Bull. Egypt. Soc. Physiol. Sci. 38(2),210-222

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Oxytocin versus Alendronate in Treating Postmenopausal Osteoporotic Female Rats; Which is Better?

[a]Ahmed Mostafa Mahmoud, [b]Hekmat O. Abdel Aziz

[a]Physiology Department, Faculty of Medicine, Sohag University, Sohag, Egypt.
[b]Histology Department, Faculty of Medicine, Sohag University, Sohag, Egypt.

Received: 22 Dec 2017
Accepted: 21 Feb 2018
Available online: 1 June 2018

Keywords

- Osteoporosis
- Oxytocin
- Alendronate
- DEXA.
- ALP
- Ovariectomy.

Abstract

Background: post menopausal period is a critical period for each female. How to minimize the complications of this period is a matter of major concern. **Materials and Methods:** 40 females' albino rates were included in this study; they were divided into four equal groups. G1: Sham ovariectomized group, G2: ovariectomized group receiving vehicle (Ve) 1mg/kg/day for 7 weeks intraperitoneal injection (Ip) after 7 weeks from ovariectomy. G3: ovariectomized group receiving alendronat 0.1mg/kg/day for 7 weeks (Ip) after 7 weeks from ovariectomy.G4: ovariectomized group receiving Oxytocin 0.1mg/kg/day for 7 weeks (Ip) after 7 weeks from ovariectomy. Serum level of (Alkaline phosphatase, Oxytocin) was determined, Body Mass Density (BMD) was measured by (DEXA), also a histological examination of the femur and tibia was done. **Results:** Marked increase in serum levels of ALP and marked decrease of serum Oxytocin in G2 compared to G1 associated with picture of osteoporosis. Marked decrease of serum ALP with improvement in osteoporotic picture in both G3 and G4.**Conclusion:** Treatment by either alendronat or oxytocin (G3 and G4) improves the osteoporotic condition with better improvement by Oxytocin.

Corresponding author: Ahmed Mostafa Mahmoud, Physiology Department, Faculty of Medicine, Sohag University, Sohag, Egypt. Tel:+201060423663; E mail: ahmed_physiology@yahoo.com

Introduction

Menopause is a critical period of a woman's life. It is a major public health problem with 6.3 million of cases expected in 2050 (1). At this period estrogen deficiency impairs the normal bone remodeling cycle by increasing osteoclastic resorption activity without a corresponding increase in osteoblastic activity leading to what is called postmenopausal osteoporosis(OP) (2).

Osteoporosis (OP) is a "silent disease" until it is complicated by fractures following minimal trauma or, in some cases, with no trauma that places an enormous medical and economic personal burden on the affected females (3).

The main target in treating OP is to reduce fracture risk, increase survival, and improve quality of life (4). Several drugs have been developed to treat bone diseases in humans are either anti resorptive (including alendronate, selective estrogen-receptor modulators, calcitonin, and vitamin D) or anabolic agents (parathyroid hormone) (5). Unfortunately these drugs are associated with serious side effects and consequently an alternative approach to treat OP is mandatory (6)

Oxytocin (OT) is a neuropeptide predominantly synthesized within the magnocellular neurones of the hypothalamus. Interestingly, it is also synthesized in some peripheral tissues, including the reproductive system, heart, and bone. OT and its receptors are positively regulated by estrogens, through estrogen receptor beta (7).

It was found that osteoblasts in bone marrow produce abundant OT, suggesting that locally released OT may be an autocrine regulator of bone formation and bone mass. OT produced from osteoblasts in response to estrogen acts upon the OT receptors to stimulate further OT release, which amplifies estrogen action (8).

So our hypothesis in this study is that oxytocin administration may improve OP with better results than alendronate (the drug of choice in treating OP).

Materials and Methods

The present study was conducted in the physiology department, faculty of Medicine, Sohag University. This study was carried out in accordance with the guidelines of the University Animal Ethics and approved by Research Ethics Committee considering care and use of laboratory animals. 40 adult female albino rats, about (200-250) gm weight were chosen for this study. All rats were kept under observation for two weeks prior to the experiments to permit the animals to adjust to the environments. The animals were housed in standard suitable cages (20 × 32 × 20 cm for every 4 rats) at room temperature with normal light and dark cycle. They were maintained on a standard diet of commercial rat chow and tap water. Animals were subjected either to bilateral ovariectomies (OVX) from the dorsal approach or to sham surgery (in which the ovaries were not exteriorized but replaced intact), under intraperotinial sodium thiopental (40 mg/kg) anesthesia. The OVX rats were subjected to total ovariectomy, as reported previously (6). In brief, two paravertebral skin incisions were made while rats were under general anesthesia. The uterine tubes were ligated, and the incisions were closed following the removal of the ovaries. For antibiotic therapy, Ceftriaxone at a dose of 50 mg/kg was

injected immediately before and 24 and 48h after surgery. The sham operated control group have the same previous incision, but with no excision of the ovaries. 7 weeks later (bone loss period), 40 adult female rats were divided into four groups (n= 10):

1. Group I, (control group) after sham surgery, rats were received no treatment.
2. Group II, after being ovariectomized, rats were received vehicle (Ve) 1mg/kg/day intraperitoneal injection for 7 weeks.
3. Group III, after being ovariectomized, rats were received alendronate 0.1mg/kg/day intraperitoneal injection for 7 weeks.
4. Group IV, after being ovariectomized, rats were injected daily with Oxytocin 0.1 mg/kg intraperitoneal injection for 7 weeks according to the manufacture structure.

