MARINA FUZETTE AMARAL

EFEITOS DA OCLUSÃO TRAUMÁTICA NO NÚMERO DE OSTEOCLASTOS E NA DENSIDADE ÓSSEA NO OSSO ALVEOLAR

EFFECT OF TRAUMATIC OCCLUSION IN THE NUMBER OF OSTEOCLAST AND BONE DENSITY OF THE ALVEOLAR BONE

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Trabalho de conclusão de curso como parte dos requisitos para obtenção do título de Bacharel em Odontologia da Faculdade de Araçatuba, Universidade Estadual Paulista "Júlio de Mesquita Filho".

Orientador(a) : Prof. Dra. Daniela Atili Brandini

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DEDICATÓRIA

Dedico esta, bem como todas as minhas demais conquistas, aos meus amados pais, Marcos Antônio do Amaral e Elza Cristina Fuzette Amaral,que, no decorrer da minha vida, proporcionaram-me, além de extenso carinho e amor , os conhecimentos da integridade, da perseverança e de procurar sempre em Deus à força maior para o meu desenvolvimento como ser humano. Por essa razão, gostaria de dedicar e reconhecer à eles, minha imensa gratidão e amor eterno.

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EPÍGRAFE

"O sucesso nasce do querer, da determinação e persistência em se chegar a um objetivo. Mesmo não atingindo o alvo, quem busca e vence obstáculos, no mínimo fará coisas admiráveis."

José de Alencar

MARINA, M.F. **EFEITOS DA OCLUSÃO TRAUMÁTICA NO NÚMERO DE OSTEOCLASTOS E NA DENSIDADE ÓSSEA NO OSSO ALVEOLAR .** 2013. TRABALHO DE CONCLUSÃO DE CURSO-FACULDADE DE ODONTOLOGIA, UNIVERSIDADE ESTADUAL PAULISTA, ARAÇATUBA, 2013.

RESUMO

Introdução: O papel mais recente que explora a relação entre a oclusão traumática e a doença periodontal, apontam para a necessidade de mais alguns estudos para explicar essa associação. **Objetivo**: Este estudo tem por objetivo avaliar o efeito do trauma oclusal no osso alveolar em molares de ratos por meio de análises quantitativas do número de osteoclastos em torno das raízes e no septo alveolar e a densidade do osso no septo alveolar do primeiro molar superior direito e primeiro molar inferior direito. Material e Método: Para este estudo, 40 ratos Wistar de sete semanas de idade foram divididos em um grupo controle e um grupo de oclusão traumática. Os períodos de estudo foram de 2, 5, 7 e 14 dias. No grupo experimental, a oclusal do primeiro molar inferior direito foi elevada através de um enchimento direto criando uma mesa oclusal plana e alta na cúspide oclusal mais alta. Cortes Transversais histológicos de 5µm de largura na parte superior direita do primeiro molar e tecidos circundante foram corados com hematoxilina e eosina e receberão tratamentos histoquímicos para detecção de TRAcP. Os dados foram analisados usando IBM SPSS 20.0 at $\alpha=0.05$. Teste Mann–***Whitney*** U e o teste de correlação de Pearson foram usados para análise estatística. **Resultados** Uma diferença estatisticamente significante pode ser vista entre os grupos, no número de osteoclastos no osso alveolar ao redor das raízes e no septo alveolar na maxila (2dias) e na mandíbula (2 e 7 dias).Em relação a densidade óssea no osso alveolar houve diferença estatisticamente significante na maxila em todos os períodos estudados e na mandíbula nos períodos de 2, 5 e 14 dias. A correlação entre o número de osteoclastos e a densidade óssea foi negativa na mandíbula e na maxila em todos os períodos estudados. **Conclusion:** O trauma oclusal provoca um aumento no número de osteoclastos em torno das raízes e no septo alveolar do primeiro molar superior direito e primeiro molar inferior direito, e também provoca uma diminuição da densidade óssea no septo alveolar nos períodos estudados.

Palavras-chave: oclusão traumática. Trauma oclusal. traumatismo oclusal. Interferência oclusal. Carga oclusal. Periodonto. Carga mecânica.

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ABSTRACT

Introduction: The most recent paper that explores the relationship between traumatic occlusion and periodontal disease, point out the necessity of some more studies to explain this association. **Objective:** This study aimed to evaluate the effect of occlusal trauma in the alveolar bone in rat molars through quantitative analyses of the number of osteoclast around the roots and in the alveolar septum; and bone density in the alveolar septum of the first upper and lower molar. **Material and Method:** For this study, 40 Wistar rats of seven weeks old were divided into Control group and Traumatic occlusion group. The periods of study were 2, 5, 7 and 14 days. In the experimental group, the inferior right first molar were raised by direct filling; creating a flat high occlusal table at the highest occlusal cusp. Transversal histological sections of 5 μm wide of the right lower and upper first molar and surrounding tissues were stained with hematoxylin and eosin, and histochemical treatment for TRAcP detection. The data were analyzed using IBM SPSS 20.0 at $\alpha=0.05$. Mann–***Whitney*** U test and the Pearson correlation were used for statistical analyses. **Results:** Statistical significant difference could be seem between the groups related to the number of osteoclasts in the alveolar bone around the root and septum in the upper jaw (Day 2) and lower jaw (Day 2 and 7); and bone density in the upper jaw in all periods of the experiment and in the lower jaw on 2, 5 and 14 day of the experiment. The rank correlation between number of osteoclasts and bone density was negative in the upper and lower jaw during all experimental period. **Conclusion:** The traumatic occlusion increased the number of osteoclast in the alveolar bone around the roots and in the alveolar septum of the right first upper and lower molar, and the bone density of the alveolar septum decrease.

