Dental Caries, Developmental Defects of Enamel and Enamel Microhardness Associated with Genetic Polymorphisms in the RANK/RANKL/OPG System

Calvano Küchler E*/ Maschietto Pucinelli C**/ Carpio Horta K ***/ Assed Bezerra da Silva R ****/ de Castro Costa M *****/ Rezende Vieira A******/ Nelson-Filho P*******/ Assed Bezerra da Silva L********/ Santos Antunes L *********/ Azeredo Antunes L**********

Purpose*: Recent studies have suggested that disruptions in the RANKL/RANK/OPG system might be involved in enamel conditions. The aim of this study was to test whether genetic polymorphisms in RANK, RANKL and OPG are associated with dental caries, developmental defects of enamel (DDE) and enamel microhardness.* ***Study design****: Saliva samples were collected from two subsets for the purpose of genomic DNA extraction. In the first subset, composed of 248 children, dental caries and DDE were evaluated during their clinical examination. In the second subset, composed of 72 children, enamel samples from the buccal surface of primary teeth were used for enamel microhardness analysis. Genetic polymorphisms in RANK, RANKL and OPG were genotyped by real-time polymerase chain reactions in all samples from both populations. The chi-square test was used for dental caries and DDE analysis while, one-way ANOVA with Tukey's post-test was used for microhardness analysis. Hardy-Weinberg equilibrium was also calculated. The established alpha was 5%.* ***Results:*** *Caries experience analysis demonstrated a statistically-significant difference for OPG allele distribution in primary dentition (p=0.033). The studied polymorphisms in RANK, RANKL and OPG were not associated with DDE or enamel microhardness (p>0.05).* ***Conclusion:*** *The genetic polymorphism rs2073618 in OPG is associated with dental caries experience in primary dentition.*

Keywords: *Caries, enamel, polymorphism, gene*

*Erika Calvano Küchler, DDS, MS, PhD, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto and School of Health Sciences, Positivo University, Curitiba- Brazil.

**Carolina Maschietto Pucinelli, DDS, MS, PhD student, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto- Brazil.

***Karla Carpio Horta, DDS, MS, PhD student, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto- Brazil.

****Raquel Assed Bezerra da Silva, DDS, MS, PhD, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto- Brazil.

*****Marcelo de Castro Costa, DDS, MS, PhD, Department of Pediatric Dentistry, Federal University of Rio de Janeiro, Rio de Janeiro-Brazil.

******Alexandre Rezende Vieira, DDS, MS, PhD, Department of Oral Biology, University of Pittsburgh, Pittsburgh, PA, USA.

*******Paulo Nelson-Filho, DDS, MS, PhD, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto- Brazil.

********Léa Assed Bezerra da Silva, DDS, MS, PhD, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto- Brazil.

********* Leonardo Santos Antunes, DDS, MS, PhD, Department of Specific Formation, School of Dentistry, Fluminense Federal University, Rio de Janeiro- Brazil.

**********Lívia Azeredo Antunes, DDS, Ms, PhD, Department of Specific Formation, School of Dentistry, Fluminense Federal University, Rio de Janeiro- Brazil.

Send all correspondence to:
Erika C Küchler
Department of Pediatric Clinics, Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto, SP, Brazil.
E-mail: erikacalvano@gmail.com

INTRODUCTION

The receptor activator of nuclear factor-kB (RANK), RANK Ligand (RANKL) and osteoprotegerin (OPG) system was initially discovered through parallel efforts in the late 1990s. This system was identified as important to immunity via actions on dendritic cells[1,2], and as essential to bone homeostasis through regulation of the activity of osteoclasts[3,4]. RANKL and OPG are members of the tumor necrosis factor (TNF) and TNF receptor (TNFr) superfamilies, respectively, and they bind to receptor activator of NF-kB (RANK)[5].

In the past two decades, genetically deficient mice models have demonstrated that the RANK, RANKL and OPG system plays a critical role in bone metabolism and immunity. This system also contributes to organogenesis, homeostasis and the development of many disease conditions[6]. More recent studies have also revealed that this triad is related to dental development[7-12]. Sheng *et al* [12] evaluated mice lacking OPG (*Opg*-/- mouse) and found different responses in dental mineralization and morphogenesis due to *Opg* gene deprivation. Ohazama *et al* [8] demonstrated that during mice's odontogenesis, RANK, RANKL, and OPG showed dynamic expression patterns. Furthermore, the addition of exogenous OPG to explant cultures of tooth primordial resulted in a reduction of enamel mineralization and enamel defects, such as enamel hypoplasia.

