Original Article

Expression of PTCH gene in ameloblastoma and odontogenic keratocyst: A comparative study

Sharada Srinagesh[1], Sahana NS[2], Suganya G[2], Renuga S[2], Srikant Natarajan[3], Meghashyama Kulkarni[2]

[1]Government Dental College and Research Institute, [2]Department of Oral Pathology and Microbiology, Government Dental College and Research Institute, Bengaluru, [3]Department of Oral Pathology and Microbiology, Manipal College of Dental Sciences, Mangalore, Karnataka, India

Abstract

Aim: This is a cross-sectional comparative study, aimed to quantify the expression of patched (PTCH) gene in ameloblastoma, odontogenic keratocyst (OKC) and also the comparison of both the expressions.

Materials and Methods: Genomic deoxyribonucleic acid (DNA) was extracted and quantified, and the expression of the PTCH gene was done in 17 cases of ameloblastoma and 17 cases of OKC by quantitative real-time polymerase chain reaction (RT-qPCR).

Results: It was observed that there was an overexpression of the PTCH gene in both ameloblastoma and OKC with a good mean cycle threshold (CT) value of 32.71 ± 2.432 and 34.69 ± 1.875, respectively. When comparing the PTCH expression between the two, ameloblastoma showed higher expression than the OKC and the difference is statistically significant with *P* value of 0.025.

Conclusions: Our findings suggest that there is overexpression of PTCH in ameloblastoma and OKC, but it is highly expressed in ameloblastoma when compared to OKC. Overexpression of PTCH may constitute the activation of the Sonic Hedgehog pathway and may suggest the mechanism for the development of ameloblastoma and OKC. Hence it can be used as a valuable marker for early diagnosis and in the identification of therapeutic targets.

Keywords: Ameloblastoma, mutation, odontogenic keratocyst, PTCH

Address for correspondence: Dr. Renuga S, Government Dental College and Research Institute, Victoria Hospital Campus, Kalasipalaya, Bangalore - 560 002, Karnataka, India.
E-mail: renugasampath2795@gmail.com

Submitted: 06-May-2022, **Revised:** 08-Jun-2022 **Accepted:** 06-Jul-2022, **Published:** 13-Jul-2023

INTRODUCTION

Odontogenic epithelium under physiological conditions will form tooth but it can also give rise to diverse types of tumours in the jaws.[1] These tumours arising from odontogenic apparatus or its remnants can be classified into benign or malignant entities based on their histological presentations.[2] Amongst them, ameloblastoma is the most common benign tumour, which does not differentiate until the level of enamel formation and arises from the odontogenic apparatus. These tumours represent only 1% of all jaw tumours.[3] Numerous studies attempted to describe the pathogenesis of ameloblastoma at a molecular level. Cellular changes like proliferation, differentiation, senescence, tumourigenesis, etc., transpire through inactivation or activation of the related molecular signalling pathways. The significant signalling molecules are either under expressed or over expressed during tumourigenesis of ameloblastoma. The aetiology

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_198_22

How to cite this article: Srinagesh S, Sahana NS, Suganya G, Renuga S, Natarajan S, Kulkarni M. Expression of PTCH gene in ameloblastoma and odontogenic keratocyst: A comparative study. J Oral Maxillofac Pathol 2023;27:427.

of ameloblastoma is said to be unclear.[1] Clonality, cell cycle proliferation, apoptosis, tumour suppressor genes, osteoclastic mechanisms, matrix metalloproteinases and various signalling pathways are the postulates used to explain the pathogenesis of ameloblastoma.[4]

Philipsen was the first to describe odontogenic keratocysts (OKCs) in 1956, as benign intraosseous lesions of odontogenic origin which account for about 10% of jaw cysts.[5] Ameloblastoma and OKC are heterogeneous lesions affecting the jaw bones causing proliferation of remnants of dental lamina having an infiltrative growth pattern leading to local bone destruction with a high recurrence rate.[6]