Key words: traumatic occlusion, occlusal trauma, occlusal traumatism, occlusal interference, occlusal overload, periodontium, mechanical loading.

SUMÁRIO

1 INTRODUCTION

The most recent paper to review the literature that explores the relationship between traumatic occlusion and periodontal disease, point out the necessity of some more quantitative studies to explain this association (Fu and Yap, 2007; Deas and Mealey, 2006; Harrel, Nunn, Hallmon, 2006; LIU, JIANG and WANG, 2013). Some authors believes that occlusal trauma is a co-destructive factor for a periodontal disease (Gher, 1998; Nunn and Harrel, 2001; Harrel and Nunn, 2001) but others not (Fu and Yap, 2007).

The normal occlusal function is a mechanical stimulus that is necessary for the maintenance of the homeostasis of periodontal tissue, whereas the absence or excess of occlusal load results in a disharmonic functioning of periodontal tissues (Glickman, 1971; Rios et al., 2008).

The occlusal overload is often caused by an occlusal interference and/or parafunctional habits; as clenching and bruxism. It can cause a variety of destructive biological effects on pulp tissue, periodontium, alveolar bone, masticatory muscle, temporomandibular joint and central nervous system (Liu, Jiang and Wang, 2013).

The changes in the alveolar bone and periodontal connective tissue can occur both in the presence and in the absence of periodontitis (Gher, 1998). A study from Branschofsky et al. (2011) indicates that secondary occlusal trauma (i.e., premature and balance contacts) is frequently seen in periodontally compromised patients and is positively correlated with the severity of attachment loss.

Occlusal trauma can affect tooth mobility and clinical probing depth (Nunn and Harrel, 2001; Harrel and Nunn, 2001). The ultimate goal of successful management of mobile teeth is to restore function and comfort by establishing a stable occlusion that promotes tooth retention and the maintenance of periodontal health (Bernal, Carvajal and Muñoz-Viveros, 2002).

Histologically, several morphofunctional alterations are observed, in the face of excessive occlusal force; such as disorientation and a decrease in collagenous fibers (Palcanis, 1973; Biancu, Ericsson, Lindhe, 1995), change in the alignment of periodontal fibers (Glickman, 1971; Jabôr et al., 2003), an increase in the number of fibroblasts (Palcanis, 1973), elevated osteoclast activity (Palcanis, 1973; Biancu, Ericsson, Lindhe, 1995; Glickman, 1971), venous thrombosis (Palcanis, 1973), and cell necrosis in the periodontal ligament (Palcanis, 1973).

In the first seven days following the traumatic occlusion, an increase in the pressure of the interstitial fluid of the periodontal ligament was observed after 48 hours (Palcanis, 1973). Until the 5th day the width of the periodontal ligament space decreased, to return to normality on day 7 (Kaku et al., 2005), probably owing to bone remodeling (Glickman, 1971).

However, the influence of the traumatic occlusion on the osteogenesis and osteoclastogenesis of the alveolar still controversial (Kawamoto and Nagaoka, 2000; Kaku et al., 2005; Wan et al., 2012); even thought, the trauma occlusal concept is accepted in the academic field.

2 OBJECTIVES

This study aimed to evaluate the effect of traumatic occlusion in the number of osteoclast and bone density of the alveolar bone in rat molars through histomorphological analyses.

Specifics objectives:

a) Quantitative analyses of the number of osteoclast around the roots of the right first upper and lower molar;
b) Quantitative analyses of the number of osteoclast in the alveolar septum of the right first upper and lower molar and
c) Quantitative analyses of the bone density in the alveolar septum of the right first upper and lower molar.

3 MATERIALS AND METHODS

This study has been approved by the Animal Care Committee of the Dentistry School of Araçatuba (UNESP).

For this study, 40 Wistar rats (*Rattus Norvegicus, albinus*) of seven weeks old were used. Originating from the central bioterium of the Dentistry School of Araçatuba, the animals were transferred to the bioterium of the Department of Surgery and Integrated Clinic 5 days prior to the experiment. They were kept in cages with five animals each and given granulated food and water *ad libitum*. The environment was kept at a temperature of 22 °C (±2 ºC) and 50% (±10%) humidity, and light/dark cycles of 12/12 hours.

To address the influence of mechanical loading in the alveolar bone after occlusal trauma, the animals were divided into 2 groups: Control group and Traumatic occlusion group.

The periods of study were 2, 5, 7 and14 days, for all groups.

The groups consist of:

a. Control group (20 rats) – rats with the same age as the rats in the experimental group that won't submitted to experimental conditions, in order to compare with normal conditions.
b. Traumatic occlusion group (20 rats) – Traumatic occlusion created by ligature wire and composite filling in the right inferior first molar.

Prior to the experimental section, the animals were intramuscularly anesthetized with a solution of ketamine hydrochloride (25 mg/kg, Vetanarcol, Laboratórios König, Argentina) and xylazine (10 mg/kg, Coopazine, Coopers Brasil, Brazil). All animals will receive a single intramuscular 24,000 IU antibiotic dose (benzathine benzylpenicillin - 12,000 IU, procaine benzylpenicillin - 6,000 IU, potassium benzylpenicillin - 6,000 IU, dihydrostreptomycin sulfate - 5 mg, streptomycin sulfate - 5 mg; Fort Dodge, Animal Health Ltda., Campinas, SP, Brazil).