Developmental defects of enamel (DDE) are a result of alterations during amelogenesis and may be manifested as enamel hypoplasia, diffuse or demarcated enamel opacities or enamel hypomineralization. Clinically, developmental enamel defects often present with problems of discoloration, esthetics and tooth sensitivity[13]. DDE is a common condition and is considered to be a non-carious defect, however, some studies have speculated that DDE increases the risk of dental caries in primary and permanent dentitions[14-16].

So far, few studies[8,12] have attempted to evaluate the role of RANKL, RANK and OPG system in the enamel development and clearly suggested that disruptions in this system might be involved in enamel defects in an animal model. The enamel phenotypes observed in the models of the mentioned studies above clearly support that RANK, RANKL and OPG system might be involved in DDE phenotype and enamel microstructures alterations. These observations lead to the hypothesis that polymorphisms in genes encoding human *RANK, RANKL* and *OPG* are associated with DDE, enamel microhraness alterations and consequently higher caries susceptibility in primary and/or permanent teeth. Therefore, this study aimed to evaluate whether genetic polymorphisms in *RANK, RANKL* and *OPG* are associated with alterations in enamel microhardness and the risk of DDE and dental caries.

MATERIALS AND METHOD

Samples

The Human Ethics Committees of the School of Dentistry of Ribeirão Preto of the University of São Paulo (#35323314.7.0000.5419) and the Health Department of Federal University of Rio de Janeiro (#333.167) approved this study. Informed written consent was obtained from the parents, and an age-appropriate assent document was used for every child.

Two data sets from children that sought dental treatment at public dental schools were available for this study. The first sample set was composed of 248 biologically unrelated children who sought treatment at the School of Dentistry of Ribeirão Preto, University of São Paulo, São Paulo state, Brazil. The second sample set was composed of 72 biologically unrelated children that sought treatment at the Federal University of Rio de Janeiro, Rio de Janeiro state, Brazil. Both cities are located in the southeast region of Brazil. Because of Brazilian immigration patterns, there are several different ethnic backgrounds in this region, which are mainly European and African descendants, a small percentage of Asian descendants and Native Americans.

Saliva samples for genomic DNA extraction were collected from both data sets and extracted as previously described[17]. Briefly, patients were instructed to rinse the mouth with 10 mL of saline and expectorating the rinse in a 50 mL propylene tube. The tubes were centrifuged to pellet the buccal cells. The supernatant was discarded and 1 mL of extraction solution containing proteinase K was added. After overnight incubation the non-digested proteins were removed by adding ammonium acetate. The DNA was precipitated with isopropanol. After centrifugation, the supernatant was poured off. The DNA was re-suspended in TE and quantification of the concentration and purity of the DNA was determined by spectrophotometer (Nanodrop 1000; Thermo Scientific, Wilmington, DE, USA). The DNA was stored at -20°C until the real time polymerase chain reaction (PCR) analysis.

Information on demographic data was obtained through a questionnaire on oral hygiene habits (frequency of tooth brushing and dental flossing), and frequency of sweet intake between meals.

Clinical examination for dental caries experience and DDE

This sample included 248 children from Ribeirão Preto, with ages ranging from 4 to 12 years. Three trained, experienced Pediatric Dentists (CPM, RABS and PNF) performed the clinical examination. Dental caries was diagnosed by visual examination and registered if there was a visual evidence of loss of tooth structure (cavitation), using a modified World Health Organization protocol recommended for oral health surveys[18]. Dental caries was assessed by the dmft and DMFT indexes (decayed, missing teeth due to caries and filled teeth, for primary and permanent dentition respectively). DDE were diagnosed using the modified DDE index based on the federation dentaire internationale recommendation (FDI)[19]. The patient was considered to have DDE if he or she presented at least one primary or permanent tooth affected by DDE, as presented in Figure 1.

