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Evaluation of Periodontal Tissues and Abdominal Aorta of Rats with Induced Obesity by Monosodium Glutamate and Experimental Periodontitis

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Authors' contributions

This work was carried out in collaboration between all authors. Author KFC did the experimental studies, data and statistical analysis, manuscript preparation, editing and review. Authors MLB, VP, SP, PB, RMCB and CCLB did the experimental studies, data analysis and manuscript preparation. Authors PON and CAN performed the definition of intellectual content, design, experimental studies, data and statistical analysis, manuscript preparation, editing and review. All authors read and approved the final manuscript.

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ABSTRACT

Introduction and Aim: The aim of this study was to evaluate the behavior of experimentally-provoked-periodontitis tissues and the abdominal aorta in rats with acquired induced hypothalamic obesity.
Methods: Initially in the first 5 days of life, a cohort of twenty-eight male Wistar rats were divided

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into 2 groups; the first group [n=14] were given intradermal injections of 4 G/kg of solution of Monosodium glutamate (MSG); the second group [n=14] was given 1,25 G/kg/day of saline solution (group CTL) in the cervical region. At 70 days, each of the two groups were further divided into another 2 subgroups, and periodontitis was induced with ligature placing as the experimental variable, on the 1st lower molars, except in group 1 and 3. This created 4 groups: (1) control group without ligature (CTL n=7); (2) control group with ligature (CTLLIG n=7); (3) group MSG without ligature (MSG n=7); (4) and group MSG with ligature (MSGLIG n=7). Total n=28. After 100 days the rats were sacrificed and gingival tissue, abdominal aorta samples, and a hemi-mandible were dissected out for immunological, morphological and radiographic analysis.

Results: Both Radiography and histopathology showed significant lower alveolar bone loss when the MSGLIG, group was compared to the CTLLIG group ($p< 0.05$). In the abdominal aorta morphometric analysis there was statistically significant difference in the measurement of the thickness of the aortic wall, being the largest thickness observed in the MSGLIG group ($p< 0.05$).

Conclusion: Therefore, this study suggests that the periodontitis in association with the hypothalamic obesity may contribute to the increase of the width of the abdominal aorta walls, as well as the hypothalamic obesity may exert a protective effect on alveolar bone loss.

Keywords: Obesity; monosodium glutamate; atherosclerosis; periodontal disease.

1. INTRODUCTION

Worldwide obesity has reached epidemic proportions [1-3]. It is a medical disorder characterized by an excess of weight and their consequences for health vary from increased premature death risk to serious non-lethal diseases, but debilitating that directly affect the life quality of these individuals [4-6].

For better understanding the role of each components involved in the obesity pathophysiology, researchers make use of animal experimental models [7].

Among the neural models, the hypothalamic obesity is the most well-known, a model that mimics the clinical metabolic syndrome situation labeled as neuroendocrine obesity [8]. In rats that obesity can be achieved by subcutaneous monosodium glutamate administration (MSG), an injurious neural excitatory amino acid that affects the central nervous system, when in excessive quantities, because it damages cores belonging to the hypothalamus, in other words, it provokes changes in hypothalamic-pituitary axis [8].

Another disease that affects a major proportion of the population is periodontitis, its polymicrobial nature orchestra a complex mechanism of inflammation that is characterized by collagen fibers destruction, others matrix constituents of the periodontal ligament and alveolar bone, in conjunction with the periodontal pockets formation [9-11].

It is mainly caused by bacteria that release endotoxins that activate proinflammatory cytokines (interleukin 1, IL-1; tumor necrosis factor-alpha (TNF-α; among others) that affect the teeth´s supporting tissue [12-16]. It is suggested that obesity contributes effectively to the periodontal disease severity [17-21].

The pro-inflammatory cytokines concentration increase in the plasma could justify the relationship between obesity and periodontal disease [15]. Obesity may increase the individual susceptibility by modulating the immune system and inflammation, increasing the cytokines release, activating macrophages and thus increasing the risk for periodontitis [22].

It´s reasonable to believe that the systemic inflammation induced by periodontal pathogens may be associated with endothelial dysfunction and atherosclerosis 22 first in the small vessels, and later in larger arteries, such as the coronary arteries [23]. The increase in production of accession mediators leads to an increase in the permeability of the intimal layer and initial formation of atheroma [24].

The enthusiasm for this relationship between cardiovascular disease and periodontal disease arose from the assumption that the periodontitis, initiated by the accumulation of bacteria and perpetuated by an immune response, may contribute to a systemic inflammation linked to atherogenesis [25].

Although is suggested that there is an association between oral health and cardiovascular disease, a causal link has not been proven and the biological mechanisms

involved still remain obscure [26]. Thus, the present study was proposed to evaluate the behavior of periodontal tissues and abdominal aorta artery in rats with hypothalamic obesity associated with experimental periodontitis.