In the experimental group, the traumatic occlusion were induced by the method described by Sodeyama et al. (1996), Kawamoto e Nagaoka (2000), Kaku et al. (2005) and Wan et al. (2012). The inferior right first molar were raised by direct filling using 37% phosphoric acid etchant for enamel and dentin (FGM, Brazil), microbrush (Microbrush® International, Grafton, USA); scotchbond multi-purpose light adhesive (3M ESPE, Sant Paul, USA), Estelite e Quick composite resin (Tokuyama Dental

Corp, Japan) and photopolimerizer (Dabi Atlante, Ribeirão Preto, Brazil), as indicated by producer; creating a flat high occlusal table at the highest occlusal cusp. A piece of ligature wire 0.20mm (.008´´) (Morelli, Sorocaba, São Paulo, Brazil) were attached to the surface of the composite filling. Prior to the restoration, microretentions were made with Carbide burs FG ¼ (Beavers Dental, Canada) at high speed handpiece with water.

Animals were excluded in case they die of natural causes, and if they lose the occlusal composite filling.

After anesthesia, transcardial perfusion was performed. An intraventricular injection of heparin (0.1 ml / 5,000 U.I/ml) was administered. After 1 minute 100 ml saline were perfused via the aorta, followed by mixed solution of 200 ml of paraformaldehyde fixation solution at 4% (Sigma Chemical Co., St. Louis, MO, USA) and 200 ml phosphate buffered saline (PBS) at 0.1M, pH 7.4, 4ºC (Sigma Chemical Co., St. Louis, MO, USA).

After dissection, the specimens were washed in PBS and kept in 4% paraformaldehyde (Sigma Chemical Co., St. Louis, MO, USA) for 24 hours before decalcification with ethylene diamine tetra acetic acid disodium (EDTA) at 10% for 20 days. The specimens were processed with progressive dehydration in ethyl alcohol, diafanized in xylol, impregnated by paraffin at a low fusion temperature (56-58 ºC) for 3 hours and embedded according to standard protocols.

Transversal histological sections of 5 μm wide of the right upper and lower first molar and surrounding tissues were obtained with an automated microtome (Leica SMR 2000), transferred to a bain-marie (40-50 ºC) and then collected using silanised slides .

Some sections were stained with hematoxylin and eosin, HE using conventional methods and others were submitted to histochemical treatment for TRAcP detection.

Histochemical for TRAcP detection

To show TRAcP activity, sections were deparaffinized, rehydrated and rinsed in PBS. For reactivation were used a 0.1M Tris pH 9.0 solution, overnight at room temperature. After that, the section were incubated with a solution containing 30 mg Naphtol-AS-BI phosphate (Sigma D4254, Sigma Aldrich Chemie Gmbh, Taufkirchen, Germany), 0.5 ml Dimethylformamide (Sigma D4254, Sigma Aldrich Chemie Gmbh, Taufkirchen, Germany), 9 ml acetatebuffer in PVA , 0.1 ml $MgCl_2$. $6H_2O$, 0.1 ml KNa-tartrate $4H_2O$, 0.3 ml pararosanilin and 0.3 ml NaNO2; for 1h at 37^oC. Subsequently, the sections were rinsed in PBS and counterstained with Mayer hematoxylin.

Labeling analyses

A number of three sections of each specimen, in a distance of 40 μm between them, were selected to evaluate the histomorphology at the time-related changes throughout the experimental period and comparation between the groups.

The sections were observed with an Aristoplan light microscope (Leica - Aristoplan, Alemanha) and micrographed with an Axiocam MRc digital camera (Carl Zeiss MicroImaging GmbH, Germany).

The visual camps of the superior and inferior right first molar and surrounding tissues of each animal were collected with 20X objectives, and also analysed using the program Axionvision Rel 4.0 (Carl Zeiss GmbH, Germany). For quantitative analysis, the images were processed using ImageJ.

The sections counterstained with hematoxylin and eosin (HE) were used for qualitative and quantitative analyzes of the alveolar bone density. An area of $400\mu m^2$; magnified 100 times, at the interradicular alveolar bone between the roots of the right superior and inferior first molar were used. To calculate the surface occupied by bone, these were quantified as a percentage of the total area.

The number of osteoclasts (TRAcP positive cells) were counted around the all roots, and in the alveolar septum ($600 \times 800\mu m^2$) of the right first lower and upper molar.

The examiner were not informed to which group the images belonged, in order to avoid bias during the analysis.

Statistical Analysis

The data were analyzed using IBM SPSS 20.0 (IBM, Armonk, NY, USA) at $\alpha=0.05$. ***Mann–Whitney*** U test was used for group comparison. The Pearson correlation test was made between number of osteoclasts and bone density. Data were expressed as mean ± SD or percentage.

4 RESULTS

Representative histological observations are shown in Fig. 1, 2 and 3.

CONTROL GROUP

This analyses were limited in the cervical region of the roots and alveolar bone of the right first molar of the maxillary (Fig. 1A) and mandible (Fig. 1B), specifically in the periodontal ligament and alveolar bone.

The control group showed a normal pattern of histological characteristics during the whole experimental period. The cement was thin and showed acellular aspects with evident insertion of extrinsic collagen fibres. The cementoblasts showed morphological evidence of cells with a high synthesis metabolism and protein secretion. Areas of resorption or cement repair were rarely observed. The periodontal ligament had collagen fibres with a radial disposition, typical of the horizontal collagen fibre group of that area. An elevated quantity of cells similar to fibroblasts was found among the collagen fibres. Blood vessels and nervs were identified mainly in the alveolar third of the periodontal ligament. The alveolar bone showed basophilic intermittent concentric lines, reveling a incremental pattern of aposition of bone matrix (Fig. 1E). A lot of medular space of the alveolar bone present continuit with the periodontal ligament.

