

# Biochemical and immunohistochemical investigations on bone formation and remodelling in ovariectomised rats with tamoxifen citrate administration

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**Background:** Osteoporosis results with the imbalance between osteoblastic formation and osteoclastic resorption, resulting in susceptibility to bone fractures. Ovariectomy leads to osteoporosis by triggering alterations in bone formation and structure. Tamoxifen as an anti-oestrogen is used for adjuvant therapy especially in metastatic diseases and known to have a bone mass protective effect after ovariectomy.

**Materials and methods:** An animal model of ovariectomy induced osteoporosis after tamoxifen citrate administration was studied via biochemical and immunohistochemical methods. Female Wistar albino rats ( $n = 45$ ), selected according to their oestrous cycle, were divided into three groups; I — control, II — ovariectomy, III — ovariectomy + tamoxifen. Following ovariectomy, tamoxifen citrate (10 mg/kg) was given intraperitoneally daily for 8 weeks. At the end of the period, animals were sacrificed under anaesthesia, blood samples were taken to measure oestrogen, calcium, and alkaline phosphate. Tibia bone samples were fixed in formalin solution and decalcified with 5% ethylene-diamine tetra acetic acid. After the routine histological follow up, samples were embedded in paraffin and cut with a microtome for semi-thin sections. Primary antibodies osteonectin and osteopontin were applied to sections and examined under light microscope.

**Results:** As a consequence, when oestrogen and calcium data were compared there was a decrease in ovariectomy group with an increase in alkaline phosphatase. In ovariectomy + tamoxifen group, these values were close to the control group. Osteonectin was observed to promote bone formation by influencing collagen fibre formation, extracellular matrix development, osteoblast differentiation and the capacity to affect osteoclast activity.

**Conclusions:** It has been suggested that osteopontin, the cytokine and cell binding protein, stimulates cellular signalling pathways, induces bone remodelling and acts in osteoporosis. (Folia Morphol 2019; 78; 4: 789–797)

**Key words:** ovariectomy, bone remodelling, tamoxifen, osteonectin, osteopontin

## INTRODUCTION

Ovariectomy (OVX) is a procedure to simulate the hormonal status of postmenopausal women. Osteoporosis is a multifaceted skeletal disease characterised by decreased bone mass and impaired structure of the bone tissue, resulting in mechanical strength loss and increased fracture risk [11]. Bone resorption after ovariectomy leads to bone loss, initially exceeding bone formation. Shortly after this, bone remodelling reaches a steady state where absorption and formation are balanced [23]. Depending on the severity of the oestrogen deficiency, post-menopausal women can experience a reduction in osteoblastic activity in the bones and bone matrix, as well as a decrease in calcium and phosphate accumulation in the bones.

Tamoxifen is a well characterised breast cancer drug and prophylactic that is a selective oestrogen receptor modulator (SERM). SERMs are structurally diverse compounds that bind the oestrogen receptor and elicit ligand- and tissue-specific effects. Tamoxifen demonstrates the ability of oestrogen to increase bone mass and reduce the risk of fracture in postmenopausal women. However, it functions as an oestrogen antagonist in reproductive tissues [13, 15]. SERMs may share the stimulatory effect of oestrogen on osteoblast activity and are therefore reported to provide an alternative basis for anabolic treatment in osteoporosis. Experimental studies on rats depicted that tamoxifen may have a bone mass protective effect after ovariectomy [12]. Tamoxifen is a commonly used anti-oestrogen for adjuvant therapy and metastatic disease in postmenopausal women with breast cancer having a high risk of osteoporosis. Tamoxifen has been shown to induce the longitudinal growth rate in connection with an increase in the width of the proliferation zone in the bone epiphyseal plate. However, oestrogen was indicated to suppress the rate of longitudinal growth [30]. Tamoxifen has been shown to inhibit prostaglandin synthesis *in vitro* [41], and it was previously reported that prostaglandin E, a potent bone resorbing agent, increases in bone after immobilisation [42]. Tamoxifen has also been shown to regulate the expression of genes that control polyamine biosynthesis [36], and polyamine inhibitors have been shown to inhibit parathyroid hormone-mediated bone resorption [26]. Induction of transforming growth factor-beta by tamoxifen has also been reported [20]. Transforming growth factor-beta is believed to play an important role in intercellular communication within bone [27].