Collection of samples

At the end of the experimental period all animals were anaesthetized with thiopental sodium (40 mg/kg) intraperitoneally. Blood were being collected, centrifuged, and serum was stored at -80℃ until the determination of serum ALP and Oxytocin.

1-Biochemical Parameters

Determination of serum Oxytocin:

Serum concentration of OT (OT EIA kit) was measured using commercially available kits (Nordic Bioscience Diagnostics, Harley, Denmark) **(9)**

Determination of bone turnover biomarkers:

Serum alkaline phosphatase (ALP) was determined by ALP assay kits (DALP-250, BioAssay Systems, CA, USA) (**10).**

Assessment of bone mass density (BMD)

BMD was assessed by using DEXA machine was calibrated per manufacturer's instruction protocol. Measurements were attained by positioning the rat in prone position with knee flexed and extended hips.

2- Histological study

Following decalcification in 10% EDTA, pH 7.4, the tibiae were processed histologically for paraffin embedding, and longitudinal sections were obtained and stained with H&E ***(11).***

Morphometric studies:

Using an image analyzer (Leica version 3.7.2005-2010) in histology department, Sohag university, the numbers of osteoclast and osteoblast lining the endosteum were counted under x 400 magnification in three sections from each animal.

Statistical analyses

Demographics and baseline characteristics were summarized across treatment groups using descriptive statistics. The data from both normal and osteoporotic groups were compared using the Student's t-test. Significance was set at P value of less than 0.05 for all comparisons. Data analysis were performed using the SPSS version 20 software.

Results

All statistical data are summarized in table (1), showing the ALP levels & DEXA measurements and the oxytocin levels among all groups.

Table 1. DEXA, ALP and oxytocin levels in different groups

	DEXA (gm\cm)²	***ALP (ng\ml)***	***Oxytocin (pg\ml)***
Group I ***Sham (Control)***	0.0675 ± .007	57.0 ± 6.0	29.4±3.9
Group II (OVX)	0.009 ± .002[a]	154.3 ± 30.3[a]	16.3± 2.7 [a]
Group III ***OVX + alendronate*** ***7 weeks after surgery***	0.0648 ± .009[b]	41.3 ± 7.4[b]	21.8 ± 2.7 [b]
Group IV ***OVX + OT*** ***7 weeks after surgery***	0.0670 ± .011 [b c]	35.3 ± 6.9[b c]	28.2± 2.2 [b,c]

* Data are represented as mean ± SD, (a) Significant from Sham Group I (P < 0.05), (b) significant from OVX Group II (P < 0.05) (c) Significant from Group III (P < 0.05)

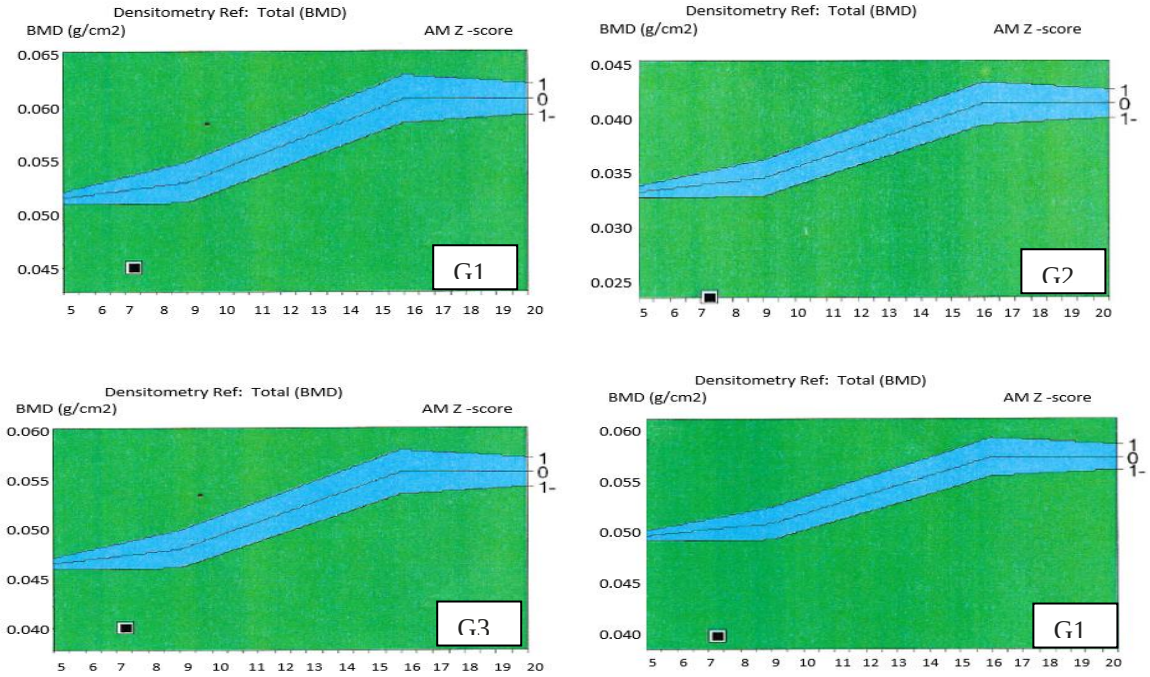


Fig (1): DEXA in different groups

1- Serum levels of ALP:

As summarized in table 1, there were highly statistical significances among all the examined groups regarding the serum levels of ALP (p=0.000). Sensitivity analysis was conducted between every 2 groups for determining the point of significance regarding the serum levels of ALP and their response on the osteoporotic rats.