Enamel microhardness analysis

This assay was performed on exfoliated primary teeth (51 molars, 15 incisors, and 6 canines) of 72 children (41 boys and 31 girls) from Rio de Janeiro with ages ranging from 4 to 12 years. The enamel samples from the buccal surface were submitted to enamel microhardness analysis as previously described by Romanos *et al* (2015)[20]. Each enamel block was submitted to microhardness analysis using a microhardness tester (IndentaMet 1100 Series, Buehler Ltd., Lake Bluff, IL, USA) with a knop diamond under a load of 25 grams for 10 seconds. Five indentations spaced 100 mm away from each other were made. The data were analyzed as continuous variables.

Normal	0
Demarcated Opacities	1
Diffuse Opacities	2
Hypoplasia	3
Other defects	4

Figure 1. Modified developmental defects of the enamel index.

Genotype analysis

The characteristics of the genetic polymorphisms in *RANK, RANKL* and *OPG* are presented in Table 1. The genetic polymorphisms in RANK and RANKL are located in intron while the genetic polymorphism in OPG is a missense polymorphism (Lysine>Asparagine). were genotyped by real-time PCR using the TaqMan assay (step OnePlus Real-Time PCR System (Applied Biosystems, Foster City, USA).

Statistical Analysis

Data were analyzed using Epi Info 7 (Epi Info 7 software, Atlanta, GA, USA) and GraphPad Prism 5.0a package (Graph-Pad, San Diego, CA, USA). For dental caries evaluation the individuals were divided into 'Caries Free' and 'Caries Experience' groups. For DDE evaluation, they were divided into 'Without DDE' and 'With DDE' groups. Comparisons between these groups were performed using the chi-square test to compare allele and genotype distributions among the groups. The odds ratio was used to calculate the probability among these groups.

The microhardness data were evaluated as continuous data. The Shapiro–Wilk test was used to verify the normality of the data. One-way ANOVA with Tukey's post-test was used for comparisons of microhardness means and standard deviations (SD) among genotypes. The established alpha was 5%.

Hardy-Weinberg equilibrium was evaluated using the chi-square test within each polymorphism.

RESULTS

Caries and DDE

Among the 248 included children, 134 were boys and 114 were girls. The mean age was 7.6 (Standard Deviation= 2.8 years). One hundred sixty-six children had caries experience in at least one primary tooth and 115 children had caries experience in at least one permanent tooth. The mean dmft score was 5.0 (SD 3.8) and scores ranged from 0 to 23. All the children used fluoride tooth paste. Sweet consumption between meals was reported in 50.4% (n=125) of the children. Thirty-five (16.4%) brushed their teeth once a day and 213 (83.6%) two or more times per day. Two hundred and one (81.1%) children brushed their teeth before sleep. Eighty-nine (35.9%) children used dental floss daily.

Age, ethnicity, use of dental floss and sweet intake between meals showed no statistically-significant difference between the caries free and caries experience groups (p>0.05). DDE was not associated with dental caries (p>0.05).

Table 2 demonstrates genotype and allele distribution for *RANK, RANKL* and *OPG* polymorphisms for primary and permanent dentition. A statistically-significant difference was observed only for *OPG* (rs2073618) allele distribution in primary dentition, in which the C allele was associated with caries free (p=0.033; OR=0.59, CI 95% 0.36- 0.96). The genotype and allele distributions of the genetic polymorphisms rs3826620 in *RANK* and rs9594738 in *RANKL* were not associated with caries experience, neither in primary dentition, nor in permanent dentition (p>0.05).

Fifty-five (22.17%) children presented DDE in at least one tooth. The most affected tooth was first permanent molars (31 children had at least one permanent first molar affected by DDE), followed by permanent incisors (17 children had at least permanent incisor affected by DDE). Seven children had at least one primary tooth affected by DDE. Forty-six children had two or DDE affected teeth. Table 3 shows the genotype and allele distribution among the groups with DDE and without DDE. The genotype and allele distributions of the studied polymorphisms rs9594738 in *RANK,* rs9594738 in *RANKL* and rs2073618 in *OPG* were not statistically significant associated with DDE (p>0.05).

