



# RANK, RANKL, and OPG in Dentigerous Cyst, Odontogenic Keratocyst, and Ameloblastoma: A Meta-Analysis

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The aim of this study was to assess and compare RANK, RANKL, and OPG immunoeexpression in dentigerous cyst, odontogenic keratocyst, and ameloblastoma. The protocol was registered in PROSPERO (CRD42018105543). Seven databases (Embase, Lilacs, LIVIVO, PubMed, Scopus, SciELO, and Web of Science) were the primary search sources and two databases (Open Grey and Open Thesis) partially captured the "grey literature". Only cross sectional studies were included. The JBI Checklist assessed the risk of bias. A meta-analysis with random effects model estimated the values from the OPG and RANKL ratio reported by the individual studies and respective 95% confidence intervals. The heterogeneity among studies was assessed with I<sup>2</sup> statistics. Only nine studies met the inclusion criteria and were considered in the analyses. The studies were published from 2008 to 2018. Two studies presented low risk of bias, while seven studies presented moderate risk. The meta-analysis showed the highest OPG>RANKL ratio for dentigerous cyst (ES=43.3%; 95% CI=14.3-74.8) and odontogenic keratocyst (ES=36.8%; 95% CI=18.8-56.7). In contrast, the highest OPG<RANKL ratio was found for ameloblastoma (ES=73.4%; 95% CI=55.4-88.4) and it was higher in the stromal region compared to the odontogenic epithelial region. The results may explain the aggressive potential of ameloblastoma from the higher OPG<RANKL ratio in this tumor, while it was lower for dentigerous cyst and odontogenic keratocyst.

## Introduction

Odontogenic cysts and tumors constitute one of the most important lesion groups of the oral and maxillofacial complex (1). Odontogenic tumors are rare and manifest from various clinical features, as well as different histopathological presentations of the epithelium and odontogenic ectomesenchyme (2). On the other hand, cysts originate from Malassez epithelial rests remaining from the dental blade or the reduced enamel epithelium (3). One of the most significant biological events in the pathogenesis of these lesions is osteoclastic cell activation, which results in bone resorption (4).

The balance between bone formation and resorption is required for bone homeostasis, so the tissue may fully function (5,6). Hence, the unbalance of this system has been associated with several bone neoplasias (7). Among the regulating factors of bone resorption, the system that stands out the most is composed by the receptor activator triad of nuclear factor kappa B (RANK), nuclear factor kappa B ligand (RANKL), and osteoprotegerin (OPG) (8). The RANK works as a signaling receptor of RANKL, while the latter is expressed in the osteoblastic cells of the periodontal ligament (9), binding to RANK and activating osteoclasts to promote bone resorption (10). On the other hand, OPG

inhibits the osteoclastogenic process, blocking the RANKL and RANK binding (11).

A tumor may invade bone tissue and affect the balance between resorption and apposition (12). Several studies (4,12-14) have been focused on investigating the correlation of the immunologic expression of RANK, RANKL, and OPG to the development of odontogenic cysts and tumors. However, the scientific literature is still controversial regarding the expression of such proteins in the development of these lesions. Therefore, this study aimed to assess and compare, through a systematic review of the literature, the immunoeexpression of RANK, RANKL, and OPG on dentigerous cyst, odontogenic keratocyst, and ameloblastoma, in order to verify whether the odontogenic keratocyst is more similar to the neoplastic or the cystic profile. The authors tested the following hypothesis: the odontogenic keratocyst will be more similar to the cystic profile.

## Material and Methods

### Protocol and Registration

This systematic review was performed according to the list of PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols) recommendations

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(15) and the Cochrane guidelines (16). The systematic review protocol was registered in the PROSPERO database under no. [CRD42018105543](#).

### *Study Design and Eligibility Criteria*

This study was a systematic review that aimed to answer the following guiding question: "Is the profile of the RANKL/OPG ratio in the odontogenic keratocyst, assessed by the immunoexpression of RANK, RANKL, and OPG, more similar to the neoplastic or the cystic profile?"

The studies included assessed the expression of the osteoclastogenic factors RANK, RANKL, and OPG in ameloblastoma, dentigerous cyst, and non-syndromic odontogenic keratocyst by immunohistochemistry, without restrictions of year, language, or publication status (In Press).

The following were excluded: 1) Studies involving inflammatory odontogenic cysts; 2) Studies not related to the topic; 3) Review studies, case reports, letters to the editor or editorials, congress abstracts, personal opinions, and books and/or book chapters; 4) Studies with high risk of bias.

### *Sources of Information and Search*

The Embase, Latin-American and Caribbean Health Sciences Literature (LILACS), LIVIVO, PubMed (including MedLine), SciELO, Scopus, and Web of Science databases were the primary study sources. OpenThesis and OpenGrey partially captured the "grey literature". A manual search was also performed through a systematized analysis of the references of the eligible articles. All steps aimed to minimize selection and publication biases.

The MeSH (Medical Subject Headings), DeCS (Health Sciences Descriptors), and Emtree (Embase Subject Headings) resources were used to select the search descriptors. The Boolean operators "AND" and "OR" enhanced the research strategy through several combinations (Table 1). The bibliographic research was performed in March 2019. The results obtained were exported to the EndNote Web™ software (Thomson Reuters, Toronto, Canada), in which duplicates were removed. The remaining results were exported to Microsoft Word™ 2010 (Microsoft™ Ltd, Redmond, WA, USA), in which the remaining duplicates were manually removed.

### *Study Selection*

The studies were selected in three different phases. In the first phase, as a calibration exercise, the reviewers discussed the eligibility criteria and applied them to a sample of 20% of the studies retrieved, so to determine inter-examiner agreement. After achieving a proper level of agreement ( $Kappa \geq 0.81$ ), two eligibility reviewers [IFPL

and FRM] performed a methodical analysis of the titles of the studies, independently. The reviewers were not blind to the names of authors and journals. Titles not related to the topic were eliminated in this phase. In phase 2, two reviewers [IFPL and FRM] also analyzed the abstracts systematically. Studies not related to the topic, review studies, case reports, letters to the editor or editorials, congress abstracts, personal opinions, and books and/or book chapters were excluded. The studies related to the topic, but without abstracts available were fully analyzed in the third phase.

In this phase, the full texts of preliminary eligible studies were analyzed to verify whether they fulfilled the eligibility criteria. When there was no agreement in the assessment, a third reviewer [LRP] was consulted to make a final decision. The studies rejected were registered separately, explaining the reasons for exclusion.

### *Process of Data Collection and Extraction*

After the selection, the studies were analyzed and two reviewers [IFPL and FRM] extracted their data for the following information: Identification of the study (author, year of publication, and study location); sample characteristics (number of cases); cysts and tumors assessed; ethical criteria involved; specimen fixation; diagnostic method used; immunoexpression of RANK, RANKL, and OPG; and correlation of RANKL and OPG.

To ensure the consistency among reviewers, both reviewers [IFPL and FRM] performed a calibration exercise, in which information were extracted jointly from an eligible study. The reviewers solved any disagreement through discussions, and when both reviewers disagreed, they consulted a third one [LRP] for a final decision.

### *Risk of Individual Bias of the Studies*

The risk of bias of the studies selected was assessed by the Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews Checklist for Analytical Cross Sectional Studies (17). Two authors [IFPL and FRM] assessed each domain independently and systematically regarding their potential risk of bias, as recommended by the PRISMA-P (15). The reviewers solved any disagreement through discussions, and when both reviewers disagreed, they consulted a third one [LRP] for a final decision.

