



Age as a Progression Factor of Alveolar Bone Loss in Rats

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Abstract

Objective: The aim of this study was to evaluate the presence and progression of loss of periodontal bone support (PBS) in male rats as a function of age.

Materials and Methods: The animals were reproduced and kept in the institution vivarium under controlled conditions and standard diet. Seventeen male Wistar rats were evaluated totaling 34 hemi-mandible pieces, divided into three groups according to their age: group 1 (6 months) n = 12; group 2 (12 months) n = 14 and group 3 (18 months) n = 8. Bone loss was assessed by means of digitized radiographs. Three points were considered in the selected molar: the distal root Apex, distal cusp tip and the bone defect fundus. The results were statistically analyzed using mean (\bar{X}) and standard error (SE), followed by the TUKEY test.

Results: Comparison of the observed indices showed significant difference of bone loss between groups 1 and 3 and between 2 and 3. Comparing groups 1 and 2 no significant changes were found.

Conclusion: This study showed that age was determining factor for the progression of bone loss in the animals studied.

Keywords: Aging, Alveolar bone loss, Periodontal bone support, Periodontal disease

INTRODUCTION

The population aging process is a worldwide phenomenon [1]. International organizations predict that, in 2025, there will be 1.2 billion people over the age of 60, with older elderly people aged 80 or over constituting an age group of significant numerical importance [2]. Aging is characterized as a dynamic, progressive and irreversible process, closely linked to biological, psychological and social factors [3]. The improvement in living conditions, progress in health care and the implementation of public health measures have been considered the main causes for the demographic growth of this age group. However, this situation has also led to an increase in diseases associated with aging, disabling conditions and chronic diseases [4,5]. In the aging process, changes in orofacial structures occur due to the deterioration of physiological processes and cellular functions, also leading to anatomical changes. These changes can be related to aging and can be related to diseases, or be the combination of both [2].

Bone is a living and dynamic tissue which undergoes remodeling throughout the life of the organism. Its main component is hydroxyapatite that corresponds to about 60% of its gross weight [6].

In the course of aging, bone loss varies from one individual to another; as the remodeling process, which consists of resorption and new formation of bone trabeculae, can be

modulate by factors such as diet, drugs and hormones, in addition to aging [6-10].

The physiological process of normal bone remodeling consists of the balance between bone resorption and bone neoformation. During the aging process, there is an imbalance in this process, with resorption predominating over the new bone formation, culminating in bone loss [11].

To understand what factors are associated with loss of alveolar bone, Huttner [12] analyzed the effect of human aging on periodontal tissues and observed that the changes in these tissues are due to molecular changes in cells and that they are related to the intensity of bone loss in the elderly. They concluded that this bone loss may be related to changes in the differentiation and proliferation of osteoblasts and osteoclasts, secretion of cytokines involved in the bone resorption process and endocrine changes commonly found in elderly individuals.

In addition to human studies, several studies have been

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carried out using animal models [13-15]. Therefore, the objective of this work was to show periodontal bone support in naturally aged Wistar rats not showing systemic or oral diseases.

MATERIALS AND METHODS

This is an experimental study performed on an animal model in rats, with randomized samples, blinding and examiner calibration and comparison of groups. In the present study, 17 adults male Wistar rats (*Rattus norvegicus*) were used, aged 6 (n = 6), 12 (n = 7) and 18 (n = 4) months, supplied and kept in the institutional vivarium. Animals suffering from periodontal disease were not included in the study. Throughout the period all animals were monitored for systemic and oral health. The experimental protocols proposed in this study are in accordance with that established by the Brazilian College of Animal Experimentation-COBEA and were approved by the Ethics Committee on Animal Experimentation - FUNVIC.

These animals were divided into three groups according to age kept each group in a polypropylene housing box (60x50x22 cm) at a controlled temperature ($22 \pm 2^\circ\text{C}$), in a 12-hour controlled light regime. The three groups were offered a solid routine diet with commercial feed Nuvilab CR-1 (Nuvital®, Dois Vizinhos-PR) and water *ad libitum*.

The animals in each batch had their deaths induced by anesthetic deepening with 0.2 ml / 100 g of live weight of a 1: 1 solution of 2% xilasine hydrochloride and 10% ketamine hydrochloride and necropsied to remove the mandibles.

