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Original Article

Ki67 as a proliferation marker: A study on odontogenic keratocysts and radicular cysts

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ABSTRACT

Ki67 as a proliferation marker plays a critical role in assessing cellular activity in various pathological tissues, including cystic lesions. Odontogenic keratocysts (OKCs) and radicular cysts (RCs) are two common types of jaw cysts that exhibit distinct biological behaviors, particularly in their proliferative patterns. OKCs are known for their aggressive nature and high recurrence rate, while RCs are typically less aggressive and exhibit lower recurrence risk. By evaluating the expression of Ki67, a nuclear protein linked to cell proliferation, researchers can gain valuable insights into the growth potential and recurrence tendencies of these cysts.

The study involved analyzing 32 biopsy samples from patients with OKCs and RCs, using immunohistochemical techniques to assess Ki67 expression levels. These samples were surgically removed and prepared for histological examination, with Ki67-positive cells quantified in both basal and suprabasal layers of the cystic epithelium. The findings revealed that OKCs exhibited a higher concentration of Ki67-positive cells in the basal layers, while RCs showed proliferative activity in both basal and suprabasal layers. This differential pattern highlights the more aggressive proliferative behavior of OKCs.

The statistical analysis confirmed significant differences in Ki67 expression between the two cyst types, underscoring the importance of this marker in differentiating OKCs from RCs. The confined expression of Ki67 in the basal layer of OKCs, as opposed to the broader distribution in RCs, suggests that OKCs have a higher proliferative potential, contributing to their increased recurrence rates.

1. Introduction

Odontogenic keratocysts (OKCs) are a unique type of cystic lesion found in the jaw, originating from the dental lamina. They are characterized by their aggressive behavior and high recurrence rate. Cysts are pathological entities found in both hard and soft tissues, characterized by a central cavity lined with an epithelial layer supported by a fibrous capsule. This cavity can contain various substances, such as liquids, a mix of desquamated cells, mucus, and keratin. Cysts can form in numerous body locations and can vary significantly in their behavior and clinical implications [1].

The term "keratocyst" was first introduced by Philipsen in 1956 [2]. In the following years, numerous authors reported extensive cases related to this pathological entity, also known as keratinous cysts, epidermoid cysts, cholesteatomas, and primordial cysts [3]. Odontogenic keratocysts (OKCs) are a distinct type of jaw cyst known for their aggressive behavior and high recurrence rates. These cysts arise from the remnants of the dental lamina and are lined by keratinizing epithelium.

The World Health Organization (WHO) reclassified OKCs as keratocystic odontogenic tumors in 2005 due to their neoplastic nature. The 2017 classification restored the original terminology of odontogenic keratocyst (OKC), recognizing that the neoplastic designations of the 2005 classification were not fully accepted because research clarified their pathology and behavior [4].

Understanding the proliferative activity within these cysts can provide insights into their biological behavior and potential for recurrence.

OKCs present unique clinical challenges due to their tendency to recur after treatment and their potential association with nevoid basal cell carcinoma syndrome (NBCCS).

Ki67, a nuclear protein, serves as a reliable marker for cell proliferation, as its expression is linked to all active phases of the cell cycle except the G0 phase. This makes it a useful tool for evaluating

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proliferative activity in various tissues, including cystic lesions. In the context of OKCs, assessing Ki67 expression provides valuable insights into the biological aggressiveness and potential for recurrence of these cysts. By comparing Ki67 levels in OKCs and radicular cysts (RCs), we can better understand the differences in their proliferative behavior and their respective risks of recurrence [5].

This immunohistochemical study aims to conduct a thorough evaluation of the expression levels of Ki67, as marker of cell proliferation, specifically in odontogenic keratocysts (OKCs) in comparison to radicular cysts (RCs). The investigation will delve into the differences in Ki67 expression between these two types of cystic lesions, providing insights into their proliferative activity. By examining the varying levels of Ki67, we hope to contribute to a better understanding of the biological behavior of OKCs relative to RCs, which may ultimately inform clinical approaches to diagnosis and treatment.