2. METHODOLOGY

2.1 Animals

Pregnant *Wistar* Rats were obtained from Central Animal Lab of the University of the West of Parana (UNIOESTE), Cascavel Campus, and maintained in the Laboratory Sector of the Laboratory of Human Physiology under controlled conditions of temperature (21 + /- 2 degrees) and light (12-hour cycle of c &Light clustered dither and 12 hours of darkness - 7:00 - 19:00 h). At birth, the pups were separated by sex and only 7 males for each brood, making a total of 28 animals *Wistar* rats. Later these animals were weaned at 21 days and received a standard diet and water ad libitum throughout the experimental period. All experimental protocols were approved (April 09, 2013) by the UNIOESTE´s Ethics Committee for Animal Experimentation and Practical Classes (CEEAAP).

2.2 Obesity Induction

The animals were divided into 2 groups, with 14 rats each one and then submitted, during the first five days of life, with initial body weight between 6 g and 7 g, to intradermal injections, in the cervical region, of monosodium glutamate at a dose of 4g/kg of body weight (group MSG) or saline at a dose 1.25 g/kg of body weight (CTL) [27].

2.3 Periodontal Disease Induction

At 70 days of life the animals from MSG and CTL groups were subdivided into two groups of 7 animals each, these animals received ligature of cotton number 40 (Coats Corrente Ltda., SP, Brazil) around the first molars in a position sub marginal to induce experimental periodontitis, in accordance with the methods proposed by Nassar et al. [28].

The anesthesia was induced by intramuscular administration of 0.08 ml/100 g body weight of ketamine (Francotar, Virbac Brazil Ind. AND With. Ltda., Sao Paulo, SP, Brazil) 0.04 ml/100 g body weight of xylazine (Virbac Brazil Ind. and With. Ltda, Sao Paulo, SP, Brazil). After this procedure, 4 groups were created (n= 7): control without ligation (CTL), control with ligation (CTLLIG), obese without ligation (MSG) and obese with ligation (MSGLIG). This ligation acted as irritating gum for 30 days and favored the accumulation of plaque [28].

2.4 Obesity Avaliation

After 100 days of life, the animals were weighed and was obtained the naso-anal length for calculation of the index of Lee (cube root of body weight (g) / length naso-anal (cm^3) X 100) [29].

2.5 Obtaining Samples

After 100 days of the total time of the experiment, the rats of all groups were subjected to a fasting for 8 hours prior to sacrifice, being provided water ad libitum.

The animals were desensitized with carbon dioxide and then the sacrifice made by decapitation. After the sacrifice, blood samples were collected and centrifuged at 300xg for 15 min at 4C and stored in a freezer at -80℃, for output of the triglycerides and total cholesterol analysis using commercial kits according to the manufacturer's instructions (Conjugate (Laborclin®, Bioliquid, PR, BR).

The periodontal tissues samples of the right side hemimandible and abdominal aorta for analysis were reduced and pre-fixed in metacarn (70% methanol, 20% chloroform and 10% glacial acetic acid) with constant drips, during the reduction.

In left side hemimandible, the gum which involved the tooth, affected by experimental periodontitis, was removed and, as well as a portion of the abdominal aorta, were allocated in ependorfs and stored in frezer - 80C, and subsequently, the left hemi-jaw was dissected and fixed in 10% formalin for subsequent x-ray analysis.

2.6 Analysis of Theinterleukin 6 and Tumor Necrosis Factor Alpha Expression

Samples of gingival tissue around the teeth of left hemimandible and animals abdominal aorta of all experimental groups, were removed and used for the analysis, by Linked Immunosorbent Assay

Coupled to Enzymes (ELISA - INVITROGEN® - Waltham, Massachusetts, USA) for the cytokines interleukin 6 and tumor necrosis factor-alpha presence respectively.

Total proteins were extracted from samples of gingival tissue of the animals and abdominal aorta using an extraction buffer total base of detergent containing a protease inhibitor cocktail. After this, was performed protein quantification by the Bradford method.

2.7 Radiographic Analysis

Soon after the sacrifice, the left hemimandible of each animal was removed, fixed in buffered formalin (pH 7.2) for 48 hours. The hemimandible were placed with the lingual side on the radiographic film periapical (AGFA REDEMPTUS®, Ultraspeed disc - Agfa Gevaert N. V., Belgium) and positioned so that the buccal and lingual leaflets of first molars fell in the same vertical plane.

