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The Relationship of Mandibular Bone Density Level to Femur Bone Density Level in Ovariectomized Female Rats given Isoflavones and 17β-estradiol

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***Abstract*–Osteoporosis is a multifactorial disease, and the most common type of osteoporosis is involutional osteoporosis associated with aging and menopause. Estrogen deficiency in menopause and post-menopause is a major pathogenic factor in bone loss. Osteoporosis in the skeletal bone of the body also occurs in the mandible bone. The purpose of this study was to investigate the correlation between mandibular bone density level and femoral bone density level in ovariectomized female rats either given estrogen or phytoestrogen therapy or not given any replacement therapy. The subject of this study was a 12-month-old Sprague Dawley female rat which was ovariectomized with 50-days study duration. Examination of bone density was based on the grayscale values of digital radiographs in pixels. Pearson correlation test showed that there was a strong correlation between mandibular and femoral neck (r = 0.593); mandibular with major trochanter (r = 0.576) and mandibular with Ward's triangle (r = 0.655). There is a strong correlation between mandibular bone density levels and femoral bone density levels in the femoral neck area, major trochanter and Ward's triangle in ovariectomized rats.**

***Keywords*–bone density level, mandibular, femur**

I. INTRODUCTION

Osteoporosis is defined as a disease characterized by low bone mass and microarchitectural decline of bone tissue leading to increased bone fragility with the consequent increasing the risk of fracture [1]. After the growth of the body stopped, maintenance of normal bone mass in humans is based on balancing the bone formation by osteoblasts and bone resorption by osteoclasts. In the third decade of life, after the peak bone mass is achieved, there will be a slow and continuous bone loss. Bone loss also occurs in all mammalian species [2]. Although this disease has many causes, the most common type of osteoporosis is involutional osteoporosis that primarily associated with aging and menopause. Women experience more osteoporosis than men. Estrogen deficiency in menopause and post-menopause is a major pathogenesis factor in bone loss [3,4].

Decreased estrogen activity initiates a significant increased in plasma calcium, leading to hypercalciuria and negative calcium balance. Several studies found an increase in PTH along with age. Long-term estrogen therapy lowers the rate of bone resorption becomes very low. This indicated that after menopause, interference in the PTH regulation mechanism in bone replacement, the absence of gonadal steroids and abnormalities in the intrinsic function of bone cells had an effect in the pathogenesis of osteoporosis [5,6].

HRT (Hormone Replacement Therapy) in postmenopausal women had an anti-osteoporosis effect, because of estrogen's ability to interact with bone cells and regulates the circulation of cytokines in controlling bone remodeling [7]. The main issue in HRT is the risk of endometrial and breast cancer [8,9,10] so that lots of research were done on phytoestrogens. Phytoestrogens had estrogenic and anti-estrogenic action; in the reproductive tissues (mammary glands, ovaries, endometrium, and prostate), phytoestrogens work as anti-estrogens, but in bone, they had estrogenic activity [11]. The largest concentration of phytoestrogens in human's diet is isoflavones, which contained in the soybean meal [12,13].

The association of osteoporosis in the skeletal bone of the body with bone loss in the oral cavity has been widely investigated. The loss of bone mass in the trabecular bone is higher than in compact bone. In osteopenia condition in maxilla and mandibular, it showed a very fine trabecular pattern, called ground glass. Lamina dura is lost by thinning or loss of cortical bone thickness at the mandibular lower edge [14,15]. The purpose of this study was to investigate the correlation of mandibular bone density level with femoral bone density level in ovariectomized female rats either given estrogen or phytoestrogen therapy or not given any replacement therapy.

II. MATERIALS AND METHODS

This study was an experimental design with pre-post treatment and control group. Subjects were a 12-month-old Sprague Dawley female rats divided into four treatment groups: no ovx groups as a standard reference for normal bone density groups; ovx groups as negative control groups; ovx groups given 17β-estradiol; and ovx groups given isoflavones added to the soy flour. The duration of this study was 50 days.

During the study, rats were given calcium supplements and basal feeds in accordance with AOAC (1990) [16]. The doses of calcium, isoflavones, and estradiol were administered according to the interspecies conversion table [17]. The ovariectomy technique used in this research was modified from Waynforth [18]. Samples of rat bone were dried at 450^{o}C for 24 hours.

Periapical X-ray photograph was used to determine the rate of bone density. All bones were photographed in the anterior-posterior direction, with 7.6 cm conical distance and radiation length 0.14 seconds. As the whitest standard histogram, a non-translucent X-ray metal steel is placed on top of the film. Examination of bone density was based on the grayscale values of digital radiographs in pixels. In mandibular bone, measurements were made on mandibular alveolar bone between the root of the first and second molars. Femur bone density measurements were performed on 3 ROIs (Rest of Interests) including femur neck, major trochanters, and Ward's triangle.

