UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA

ANDRESSA GASPARETTO MOREIRA

O EFEITO DA OBESIDADE E/OU DA PERIODONTITE INDUZIDA SOBRE A ESPESSURA DAS AORTAS DE RATOS WISTAR

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RESUMO

As doenças cardiovasculares são tratadas como uma condição altamente prevalente e é a principal causa de morbidade e mortalidade no mundo. A literatura atual tem sugerido que a obesidade e a periodontite poderiam ser fatores de risco para essa condição. Assim, o presente estudo tem como objetivo avaliar a espessura da parede de aortas depois da indução de modelos de doença periodontal e/ou obesidade em ratos Wistar. Sessenta ratos foram randomicamente divididos em 4 grupos experimentais: controle (CT), doença periodontal (DP), obesidade (OB), e obesidade e doença periodontal (OB+DP). Grupos OB e OB+DP receberam dieta de cafeteria (hiperlipídica/hipercalórica) por 17 semanas. Depois de adquirir obesidade, na semana 12, a doença periodontal foi induzida por meio de ligadura no segundo molar superior direito dos grupos DP e OB+DP. Durante o período experimental, o peso corporal e o índice de Lee foram avaliados. Ao final do período experimental, os animais foram mortos. Todas as seguintes analises foram feitas por um examinador cego. A média de perda óssea alveolar (POA) foi avaliada, e as aortas preparadas para analises histotécnicas da parede aórtica (em μm) por meio do software ImageJ por um examinador cego. Os ratos expostos à dieta de cafeteria mostraram um aumento no peso corporal e no índice de Lee. Foi observada uma média maior de POA nos grupos DP e OB+PD em comparação ao controle e OB (0,71±0,08 e 0,84±0,11 vs. 0,42±0,05 e 0,45±0,08, respectivamente). O grupo OB+DP, quando comparado ao DP, apresentou uma POA 18% maior, e essa diferença foi estatisticamente significativa ($p<0,001$). Os grupos OB e OB+DP exibiram maiores espessuras comparados aos grupos controle e DP, respectivamente (2,31 ± 0,28 e 2,33 ± 0,29 vs. 2,18 ± 0,26 e 2,14 ± 0,27). Diferenças significativas foram encontradas na comparação entre os grupos OB e controle ($p=0,036$) e OB+DP e OB comparados com o grupo DP ($p=0,004$ e $p= 0,001$, respectivamente). Pode-se concluir que o modelo de indução de obesidade parece alterar a espessura da parede de aortas em ratos Wistar. Entretanto, a presença de doença periodontal não teve efeito sobre a espessura da parede aórtica.

Palavras-chave: Obesidade. Periodontite experimental. Dieta de cafeteria. Aterosclerose. Ratos.

ABSTRACT

Cardiovascular diseases are a highly prevalent health condition and considered the main cause of morbidity and mortality worldwide. Studies have been suggesting that obesity and periodontitis may be risk factors for this disorder. This study aimed to evaluate the aortic wall thickness after periodontal disease (PD) and/or obesity (OB) induction models in Wistar rats. Sixty Wistar rats were randomly divided in four groups: control (CT), periodontal disease (PD), obesity (OB), and obesity plus periodontal disease (OB+PD). Groups OB and OB+PD received cafeteria diet (high fat/hypercaloric diet) throughout 17 weeks. After acquiring obesity, week 12, periodontal disease was induced by placing a silk ligature on the maxillary upper right second molar of groups PD and OB+PD. During the experimental period, body weight and Lee index were assessed. At the end of the experiment, animals were killed. All of the following analyzes were done by a blind examiner. Mean alveolar bone loss (ABL) was evaluated, and aortas prepared for histometric analysis of the aortic wall (in μm) by ImageJ software. Rats exposed to cafeteria diet showed an increase of body weight and Lee index. A higher mean ABL was observed in groups PD and OB+PD compared to control and OB (0.71±0.08 and 0.84±0.11 vs. 0.42±0.05 and 0.45±0.08, respectively). Group OB+PD, when compared to PD, presented 18% higher ABL, and this difference was statistically significant ($p<0.001$). Groups OB and OB+PD exhibited a higher thickness compared to control and PD groups, respectively (2.31 ± 0.28 and 2.33 ± 0.29 vs. 2.18 ± 0.26 and 2.14 ± 0.27). Significant differences were found in comparisons of group OB and control ($p=0.036$), and OB+PD and OB compared to PD group ($p=0.004$ and $p= 0.001$, respectively). It may be concluded that obesity induction model seems to alter aortic wall thickness in Wistar rats. However, the presence of periodontal disease did not affect the aortic wall thickness.

Keywords: Obesity. Experimental Periodontitis. Cafeteria Diet. Atherosclerosis. Rats.