Osteonectin is a glycoprotein abundantly expressed in bone undergoing active remodelling. Osteonectin is synthesised by cells of the osteoblastic lineage; binds hydroxyapatite, calcium, and type I collagen; and inhibits mineralisation *in vitro* [19]. Osteopontin (OPN) is one of the major non-collagen proteins in extracellular bone matrix; it has a role in osteoclast-mediated bone resorption [21]. OPN is produced by differentiated osteoblasts and osteocytes, and also by osteoclasts. OPN has resorptive activity with the formation, migration and attachment of osteoclasts [33]. During bone remodelling, bone cells secrete OPN physiologically. It was noted that OPN acts as a pro-inflammatory cytokine and plays an important role in regulating the inflammatory process [34].

The aim of this study was to investigate the expressions of osteonectin and osteopontin proteins in tamoxifen citrate administrated in OVX rats.

## MATERIALS AND METHODS

### Animals

Every single surgical methodology and the consequent care and healing of the animals utilised as a part of this investigation was in strict understanding with the National Institutes of Health (NIH Publications No. 85-23) rules for animal care. The Experimental Animal Laboratory Institute of Dicle University supplied 45 healthy adult female Wistar rats (280–310 g), which were selected according to their oestrous cycle. This study was approved by the Ethics Committee for Animal Experimentation of the Faculty of Medicine at Dicle University, Turkey. The rats were housed in separate cages at  $26 \pm 2^\circ\text{C}$  with 40–60% humidity and were exposed to 10–12 h of daylight. They were fed a standard laboratory diet and tap water *ad libitum*. All rats at the end of experiment were healthy and no difference in food/water consumption and body weight gain between experimental and control rats were observed.

### Experimental procedure

Three groups (15 rats per group) were arranged as below:

- **Control group:** animals were fed only standard rat chow and drinking water for 8 weeks;
- **Ovariectomy group:** prior to surgery, the abdominal areas of the rats were cleaned and removed from the ovarian cervical horns by making a 2 cm incision. The bilateral ovaries were removed from

the uterine apex with silk thread. The ovariectomy was performed with two dorsolateral incisions according to the method by Park et al. [29]. The uterus was left in the abdominal cavity, and the abdominal area was closed with a sterile suture;

— **Ovariectomy + tamoxifen group:** Following the bilateral ovariectomy, the animals were given intraperitoneally 10 mg/kg/day tamoxifen citrate for 8 weeks.

All animals were anaesthetised using 50 mg/kg of Ketalar® Vial M (ketamine HCl-Pfizer) and 10 mg/kg Rompun 25 mL 2% w/v solution for intramuscular injection (xylazine hydrochloride; Bayer). Blood samples were taken for oestrogen, calcium, and alkaline phosphatase levels. Then, the tibia bones were dissected bilaterally for the basic histology and immunohistochemistry.

#### Histopathological evaluation

The tibial bone samples were fixed with 10% neutral buffered formalin solution and decalcified with 5% ethylenediaminetetraacetic acid (EDTA). After preservation, the tibial bone samples were directly dehydrated in a graded series of ethanol and embedded in paraffin wax. Next, 4–6 µm sections of the tibia bones were cut with a microtome (Rotatory Microtome, Leica, RM 2265, Germany) and mounted on coated slides. Bone sections were stained with haematoxylin and eosin (H&E) for observation by light microscopy (Nikon Eclipse 80i).

#### Biochemical analysis

Serum calcium, oestrogen and serum alkaline phosphatase (ALP) levels were measured using standard colorimetric methods with commercial kits (Sigma Chemical Co., Saint Louis, MA).

