Investigation of Changes in Dental Pulp Tissue in Rats with Bilateral Ovariectomy by Histopathological Methods

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Abstract: In this study, we aimed to investigate the histopathological changes on 14th and 28th days in the dental pulp of ovariectomized rats. Twenty 12-week-old 21 female Sprague Dawleys were divided into 3 groups. Group 1: No procedure was applied to the animals. All teeth were excised at the end of the experiment (at the 14th day of experiment). Group 2: The abdomen was opened and the ovary tied with 3/0 silk sutures to block blood supply. The ovaries were placed in its original position. Teeth of ovariectomized rats were excised at the 14th day of experiment. Group 3: Similar procedure was applied as in Group 2. The teeth were removed on 28th days of experiment. The dental tissues taken after the experiment were processed for routine paraffin wax tissue preparation. Hematoxylin-Eosin staining was applied to sections of 4 µm thickness. As a result, when compared to the dental pulp of control group, degenerated dental pulp tissue, vascular dilatation and pyknotic osteoblast cells were observed in ovariectomized rats, however the pathological intesity was higher in ovariectomized rats for 28 days than ovariectomized rats for 14 days.

Keywords: Ovariectomy, Dental Pulp, Rat, Osteoporosis, Estrogen.

INTRODUCTION

Ovariectomy, the removal of ovaries, causes decreased estrogen levels and bone loss, observed in studies with experimental animals. Due to the development of osteoporosis, bone fragility increases [1]. Estrogen plays the most critical path in the pathogenesis of osteoporosis [2]. Osteoporosis not only causes deterioration in the bone structure, but also causes changes in the structure of the tooth and alveolar bone attached to the tooth [3]. In the light of these data, it is assumed that there are changes in tooth and bone structure in estrogen deficiency. Bone mineral density (BMD) changes have an impact on oral health. Studies with various methodologies have found a link between estrogen deficiency and changes in mandible measurements and other bone areas. It is assumed that estrogen has an effect on the amount and quality of bone in the maxilla and mandible [4]. There are studies on bone and post menopause in rats with ovaries removed, approved by the American Food and Drug Administration (FDA). The most widely approved model is the 12-week-old female Sprague-Dawley rat [5]. After removal of the ovaries in female rats, osteonectin content in the pulp tissue decreased and changes were observed in the predentin areas [6].

Especially osteoporosis and bone development were studied in ovariectomized rats. However, studies on teeth and dental pulp are scarce in the literature. In almost all animal experiment studies and clinical use studies on pulp histopathology in restorative dental treatment, in order to observe the dentin development, dentin formation stages and pulpal response of the active substance or invasive procedure, on the 7th, 14th, 21st, 28th, 60th days after the procedure were examined. Especially osteoporosis and bone development were studied in ovariectomized rats. However, studies on teeth and dental pulp are scarce in the literature. Due to the use of experimental animals in our study, it was thought that using the least number of animals, it was planned to compare only two time periods.

In our study, we aimed to investigate the histological changes in the dental pulp within 14 and 28 days in 12-week-old rats that underwent bilateralovariectomy.

MATERIAL AND METHOD

Ethical Approval and Experiment Procedure

All experimental procedures were approved by the Dicle University Animal Experimentation Local Ethics Committee (ethical approval number: 2020/16). This work was performed in Dicle University Experimental Animals Care Unit. Before starting the experimental procedure; animals were given general anesthesia with

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E-ISSN: 2311-8695/21

90 mg/kg intramuscular ketamine hydrochloride and 8 mg/kg xylazine. In the experimental protocol, 12-week-old female Sprague Dawley rats (n:21) were used. Experimental animals were obtained from Dicle University Health Sciences Research and Application Center (DÜSAM). This study is a part of Elif Pınar BAKIR's doctoral thesis, was supported by Dicle University Scientific Research Projects (TIP.21.004). Subjects were housed in clean cages with free access to water and food, on a 12-hour dark, 12-hour light cycle at room temperature. For the first two days, 25 mg/kg metamizole sodium (Devaljin®) was administered twice a day to all groups operated to provide analgesia.