SUMÁRIO

1 APRESENTAÇÃO

A doença periodontal é uma doença multifatorial de origem infecciosa/ imunológica crônica que afeta os tecidos de suporte e sustentação dos dentes, e com a sua progressão, pode levar a perda dos mesmos. Além disso, é influenciada por alguns fatores de risco que são as condições locais e/ou sistêmicas que aumentam a probabilidade de desenvolver ou agravar a doença (SLOTS, 2013). Dentre os fatores de risco sistêmicos estão incluídos os fatores comportamentais, como tabagismo, condições médicas (diabetes descompensada), indicadores de risco como a obesidade, estresse, osteoporose e baixo consumo de cálcio e vitamina D (GENCO; BORGNAKKE, 2013). A partir disso, na prática clínica indicamos ao paciente que abandone ou modifique esses hábitos para o tratamento da doença. Existem outros fatores de risco como fatores genéticos, mas esses não podem ser modificados (GENCO; BORGNAKKE, 2013). Entretanto, saber quais pessoas tem esse risco de resultados adversos nos fornece um meio para direcionar a intervenção.

É sabido que algumas doenças podem influenciar a severidade da doença periodontal, como é o caso do diabetes mellitus. Nesse contexto, o termo Periodontia Médica, o qual Offenbacher, em 1996, definiu como a relação entre saúde/doença periodontal e saúde/doenças sistêmicas, no qual podemos entender que as infecções bucais agravam quadros de doenças sistêmicas e vice-versa (OFFENBACHER et al., 1996). Em uma revisão de literatura recentemente publicada, Cullinan e Seymour, observaram evidências para associação entre doença periodontal e doença cardiovascular, diabetes e parto prematuro (CULLINAN; SEYMOUR, 2013). Assim, uma nova forma de entender as doenças periodontais tem demostrado uma provável relação entre as mesmas e inflamação sistêmica de baixa intensidade (CAIRO et al., 2010).

Segundo Paquette e colaboradores, a relação entre doença periodontal e doença sistêmica implica que a periodontite pode fornecer uma pista sobre outras doenças sistêmicas, mas não está claro se a periodontite é um determinante etiológico destas (PAQUETTE; BRODALA; NICHOLS, 2007). Por isso, os pesquisadores veem a necessidade de definir o tipo de evidência que será suficiente para aceitar (ou rejeitar) o conceito de um efeito causador da periodontite sobre as principais doenças sistêmicas.

Dito isso, por se tratar de objeto de estudo da presente investigação dar-se-á destaque às doenças cardiovasculares (DCV), as quais segundo a OMS, são definidas como um grupo de distúrbios do coração e vasos sanguíneos incluindo hipertensão arterial, doenças

coronarianas, acidentes vasculares cerebrais, falência congestiva do coração e infarto do miocárdio. As DCV são tratadas como uma condição altamente prevalente na população e com altas taxas de morbidade e mortalidade (WORLD HEALTH ORGANIZATION, 2017).

É consenso na área médica que as doenças cardiovasculares são causadas por um fenômeno denominado aterosclerose. A mesma se trata de uma forma de arteriosclerose causada por depósitos ateromatosos e fibrose da camada mais interna do endotélio dos vasos sanguíneos, observável após a alimentação de um elevado teor de gordura, ou seja, uma dieta de colesterol alto. Além disso, é considerada uma doença inflamatória e existe evidência demonstrando que outros processos inflamatórios do organismo, incluindo a periodontite e a obesidade poderiam propagar esse processo (PIRES et al., 2014).

Nesse contexto, a Organização Mundial de Saúde definiu obesidade como uma doença crônica de origem multifatorial e é caracterizada pelo acúmulo anormal ou excessivo de gordura que representa risco à saúde (WORLD HEALTH ORGANIZATION, 2000). Dentre os fatores etiológicos destaca-se fatores genéticos e comportamentais, os quais proporcionam o acúmulo excessivo de energia sob a forma de gordura decorrente de uma dieta rica em açúcares, gorduras e carboidratos, e associada à inatividade física (SMITH, K. B.; SMITH, M. S., 2016). O método mais amplamente utilizado para mensurar obesidade é através do índice de massa corporal (IMC), no qual para ser considerado obeso o indivíduo deve ter um valor $\geq$ 30 kg/m^2 (WORLD HEALTH ORGANIZATION, 2000). Este é calculado através da divisão do peso corporal em quilogramas pelo quadrado da altura em metros.

Estudos mostram que a tendência global da obesidade continua em ascensão, embora haja variações de sexo, regiões do mundo e países. Mundialmente, a prevalência de sobrepeso e obesidade aumentou cerca de 27% para adultos e 47% para crianças entre 1980 e 2013 (NG *et al.*, 2014). Este aumento considerável na prevalência de obesidade pressupõe um aumento na carga global da doença cardiovascular (DCV), pois acredita-se que a obesidade causa uma série de fatores de risco estabelecidos para essa doença, como hipertensão, dislipidemia, diabetes tipo 2, hiperinsulinemia e aterosclerose (SMITH, K. B.; SMITH, M. S., 2016). Alguns autores têm apontado que a relação da obesidade com aterosclerose, se daria pelo processo inflamatório responsável pelo desenvolvimento e complicações de ambas as doenças (ROCHA; LIBBY, 2009). Assim uma dieta rica em gordura pode promover a disfunção endotelial, aumentar o estresse oxidativo e a aterogênese (ROCHA; LIBBY, 2009). Desse modo, a inflamação resultante desses processos pode originar formação das placas ateromatosas na túnica intima da artéria, localizada entre o endotélio e a túnica média

(FREDIANI BRANT et al., 2014).

