The Effect of Anti-Oxidant and Iron Chelator on Metabolic E Bone Turnover in Ovariectomized Rats

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Abstract: Background: Osteoporosis affects women a lot especially older age after menopause. It may lead to bad back draws that may prone to considerable pathological conditions e.g. fracture and sever pain. Objective: This study aims to identify the effect of anti-oxidant and iron chelator on metabolic bone turnover in ovariectomized rats. Materials and Methods: The rats were divided into five groups, negative control, positive control, desferal treated, vitamin E treated and desferal + vitamin E treated groups. determination of serum estrogen, ferritin, ALP, TNF α and osteocalcin at the end of experiment. Also, morphology of bone in female rats were evaluated at the end of experiment. Results: The ovariectomy lead to decrease in serum estrogen level and increase serum level of ferritin, ALP, TNF α and osteocalcin. On the other hand usage of desferal and vitamin E lead to decrease serum level of ferritin, ALP, TNF α and osteocalcin. Histological picture of bone, our results showed that abnormal structure of bone of untreated group versus to improvement in histological picture of treated groups. Conclusion: The female rats alleged to ovariectomy and suffered from osteoporosis will be showed improved in her bone by using desferal and vitamin E alone or in combination. Keywords: Ovariectomy; Vitamin E; Desferal; Bone remodeling.

Introduction
Bone is a metabolically active tissue composed of several types of cells. These cells include osteoblasts, which are involved in the creation and mineralization of bone tissue, osteocytes, and osteoclasts, which are involved in the reabsorption of bone tissue[1].

The bone remodeling cycle involves a series of highly regulated steps that depend on the interactions of two cell lineages, the mesenchymal osteoblastic lineage and the hematopoietic osteoclastic lineage. The balance between bone resorption and bone deposition is determined by the activities of these two principle cell types, namely, osteoclasts and osteoblasts[2].

Osteoporosis was defined as a progressive systemic skeletal disease characterized by low bone mass and micro architectural disturbance in bone tissue, with a consequent increase in bone fragility and susceptibility to fracture[3]. Postmenopausal osteoporosis, the most frequent form of osteoporosis, is caused by a reduction of estrogen secretion accompanied by a decrease in ovarian function. As estrogen maintains bone density by suppressing bone resorption[4].

Iron overload is a risk factor for osteoporosis. In women, the levels of iron in the form of ferritin (an iron storage protein) have been observed to increase markedly following menopause[5].

A role of oxidative stress in estrogen-deficiency-induced bone loss has also been demonstrated as ovariectomy significantly reduces two of the antioxidants, glutathione
and thioredoxin, in osteoclasts [6].

The present work was undertaken to evaluate the possible effects of anti-oxidant and iron chelator on metabolic bone turnover in ovariectomized rats.

Materials and Methods

Fifty adult female albino rats (weighting 100-125 gm) of local strain were chosen as an animal model for this study. The animals were randomly divided into five groups. Each group comprised ten rats:

**Group 1**: (Negative Control Group): non-ovariectomized rat that were received a single daily intraperitoneal injection of sterile physiological saline solution daily for four weeks.

**Group 2**: (Positive Control Group): Untreated ovariectomized rats that were received a single daily intraperitoneal injection of sterile physiological saline solution for four weeks.

**Group 3**: (Desferal Treated Group): Ovariectomized rats that were received exogenous desferal (50 mg/kg/day) dissolved in physiological saline 0.9% for four weeks.

**Group 4**: (Vit E Treated Group): Ovariectomized rats that were received vitamin E at a dose of 100 mg /kg/ day. Vitamin E dissolved in olive oil and was given daily orally for four weeks.

**Group 5**: (Desferal + Vit E Treated Group): Ovariectomized rats that were received both desferal (50 mg/kg/day) dissolved in physiological saline 0.9% and vitamin E at a dose of 100 mg /kg/ day for four weeks.

**The selected rats were subjected to:**

I–** Blood sampling**: At end of the experiment, blood was withdrawn by retro-orbital puncture from each rat. Determination of estrogen, osteocalcin, ferritin, TNF α and alkaline phosphatase enzyme in serum.

II–** Histopathological study**: At the end of experiment morphology of bone in female rats were evaluated at the end of experiment.

III–** Statistical analysis**: The quantitative data were presented in the form of mean ± standard error (S.E). One way analysis of variance (ANOVA) followed by Tukey-Kramer as a post-hoc test was done to compare between the studied groups. All statistical analysis were performed using analysis of variance technique by means of computer software package.

**Results**

Rats of positive control group showed significant decreased in serum level of estrogen but showed significant increased in serum level of ferritin, ALP, TNF α and osteocalcin compared to negative control group. Inversely to desferal and desferal + vitamin E treated groups which showed significant decreased in serum level of ferritin, ALP, TNF α and osteocalcin compared to positive control group. Also, vitamin E treated group which showed significant decreased in serum level of ALP, TNF α and osteocalcin compared to positive control group. But failed to produce any significant alteration in serum ferritin compared to positive control group (Table 1 and 2).