TRAUMATIC OCCLUSION GROUP

Periodontal Ligament

On Day 2 the main signal was the presence of enlarged blood vessel in the alveolar third of the periodontal ligament. There were also, some few areas with possible disorganization of the collagenos fibers of the periodontal ligament near the alveolar bone on Day 2, 5, 7 and 14.

Alveolar Bone

Concerning the bone resorption parameters, contrariwise, the distal side showed significantly higher numbers of osteoclasts than the mesial side, in both groups during all experimental period.

On Day 2 the alveolar bone septum show clearly lower boné density (Fig. 2F e 3F), many áreas showing continuity between the periodontal ligament and the adjacents roots. In these areas were possible show active osteoclasts in the alveolar surfice and medular spaces (Fig. 2B e 3B). On Day 5 the persistence of alveolar bone reabsoption was seen, mainly in the alveolar septum (Fig. 2D e 3D). On days 7 and 14 (Fig. 2H and 3H) the remodeling of alveolar bone still exists in a moderate intensity, with a sign of smaller density and higher bone reabsorption, alghout in a minor degree than the prior groups.

In the Traumatic occlusion group, TRAcP expression is higher in the osteoclast located in the alveolar around the roots and in the alveolar septum (Fig 2B, 2D, 3B and 3D). A statistical significant difference should be seen in the number of osteoclast in the alveolar bone around the roots and in alveolar bone septum on day 2 in the upper molar and days 2 and 7 in the lower molar (Table 1). The bone density showed significatively decreased in the Traumatic occlusion group during all experimental period in the maxilla (Figure 2F e 2H) and mandible (Figura 3F e 3H) (Table 1 and 2).

Differences between Upper and Lower Jaw

Traumatic occlusion group show higher number of enlarged blood vessel, disorganization of collagens fibers in the periodontal ligament and alveolar bone reabsorption in the lower jaw.

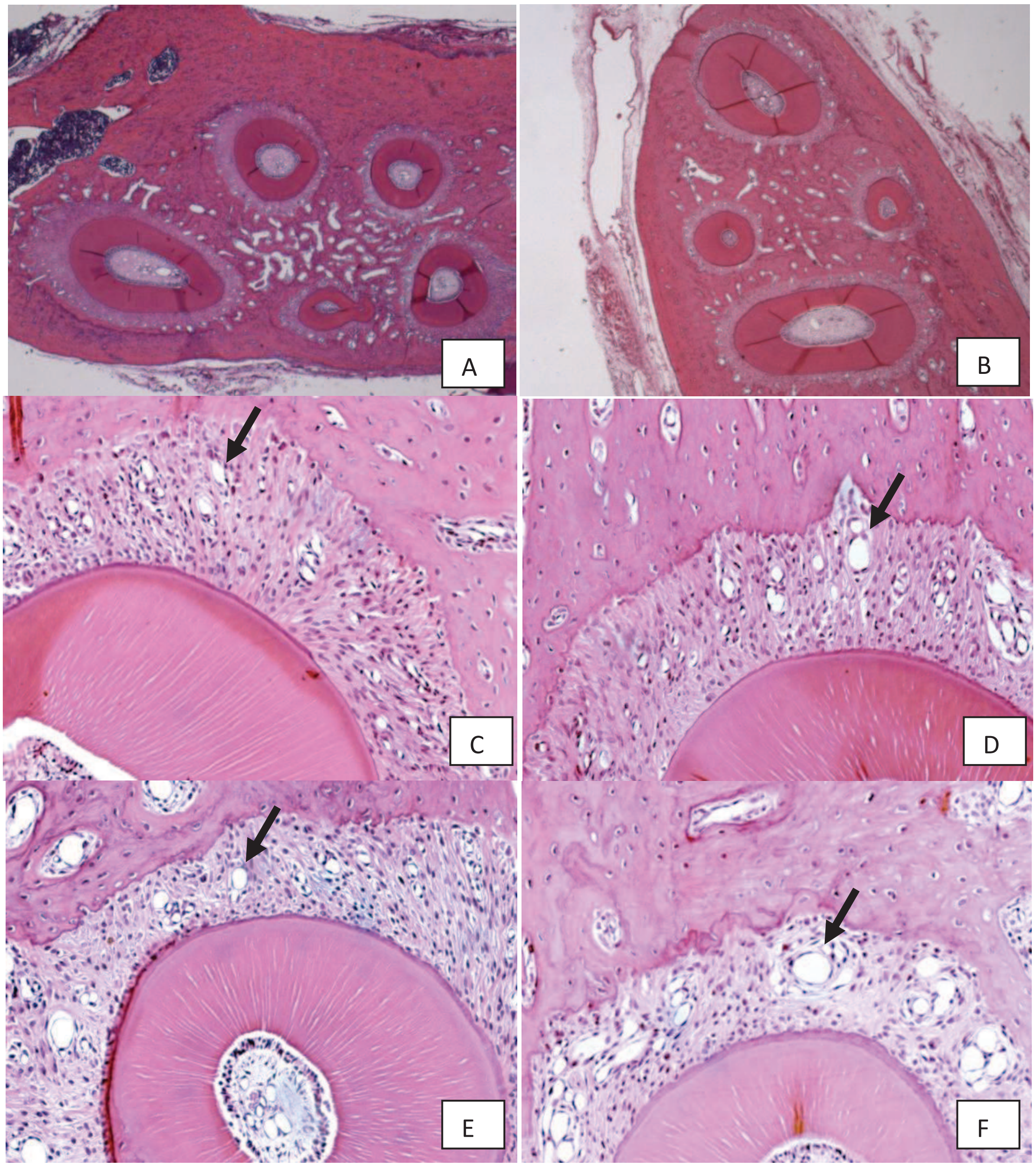

Figure 1. Transversal histological sections of the right upper (1A) and lower (1B) first molar and surrounding tissue were stained with hematoxilin and eosine. The right upper molar show five roots (1A) and the right lower molar four roots (1B). Blood vassels (arrow) are more enlarged blood vessels in the Trumatic occlusion group in the maxila (1D) and mandible (1F), whem compared with control group, respectively (1C e 1E). Desorganization of the colagens fibers of the periodontal ligamento in the Traumatic Oclusion group (*).