Assim a obesidade e a periodontite tornam-se um problema de saúde pública, pois são possíveis fatores de risco para as DCV (PIRES et al., 2014), as quais causam um impacto econômico de, aproximadamente, R$ 7.397.958,84 por ano para o Sistema Único de Saúde no Brasil (MORAIS; PONTES; MARTINS, 2011). Desse modo é importante o controle ou prevenção da obesidade e da periodontite, a fim de prevenir as DCV.

Nesse sentido, o presente trabalho de conclusão de curso avaliará a espessura da parede de aortas de ratos submetidos a modelos de doença periodontal induzida e/ou obesidade. O presente estudo consiste em um artigo científico intitulado “Effect of Obesity and/or Ligature-Induced Periodontitis in Aortic Wall Thickness in rats” que será submetido para publicação na revista Archives of Oral Biology.

2 ARTIGO

Effect of Obesity and/or Ligature-Induced Periodontitis in Aortic Wall Thickness in rats.

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Abstract:

Objective: to evaluate the aortic wall thickness after periodontal disease (PD) and/or obesity (OB) induction models in Wistar rats.

Design: Sixty Wistar rats were randomly divided in four groups: control (CT), periodontal disease (PD), obesity (OB), and obesity plus periodontal disease (OB+PD). Groups OB and OB+PD received cafeteria diet (high fat/hypercaloric diet) throughout 17 weeks. After acquiring obesity, week 12, periodontal disease was induced by placing a silk ligature on the maxillary upper right second molar of groups PD and OB+PD. During the experimental period, body weight and Lee index were assessed. At the end of the experiment, animals were killed. All of the following analyzes were done by a blind examiner. Mean alveolar bone loss (ABL) was evaluated, and aortas prepared for histometric analysis of the aortic wall (in mm) by ImageJ software.

Results: Rats exposed to cafeteria diet showed an increase of body weight and Lee index. A higher mean ABL was observed in groups PD and OB+PD compared to control and OB (0.71±0.08 and 0.84±0.11 vs. 0.42±0.05 and 0.45±0.08, respectively). Group OB+PD, when compared to PD, presented 18% higher ABL, and this difference was statistically significant ($p<0,001$). Groups OB and OB+PD exhibited a higher thickness compared to control and PD groups, respectively (2.31 ± 0.28 and 2.33 ± 0.29 vs. 2.18 ± 0.26 and 2.14 ± 0.27). Significant differences were found in comparisons of group OB and control ($p=0,036$), and OB+PD and OB compared to PD group ($p=0.004$ and $p= 0.001$, respectively).

Conclusions: It may be concluded that obesity induction model seems to alter aortic wall thickness in Wistar rats. However, the presence of periodontal disease did not affect the aortic wall thickness.

Keywords: Obesity; Experimental Periodontitis; Cafeteria Diet; Atherosclerosis; Rats

INTRODUCTION

Obesity is defined as a chronic and multifactorial disease and is characterized by an abnormal or excessive fat accumulation that presents a risk to health (World Health Organization, 2000). Genetic and behavioral causes are the highlights within the etiological factors, which can lead to an excessive increase of energy in the form of fat tissue due to diet rich in sugars, fats and carbohydrates, and physical inactivity (Smith & Smith, 2016).

According to WHO, cardiovascular diseases (CVD) are the main cause of death worldwide (Mendis et al., 2011). CVD pathogenesis may be triggered by systemic and vascular inflammatory progressions, also called atherosclerosis (Ross, 1999). Atherosclerosis is a progressive multifactorial vascular disease characterized by the deposition of fat particles and fibrosis on the arterial wall. Studies suggest that periodontitis may influence development of atherosclerosis (Kinane & Lowe, 2000; Teng et al., 2002), and obesity might be a risk factor for developing CVD (Kappus et al., 2014; Yatsuya et al., 2014). In addition, studies report that obese patients with periodontal disease may have an increased risk to cardiovascular events, which is approximately 9 percent higher for developing heart disease, in a range of 10 years, compared to those without chronic periodontitis (Pires et al., 2014; Pires JR, Dezem TU, Barroso EM, Toledo BE, Monteiro SC, Martins AT, Zuza EP, 2013).

The biological plausibility is that obesity and periodontal disease lead to a prothrombotic and proinflammatory condition (Chistiakov, Orekhov, & Bobryshev, 2016; Humphrey, Fu, Buckley, Freeman, & Helfand, 2008; Rocha & Libby, 2009). However, there are ethical issues that hinder the ongoing studies on humans involving these problems, especially due to important mortality rates. Moreover, there are also some animal studies which evaluated the correlation between periodontal disease and obesity regarding atherosclerotic events (Ekuni et al., 2010; Jain, Batista, Serhan, Stahl, & Van Dyke, 2003). Ekuni et al. (2010) found that periodontal disease in obese rats stimulated the early stages of atherosclerosis. These authors held that lipid deposits in the aorta were increased in obese rats with periodontitis compared to those which were obese but without periodontitis.

This study aimed to evaluate the aortic wall thickness of rats exposed to induced periodontal disease (DP) and/or obesity models (OB). The hypothesis under study is that periodontal disease could be an additional risk factor for the development of atherosclerosis.