#### Immunohistochemical staining

Sections were brought to distilled water and washed in 3 × 5 min phosphate buffered saline (PBS) (Catalogue number 10010023, Thermo Fischer Scientific Fremont, CA, USA). Antigen retrieval was done in microwave (Bosch®, 700 watt) for 3 min × 90°C. They were subjected to a heating process in a microwave oven at 700 watts in a citrate buffer (pH 6) solution for proteolysis. Sections were washed in 3 × 5 min PBS and incubated with hydrogen peroxide [K-40677109, 64271 Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) Dortmund+Germany, MERCK] (3 mL 30% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) + 27 mL methanol) for 15 min. Ultra V

block (lot: PHL150128, Thermo Fischer, Fremont, CA, USA) was applied to the sections for 10 min prior to addition of the primary antibodies, which were left on overnight (Osteonectin (SPARC), Catalogue #:33-5500, 1:100, Thermo Fischer, Fremont, CA, USA, Osteopontin monoclonal antibody 1:100, MA5-17180, Thermo Fischer, Fremont, CA, USA). Sections were washed in 3 × 5 min PBS and then incubated with Biotinylated Secondary Antibody (lot: PHL150128, Thermo Fischer, Fremont, CA, USA) for 20 min. After washing with PBS, Streptavidin Peroxidase (lot: PHL150128, Thermo Fischer, Fremont, CA, USA) was applied to sections for 20 min. Sections were washed in 3 × 5 min PBS and diaminobenzidine (DAB) (lot: HD36221, Thermo Fischer, Fremont, CA, USA) were applied to sections up to 20 min. DAB was used as a chromogen. Control slides were prepared with the same procedure, without primary antibodies. Counter staining was done with Harris's haematoxylin for 45 s, dehydrated through ascending alcohol and cleared in xylene (Product Number: HHS32 SIGMA, Haematoxylin Solution, Harris Modified, Sigma-Aldrich, 3050 Spruce Street, Saint Louis, MO 63103, USA). Slides were mounted with Entellan® (lot: 107961, Sigma-Aldrich, St. Louis, MO, USA) and examined under light microscope (Nikon Eclipse 80i).

#### Statistical analysis

Statistics and analyses were performed using the SPSS 22.0 for Windows computer package programme. In the analysis of the data, Kruskal-Wallis and Mann-Whitney U non-parametric statistical tests were used in the intergroup comparisons depending on the variables and the results were given as the mean ± standard deviation and mean rank. And, the results were considered statistically significant for  $p = 0$  with Kruskal-Wallis test, and  $p < 0.05$  with Mann-Whitney U test (Table 1).

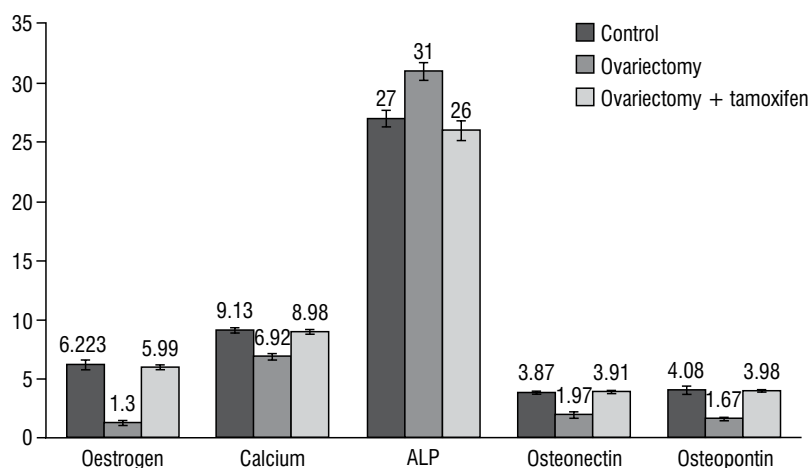
## RESULTS

#### Biochemical findings

It was found that oestrogen level decreased significantly in the ovariectomy group. However, it was determined that oestrogen level was close to the control group with tamoxifen application in ovariectomised rats ( $p = 0$  with Kruskal-Wallis test,  $p < 0.05$  with Mann-Whitney U test). Calcium levels were statistically lower in the ovariectomy group as in oestrogen level. On the other hand, a significant increase in the calcium levels in the ovariectomised group with tamoxifen