Microhardness Analysis

The mean enamel microhardness according to the genotype is shown in Figure 2. In *RANK*, the mean enamel microhardness was 245.4 Kg/mm^2 (SD 67.8) in GG genotype, 233.1 Kg/mm^2 (SD 68.2) in GT genotype, and 207.8 Kg/mm^2 (SD 56.0) in TT genotype (p>0.05). In *RANKL*, the mean enamel microhardness was 231.1 Kg/mm^2 (SD 79.3) in CC genotype, 240.5 Kg/mm^2 (SD 81.6) in CT genotype and 243.5 Kg/mm^2 (SD 37.2) in TT genotype. In *OPG*, the mean enamel microhardness was 244.0 Kg/mm^2 (SD 65.8) in CC genotype, 235.0 Kg/mm^2 (SD 65.8) in CG genotype and 220.3 Kg/mm^2 (SD 92.2) in GG genotype. The mean enamel microhardness was not associated with any genotype of the studied polymorphisms (p>0.05).

Table 1. Description of the studied genetic variations.

Gene	Variant	Chromosome Location	Base changed	Average heterozygosity standard error
RANK	rs3826620	18q21.33	G/T	0.454 +/- 0.144
RANKL	rs9594738	13q14.11	C/T	0.407 +/- 0.194
OPG	rs2073618*	8q24.12	C/G	0.444 +/- 0.157

Note: Obtained from databases: http://www.ncbi.nlm.nih.gov; http://genome.ucsc.edu. MAF: Minor allele frequency; bold form indicates mutant allele. *Missense variant (Lys>Asn).

Table 2. Genotype and allele distribution among caries free and caries experience groups in primary and permanent teeth.

Dentition	Groups	Genotype n (%)				Allele n (%)		
		AA	Aa	aa	p-value	A	a	p-value
				RANK rs3826620				
Primary teeth	Caries Free	23 (48.9)	21 (44.7)	3 (6.4)	0.580	67 (71.3)	27 (28.7)	0.408
	Caries experience	70 (43.2)	74 (45.7)	18 (11.1)		214 (66)	110 (34)	
Permanent teeth	**Caries Free**	38 (53.5)	27 (38.0)	6 (8.5)	0.282	103 (72.5)	39 (27.5)	0.166
	Caries experience	47 (41.6)	53 (46.9)	13 (11.5)		147 (65.0)	79 (35.0)	
				RANKL rs9594738				
Primary teeth	**Caries Free**	19 (40.4)	19 (40.4)	9 (19.1)	0.445	57 (60.6)	37 (39.4)	0.962
	Caries experience	59 (35.8)	83 (50.3)	23 (13.9)		201 (60.9)	129 (39.1)	
Permanent teeth	**Caries Free**	28 (38.9)	33 (45.8)	11 (15.3)	0.868	89 (61.8)	55 (38.2)	0.760
	Caries experience	40 (35.1)	56 (49.1)	18 (15.8)		136 (59.6)	92 (40.4)	
				OPG rs2073618				
Primary teeth	**Caries Free**	23 (48.9)	19 (40.4)	5 (10.6)	0.132	65 (69.1)	29 (30.9)	0.033*
	Caries experience	59 (35.8)	70 (42.4)	36 (21.8)		188 (57.0)	142 (43.0)	
Permanent teeth	**Caries Free**	28 (39.4)	29 (40.8)	14 (19.7)	0.939	85 (59.9)	57 (40.1)	0.978
	Caries experience	47 (40.3)	44 (38.3)	24 (20.9)		138 (60.0)	92 (40.0)	

Note: AA means the common homozygotic, Aa the heterozygotic, and aa the uncommon homozygotic.
*Means statistical significance difference ($p<0.05$).

Table 3. Genotype and allele comparisons between DDE and without DDE groups.

Groups	Genotype n (%)				Allele n (%)		
	AA	Aa	aa	p-value	A	a	p-value
			RANK rs3826620				
Without DDE	80 (42.3)	90 (47.6)	19 (10.1)	0.323	250 (66.1)	128 (33.9)	0.410
With DDE	29 (52.7)	20 (36.4)	6 (10.9)		78 (70.9)	32 (29.1)	
			RANKL rs9594738				
Without DDE	74 (38.5)	91 (47.4)	27 (14.1)	0.416	239 (62.2)	145 (37.8)	0.405
With DDE	16 (29.1)	31 (56.4)	8 (14.5)		63 (57.3)	47 (42.7)	
			OPG rs2073618				
Without DDE	72 (37.5)	83 (43.2)	37 (19.3)	0.956	227 (59.1)	157 (40.9)	0.996
With DDE	20 (36.4)	25 (45.5)	10 (18.2)		65 (59.1)	45 (40.9)	

Note: AA means the common homozygotic, Aa means the heterozygotic, and aa means the uncommon homozygotic.