METHODS

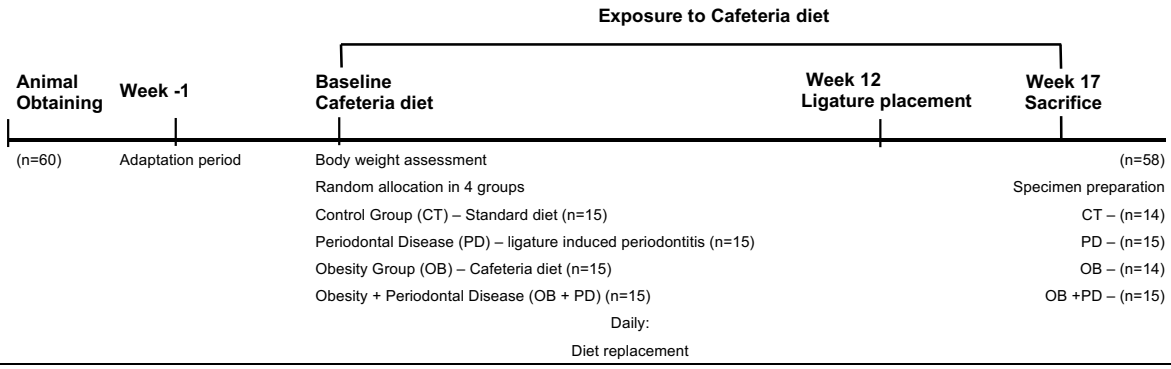
Study design

This is a blinded, randomized and controlled animal model study. The Animal Research: Reporting In Vivo Experiments (ARRIVE) guideline was followed (Kilkenny, Browne, Cuthill, Emerson, & Altman, 2010). It is important to emphasize that the present study is a secondary data analysis of a project that aimed mainly to evaluate the effects of a cafeteria diet on the periodontal parameters and some markers of systemic inflammation (Cavagni et al., 2016). In this sense, some data presented here have already been published, however it is necessary to present them again for a better understanding and evaluation of the penetrance of periodontal disease and obesity models.

The research protocol was approved by the Animal Research Ethics Committee of the Hospital de Clínicas de Porto Alegre, Brazil (protocol number 110051 at 26/04/2011) (in Anexo).

The protocol complies with the regulations set down by the Universal Declaration of Animal Rights (UNESCO – January 27, 1978) and the International Ethical Guidelines for Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences – CIOMS). Figure 1 demonstrates the study flowchart.

Figure 1. Study flowchart



Animals

In the present study, 60 male 60-days-old Wistar rats, with approximately 350g each were enrolled. All necessary procedures to minimize pain and discomfort were carried out by

experienced researchers. Therefore, animals were housed in groups of 4-5 under a light/dark cycle of 12 hours at room temperature (22°C ± 2°C) with free access to water and the assigned diet.

Randomization and group allocation

Animals were randomly divided into 4 experimental groups according to body weight. This was done by draw within these categories in the following groups:

Control (CT): received standardized rat diet and water. No additional experimentation was performed in the animals

Periodontal Disease (PD): exposed to ligature-induced periodontal disease in the upper right 2nd molar, with the same diet as CT.

Obesity (OB): obesity induction using a high fat and hypercaloric cafeteria diet (CAF).

Obesity and Periodontal Disease (OB + PD): exposed to obesity and periodontal disease induction models, as described in OB and PD.

Cafeteria diet-induced obesity

The CAF diet was used to induce obesity in rats throughout 17 weeks, and it was composed of 55% carbohydrates, 20% lipids, 20% protein and 5% other constituents (sodium, calcium, vitamins, preservatives, minerals among others) (Estadella, Oyama, Dâmaso, Ribeiro, & Oller Do Nascimento, 2004). This high caloric diet included condensed milk, soda, sandwich cookies, wafer, sausage, cheese and ham snacks. The control group animals were fed with standard diet. Water was available ad libitum for all groups. The CAF diet model is able to rise body weight, glucose levels, and cause hyperlipidemia (Sampey et al., 2011). This model is relevant considering the current deprived human diet, also called fast food.

Body weight and Lee index assessments

Throughout the 17-week experimental period, the animals were weighed (in grams) on an electronic scale. Also, the Lee Index method was assessed, in order to accurately and rapidly measure obesity in the groups that were submitted to the cafeteria diet. The Lee Index was calculated with the naso-anal length (in cm) and body weight (in grams), which consists

of dividing the cubic root of the weight in grams by the naso-anal length in centimeters and multiplying by ten (Bernardis & Patterson, 1968).

Periodontal disease model

After acquiring obesity to group OB+PD, at week 12, periodontal disease was induced by placing a silk ligature on the maxillary right second molar of the rats, which belonged to PD and OB+PD groups. The contralateral molar was intra-group control (Galvão, Chapper, Rösing, Ferreira, & de Souza, 2003; Sallay et al., 1982). A veterinarian ensured the general anesthesia of the animals; hence the ligature placement was performed.

Sacrifice and specimen preparation

A trained researcher performed the decapitation technique 30 days after placing the ligature. The maxillary jaws were removed, sectioned, and defleshed in 9% sodium hypochlorite for 2 hours until they could be analyzed. The aortic arteries were carefully removed and washed abundantly with saline solution and fixed with 10% buffered formalin until the moment of histological processing.