Morphometric Analysis of Alveolar Bone Loss

After being collected, the mandibles were carefully dissected by means of a procedure that included immersion in sodium hypochlorite solution for one hour and dissected with mechanical removal of all remaining soft tissue [14].

The right and left mandibles were radiographed by the X-ray machine Gnatus Equipamentos Médico-odontológico LTDA TIMEX 70E WALL 220V + 4% - 60 HZ, with an exposure time of 0.64s. The images were captured using the Soredex Digora 2.1.2 program. The digital radiological sensor was fixed on a table and the cylinder was positioned at a focal distance of 30 cm, the hemimandibles, with the vestibular face facing the x-ray cylinder. The pieces were fixed with the aid of utility wax. The sensor showed a parallel relationship with the free faces of the teeth and a perpendicular relationship with the occlusal faces. And to measure the percentage of periodontal bone support (PPBS) in the interproximal region, the program ImageJ 1.52a was used. The PBS values were obtained using the formula: $\text{PBS} = \text{AB} \div \text{AC} \times 100$ (Figure 1) [14].

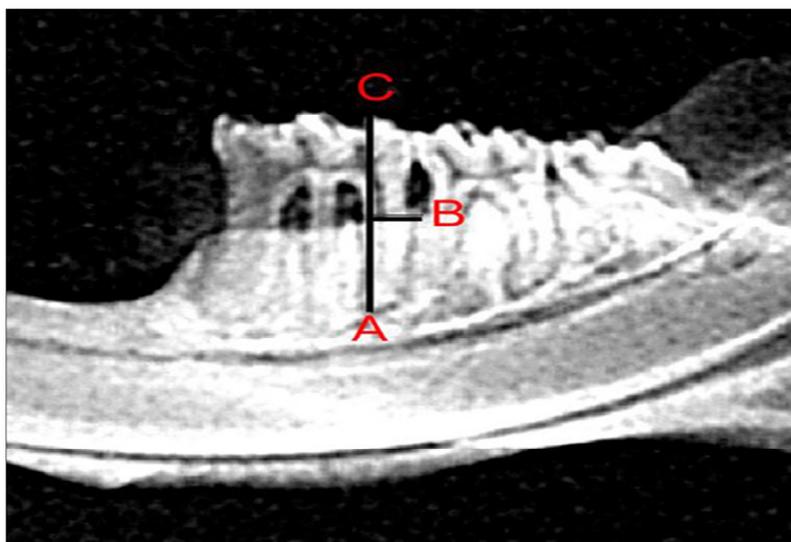


Figure 1. Illustration of points A (apex of the distal root of the lower 1st molar), B (alveolar bone crest) and C (apex of the highest tooth cusp). The remaining PBS was obtained by $\text{PBS formula} = \text{AB} \div \text{AC} \times 100$.

Statistical analysis

On statistical analysis, mean (\bar{X}), standard error (SE), minimal (Min) and maximal (Max) values were calculated; t-test (p) was used to test differences in the means (\bar{X}) between the three age groups followed by the TUKEY test, with a significance level of 5%.

RESULTS

One day before euthanasia, each animal had its oral cavity evaluated and no periodontal disease was found.

The results of bone support measurements showed a higher ($p < 0.05$) bone loss percentage in the 18 m age group ($57.01 \pm 5.68\%$) than in the 12 m group ($33.77 \pm 4.96\%$) and the 6 m age group ($31.49 \pm 2.03\%$). There was no significant difference of bone loss percentage between the groups 6 m and 18 m age (Figure 2).