The Drosophila polarity patched gene (PTC) encodes an integral membrane I protein with 12 putative transmembrane domains (Hooper and Scott, 1989). This protein participates in segment polarity in Drosophila development and is implicated in the Hedgehog (HH) signal transduction pathway in arthropods and vertebrates. The human homologue of the PTC gene (PTCH) has been described and mapped to chromosome 9q22.3-q-3 1.[7] PTCH expression is intended to down-regulate and stop the signalling triggered by the arrival of the Sonic Hedgehog (SHH) secreted protein at the cell membrane.[4,5] This auto-regulating PTCH role, which ensures restricted programmed proliferation, would fail if the gene is altered by deletions or mutations. The result is uncontrolled cell proliferation, as well as a continuous and useless synthesis of the non-functional PTCH protein.[1] Aberrant activation of PTCH via SHH signalling pathway during adult life is related to tumour formation and disturbances in this act as a potential factor involved in the pathogenesis of odontogenic tumours.[8]

PTCH is only expressed during embryonic development.[9] Later, it will be down regulated and their expression will not be detected. As all the known mutations in PTCH affect the function but not the stability of its protein.[10] It is believed that this antibody mainly detects the mutant PTCH in adult tissue.

This study aims at evaluating the potential role of PTCH in OKC, ameloblastoma and comparing the same with each other thereby providing novel information about diagnostic as well as treatment modalities. It would help in specific targeted therapy and serve as a novel target for chemotherapeutic intervention or other molecular targeted therapy to prevent or limit the extensive surgical intervention and prognosis. Hence, PTCH expression may prove useful in assessing in comparing the biological behaviour of these odontogenic lesions.

MATERIALS AND METHODS

The study was conducted at the Department of Oral Pathology and Microbiology, Government Dental College and Research Institute, Bangalore, India between 5 January and 1 March 2022.

The study protocol was approved by the Institutional Ethical Committee of Government Dental College and Research Institute, Bangalore, India Country (Protocol No. GDCRI/IEC-ACM[2]/11/2020-2021).

Sample

A total of 34 cases were selected from the archives of the Department of Oral Pathology, GDCRI Bangalore. Cases were divided into two groups.

Group I: 17 histopathologically diagnosed cases of ameloblastoma and Group II: 17 histopathologically diagnosed cases of OKC.

Method: 10 µm sections were cut from each formalin-fixed paraffin-embedded tissue block using a microtome [Figure 1] and subsequently placed in 1.5 ml Eppendorf tubes [Figure 2]. The sections were then deparaffinized by keeping the tissue section in the microwave oven at 65°C for 15 min [Figure 3]. Later 1 ml of xylene was added to the tissue sections in the Eppendorf tubes to dissolve the paraffin and centrifuged at 14,000 rpm for 3 min after which the supernatant was carefully pipetted out and the process was repeated thrice. Residual xylene was removed and tissue rehydration was done by adding 1 ml of absolute ethanol centrifuged at 14,000 rpm for 3 min, later the supernatant was removed by pipetting and this process was also repeated thrice. The pellets were vacuum dried completely. The tissue pellets then were re-suspended by adding 180 microlitre

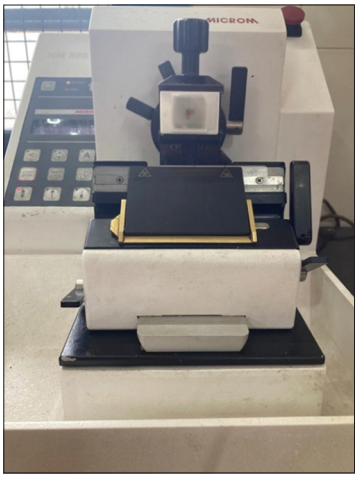


Figure 1: Microtome machine

of lysis solution (AL) (DS0015) and incubated at room temperature for 30 min.