Histometric Analysis

The aortas of the animals were sectioned in four parts and paraffin-embebbed. Sections of 3μm were obtained for hematoxylin and eosin staining.

The stained slides were captured by CX41RF model binocular microscope (Olympus Latin America Inc., Miami, FL, USA) coupled to Qcolor 5 camera, Coolet, RTV (Olympus Latin America Inc.), at 100x magnification. The aortic wall (including intima and media) was measured (μm) by means of (ImageJ) software. The examiner was calibrated by an experienced pathologist. The thickest point of each section was selected for measurement.

Quality control

Blinding – The examiner was blinded to group allocation during histometric analysis and the codes kept by an external researcher.

Reproducibility - Prior to the beginning of the histometric analysis, calibration of the examiner took place, with 20% of the aorta slides randomly chosen to be doubly measured, with a one-week interval, and their means were compared Pearson's correlation coefficient. Absence of a statistically significant difference between the means was considered an indication of reproducibility. The intra-class correlation coefficient was also performed and the calculated value was 0.61.

Statistical analysis

For each evaluated parameter, normality was tested by means of Shapiro-Wilk test, and the appropriate statistical test was selected according to this assumption. Mean and standard deviation (SD) of body weight and Lee index were compared by Repeated Measures one-way ANOVA followed by Bonferroni. Alveolar bone loss did not exhibit a symmetric distribution and therefore the data was evaluated by Kruskall Wallis followed by Dunn multiple comparison test and the data expressed in median and interquartile range. The main outcome of the present study (aortic wall thickness) compared by one-way ANOVA, followed by Bonferroni multiple comparison's test.

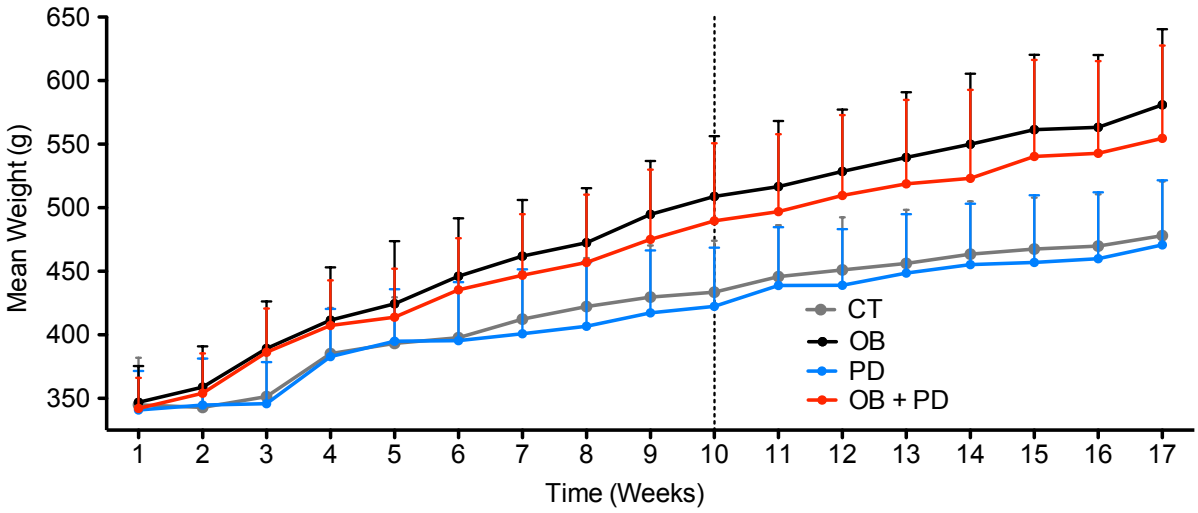
RESULTS

The results is presented based on the sample that completed the study. Two animals were lost during the experimental period, being one of the CT group and another of the OB, for reasons not related to the protocol, as confirmed in necropsy.

Obesity parameters

All experimental groups had a body weight increase, but there was an alteration in the weight gain pattern in rats exposed to CAF (Fig 2). From week 10 until the end of experimental period, a significant association between time and group was seen. OB and OB+PD groups displayed a statistically significant higher body weight as compared to CT and PD groups.

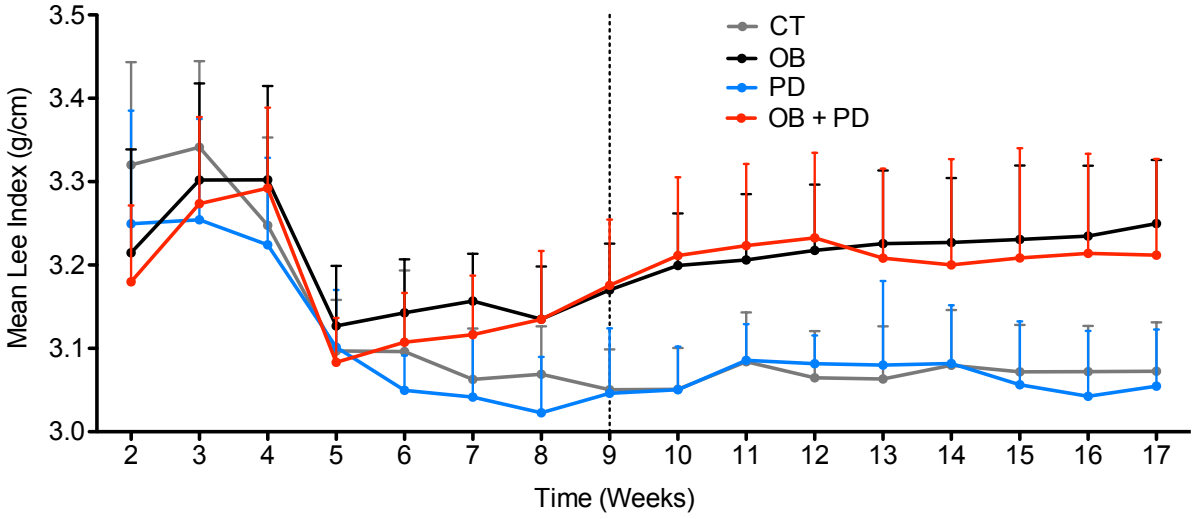
Figure 2. Mean (SD) body weight according to experimental groups throughout study