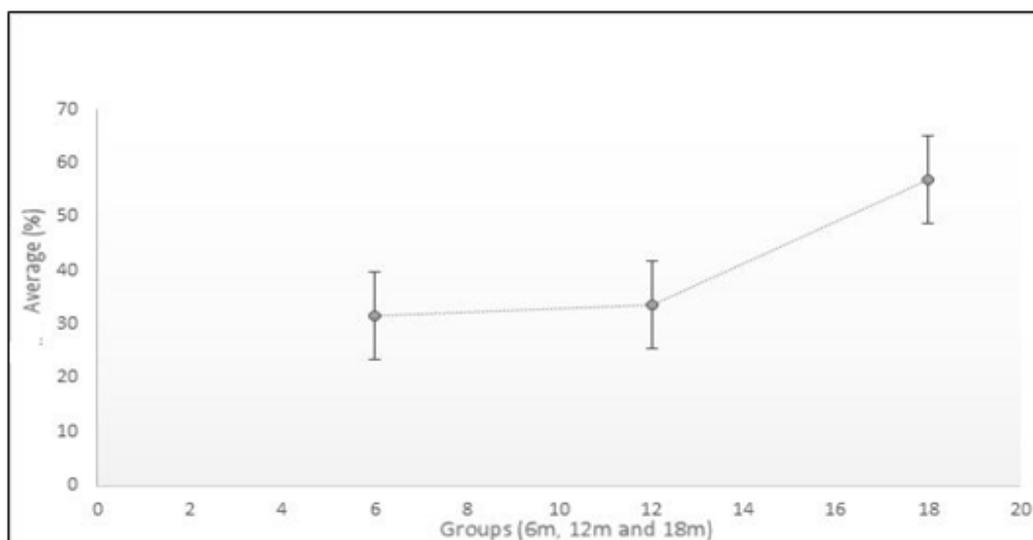


Figure 2. Mean and standard deviation of the periodontal bone loss (mm) in each group.

**Statistically significant (ANOVA and Tukey test; $p < 0.05$).*

DISCUSSION

This study used the PBS method, which evaluated periodontal bone support using the radiographic method, measuring two points: from the apex of the distal root of the lower 1st molar to the alveolar bone crest, and from the apex of the distal root of the lower 1st molar to the apex of the largest cusp of the same tooth. Our group has used this method as a method of choice for some time [13-15]. According to Beck [16] the consideration of a limited number of teeth seems to provide a satisfactory estimate of the decrease in bone level.

The loss of periodontal bone support may be associated with age [17,18]. Epidemiological studies of periodontal health have revealed that in a healthy adult, the age alone does not lead to a critical loss of periodontal support, although moderate loss of alveolar bone is common in the elderly [19]. However, we still cannot affirm this involvement, due to the difficulty of carrying out studies in humans, because there are several confounding factors. Thus, an alternative to assess these conditions is to conduct studies in rats in a controlled manner. It is known that the physiological conditions are very close to that of humans, justifying the choice of this experimental model [20-22].

Some studies relate bone loss to imbalance in the bone remodeling process, where resorption prevails over bone neoformation. These, also evaluated that there is bone loss in volume, density and height of the alveolar crest. Some risk factors are associated with this bone loss; however, studies highlight the chronic inflammatory process as a predominant factor [7,9-11,20-22]. In this study, no gingival inflammatory process was observed. Huttner [12] studied the effect of aging on periodontal tissues. They concluded that aging alone does not cause a significant loss of alveolar bone

in healthy elderly people, but rather, molecular changes such as changes in bone cell differentiation and proliferation, in the secretion of chemical molecules involved in bone resorption and endocrine changes may be related to bone loss. Corroborating these findings, Laurent [9] suggested that with aging there is a reduction in bone mass and a degeneration of bone tissue microarchitecture, predisposing to loss of alveolar bone. Ruquet [18] analyzed the possible association of coronary heart disease and or severe vascular disease with alveolar bone loss and aging. They concluded that there is a statistically significant relationship between cardiovascular disease and alveolar bone loss in elderly individuals [18].

The present study evaluated the presence and progression of loss of periodontal bone support in male rats as a function of age, showing a significant difference between the loss observed in animals aged 6 months when compared to animals aged 18 months and in animals aged 12 months when compared to the 18-month group. But there was no significant variation between 6 and 12 months. This observation suggests that the implantation of the loss occurs with the onset of aging and then continues. Alveolar conditions are one of the dental changes that is associated with the process of aging [17] Alveolar resorption as a process of physiological involution is difficult to distinguish from alveolar resorption as a pathophysiological process of infection, inflammation and destruction of damaged periodontium [10-16]. For this reason, it was important to monitor the animals' oral cavity throughout the experiment.

CONCLUSION

The present finds demonstrate that aging can induce alveolar bone loss in rats in the absence of any other factor inducing periodontal disease.