Deoxyribonucleic acid (DNA) extraction

DNA was extracted from deparaffinized tissue pellets by adding 20 microlitre of proteinase K solution (20 mg/ml) and mixed by vortexing and incubated at 56°C overnight. After overnight incubation, it was centrifuged at the speed of 20,000*g* at 15,000 rpm for 5–10 min and the supernatant was transferred into a new 2.0 ml capped microcentrifuge tube [Figure 4]. 20 microlitre of RNase A solution (DS0003) was added and mixed by pulse vortexing thoroughly for 15 s and incubated for 2 min at room temperature. 200 microlitre of lysis solution (C1) (DS0010) was added and mixed by vortexing thoroughly for 15 s and incubated at 70°C for 10 min. Later 200 microlitre of absolute ethanol was added to the lysate and mixed thoroughly by vortexing for 15s. The lysate obtained was transferred to the HiElute Miniprep spin column and incubated at room temperature for 5 min. Later the lysate was centrifuged at 6,500*g* at 10,000 rpm for 1 min. After this, centrifugation flow was reloaded through the liquid onto the column again and centrifuged at 10,000 rpm for 1 min. The flow-through was discarded and paced into the same column. Wash solution is prepared, and 500 microlitre of wash solution was added to the column and centrifuged at 10,000 rpm for 1 min [Figure 5]. The flow-through liquid was discarded, and the column was placed in the same collection tube. Later another 500 microlitre of diluted wash solution was added to the column and centrifuged at 20,000*g* (15,000 rpm) for 3 min. The collection tube containing flow-through liquid was discarded, and the column was placed in a new 2.0 ml uncapped collection tube. DNA elution was done by adding 50–100 microlitre of the elution buffer directly onto the column and incubated for 5 min at room temperature and centrifuged at 6,500*g* (10,000 rpm) for 1 min.

DNA amplification

The specific primer sequences of both forward (F) and reverse (R) primer were commercially obtained. A total

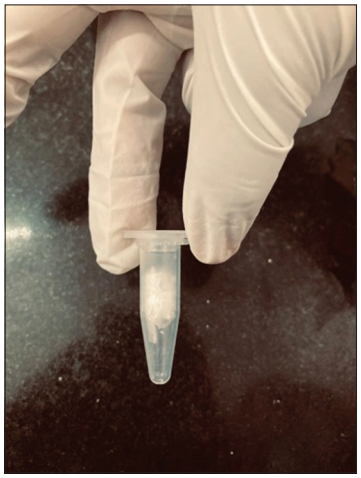


Figure 2: 10 µm tissue section placed in 1.5 ml Eppendorf tube

Figure 3: Deparaffinization in a hot air oven

Figure 4: Addition of proteinase K

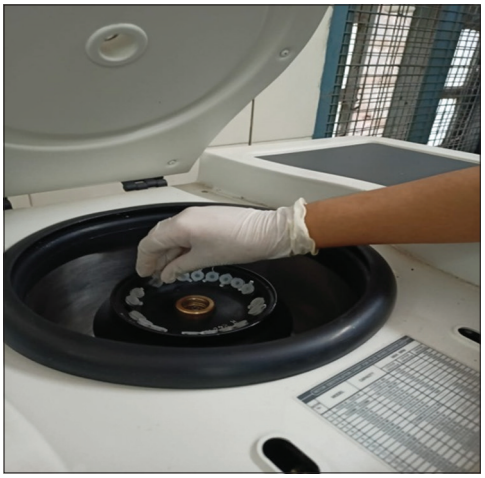


Figure 5: Centrifuge machine

of 10 µl reaction volume per well was added to the 96 well-plate. The reaction volume of 10 µl constitutes 1 µl primer, 1 µl extracted DNA, 5 µl of master mix (SYBR Green) and 3 µl of nuclease-free water. The 96 well-plate with samples was sealed and loaded in an Applied Biosystem Step One Plus™ Real-Time PCR system Thermal Cycling Block (S/N: 2720010242) [Figure 6]. Initial denaturation at 98°C for 5 min, then 35 cycles of final denaturation at 94°C for 30 s, annealing at 58°C, extension at 72°C for 45 s, and final extension at 72°C for 5 min followed by cooling for 5 min was set to run.

Statistical analysis

Parametric data of cycle threshold (CT) value of PTCH gene expression in ameloblastoma and OKC were expressed as mean (M) and standard deviation (SD). And for comparison of PTCH expression between ameloblastoma and OKC independent t-test was applied. The statistical significance level was defined at $P = 0.05$.

RESULTS

To characterise the expression of PTCH gene in ameloblastoma and OKC, we used qRT-PCR on genomic DNA isolated from 17 cases of ameloblastoma and 17 cases of OKC. Both ameloblastoma and OKC expressed PTCH gene with good mean CT value of 32.71 ± 2.432 and 34.69 ± 1.875, respectively [Graph 1a-c]. In comparison between the two lesions, the expression PTCH gene is more in ameloblastoma with good CT value than in OKC [Figure 7]. And the difference is statistically significant with the *P* value 0.025 [Tables 1 and 2].

DISCUSSION

Many genes through signalling pathways participate actively in the formation of human embryonic tissues which disappear in adult cells, when there is an aberrant activation of these genes, it leads to the tumour formation.