There were a statistically significant increase in serum levels of ALP in group II compared to the other groups, especially on comparing between the group I and group II in the levels of ALP (57.0±6.0 vs 154.3±30.3, P=0.002), respectively.

Also There were a statistically significant decrease in serum ALP levels in both group III (OVX+ alendronate) and group IV(OVX+ Oxytocin) in

comparison with group II (OVX) (41.3±7.4, 35.3±6.9 vs. 154.3±30.3, P=0.004) respectively.

A slight decrease in serum ALP levels in favor of group IV (41.3±7.4 vs. 35.3±6.9, P=0.809), when comparing group III with group IV.

2- DEXA measurements:

Highly statistical significances between all the examined groups regarding the DEXA measurements (p=0.000) as summarized in table 1 and figure 1 with the lowest values in group II in comparison with the other groups.

Marked deterioration in the DEXA measurements were observed in group II when comparing between group I and group II (0.0675±0.007 vs. 0.009 ± .002, P=0.000).

There were a statistically significant improvement in the DEXA measurements in both group III (OVX+ Alendronate) (0.0648±0.0090 vs. 009±0.002, P=0.001) and group IV(OVX+ Oxytocin) in comparison with group II, with much more improvement in group IV on comparing between group II and group IV(0.009±0.002 vs. 0.0670±0.011, P=0.000).

Also, there were near normal improvement in group III and group IV on comparing between group I vs. group III and group I vs. group IV in the DEXA measurements (0.0675±0.007 vs. .0648 ± .009, P=0.518), (0.0675±0.007 vs. 0.0670 ±0.011, P=0.102)respectively.

Also, there were more improvement in the DEXA measurements on comparing between group III and group IV (0.0648±0.009 vs. 0.0670±.011, P=0.724) in favor of group IV.

3 - Serum Oxytocin level:

There were a highly statistical significance among all the examined groups regarding the OT levels (p=0.000).

There were a statistically significant decrease in the serum levels of OT in group II compared to group I (16.3±2.7 vs 29.4±3.9, P=0.313), respectively.

Also a statistically significant increase in the serum levels of OT were noticed in group IV compared to group II (28.2± 2.2 vs. 16.3± 2.7, p = 0.000) respectively.

4- Morphometric and histological results:

1- Control group:

Examination of H & E stained sections from the control group showed normal architecture of the cancellous bone; the tissue arranged as trabeculae which appear eosinophilic, numerous interconnecting bone marrow spaces of various sizes were present between the bone tissues (Fig. 1a). Osteocytes were seen as basophilic cells inside their lacunae in the bone lamellae. Osteoblasts appeared with their large size, polygonal shape; with heavily basophilic cytoplasm (Fig. 1a_2, 1b).The mean number of osteocytes was 40.4±4.8 cell/HPF. The mean number of osteoblasts was 20.6±1.9 cell/HPF.

2- Group II (OVX+ saline):

In group II the bone showed picture of osteoporosis in the form of; the bone lost its normal architecture, trabeculae were thin, reduced in number and sometimes fragmented and fractured. They were separated with wide marrow spaces (Fig. 2a). There was a marked decrease in number of osteocytes, without lacunae (Fig.2a_2,

2b).The mean number of osteocytes was 5.7±1.4 cell/ HPF and the mean number of osteoblasts was 2.0±0.8 cell/HPF. There was an apparent significant decrease in the number of osteocyte and osteoblast between group I and group II (40.4±4.8 vs 5.7±1.4,P=0.028), (20.6±1.9 vs 2.0±0.8, P=0.048) respectively.

3- Group III (OVX + Alendronate 7 weeks after OVX)

Rats treated with alendronate in group III showed that; the bone was nearly normal. Trabeculae were thick with large number; no fractures or fragmentation were seen (Fig.4a).

With high magnification; osteocytes were seen with large nucleus, filling the lacunae. Osteoblasts were seen with numerous amounts (Fig.4b).The mean number of osteocytes was 41.7±6.3 cell/HPF and the mean number of osteoblasts was 17.7±1.9 cell/HPF. Marked difference was noticed between the group II and group III with apparent increase in the number of osteocytes and osteoblasts (P=0.002 and P=0.018) respectively.

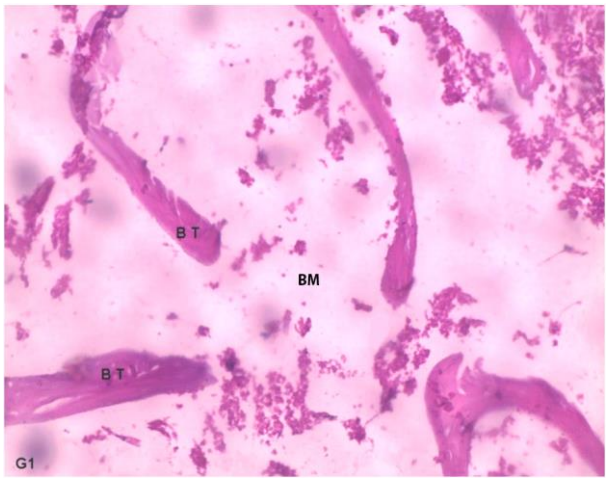
4- Group IV (OVX + OT 7 weeks after OVX):