III. RESULTS

Research on the femur bone was done on the femoral neck, major trochanter, and Ward's triangle. In Table I, we can see the trabeculation value of the alveolar bone of the mandible, femoral neck, major trochanter and Ward's triangle.

TABLE I. THE VALUE OF TRABECULATION OF MANDIBULAR BONE AND FEMORAL BONE

Treatment Group	N	Mean of Mandibula	Mean of femur bone		
			Femur Neck	*Major Trochanter*	*Ward's Triangle*
No ovx	8	79.6369 ± 3.5783	83.1213 ± 3.8229	80.0200 ± 3.3219	78.1056 ± 2.9362
Ovx	8	71.8979 ± 5.1223	74.6129 ± 4.4015	74.0979 ± 4.3451	70.0743 ± 5.8534
Ovx + Isoflavone	8	75.3019 ± 5.5106	75.4525 ± 4.2871	76.3919 ± 3.9177	71.9563 ± 3.7227
Ovx + estradiole	8	75.5850 ± 2.6321	77.4081 ± 2.8444	78.5119 ± 3.7694	74.7187 ± 3.6911
Total	32	75.7250 ± 4.9486	77.7466 ± 4.9889	77.3573 ± 4.2686	73.8311 ± 4.9588

The Pearson correlation test results showed a strong correlation between mandibular and femur neck (r = 0.593); mandibular with major trochanter (r = 0.576); and mandibular with Ward's triangle (r = 0.655).

IV. DISCUSSION

The high proportion of low bone density in the ovariectomized group of rats may be due to accelerated bone remodeling after cessation of ovarian function [19]. Cecchini et al stated that large bone resorption in skeleton reached 40% within the first week after ovariectomy was monitored based on pre-label tetracycline [20]. Associated with the manifestation of estrogen depletion in experimental animal bones, found an increased in IL-1, IL-6, and TNF in osteoblasts stimulate osteoclast synthesis with accelerated bone resorption [21]. Woo et al stated that induction of ovariectomy caused a dramatic bone loss in both epiphysis and metaphysis of trabecular bone of rats. The decreased in bone density in the 4th lumbar continue until the 56th week after ovariectomy [22].

Several studies have shown a correlation between changes in bone mass of the vertebrae and femoral neck with bone changes in the mandible [23]. Rigsby et al stated that the mandibular trabecular bone strength is lower than trabecular bone in the proximal femur. In long bones, as in the femur and tibia, the load direction was on the primary axis. The muscle load in mandibular may be larger and run in the dorsoventral direction located around the mandibular and transverse long axis. The magnitude of the load increases from the posterior to the anterior of mandibular [24].

The rat femur is a long bone, the head is round and attached to the medial side of the proximal end of the bone shaft by the femur neck. The head lies fitting inside the acetabulum to form the hip joint. Near the head found three trochanters for the attachment of pelvic muscles [25].

In this study, the value of bone trabeculation in femoral neck in the non-ovariectomized group was higher than in other groups. The lowest value of bone trabeculation was in the ovx group. Estrogen and androgens play an important role in skeletal maturation in growing individuals and preventing loss of age. In vivo, estrogen decreased bone resorption and acted indirectly to inhibit osteoclast function [26]. Estrogen showed to decrease cytokine synthesis such as IL-6, an estrogen action mechanism to decrease bone resorption [26,27]. The results of this study indicated that the administration of 17β-estradiol is more effective to maintain bone density compared with isoflavone administration.

Ovariectomy in rats significantly induced a decrease in bone mechanical strength, which was higher in femoral neck than other long bones such as humerus. It can be explained, however, that the mechanical strength of the femoral neck is largely determined by its trabecular bone which is more susceptible to estrogen deficiency compared with cortical bone [28]. Peng et al used old rats and found a decreased in the maximum loading of bending forces on tibia shaft (8.7%) and femoral neck (15.8%) [29]. Bagi et al found a decreased in stiffness and strength of femoral neck in ovariectomized rats compared with control rats [30,31].

The trabecular strength of the femoral neck was to give body support in rats, but not as important as the tremendous bone strength of the femoral neck in humans. The femoral neck of rats contains more cortical bone than the human femoral neck [32]. The metabolic activity of cortical bone was lower than trabecular bone [33]. Associated with the results of this study, it was understandable why the femoral neck response to 17β-estradiol ($t = 2.955$) is not as well as major trochanter ($t = 0.786$) and Ward's triangle's response ($t = 1.644$).

Immediately after ovariectomy in rats, there was a sharp decrease in estrogen that leads to decrease in bone density. There was a strong relationship between mandibular bone density levels and the density level of the femoral bone. Decreased levels of bone density also affect bone biomechanical strength and that can significantly be suppressed by estrogen therapy or phytoestrogen therapy.

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