The risk of bias was ranked as High when the study reached up to 49% of "yes" score, Moderate when the study reached from 50% to 69% of "yes" score, and Low when the study reached over 70% of "yes" score.

### *Summary Measures and Syntheses of Results*

A meta-analysis using random effects estimated the pooled values calculated by the ratio between OPG and

Table 1. Search strategies in the databases

Databases	Search strategies (March, 2019)	Results
PubMed <a href="http://www.ncbi.nlm.nih.gov/pubmed">http://www.ncbi.nlm.nih.gov/pubmed</a>	("Odontogenic Tumors"[MeSH Terms] OR "Odontogenic Tumors"[All Fields] OR "Odontogenic Tumor"[All Fields] OR "Dental Tissue Neoplasms"[All Fields] OR "Odontogenic Cysts"[MeSH Terms] OR "Odontogenic Cysts"[All Fields] OR "Cyst, Odontogenic"[All Fields]) AND ("RANK Ligand"[MeSH Terms] OR "RANK Ligand"[All Fields] OR "Osteoclast Differentiation Factor"[All Fields] OR "RANKL Protein"[All Fields] OR "Osteoprotegerin"[MeSH Terms] OR "Osteoprotegerin"[All Fields] OR "OPG"[All Fields] OR "Osteoclastogenesis Inhibitory Factor"[All Fields] OR "RANKL"[All Fields])	46
LILACS <a href="http://lilacs.bvsalud.org/">http://lilacs.bvsalud.org/</a>	(Odontogenic Tumors AND RANK) AND (instance:"regional") AND ( db:("LILACS"))	8
	(Odontogenic Tumors AND RANKL) AND (instance:"regional") AND ( db:("LILACS"))	1
	(Odontogenic Tumors AND Osteoprotegerin) AND (instance:"regional") AND ( db:("LILACS"))	0
	(Odontogenic Tumors AND OPG) AND (instance:"regional") AND ( db:("LILACS"))	1
	(Odontogenic Cysts AND RANK) AND (instance:"regional") AND ( db:("LILACS"))	5
	(Odontogenic Cysts AND RANKL) AND (instance:"regional") AND ( db:("LILACS"))	1
	(Odontogenic Cysts AND Osteoprotegerin) AND (instance:"regional") AND ( db:("LILACS"))	1
	(Odontogenic Cysts AND OPG) AND (instance:"regional") AND ( db:("LILACS"))	1
	(Dental Tissue Neoplasms AND RANK) AND (instance:"regional") AND ( db:("LILACS"))	7
	(Dental Tissue Neoplasms AND RANKL) AND (instance:"regional") AND ( db:("LILACS"))	1
(Dental Tissue Neoplasms AND Osteoprotegerin) AND (instance:"regional") AND ( db:("LILACS"))	0	
(Dental Tissue Neoplasms AND OPG) AND (instance:"regional") AND ( db:("LILACS"))	1	
Embase <a href="https://www.embase.com/">https://www.embase.com/</a>	('odontogenic tumors'/exp OR 'odontogenic tumors' OR 'odontogenic tumor'/exp OR 'odontogenic tumor' OR 'dental tissue neoplasms' OR 'odontogenic cysts'/exp OR 'odontogenic cysts' OR 'cyst, odontogenic'/exp OR 'cyst, odontogenic') AND ('rank ligand'/exp OR 'rank ligand' OR 'osteoclast differentiation factor'/exp OR 'osteoclast differentiation factor' OR 'rankl protein' OR 'osteoprotegerin'/exp OR 'osteoprotegerin' OR 'opg' OR 'osteoclastogenesis inhibitory factor'/exp OR 'osteoclastogenesis inhibitory factor' OR 'rankl'/exp OR 'rankl')	50
Scopus <a href="https://www.scopus.com/">https://www.scopus.com/</a>	( ("Odontogenic Tumors" OR "Dental Tissue Neoplasms" OR "Odontogenic Cysts" ) AND ( "RANK Ligand" OR "Osteoclast Differentiation Factor" OR "RANKL Protein" OR "Osteoprotegerin" OR "OPG" OR "Osteoclastogenesis Inhibitory Factor" OR "RANKL" ) )	49
LIVIVO <a href="https://www.livivo.de">https://www.livivo.de</a>	("Odontogenic Tumors" OR "Odontogenic Tumor" OR "Odontogenic Cysts" OR "Odontogenic Cyst" OR "Dental Tissue Neoplasms") AND ("RANK Ligand" OR "RANKL Protein" OR "Osteoprotegerin" OR "OPG")	34
SciELO <a href="http://www.scielo.org/">http://www.scielo.org/</a>	Dental Tissue Neoplasms AND RANK Ligand	0
	Dental Tissue Neoplasms AND RANKL	0
	Dental Tissue Neoplasms AND Osteoprotegerin	0
	Odontogenic Tumors AND RANK Ligand	0
	Odontogenic Tumors AND RANKL	0
	Odontogenic Tumors AND Osteoprotegerin	0
	Odontogenic Cysts AND RANK Ligand	0
Odontogenic Cysts AND RANKL	0	
Odontogenic Cysts AND Osteoprotegerin	0	
Web Of Science <a href="http://apps.webofknowledge.com/">http://apps.webofknowledge.com/</a>	("Odontogenic Tumors" OR "Dental Tissue Neoplasms" OR "Odontogenic Cysts" OR "Cyst, Odontogenic") AND ("RANK Ligand" OR "Osteoclast Differentiation Factor" OR "RANKL Protein" OR "Osteoprotegerin" OR "OPG" OR "Osteoclastogenesis Inhibitory Factor" OR "RANKL")	12
OpenGrey <a href="http://www.opengrey.eu/">http://www.opengrey.eu/</a>	"Odontogenic Tumors" OR "Odontogenic Cysts"	1
	("Odontogenic Tumors" OR "Odontogenic Tumor") AND ("RANK" OR "RANK Ligand")	0
	("Odontogenic Tumors") AND ("RANK")	0
	("Odontogenic Tumors") AND ("RANKL Protein")	0
	("Odontogenic Tumors") AND ("Osteoprotegerin" OR "OPG"))	0
OpenThesis <a href="http://www.openthesis.org/">http://www.openthesis.org/</a>	("Odontogenic Cysts") AND ("RANK")	0
	"Odontogenic Tumors" OR "Odontogenic Cysts"	25
	("Odontogenic Tumors" OR "Odontogenic Tumor") AND ("RANK" OR "RANK Ligand")	0
	("Odontogenic Tumors" OR "Odontogenic Tumor") AND ("RANKL" OR "RANKL Protein")	0
	("Odontogenic Tumors" OR "Odontogenic Tumor") AND ("Osteoprotegerin" OR "OPG")	0
OpenThesis <a href="http://www.openthesis.org/">http://www.openthesis.org/</a>	("Odontogenic Cysts" OR "Odontogenic Cyst") AND ("RANK" OR "RANK Ligand")	1
	("Odontogenic Cysts" OR "Odontogenic Cyst") AND ("RANKL" OR "RANKL Protein")	0
	("Odontogenic Cysts" OR "Odontogenic Cyst") AND ("Osteoprotegerin" OR "OPG")	0
Total		245

RANKL, which were reported as percentages along with the respective 95% confidence intervals and DerSimonian and Laird weights (16,18). The random model was used to minimize the heterogeneity effect between the studies (16,18). The variance of crude estimates was stabilized using the Freeman-Tukey double arcsine transformation (19). The heterogeneity among studies was assessed with  $I^2$  statistics and classified as follows: low ( $I^2 < 25\%$ ), moderate ( $I^2 = 50\%$ ), and high ( $I^2 > 75\%$ ) (20). The analyses were performed according to three different OPG and RANKL ratios (OPG > RANKL, OPG < RANKL, and OPG = RANKL), and according to two tissues (epithelium, and stroma). All estimates were assessed regarding the type of lesion. The analyses were performed with the Stata, version 15.1 (Stata Corp., College Station, USA).

