffin-embedded cases: thirty-four odontogenic keratocysts, twenty-nine radicular cysts, and twenty-five ameloblastomas. We performed routine IHC staining to evaluate and compare CDK1 expression across these three types of lesions. The results showed that CDK1 expression was significantly higher in the suprabasal layers of odontogenic keratocysts compared with radicular cysts, with low immunoreactivity ($P < 0.001$). Also, there was no significant statistical difference between the staining intensity of follicular and plexiform histological types of ameloblastoma (p -value=0.94). The results of this study reveal that CDK1 exhibits increased staining intensity in odontogenic keratocysts compared to AM and RC. This likely explains why these cysts tend to be more aggressive and grow faster. In contrast, the reactivity of CDK1 was negligible in the lining of radicular cysts, whereas it was prominent in the stromal inflammatory cells of these cysts. This observation appears more associated with inflammation than with actual neoplastic development. Samples from AM suggest that the difference in staining patterns between follicular and plexiform types may indicate changes in tumour architecture and growth dynamics.

Introduction

Odontogenic cysts and odontogenic tumours originate from remnant odontogenic tissue that was formerly involved in the formation of the tooth. Under certain stimuli, these remnants retain the potential for clinical proliferation, ultimately leading to the development of various odontogenic cysts and tumours [1]. Odontogenic cysts are classified into developmental and inflammatory types, which display distinct clinical presentations and biochemical profiles. Inflammatory radicular cysts arise from the epithelial remnants of Malassez. Conversely, odontogenic keratocysts are developmental in origin, deriving from remnants of the dental lamina. OKCs display neoplastic-like characteristics characterised by a strong intrinsic growth capacity compared to inflammatory cysts. This feature distinguishes them from other odontogenic cysts and explains their more aggressive clinical behaviour.

Multiple studies on the odontogenic keratocyst indicate that its heightened mitotic index, relative to other odontogenic cysts, further supports the theory that OKCs are benign cystic neoplasms rather than odontogenic cysts [2,3]. The odontogenic keratocyst has been one of the most controversial developmental anomalies over the past decade, with its fundamental concept and related terminology undergoing substantial transformations and ongoing review. This discrepancy is attributed to the lesion's erratic and unpredictable clinical behaviour, which has sparked widespread controversy over its nomenclature.

Consequently, the proposed classifications for the OKC have been characterised by complexity and ambiguity, leading to significant conceptual confusion among both clinicians and pathologists [4]. Ameloblastoma is a histologically benign yet aggressive odontogenic neoplasm originating from remnants of dental epithelium, representing 63.2% of all odontogenic tumours and 3.05% of oral-maxillofacial disorders. AM is locally invasive and non-metastatic, although conservative therapy results in recurrence rates of up to 50%. The tumour exhibits a preference for the jawbones, particularly the posterior part of the mandible, especially the molar-ramus area. The latest 2022 WHO classification identifies five principal histopathological variants: conventional (solid/multicystic), unicystic, extraosseous (or peripheral), metastatic, and adenoid ameloblastoma [5,6]. Uncontrolled cellular proliferation is a characteristic feature of oncogenesis. Under physiological conditions, the cell cycle proceeds through a highly coordinated, chronological sequence regulated at critical checkpoints by an intricate biochemical network of cyclin-dependent kinases (CDKs).

To date, twenty distinct members of the CDK family have been identified, and they are key regulators of the cell cycle, transcription, and RNA splicing. These kinases function within well-defined pathways to ensure high-fidelity DNA replication and equal segregation of genetic material to daughter cells in the process of mitotic division [7,8]. The first member of the

cyclin-dependent kinase (CDK) family to be identified was cyclin-dependent kinase 1 (CDK1). CDK1 is an essential molecule in the transition from G2 to M phase, with the cyclin B1 complex enabling this progression, hence earning the title of maturation-promoting factor (MPF). This combined activity orchestrates the dramatic structural overhaul required for mitosis, including chromatin condensation, nuclear envelope disintegration, fragmentation of the Golgi apparatus and endoplasmic reticulum, and spindle formation via microtubule instability. In late G2, the activity of CDK1/cyclin B1 increases and continues into prometaphase until the spindle assembly checkpoint is satisfied and the cell is allowed to move into metaphase [9,10].