**Table (1):** Serum level of estrogen, osteocalcin and ferritin in the different studied groups (Mean ± standard error).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estrogen (pg/ml)</th>
<th>Osteocalcin (ng/ml)</th>
<th>Ferritin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Groups</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative Control</td>
<td>29.14 ± 0.911</td>
<td>20.07 ± 2.15</td>
<td>116.70 ± 1.01</td>
</tr>
<tr>
<td>Positive Control</td>
<td>17.57 ± 1.525 a</td>
<td>44.14 ± 1.81 a</td>
<td>134.90 ± 1.72 a</td>
</tr>
<tr>
<td>Desferal treated</td>
<td>16.43 ± 0.84 a</td>
<td>33.01 ± 0.64 ab</td>
<td>119.6±1.44 b</td>
</tr>
<tr>
<td>Vit. E treated</td>
<td>15.71 ± 1.47 a</td>
<td>33.00 ± 0.62 ab</td>
<td>132.00 ± 1.32</td>
</tr>
<tr>
<td>Desferal + Vit. E treated</td>
<td>17.00 ± 0.92 a</td>
<td>25.09 ± 0.58bc</td>
<td>119.40 ±1.320 b</td>
</tr>
</tbody>
</table>
Table (2): Serum level of Alkaline phosphates and TNF α in the different studied groups (Mean ± standard error).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TNF α (pg/ml)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>23.07 ± 0.71</td>
<td>98.57 ± 0.84</td>
</tr>
<tr>
<td>Positive Control</td>
<td>34.87 ± 1.07</td>
<td>158.00 ± 2.18</td>
</tr>
<tr>
<td>Desferal treated</td>
<td>28.96 ± 1.10</td>
<td>135.00 ± 2.77</td>
</tr>
<tr>
<td>Vit. E treated group</td>
<td>31.31 ± 0.65</td>
<td>130.70 ± 1.98</td>
</tr>
<tr>
<td>Desferal + Vit. E treated</td>
<td>23.29 ± 0.28</td>
<td>116.00 ± 2.18</td>
</tr>
</tbody>
</table>

- Data are expressed as means ± SEM of ten rats per groups.
- Multiple comparisons were accomplished using one way ANOVA followed by Tukey-Kramer as a post-hoc test.
  - a: Significantly different from negative control group at P ≤0.05.
  - b: Significantly different from positive group at P ≤0.05.
  - c: Significantly different from Desferal and Vitamine E treated group at P ≤0.05.

Histopathological examination:
Bone section of negative control group showed normal, structure of bone, i.e. intact cortex healthy epiphyseal plate (cartilage) (fig. 1).

Inversely to Positive control group showed abnormal structure of bone that consisted of discrete cortex and epiphyseal plate (cartilage). The trabecular plates are thin, irregular and widely spaced (multiple pores) (fig. 2).

Also, Desferal treated group showed increased trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (arrow)(fig. 3).

Furthermore, Vitamin E treated group showed increased trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (arrow) (fig. 4).

Finally, Desferal + Vitamin E treated group showed nearly normal trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (arrow) (fig. 5).
Fig. 1: Histologic section of the femur of negative control group showing periosteum (arrow), endosteum and bone matrix containing osteocytes (arrow) (H&E X100).

Fig. 2: Histologic section of the femur of positive control group showing non homogenous matrix with multiple osteoporotic cavities (arrow) filled with a granulation tissue (H&E X100).
Fig. 3: Histologic section of the femur of desferal treated group showing increased trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (arrow) (H&E X100).

Fig. 4: Histologic section of the femur of vitamin E treated group showing increased trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (arrow) (H&E X100).
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Discussion

Estrogen deficiency is regarded as the main causative factor in PMO. Withdrawal of estrogen or estrogen deficiency stimulates bone resorption by 90%, while increasing bone formation by 45%, as measured using biochemical markers [7].

Iron overload has recently been linked to abnormalities in bone metabolism, including osteopenia, osteoporosis, and osteomalacia. Most importantly, iron accumulation, a recently observed clinical phenomenon in postmenopausal women, might be involved in the pathogenesis of PMO [8].

In the present work, results revealed that, there was significant reduction in level of serum estrogen in all groups compared to the negative control group. This results may be explained by that, decrease in estrogen serum level in all groups compared to the negative control group due to process of ovariectomy. This result is compatible with [9] who observed that ovariectomy, irrespective of the time of hormonal estimation caused significant increase in the levels of testosterone and decrease in estradiol levels.

In the present work, results revealed that, there was significant increase in serum osteocalcin level in positive control group compared to negative control group and significant reduction in value of serum osteocalcin in desferal and vit E treated groups compared to positive control group. Also there was significant reduction in serum osteocalcin level in desferal + vit E treated group compared to positive control group and both desferal and vit E treated group.