The X-ray equipment used was the GE 1000® (General Electric Co, Milwaukee, WI, USA), set to 15mA, 65Vp, 18 pulses, distance focus/film of 50 centimeters with perpendicular incidence of X-ray to parts. For films processing were used developer and fixer Kodak® (Kodak Amazon's Industry and Trade Ltda, Manaus, Amazon, Brazil) in its processing time/temperature and digitalized by a "scanner" to devices (Polaroid Sprint Scan 35 Plus, Polaroid, Sao Paulo, Brazil).

The scanned images were analyzed using 3 measures in the Image Tools 3.0 program (The University of Texas Health Science Center, San Antonio, TX, USA) and made the average between them, by means of a linear measure, that has traveled the distance from junction cement-enamel up to the mesial side crest alveolar bone of the lower first left molar of the mouse, with measurements in pixels [30].

2.8 Histological Processing

The right hemimandible were decalcified with trichloroacetic acid (TCA) 5%, 10℃ for 27 days. The pieces were evaluated to assess the expected degree of descaling, with renewal of the solution of 25 TCA every 5 days.

After descaling, tissues were immersed in sodium sulphate 5% during approximately 2 hours to neutralize the TCA, washed in water for two hours, kept in 70% alcohol until the histological processing for inclusion in paraffin (Purified Paraffin, code 1228, lot 1008459, Vetec Fine Chemistry, Rio de Janeiro, Brazil)

The fragments of the abdominal aorta after withdrawals from metacarn were kept in 70% alcohol until the histological processing. The fragments of hemi-jaws and abdominal aortas were dehydrated in series ascending alcohol, cleared in xylene and embedded in paraffin.

The paraffin blocks were cut in manual microtome (Olympus, CUT 4055 - Charleston, South Carolina, USA) to obtain sections of 7µm thickness, which were assembled in histological slides and stained with Hematoxylin and Eosin (HE).

2.9 Microscopic Observation

The microscopic analysis was performed by a single examiner through the evaluation of the histological sections stained with HE. The slides were analyzed with the aid of a light microscope transmitted commonly (Leica Microsystems, Switzerland) for morphological observations of gingiva and alveolar process hemi-jaws of rats of all experimental groups.

2.10 Bone Morphometry

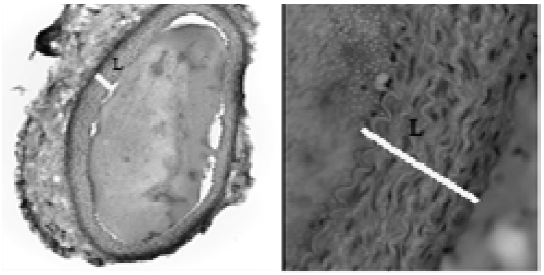
After obtaining the histological slides was performed the osteocytes, osteoblasts and osteoclasts quantification present in five consecutive fields of vestibular alveolar bone crest starting from the highest point of the crest. For the observation was used the increase of 400 times under the microscope. Were made two observations per field, and then done the average of the values for each animal and each group.

The measure of alveolar bone crest was performed through a microscope coupled to a computer, which allows capturing images, through the Laz Ez® software program (accounting System and capture LAS V4.2 Leica microsystems). There was a measurement of the smallest distance between the apex of vestibular alveolar bone crest and the cement-enamel junction. The measurements were repeated twice a day, on three different days, and then was made the average between the values.

2.11 Morphometric Analysis of the Abdominal Aorta

The aorta is an elastic artery that originates in the heart and goes to the abdominal region. It is divided into ascending aorta (region that communicates with left ventricle); aortic arch; descending aorta - in this region is called thoracic aorta and finally, abdominal aorta, this starts at the level of the 12TH thoracic vertebra and ends at the height of the 4th lumbar vertebra.

The arteries are composed of three layers, the outer most call adventitia or external, formed mainly by connective tissue; intermediate is the middle layer or muscular, formed of elastin, collagen and smooth muscle cells. The innermost layer is known as intimate and formed by a thin layer of endothelial cells, some fibroblasts and macrophages, this layer is the region of interest in atherosclerosis, maintains direct contact with the blood flow and when there is endothelial dysfunction is in this region that results in the formation of atherosclerotic plaque (Figs. 1A and B).



A **B**

Fig. 1. Representative photomicrographs of measures the thickness of the walls of abdominal aortas arteries in CTL

The same measures were made in the others groups. A: increase of 40X; B: increase of 400X (Hematoxylin and Eosin) animal groups

After obtaining the histological slides of the animal´s abdominal aorta, a linear measure of the widths of the walls´artery was performed through a microscope coupled to a computer, which allow capturing images, through the Laz Ez® software program (accounting System and capture LAS V4.2 Leica microsystems).

A measurement of the thickness of the aortic wall was then realized. The measurements were repeated twice a day, on three different days, and then, the average between the values was made.