## Results

### Study Selection

The first selection phase resulted in 245 studies distributed in nine electronic databases. After removing duplicates, 120 studies remained for the analysis of titles and abstracts. Then, after reading the titles, 15 studies continued to the analysis of abstracts. After analyzing the abstracts, only 11 studies were considered eligible for the full text reading. The references of the 11 studies were carefully assessed to check for studies retrieved through the main search strategy, but none was found. From the 11 studies included in this phase, two were removed for the following reasons: 1) use of Polymerase Chain Reaction (PCR) for the analysis; and 2) diagnostic study. Therefore, nine studies continued to the qualitative analysis of results. Figure 1 reproduces the process of search, identification, inclusion, and exclusion of articles.

### Study Characteristics

Table 2 shows a summary of the main characteristics of the studies. Most part of the studies (five) (13,14,21-23) were performed in Brazil, while the other four studies were conducted in Mexico (24), Turkey (4), Greece (25), and Malaysia (26). The analysis of the nine studies resulted in a total sample of 285 specimens. From the nine studies analyzed, only three (14,21,22) mentioned the ethical criteria involved. As for specimen fixation, five studies (13,14,21,23,25) used 10% formaldehyde and four studies (4,22,24,26) did not mention the means of fixation. All studies (4,13,14,21-26) used immunohistochemistry as the diagnostic method.

Four studies (13,14,22,23) used anti-OPG (N-20; Santa Cruz Biotechnology) and anti-RANKL (N-19; Santa Cruz Biotechnology) primary polyclonal

antibodies from rabbits, while two studies (21,25) used anti-OPG (H-249, sc1383, Santa Cruz Biotechnology) and anti-RANKL (FL-327, sc9073, Santa Cruz Biotechnology) primary polyclonal antibodies from rabbits. One study (24) used anti-OPG (clone ab73400 Abcam laboratories), anti-RANK (clone 64C1385 Abcam laboratories), and anti-RANKL (clone 12A668 Abcam laboratories) antibodies. One study (26) used primary antibodies from mice for RANK (AB13918 - Abcam Inc. Cambridge) and RANKL (AB45039 - Abcam Inc. Cambridge), and primary antibodies from rabbits for OPG (AB9986 - Abcam Inc. Cambridge). Moreover, five studies (13,14,22,23,26) used the central lesion of giant cells as positive control. For negative control, all studies (4,13,14,21-26) replaced the primary antibody.

### Risk of Bias of Studies

Table 3 shows information regarding the risk of bias and individual quality of the studies included in this systematic review. According to the analysis of the JBI Critical Appraisal Checklist for Analytical Cross Sectional Studies (17), two studies (24,25) presented low risk of bias, while seven studies (4,13,14,21-23,26) presented moderate risk.

### Individual Results of Studies

From the nine studies included in this systematic review, six (4,21,13,23,24,26) assessed the immunorexpression of RANK and RANKL, while all studies (4,13,14,21-26) assessed

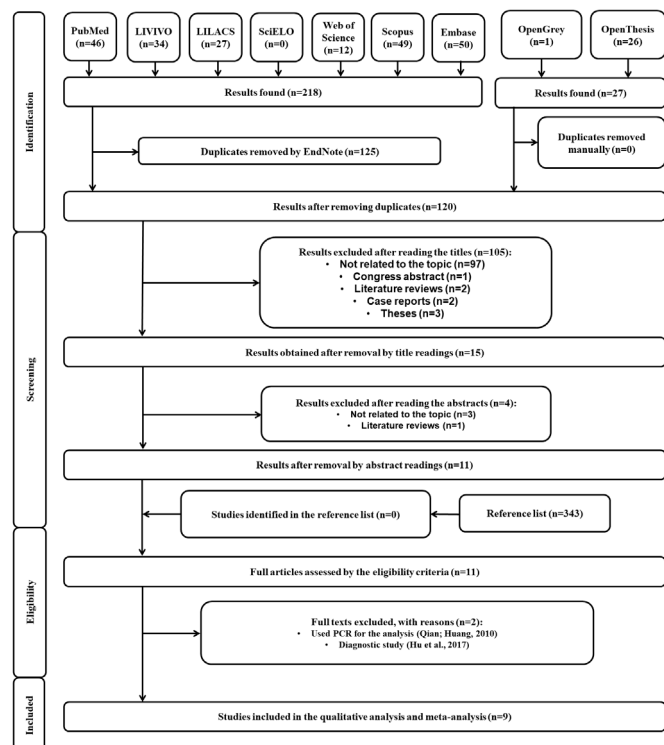


Figure 1. Flowchart of the process of literature search and selection, adapted from the PRISMA statement.

Table 2. Summary of the main characteristics of the eligible studies for qualitative analysis

Author and year	Country	Sample (n)	Cysts and tumors assessed	Ethics committee	Specimen fixation	Diagnostic method
da Silva et al. (21)	Brazil	40	OK, AB, and DC	Yes	10% formaldehyde	Immunohistochemistry
Tekkesin et al (4)	Turkey	40	OK and AB	*	*	Immunohistochemistry
de Moraes et al. (13)	Brazil	20	DC	*	10% formaldehyde	Immunohistochemistry
Nonaka et al. (22)	Brazil	22	OK	Yes	*	Immunohistochemistry
de Moraes et al. (23)	Brazil	20	DC	*	10% formaldehyde	Immunohistochemistry
de Matos et al. (14)	Brazil	58	OK, DC, and AB	Yes	10% formaldehyde	Immunohistochemistry
Iakovou et al. (25)	Greece	29	AB	*	10% formaldehyde	Immunohistochemistry
Siar et al. (26)	Malaysia	15	AB	No	*	Immunohistochemistry
Brito-Mendoza et al. (24)	Mexico	41	OK and DC	*	*	Immunohistochemistry

\*Data not informed by the authors; AB=ameloblastoma; DC=dentigerous cyst; OK=odontogenic keratocyst.

Table 3. Risk of bias assessed by the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies.