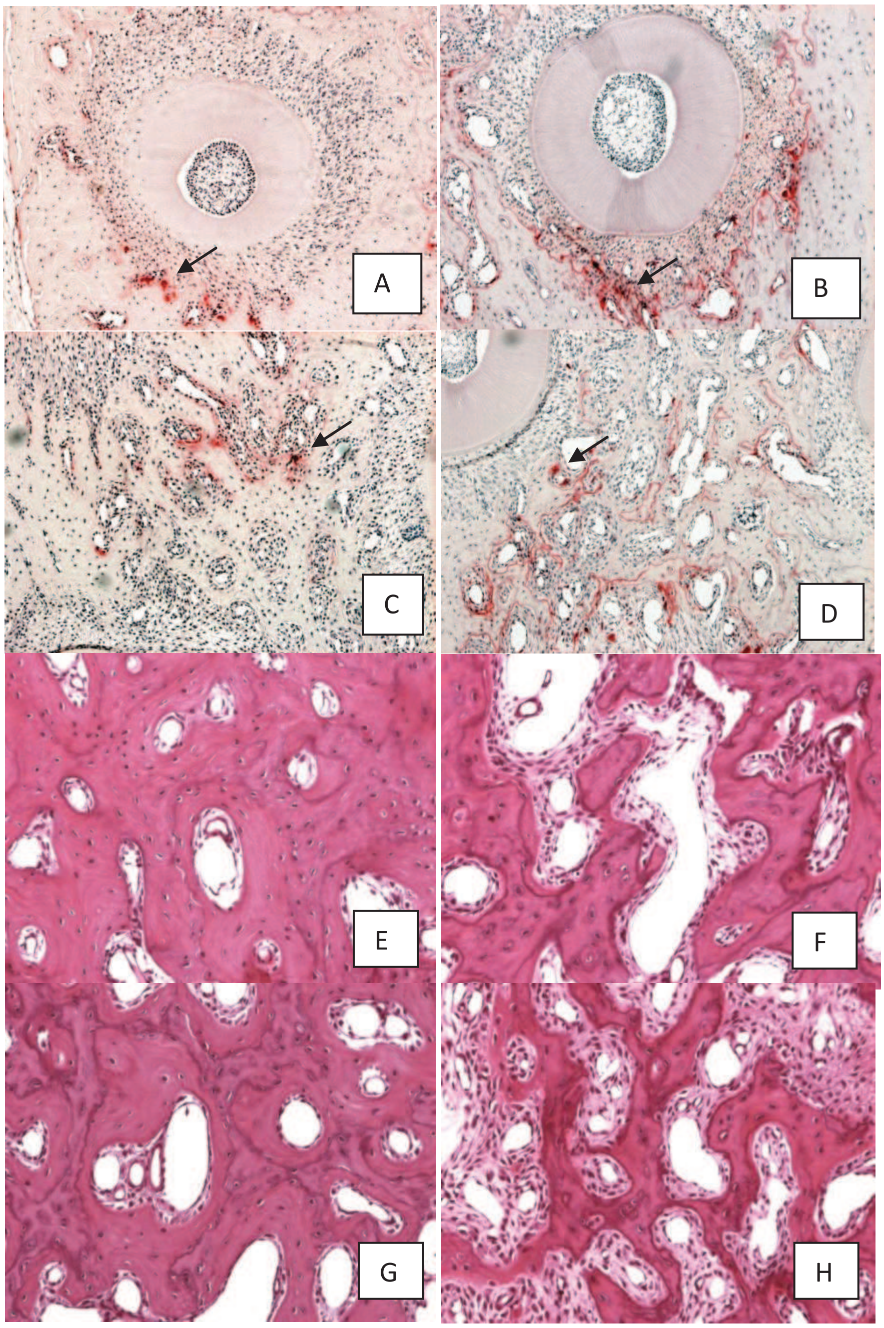

Figure 2. Transversal histological sections of the right upper first molar and surrounding tissue were stained for TRAcP detection and hematoxilin and eosine. Detection of TRAcP positive osteoclasts (arrows) on day 2 (2A and 2B) and 5 (2C and 2D) of the experiment in the Traumatic occlusion group (2B and 2D) is in great number than Control group (2A and 2C) around the root and also in the alveolar bone septum (Magnification 100x). On day 5, the bone the bone reabsorption and number of osteoclast (arrow) can be seen in the in the unloaded (2C) and loaded group (2D). The presence of bone reasorption (2F and 2H) in the Traumatic occlusion group in comparation with the bone density in the Control group (2E e 2G) on days 2 and 14, respectively (Magnification 200x).