For the statistical analysis, all numerical values were expressed as mean standard deviation. In a first moment, through the Bioestat 5.3 program (Institute Mamiraua, Amazonas, Brazil), was carried out the Shapiro-Wilk test to evaluate the distribution of the data normality. After checking the data normality, were made the ANOVA test and consequently the Tukey test with $p<0.05$ to assess the difference between the groups in every parameters.

3. RESULTS

3.1 Effect on the Development of Obesity in Rats with and without Periodontitis Induced

The administration of MSG caused an increase in the index of Lee when compared treated groups (MSG and MSGLIG) with non-treated groups (CTL and CTLLIG) ($p< 0.05$), this difference was not observed when compared between the obese groups (MSG and MSGLIG) ($p>0.05$). The animals treated with neonatal MSG shown a significant decrease in body weight of rats ($p< 0.05$) (Table 1).

3.2 Plasma Concentrations of Total Cholesterol and Triglycerides

The results show that groups with obesity had a significant increase in the concentrations of triglycerides and total cholesterol ($p< 0.05$), suggesting an effect of obesity induced on these analyzed parameters (Table 2).

3.3 Analysis of the IL-6 Presence in Gingival Tissue

The results show that the mean IL-6 concentration was significantly higher in the CTLLIG group when compared with the other groups ($p< 0.05$), in other words, there was a significant increase of this cytokine (Table 3).

3.4 Analysis for the Presence of TNF- α in the Samples in the Tissue of the Abdominal Aorta

The TNF-α mean concentration was greater in the CTLLIG group ($p< 0.05$). Furthermore when the groups with ligation were compared with the groups that did not have the ligature-induced, the results showed a significant increase of this cytokine in the abdominal aorta ($p< 0.05$) (Table 4).

Table 1. Neonatal treatment effect with MSG on body parameters of CTL, CTLLIG, MSG and MSGLIG rats. The values represent mean standard deviation

	CTL	CTLLIG	MSG	MSGLIG
Lee Index (g/cm^3)	32.72±1.22A	35.50±0.79B	36.60±1.00C	36.9±0.17C
Animals final weight (g)	385.57±29.28A	356.14±17.07A	251.81±61.83B	223.16±23 .68B

Different Letters, mean that the data are statistically different, within the same parameter, with p<0.05

Table 2. Total cholesterol and triglycerides concentration in the rat's blood in the experimental groups. The values represent mean standard deviation and are expressed in mg/dL

	Total cholesterol	Triglycerides
CTL	91.15 ± 6.07 A	150.5 ± 29.0 A
CTLLIG	79.48 ± 6.02 A	158.8 ± 30.4 A
MSG	127.78 ± 2.38 B	292.5 ± 65.5 B
MSGLIG	134.85 ± 3.05 B	352.6 ± 81.2 B

Different Letters, mean that the data are statistically different, within the same parameter, with p<0.05

Table 3. IL-6 concentration in rats gingival samples of experimental groups. The values represent mean standard deviation and are expressed in pg/mL

Groups	Means	
CTL	9.75 ± 2.70	A
CTLLIG	15.33 ± 1.50	B
MSG	7.78 ± 3.19	A
MSGLIG	12.78 ± 1.03	C

Different Letters, mean that the data are statistically different at p<0.05

3.5 Radiographic Analysis of the Average Distance from Cement-enamel Junction until the Alveolar Bone Crest of the Left Lower First Molar

In radiographic analysis, it was found that there was a decrease of insertion in animals exposed to experimental periodontitis (p< 0.05), demonstrating the effectiveness periodontal disease induction on alveolar bone tissue, but in CTLLIG group this loss was more pronounced than MSGLIG (Table 5).

3.6 Right Hemymandible Histological Analysis

3.6.1 Control group

In the histological evaluation of CTL group, it was possible to observe the normal oral epithelia, junctional, sulcular and connective tissue, without observation of inflammatory aspect on these tissues. The alveolar bone was intact, compact and regular, with a normal appearance of cancellous central bone.

Table 4. TNF-alpha Concentration of the abdominal aorta of rats in the experimental groups. The values represent mean standard deviation and are expressed in pg/mL

Groups	Means	
CTL	42.57 ± 9.0	A
CTLLIG	107.58 ± 8.45	B
MSG	56.39 ± 12.3	A
MSGLIG	83.20 ± 6.15	C

Different Letters, mean that the data are statistically different at p<0.05.