Materials and Methods

Tissue Samples

A total of eighty-eight cases of odontogenic cysts and tumours were included in this retrospective study, utilising archival formalin-fixed, paraffin-embedded (FFPE) tissue blocks. The sample comprised 34 odontogenic keratocysts (OKC), 29 radicular cysts (RC), and 25 cases of ameloblastoma (AM) (Figure 1). Negative cases were included in the overall positivity analysis but excluded from the subsequent subgroup evaluation of staining intensity (Tables II, III). The samples were retrieved retrospectively from the archives of the University Medical Center. All included specimens had a definitive histopathological diagnosis and demonstrated adequate epithelial representation for analysis. Relevant baseline clinicopathological data, including patients' age, sex, and lesion site, were systematically collected from the corresponding medical records.

Immunohistochemical Staining

The primary antibody used for immunohistochemical analysis was a mouse monoclonal antibody to human CDK1 fusion protein (1:50 dilution, Fuzhou Maixin Biotechnology Development Co., Ltd., China), prepared for staining. Serial sections of 4 µm thickness were sliced from the paraffin-embedded blocks and mounted onto positively charged slides. To prepare the tissue for primary antibody incubation, the sections underwent a standard deparaffinization and rehydration sequence through a graded series of alcohol concentrations. Endogenous peroxidase activity was inhibited by incubating the slides in a 0.3% hydrogen peroxide methanol solution for 10 minutes. After pre-treatment, the sections were treated with the anti-CDK1 primary antibody. Detection was subsequently carried out by uniformly applying a streptavidin-peroxidase conjugate to each slide under identical incubation conditions. Finally, the cell nuclei were counterstained with hematoxylin, and the slides were permanently mounted for microscopic evaluation.

Interpretation of Staining

All slides were visually examined by two senior pathologists using light microscopy (Olympus BX41TF, Tokyo, Japan) in a randomly selected sequential high-power (×400 magnification) field. The expression of CDK1 was assessed as staining intensity in epithelial cells using a three-tier grading system: 1 (weak staining), 2 (moderate), and 3 (strong staining).

Statistical Analysis

Statistical analyses were performed on the clinicodemographic and immunohistochemical data. A One-Way ANOVA test was used to compare the mean ages for the different lesion groups. The Chi-square test was used to compare positive and negative CDK1 expression and the distribution of gender and tumour site among the lesions. The expression intensity of CDK1 was compared between the three main lesion groups by the Kruskal-Wallis test. Finally, the Mann-Whitney U test was utilised to compare CDK1 expression intensity between the histological variants of ameloblastoma. Statistical significance was established at $P \leq 0.05$.

Results

Patient general data

All specimens were assessed using hematoxylin and eosin staining and subsequently confirmed by senior pathologists upon reevaluation. Baseline characteristics about age, gender, and lesion location in each group are presented in (Table 1).

Immunohistochemical expression of CDK1 across lesions AM, OKC, and RC

Odontogenic keratocyst

Among the positive cases of OKC, twenty-four samples (75%) exhibited strong positive reactivity to CDK1, localised to the suprabasal layers of the epithelium (Figure 1), while weak reactivity was observed in only two cases (6.2%). Conversely, six cases of OKC (18.8%) demonstrated moderate staining extending from the parabasal to superficial layers of the epithelium (Figure 2) (Table 3).

Radicular Cyst

The immunohistochemical analysis revealed that the majority of radicular cyst cases, specifically twenty-two samples (88%), displayed weak CDK1 positivity in the epithelial lining, while moderate expression was observed in only three cases (12%). It was worth mentioning that the stromal inflammatory cells showed a positive diffuse reaction for CDK1 in all studied cases (Figure 3 and Table 3).

Ameloblastoma

The immunohistochemical expression of CDK1 was compared between the follicular and plexiform histological variants of ameloblastoma among the confirmed positive cases (n = 23). Within the follicular variant (n = 12), weak immunopositivity was noted in 4 cases (33.3%), moderate staining in 7 cases (58.3%), and strong expression was observed in only 1 case (8.3%). A similar distribution was identified in the plexiform variant (n = 11), where 4 samples (36.4%) showed weak intensity, 6 cases (54.5%) showed moderate intensity, and 1 case (9.1%) showed strong staining intensity. The statistical analysis comparing staining intensity between these two histological variants indicated no statistically significant differences (P=0.94) (Figures 4 & 5) & (Table 4).