**Table 1.** Biochemical and immunohistochemical expression data. Data are expressed as the mean  $\pm$  standard deviation (SD) and mean rank (\* $p = 0$  with Kruskal-Wallis test, \*\* $p < 0.05$  with Mann-Whitney U test, \* and \*\* statistically significant result)

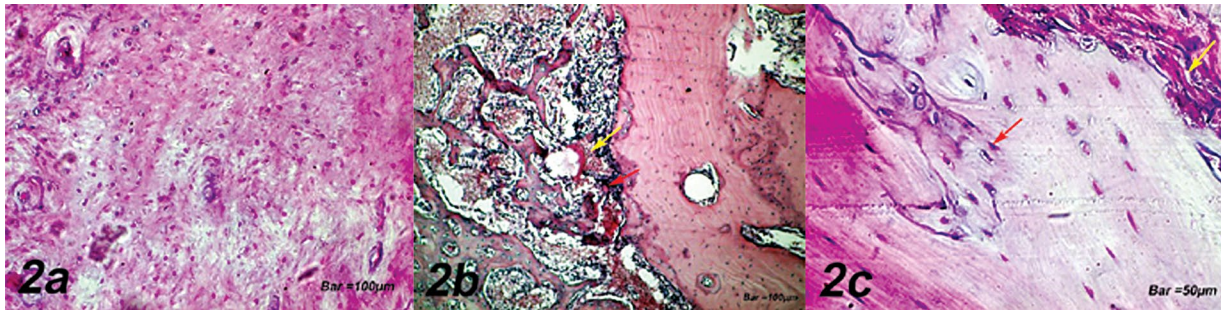
Parameter	Groups	N	Mean $\pm$ SD	Mean rank	Kruskal-Wallis test value	Multiple comparisons for groups ( $p < 0.05$ )
Oestrogen [mg/mL]	(1) Control	15	6.23 $\pm$ 0.38	17.6	15.825 * $p = 0$	(2)**
	(2) Ovariectomy	15	1.30 $\pm$ 0.18	4.50		(1)** (3)**
	(3) Ovariectomy + tamoxifen	15	5.99 $\pm$ 0.19	15.3		(2)**
Calcium [mg/dL]	(1) Control	15	9.13 $\pm$ 0.20	18.1	16.226 * $p = 0$	(2)**
	(2) Ovariectomy	15	6.92 $\pm$ 0.26	4.50		(1)** (3)**
	(3) Ovariectomy + tamoxifen	15	8.98 $\pm$ 0.20	14.8		(2)**
Alkaline phosphatase [mg/dL]	(1) Control	15	27.0 $\pm$ 0.74	11.0	17.383 * $p = 0$	(2)** (3)**
	(2) Ovariectomy	15	31.0 $\pm$ 0.75	20.5		(1)** (3)**
	(3) Ovariectomy + tamoxifen	15	26.0 $\pm$ 0.79	6.0		(1)** (2)**
Osteonectin	(1) Control	15	3.87 $\pm$ 0.12	15.5	15.689 * $p = 0$	(2)**
	(2) Ovariectomy	15	1.97 $\pm$ 0.26	4.50		(1)** (3)**
	(3) Ovariectomy + tamoxifen	15	3.91 $\pm$ 0.10	17.4		(2)**
Osteopontin	(1) Control	15	4.08 $\pm$ 0.31	16.8	15.389 * $p = 0$	(2)**
	(2) Ovariectomy	15	1.67 $\pm$ 0.13	4.50		(1)** (3)**
	(3) Ovariectomy + tamoxifen	15	3.98 $\pm$ 0.12	16.1		(2)**

**Figure 1.** Biochemical and immunohistochemical expression data graphs of all groups. The quantification of osteonectin and osteopontin expressions: 0 — no change, 1 — too weak, 2 — week, 3 — middle, 4–5 — strong (Scoring of osteonectin and osteopontin expressions were determined by examining histological parameters in 15 different regions within the microscope field); ALP — alkaline phosphatase.

application ( $p = 0$  with Kruskal-Wallis test,  $p < 0.05$  with Mann-Whitney U test) were seen. ALP level was statistically higher in the ovariectomy group however, decreased in rats with ovariectomy and tamoxifen treatment ( $p = 0$  with Kruskal-Wallis test,  $p < 0.05$  with Mann-Whitney U test). The above-mentioned findings were shown in detail in Table 1 and Figure 1.