Group 1 (Control)(n=7): No treatment was applied to the animals. Rats were sacrificed by cervical dislocation and lower central teeth were removed.

Group 2 (Ovariectomy – 14 days)(n=7): Before surgical procedures, 90 mg/kg intramuscular ketamine hydrochloride (Ketalar; Pfizer, İstanbul, Turkey) and 8 mg/kg xylazine (Rompun; Bayer, İstanbul, Turkey) general anesthesia were administered. All rats were fixed on the operating table after anesthesia, in the supine position, the abdomen was shaved and cleaned with 10% povidone-iodine, and the abdomen was opened with a 1 cm lower midline incision. The bilateral ovaries at the tip of the uterus was found and ligated from the bottom with 3/0 catgut and cut without blood supply. After the ovaries were cut with the help of surgical scissors, the uterus was placed in its original position. Before wound closure, ciprofloxacin 10 mg/kg was given. Finally, the subcutaneous tissues and then the skin were sutured with 3/0 catgut and the wound was closed. Experimental animals were placed in their cages by controlling the wounds. At the end of the 14th day, the rats and xylazine (Rompun; Bayer, Istanbul, Turkey) were sacrificed by cervical dislocation under sedation and their lower central teeth were removed.

Group 3 (Ovariectomy – 28 days)(n=7): The protocol applied in the 2nd group was applied in this group as well. Unlike the 2nd group, at the end of the 28th day, the rats (Rompun; Bayer, Istanbul, Turkey) were sacrificed by dislocation under sedation and their lower central teeth were removed.

Anesthesia and Surgical Procedures

At the end of the experiment, rats were sacrificed under anesthesia intramuscular ketamine hydrochloride (75 mg / kg) and 10 mg/kg xylazine. Teeth were taken with 10% neutral zinc-formalin solution for routine histological follow-up

Histological Examination

5 µm thick sections were taken from paraffin-embedded teeth tissues. After the deparaffinization process, the sections were passed through alcohol series and taken into distilled water. After the deparaf through alcohol series they were taken into distilled water. Hematoxylin Eosin (HE) staining was applied to sections. The stained sections were washed with distilled water, then passed through alcohol series and dehydrated. They were covered with Entellan and examined under a light microscope (Zeiss, Imager A2, Germany).

RESULTS

Histopathological Findings

Hematoxylin-eosin sections of the groups were shown in Figure **1**. When the sections of the control group were examined, it was observed that the pulp structure was normal. Odontoblasts were found to have normal appearances in the basement membrane close to the predentine. No damage was observed in the connective tissue and blood vessels in the pulp (Figure **1**.**a**). When the sections taken from the 14th-day ovariectomy group are examined, inflammation is observed in the cells in the pulp and in the connective tissue (Figure **1**.**b**). In the 28th-day ovariectomy group, there was a clear separation in the pulp structure. A significant dilatation is observed in the blood vessels. In addition, deformations in the tissue structure were observed in skin. Pyknosis was seen due to the narrowing of the cytoplasm of the odontoblast cells (Figure **1**.**c**).

DISCUSSION

Estrogen is effective in both the development and growth of bones. Post-osteopenia is observed in experimental rat ovariectomy models [7]. However, there are question marks about the effect of this situation on the dental pulp. Ovariectomized rats have bone loss due to lack of estrogen [8]. Tissue loss is observed in the femoral neck of rats undergoing ovariectomy [9]. In cell culture studies, it was clearly seen that estrogen plays a role in the differentiation and proliferation of osteoblast cells [10, 11]. These results are similar to our study.In our study, we observed that rats that underwent ovariectomy for 28