Authors	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	%yes/risk
da Silva et al. (21)	√	--	√	√	--	--	√	√	62,5% (Moderate)
Tekkesin et al (4)	√	√	--	√	--	--	√	√	62.5% (Moderate)
de Moraes et al. (13)	√	√	--	√	--	--	√	√	62.5% (Moderate)
Nonaka et al. (22)	√	--	√	√	--	--	√	√	62.5% (Moderate)
de Moraes et al. (23)	√	--	√	√	--	--	√	√	62.5% (Moderate)
de Matos et al. (14)	√	--	√	√	--	--	√	√	62.5% (Moderate)
Iakovou et al. (24)	√	√	√	√	--	--	√	√	75.0% (Low)
Siar et al. (25)	√	√	--	√	--	--	√	√	62.5% (Moderate)
Brito-Mendoza et al. (24)	√	√	√	√	--	--	√	√	75.0% (Low)

Q.1) Were the criteria for inclusion in the sample clearly defined?; Q.2) Were the study subjects and the setting described in detail?; Q.3) Was the exposure measured in a valid and reliable way?; Q.4) Were objective and standard criteria used for measuring the condition?; Q.5) Were confounding factors identified?; Q.6) Were strategies to deal with confounding factors stated?; Q.7) Were the outcomes measured in a valid and reliable way?; Q.8) Was appropriate statistical analysis used? / √ - Yes; -- - No.

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the immunoexpression of OPG. The three lesions assessed were stained for RANK in both the odontogenic epithelium and stroma. However, the immunoexpression of RANKL and OPG ranged from 0% to 100% in the odontogenic epithelium and the stroma (Table 4). The OPG>RANKL ratio ranged from 0% to 70% in the odontogenic epithelium and from 0% to 77.8% in the stroma (Fig. 2), while the OPG<RANKL ratio ranged from 0% to 50.0% in the odontogenic epithelium and from 0% to 75% in the stroma (Fig. 3). The OPG=RANKL ratio ranged from 20.0% to 60% in the odontogenic epithelium and from 0% to 90.0% in the stroma (Fig. 4).

### Synthesis of Results and Meta-Analysis

Considering the OPG>RANKL ratio (Fig. 2), the highest estimate was perceived for dentigerous cyst (ES=43.3%; 95% CI=14.3-74.8) analyzed in the epithelium, although bigger, this ratio was statistically similar to that calculated for odontogenic keratocyst (ES=36.8%; 95% CI=18.8-56.7). In contrast, the highest OPG<RANKL ratio was found in

ameloblastoma analyzed in the stroma tissue (ES=73.4%; 95% CI=55.4-88.4). The OPG<RANKL ratio (Fig. 3) was significantly higher for ameloblastoma in the stromal region compared to the estimate for the odontogenic epithelial region (ES=46.7%; 95% CI=28.5-65.2). Regardless of the type of lesion, the degree of heterogeneity between the studies ranged from low ( $I^2=18.8%$ ), when the OPG=RANKL ratio was evaluated in the epithelial region, to high moderate to high ( $I^2=92.8%$ ), when the same ratio was analyzed for the stromal region (Fig. 4).

## Discussion

Odontogenic cysts and tumors are lesions of the oral cavity with the potential to affect the balance of the bone resorption and apposition system (12). However, the scientific literature is still controversial regarding the expression of RANK, RANKL, and OPG in the development of such lesions.

The dentigerous cyst is one of the most common odontogenic cysts of the oral and maxillofacial complex.

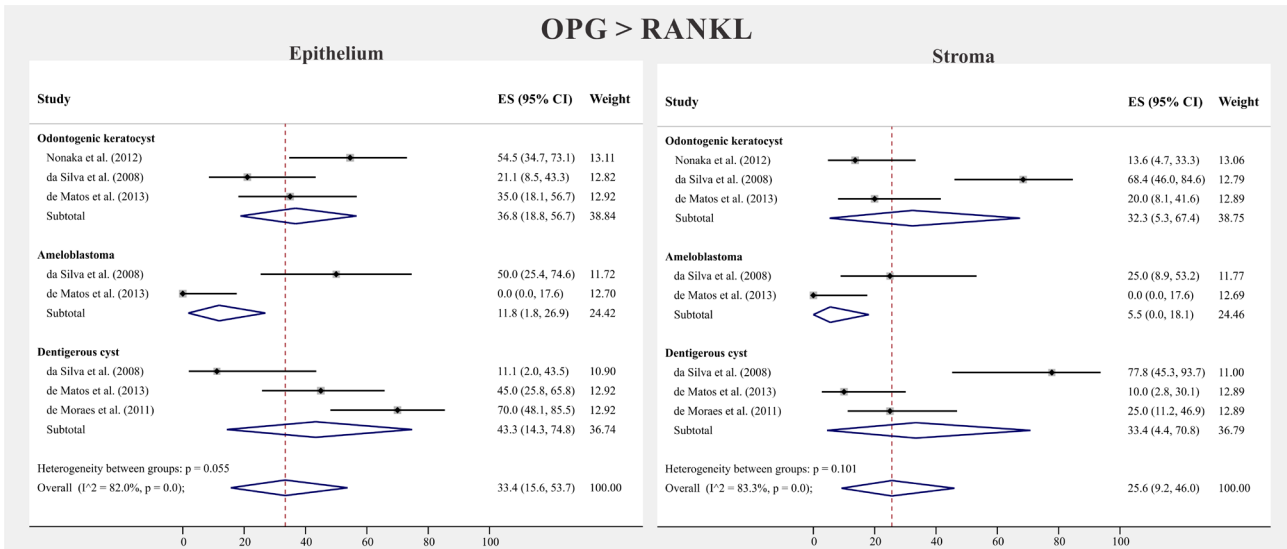


Figure 2. Forest plot showing pooled estimates for the OPG>RANKL ratio according to tissue and type of lesion, considering an effect size (ES) of 95%.

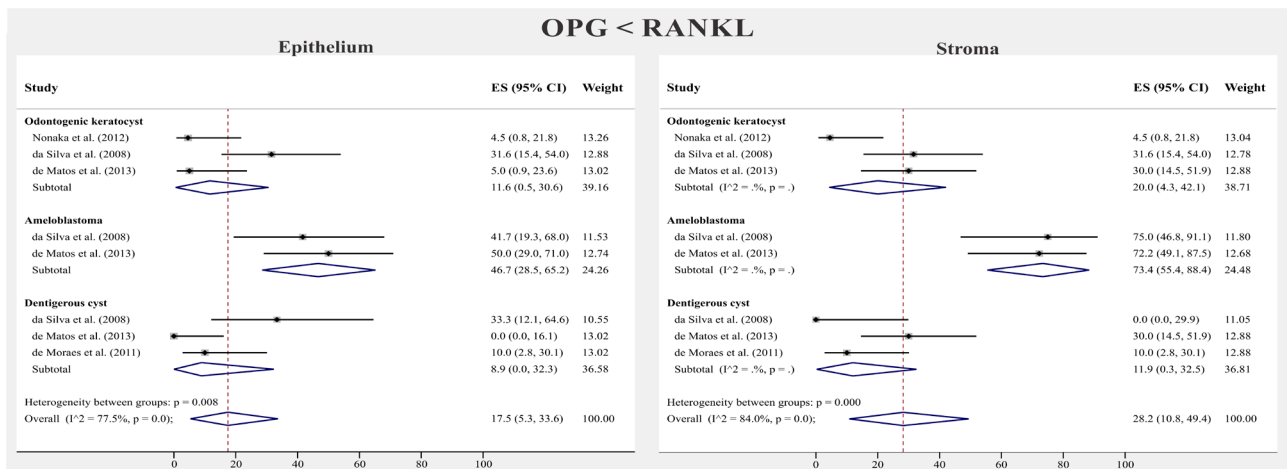


Figure 3. Forest plot showing pooled estimates for the OPG<RANKL ratio according to tissue and type of lesion, considering an effect size (ES) of 95%.