Dashed lines indicate the time from which significant difference occurred between OB and OB + PD) groups compared to CT and PD groups (**Repeated Measures ANOVA – Bonferroni)**

Until week 12, OB and OB+PD groups presented a statistically significant increase in Lee Index (Fig 3). In addition, a significant interaction among groups and between time and groups ($P \leq 0.01$) for this index was demonstrated by Repeated Measures ANOVA.

Figure 3. Mean (SD) Lee Index according to experimental groups throughout study.

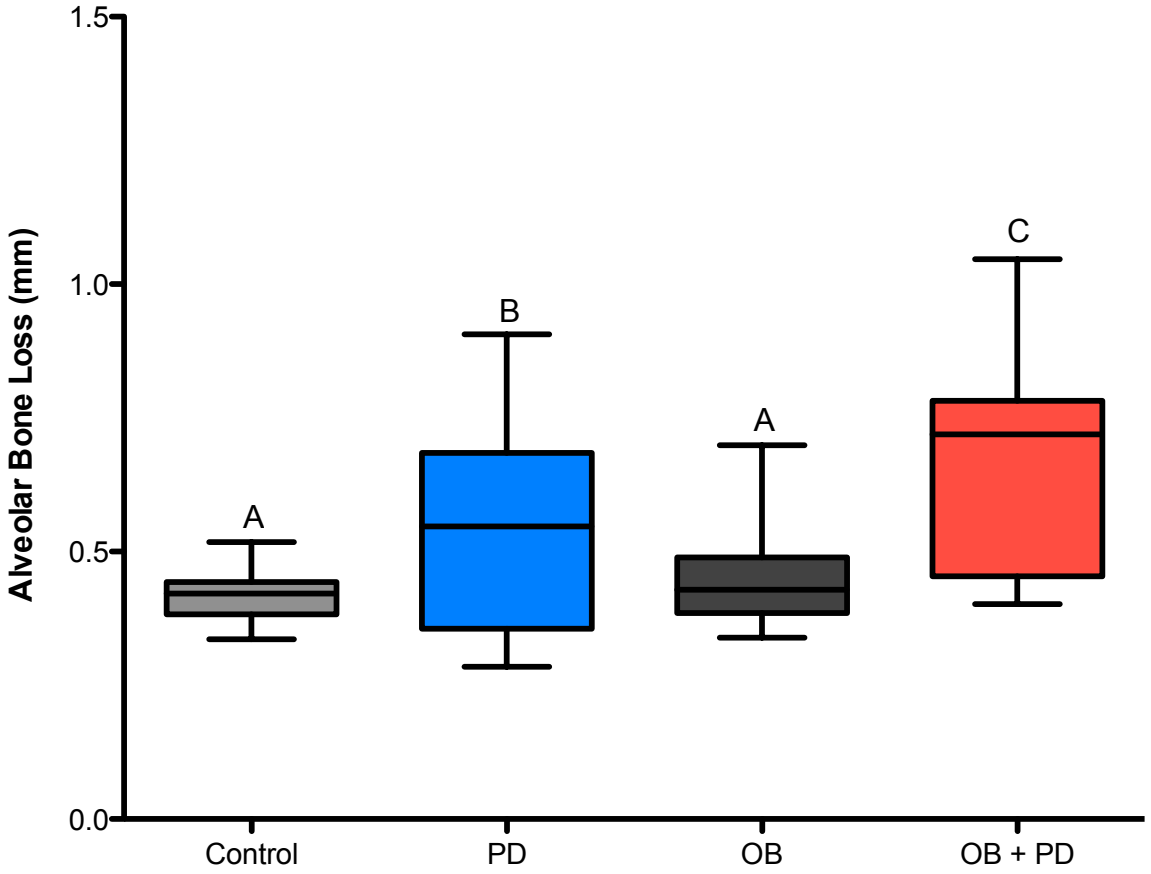


Dashed lines indicate the time from which significant difference occurred between OB and OB + PD) groups compared to CT and PD groups (**Repeated Measures ANOVA – Bonferroni)**

Alveolar bone loss

Figure 4 shows that alveolar bone loss was significantly higher in groups in which ligature was placed (PD and OB+PD) as compared with groups Control and OB. Interestingly, the OB+PD group compared to PD had increased 18% of ABL in the sites with ligature and it was statistically significant ($p<0.001$).

