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A Comparative Study for Localization of Odontoclast in Crown and Root of Physiological Resorbed Primary Teeth

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Key words
odontoclast, resorption, oral physidogy.

Abstract

Root and crown resorption is a physiologic event for the primary teeth. It is still unclear whether odontoclasts, the cells which resorb the dental hard tissue, are different from the osteoclasts, the cells that resorb bone. Dental tissue resorption seems to be initiated and regulated by the stellate reticulum and the dental follicle of the underlying permanent tooth via the secretion of stimulatory molecules, i.e. cytokines and transcription factors. The primary teeth resorption process is regulated in a manner similar to bone remodeling, involving the same receptor ligand system known as RANK/RANKL (receptor activator of nuclear factor-kappa B/ RANK Ligand), which represent two cytokine-like proteins of the tumor necrosis factor superfamily, are localized on bone cells and dental cells .They are crucial for the regulation of osteoclastic/odontoclast cell differentiation and also for the upregulation of mature osteoclasts/odontoclasts mediated by cell-to-cell contact and a subsequent cascade of diverse intracellular signaling processes .The aim of the present study was to localize and compare the IHC reactions for RANKL along root surface and the crown of human phsiological resorbed primary teeth. Fifteen human upper deciduous (second molar) teeth ,undergoing root and crown resorption were used for immunohistochemical study to identify RANKL expression. The results demonstrated a high mean of expression of RANKL in root as compared with crown in human primary shedding teeth. The present study concludes that RANKL play a role in resorption process of the primary teeth.

Introduction

Resorption is an important part of a multitude of physiological and pathological processes in the human body. Resorption can affect hard tissues such as bone and dental hard tissues[1]. But it can also involve soft tissue and foreign material such as necrotic pulp tissue or materials used in pulp capping or root filling extruded through the apical foramen [2]. A well known example of physiological hard tissue resorption is resorption of primary teeth. A complex network of events on a cellular level including several activating and inhibiting cytokines and other compounds is required to direct the resorption of the primary tooth[3].Cells resorbing dental hard tissues are odotoclast cells .The multinuclear cells are formed by fusion of mononuclear cells. Several mononuclear odontoclast precursor cells may undergo fusion simultaneously with each other and form multinuclear cells. Mononuclear odontoclasts can also actively resorb dental hard tissue, although during progressive resorption most cells have several nuclei [4]. Shedding of human primary teeth expressed key mediators

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of hard tissue resorption through RANK/RANKL – system. Therefore odontoclast found as long as the roots were actively resorbed until root resorption was nearly finished, they were detected in the pulp .Then, odontoblasts began to degenerate, multinucleate odontoclasts appeared on the coronal dentin surface, and resorption proceeded from the predentin to the dentin [5]. Therefore the present study was designed to identify a positive reaction of RANKL by odontoclast in root and crown of resorbed primary tooth.

Materials and Methods

Fifteen deciduous second molar teeth ,at normal time of exfoliation were collected in the dental clinic , fixed, decalcified (using 10% formic acid ,changed every 3 days), and embedded in paraffin wax.Sections (5µ) were prepared for immunohistochemical (IHC) observations of RANKL marker.

Materials and Methods of Immunohistochemical Study

1.Monoclonal antibody for RANKL R1075-11A US Biological RANKL (immunogene,recombinant mouse RANKL,crossreactivity ,human.
2.Detection Kit 17506-06 US biological immunohistochemistry detection kit
Immunohistochemistry (Formalin fixed paraffin-embedded sections). Tissue underwent heat mediated antigen retrieval in sodium citrate buffer (pH 6.0). The primary antibody was used at 0.25 ug/ml and incubated with sample at 4°C overnight. A HRP-labeled polymer detection system was used with a DAB chromogen. Positive tissue control for RANKL was human giant cell granuloma.

Immunohistochemical Scoring of RANKL

The scoring was done under light microscope and the immunoreactive score (IRS, staining intensity) includes negative(0), weak(+), moderate(++), and strong(+++) represent mean of positive cells in 4 quartiles) and ranged from 0 to 12;[6] the
0–2 were considered negative
3–5 weak
6–8 moderate
and 9–12 strong staining .

Statistic Analysis

ANOVA test was used to identify differences in mean value.