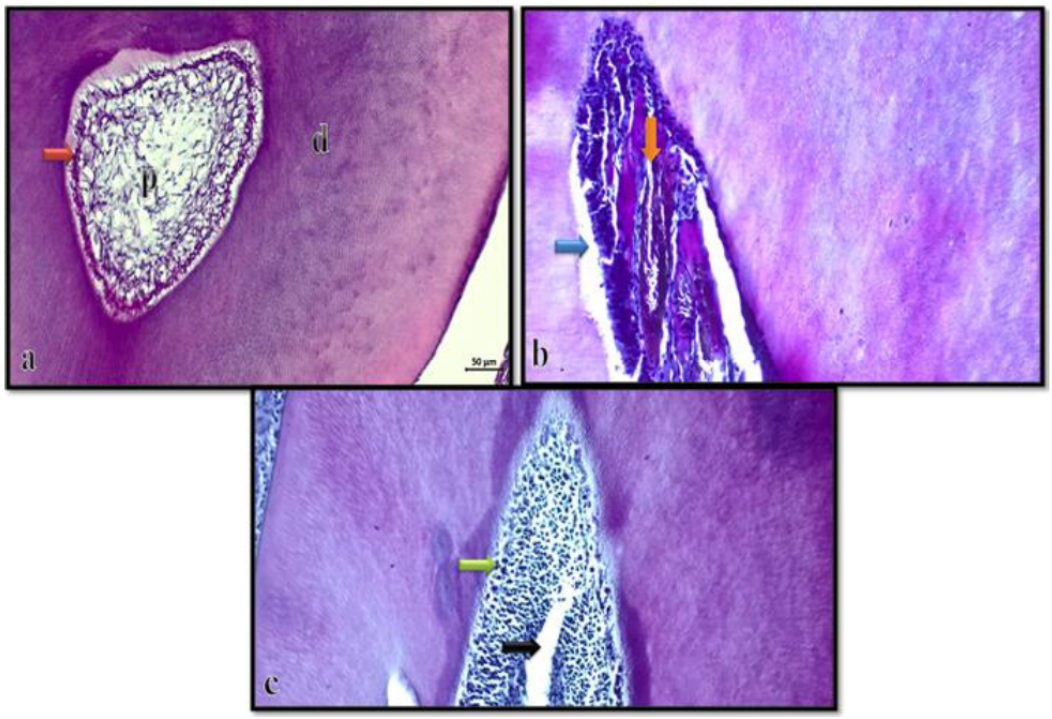

Figure 1: a) Group 1. Pulp(p), odontoblast (red arrow), dentin (d). **b) Group 2.** Odontoblast (blue arrow), pulpa connective tissue (orange arrow). **c) Group 3.** Odontoblast (green arrow), pulpa connective tissue (black arrow) (H&E, 50 µm).

days had more pyknosis of osteoblasts and tissue loss in the pulp than rats who underwent ovariectomy for 14 days. Also osteonectin is a protein secreted by osteoblasts during tooth and bone development [12]. It is known that some changes occur in both bone structure and tooth structure due to postmenopausal hormones in women [13]. These hormonal changes occur not only in the bones but also in the dental pulp. In our study, tissue loss and changes due to hormonal changes in the dental pulp were observed (Figure **1**.**c**). Estrogen causes both tissue and cellular changes on the skeletal system [14]. In our study, degenerations in tissue and cell level were observed on the dental pulp as a result of inhibition of estrogen with the ovariectomy model. In the 28-day follow-up group, more degeneration and pyknosis had occurred in the dental pulp. Xu *et al*. reported that pre-dentin and pulp tissue can provide a supportive mechanical function [15]. In our study, no thinning of the predentin was observed on the 28th day of the ovariectomy group.

We may suggest that with the effect of bilateral ovariectomy, inhibition of estrogen that plays a role in bone and tooth development, pershaps led to the deterioration of osteonectin structure and tissue regeneration. In our study, it was observed that the pulp structure was completely degenerated and the odontoblast cells became pyknotic in rats who underwent ovariectomy for 28 days.

CONCLUSION

We think that osteoblastic activity and vasculogenesis were adversely affected by ovariectomy, causing inflammation in dental pulp.

ACKNOWLEDGEMENT

This study is a part of Elif Pınar Bakır's doctoral thesis and was supported Research Projects (DUBAP) with the by Dicle University Scientific Projects number TIP.21.004

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Received on 31-10-2021 Accepted on 25-11-2021 Published on 09-12-2021

DOI: https://doi.org/10.12974/2311-8695.2021.09.3