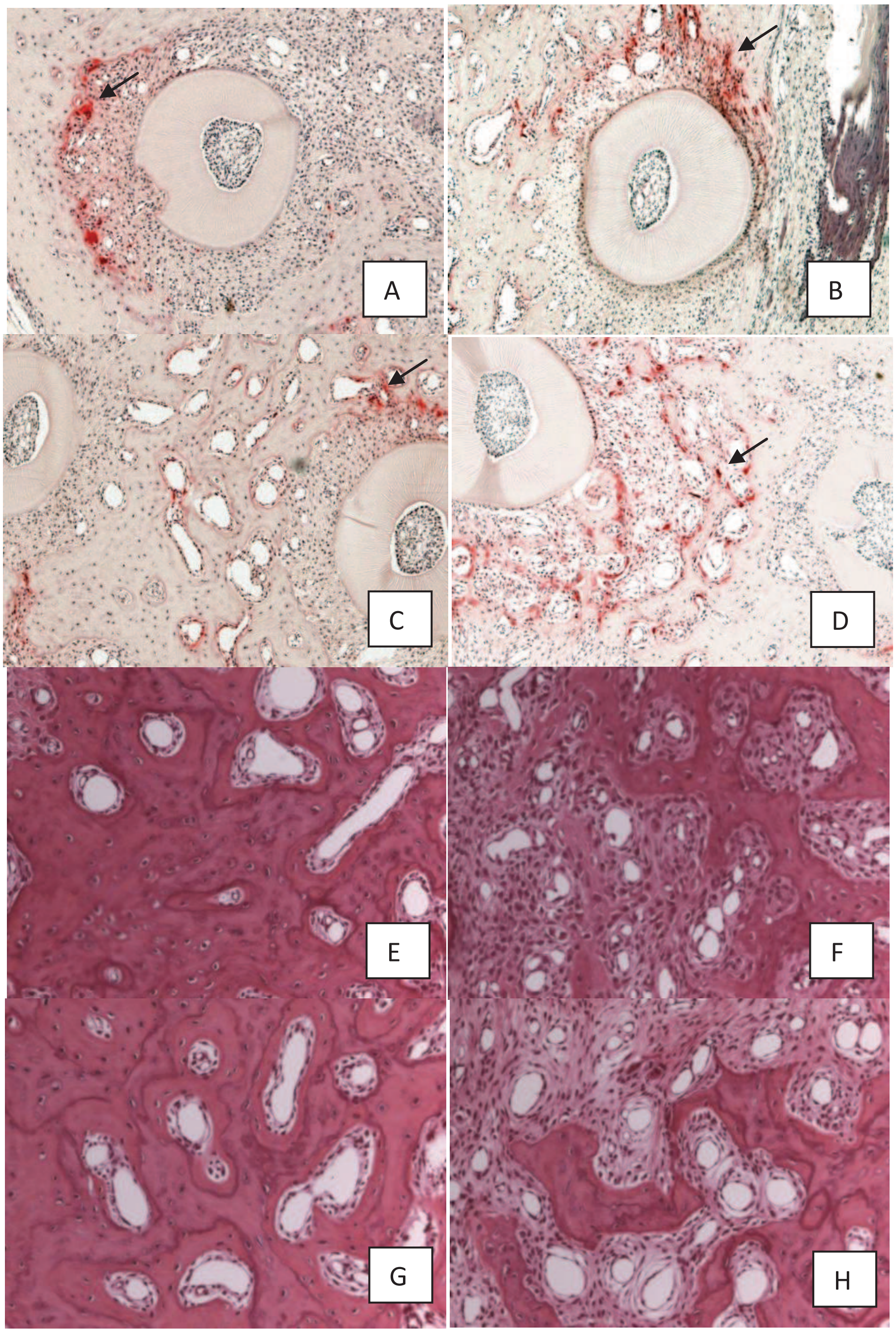

Figure 3. Transversal histological sections of the right lower first molar and surrounding tissue were stained for TRAcP detection and hematoxilin and eosine. Detection of TRAcP positive osteoclasts (arrows) on day 2 and 5 of the experiment in the Traumatic occlusion group (3B and 3D, respectively) is in great number than Control group (3A and 3C, respectively) around the root and also in the alveolar bone septum (Magnification 100x). The presence of bone reasorption (3F and 3H) in the Traumatic occlusion group in comparation with the bone density in the Control group (3E e 3G) on days 2 and 14, respectively (Magnification 200x).

Table 1. Bone remodeling in the upper right first molar front of traumatic occlusion.

Right first upper molar						
	Days of the Experiment	Control		Traumatic Occlusion		P value
		mean	(SD)	mean	(SD)	
Mononuclear Osteoclasts Around the roots	2	19,67	8,11	57,17	13,01	0.029*
	5	14,33	6,30	56,56	25,43	0.057
	7	13,67	6,13	47,56	6,35	0.200
	14	21,00	1,86	50,17	10,61	0.200
Multinuclear Osteoclasts Around the roots	2	10,58	4,37	26,25	7,26	0.029*
	5	8,33	5,35	36,50	19,84	0.057
	7	8,00	5,66	31,00	7,02	0.200
	14	14,33	4,26	39,17	4,48	0.200
Osteoclasts total Around the root	2	30,25	12,30	83,42	20,19	0.029*
	5	22,67	11,58	93,06	45,26	0.057
	7	21,67	11,79	78,56	13,37	0.200
	14	35,33	5,36	89,33	15,08	0.200
Mononuclear Osteoclasts in the alveolar bone septum	2	3,42	1,23	12,92	4,91	0.029*
	5	1,58	1,20	11,11	0,69	0.057
	7	1,50	0,24	12,22	2,83	0.200
	14	4,33	0,58	11,44	2,22	0.100
Multinucleated Osteoclasts in the alveolar bone septum	2	2,13	0,69	9,50	5,09	0.029*
	5	1,00	0,47	8,56	1,92	0.057
	7	1,33	0,47	7,56	1,50	0.200
	14	1,56	0,19	9,11	1,26	0.100
Osteoclasts total in the alveolar bone septum	2	5,54	1,66	22,42	7,44	0.029*
	5	2,58	0,88	19,67	2,60	0.057
	7	2,83	0,71	19,78	4,14	0.200
	14	5,89	0,69	20,56	3,20	0.100
Bone density in the alveolar bone septum	2	82,16	5,72	32,37	14,29	0.029*
	5	78,25	13,09	29,39	15,70	0.029*
	7	80,19	9,18	24,64	13,22	0.029*
	14	72,58	4,48	28,62	6,46	0.029*

P values are for comparison between the two groups. ***Mann–Whitney*** U test were used for continuous variables. Data are presented as the mean ± standard deviation.*P<.05; **P<.001.