H & E sections from group IV, that was treated with OT as a curative thereby showed an apparent increase in the thickness of cortical bone similar to the normal bone. No cavities were seen in the part of bone near the medullary cavity, which was occupied by red bone marrow (Fig. 5a). Osteocytes were seen with large nucleus, also osteoblasts were observed with numerous amounts. The mean number of osteocytes was 42.0±6.4 cell/ HPF, the mean number of osteoblasts was 25.5±23.1 cell/HPF. Highly significant increase was observed on counting the number of osteocyte and osteoblast when comparing group II with group IV (P=0.004 and P=0.047) respectively. Slight difference was noticed between group III and group IV regarding the number of osteocyte and osteoblast in favor of group IV (42.0±6.4 vs 41.7±6.3, P=0.770), (25.5±23.1 vs 17.7±1.9, P=0.779) respectively.

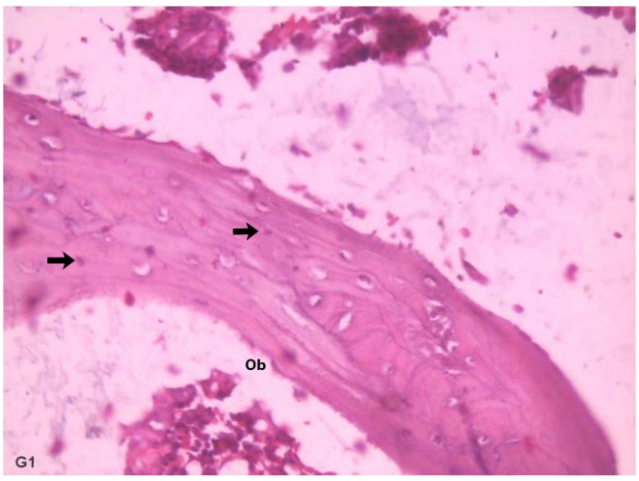
Table (2): showing the number of osteocytes and osteoblasts in section of the head of the tibia in all the examined groups:

	Osteocyte	***Osteoblast***
Group *I* ***No OVX + Sham*** ***No treatment***	40.4±4.8	20.6±1.9
Group *II* ***OVX + Ve*** ***7 weeks after surgery***	5.7±1.4[a]	2.0±0.8[a]
Group *III* ***OVX + Alendronate*** ***7 weeks after surgery***	41.7±6.3[b]	17.7±1.9[b]
Group *IV* ***OVX + OT*** ***7 weeks after surgery***	42.0±6.4[b]	35.3 ± 6.9 [bc]

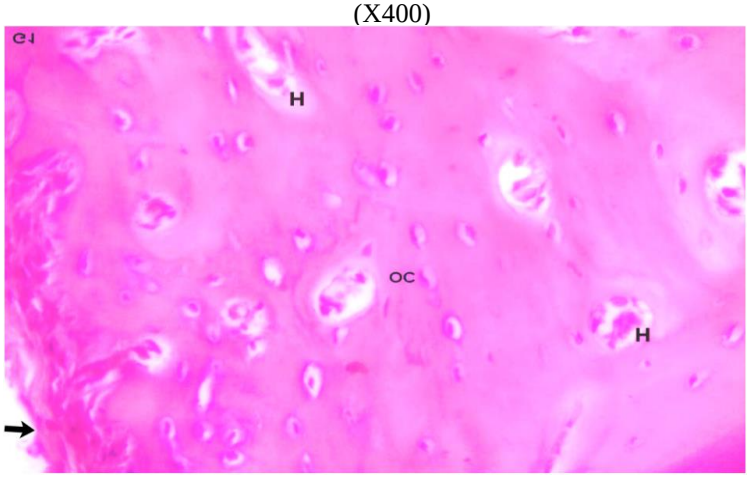
* Data are represented as mean ± SD, (a) Significant from Sham Group I (P < 0.05); (b) Significant from OVX Group II (P < 0.05), (c) Significant (P < 0.05) vs Group III; (n =10 rats per group).



(Fig. 1a): A photomicrograph of a section of a control group (G1) at the head of the tibia showing: Irregular cancellous bone trabeculae (BT) of the metaphysis and bone marrow spaces (BM) in between the trabeculae. H&E (X 200)



(Fig.1a$_2$): A higher magnification of a section of a control group at the head of the tibia showing: Osteocytes seen inside their lacunae in the bone lamella (↑). Osteoblasts (Ob) are seen covering the surface of the lamellae. H & E (X400)



(Fig. 1b): A photomicrograph of a section from a control rat showing the outer part of the cortex of tibia. The bone is covered from outside by the periosteum (↑). The compact bone tissue is well organized; showing concentric lamellae arranged around the Haversian canals (H).Osteocytes (oc) inside the lacunae are shown in between the bone lamellae. (H & E X 400)