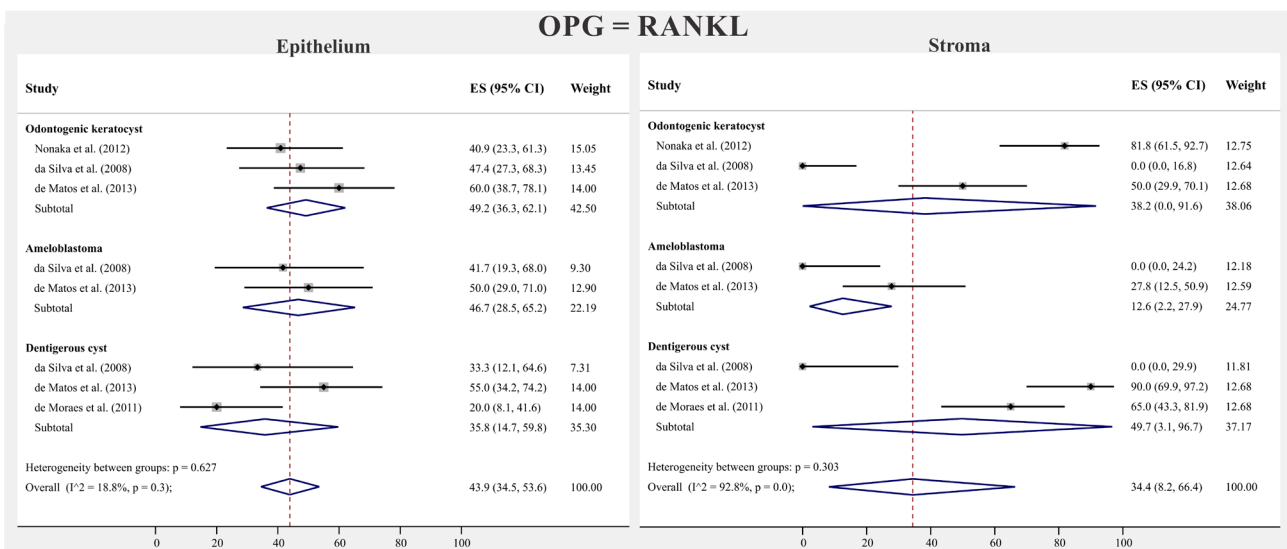


Figure 4. Forest plot showing pooled estimates for the OPG=RANKL ratio according to tissue and type of lesion, considering an effect size (ES) of 95%.

Although it is asymptomatic and rarely aggressive, in some cases it may cause significant bone expansion and teeth dislocation (27). On the other hand, the ameloblastoma and the odontogenic keratocyst present a more invasive biological behavior with a potential destructive growth (28). Hence, several studies (4,13,14,21) have been focused on investigating the correlation of the immunologic expression of the osteoclastogenic factors RANK, RANKL, and OPG in the development of such pathologies. The present study proposed to verify whether the profile of the odontogenic keratocyst is more similar to a cystic or neoplastic lesion, which excluded inflammatory cysts from the analysis. It is worth noting that this study extracted only the results from non-syndromic odontogenic keratocysts, considering that other variables may affect the expression of RANK, RANKL, and OPG in the syndromic odontogenic keratocyst.

Immunohistochemistry is a diagnostic method that uses antibodies as specific reagents for detecting antigens in tissue and cell cut-offs, and it is often used for diagnosing neoplasias (29). This type of technique depends on the means of specimen fixation (30), for which 10% formaldehyde is mostly recommended (31). All the eligible studies in the present systematic review performed the immunohistochemistry technique for analyzing the expression of RANK, RANKL, and OPG, agreeing the recommendations of the scientific literature. However, only five studies (13,14,21,23,25) reported specimen fixation in 10% formaldehyde (Table 2).

DNA amplification reactions, as in PCR, are one of the most important molecular biology techniques today

(32). Despite this, it is a high-cost method, which involves extraction of genetic material and need experienced professionals to avoid errors inherent to the sensitivity of the technique, such as failure in amplification (33). As a consequence, several laboratories find it difficult to apply it in their routine (34). Based on this, we only included studies that used immunohistochemistry to evaluate the immunoexpression of RANK, RANKL and OPG, because, despite being a technique with less precision than PCR, it is widely used in laboratories.

Most studies (13,14,21-23,25) used polyclonal antibodies to perform the immunohistochemical reaction. Polyclonal antibodies have low specificity, as they bind to different epitopes, increasing the chances of promoting a cross reaction and, consequently, generating unspecific markings (35). The widespread use of polyclonal antibodies in studies the present systematic review may have occurred due to their low cost, when compared to monoclonal antibodies. Therefore, we believe - and strongly suggest - that monoclonal antibodies should be chosen for immunohistochemical reactions, whenever possible.

The RANK is a member of the tumor necrosis factor (TNF) receptor family (36) that works as a signaling receptor of RANKL, while the latter is expressed in the osteoblastic cells of the periodontal ligament (9), binding to RANK and activating osteoclasts to promote bone resorption (10). Thus, the RANKL, which is a protein that also belongs to the TNF family, works by regulating osteoclastic activity (37). Confirming such affirmation, six studies (4,13,21,23,24,26) of the present systematic review

Table 4. Immunoexpression of RANK, RANKL, and OPG

Authors	RANK			RANKL			OPG		
	OK	AB	DC	OK	AB	DC	OK	AB	DC
da Silva et al. (21)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)
Tekkesin et al (4)	100% (S) 100% (OE)	100% (S) 100% (OE)	--	100% (S) 100% (OE)	100% (S) 100% (OE)	--	100% (S) 100% (OE)	100% (S) 0% (OE)	--
de Moraes et al. (13)	--	--	100% (S) 100% (OE)	--	--	100% (S) 100% (OE)	--	--	100% (S) 100% (OE)
Nonaka et al. (22)	--	--	--	100% (S) 100% (OE)	--	--	100% (S) 100% (OE)	--	--
de Moraes et al. (23)	--	--	100% (S) 100% (OE)	--	--	100% (S) 100% (OE)	--	--	100% (S) 100% (OE)
de Matos et al. (14)	--	--	--	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)	100% (S) 100% (OE)
Iakovou et al. (25)	--	--	--	--	100%*	--	--	100%*	--
Siar et al. (26)	--	100% (S) 100% (OE)	--	--	0% (S) 0% (OE)	--	--	0% (S) 100% (OE)	--
Brito-Mendoza et al. (24)	100% (S) 100% (OE)	--	100% (S) 100% (OE)	100% (S) 100% (OE)	--	100% (S) 100% (OE)	100% (S) 100% (OE)	--	100% (S) 100% (OE)

AB=ameloblastoma; DC=dentigerous cyst; OE=odontogenic epithelium; OK=odontogenic keratocyst; S=stroma; \*=the authors did not present the results divided by stroma and odontogenic epithelium.

assessed the immunoeexpression of RANK in dentigerous cyst, odontogenic keratocyst, and ameloblastoma, and all of them presented positive expression of RANK in both the odontogenic epithelium and stroma, reinforcing the action of this protein in the osteolytic process (Table 4).