Table 1. Clinicodemographic Characteristics of the Study Population

Type of lesions	Total number	Age	P-v	Sex		P-v	Site		P-v
		Mean±SD	0.214	Female	Male	0.01	Maxilla	Mandible	P<0.0001
Odontogenic Keratocyst	34	30.3 ±10.4		10	24		12	22	
Radicular Cyst	29	32.7±14.3		19	10		25	4	
Ameloblastoma	25	33.3±12.1		10	15		5	20	

Table 2. Frequency and percentage distribution of CDK1 immunohistochemical expression across the studied odontogenic cysts and tumours

Type of lesions	Number of positive cases (%)	Number of negative cases (%)	P-value
OKC	32 (94.1%)	2 (5.9%)	0.56
RC	25 (86.2%)	4 (13.8%)	
AM	23 (92%)	2 (8%)	

Chi-Square test ($\chi^2 = 1.2$), no statistically significant difference.

Table 3. Immunoreactivity of CDK-1 in different lesions

Positivity reactivity of CDK1	OKC	RC	AM	P-value
weak	2 (6.2%)	22 (88%)	8 (34.8%)	P<0.001
Moderate	6 (18.8%)	3 (12%)	13 (56.5%)	
Strong	24 (75%)	0	2 (8.7%)	
Total	32 (100%)	25 (100%)	23(100%)	

The Kruskal-Wallis H-test: a highly significant difference, H value (41.94)

Table 4. Comparison of CDK1 expression intensity between the histological variants of ameloblastoma

Types of AM		P-Value
Follicular	Plexiform	0.94
4 (33.3%)	4 (36.4%)	
7(58.3%)	6(54.5%)	
1 (8.3%)	1 (9.1%)	

The Mann-Whitney U test was not statistically significant, with a U value of 62.0.