Results

Immunohistochemical findings illustrate Positive RANKL expression by odontoclast near by subodontoblastic layer of the pulp ,the odontoblast cell shows positive expression with obvious displacement figure(1).Positive expression of RANKL by multinucleate odontoclast in coronal resorbed dentin figure(2).Detached odontoclast after dentin resorption can be recognized in pulp figures (3,4). Figure(5) illustrates pay like resorbed area in cementum with detection of resorbed material. Statistical analysis of the data for positive expression of RANKL by odontoclast in crown and root of resorbed primary teeth revealed a high significant difference value when compaired between two ,tables(1,2,3).

Discussion

The process of tooth resorption in the present study was showed many proceeding stages depending on immunohistochemical evaluation of positive expression of RANKL by odontoclast and may represent life cycle of the cell and include the followings:

1)Preresorptive wall of the dental pulp is covered with an odontoblast layer, although displacement of odontoblastic cell layer were detected .
2)RANKL -positive multinucleate odontoclasts are present near the subodontoblast layer ,while the rest of the pulp surface is still covered with an displaced -odontoblast layer which showed positivity too.

3)Mature multinucleate odontoclasts with positive expression of RANKL detected near resorbed dentin and cementum

4)Final resorption ,Odontoclasts are usually detached from the resorbed surface, and show signs of degeneration . Resorbed concavity areas in both coronal ,radicular dentin and in cementum were detected with resorbed material accumulate underneath ,which indicates that the process of exfoliation start first in root and it include periodontal ligament of the teeth[7]. Fuenzalid etal[8] 1999 found resorption of pulp surface with presences of positive multinucleate odontoclast cell detected by TRAP activity While. Kimura[9] 2003 studied histochemical and histometric analyses utilizing the positive tartrate-resistant acid phosphatase (TRAP) reaction by odontoclast in root resorption.

The present results of statistic analysis for the mean of positive odontoclast in root and crown show a high significant difference value.That 's result could be related to starting time of physiological resorption occurs firstly in root region and then proceed toward resorption of the crown therefore odontoclasts seemed to be more abundant in root than in crown and it also a time dependent with collection of tooth samples from different subjects. These results coincide with findings of Sahara etal[10]1996 who found that TRAP-positive mononuclear cells were detected in the pulp chamber as root resorption neared completion. Multinucleate odontoclasts can resorb dentine as well as cementum in the same way as osteoclasts resorb bone[11,12].

Conclusion

Resorption of root start first in shedding teeth and expressed RANKL which plays important role in resorption process.

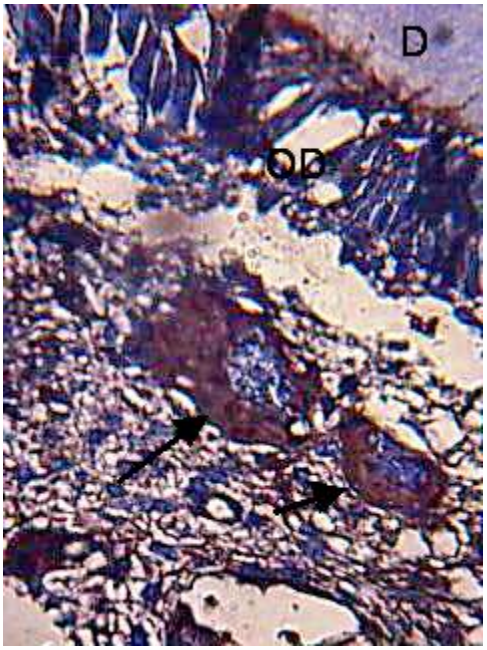

Fig.(1):- Immunohistochemical view for positive expression of RANKL by odontoclast(arrow),odontoblast(OD),Dentin(D) showed negative.DAB stain with hematoxylin counter stain ×20.

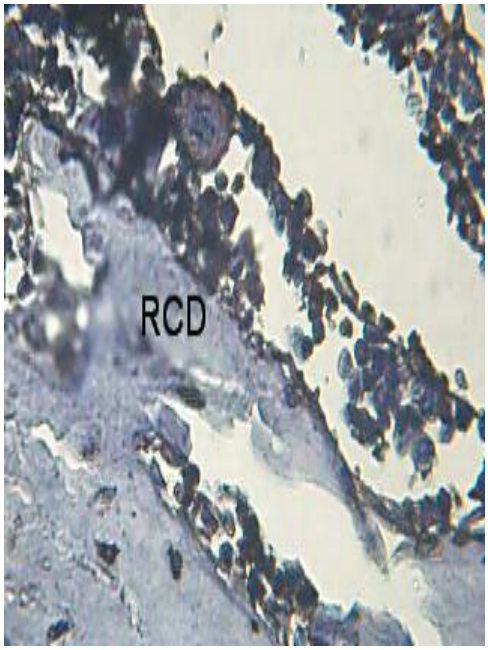

Fig. (2):- Immunohistochemical view for positive expression of RANKL by odontoclast (arrow)near by resorbed coronal dentin(RCD).DAB stain with hematoxylin counter stain ×20.