The immunoeexpression of RANKL was assessed in all the studies included in this systematic review. From the nine studies included, eight (4,13,14,21-25) presented positive expression of RANKL in dentigerous cyst, odontogenic keratocyst, and ameloblastoma in both the odontogenic epithelium and stroma. This result corroborates the findings of the study by Qian and Zuang (38), which assessed 24 ameloblastoma specimens and verified that RANKL was the key factor for the development of osteoclastogenesis. However, in the present systematic review, only one study (26) did not present positive expression of RANKL for ameloblastoma, which is incompatible with the biological behavior of this pathology (Table 4). Ameloblastomas are known to be aggressive and locally invasive/destructive tumors. In some cases, bone expansion is so significant that radical resection is the only form of treatment (38). The referred authors of the study (26) suggest that the expression of RANKL may be absent because either the sample studied was a group of indolent tumors or the undergoing dynamic process was associated with bone remodeling induced by the tumor.

The OPG is a receptor of the TNF family and it is secreted by a number of cell types, including osteoblasts (39). This protein works by inhibiting the osteoclastogenic process, thus blocking the RANKL and RANK binding (11). Hence, studies (40,41) have been developed aiming to investigate its potential as a therapeutic agent for bone diseases. All the studies included in the present systematic review assessed the immunoeexpression of OPG, and immunoreactivity was not positive in only two of them (4,26), whereas both verified OPG in the ameloblastoma (Table 4).

Studies (14,25) have shown that assessing the ratio of immunoeexpression of RANKL and OPG is particularly important, considering it may indicate the biological activity and the osteolytic potential of the odontogenic cyst or tumor. From the nine studies included in this systematic review, five (13,14,21,22,25) assessed such ratio. Thus, the highest OPG>RANKL ratio was found for odontogenic keratocyst and dentigerous cyst. These findings agree with the biological behavior of the odontogenic keratocyst and the dentigerous cyst, considering these are lesions with lower bone resorption ability than ameloblastoma, which is a very aggressive tumor (42). It is known that RANKL binds to RANK in the surface of osteoclast precursors, recruiting and activating the tumor necrosis factor receptor-associated factor-6 (TRAF-6). Thus, the TRAF-6 stimulates the activation of the nuclear factor- $\kappa$ B (NF- $\kappa$ B) through the interaction of p62

and the atypical protein kinase C (aPKC), which triggers the transcription of osteoclastogenic genes (43). The NF- $\kappa$ B signaling is essential for osteoclastogenesis (44). Thus, the OPG inhibits the osteoclastogenic events by not allowing the binding of RANKL to RANK and preventing the entire activation cascade of NF- $\kappa$ B from being activated (45). Confirming such findings, authors (12) verified a similar ratio with higher prevalence of OPG than RANKL in the calcifying odontogenic cyst, reinforcing the proposal of the cystic nature of the odontogenic keratocyst. Such ratio may suggest that OPG may be involved in other different biological processes of bone remodeling (22).

However, the pathogenesis of the odontogenic keratocyst is still uncertain (46). The Sonic Hedgehog (Shh) activation has been indicated as one of the main mechanisms involved in the progression of this pathology (47). In normal cells, the Patched (PTCH) transmembrane receptor hinders Shh activation (46). However, in neoplastic cells, the Shh protein binds to the PTCH1 receptor, activating the Smoothed (Smo) transmembrane protein and inducing cell proliferation from the expression of Glioma cytoplasmic proteins (Gli-1 and Gli-2) (48). Consequently, the NF- $\kappa$ B is activated, which is a signaling pathway that participates directly in the osteolytic process (44). It is known that PTCH1 works as an inhibitor of Smo in the absence of the Shh ligand. A study (49) featuring the treatment of cerebral ischemia in rats using polydatin verified an overexpression of PTCH1 and a reduction of NF- $\kappa$ B, which may explain indirectly the OPG>RANKL ratio found in the odontogenic keratocyst, considering that the lower NF- $\kappa$ B activation implies in lower expression of RANKL and, consequently, lower osteolytic potential.

In contrast, the OPG<RANKL ratio was higher for ameloblastoma, considering the stromal region is significantly larger. The results agree with the ameloblastoma activity, considering the stromal region participates actively in the invasion and proliferation of tumor cells (50). A study (51) performed with the immunohistochemical marker for detecting myofibroblasts and anti- $\alpha$ -actin smooth muscle antibody ( $\alpha$ -SMA) verified that from the 15 cases of solid ameloblastomas examined, only one did not express  $\alpha$ -SMA, indicating a high myofibroblast activity in the development of ameloblastomas. Such aggressiveness from the stroma may be explained by the potential changes in the components of the mitogen-activated protein kinase (MAPK), especially in the BRAF gene, which may be activated by the fibroblast growth factor (FGF) (52). The expression of FGF in ameloblastomas, especially FGF1, FGF2, and FGF3, are important for the positive MAPK regulation (53). The MAPK activation allows the phosphorylation of Raf, MEK, and ERK proteins, inducing cell proliferation (54).

Moreover, a recent study (55) suggested that the



transforming growth factor- $\beta$  (TGF- $\beta$ ) and interleukin- $1\alpha$  (IL- $1\alpha$ ) have the ability to induce the expression of RANKL in stromal fibroblasts of ameloblastomas. The positive regulation of RANKL is associated with a negative regulation of OPG, which causes the system to work in favor of osteoclastogenesis (43). The higher expression of RANKL allows its binding to RANK and consequently the activation of NF- $\kappa$ B activation, which is an essential transcription factor for the osteolytic potential of tumor lesions (44). Reinforcing these findings, authors (56) verified a higher immunexpression of RANKL than OPG in osteosarcoma, which is a tumor similar to ameloblastoma regarding its aggressive and osteolytic behavior. Similarly, Sambandam et al. (57) studied a squamous cell carcinoma sample and verified a positive regulation of RANKL, suggesting that this protein works in osteoclastic differentiation and bone destruction.

The present study is not free of limitations since immunohistochemistry is a semi-quantitative technique that, in some cases, may not be objective. Besides, there is no way to control the intensity of staining among the studies evaluated. Because of this, our systematic review did not measure the levels of staining intensity, but only the simple presence of staining. However, this study has strengths that should be considered. This is an original systematic review and meta-analysis, which used an extensive search strategy including the "grey literature", without restrictions of language or publication status. Also, the recent classification of odontogenic cysts and tumors proposed by the WHO (58) establishes that the dentigerous cyst and the odontogenic keratocyst are considered odontogenic cysts, while ameloblastoma is considered a benign odontogenic tumor. Thus, this systematic review corroborates the classification proposed by the WHO. This is particularly important in clinical practice since the odontogenic keratocyst is more similar to the cystic profile, requiring greater care to perform systematic curettage technique after removal of the lesion, in order to reduce the chances of recurrence.

The results of the present systematic review suggest that the OPG<RANKL ratio was higher for ameloblastoma, which may explain its aggressive potential, and it was lower for dentigerous cyst and odontogenic keratocyst, reinforcing the WHO classification of odontogenic keratocyst as an odontogenic cyst.