2. Materials and methods

2.1. Biopsy samples and protein quantification

From January 2015 to February 2020, a total of 32 cystic lesions were surgically removed from patients treated at the Unit of Oral Pathology, Dental Clinic, Department of Life, Health and Environmental Sciences of the University of L'Aquila and at San Salvatore Hospital, L'Aquila, Italy. These included 16 odontogenic keratocysts (OKCs) and 16 radicular cysts (RCs). Clinical data such as patient gender, age, lesion location, and recurrence information over a 5 to 7-year period were collected. Patient demographics, including mean age, standard deviation (SD), and gender distribution (% male and female), are summarized in Table 1 for a clear comparison between the two groups (Table 1).

The anatomical samples were fixed in 10 % buffered formalin immediately post-extraction and subsequently embedded in paraffin [6,7]. Sections were then prepared for both conventional microscopy and immunohistochemical analysis. For histological analysis, the samples were stained with hematoxylin and eosin to assess their cellular architecture.

For immunohistochemical analysis, immunohistochemical staining for Ki67 was performed using a monoclonal anti-Ki67 antibody (1:100, Dako Mib-1, Italy) for 60 min at room temperature. This was followed by a 30-minute incubation with a streptavidin-biotin-peroxidase complex, resulting in a characteristic brown staining of Ki67-positive cells [8]. The slides were then counterstained with hematoxylin, dehydrated, and mounted for microscopic evaluation.

The evaluation involved identifying the most intensely stained epithelial areas at low magnification (30–40x) and counting the positive nuclei at high magnifications (100–200x) (Fig. 1). The percentage of Ki67-positive cells was determined by counting at least 1000 cells in representative areas, focusing on non-inflamed regions for OKCs).

2.2. Data treatment and statistical analysis

Each biopsy sample was meticulously examined to determine the percentage of Ki67 positive cells within the epithelium [9]. These percentages were then categorized into rank classes as follows:

- Rank 1: 0–4 s (-)
- Rank 2: 5–10 % (±)
- Rank 3: 11–25 % (+)
- Rank 4: 26–50 % (++)
- Rank 5: >50 % (+++)

Only samples with >5 % Ki67-positive cells were considered true positives [10]. Data on the percentages of Ki67-positive cells were presented as mean ± SD, while those derived from rank assignments were presented as median, 25th, and 75th percentiles.

Data were statistically analyzed using the Mann-Whitney test with Bonferroni corrections, comparing the percentages of Ki67-positive cells between the different types of cysts and their site of origin.

Table 1
Patient demographics, including mean age, standard deviation (SD), and percentage of male and female patients in each group.

	Mean Age	SD	% Male	% Female
Odontogenic keratocysts (OKCs)	53,44	8,95	56 %	44 %
Radicular cysts (RCs)	59,66	9,34	62 %	38 %
Total	56,55	9145	59 %	41 %

3. Results

The epithelium of keratocysts exhibited uniform thickness (6–8 cells) with correct polarization; in some areas, it appeared parakeratinized and often had a corrugated surface [11]. Daughter cysts or epithelial islands were frequently observed in the cystic wall, while inflammatory cells were consistently absent. In cases where significant inflammation was present, the characteristic features of the epithelium were lost to the extent that they were comparable to those of a radicular cyst (RC). The epithelium of the radicular cyst (RC) appeared as a variable thickness, sometimes interrupted, non-keratinized stratified squamous epithelium. The morphology of the epithelium always depended on the degree of inflammation; when present, the epithelium appeared rich in proliferating processes. The fibrous walls often contained cholesterol crystals, deposits of hemosiderin with an associated foreign body reaction. The walls of radicular cysts showed a significant inflammatory infiltrate in which plasma cells were either relevant or predominant [12] (Fig. 2).