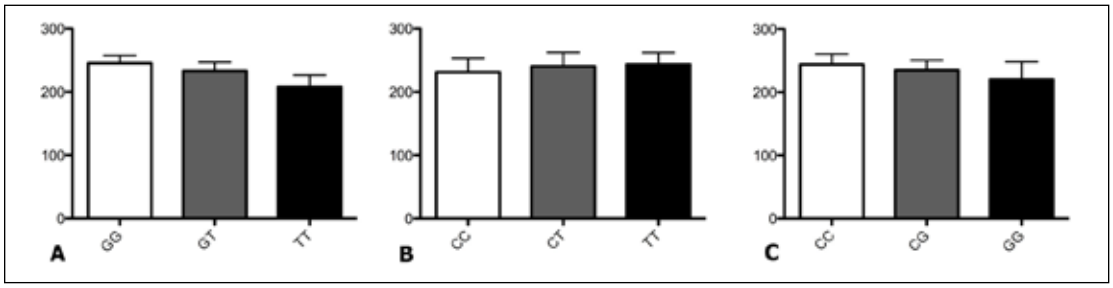

Figure 2. Enamel microhardness (Kg/mm2) distribution according to the genotypes: A- RANK; B-RANKL; and C- OPG genes.

DISCUSSION

In the present study, an association between the genetic polymorphism rs2073618 in OPG and caries experience in primary dentition was observed. This polymorphism is a leads to a Lysine aminoacid replacement by an Asparagine. This aminoacid substitution could increase the primary enamel susceptibility to caries. The critical role of the RANK/RANKL/OPG system in development is reinforced by cases of genetic alterations observed in patients, such as juvenile Paget's disease[21] and idiopathic hyperphosphatasia[22]. In a case report presenting juvenile Paget's disease with heterozygous duplication in *RANK*, the patient presented remarkable dental alterations in primary and permanent dentitions. Both dentitions were described with shape alterations, large pulp chambers and poorly mineralized tissues, and some teeth also presented DDE[23]. In individuals with chronic idiopathic hyperphosphatasia, dental alteration descriptions included generalized enamel hypoplasia[24]. These dental findings could be explained by the disruption of the interaction between RANK and RANKL which affects the temporal program of odontogenesis[8].

OPG, RANK and RANKL are closely linked with each other, in which OPG acts as a soluble decoy receptor and competes with RANK for binding to RANKL[3]. In molars from mice embryos, OPG and RANK were expressed from the internal and the external enamel epithelium when the bud epithelium progressively takes the form of the cap configuration and develops into the internal and the external enamel epithelium[8]. Additionally, the cell culture result has shown that RANKL is expressed in dental follicle cells of mice[7]. A study of primary human teeth demonstrated, through immunohistochemical study, that RANKL is presented in odontoblasts, odontoclasts, periodontal ligament cells, and pulp fibroblasts[25]. Our results also support the role of this system in human primary tooth enamel development in an allele dependent manner, once the polymorphism in *OPG* was associated with caries, in which children carrying the C allele had a lower chance of having caries experience. The genetic polymorphism in *OPG* evaluated here is a missense mutation. Our results suggest that the change of Lysine to Asparagine could alter the enamel, favoring the increase of caries susceptibility in primary teeth.

In a previous study with *opg*-/- knockout mice, the micro-computed tomography analysis showed significantly lower bone mineral density of alveolar bone in comparison with wild type mice[12]. Another study that evaluated the effects of OPG on the mechanical properties of rat bone also demonstrated that OPG increased femoral mineralization and strength indices[26]. In our study, an association between enamel microhardness and polymorphisms in *RANK, RANKL* and *OPG* was not observed. However, the wide range of age of the microhardness sample could be a limitation of our study, once the maturity of enamel changes with age[27]. Interestingly, although Sheng et al. (2010)[12] found lower bone mineralization in *Opg*-/- knockout mice, higher degrees of mineralization were observed in the enamel and dentin analysis. More functional and epidemiological studies should be performed in order to better understand the role of *RANK, RANKL* and *OPG* in the enamel and dentin mineralization in humans.