The bone crests were thick and high (the cervical third of the root), data obtained by measuring the distance between enamel-cementum junction and bone crest. The presence of osteoblast and osteoclast was also observed indicating the resorption and bone formation, but within the normal range. Cementoenamel junction, cementum and periodontal ligament showed normal characteristics. (Fig. 2.A)

3.7 Ligation Control Group

In CTLLIG, group, the image showed the abnormality on the morphology of oral epithelia, junctional, sulcular, with migration to the apical region and connective tissue with predominance of acute inflammatory state (Fig. 2.B). The bone crest presented an irregular manner, with extensive alveolar bone loss, causing the exposure of the cervical third of the root, with strong presence of osteoclast revealing the activity of bone resorption. We also observed changes in cementum and periodontal ligament.

3.8 MSG Group

After the monosodium glutamate treatment, the MSG group continued showing regularity in morphological differentiation between the oral

epithelia, junctional, sulcular and connective tissue without inflammatory aspect. The level of crest bone and the distance to the cement-enamel junction was similar to that found in CTL group. (Fig. 2.C).

Table 5. X-ray analysis of distance from enamel –cement junction until the alveolar bone crest of the left lower first molar mesial side of experimental groups rats. The values represent mean standard deviation and are expressed in pixels

Groups	Means
CTL	64.46 ± 1.01 A
CTLLIG	80.62 ± 3.95 B
MSG	62.03 ± 0.84 A
MSGLIG	71.00 ± 0.56 C

Different Letters, mean that the data are statistically different at p<0.05

3.9 Ligation MSG Group

It has been observed in the MSGLIG group a small irregularity in oral epithelia, junctional and sulcular, with connective tissue presenting inflammatory aspect. The bone crest, although it is at a time close to the level of cervical root, shows irregularities, with bone loss less pronounced than that observed in the CTLLIG. group. There was an increased osteoclast presence in relation to MSG group, revealing the activity of bone resorption. There are also observed changes in cementum and periodontal ligament (Fig. 2.D).

The Fig. 3 demonstrate, the bone crest region, in (A) CTL group presence of osteoblasts in the periphery of the bone, forming a epithelium, and presence of osteocytes in the central region of the bone. The same is observed in (C) MSG group. (B) CTLLIG group already noticed the presence of osteoclastic areas indicating evidence of bone resorption. The same is repeated in (D) MSG group, with presence of incremental lines observed in all images.

3.10 Right Hemymandible Morphometric Analysis

The administration of MSG caused statistical difference as regards the number of osteoblasts when compared groups CTL with MSG ($p < 0.05$), there was no statistical difference in the count of osteocytes ($p > 0.05$) and in relation to osteoclasts this difference was significant between the groups with ligation ($p < 0.05$) (Table 6).

3.11 Abdominal aorta Morphometric Analysis

The average walls arteries width showed a significant difference in all the groups, and the CTL group showed the lowest thickness, and the MSGLIG group presented a greater wall thickness ($p < 0.05$) (Table 7).

4. DISCUSSION

The MSG neonatal treatment induces obesity through injury of neural cells of the arcuate nucleus and hypothalamus medium eminence [5,31,32]. The MSG administration caused an increase in the index of Lee of obese groups (Table 1), with a significant difference between the treated groups, but there was no difference between the MSG groups, this variable is evident in this model of obesity due to this treatment could result in numerous endocrine abnormalities and behavioral, such as disturbances of growth, production of glucocorticoids, obesity and hypogonadism [4].

Table 6. Morphometric analysis of right hemi-jaw of rats in the osteocytes, osteoblasts and osteoclasts in the experimental groups for quantification of osteocytes, osteoblasts and osteoclasts and measuring the distance from the crest alveolar bone to splice cementoesmaste (JCE-Crest). The values represent mean standard deviation and are expressed in units for the osteoblast and osteocyte and osteoclast and expressed in pixels for JCE-Crest

	Osteoblast	Osteocyte	Osteoclast	JCE -Crest
CTL	16.80±1.01 A	221.50±41.41A	2.00±0.81 A	1.26±0.01 A
CTLLIG	14.71±0.60 A	206.57±86.13A	5.20±0.62B	2.32±0 .06B
MSG	20.33±1.01 B	175.83±57.40A	1.50±0.25[a]	1.27±0.02 A
MSGLIG	20.66±0.80 B	149.00±45.40A	3.50±0.15B	1.37±0 .04C

Different Letters, mean that the data are statistically different, within the same parameter, with p<0.05.