Immunohistochemical analysis revealed distinct patterns of Ki67 expression between OKCs and RCs. In OKCs, Ki67 positivity was confined to the basal layer, with similar patterns observed in daughter cysts [13]. Ki67-positive cells in RCs were predominantly located in the basal layers, though they were also present in suprabasal layers [14]. This differential distribution indicates distinct proliferative profiles between RCs and OKCs, aiding in their differentiation even in the presence of inflammation. The linear and suprabasal distribution of Ki67+ cells in RCs serves as a reliable marker to distinguish inflammatory cysts from odontogenic ones, including OKCs.

The results obtained from the Mann-Whitney test with Bonferroni corrections provided a comprehensive statistical comparison of the percentages of Ki67-positive cells among the various types of cysts and their respective sites of origin. This analysis led to the data presented in Tables 2 and 3, which summarize the significant differences in Ki67 expression levels. The findings in these tables illustrate how the proliferative activity varies between the different cyst types, offering insights into their biological behavior based on their anatomical locations. (Table 2 and Table 3).

4. Discussion

Ki67 is a well-established and widely recognized marker of cell proliferation. It is present in all the active phases of the cell cycle, from the G1 phase to the M phase, and is notably absent during the quiescent G0 phase of the cell cycle. The expression of Ki67 increases progressively as cells advance through the different stages of the cycle, with a significant peak in its expression observed during the G2 and M phases, when the cell is preparing for and undergoing division. However, after mitosis, Ki67 levels decrease rapidly as the cells either re-enter a new cell cycle or return to a less active state. This specific expression pattern makes Ki67 an important tool in studies focused on cell proliferation, as it is exclusively present in dividing cells, providing insight into cellular dynamics. Many studies have