Figure 4. Median and interquartile range (percentile 25th and 75th) of alveolar bone loss according to experimental groups.



Different letters indicate statistically significant difference among experimental groups (Kruskall Wallis – Dunn).

Aortic wall thickness

Table 1. Mean (±SD) of aortic wall thickness according to experimental groups.

Group	Thickness (µm)
CT	**2,18 ± 0.26 A**
PD	**2,14 ± 0.27 A**
OB	**2,33 ± 0.29 B**
OB + PD	**2,31 ± 0.28 B**

Different letters in the column indicates statistically significant difference (One-way ANOVA- Boferroni)

Table 1 presents the main outcome of the present study. Aortic wall thickness was higher in groups exposed to CAF diet, independently of exposure to periodontal disease model (2.31 ± 0.28 and 2.33 ± 0.29 for OB and OB+PD vs. 2.18 ± 0.26 and 2.14 ± 0.27 for CT and PD, respectively). Statistically significant differences were found between OB and CT (p=0,036) and between OB+PD and OB compared to PD group (p=0.004 and p=0.001, respectively). No statistically significant differences were observed between CT and PD and between OB and OB+PD.

DISCUSSION

The present study evaluated aorta wall thickness and its relation to obesity and/or periodontal disease. The rationale for such a study is that the inflammation represented by periodontal disease could be an additional factor in the pathogenesis of cardiovascular diseases. The results demonstrated that obesity was able to increase aorta wall thickness, however periodontal disease did not modify this parameter.

CVD are considered a highly prevalent health condition and it with high morbidity and mortality rates worldwide (World Health Organization, 2017). In the medical literature, it is well-established that these diseases are caused by atherosclerosis. It is important that the researchers evaluate the possible risk factors, and whether they could worsen these health disorders or not, order to establish preventive and therapeutic strategies. Atherosclerosis is considered an inflammatory disease, and Orlandi et al., (2014) showed that it can be related to other inflammatory factors, such as periodontal disease and cardiovascular risk factors, which include obesity, smoking, sedentary lifestyle, dyslipidemia and diabetes. Therefore, all these elements could increase inflammatory process and change the arterial endothelial function,

which is the first stage of atherosclerosis (Hansson, 2005). Furthermore, a systematic review suggested that there is an association between overweight and obesity with periodontal disease both related to establishment and progression. (Keller, Rohde, Raymond, & Heitmann, 2015). However, studies still fail in establishing the exact biological process underlying this relationship.

Clinical studies using true outcomes such as myocardial infarction or even death are scarce and suffer from ethical restraints. Therefore, animal studies are warranted. This study used Wistar rats, which are widely used both in periodontal as well as in cardiovascular studies. The experiment induced atherosclerosis by consumption of cafeteria diet, which mimics the west diet, with a lot of carbohydrate and fat intake and has been previously used (Anandhi, Thomas, & Geraldine, 2014). In the study of pathogenesis of periodontitis and its relation to metabolic abnormalities, these models have also been utilized in the literature (Oz & Puleo, 2011; Verzeletti, Gaio, Linhares, & Rösing, 2012).

In the first 12 weeks, before ligature placement, OB and OB + DP groups were fed by cafeteria diet, which led to obesity (Sampey et al., 2011; Verzeletti et al., 2012; Cavagni et al., 2013). Body weight and Lee index values were used as methods to infer obesity. At baseline, the sixty Wistar rats had approximately 350g weight and no statistically significant difference among these groups was observed. All groups increased body weight, however, from week 10, rats which received CAF diet displayed a statistically significant higher body weight compared to CT and PD groups. Additionally, Lee index presented a statistically significant increase in groups fed by cafeteria diet. Even though studies suggest that periodontitis may stimulate obesity by secretion of proinflammatory cytokines (Pischon et al., 2007), no significant differences were observed between OB and OB+PD, with regard to body weight or Lee index. However, body weight and Lee index results established that obesity was achieved in the CAF diet exposed groups.

In the analysis of periodontal disease induction, the mean values for alveolar bone loss were expressively higher in the groups in which ligature was placed. Therefore, it can be assumed that the periodontal disease induction model was effective. Furthermore, higher alveolar bone loss was observed in OB+PD in contrast to PD groups. Similarly, a study showed that obesity may influence the progression of ABL in rats with induced periodontal disease compared to non-obese rats (Verzeletti et al., 2012). These data suggested that in the presence of ligature, obesity acted as a synergistic factor in ABL. A possible explanation for this increased ABL, it is the inflammatory process in obesity, which may result an increase in local inflammation of the gingiva and spread of bacteria in the tooth root by an

overproduction of proinflammatory cytokines by adipose tissue (Pischon et al., 2007).