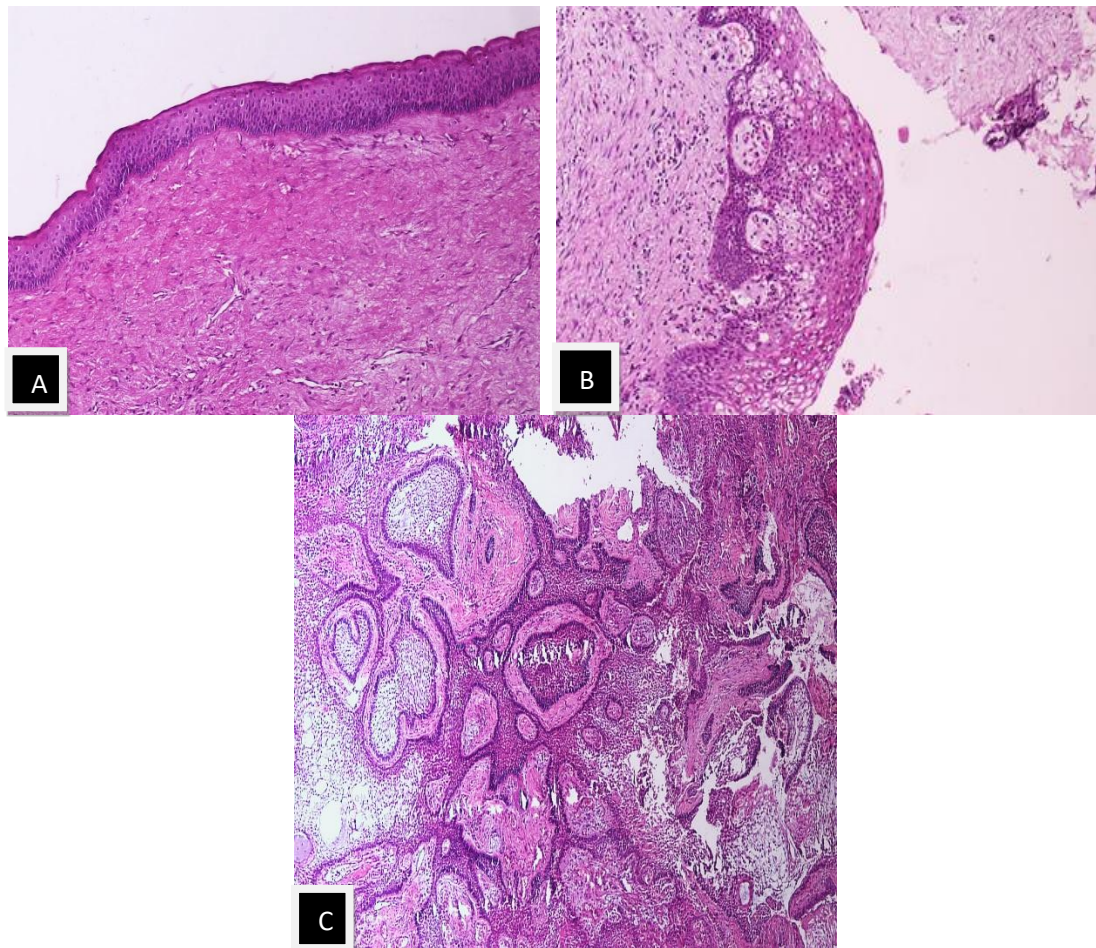


Figure 1. Histological features of odontogenic keratocyst, radicular cyst, and ameloblastoma

A) An odontogenic keratocyst exhibiting squamous epithelium that is stratified, a parakeratotic surface, and a palisaded hyperchromatic basal cell layer (100x). B) A radicular cyst is characterised by a fibrous wall lined with hyperplastic stratified squamous epithelium organised in interconnecting rings (100x). C. Ameloblasts are arranged in a follicular morphology, bounded by columnar ameloblastic-like cells in the peripheral region and stellate reticulum-like cells in the central region (100x).

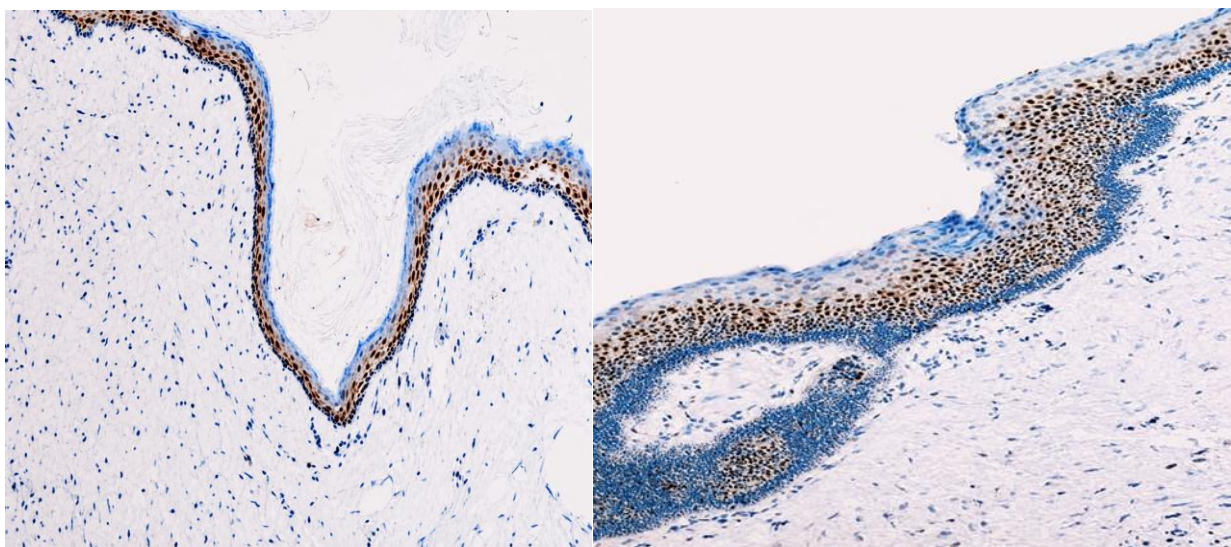


Figure 2. Photomicrograph of OKC showing strong intensity in parabasal layers of the epithelium (anti-CDK1 ×100)

