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Hypoestrogenism during the Pubertal Stage Affects Alveolar Bone Loss

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Abstract

Background/Purpose: There are some evidence suggesting that (hypoestrogenism) estrogen deficiency may increase alveolar bone loss during adulthood. Therefore, the objective of this work was to evaluate, if hypoestrogenism during puberty impacts on alveolar bone loss.

Material and Methods: Wistar female rats were equally distributed into ovariectomy and control groups (12 rats per group). At an age of 21 days (prepubertal stage) hypoestrogenism was induced via bilateral ovariectomy in the ovariectomy group, while placebo surgery was executed in the control group. At the age of 63 days, the animals were euthanized. A micro-computed tomography analyses was performed in the mandibles. Alveolar bone loss was assessed morphometrically by linear measurements from the cemento-enamel junction (CEJ) to the alveolar bone crest (ABC) along the root axes and surfaces at the mandibular first right molar. Student's t-test was used for statistical comparisons.

Results: At the lingual site, alveolar bone loss (CEJ-ABC) in the hypoestrogenism group was significantly more pronounced than in the control group at the medial root. At buccal sites, significantly more alveolar bone loss was also observed in the hypoestrogenism group compared to the control group at the medial and distal roots ($p \leq 0.05$).

Conclusion: Hypoestrogenism during puberty is involved in alveolar bone loss in teenagers and young adults.

Keywords: Bone loss; Estrogen; Periodontal disease

Introduction

Microorganisms of the subgingival biofilm are associated with periodontal inflammation and its progression involves interactions between host inflammatory and immune system and pathogenic microbiota [1]. Periodontal diseases are complex diseases that should be treated based on their multifactorial nature and variability identifying some risk factors, such as lifestyle, medication, and systemic conditions [2,3], such as hormonal imbalance [4].

Estrogen is a widely studied hormone that is known to play a substantial role in inhibiting bone resorption [5] and stimulating bone formation, thus leading to a steady or slightly increased bone mineral density [6]. Estrogen-deficiency (hypoestrogenism) induces expression of receptor activator of nuclear factor kappaB ligand (RANKL) and decreases the level of osteoprotegerin, causing increased osteoclastogenesis [7]. Endogenous levels of estrogen can change according to age and gender [8]. For instance, during puberty, there is an increase in estrogen levels [9]. Hypoestrogenism can appear naturally after menopause

[10,11], but may also occur during the pubertal stage in some conditions [12-17]. In animal models, bilateral ovariectomy is a common procedure to stimulate hypoestrogenism [18].

Previous works have shown that hypoestrogenism in adulthood may increase alveolar bone loss [3,7,19-21], negatively influencing the periodontium and alveolar bone resorption, leading to a tooth loss in rats [11] and human studies [22]. Considering the important role of estrogen during female puberty, in this study we evaluated, if hypoestrogenism during puberty impacts alveolar bone loss.

Materials and Methods

Ethical aspects

The Committee of Ethics in the Use of Animals (n° 2014.1.721.58.7) approved this study. The ARRIVE (Animal Research Reporting of *in vivo* Experiments) [23] protocol was followed considering the methodological orientations regarding blinding, calibration of examiners, and animal welfare.

Sample

Wistar female rats were equally divided into ovariectomy (OVX-hypoestrogenism) and Sham-operated (Control) groups (12 rats per group). Succinctly, the rats were anesthetized using an intraperitoneal injection of 10% ketamine hydrochloride (55 mg/kg of gross body weight) and 2% xylazine hydrochloride (10 mg/kg of gross body weight), when they were at 21 days old (which is the female rat's prepubertal period). Bilateral ovariectomy was conducted in the hypoestrogenism group, while a placebo surgery was conducted in the control group. At the age of 63 days (young adult period for female rats), the animals were euthanized for analysis of bone loss. As previously described by Chen et al [24] and Omori et al [18], body and uterus weight were significantly higher in the hypoestrogenism animals than in control animals in the adult phase ($p<0.05$), confirming the success of ovariectomy.

Micro-computed tomography (µCT) and morphometric analysis

µCT analyses were performed in mandibles as previously described [25,26]. For the morphometric measurements, we used the images from the µCT, VGSTUDIO MAX 3.3 software. Alveolar bone loss was evaluated morphometrically by linear measurements at the tooth root surface along the respective root axis from the cemento-enamel junction (CEJ) to the alveolar bone crest (ABC) at lingual and buccal sites for each tooth root of the mandibular first right molars as demonstrated in (Figure 1). The linear dimensions (mm) were measure using Image J software.

Statistical Analysis

Sample normality was analyzed by Shapiro-Wilk tests. Comparative analysis was performed by Student's t-tests to verify the difference in the measurements between hypoestrogenism and control groups. The results were presented as means and standard deviations (SDs). Statistical significance was assumed at $p \leq 0.05$. All analyses were performed using the Prism 8 software (Graph Pad Software Inc., San Diego, California, USA).