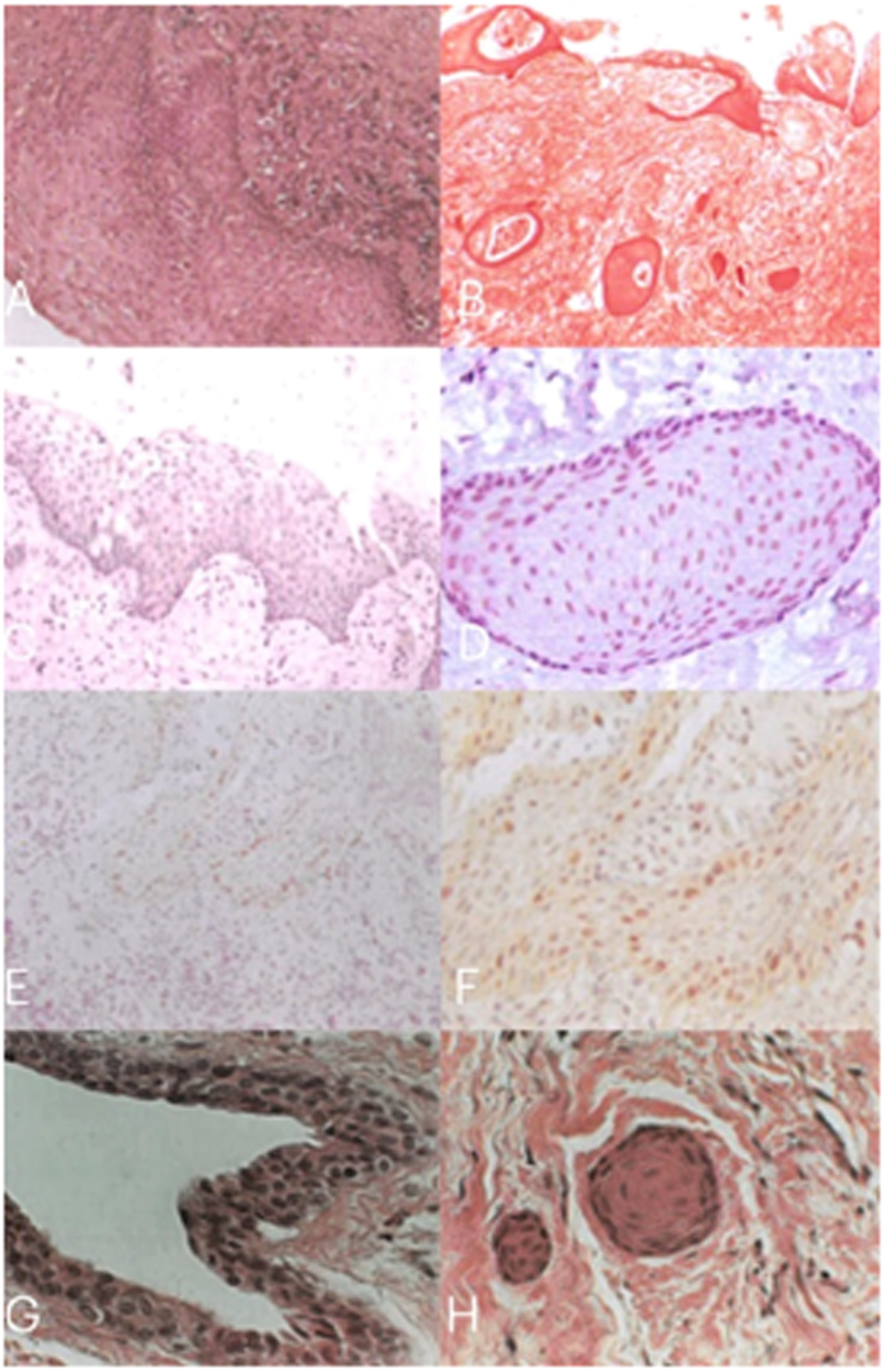

Fig. 1. **[A]** RC uniform epithelial thickness; areas of parakeratosis - Hematoxylin-eosin, 100x. **[B]** OCK stratified keratinized squamous epithelium of variable thickness; epithelial morphology: depends on the degree of inflammation; fibrous wall, cholesterol crystals, hemosiderin deposits. Hematoxylin-eosin, 100x. **[C]** OCK ki-67 positive cells exclusively in the basal layer, 100x. **[D]** OCK epithelial island: ki-67 positive cells predominantly located in the basal layers, but they are often found in the suprabasal layers as well, 200x. **[E]** RC ki-67 positive cells predominantly located in the basal layers, but they are often found in the suprabasal layers as well. Hematoxylin-eosin, 100x. **[F]** RC detail at 200x of figure E showing the presence of ki-67 (brown areas). **[G]** OCK epithelial layer lining the cystic lumen. Hematoxylin-eosin, 630x. **[H]** OCK detail of keratin pearls. Hematoxylin-eosin, 100x.

demonstrated that the Ki67 antigen can be reliably detected in a variety of tissue types, including fresh tissue samples and frozen sections, as well as in tissue samples that have been treated with formalin for fixation and then subsequently processed. The versatility of Ki67 detection across different tissue preparation techniques further underscores its utility as a dependable marker of cell proliferation. Importantly, the presence of the Ki67 antigen is widely considered a valid and accurate indicator of proliferative activity in cells, particularly in pathological conditions where altered cell division rates are a hallmark. The qualitative and quantitative differences observed in the proliferative activity of various epithelial tissues, such as the cystic epithelium, are indicative of the intrinsic growth potential of these