### Histopathological findings

Histological examination of the tibia revealed that the bone lamellae were distributed regularly, and the osteocytes were located radially around the osteon structures in the control group. The Havers and Volkmann channels were located in parallel, the matrix was densely distributed, and the collagen fibres were irregular (Fig. 2a). Tibia bone sections of the ovariec-



**Figure 2.** a. Haematoxylin and eosin (H&E) staining (control group). Tibial bone lamellas are regular, distribution of osteocytes around osteon structures, the matrix dense and irregular distribution of collagen fibres can be seen; b. H&E staining (ovariectomy group). An increase in inflammatory cell infiltration, congestion in blood vessels (red arrow) and osteoclastic cells with degenerative changes in the periphery of compact bone, osteon structures loss and loss of lamellar bone structure and degeneration (yellow arrow) can be seen; c. H&E staining (ovariectomy + tamoxifen group). An increase in collagen fibres in the periphery of the compact bone (yellow arrow), osteoblastic activity, progression of matrix release and decrease in osteoclast cells can be seen. A significant maturation of osteocytes in the compact bone and the osteon lines (red arrow) are visible.

tomised group showed an increase in inflammatory cell infiltration, congestion in blood vessels and increased osteoclastic cells with degenerative changes in the periphery of compact bone. In bone trabeculae, loss of osteon structures and lamellar bone structure and degeneration were observed (Fig. 2b). Tamoxifen citrate administration after ovariectomy group revealed, increased collagen fibres in the periphery of the compact bone, increased osteoblastic activity, progression of matrix release and osteocyte differentiation decreased osteoclast cells. A significant maturation of osteocytes in the compact bone and the osteon lines in which the Havers channels were concentrated around the lamellar bone (Fig. 2c).

#### Immunohistochemical findings

In the control group sections, osteonectin expression was positive in osteoblast cells in the endosteum region near the bone marrow, in osteocyte cells resident of lacuna, and in Havers and Wolkmann channels (Fig. 3a). In the ovariectomised group, osteonectin expression in the collagen fibres, osteoclastic cells and hyperplastic osteoblast cells around the blood vessels located within the degenerative area was reduced in a positive reaction (Fig. 3b). In the tamoxifen administered group after ovariectomy, osteonectin expression was positively observed in collagen fibres, increased osteoblast cells and osteocyte cells embedded in lacuna in the periphery of wide bone trabeculae (Fig. 3c).

In the control group sections, osteopontin expression was observed around the osteoblast cells in the periphery of the bones, the osteocytes in the lacuna and some of the matrix areas around the osteon