This results may be explained by that, increase in osteocalcin serum level in positive control group was due to ovariectomy leaded to decrease in estrogen level which in turn defects in bone remodeling. It's appear through imbalance between osteoclast and osteoblast cell in the bone.

Osteoclast number was increased in expanse of osteoblast, which was secreted osteocalcin in serum, on the other hand decreased in serum osteocalcin in all treated groups due to efficacy of vitamin E and desferal. This result is compatible with [10] who stated that, the osteocalcin level in

Fig.5: Histologic section of the femur of desferal + vitamin E treated group showing nearly normal trabecular thickness (arrows) and narrowing of the widened spaces with newly formed osteoid tissue (arrow) (H&E X400).
vitamen E treated rats was significantly reduced compared with the ovariectomy control group. The osteocalcin level, which is a marker of bone formation, was also increased. This is consistent with previous findings that bone loss in ovariectomy is due to a high bone turnover rate with resorption exceeding formation\cite{11}.

In the present work, results revealed that, there was significant increase in serum ferritin level in positive control group compared to negative control group and significant reduction in value of serum ferritin in desferal and desferal + vit E treated treated groups compared to positive control group, inversely to vit E treated treated group which failed to produce any significance with positive control group. This results may be explained by that, increase in ferritin serum level in positive control group was due to ovariectomy leaded to menopause. Following menopause, iron is no longer lost through menstruation, and the metal iron increased and accumulated in the body, on the other side decreased in ferritin serum level in desferal and desferal + vit E treated treated, which happened by the effects of desferal. This result is compatible with\cite{12}

who demonstrated a negative correlation between ferritin and estrogen levels during the menopausal transition period. With regard to the changes in ferritin and testosterone levels in men, asynchronized pattern was observed as the men age, in which ferritin levels decreased gradually following ‘andropause’. Also menstruation is a key process in women of a reproductive age, which is characterized by periodic fluctuations in estrogen and the discharge of blood. For menstruating women, the excretion of endogenous iron occurs through blood loss, resulting in reduced levels of ferritin and increased prevalence of iron deficiency\cite{13}.

In the present work, results revealed that, there was significant increase in serum TNF α level in positive control group compared to negative control group and significant reduction in value of serum TNF α in desferal and vit E treated groups compared to positive control group. Also there was significant reduction in serum TNF α level in desferal + vit E treated group compared to positive control group and both desferal and vit E treated group.

This results may be explained by that, increase in TNF α serum level in positive control group was due to ovariectomy leaded to decrease in estrogen level and increase in ferritin level which in turn defects in bone mineralization. This imbalance lead to increase Oxygen-derived free radicals such as and TNF α. on the other hand decreased in serum TNF α in all treated groups due to efficacy of vitamin E and desferal. This explanation is compatible with\cite{14}
suggested that the level of blood cytokines increased and parallel with the condition of elevated bone resorption in ovariectomized women. Additionally, another study demonstrated that serum cytokines caused a differentiation of osteoclast and cytokines secreted free radicals and inhibited the differentiation of osteoblasts. From these cytokines, TNF-α and IL-1β stimulated osteoclast formation and bone resorption\cite{15}.

Also, in postmenopausal osteoporosis, estrogen deficiency leads to increased expression of bone-resorbing cytokines, such as M-CSF, tumor necrosis factor-α (TNF-α), interleukin-1 and interleukin-6; and a direct effect on osteoclasts via the inhibition of apoptosis and an increase in the differentiation of osteoclast precursors into mature osteoclasts\cite{16}.

In the present work, results revealed that, there was significant increase in serum ALP level in positive control group compared to negative control group and significant reduction in value of serum ALP in desferal and vit E treated groups compared to positive control group. Also there was significant reduction in serum ALP level in desferal + vit E treated group compared to positive control group and both desferal and vit E treated group. This results may be explained by that, increase in ALP serum level in positive control group was due to ovariectomy leaded to defect in osteoblast
activity which liberate alkaline phosphates enzyme. on the other hand decreased in serum ALP in all treated groups due to efficacy of vitamin E and desferal.

This explanation is compatible with\textsuperscript{[17]} stated that, The highest total ALP values have been attributed to an increased bone isoenzyme level due to bone disease e.g. osteomalasia. The enzyme activity, which is localized in the plasma membrane of osteoblasts before extracellular release, correlates with the extent of the disease on skeletal surveys and with parameters of bone resorption.

Our result is incompatible with\textsuperscript{[5]} who stated that, vitamin E was able to dose-dependently increase serum ALP concentrations, a nonspecific biomarker of bone formation expressed in the osteoblastic plasma membrane.

**Conclusion**

Despite of this result it is difficult to confirm that, the female rats alleged to ovariectomy and suffered from osteoporosis will be cured by using desferal and vitamin E alone or in combination. but in fact we noticed appropriate improvement in treated female rats in compared to diseased rats.

**References**

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