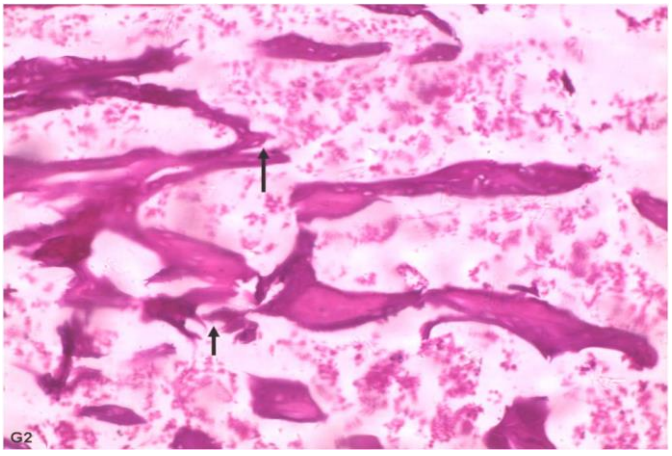


Fig.(2a): A photomicrograph of a section of ovariectomized group at the head of the tibia showing: Bone trabeculae appeared thin and fragmented (↑). H&E (X200)

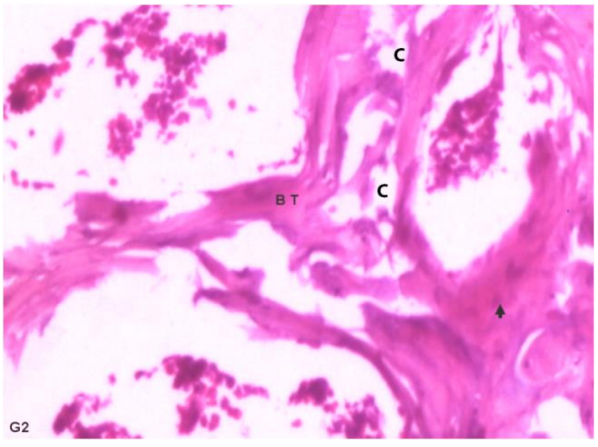


Fig.(2a$_2$): A higher magnification of a section of ovariectomized group at the head of the tibia showing: Appearance of cavities (C) in the bone trabeculae near the medullary cavity. There is also decrease number of the osteocyte (↑). H&E (X400).

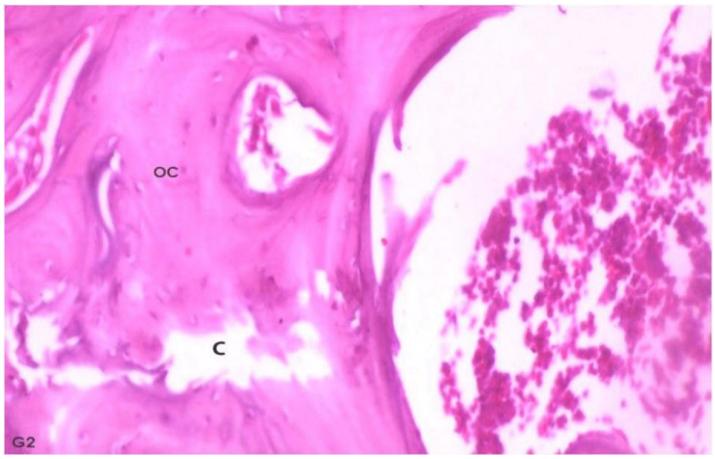
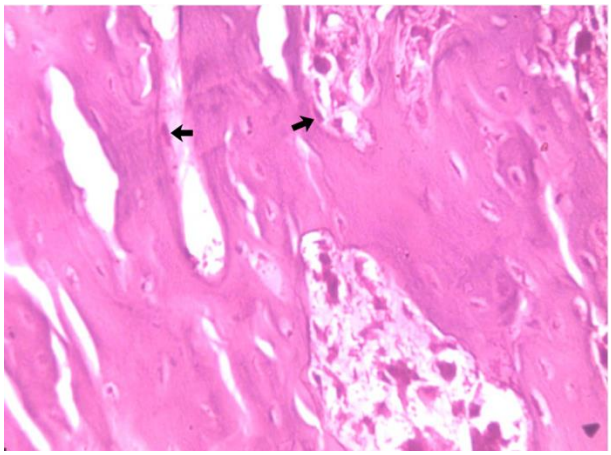
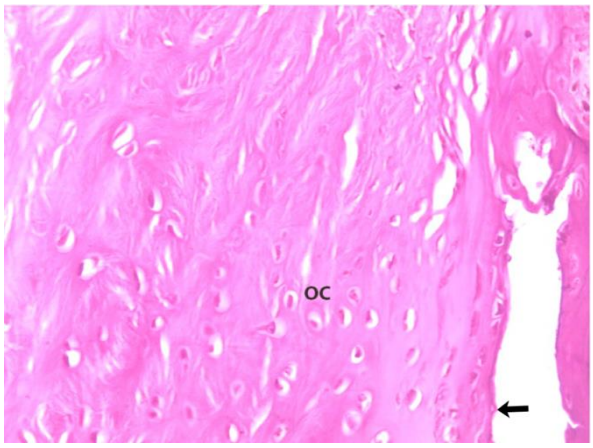


Fig.(2b): A photomicrograph of a section of ovariectomized group at the head of the tibia showing: Appearance of cavities (C) in the compact bone. osteocytes showing apparent decrease in number (OC). H&E X400



(Fig. 3a): A photomicrograph of a section of group III at the head of the tibia showing:the bone was nearly normal, trabeculae were thickened, no fragmentation were seen. Osteoblasts (↑) were lining the trabeculae with numerous amounts. H&E (X 400)