Table 2. Bone remodeling in the lower right first molar front of traumatic occlusion.

Right first inferior molar						
	Days of the Experiment	Control		Traumatic Occlusion		P value
		mean	(SD)	mean	(SD)	
Mononuclear Osteoclasts Around the roots	2	15,83	6,76	73,17	6,92	0.029*
	5	15,04	2,11	81,67	29,29	0.057
	7	17,50	4,24	56,27	15,08	0.016*
	14	18,94	18,83	67,83	18,82	0.057
Multinuclear Osteoclasts Around the roots	2	8,33	4,28	25,54	6,25	0.029*
	5	11,58	1,64	27,67	11,05	0.057
	7	14,75	2,30	20,27	5,13	0.111
	14	8,11	9,45	27,92	8,70	0.057
Osteoclasts total Around the root	2	24,17	10,90	98,71	11,76	0.029*
	5	26,63	3,44	109,33	39,84	0.057
	7	32,25	6,36	76,53	19,45	0.016*
	14	27,06	28,16	95,75	25,29	0.057
Mononuclear Osteoclasts in the alveolar bone septum	2	2,42	0,79	15,79	4,52	0.029*
	5	2,63	1,72	26,67	21,76	0.057
	7	3,00	1,28	24,00	6,86	0.016*
	14	2,56	0,69	16,54	4,22	0.057
Multinucleated Osteoclasts in the alveolar bone septum	2	0,33	0,27	9,04	3,95	0.029*
	5	0,58	0,79	10,22	12,10	0.114
	7	0,92	0,63	9,00	4,50	0.016*
	14	0,44	0,77	5,71	1,76	0.057
Osteoclasts total in the alveolar bone septum	2	2,75	0,92	24,83	7,52	0.029*
	5	3,21	1,97	36,89	33,84	0.057
	7	3,92	1,89	33,00	10,63	0.016*
	14	3,00	1,45	22,25	4,98	0.057
Bone density in the alveolar bone septum	2	69,43	7,03	23,04	2,83	0.029*
	5	64,68	12,53	38,81	11,59	0.016*
	7	73,63	6,02	43,27	10,40	0.057
	14	65,06	15,66	30,80	13,09	0.010*

P values are for comparison between the two groups. ***Mann–Whitney*** U test were used for continuous variables. Data are presented as the mean ± standard deviation.*P<.05; **P<.001.

Table 3. Nonparametric correlation between number of osteoclast and bone density in the upper jaw, presented by rank correlation coefficient.

MAXILA			
Number of osteoclast in the alveolar bone septum x Bone density			
Day 2	**Day 5**	**Day 7**	**Day 14**
-,833 ,010*	-,750 ,052	-,800 ,104	-,900 ,037*

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

Table 4. Nonparametric correlation between number of osteoclast and bone density in the lower jaw, presented by rank correlation coefficient.

MANDIBULA			
Number of osteoclast in the alveolar bone septum x Bone density			
Day 2	**Day 5**	**Day 7**	**Day 14**
-,905 ,002**	-,775 ,041*	-,750 ,052	-,786 ,036*

** Correlation is significant at the 0.01 level

* Correlation is significant at the 0.05 level

5 DISCUSSION

This study evaluated the action of traumatic occlusion in the periodontium, and the null hypothesis was not proven because there were differences between the groups.

Localized, primary occlusal trauma most typically is related to a "high" restoration, a common sequela of the placement of a new restoration in an individual tooth that has been extensively instrumented. Some authors believe that occlusal trauma is a co-destructive factor (Gher, 1998; Nunn and Harrel, 2001; Harrel and Nunn, 2001) for a periodontal disease, but for others it still inconclusive (Gher, 1998; Fu and Yap, 2007).

For the examination of the influence of occlusion status on tooth-supporting tissue, experimental studies in animals have been extensively performed. In some studies, experimental traumatic occlusion was simulated by raising the vertical dimension of occlusion (Bhaskar & Orban, 1955; Wentz, Jarabak & Orban, 1957; Safavi et al., 1974; Jorgensen, 1980). The destruction of the alveolar bone proper by traumatic occlusion was assessed by radiographic or histologic methods. However, there have been a few quantitative analyses of occlusal trauma in bone tissues. Young rats, seven weeks old were used in the study. The osteoclasts could be seen in the distal side of the roots in both groups, In front a traumatic occlusion, there were a increase of the number of the osteoclasts, although it still concentrated in the distal side of the root.

Misawa et al (2007). demonstrated that the specific changes that occur in the alveolar bone with advancing age are related to both systemic factors as with local factors that may be involved in the regulation of bone metabolism alveolar wall. The teeth appeared to move distally throughout observation weeks, although the rate of movement rapidly decreased with age. Rat molars are known to drift distally through the alveolar bone, and both resorptive activity and depository activity are detected in the socket through out the life of the animal. Misawa et al, (2007); Sicher and Weinmann, (1944) suggested that the continuous growth of rat jaws and their posteriorior site of lengthening are responsible for the distal movement.