The protein patched homologue 1 (PTCH-1), a member of SHH pathway plays an inevitable role in tooth development and is also involved in the pathogenesis of odontogenic tumour. They are also involved in the induction or promotion of carcinogenesis in organs. The study on the high expression of PTCH is significant because certain therapeutic compounds have been proven as effective antagonists of the

Table 1: Individual P value of CT value of Ameloblastoma and OKC

Group	Kolmogorov–Smirnov			Shapiro–Wilk		
	Statistic	df	Sig.	Statistic	df	*P*
Ameloblastoma CT value	0.179	15	0.200*	0.903	15	0.104
OKC	0.143	13	0.200*	0.945	13	0.528

*This is a lower bound of the true significance. Lilliefors Significance Correction

Table 2: Statistically significant p value on comparison of CT value of Ameloblastoma and OKC

	Mean±SD		*t*	*P*
	Ameloblastoma (*n*=15)	OKC (*n*=13)		
CT value	32.72±2.43	34.69±1.88	−2.373	0.025

Independent *t*-test. A comparison of the CT value between the two groups shows that CT value is higher in the OKC group with a *t*-value of-2.373 and is statistically significant with a *P* value of 0.025.
Note: lower the CT, the greater the expression will be

Figure 6: RT-PCR machine

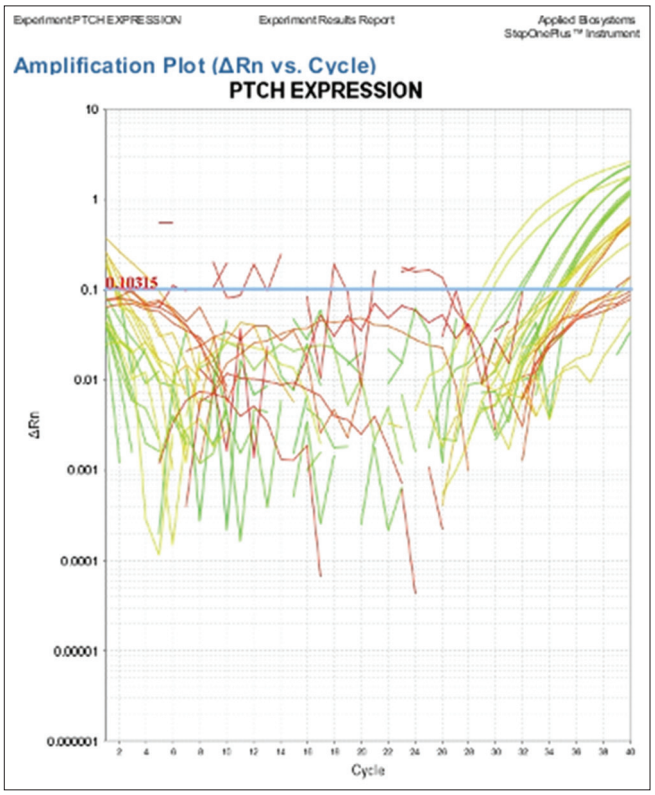
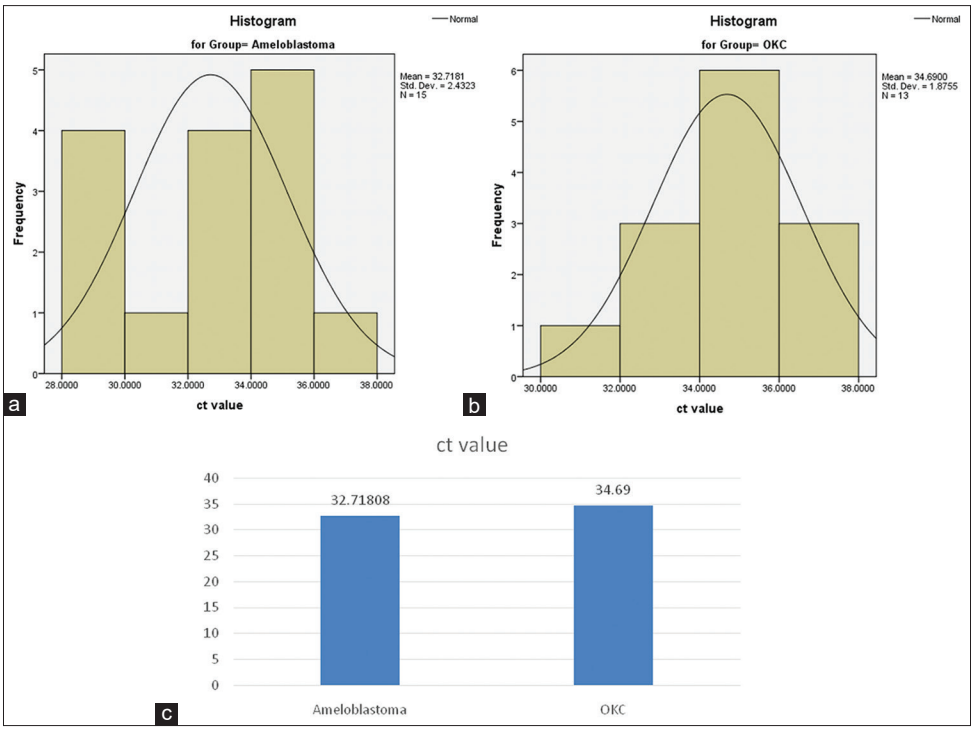