(Fig.3b): A photomicrograph of a section of group III at the head of the tibia showing:Osteocytes (OC) were seen with large number, prominent nucleus filling the lacunae. Osteoblasts (↑) were normally distributer, lining endosteum with numerous amounts. H&E (X 400)

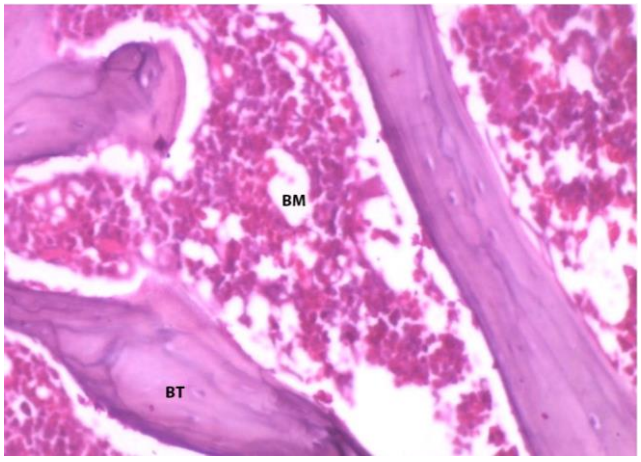
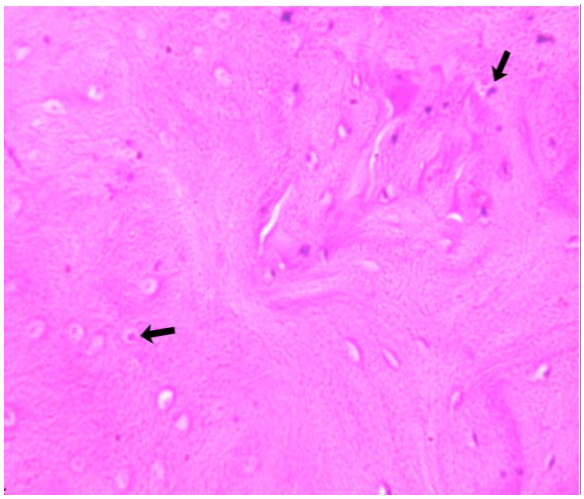


Fig. 4a: A photomicrograph of a section of group IV at the head of the tibia showing: An apparent increase in the thickness of bone trabeculae (BT), separated by red bone marrow (BM). No cavities were seen with an improved structure, more or less similar to normal bone.



(Fig. 4b): A photomicrograph of a section of group IV at the head of the tibia showing: Osteocytes (↑) were seen with prominent nucleus, increased in number with normally distributed matrix with no cavities appeared. H&E (X 400)

Discussion

Menopause is known to be associated with numerous physiological and biochemical changes affecting bone mineral metabolism. Our results from this study have revealed a statistically increase in serum levelS of the bone turnover marker (ALP) in group II in comparison to group I. These results are in accordance with (12, 10, 13) they found an elevation of serum ALP with post-menopausal females.

Lack of inhibiting activity of estrogen on osteoclasts caused the increase in bone resorption. It was noticed that ALP measurements correlate with the rates of bone mineralization (12). Group II (OVX rats) had developed osteopenia with high level of ALP and very low BMD by DEXA scan after 7 weeks of the surgery which were confirmed histologically Fig 2a,a_2,b (the bone lost its normal architecture; the trabeculae became thin and associated with almost no osteoblastic cover like the osteoporotic picture seen in (14).

Interestingly, the OT value was significantly decreased in group II when ovariectomy was performed and a state of hypogonadism was triggered.

The decreased OT level in group II may be attributed to that transcription of the oxytocin and oxytocin receptor genes are under control of estrogens. Therefore, as the estrogen levels were decreased

in group II rats, the levels of OT were lower as well (5). Also oral administration of estrogen induces an increase in OT serum levels within 12 hours in humans (15).

These results of low OT level in group II comes in agreement with(5, 16,7) they found that low OT serum levels was significantly associated with severe OP, independently of other factors associated with OP or known to regulate OT serum levels, such as age, estradiol, or leptin (7).

The main target in treating OP is to reduce fracture risk, increase survival, and improve quality of life (4). In our study after alendronate (the drug of choice in OP treatment) was given for 7 weeks after 7 weeks of surgery in group III, there were a marked decrease in the serum ALP levels associated with increased BMD on DEXA scan. Histologically (Fig $3_{a,\ b}$), the results supports the alendronate action on bone in the form of increasing the bone thickness. Osteoblasts and osteocytes were noticed with numerous amounts in this group. These data confirmed the well-known role of alendronate in the treatment of OP (17, 18, and 19).

Alendronate inhibits osteoclastic bone resorption by attaching to hydroxyapatite binding sites on bony surfaces, especially surfaces undergoing active resorption. When osteoclasts begin to resorb bone that is impregnated with alendronate, the alendronate released during resorption impairs the ability of the osteoclasts to form the ruffled border, to adhere to the bony surface, and to produce the protons necessary for continued bone resorption [20]. Also by decreasing osteoclast progenitor development and recruitment and by promoting osteoclast apoptosis, alendronates can reduce osteoclastic activity [21]. In addition to their inhibitory effect on osteoclasts, alendronates appear to have a beneficial effect on preventing osteoblasts apoptosis (22)

Almost all therapies which are currently used in OP treatment whether anti resorptive, such as alendronates or anabolic ,such as Parathormone hormone (PTH) have serious adverse side effects like osteonecrosis, dysphagia, esophagitis, headache, nausea, arthralgia, dizziness, and others (23). Unfortunately; the alendronate therapy has many adverse effects on the gastrointestinal tract. All bisphosphonates (including alendronates) can affect renal function and are contraindicated in patients with estimated GFR below 30-35 ml/min (3).