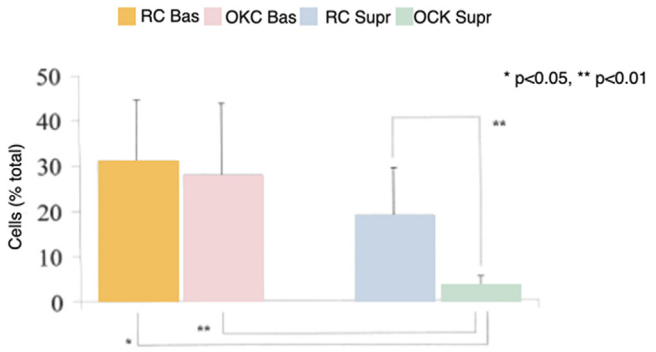

Fig. 2. Cell positive for Ki67 in relation to the lesion and the originating layer. Mean ± SD.

tissues. This intrinsic potential plays a crucial role in shaping the biological behavior and evolution of lesions, particularly in conditions involving cyst formation and progression [5,15].

In the context of our study, our findings are consistent with the existing body of research that shows a marked difference in the distribution of Ki67+ cells between radicular cysts (RCs) and odontogenic keratocysts (OKCs). Specifically, Ki67+ cells are more commonly found in the basal and suprabasal layers of the epithelium in RCs, whereas in OKCs, the Ki67+ cells are primarily restricted to the basal layer of the cystic epithelium. This distinctive pattern of cell proliferation in the basal layer of OKCs as compared to RCs is reflective of the biological differences between these two types of cysts. The higher prevalence of proliferating cells in the basal layer of OKCs could be a contributing factor to the aggressive behavior and higher recurrence rate of OKCs. This proliferative activity, particularly in the basal layer, might also explain the difficulty in completely eradicating OKCs, as remnants of proliferative epithelial islands are often left behind following surgical excision. The presence of these residual epithelial cells could potentially drive recurrence, making it a significant consideration in the clinical management of OKCs [16,17].

The complex relationship between cell proliferation and the recurrence of cysts has been a topic of substantial interest and debate within the scientific community. Numerous studies have explored whether increased cell proliferation directly correlates with the likelihood of cyst recurrence. Some research suggests that there is no direct correlation between recurrence and heightened proliferative activity, while other studies have presented data indicating that OKCs exhibit higher rates of cell proliferation compared to RCs. This increased proliferative potential in OKCs may be linked to the cyst's propensity for recurrence, particularly when epithelial remnants are left behind post-surgery. The ongoing debate highlights the need for further research into the mechanisms that drive cyst recurrence and the role of cell proliferation in this process [18–20].

However, the immunohistochemical analysis carried out in this experimental study provides compelling evidence that odontogenic keratocysts can be clearly differentiated from radicular cysts based on their proliferative profiles. The use of Ki67 as a proliferation marker in this context has been instrumental in identifying these differences, reinforcing the notion that OKCs have a greater intrinsic proliferative potential than RCs [21,22]. This finding is particularly relevant when considering the biological behavior of daughter cysts, which have been shown to exhibit an even higher proliferative potential than their parent cysts. This phenomenon of enhanced proliferative activity in daughter cysts could be a key factor underlying the high recurrence rate of OKCs, especially when epithelial islands are inadvertently left behind during surgical excision. Therefore, careful consideration should be given to the complete removal of these cystic lesions to minimize the risk of recurrence and prevent the further proliferation of residual epithelial cells. Contrary to expectations, our results showed significantly fewer Ki-67-positive cells in odontogenic keratocysts (OKCs) compared to radicular cysts (RCs), suggesting that cellular proliferation alone does not explain the aggressive behavior and high recurrence rate of OKCs. While Ki-67 is a widely recognized marker of cellular proliferation, several studies indicate that additional molecular mechanisms play a crucial role in OKC behavior. The remodeling of the extracellular matrix, driven by increased expression of metalloproteinases such as ADAMTS-1, and proteoglycans like versican and aggrecan, has been implicated in tissue invasion and tumor progression [23]. Furthermore, chronic inflammation and the presence of myofibroblasts in the stromal environment contribute to the destructive potential of OKCs, promoting recurrence independent of their proliferation rate. A recent study by Saif et al. [24] specifically examined the immunohistochemical expression of Ki-67 in OKCs and found that although most cases exhibited low Ki-67 expression, certain foci displayed strong expression in areas of epithelial atypia. However, the study concluded that there was no significant association between Ki-67 expression and the histological characteristics of OKCs, reinforcing the idea that other molecular factors contribute to their aggressive behavior. Thus, while Ki-67 is a useful tool for assessing proliferation, further molecular pathways must be explored to fully understand the biological behavior of this pathology.