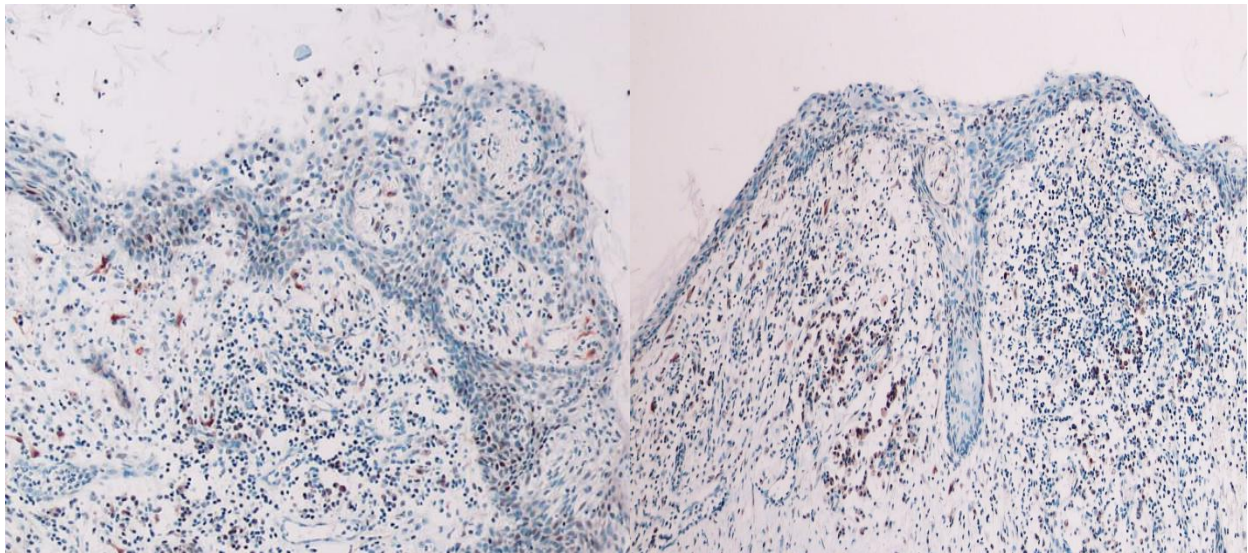


Figure 3. Photomicrograph of a radicular cyst showing weak intensity in the lining epithelium, whereas stromal inflammatory cells show scattered immunostaining reaction (anti-CDK1 ×100)

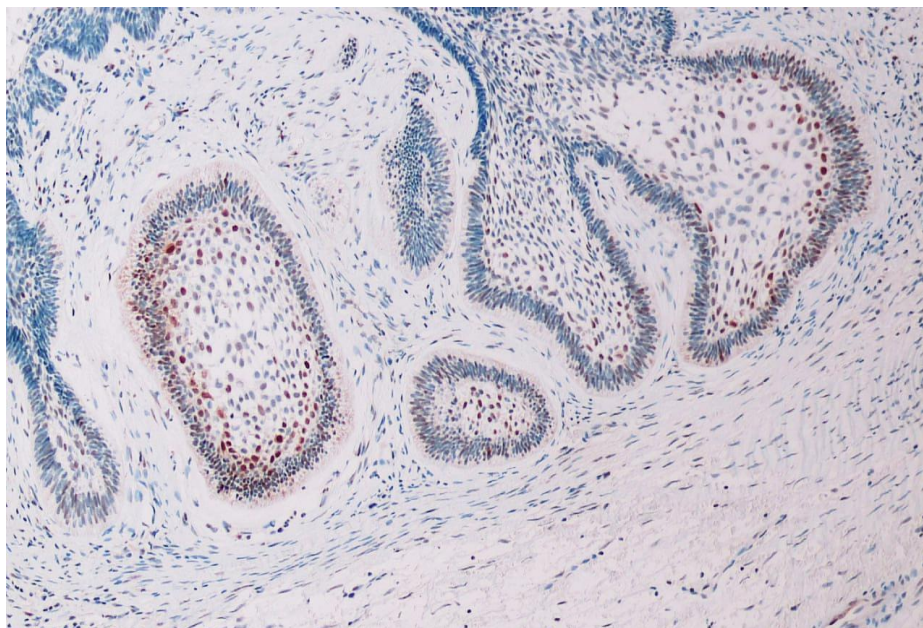


Figure 4. Follicular ameloblastoma displayed a diffuse moderate nuclear expression of CDK1 in the ameloblast-like cells and some of the stellate reticulum-like cells (anti-CDK1 ×100)