Ohazama *et al* (2004)[8] observed that the addition of exogenous OPG to explant cultures of E12 mice tooth primordial led to a delay in tooth development. When the explants were transplanted under kidney capsules to allow full tooth development, the OPG-treated teeth showed thinner dentin and enamel, with enamel defects such as hypoplasia. Enamel hypoplasia is a quantitative defect associated with the reduction of enamel thickness formed during the secretory stage of amelogenesis[28,29]. We did not observe an association between genetic polymorphisms in *RANK, RANKL* and *OPG*, but it is possible that other genetic polymorphisms in these genes may be associated with DDE.

Socio-demographic and behavioral variables were not associated with caries experience in the present study. However, it is possible that the lack of association was due to the fact that the socio-demographic and behavioral variables were collected through a questionnaire, which could be a limitation or bias in our study due to the reporting reliability of the parents and guardians. Other important limitation of our study is the lack of information regarding pre-peri-and post-natal conditions and their association with DDE. Although the results from previous studies are conflicting[30], it is possible that prenatal or early childhood health factors are involved with DDE only in individuals that carry a specific genetic background.

Researchers have been discovering new phenotypes related to the triad RANK/RANKL/OPG. Therefore, further studies should be performed in order to evaluate this pathway in oral and dental phenotypes.

CONCLUSION

The genetic polymorphism rs2073618 in *OPG* gene was associated with dental caries experience in primary dentition, while *RANK* rs3826620 and *RANKL* rs9594738 polymorphisms did not show any association with dental caries. The studied polymorphisms in *RANK, RANKL* and *OPG* were not associated with DDE nor microhardness.