Future research should focus on the regulatory mechanisms governing cell proliferation in cystic lesions, exploring the roles of key

Table 2
Significance of the differences in KI67 levels from the cysts under examination. Data presented as mean ± SD of the % of positive cells (n = 16). Significance levels for the Mann-Whitney test: $^{*}p < 0.05$, $^{**}p < 0.01$.

Site	Radicular cysts	Odontogenic keratocysts	Radicular cysts vs Odontogenic keratocysts	P-value for Mann-Whitney test
Basal	31.1 ± 13.7	27.9 ± 15.9	*	$P < 0.05$ =*
Suprabasal	18.9 ± 10.4	3.3 ± 2.0	**	$P < 0.01$ = **

Table 3
Significance of the differences in Ki67 levels from the cysts under examination. Data calculated after assigning the respective ranks (n = 16). Significance levels for the Mann-Whitney test: $^{*}p < 0.05$, $^{**}p < 0.01$.

Site	Radicular cysts			Odontogenic keratocysts			
	Median	25th p	75th p	Median	25th p	75th p	RC vs OKC
Basal	4	3	4	3	3	4	*
Suprabasal	3	3	3	1	1	1,25	**
Basal vs Suprabasal	*			**			

markers such as Ki67, p53, and bcl-2 in cellular homeostasis and pathology. Such studies will enhance our understanding of the pathophysiology of cystic lesions and improve diagnostic and therapeutic approaches.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Maurizio D'Amario: Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **Tommaso Pizzolante:** Writing – original draft, Investigation, Formal analysis, Data curation. **Claudio Magnacca:** Methodology, Investigation, Data curation. **Ilaria Mariani:** Writing – original draft, Investigation, Data curation. **Mario Capogreco:** Writing – review & editing, Validation, Conceptualization. **Ettore Lupi:** Writing – review & editing, Validation, Supervision.