Figure 7: Amplification graph for PTCH expression



Graph 1: (a) Histogram depicting the mean CT value of PTCH gene expression in ameloblastoma. (b) Histogram depicting the mean CT value of PTCH gene expression in OKC. (c) Comparison of mean CT value of PTCH gene expression in ameloblastoma and OKC

PTCH in the SHH pathway. Cyclopamine, for example, acts at the level of SHH signalling and it is effective in reducing the viability of cancer cells by blocking the activation of the SHH response pathway and abnormal cell growth.[11,12]

A study by DeVilliers *et al*.[10] investigated microgenomic profile of ameloblastoma via several molecular methods reported that most members of the SHH pathway were under expressed except for PTCH which was over expressed by two-folds. They additionally validated the protein expression in their study by performing immunohistochemistry. They also suggested that overexpression of PTCH could be utilised for chemotherapeutics. In contrast, Heikinheimo *et al*.[11] investigated ameloblastoma through diverse molecular methods and reported that there exists under-expression of PTCH and other SHH pathway members. Shiori Kanda *et al*. and Ren *et al*.[12,13] documented that the proliferation of the ameloblastoma AM-1 cell line was significantly inhibited in the presence of SHH neutralising antibody and cyclopamine. OKC, which is in the same odontogenic tumour group as ameloblastoma, also has PTCH dysfunction implicated in its pathogenesis, and it has been shown to respond significantly to cyclopamine in a dose-dependent manner by targeting the SHH pathway. Rosenstein *et al*. and Samuel Ebele Udeabor *et al*. suggested that there exists strong expression for PTCH in ameloblastoma and it is comparable to the expression in other neoplastic odontogenic cysts documented that the percentage of cells in the cell cycle was higher in cystic ameloblastoma compared to the solid variant.[14] Although cystic ameloblastoma may appear innocuous, it has higher biological behaviour at the ameloblast cellular level and may thus have a higher expression of PTCH which is a gene related to proliferation.[15] Johnson *et al*. reported that the PTCH gene can be considered as a candidate gene to treat basal cell nevus syndrome, otherwise called Gorlin syndrome, and in other abnormalities which are linked to mutation and/or overexpression of PTCH in conditions such as colonic neoplasia, OKCs and ameloblastomas.[16] It is suggested that expression of the tumour suppressor PTCH and the oncogene SHH may cause loss of PTCH function and so lead to OKC development by stimulating continuous proliferation of dental lamina cells instead of their normal degeneration.[6,17] HoyosCadavid *et al*.[18] suggested that all major subtypes of odontogenic cysts might express PTCH. Through our study, we are reporting that in both ameloblastoma and OKC, the PTCH is expressed significantly with a good CT value. In comparison to the level of expression, ameloblastoma showed higher expression of PTCH when compared to OKC. One of the reasons for the recurrence of these lesions is attributed to genetic mutation of the PTCH gene; hence, targeted therapies in this direction can help in the management of the large recurrent lesions.

Limitations of the study

The present study was performed in a lesser degree with small sample groups. More molecular studies in different

geographical areas should be encouraged for a better understanding of the cysts and tumours' pathogenesis to provide a targeted therapeutic approach.