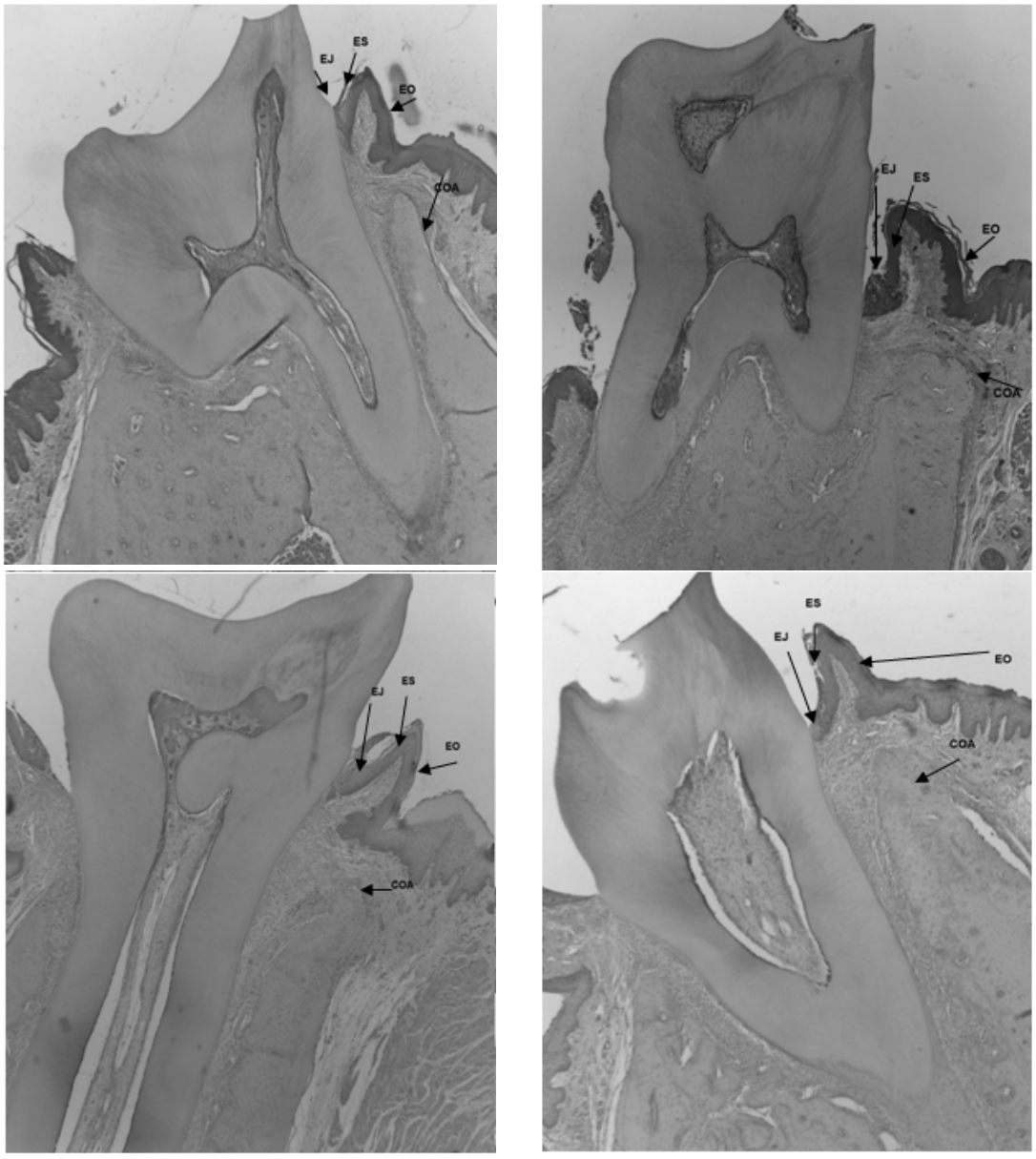


Fig. 2. Representative photomicrography of an animal in the CTL group

COA, alveolar bone crest; EJ, junctional epithelium; EO, oral epithelium; ES, groove epithelium (A); Representative photomicrography of An Animal of CTLLIG group. COA, alveolar bone crest; EJ, junctional epithelium; EO, oral epithelium; ES, groove epithelium (B); Representative Photomicrography of an animal in MSG group\. COA, alveolar bone crest; EJ, junctional epithelium; EO, oral epithelium; ES, groove epithelium (C); Representative photomicrography of an animal of MSGLIG group. COA, alveolar bone; EJ crest, junctional epithelium; EO, oral epithelium; ES, sulcular epithelium (D) (Hematoxylin and Eosin, 40X)

However the lower final weight of rats treated with MSG, also shown in Table 1, can probably be attributed to the smaller size of these 30 animals, since between the endocrine abnormalities described in this model is the reduction in the secretion of growth hormone 8 In relation to the lipid profile, this study presents concentrations of total cholesterol and triglyceride levels increased in both obese groups when compared with the control groups (Table 2).