References

[1] Kaplan I, Anavi Y, Hirshberg A. Glandular odontogenic cyst: a challenge in diagnosis and treatment. Oral Dis 2008;14(7):575–81 Oct.
[2] Philipsen HP, Reichart PA. Classification of odontogenic tumours. A historical review. J Oral Pathol Med 2006;35(9):525–9 Oct.
[3] Kumar P, Malhotra R, Urs AB, Augustine J, Mohanty S. Epidermoid cysts of the orofacial region: a clinico-pathological study of 13 cases with review of literature. Indian J Pathol Microbiol 2023;66(3):568–72 Jul-Sep.
[4] Thompson L. World health organization classification of tumours: pathology and genetics of head and neck tumours. Ear Nose Throat J 2006;85(2):74. Feb.
[5] Sasaki K, Murakami T, Kawasaki M, Takahashi M. The cell cycle associated change of the ki-67 reactive nuclear antigen expression. J Cell Physiol 1987;133(3):579–84 Dec.
[6] Thavarajah R, Mudimbaimannar VK, Elizabeth J, Rao UK, Ranganathan K. Chemical and physical basics of routine formaldehyde fixation. J Oral Maxillofac Pathol 2012;16(3):400–5 Sep.
[7] Titford M. Progress in the development of microscopical techniques for diagnostic pathology. J Histotechnol 2009;32(1):9–19.
[8] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000;182(3):311–22 Mar.
[9] Li LT, Jiang G, Chen Q, Zheng JN. Ki67 is a promising molecular target in the diagnosis of cancer (review). Mol Med Rep 2015;11(3):1566–72 Mar.
[10] Urruticoechea A, Smith IE, Dowsett M. Proliferation marker ki-67 in early breast cancer. J Clin Oncol 2005;23(28):7212–20 Oct 1.
[11] Urs AB, Kumar P, Singh S, Mohanty S, Chaudhary Z. Odontogenic keratocysts: a retrospective histopathological study. Natl J Maxillofac Surg 2024;15(1):136–41 Jan-Apr.
[12] Pylkkö J, Willberg J, Suominen A, Laine HK, Rautava J. Appearance and recurrence of odontogenic keratocysts. Clin Exp Dent Res 2023;9(5):894–8 Oct.
[13] Slootweg PJ. p53 protein and ki-67 reactivity in epithelial odontogenic lesions. An immunohistochemical study. J Oral Pathol Med 1995;24(9):393–7 Oct.
[14] Macagno N, Sohier P, Kervarrec T, Pissaloux D, Jullie ML, Cribier B, Battistella M. Recent advances on immunohistochemistry and molecular biology for the diagnosis of adnexal sweat gland tumors. Cancers (Basel) 2022;14(3):476. Jan 18.
[15] Alsaegh MA, Mahmoud O, Varma SR, Mathew A, Altaie AM, Zhu S. P63 and ki-67 expression in radicular cyst. J Oral Biol Craniofac Res 2023;13(5):575–80 Sep-Oct.
[16] Modi TG, Chalishazar M, Kumar M. Expression of Ki-67 in odontogenic cysts: a comparative study between odontogenic keratocysts, radicular cysts and dentigerous cysts. J Oral Maxillofac Pathol 2018;22(1):146. Jan-Apr.
[17] Li TJ, Yu SF. Clinicopathologic spectrum of the so-called calcifying odontogenic cysts: a study of 21 intraosseous cases with reconsideration of the terminology and classification. Am J Surg Pathol 2003;27(3):372–84 Mar.
[18] Kaczmarzyk T, Mojsa I, Stypulkowska J. A systematic review of the recurrence rate for keratocystic odontogenic tumour in relation to treatment modalities. Int J Oral Maxillofac Surg 2012;41(6):756–67 Jun.
[19] Mateus GC, Lanza GH, de Moura PH, Marigo Hde A, Horta MC. Cell proliferation and apoptosis in keratocystic odontogenic tumors. Med Oral Patol Oral Cir Bucal 2008;13(11):E697–702 Nov 1.
[20] Soluk Tekkeşın M, Mutlu S, Olgaç V. Expressions of bax, bcl-2 and ki-67 in odontogenic keratocysts (Keratocystic Odontogenic Tumor) in comparison with ameloblastomas and radicular cysts. Turk Patoloji Derg 2012;28(1):49–55.
[21] Stoll C, Stollenwerk C, Riediger D, Mittermayer C, Alfer J. Cytokeratin expression patterns for distinction of odontogenic keratocysts from dentigerous and radicular cysts. J Oral Pathol Med 2005;34(9):558–64 Oct.
[22] Kamath KP, Vidya M. Cytokeratin 19 expression patterns of dentigerous cysts and odontogenic keratocysts. Ann Med Health Sci Res 2015;5(2):119–23 Mar-Apr.
[23] de Souza Neto OR, de Moraes ATL, Fuzii HT, Maneschy Faria AG, Freitas VM, da Silva Kataoka MS, de Melo Alves Jr S, de Jesus Viana Pinheiro J. Gene expression and immunohistochemistry analysis of ADAMTS-1 and its substrates in odontogenic keratocyst. Diagn Pathol 2025;20(1):1. Jan 7.
[24] Saif R, Jehangir HM, Nagi AH, Naseem N, Rizvi Z, Rasul F. Immunohistochemical expression of ki-67 in odontogenic keratocyst: evidence of aggressive behavior. Professional Med J 2020;27(1):74–9.