REFERENCES

1. Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME, Roux ER, Teepe MC, DuBose RF, Cosman D, Galibert L. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. Nature; 13;390(6656):175-9. 1997.
2. Wong BR, Josien R, Lee SY, Sauter B, Li HL, Steinman RM, Choi Y. TRANCE (tumor necrosis factor [TNF]-related activation-induced cytokine), a new TNF family member predominantly expressed in T cells, is a dendritic cell-specific survival factor. J Exp Med; 186(12):2075-80. 1997.
3. Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR, Burgess T, Elliott R, Colombero A, Elliott G, Scully S, Hsu H, Sullivan J, Hawkins N, Davy E, Capparelli C, Eli A, Qian YX, Kaufman S, Sarosi I, Shalhoub V, Senaldi G, Guo J, Delaney J, Boyle WJ. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell; 93(2):165-76. 1998.
4. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci ; 95(7):3597-602. 1998.
5. Silva I, Branco JC. Rank/Rankl/opg: literature review. Acta Reumatol Port; 36(3):209-18. 2011.
6. Walsh MC, Choi Y. Biology of the RANKL-RANK-OPG System in Immunity, Bone, and Beyond. Front Immunol; 20;5:511. 2014.
7. Nakchbandi IA, Weir EE, Insogna KL, Philbrich WM, Broadus AE. Parathyroid hormone-related protein induces spontaneous osteoclast formation via a paracrine cascade. Proc Natl Acad Sci; 97:7296–7300. 2000.
8. Ohazama A, Courtney JM, Sharpe PT. Opg, Rank, and Rankl in tooth development: co-ordination of odontogenesis and osteogenesis. J Dent Res. 2004; 83(3):241-4. PubMed PMID: 14981127.
9. Suzuki T, Suda N, Ohyama K. Osteoclastogenesis during mouse tooth germ development is mediated by receptor activator of NFKappa-B ligand (RANKL). J Bone Miner Metab; 22:185–91. 2004.
10. Yao S, Ring S, Henk WG, Wise GE. In vivo expression of RANKL in the rat dental follicle as determined by laser capture microdissection. Arch Oral Biol.; 49(6):451-6. 2004,
11. Heinrich J, Bsoul S, Barnes J, Woodruff K, Abboud S. CSF-1, RANKL and OPG regulate osteoclastogenesis during murine tooth eruption. Arch Oral Biol; 50:897–908. 2005.
12. Sheng ZF, Ye W, Wang J, Li CH, Liu JH, Liang QC, Li S, Xu K, Liao EY. OPG knockout mouse teeth display reduced alveolar bone mass and hypermineralization in enamel and dentin. Arch Oral Biol.; 55(4):288-93. 2010.
13. Seow WK. Developmental defects of enamel and dentine: challenges for basic science research and clinical management. Aust Dent J;59 (1):143-54. 2014.
14. Pascoe L, Seow WK. Enamel hypoplasia and dental caries in Australian aboriginal children: prevalence and correlation between the two diseases. Pediatr Dent; 16(3):193-9. 1994.
15. Li Y, Navia JM, Bian JY. Caries experience in deciduous dentition of rural Chinese children 3-5 years old in relation to the presence or absence of enamel hypoplasia. Caries Res; 30(1):8-15. 1996.
16. Hong L, Levy SM, Warren JJ, Broffitt B. Association between enamel hypoplasia and dental caries in primary second molars: a cohort study. Caries Res; 43(5):345-53. 2009
17. Küchler EC, Tannure PN, Falagan-Lotsch P, Lopes TS, Granjeiro JM, Amorim LM. Buccal cells DNA extraction to obtain high quality human genomic DNA suitable for polymorphism genotyping by PCR-RFLP and Real-Time PCR. J Appl Oral Sci; 20(4):467-71. 2012.
18. World Health Organization (WHO). Oral health surveys: basic methods. 5th ed. Geneva; 2013.
19. FDI. A review of the developmental defects of enamel index (DDE Index). Commission on Oral Health, Research & Epidemiology. Report of an FDI Working Group. Int Dent J; 42(6):411-26. 1992.
20. Romanos HF, Antunes LS, Lopes LB, Sabóia Tde M, Tannure PN, Lips A, Antunes LA, Abreu FV, Deeley K, Alves G, Granjeiro JM, Vieira AR, Costa MC, Küchler EC. BMP2 Is Associated with Caries Experience in Primary Teeth. Caries Res; 49(4):425-33. 2015.
21. Naot D, Choi A, Musson DS, Simsek Kiper PÖ, Utine GE, Boduroglu K, Peacock M, DiMeglio LA, Cundy T. Novel homozygous mutations in the osteoprotegerin gene TNFRSF11B in two unrelated patients with juvenile Paget's disease. Bone; 68:6-10. 2014.
22. Cundy T, Hegde M, Naot D, Chong B, King A, Wallace R, Mulley J, Love DR, Seidel J, Fawkner M, Banovic T, Callon KE, Grey AB, Reid IR, Middleton-Hardie CA, Cornish J. A mutation in the gene TNFRSF11B encoding osteoprotegerin causes an idiopathic hyperphosphatasia phenotype. Hum Mol Genet; 11(18):2119-27. 2002.
23. Whyte MP, Tau C, McAlister WH, Zhang X, Novack DV, Preliasco V, Santini-Araujo E, Mumm S. Juvenile Paget's disease with heterozygous duplication within TNFRSF11A encoding RANK. Bone; 68:153-61. 2014.
24. Sreejan CK, Gopakumar N, Subhas Babu G. Chronic idiopathic hyperphosphatasia with unusual dental findings–A case report. J Clin Exp Dent.; 4(5):e313-6. 2012.
25. Lossdorfer S, Gotz W, Jager A. Immunohistochemical localization of receptor activator of nuclear factor kappaB (RANK) and its ligand (RANKL) in human deciduous teeth. Calcif Tissue Int; 71:45–52. 2002.
26. Ross AB, Bateman TA, Kostenuik PJ, Ferguson VL, Lacey DL, Dunstan CR, Simske SJ. The effects of osteoprotegerin on the mechanical properties of rat bone. J Mater Sci Mater Med; 12(7):583-8. 2001.
27. Kunin AA, Evdokimova AY, Moiseeva NS. Age-related differences of tooth enamel morphochemistry in health and dental caries. EPMA J; Jan 29;6(1):3. 2015.
28. Suckling GW. Developmental defects of enamel—historical and present-day perspectives of their pathogenesis. Adv Dent Res. 1989; 3(2):87-94.
29. Seow WK. Enamel hypoplasia in the primary dentition: a review. ASDC J Dent Child; 58(6):441-52. 1991.
30. Silva MJ, Scurrah KJ, Craig JM, Manton DJ, Kilpatrick N. Etiology of molar incisor hypomineralization–A systematic review. Community Dent Oral Epidemiol;44(4):342-53. 2016.