## Resumo

O objetivo deste estudo foi avaliar e comparar a imunexpressão de RANK, RANKL e OPG em cisto dentífero, ceratocisto odontogênico e ameloblastoma. O protocolo foi registrado no PROSPERO (CRD [Oculto]). Sete bancos de dados (Embase, Lilacs, LIVIVO, PubMed, Scopus, SciELO e Web of Science) foram as principais fontes de pesquisa e duas bases de dados (Open Grey e Open Thesis) capturaram parcialmente a "literatura cinza". Apenas estudos transversais foram incluídos. A ferramenta

JBI avaliou o risco de viés. Uma metanálise com modelo de efeitos aleatórios estimou os valores da razão OPG e RANKL relatados pelos estudos individuais e seus respectivos intervalos de confiança de 95%. A heterogeneidade entre os estudos foi avaliada por meio do teste I<sup>2</sup>. Apenas nove estudos preencheram os critérios de inclusão e foram considerados nas análises. Os estudos foram publicados entre 2008 e 2018. Dois estudos apresentaram baixo risco de viés, enquanto sete estudos apresentaram risco moderado. A meta-análise mostrou a maior razão OPG>RANKL para cisto dentífero (ES=43,3%; IC95%=14,3-74,8) e ceratocisto odontogênico (ES=36,8%; IC95%=18,8-56,7). Por outro lado, a maior razão OPG<RANKL foi encontrada para ameloblastoma (ES=73,4%; IC95%=55,4-88,4) e foi maior na região estromal em comparação com a região epitelial odontogênica. Os resultados podem explicar o potencial agressivo do ameloblastoma devido a uma maior proporção OPG<RANKL nesse tumor, enquanto tal proporção foi menor no cisto dentífero e no ceratocisto odontogênico.

## References

- Kouhsoltani M, Abdolhosseinzadeh M, Bahramian A, Vakili Saatloo M, Dabbaghi Tabriz F, Pourlak T. A. A Comparative Study of Macrophage Density in Odontogenic Cysts and Tumors with Diverse Clinical Behavior. *J Dent* 2018;19:150-154.
- Philipsen HP, Reichart PA. Classification of odontogenic tumors. A historical review. *J Oral Pathol Med* 2006;35:525-529.
- Hume WJ, Moore JK, Main DM. Differences in in vitro growth of epithelium from inflammatory and developmental odontogenic cysts. *Br J Oral Maxillofac Surg* 1990;28:85-88.
- Tekkesin MS, Mutlu S, Olgac V. The Role of RANK/RANKL/OPG Signalling Pathways in osteoclastogenesis in odontogenic keratocysts, radicular cysts, and ameloblastomas. *Head Neck Pathol* 2011;5:248-253.
- Clarke B. Normal bone anatomy and physiology. *Clin J Am Soc Nephrol* 2008;3:5131-139.
- Boyce BF, Xing L. Functions of RANKL/RANK/OPG in bone modeling and remodeling. *Arch Biochem Biophys* 2008;473:139-146.
- Hofbauer LC, Neubauer A, Heufelder AE. Receptor activator of nuclear factor kappa B ligand and osteoprotegerin: potential implications for the pathogenesis and treatment of malignant bone diseases. *Cancer* 2001;92:460-470.
- Yamaguchi M, Aihara N, Kojima T, Kasai K. RANKL increase in compressed periodontal ligament cells from root resorption. *J Dent Res* 2006;85:751-756.
- Ogasawara T, Yoshimine Y, Kiyoshima T, Kobayashi I, Matsuo K, Akamine A, et al. In situ expression of RANKL, RANK, osteoprotegerin and cytokines in osteoclasts of rat periodontal tissue. *J Periodontol Res* 2004;39:42-49.
- Stejskal D, Bartek J, Pastorková R, Ruzicka V, Oral I, Horalik D. Osteoprotegerin, RANK, RANKL. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2001;145:61-64.
- Suda T, Takahashi N, Udagawa N, Jimi E, Gillespie MT, Martin TJ. Modulation of osteoclast differentiation and function by the new members of the tumor necrosis factor receptor and ligand families. *Endocr Rev* 1999;20:345-357.
- Andrade FR, Sousa DP, Mendonça EF, Silva TA, Lara VS, Batista AC. Expression of bone resorption regulators (RANK, RANKL, and OPG) in odontogenic tumors. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;106:548-555.
- de Moraes M, de Lucena HF, de Azevedo PR, Queiroz LM, Costa Ade L. Comparative immunohistochemical expression of RANK, RANKL and OPG in radicular and dentigerous cysts. *Arch Oral Biol* 2011;56:1256-1263.
- de Matos FR, de Moraes M, das Neves Silva EB, Galvão HC, de Almeida Freitas R. Immunohistochemical detection of receptor activator nuclear kb ligand and osteoprotegerin in odontogenic cysts and tumors. *J Oral Maxillofac Surg* 2013;71:1886-1892.
- Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* 2015;4:1.
- Higgins JPT, Green S. *Cochrane handbook for systematic reviews of interventions* version 5.1.0 [updated in March 2011]. The Cochrane