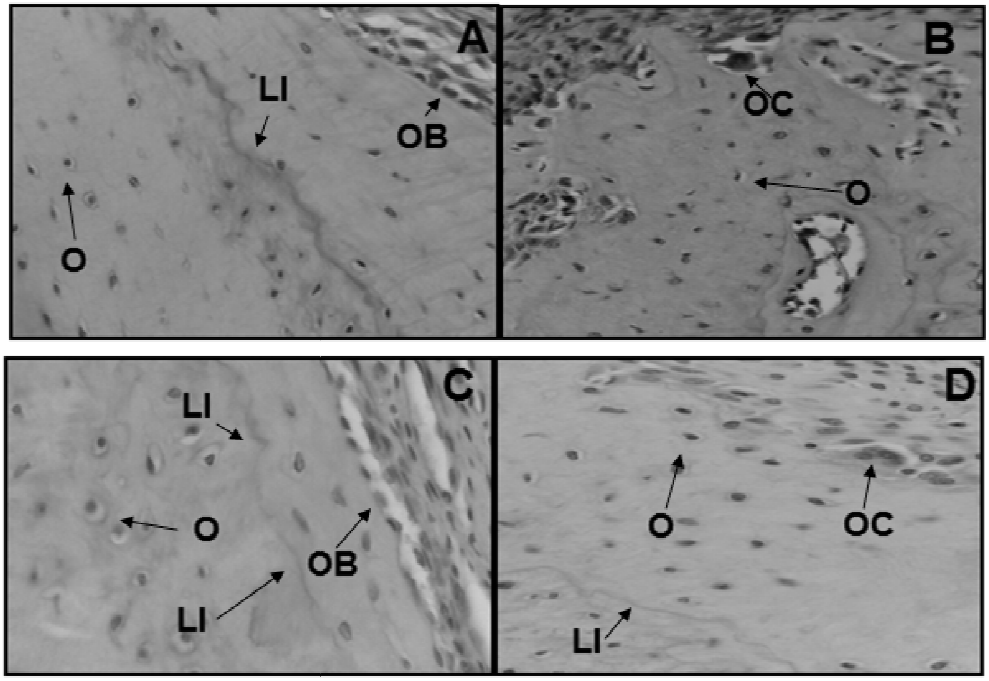


Fig. 3. Representative photomicrographs of animals in CTL (A), CTLLIG (B), MSG (C) and MSGLIG (D) groups. The osteocyte; OB, osteoblast; OC, osteoclast; LI, incremental lines (Hematoxylin and Eosin, 400X)

Table 7. Aorta abdominal morphometric analysis to measure rats artery walls in the experimental groups. The values represent mean standard deviation and are expressed in pixels

Groups	Means	
CTL	0.078 ± 0.001	A
CTLLIG	0.085 ± 0.001	B
MSG	0.093 ± 0.004	C
MSGLIG	0.104 ± 0.001	D

Different Letters, mean that the data are statistically different, within the same parameter, with p<0.05

However, this statement is still uncertain and currently there is no consensus within the Dentistry/Periodontics on these findings [33]. In periodontitis, the microorganisms cell wall that colonize the plaque contains endotoxin, also known as lipopolysaccharide (LPS), which induce the inflammatory and immune response of the host.

These changes are associated with tissue inflammatory response and the periodontal attachment loss, whether relating to the aggregation and platelet adhesion and with the cholesterol levels elevation [34].

The results of these studies are contradictory, since an increase in the triglycerides levels and cholesterol was observed in obese groups, regardless of the presence or not of experimental periodontitis, suggesting that the periodontitis does not influence the increase in these parameters, corroborated by the study of Shridar et al. [35], which also failed to demonstrate the influence of periodontal disease in these parameters.

For many years it was believed that the adipose tissue was a dummy component for storage of triglycerides, without relevant metabolic functions. Today it is clear that the adipose tissue is a complex endocrine organ, metabolically active, which secrete more than fifty bioactive molecules. Some of them are classic cytokine of the inflammatory process such as TNF- α and IL-6.

The increase of these cytokines in the bloodstream can cause a subclinical inflammatory process, which interferes with other pre-existing inflammatory processes [36] (Tables 3 and 4). The TNF- high concentration can exacerbate the pre-existing periodontitis through the fibroblasts stimulation which promote

the enzymes synthesis and by osteoclasts stimulation that activate bone resorption [15].

Nevertheless, the pro inflammatory cytokines levels are proportional to BMI, particularly in individuals with visceral obesity, so that an increase in fat mass can induce a hiper inflammatory response in periodontal disease. Obesity can affect the immune and vascular response of the host due to the decrease in blood flow [37].

The periodontitis is directly involved in the cardiovascular disease pathogenesis, both because of the oral bacteria, and because the response of the host, because it can release cytokines (IL-6, TNF-α that can initiate a cascade of biochemical reactions and endothelial damage and facilitate the fixing of cholesterol plaques [38].

Both inflammatory markers were increased in the present study (Tables 3 and 4). Our results showed a loss of sharp bone in groups with induced periodontitis, this difference was more significant in CTLLIG group (Table 5).