REFERENCES

1. Kinane DF (2001) Causation and pathogenesis of periodontal disease. *Periodontology* 25: 8-20.
2. Irie K, Tomofuji T, Tamaki N, Sanbe T, Eruni D, et al. (2008) Effects of ethanol consumption on periodontal inflammation in rats. *J Dent Res* 87: 456-460.
3. Sodek J (1997) A comparison of the rates of synthesis and turnover of collagen and non-collagen protein in adult rat periodontal tissues and skin using a microassay. *Arch Oral Biol* 22: 655-657.
4. Bartold PM, Walsh LJ, Narayanan AS (2000) Molecular and cell biology of the gingiva. *Periodontol* 24: 28-31.
5. Tezal M, Grossi SG, Ho AW, Genco RJ (2001) the effect of alcohol consumption on periodontal disease. *J Periodontol* 72: 183-189.
6. Souza DM, Ricardo LH, Kantoski KZ, Rocha RF (2009) Influence of alcohol consumption on the alveolar bone level associated with ligature-induced periodontitis in rats. *Braz Oral Res* 23: 326-332.
7. Tezal M, Grossi SG, Ho AW, Genco RJ (2004) Alcohol consumption and periodontal disease. The third national health and nutrition examination survey. *J Clin Periodontol* 31: 484-488.
8. Khocht A, Janal M, Schleifer S, Keller S (2003) The influence of gingival margin recession on loss of clinical attachment in alcohol-dependent patients without medical disorders. *J Periodontol* 74: 485-493.
9. Laurent MR, Dedeyne L, Dupont J, Mellaerts B, Dejaeger M, et al. (2019) Age-related bone loss and sarcopenia in men. *Maturitas* 122: 51-56.
10. Newman MG, Takei H, Carranza FA (2007). *Periodontia clínica*. 10 ed. Rio de Janeiro: Saunders Elsevier. pp: 10-12.
11. Loe H, Theilade E, Jense SB (1965) Experimental gingivitis in man. *J Periodontol* 36: 177-187.
12. Huttner EA, Machado DC, Oliveira RB, Antunes AGF, Hebling E (2009) Effects on human aging of periodontal tissues. *Spec Care Dentist* 29(4): 149-155.
13. Gonçalves AF, Ribeiro DC, Zanin MT, Carvalho C, Souza DM (2017) Efeito da obesidade no nível ósseo alveolar de ratos. *Rev Ciên Saúde* 2(1): 22-28.
14. Souza DM, Rodrigues VA, Silva AA, Gonsalves VS, Pereira KA, et al. (2017) Alveolar bone loss induced by alcohol consumption in rats. *Braz Dent Sci* 20(4): 42-48.
15. Souza DM, Silva AA, Pereira KA, Gonsalves VS, Rodrigues VA, et al. (2018) Influence of diferente alcohol intake frequencies on alveolar bone loss in adult rats: A SEM study. *J Clin Exp Dent* 10(9): e852-857.
16. Beck JD, Caplan DJ, Preisser JS, Moss K (2006) reducing the bias of probing depth and attachment level estimates using random partial-mouth recording. *Community Dent Oral Epidemiol* 34(1): 1-10.
17. Sarajlic N, Topic B, Brkic H, Alajbeg I Z (2009) Aging Quantification on Alveolar Bone Loss. *Coll Antropol* 33(4): 1165-1170.
18. Ruquet M, Saliba-Serre B, Tardivo D, Foti B (2015) Estimation of Age Using Alveolar Bone Loss: Forensic and Anthropological Applications. *J Forensic Sci* 60(5): 1305-1309.
19. Burt BA (1994) Periodontitis and aging: reviewing recent evidence. *JADA* 125: 273-279.
20. Souza DM, Ricardo LH, Rocha RF, Prado MA, Prado FA (2006) The effect of alcohol consumption on periodontal bone support in experimental periodontitis in rats. *J Appl Oral Sci* 14(6): 443-447.
21. Souza DM, Ricardo LH, Rocha RF (2014) Effects of alcohol intake in periodontitis progression in female rats: a histometric study. *Bras J Oral Sci* 13(3):229-234.
22. Bannach SV, Teixeira FB, Fernandes LMP, Ferreira RO, Santana LNS, et al. (2015) Alveolar bone loss induced by chronic ethanol consumption from adolescence to adulthood in Wistar rats. *Indian J Experi Biol* 53: 93-97.