The most important function of the alveolar wall around the tooth socket is mechanical support of the tooth, and this wall interacts with collagen fibers of the periodontal membrane, which transmit mechanical strain from the tooth to the alveolar bone. Therefore, unlike bone at other sites, the alveolar wall has a unique pattern of continuous remodeling that may respond to systemic as well as local factors such as tooth movement and mechanical stress (Misawa et al,2007).

Excessive mechanical loading can that cause a decrease in the space of the periodontal ligament. First reaction of the organism is the remodelation of the bone to reestablish the periodontal ligament space (GREEN and. LEVINE. 1996). If the boné remodeling can not follow the destruction cause by the traumatic occlusion, there are some changes in the periodontum aiming to criate a structural relation where the loading will not be seen as a damage. .It results in a larger space of the periodontal ligament like a funnel in the crest, without bone pocket.

Bone remodeling in response to force requires the coordinated action of osteoblasts, osteoclasts, osteocytes, and periodontal ligament cells. Coordination among these cells may be mediated, in part, by cell-to-cell communication via gap junctions (Su, 1997). Loaded mechanical stress is converted to a series of biochemical reactions, and finally activates osteoclasts and osteoblasts to cause bone resorption and formation (Nomura and Takano-Yamamoto; 2000). These communication occurs between cells in short or long distance; the remodeling happens in the alveolar bone round the root and in the septum by this mechanism, as was seen in research.

The bone resorption activity is very difficult to evaluate and quantify due to the imbalance with bone formation. The measurement of active resorption surface, number of osteoclasts and eroded surface can be optimized by a specific staining for osteoclasts, as TRACP.

Osteoclasts are readily distinguished from macrophages by the presence of TRAP in their cytoplasm (Phan, Xu, Zheng, 2004). This type-V isoenzyme of acid phosphatase (Li CY, 1970) presents an intense activity in osteoclasts, being considered a specific marker for osteoclasts (Minkin, 1982 and Phan, Xu, Zheng, 2004)

TRAP staining is widely used to identify osteoclasts in vivo and in vitro, because of its simplicity and ease of manipulation (Minkin, 1982 and Takahashi et al, 2007). Although its exact role in osteoclast function is not known, TRAP is thought to be closely involved in bone resorption. There are two hypotheses on the secreted TRAP by osteoclasts: One is that TRAP is incorporated into in transcytotic vesicles and secreted from the functional secretory domain (Halleen et al, 2006 and Alatalo et al, 2004), and the other is that TRAP is directly secreted from the ruffled border (Kirstein, Chambers, Fuller, 2006 and Fuller et al, 2010).

It has been suggested that progenitor cells show TRAP activity during their differentiation to osteoclasts through interaction with stromal cells (Udagawa et al., 1989). Hence, TRAP-positive cells are thought to be committed osteoclast precursors.

TRAP-positive cells were generally distributed in perivascular areas, alveolar bone surfaces and between these areas. Kawamoto et al.(2002) suggest that osteoclast precursors are recruited via blood vessels in the periodontium, which leads to their differentiation into osteoclasts, migration towards the bone surface with expression of TRAP activity and multinucleation preceding bone resorption.

The difference in the number of osteoclasts and bone density found in this study can be explained by the bone quality. With regard to bone quality, the maxilla has predominantly bone with wide medullary spaces and little cortical width, classified as bone type III and IV. In the mandible there is a predominance of bone with small medullary spaces and thick cortical (type I and II), and in some areas more corticalisation and little medullary bone tissue. The internal and external osseous lamina of the alveolars is much stronger and resistant than the maxilla, a characteristic present in the whole mandibular bone compact. As the resistance of a bone to compression forces is proportional to the square of its density, the elasticity module and, consequently, the mechanical resistance of the cortical bone can reach 10 times that of the spongious bone. On the other hand, the spongious bone occupies approximately 20 times more surface area per volume unit than cortical bone, and therefore its cells can be more easily and directly influenced by medullary bone cells. Due to this fact, and considering its organisation, the spongious bone has a higher metabolic capacity and remodelling activity. As a consequence, it responds quicker to mechanical, chemical and hormonal stimuli (Judas et al., 2012).

This study shows that occlusal forces can cause changes in the alveolar bone and periodontal connective tissue. Once the occlusal traumacause temporary or permanent changes in the periodontium, this can be considered as a specific periodontal disease requires a personalized treatment.

6 CONCLUSIONS

The traumatic occlusion increased the number of osteoclast in the alveolar bone around the roots and in the alveolar septum of the right first upper and lower molar, and the bone density of the alveolar septum decrease.

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8 APPENDIX

APPENDIX A- Certificado de Aprovação da Comissão de Ética na Experimentação Animal

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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

Comitê de Ética no Uso de Animais (CEUA)
Committee for Ethical Use of Animals (CEUA)

CERTIFICADO

Certificamos que o Projeto **"Efeito do trauma oclusal em algumas estruturas do sistema estomatognático"** sob responsabilidade da Pesquisadora **DANIELA ATILI BRANDINI DE WEERT** e colaboração de Ana Paula Farnezi Bassi e Sara Vieira Pacanaro está de acordo com os Princípios Éticos da Experimentação Animal (COBEA) e foi aprovado pelo CEUA, de acordo com o protocolo **2012-00980**.

CERTIFICATE

We certify that the research **"Effect of the occlusal trauma in some struture of the stomatognatic system"**, protocol number **2012-00980**, under responsibility of **DANIELA ATILI BRANDINI DE WEERT** and with collaboration of Ana Paula Farnezi Bassi and Sara Vieira Pacanaro agree with Ethical Principles in Animal Research (COBEA) and was approved by CEUA.

Prof. Dr. Edilson Ervolino
CEUA Vice-Coordinador

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