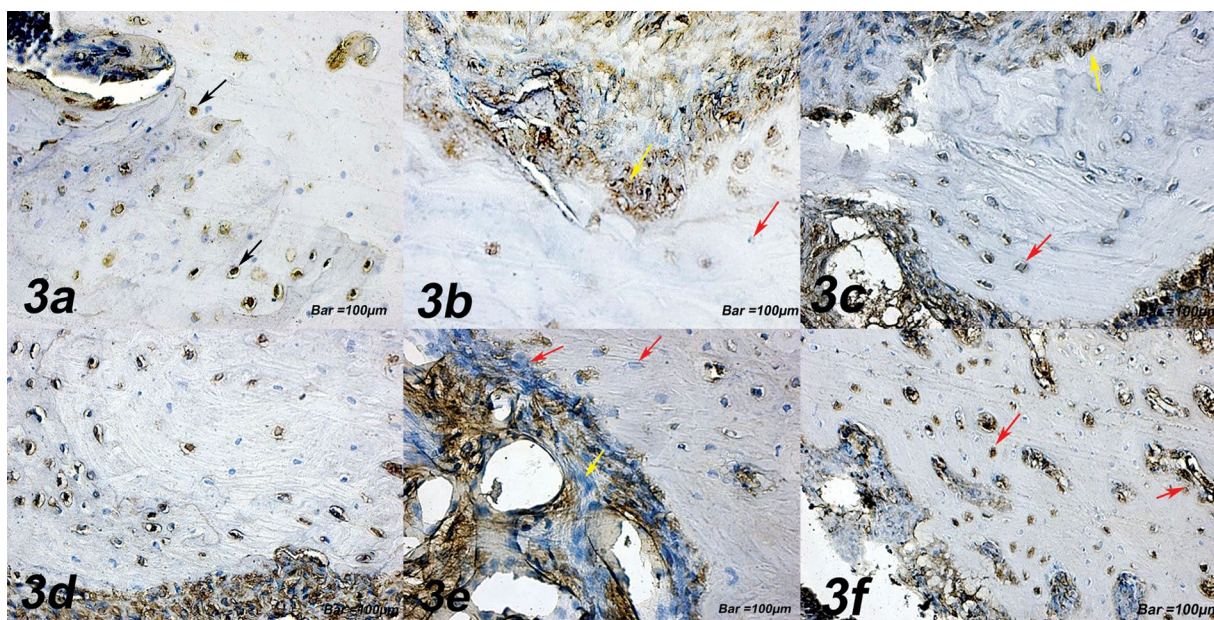
lines (Fig. 3d). In the ovariectomised group, weak osteopontin expression was observed in osteoclast cells within degenerative area around thinned bone trabeculae. Weak osteopontin expression was also observed in osteoblast cells and osteocyte cells of bone trabeculae (Fig. 3e). In the group of tamoxifen citrate after ovariectomy, positive osteopontin expression in osteoblast cells was observed in the osteocyte cells around the Havers and Volkmann channels (Fig. 3f).

Statistically, we found that osteopontin and osteonectin protein expression was decreased in the ovariectomised group ( $p = 0$  with Kruskal-Wallis test,  $p < 0.05$  with Mann-Whitney U test). However, osteonectin and osteopontin expression levels in the ovariectomised group and ovariectomy + tamoxifen group were found to be statistically significant ( $p < 0.05$  with Mann-Whitney U test). The values of the control group supported this analysis.

## DISCUSSION

Osteoporosis is defined as low bone mass and bone structure with high fracture risk [45]. Bone fragility occurs due to excessive resorption and decreased bone formation [8]. It is reported that osteoporosis affects about 25 million individuals in the United States alone, and it is estimated that women > 50 years old possess an 11–18% risk of suffering a hip fracture [6]. The most common form of osteoporosis is postmenopausal bone loss associated with ovarian hormone deficiency [18]. Previous studies have suggested that OVX reduces bone mass in rat models and modified micro-architecture in different skeletal sites containing trabecular bone [24], and





**Figure 3.** a. Osteonectin immunostaining (control group). Positive osteonectin expression in osteoblast cells in the endosteum region near the bone marrow and osteocyte cells within osteon area (black arrow) can be seen; b. Osteonectin immunostaining (ovariectomy group). Osteonectin expression in the collagen fibres (yellow arrow), osteoclastic cells and hyperplastic osteoblast cells around the blood vessels, decrease in osteonectin expression within trabecular bone (red arrow) can be seen; c. Osteonectin immunostaining (ovariectomy + tamoxifen group). Positive osteonectin expression in osteoblast cells (yellow arrow), and osteocyte cells in the periphery of wide bone trabecular (red arrow) are visible; d. Osteopontin immunostaining (control group). Positive osteopontin expression around the osteoblast cells in the periphery of the bones, the osteocytes in the lacuna and some of the matrix areas around the osteon lines can be seen; e. Osteopontin immunostaining (ovariectomy group). Weak osteopontin expression in osteoclast cells around thin bone trabeculae (yellow arrow), osteoblast cells and osteocyte cells of bone trabeculae (red arrow) are visible; f. Osteopontin immunostaining (ovariectomy + tamoxifen group). Positive osteopontin expression in osteoblast cells, osteocyte cells around the Havers channels and Volkmann channels (red arrow) can be seen.