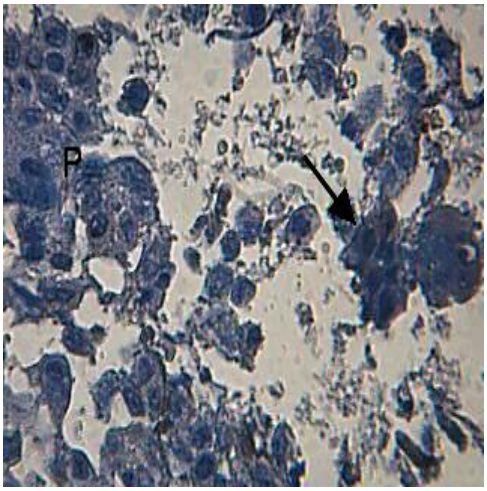

Fig. (3):- Immunohistochemical view for positive expression of RANKL by odontoclast (arrow)detached from resorbed dentin and be free in pulp(P) .DAB stain with hematoxylin counter stain ×20.

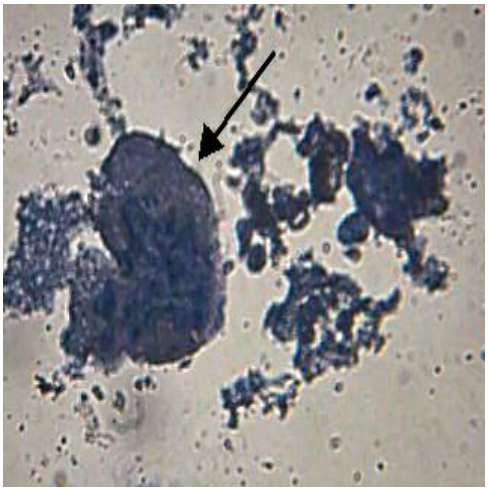

Fig.(4):- Magnifying view for previous figure (2) shows detached odontoclast (arrow)in the pulp. DAB stain with hematoxylin counter stain ×40.

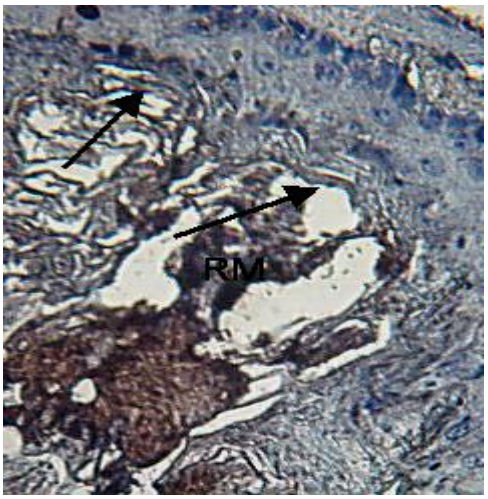

Fig.(5):-Immunohistochemical view for resorbed cellular radicular cementum (arrow), resorbed material(RM)can be detected.DAB stain with hematoxylin counter stain ×20.

Table(1):-Scoring of RANKL expression by odontoclast in crown and root of resorbed primary teeth.

Studied resorbed site	No.of specimens	Negative expression(-)		Weak expression(+)		Moderate expression(++)		Strong expression (+++)	
Crown	**15**	**6**	**40%**	**8**	**53.3%**	**1**	**6.6%**	**0**	**0%**
Root	**15**	**2**	**13.3%**	**5**	**20%**	**8**	**53.3%**	**0**	**0%**

Table(2):-statistics for positive RANKL expression in crown and root of resorbed primary teeth.

Studied resorbed site	NO. Of Specim.	Mean	Std.Dev	Std.Erro.	95% confidence interval for mean		Min.	Max.
					Lower Bound	Upper Bound		
Crown	15	2.6	0.33	0.23	1.8	3.4	2.3	3.1
Root	15	5.25	0.78	0.34	4.10	6.40	4.67	5.77

Table (3):-Equality of variance and equality of means of positive expression of RANKL value by ANOVA.

Studied resorbed site	Test of Homogencity of variance		ANOVA		C.S. P value
	Levene Statistic	Sig	F	Sig.	
Root/Crown	1.103	0.0015	5.29	0.004	HS

HS: Highly significant P<0.01

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