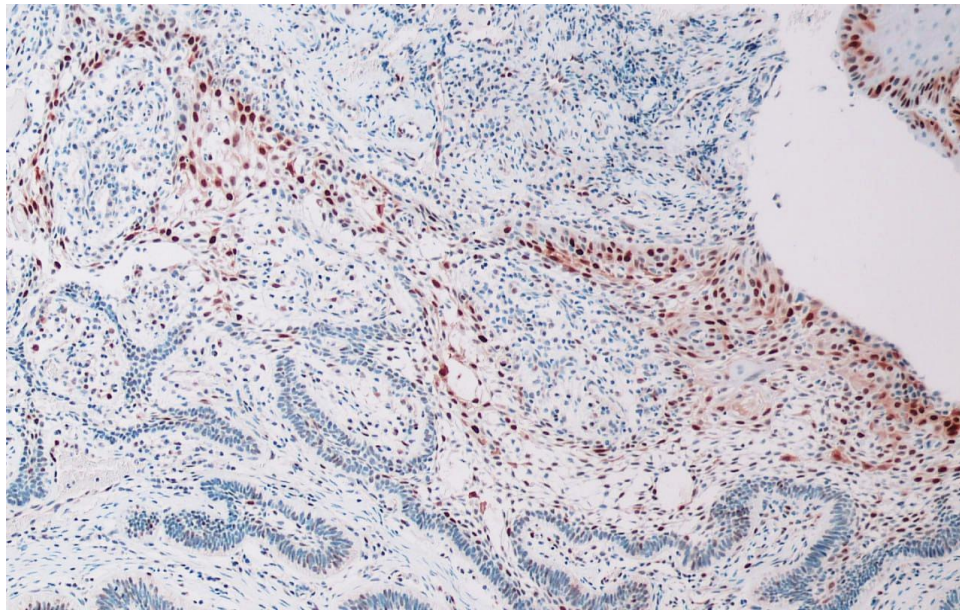


Figure 5. Photomicrographs of plexiform ameloblastoma showing CDK1 immunostaining at the central part (stellate reticulum-like cells) and decreased or negative staining toward the periphery of neoplastic cells (anti CDK1×100)

Discussion

The cell cycle comprises the mitotic (M) phase and the interphases: G1, S, and G2. CDK1 is a serine/threonine kinase that acts throughout the cell cycle by phosphorylating multiple substrates, serving as the fundamental protein kinase that propels cells into mitosis. The deregulation of CDK1 has been associated with accelerated division, heightened proliferative activity, and more histologic aggressiveness. In this study, we employed immunohistochemical staining of CDK1 in OKC, RC, and ameloblastoma to illustrate differences in behaviour and aggressiveness among odontogenic cysts and tumours [11].

The odontogenic keratocyst is classified as a developing cyst in the 2022 WHO odontogenic classification. Although it exhibits clinical characteristics of a locally aggressive lesion with a significant recurrence rate following excision, the evidence supporting its neoplastic nature remains inadequate, hence generating considerable interest in its investigation [12]. The present investigation revealed notably higher levels of CDK1 in odontogenic keratocysts compared with radicular cysts and ameloblastoma. This may be attributed to the increased mitotic activity and rapid proliferation, thus contributing to their aggressive clinical behaviour and high recurrence rate. Also, in our study, the epithelium lining of OKCs showed mainly CDK1 in the suprabasal layer; this may be a zone of proliferative activity limited to the suprabasal layer, representing epithelial disorganisation and abnormal regulation of the cell cycle. Although this high proliferative drive and aggressive clinical behaviour strongly evoke a neoplastic nature, the entity remains officially classified as an odontogenic cyst according to the latest WHO guidelines. This finding correlates with the work of Massacci et al. [13], who highlighted the kinase's pivotal role in cellular proliferation, as well as its documented involvement in driving cyst growth in other non-neoplastic pathologies [14].