postmenopausal osteoporosis is commonly used animal models [38]. Rats are commonly used in OVX model, as because subjects like primates that are most similar to human, there are limits such as high cost of maintenance, reduced availability in experimental labs, ethical permissions etc. But there is a contrary on the issue that use of rat models for osteoporosis is the lack of Haversian remodelling in the rat skeleton. According to Lelovas et al. (2008) [23]; in humans, increased Haversian remodelling is the main cause of cortical porosity, but rats do not have a well-developed Haversian remodelling system. On the other hand, ovariectomy of skeletally mature rats leads to a condition similar to menopause, in that the surgery leads to cancellous and endocortical bone loss by increasing the overall rate of bone remodelling and by altering the balance between bone formation and bone resorption, such that resorption predominates at selected skeletal sites [14].

Takayama et al. [35] also carried out that ovariectomised rats exhibit the same hormonal changes as observed in humans with osteoporosis. It was report-

ed that the bone loss caused by ovariectomy depends on the degree of skeletal area and the time since ovariectomy. Cao et al. [4] examined the effect of icariin on fracture healing in an ovariectomised patient and reported that the use of icariin may help to improve fracture healing in postmenopausal osteoporosis and can be improved as an alternative therapy. The effect of oestrogen deficiency was reduced bone mass due to increased bone resorption and increased osteoclastic activity and also decreased new bone formation due to decreased osteoblastic life. Ovariectomy produced significant increases in medullary area, periosteal bone formation rate, and periosteal bone apposition rate compared to values in sham operated animals but, did not change endosteal bone formation rate. The increase in medullary area resulted from an increase in osteoclast number and resorbing surface length. Although endosteal forming surface length decreased, this was compensated by an increase in the apposition rate. 17- $\beta$ -oestradiol and tamoxifen each prevented the increase in bone formation rate and medullary area in ovariectomised rats [17].

Tamoxifen reduced the length of the resorbing surface and osteoclast number to values observed in control operated animals. The findings demonstrate that in the rat, tamoxifen acts as an oestrogen agonist by preventing the skeletal alterations that result from ovarian hormone deficiency [40]. In our study, osteonectin expression in collagen fibres and osteoclastic cells scattered among thinner bone trabeculae and osteonectin expression in osteoblast cells with hyperplasia was found to be decreased in the ovariectomised group.

In contrast to the oestrogen-antagonistic effect in human breast cancer, studies in rat [16, 39], confirmed in human [25, 37], demonstrated that tamoxifen is an oestrogen agonist in bone, Baloglu and Deveci [1] reported that clomiphene citrate administration after ovariectomy, it was observed that an increase in the number of osteoprogenitor cells and osteoblasts, and a decrease in the number of osteoclast cells in the region of the bone trabeculae. They stated that osteoblasts support the synthesis of bone matrix and the construction of bone trabeculae and bone repair. Bone remodelling is a constant and dynamic process in which osteoclasts resorb old bone and osteoblasts form new bone [5, 28]. Remodelling of the bone is a complex condition involving the proliferation of osteoblast cells, the formation of matrices and the emergence of mature bone cells and the emergence of bone trabeculae. Celik et al. [7] reported that oestrogen deficiency occurring 30 days after bilateral ovariectomy in adult female rats causes changes in bone metabolism, which is associated with a loss of bone mass. Wronski et al. [43] investigated the long-term effects of ovariectomy on proximal tibial metaphyseal trabecular bone histomorphometry in ovariectomised rats. Turner et al. [39] suggested that tamoxifen may have an effect on the bone microscopic structure by decreases in both the number and activity of osteoclasts, and net loss of trabecular bone. Tamoxifen and oestrogen have similar actions in the diaphysis and metaphysis of the tibia, suggesting an agonist or partial agonist activity for tamoxifen on bone [2]. Our study showed an increase in osteoporosis activity after ovariectomy. Trabecular bone thinning and degenerative changes were observed and osteoclastic activity was decreased. As a result of tamoxifen citrate application, increased osteoblastic activity was found to be replaced with the degenerative changes induced by new bone formation and accelerating osteocyte development.