- Collaboration. Available at: <www.handbook.cochrane.org>. Access on March 10, 2018.
17. The Joanna Briggs Institute. The Joanna Briggs Institute Critical Appraisal tools for use in JBI Systematic Reviews. Checklist for Analytical Cross Sectional Studies. Available at: [https://jbi.global/sites/default/files/2019-05/JBI\\_Critical\\_Appraisal-Checklist\\_for\\_Analytical\\_Cross\\_Sectional\\_Studies2017\\_0.pdf](https://jbi.global/sites/default/files/2019-05/JBI_Critical_Appraisal-Checklist_for_Analytical_Cross_Sectional_Studies2017_0.pdf). Latest access February 2, 2021.
  18. Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. *Arch Public Health* 2014;72:39.
  19. Freeman MF, Tukey JW. Transformations related to the angular and square root. *Ann Math Stat.* 1950; 21: 607-611.
  20. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;21:1539-1558.
  21. da Silva TA, Batista AC, Mendonça EF, Leles CR, Fukada S, Cunha FQ. Comparative expression of RANK, RANKL, and OPG in keratocystic odontogenic tumors, ameloblastomas, and dentigerous cysts. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:333-341.
  22. Nonaka CF, Cavalcante RB, Nogueira RL, de Souza LB, Pinto LP. Immunohistochemical analysis of bone resorption regulators (RANKL and OPG), angiogenic index, and myofibroblasts in syndrome and non-syndrome odontogenic keratocysts. *Arch Oral Biol* 2012;57:230-237.
  23. de Moraes M, de Matos FR, de Souza LB, de Almeida Freitas R, de Lisboa Lopes Costa A. Immunoperoxidation of RANK, RANKL, OPG, VEGF, and vWF in radicular and dentigerous cysts. *J Oral Pathol Med* 2013;42:468-473.
  24. Brito-Mendoza L, Bologna-Molina R, Irigoyen-Camacho ME, Martínez G, Sánchez-Romero C, Mosqueda-Taylor A. A comparison of Ki67, Syndecan-1 (CD138), and molecular RANK, RANKL, and OPG triad expression in odontogenic keratocysts, unicystic ameloblastoma, and dentigerous cysts. *Dis Markers* 2018;2018:7048531.
  25. Iakovou M, Chrysomali E, Piperi E, Fanourakis G, Sklavounou A, Vlachodimitropoulos D, et al. A comparative study of bone remodeling molecules expression in different types of jaw ameloblastoma. *J Oral Pathol Med* 2015;44:543-551.
  26. Siar CH, Tsujigiwa H, Ishak I, Hussin NM, Nagatsuka H, Ng KH. RANK, RANKL, and OPG in recurrent solid/multicystic ameloblastoma: their distribution patterns and biologic significance. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2015;119:83-91.
  27. Ko KS, Dover DG, Jordan RC. Bilateral dentigerous cysts - report of an unusual case and review of the literature. *J Can Dent Assoc* 1990;65:49-51.
  28. Philipsen HP, Reichart PA, Sloomweg PJ. Odontogenic tumors. In: Barnes L, Eveson JW, Reichart P, Sindrinsky D. (eds): *Pathology and genetics head and neck tumours*. World Health Organization Classification of Tumours. Lyon: IARC Press 2005;283-328.
  29. Werner B, Campos A, Nadji M, Torres LFB. Practical use of immunohistochemistry in surgical pathology. *J Bras Patol Med Lab* 2005; 41:353-364.
  30. Jaffer S, Bleiweiss IJ. Beyond hematoxylin and eosin - the role of immunohistochemistry in surgical pathology. *Cancer Invest.* 2004;22:445-465.
  31. Sampaio SAP, Rivitti EA. Histopathological examination, glossary and histopathological patterns. In: *Dermatology*. São Paulo: Artes Médicas 2007;123.
  32. Martin J. Updating PCR. *Biotechniques* 2019;67:3-5.
  33. Assunção JGF, Correia AKA. Comparative analysis of molecular biology techniques for genotyping human papillomavirus - HPV. *Revista Científica da Escola da Saúde* 2014;3.
  34. Lloveras B, Lorincz A, Ejarque M, Font R, Bosch FX, Sanjosé S. Evaluación de las técnicas de detección del VPH em los programas de cribado para cáncer de cuello uterino. *Salud pública de México* 2006;48:373-378.
  35. Bayry J, Lacroix-Desmazes S, Kazatchkine MD, Kaveri SV. Intravenous immunoglobulin for infectious diseases: back to the pre-antibiotic and passive prophylaxis era? *Trends Pharmacol Science* 2004;25:306-310.
  36. Cheng X, Kinoshita M, Murali R, Greene MI. The TNF receptor superfamily: role in immune inflammation and bone formation. *Immunol Res* 2003;27:287-294.
  37. Kobayashi Y, Udagawa N, Takahashi N. Action of RANKL and OPG for osteoclastogenesis. *Crit Rev Eukaryot Gene Expr* 2009;19:61-72.
  38. Qian Y, Huang HZ. The role of RANKL and MMP-9 in the bone resorption caused by ameloblastoma. *J Oral Pathol Med* 2010;39:592-598.
  39. Pereira IA, Pereira RMR. Osteoporosis and focal bone erosions in rheumatoid arthritis: from pathogenesis to treatment. *Rev Bras Reumatol.* 2004;44:347-354.
  40. Ando K, Mori K, Rêdini F, Heymann D. RANKL/RANK/OPG: key therapeutic target in bone oncology. *Curr Drug Discov Technol.* 2008;5:263-268.
  41. Taylan A, Birlilik M, Kenar G, Toprak B, Gundogdu B, Gurler O, et al. Osteoprotegerin interacts with biomarkers and cytokines that have roles in osteoporosis, skin fibrosis, and vasculopathy in systemic sclerosis: A potential multifaceted relationship between OPG/RANKL/TRAIL and Wnt inhibitors. *Mod Rheumatol* 2018;25:1-6.
  42. Pozo JA, Espinoza J. Ameloblastoma uniuístico, bases del tratamiento conservador. Presentación de caso clínico y actualización bibliográfica. *Revista Española de Cirugía Oral y Maxilofacial* 2010;32:88-91.
  43. Boyce BF, Xing L. Biology of RANK, RANKL, and osteoprotegerin. *Arthritis Res Ther* 2007;9:1-7.
  44. Park JH, Lee NK, Lee SY. Current understanding of RANK signaling in osteoclast differentiation and maturation. *Mol Cells* 2017;40:706-713.
  45. Takayanagi H. Inflammatory bone destruction and osteoimmunology. *J Periodontol Res.* 2005;40:287-293.
  46. Hoyos Cadavid AM, Kaminagakura E, Rodrigues MFSD, Pinto CAL, Teshima THN, Alves FA. Immunohistochemical evaluation of Sonic Hedgehog signaling pathway proteins (Shh, Ptch1, Ptch2, Smo, Gli1, Gli2, and Gli3) in sporadic and syndromic odontogenic keratocysts. *Clin Oral Investig* 2019; 23:153-159.
  47. Chari NS, McDonnell TJ. The sonic hedgehog signaling network in development and neoplasia. *Adv Anat Pathol* 2007;14:344-352.
  48. Adolphe C, Narang M, Ellis T, Wicking C, Kaur P, Wainwright B. An in vivo comparative study of sonic, desert and Indian hedgehog reveals that hedgehog pathway activity regulates epidermal stem cell homeostasis. *Development* 2004;131:5009-5019.
  49. Ji H, Zhang X, Du Y, Liu H, Li S, Li L. Polydatin modulates inflammation by decreasing NF- $\kappa$ B activation and oxidative stress by increasing Gli1, Ptch1, SOD1 expression and ameliorates blood-brain barrier permeability for its neuroprotective effect in pMCAO rat brain. *Brain Res Bull.* 2012; 87:50-59.
  50. Fuch Fuchigami T, Koyama H, Kishida M, Nishizawa Y, Iijima M, Kibe T. Fibroblasts promote the collective invasion of ameloblastoma tumor cells in a 3D coculture model. *FEBS Open Bio.* 2017;7:2000-2007.
  51. Kouhsoltani M, Halimi M, Jabbari G. Immunohistochemical evaluation of myofibroblast density in odontogenic cysts and tumors. *J Dent Res Dent Clin Dent Prospects* 2016;10:37-42.
  52. Sweeney RT, McClary AC, Myers BR, Biscocho J, Neahring L, Kwei KA, et al. Identification of recurrent SMO and BRAF mutations in ameloblastomas. *Nat Genet* 2014;46:722-725.
  53. Nagi R, Sahu S, Rakesh N. Molecular and genetic aspects in the etiopathogenesis of ameloblastoma: An update. *J Oral Maxillofac Pathol* 2016;20:497-504.
  54. Hardy KM, Yatskievych TA, Konieczka J, Bobbs AS, Antin PB. FGF signalling through RAS/MAPK and PI3K pathways regulates cell movement and gene expression in the chicken primitive streak without affecting E-cadherin expression. *BMC Dev Biol* 2011;11:20.
  55. Yamada C, Aikawa T, Okuno E, Miyagawa K, Amano K, Takahata S, et al. TGF- $\alpha$  in jaw tumor fluids induces RANKL expression in stromal fibroblasts. *Int J Oncol* 2016;49:499-508.
  56. Elias LS, Costa RF, Carvalho MA, Batista AC, Silva TA, Leles CR, et al. Markers of bone remodeling in neoplastic and bone-related lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;110:624-631.
  57. Sambandam Y, Ethiraj P, Hathaway-Schrader JD, Novince CM, Panneerselvam E, Sundaram K, et al. Autoregulation of RANK ligand in oral squamous cell carcinoma tumor cells. *J Cell Physiol* 2018;233:6125-6134.
  58. El-Naggar AK, Chan JKC, Grandis JR, Takata T, Sloomweg PJ. *World Health Organization Classification of tumors. Pathology and Genetics of Head and Neck tumors*. 4th Lyon: International Agency for Research on Cancer Press 2017; 347.

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