One of the most important results recently obtained in bone research has been that the pituitary hormones including the posterior pituitary hormone oxytocin (OT) have profound effect on bone(6).

A high statistically significance decrease in serum ALP levels associated with marked BMD improvement by (DEXA) were obtained in group IV compared to group II (P=0.004, P=0.000), respectively when OT was introduced to group IV as a curative therapy for OP . The ALP level had reached the normal level and an obvious improvement in the BMD with DEXA scan was noticed. These results coincide with (5, 24, 25, 26 and 27) they found that OT can restore osteopenia by direct effect on bone cells independently on estrogen anabolic effect on bone.

Also a highly significant increase in the levels of the serum OT hormone were noticed in this group (OVX+OT) when compared with group II (p = 0.000). Histologically (Fig $4_{a,b}$) there were increased number of osteoblasts and osteocytes. Moreover, bone trabeculae were thickened with no cavitation and more or less similar to normal bone. Previous data strongly suggested that hypogonadal-induced bone loss was linked to low OT circulating levels, and that restoring OT levels could therefore reverse osteopenia (25, 26).

The action of OT on the skeleton is mainly mediated not only through its stimulation of osteoblast differentiation but also through a modulation of osteoclast formation and function. At the same time, OT stimulated osteoclast differentiation by increasing ratio of RANKL and OPG, while inhibited bone resorption by triggering cytosolic Ca^{2+} release and nitric oxide synthesis (25).

Another possible mechanism for OT is that it has been shown that (i) OT stimulates PGE2 synthesis in both undifferentiated and differentiated human osteoblastic cells, (ii) PGE2 increases bone turnover favoring bone formation, and (iii) PGE2 inhibits adipocyte differentiation. Taken together, these observations are in favor of OT exerting its effects through PGE2 (5).

Other studies speculated that high circulating OT levels might mediate the anabolic skeletal recovery (27).

To the best of our knowledge this is the first study to compare between alendronate (the drug of choice) and OT, so when comparing between the role of OT and alendronate therapy as a curative role among the group III and IV, we identified that no statistical difference between the two drugs in the treatment of OP. Nearly similar results were detected between these 2 groups in reversal of hypogonadal OP, concluded by decreased ALP to

the normal levels (P=0.809), improvement of BMD measured by DEXA scan (P=0.724), and confirmed histologically by increased number of osteocytes (P=0.770) and osteoblasts (P=0.779).

Conclusion:

These beneficial observations, which are discussed above, clearly showed that OT is involved in regulation of metabolic and bone balance and are opening a new window for drug development in osteoporosis therapeutics. Considering that OT is a FDA-approved drug, it would be extremely useful as skeletal anabolic agent to treat osteoporosis. Beside this marvelous effect of OT, it is one of the happiness hormones which can improve the mood changes (e.g. Depression) associated with OP. Further studies are needed to clarify if there are unwanted side effects of OT.

References:

1- Beranger G.E., Djedaini M., Battaglia S., Roux C.H., Scheideler M., Heymann D., Amri E. and Pisani D.: Oxytocin reverses osteoporosis in a sex-dependent manner. *Front Endocrinol (Lausanne)*. 6: 81, 2015.

2- Tella S.H. and Gallagher J.C.: Prevention and treatment of postmenopausal osteoporosis. *J Steroid Biochem Mol Biol*. 142: 155-170, 2014.

3- Cosman F., de Beur S.J., LeBoff M.S., Lewiecki E.M., Tanner B., Randall S. and Lindsay R.: Clinician's Guide to Prevention and Treatment of Osteoporosis. *Osteoporos Int*. 25(10): 2359-2381, 2014.

4- Ip T.P., Cheung S.K., Cheung T.C., Choi T.C., Chow S.L., Ho Y.Y., Kan S.Y., Kung W.C., Lee K.K., Leung K.L., Leung Y.Y., Lo S.T., Sy C.T. and Wong Y.W.: The Osteoporosis Society of Hong Kong (OSHK): 2013 OSHK guideline for clinical management of postmenopausal osteoporosis in Hong Kong. *Hong Kong Med J*. 19 Suppl 2: 1-40, 2013.

5- Elabd C., Basillais A., Beaupied H., Breuil V., Wagner N., Scheideler M., Zaragosi L.E., Massiera F., Lemichez E., Trajanoski Z., Carle G., Euller-Ziegler L., Ailhaud G., Benhamou C.L., Dani C. and Amri E.Z.: Oxytocin controls differentiation of human mesenchymal stem cells and reverses osteoporosis. *Stem Cells*. 26(9): 2399-2407, 2008.

6- Fallahnezhad S., Piryaei A., Darbandi H., Amini A., Ghoreishi S.K., Jalalifirouzkouhi R. and Bayat M.: Effect of low-level laser therapy and oxytocin on osteoporotic bone marrow-derived mesenchymal stem cells. *J Cell Biochem*.119(1):983-997, 2018.