The osteonectin protein was localised in mineralised bone trabeculae and was found to be at a higher level in the matrix than in the bone cells. Osteonectin helps to connect bone mineral and collagen fibrils to each other. Meanwhile, oestrogens increase the differentiation of osteoblast cells and stimulate bone matrix mineralisation, arrange the expression of non-collagenous proteins such as type I collagen and osteopontin, osteocalcin, osteonectin and the like [3].

Osteonectin is a protein known to be involved in cell-matrix interactions and angiogenesis. Osteonectin inhibits cell adhesion, cell cycle, and response to specific growth factors. It regulates the production of extra cellular matrix and matrix metalloproteinases [44].

The new vessel formation by affecting angiogenesis is important for normal ossification [32]. Lacin et al. [22] examined the effect of formaldehyde application on bone and observed a decrease in osteonectin expression after injury. Considering the association of osteonectin, a calcium-binding protein, involved in cell adhesion, it is suggested that osteonectin expression was increased in osteoblast and osteocyte cells due to tamoxifen effect which may stimulate the induction of bone remodelling. In our study, osteonectin expression was observed in osteoclast cells in collagen fibres around thinned bone trabeculae due to the effect of osteoporosis in ovariectomy group, whereas osteoinductive effect was decreased in osteoblast and osteocyte cells with weak osteonectin protein. In the tamoxifen group, the development of new bone formation was accelerated by osteoinductive effect due to increased osteoblastic activity. Osteonectin expression in osteoblast and osteocyte cells showed a positive reaction. Osteopontin has been shown to be expressed in osteoclast-mediated bone resorption sites, in synovial tissues of patients with rheumatoid arthritis, predominantly by fibroblastic cells and in pannus spreading regions into cartilage [31]. One study revealed that osteopontin expression was clearly observed in the periodontal ligament and alveolar bone in ovariectomised rats. The authors hypothesised that oestrogen deficiency, due to ovariectomy, may cause the destruction of periodontal ligaments [9]. In our study, osteopontin expression was observed in inflammatory cells in osteoclast cells as a result of osteoporosis change in bone trabecula after ovariectomy. In the tamoxifen group, initiation of osteocyte cells by stimulating osteoblast cells and increased osteopontin expression in these cells induced new bone formation.

Despite the results, there are a few limitations. It is a notable point whether the prevailing activity in the rat skeleton is modelling or remodelling. In the adult human skeleton, bone formation has following the sequence of activation–resorption–formation called remodelling. But, modelling is the formation and resorption of bone in a specific site and the process is activation–formation and activation–resorption. Proximal tibial metaphysis is seen in the rat skeleton, after the age of 12 months [10, 14] is remodelling but we used almost younger rats. However, many therapeutic advances in the management of osteoporosis were previously studied first in animal models and then passed to clinical practice for example; oestrogen administration prevented osteopenia and decreased bone turnover in the ovariectomised rat model, a very convenient example with the skeletal effects of oestrogen therapy in postmenopausal women studied by Lelovas et al. [23]. All combined, authors gave the decision of OVX model in rats is an appropriate model for the research of osteoporosis to provide information for preventive or therapeutic strategies to treat and manage osteoporosis.

## CONCLUSIONS

Depending on the effect of tamoxifen, osteonectin was thought to promote bone formation by influencing collagen fibres formation, extracellular matrix development, osteoblast differentiation and the capacity to affect osteoclast activity. It has been suggested that osteopontin, the cytokine and cell binding protein, stimulates cellular signalling pathways, induces bone remodelling and acts in osteoporosis.

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