The current literature demonstrates a relationship between obesity and periodontitis, in which the insertion loss is greater in obese groups, a situation that can vary with the time of exposure of the animal to diet or the type of obesity experimental model [38,39].

The study in cell culture of fat in obese rats, shows a significant increase in the release of TNF-α when compared with not obese rats. The large amount of pro-inflammatory cytokines affects tissues that support the teeth and can lead to loss of alveolar bone, cementum and periodontal ligament, thus influence and contribute effectively to the periodontitis increase or its progression [38].

Nascimento et al. [39] conducted a study in which the animals were submitted to cafeteria diet and showed significant difference in bone loss between the groups, being that the ligated groups lost more alveolar bone than their counterparts.

In the other hand Brandelero et al. [32] demonstrated that through hypothalamic obesity, you can have an opposite and protector effect when periodontitis is induced. In this study, in which the model of obesity was the induction through MSG also showed a smaller alveolar bone loss in MSGLIG group when compared with the CTLLIG group (Tables 5 and 6).

Obesity can have beneficial factors facing the bone resorption, as demonstrated by Brandelero et al. [32] who observed a protective effect of hypothalamic obesity on the alveolar bone loss. Yet, despite the mechanism by which obesity may promote bone formation is still unknown, hypotheses have been proposed, through the 32 note that in obese patients, with a high body weight, can establish a greater mechanical load on the bone [40-42].

The increased mechanical load would promote some stimuli on the skeleton, such as reduction of apoptosis, increased osteoblast differentiation and bone matrix stimulation [43].

Others evidences show that leptin, a hormone secreted by adipose tissue, is capable of stimulating the differentiation of bone marrow stromal cells into osteoblasts, leading to an increase in extracellular matrix mineralization [44,45]. In addition, leptin can reduce the expression of receptor activator of nuclear factor kB (RANK), stimulate the expression of proteasome (EPG), inhibit the differentiation of osteoclasts, leading to a reduced bone and favoring of bone formation [46,47].

However leptin can also negatively affect the bone metabolism of animals subjected to a diet with a high fat content [39]. Obesity can act on the bone tissue, through the high amount of infiltrated macrophages in adipose tissue. Such cells are considerable sources of proinflammatory cytokines, which in turn, can stimulate bone resorption [48,49].

In our study, we also observed a significant difference in the width of the abdominal aorta walls, corroborating with other studies that have suggested that vascular remodeling is an early event in atherogenesis, appearing before the inflammatory response and may be connected with the cardiovascular events incidence [50,51].

Pathologically, the atherosclerosis occurs due to an endothelial injury, changing the permeability of the vascular endothelium of the intimal layer, allowing the entry of lipids and inflammatory cells in this range inter endothelial [50,51].

Normally, happens low-density lipoproteins (LDL) deposition in endothelial cells. These electronegative charge are hydrolysed in

phospholipids, triglycerides, proteins and cholesterol. After hydrolysis, some receptors are expressed in the cell membrane and other products are used during the cellular membrane restoration, as is the case of the cholesterol [50-53].

In the presence of hypercholesterolemia or situations that aggravate the deposition of LDL, occurs greater consumption of nitric oxide (NO) in the endothelial cell and increased production of free radicals, causing a dysfunction in the metabolism of fatty acids, of 33 apoproteins, lecithin and protein G. The final result is the inability of the endothelium to respond adequately to systematic attacks [52,53]. The association between periodontitis and atherosclerotic cardiovascular disease has presented some hypotheses: 1) Inflammatory process causes endothelial damage due to the pro-inflammatory cytokines release; 2) presence of infectious agents can induce or accelerate atherosclerosis such as: promoting the local lymphocytes, macrophages increase, growth factors tissue production; endotoxin local release (LPS) and molecular mimicry of microbial protein with the human inducing an autoimmune reaction. 3) Systemic cytokine increase with inflammatory markers activation and procoagulants stimulation and may cause thrombosis and acute ischemia, in addition to induction of changes in lipoproteins resulting in pre-atherosclerotic conditions [54]. Our study corroborates these assumptions, because the results in Table 7 show an increase in width of the abdominal aorta walls, in the groups that experimental periodontitis is present, as well as with a larger width when associated with obesity (MSGLIG).

5. CONCLUSION

In conclusion, within the limitation of this study, although the mechanisms of influence of periodontitis on obesity and atherosclerosis are not elucidated, and there is no agreement on this association, this study showed significant differences in the parameters evaluated. Therefore, it is suggested that the periodontitis in association with the hypothalamic obesity may contribute to the width of the abdominal aorta walls, as well as the hypothalamic obesity may exert a protective effect on alveolar bone loss.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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