Immunohistochemical analysis showed weak immunopositivity for the CDK1 antibody in most of the cases of radicular cysts (88%). Notably, positive staining was observed in the inflammatory cells of the stroma. This finding may be explained by the secretory products, including cytokines and growth factors, secreted by stromal inflammatory cells in the stromal tissue, which can trigger the epithelial lining of the radicular cyst, whereby heightened tissue inflammation is directly linked to upgraded proliferative activity. Thus, the CDK1 immunoreactivity of the stromal inflammatory cells may correlate with the intensity of the inflammatory response. Ameloblastoma is a benign but locally aggressive odontogenic tumour characterised by variable histopathological patterns, most commonly follicular and plexiform types [15]. Tumour growth and invasive behaviour are closely related to dysregulation of the cell cycle, in which cyclin-dependent kinases (CDKs) play a central role. This work assessed the immunohistochemical expression patterns of CDK1 across different histological variants of ameloblastoma, with particular emphasis on the follicular and plexiform types. The findings demonstrated distinct expression profiles between these variants, indicating variations in their proliferative behaviour and biological characteristics.

Follicular ameloblastoma exhibited nuclear expression of CDK1 predominantly localised within the ameloblast-like cells of the tumour in the peripheral region, while the central stellate reticulum-like cells showed reduced or focal staining. Such a pattern may indicate ongoing cell-cycle progression in the peripheral layer, in keeping with the aggressive biological growth patterns of the follicular variants. On the other hand, plexiform ameloblastoma showed a localised or focal reactivity of CDK1 in certain areas of the central stellate reticulum-like cells, whereas staining was reduced or negative in the

peripheral ameloblast-like cells. Although differences in the spatial distribution of CDK1 staining were observed between the follicular and plexiform variants of ameloblastoma, statistical analysis comparing the overall staining intensity between these two histological subtypes revealed no statistically significant variance. Therefore, the variation lies within the topographic compartmentalisation of expression within the tumour architecture and growth dynamics affecting cellular proliferation rather than a quantitative difference in total protein expression.

It was worth mentioning that the stromal inflammatory cells showed a positive diffuse reaction for CDK1 in all studied cases, revealing no statistically significant differences between the overall staining intensity of these two variants. The comparison of the CDK1 expression between the three different lesions provided useful information on the biological behaviour of the lesions. The marked CDK1 reactivity in odontogenic keratocysts (OKCs), especially in the suprabasal layers, reflects their high proliferative potential and aggressive features. In particular, this pattern of expression adds to the existing evidence favouring a neoplastic rather than purely cystic nature of OKC. In contrast, radicular cysts exhibited very little positive epithelial staining, and the positive reactivity was limited to inflammatory cells in the stroma, suggesting a more significant relationship with inflammatory responses. Conversely, ameloblastoma shows moderate expression with variable topographic patterns of histological variants with changes in tumour architecture and not in total immunoreactivity. Thus, the significantly increased expression of CDK1 in OKCs compared to other odontogenic lesions emphasises the possible role of this protein as a reliable immunohistochemical marker in evaluating biological aggressiveness and clinical behaviour of different odontogenic cystic lesions.

A significant challenge encountered in this study was the scarcity of established literature investigating Cyclin-dependent kinase 1 expression within odontogenic cysts and tumours. To the best of our knowledge, no prior studies have directly correlated CDK1 expression with the biological behaviour of odontogenic keratocysts, radicular cysts, and ameloblastoma. Consequently, interpreting our immunohistochemical findings and contextualising the observed expression patterns required a careful approach, as standard comparative data were unavailable. This highlights the novelty of our research and emphasises the need for future exploration to establish CDK1 as a reliable biomarker in these specific oral pathologies.

Conclusion

In conclusion, these findings validate the neoplastic characteristics of odontogenic keratocysts, emphasising their intrinsic growth potential. Consequently, CDK1 is a dependable marker for evaluating the biological aggressiveness of different odontogenic lesions.

Conflict of Interest

No conflicts of interest exist.

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