7- Breuil V., Panaia-Ferrari P., Fontas E., Roux C., Kolta S., Eastell R., Ben Yahia H., Faure S., Gossiel F., Benhamou C.L., Euller-Ziegler L. and Amri E.Z.: Oxytocin, a new determinant of bone mineral density in post-menopausal women: analysis of the OPUS cohort. *J Clin Endocrinol Metab*. 99(4): E634-641, 2014.

8- Colaianni G., Tamma R., Di Benedetto A., Yuen T., Sun L., Zaidi M. and Zallone A.: The oxytocin-bone axis. *J Neuroendocrinol*. 26(2): 53-57, 2014.

9- Szeto A., McCabo P., Nation D, et al.: Evaluation of enzym immunoassay and radioimmunoassay methods for the meassurment of plasma oxytocin.Psychosom.Med.73(5):393-400, 2011.

10- Mukaiyama K., Kamimura M., Uchiyama S., Ikegami S., Nakamura Y. and Kato H.: Elevation

of serum alkaline phosphatase (ALP) level in postmenopausal women is caused by high bone turnover. *Aging Clin Exp Res*. 27(4): 413-418, 2015.

11- Oyhanart S., Escudero N. and Mandalunis P.: effect of alendronate on the mandible and long bones: an experimental study *in* viv**o.** Pediatric ReSeARCH.78 (6):618-625, 2015.

12- Parra-Torres A.Y., Valdés-Flores M., Orozco L. and Velázquez-Cruz R.: Molecular Aspects of Bone Remodeling. Topics in Osteoporosis. D. M. V.-F. (Ed.), InTech: 28, 2013.

13- Kuo T.R. and Chen C.H.: Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. *Biomark* Res. 5: 18, 2017.

14- Naim M.M.: Histological assessment of zoledronic acid (Aclasta) in protection against induced osteoporosis in female albino rats. *The Egyptian Journal of Histology*. 34: 129–138, 2010.

15- Nomura M, McKenna E, Korach KS, Pfaff DW, Ogawa S.; Estrogen receptor- regulates transcript levels for oxytocin and arginine vasopressin in the hypothalamic paraventricular nucleus of male mice. Brain Res Mol Brain Res. 109:84–94, 2002.

16- Breuil V., Amri E.Z., Panaia-Ferrari P., Testa J., Elabd C., Albert-Sabonnadiere C., Roux C.H., Ailhaud G., Dani C., Carle G.F. and Euller-Ziegler L.: Oxytocin and bone remodelling: relationships with neuropituitary hormones, bone status and body composition. *Joint Bone Spine*. 78(6): 611-615, 2011.

17- Pressman AR, Lo JC, Chandra M and Ettinger B.: Methods for assessing fracture risk prediction models: experience with FRAX in a large integrated health care delivery system. *J. Clin. Densitom.* 14: 407-415, 2011.

18- Sara p., Terenzio C., Giuseppe B. and Massimo M.: Alendronate rescued osteoporotic phenotype in a model of glucocorticoid-induced osteoporosis in adult zebrafish scale.International Journal of Experimental Pathology. (96); 11–20, 2015.

19- Liu C., Yuan X. and G.C. Gao G.: Effects of alendronate on osteoporosis treatment and levels of related cytokines.Genetics and Molecular Research. 16 (1): gmr16019485, 2017.

20- Fleisch H.: Bisphosphonates: mechanisms of action. Endocr Rev; 19:80, 1998.

21- Hughes D., Wright K., Uy HL, et al.: Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. J Bone Miner Res; 10:1478, 1995.

22- Plotkin L., Lezcano V., Thostenson J, et al.: Connexin 43 is required for the anti-apoptotic effect of bisphosphonates on osteocytes and osteoblasts in vivo. J Bone Miner Res; 23:1712, 2008.

23- Yoo J.H., Moon S.H., Ha Y.C., Lee D.Y., Gong H.S., Park S.Y. and Yang K.H.: Osteoporotic Fracture: 2015 Position Statement of the Korean Society for Bone and Mineral Research. *J Bone Metab*. 22(4): 175-181, 2015.

24- Tamma R., Colaianni G., Zhu L.L., DiBenedetto A., Greco G., Montemurro G., Patano N., Strippoli M., Vergari R., Mancini L., Colucci S., Grano M., Faccio R., Liu X., Li J., Usmani S., Bachar M., Bab I., Nishimori K., Young L.J., Buettner C., Iqbal J., Sun L., Zaidi M. and Zallone A.: Oxytocin is an anabolic bone

hormone. *Proc Natl Acad Sci U S A*. 106(17): 7149-7154, 2009.

25- Colaianni G., Sun L., Zaidi M. and Zallone A.: The "love hormone" oxytocin regulates the loss and gain of the fat-bone relationship. *Front Endocrinol (Lausanne)*. 6: 79, 2015.

26- Elabd S. and Sabry I.: Two Birds with One Stone: Possible Dual-Role of Oxytocin in the Treatment of Diabetes and Osteoporosis. *Front Endocrinol (Lausanne)*. 6: 121, 2015.

27- Sun L., Tamma R., Yuen T., Colaianni G., Ji Y., Cuscito C., Bailey J., Dhawan S., Lu P., Calvano C.D., Zhu L.L., Zambonin C.G., Di Benedetto A., Stachnik A., Liu P., Grano M., Colucci S., Davies T.F., New M.I., Zallone A. and Zaidi M.: Functions of vasopressin and oxytocin in bone mass regulation. *Proc Natl Acad Sci U S A*. 113(1): 164-169, 2016.