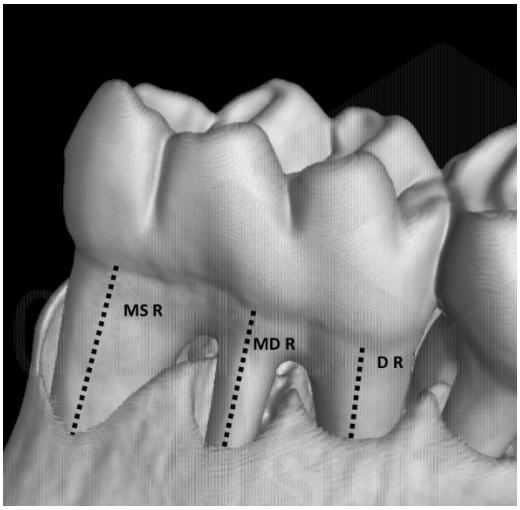

Figure 1: *Three-dimensional view of the mandibular right first molar. Linear measurements of alveolar bone loss (CEJ to ABC) at the lingual site. D R- distal root; MD R- medial root and MS R- mesial root.*

Results

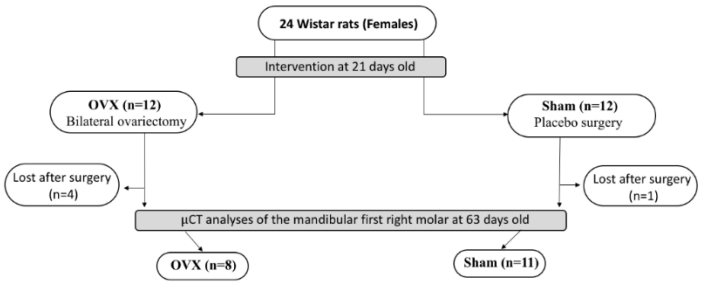

Figure 2: *Flow chart of the experiment.*

The Figure 2 demonstrated the flowchart, 5 rats died before recovering from anesthesia, therefore, 8 rats from the hypoestrogenism group and 11 rats from the control group were included in the morphometric analysis. Results of bone loss (CEJ-ABC) from the µCT analysis in hypoestrogenism and in control groups and the comparison between groups are presented in (Figure 3).

At the lingual site, a statistically significance difference between the groups at the medial root was found ($p=0.04$): in the hypoestrogenism group the CEJ-ABC mean was 0.57mm (SD = 0.14), while in the control group the CEJ-ABC mean was 0.46mm (SD = 0.07). At the linguo-distal root, a statistically significance

difference between groups (p=0.04) was observed as well: in the hypoestrogenism group the CEJ-ABC mean was 0.39mm (SD = 0.10), while in the control group the mean was 0.28mm (SD = 0.11). At the buccal area, a statistically significant difference was found between groups (p=0.05). At the medial root: in the hypoestrogenism group, the CEJ-ABC mean was 1.45mm (SD= 0.30), while in the control group the CEJ-ABC mean was 1.23mm (SD = 0.17).

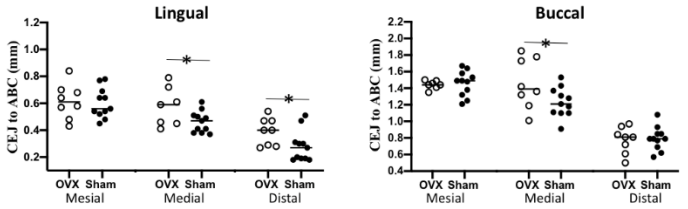

***Figure 3**: Alveolar bone loss (CEJ-ABC) according to the groups at the different measurement sites. * means statistically significant difference between groups ($p \leq 0.05$).*

Discussion

During the past decades, studies using rat models have been used to investigate periodontitis progression during estrogen-deficiency (hypoestrogenism) conditions. These were postmenopausal osteoporosis models and the results from these experiments increased our knowledge on the important role of the estrogen as a protective factor for alveolar bone loss [7,19]. Although many previous studies [3,7,19-21] evaluated the relationship between hypoestrogenism and alveolar bone loss in rodents (adult rats), our study differs from previous ones, since it focused on the pubertal stage. In humans, hypoestrogenism in young patients were reported in chromosomal conditions [12], in girls with hormonal alterations [13], including ovarian problems [14] also in undernourishments individuals [15], adolescent athlete with exercise-induced amenorrhea [16] and patients under chemotherapy treatment [17]. Therefore, we conducted an *in vivo* study in female rodents to investigate, whether hypoestrogenism in the pubertal period could impact on alveolar bone loss at molars.

The nature of the connection between periodontal disease and hypoestrogenism-induced bone loss is not completely understood. Anbinder et al. [20] reported that hypoestrogenism cannot be considered alone as a factor involved in the risk for alveolar bone amount or loss [19]. In our study, however, we observed that estrogen-deficiency during puberty is involved in alveolar bone loss in young ages without the experimental periodontitis induction. A possible lack of association between hypoestrogenism and alveolar bone loss observed in previous studies without the induction of experimental periodontitis might be due to the type of analysis performed and the age/period of the rats. In our study, with µCT analysis, we performed a more reliable analysis. µCT-based measurements have the advantage of high resolution and the ability to determine alveolar bone loss by 3D assessment.

Other studies using estrogen-deficient animals [7,21] have revealed that the osteoporosis resulting from estrogen-deficiency increases alveolar bone resorption in rats with and without ligature-induced periodontitis. The lack of estrogen induces a significant inequality in bone remodeling with bone resorption surpassing bone formation. Main characteristics of the osteoporosis induced are reduced bone mass and mineral content, alterations in bone micro-architecture and higher risk of fractures [26]. As an effect of the rising osteoporosis prevalence, clinicians and researchers from different fields have focused on studying the impact of hypoestrogenism on different bone pathologies, including the periodontal condition.

Briefly, the main power of our study is that it provides preliminary data demonstrating the influence of estrogen-deficiency in the pubertal stage on alveolar bone. General dentists, orthodontists, pediatric dentists, and periodontists must be aware of the consequences of hypoestrogenism in dental practice.

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