The main outcome of the present study was the aortic wall thickness, which was higher in OB and OB+DP groups compared to control and periodontal disease groups. This data supports the fact that obesity increases aorta wall thickness and therefore increases the chances of CVD. These data mean that obesity impacted the aortic wall thickness in this model. This method of obesity induction by hypercaloric and hyperlipidemic diet in Wistar rats presents important metabolic alterations, such as triglycerides increase. For this reason, studies suggested to use this model to investigate endothelial dysfunction (Rosini, Silva, & Moraes, 2012), which is one of the factors which impact the pathogenesis of atherosclerosis. Our measurement model proved to be effective, because it showed that there was an increase in aortic thickness of the rats which received the CAF diet. Similarly, other authors also observed greater aortic wall thickness compared to control in groups that received not a CAF diet, but a high-cholesterol one (Turkay et al., 2004). Therefore, these data suggested presence of atherosclerotic disease, because the growth of the aortic wall thickness may imply presence of plaques or atheromas, which are risk factors for cardiovascular diseases (Frediani Brant et al., 2014).

On the other hand, periodontal disease was not able in the present model to modify the effect of obesity either increasing or decreasing the aortic wall thickness. Several possible explanations could be raised and some should be further studied. First, the ligature was placed in one tooth of the rats, which could represent a low inflammatory challenge, as compared to extensive periodontal disease. Second, the aortic wall thickness is extremely variable and could difficult the observation of statistically significant differences with the numbers of analyzed animals. However, the supporting literature is sufficient to continue studying the relationship between periodontal diseases and CVD, in order to better guide preventive and therapeutic public health policies worldwide.

Conclusion

It may be concluded that obesity induction model seems to alter aortic wall thickness in Wistar rats. The additional presence of periodontal disease not affect the aortic wall thickness.

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3 CONSIDERAÇÕES FINAIS

As doenças cardiovasculares (DCV) ainda são consideradas uma condição prevalente, e causam elevadas taxas de morbidade e mortalidade no mundo. Estas são causadas por uma doença inflamatória crônica, a aterosclerose. Estudos têm sugerido que a obesidade e a periodontite (ambas doenças inflamatórias crônicas) poderiam ser fatores de risco para essa condição, e consequentemente para as DCV. Entretanto, existem questões éticas que impedem a realização de estudos em humanos, no que diz respeito a plausibilidade biológica do processo saúde-doença. Assim, o exato mecanismo da patogênese ainda é desconhecido. Além disso, existem poucos estudos que avaliam o efeito da periodontite e/ou da obesidade sobre aortas, a qual poderia demostrar alterações que sugerissem o desenvolvimento de DCV.

Por estas razões, o presente estudo objetivou acrescentar, ao corpo de evidências existentes, informações sobre o impacto da doença periodontal e/ou da obesidade sobre a espessura da parede de aortas de ratos Wistar. Além disso, a hipótese nesse estudo foi que a doença periodontal poderia ser um fator de risco adicional para o desenvolvimento da aterosclerose. No modelo avaliado, os ratos que receberam a dieta de cafeteria tiveram um aumento no peso corporal e no índice de Lee. Além disso, os grupos que foram induzidos a periodontite tiveram uma média maior de perda óssea alveolar. Assim, nossos resultados demonstraram que tanto o modelo de indução de obesidade, quanto o de doença periodontal foram efetivos. Quanto ao principal desfecho deste trabalho, foram observadas maiores espessuras nos grupos com obesidade (OB e OB+DP) comparados aos grupos controle e DP. Portanto, podemos entender que o modelo de indução de obesidade parece alterar a espessura da parede das aortas. Por outro lado, a presença de doença periodontal não teve efeito sobre aquele desfecho.

Apesar do desfecho deste estudo demonstrar que a periodontite não é um fator adicional para o desenvolvimento da aterosclerose em ratos obesos, a literatura evidencia uma associação positiva entre a doença periodontal e as DCV. Tanto a obesidade, quanto a doença periodontal, são condições prevalentes na população. Assim, é de suma importância que se dê continuidade aos estudos que avaliem esta relação, e se eles teriam efeitos sinérgicos sobre as DCV, a fim de orientar as políticas de saúde pública quanto à prevenção e tratamento em todo o mundo.

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ANEXO - APROVAÇÃO DO PROJETO DE PESQUISA PELO COMITÊ DE ÉTICA EM PESQUISA

HCPA - HOSPITAL DE CLÍNICAS DE PORTO ALEGRE
GRUPO DE PESQUISA E PÓS-GRADUAÇÃO

COMISSÃO DE ÉTICA NO USO DE ANIMAIS

A Comissão Científica e a Comissão de Ética no Uso de Animais (CEUA/HCPA) analisaram o projeto:

Projeto: 110051
Data da Versão do Projeto: 26/04/2011

Pesquisadores:
CASSIANO KUCHENBECKER ROSING
JULIANO CAVAGNI
ISABEL CRISTINA DE MACEDO
IRACI LUCENA DA SILVA TORRES

Título: IMPACTO DA SÍNDROME METABÓLICA NA PATOGÊNESE DA PERDA ÓSSEA ALVEOLAR INDUZIDA EM RATOS WISTAR

Este projeto foi APROVADO em seus aspectos éticos e metodológicos de acordo com as Diretrizes e Normas Nacionais e Internacionais, especialmente a Lei 11.794 de 08/10/2008, que estabelece procedimentos para o uso científico de animais.

- Os membros da CEUA/HCPA não participaram do processo de avaliação de projetos onde constam como pesquisadores.
- Toda e qualquer alteração do Projeto deverá ser comunicada à CEUA/HCPA.
- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao CEP/HCPA.