CONCLUSIONS

The quantification of PTCH gene expression using the RT-qPCR showed that there exists a significant expression of the PTCH gene in both ameloblastoma and OKC. Comparatively, TCH gene is expressed higher in ameloblastoma than the OKC. The quantification and elaborate molecular analysis of gene expression which is involved in the signalling pathway of pathogenesis of cysts and tumours can be utilised for prognostic or therapeutic predilection.

Financial support and sponsorship

Received UG grant from the Rajiv Gandhi University of Health Science.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Mishra P, Panda A, Bandyopadhyay A, Kumar H, Mohiddin G. Sonic hedgehog signalling pathway and ameloblastoma–a review. J Clin Diagn Res 2015;9:ZE10.
2. Wright JM, Vered M. Update from the 4th edition of the World Health Organization classification of head and neck tumours: Odontogenic and maxillofacial bone tumors. Head Neck Pathol 2017;11:68-77.
3. Onyegbula K, Onile OS, Okoje VN, Anumudu CI. Developmental signaling genes in ameloblastoma. Afr J Cancer 2015;7:16-21.
4. Gomes CC, Duarte AP, Diniz MG, Gomez RS. Current concepts ofameloblastoma pathogenesis. J Oral Pathol Med 2010;39:585-91.
5. Andrea B, Cosimo N, Caterina G. Odontogenickeratocyst: Imaging features of a benign lesion with an aggressive behavior. Insights Imaging 2018;9:883-97.
6. Barreto DC, Gomez RS, Bale AE, Boson WL, De Marco L. PTCH gene mutationsin odontogenickeratocysts. J Dent Res 2000;79:1418-22.
7. Kochaji N, Goossens A, Geerts A, Bottenberg P. PTCH expression in odontogenic cysts, a cause of pathogenesis or reason for clinical complication. Oral Oncol Extra 2005;41:284-8.
8. Dassule HR, Lewis P, Bei M, Maas R, McMahon AP. Sonic hedgehog regulates growth and morphogenesis of the tooth. Development 2000;127:4775-85.
9. Hardcastle Z, Mo R, Hui CC, Sharpe PT. The Shhsignalling pathway intooth development: Defects in Gli2 and Gli3 mutants. Development 1998;125:2803-11.
10. DeVilliers P, Suggs C, Simmons D, Murrah V, Wright JT. Microgenomics of ameloblastoma. J Dent Res 2011;90:463-9.
11. Heikinheimo K, Jee KJ, Niini T, Aalto Y, Happonen RP, Leivo I, *et al*. Gene expression profiling of ameloblastoma and human tooth germ by means of a cDNA microarray. J Dent Res 2002;81:525-30.
12. Kanda S, Mitsuyasu T, Nakao Y, Kawano S, Goto Y, Matsubara R, *et al*. Anti-apoptotic role of the sonic hedgehog signaling pathway in the proliferation of ameloblastoma. Int J Oncol 2013;43:695-702.
13. Ren C, Amm HM, DeVilliers P, Wu Y, Deatherage JR, Liu Z, MacDougall M. Targeting the sonic hedgehog pathway in keratocysticodontogenic tumor. J Biol Chem 2012;287:27117-25.
14. Rosenstein T, Pogrel MA, Smith RA, Regezi JA. Cysticameloblastoma—behavior and treatment of 21 cases. J Oral Maxillofac Surg 2001;59:1311-6.
15. Udeabor SE, Adisa AO, Lawal AO, Barbeck M, Booms P, Sader RA, *et al*. PTCH-1 and MDM2 expression in ameloblastoma from a West African sub- population: Implication for chemotherapeutics. Pan Afr Med J 2015;20:140.
16. Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, *et al*. Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 1996;272:1668-71.
17. Barreto DC, Bale AE, De Marco L, Gomez RS. Immunolocalization of PTCHprotein in odontogenic cysts and tumors. J Dent Res 2002;81:757-60.
18. HoyosCadavid AM, Kaminagakura E, Rodrigues MF, Pinto CA, Teshima TH, Alves FA. Immunohistochemical evaluation of Sonic Hedgehog signaling pathway proteins (Shh, Ptch1, Ptch2, Smo, Gli1, Gli2, and Gli3) in sporadic and syndromicodontogenickeratocysts. Clin Oral Investig 2019;23:153-9.