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PHYSIOLOGICAL CHANGES IN SEX HORMONES OF OSTEOPOROSIS WOMEN IN BASRA PROVINCE/IRAQ

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ABSTRACT
Osteoporosis is the most common bone disease in humans, it is characterized by low bone mass, deterioration of bone tissue and disruption of bone architecture, compromised bone strength and an increase in the risk of fracture. Various endocrine factors, including gonadal sex hormones influence bone growth. Estrogen deficiency accelerates the rate of bone turnover, thereby altering the balance between bone formation and bone resorption, Sex hormones have important effects on bone, especially in postmenopausal women, these hormones may be of particular significance in patients with osteoporosis disease, postmenopausal bone loss is thought to be due to the cessation of ovarian estrogen production, and cyclic estrogen replacement has been shown to prevent this bone loss. Ninety five women patients referring from urban and rural housing in Basra province aging between (50-69) years old visit al zahraa clinic in Ibnalbitar private hospital to determine the percentage of bone density by measuring the bone mineral density of lumber spine and hip by a dual energy x-ray absorptiometry (DEXA) from Lunar Prodigy (version 16) (USA). Venous blood sample was taken from each patient after diagnosing of disease by using DEXA machine, then the sex hormones include estrogen, progesterone, testosterone, FSH and LH was estimated by using VIDAS automated quantitative test. The results shows significant decrease ($p \leq 0.05$) in estrogen and progesterone and significant increase ($p \leq 0.05$) in FSH and LH while no changes occurs in testosterone compared with healthy women. In conclusion, sex hormones have significant changes in osteoporosis women in Basra province / Iraq.

KEYWORDS: osteoporosis, sex hormones, estrogen, progesterone, DEXA.

INTRODUCTION
Osteoporosis is an important disease that associated with an increased mortality after fractures (**Nguyen., et al 2009**). Also osteoporosis has been defined by (**Kanis., et al 2013**) as a disease characterized by low bone mass and microarchitectural deterioration of bone tissue leading to enhanced bone fragility and increase fracture risk.

The definition of osteoporosis by the World Health Organization (WHO) is pointed by (**Lewiecki, 2015**) about the BMD is 2.5 standard deviation (SD) or more below the mean of a young normal reference population (table 1.1**)**, the definition offers to the physician an objective standard by which to make a diagnosis and to make next management decisions. (**Lin and Lane, 2014**) also pointed that osteoporosis is also called a "silent disease" because it progresses without symptoms until a fracture occurs, the osteoporotic bone fractures are responsible for a huge pain, reduced quality of life, lost workdays, disability and some 20% of women with a hip fracture will die in the following year as an indirect result of the fracture.

Causes of osteoporosis
In childhood, bones grow and repair very quickly, but this process slows as you get older, between the ages of 16 and 18 bones stop growing in length, but continue to increase in density until you are in your age 20s, at the age of 35, you progressively lose bone density **(Saladin and Kenneth 2012).** Other things that increase the risk of developing osteoporosis include ovarian hormone deficiency, family history/genetics, females, low calcium/vitamin D intake, poor exercise, smoking, alcohol, low body weight, anorexia, hyperthyroidism, hyperparathyroidism, glucocorticoids use, liver and renal disease, low sun exposure, medications (heparin), and malignancies (metastatic disease; multiple myeloma can present as osteopenia) (**Raisz, 2015**).

Table 1.1: The World Health Organization (WHO) classification of osteoporosis.

Normal	BMD within 1 SD of young adult reference range.
Osteopenia	BMD more than 1 SD below the young adult mean but less than 2.5 SD of this value.
Osteoporosis	BMD 2.5 SD or more below the young adult mean.
Severe Osteoporosis	BMD 2.5 SD or more below young adult mean and the presence of 1 or more fragility fractures.

Types of osteoporosis

Osteoporosis can be categorized as primary and secondary.

Primary osteoporosis

It is referred to consequence of aging and decreased gonadal function not Reduction in bone mass by chronic illnesses or medication, this is subdivided into two types: postmenopausal osteoporosis (also known as type I osteoporosis) and age-related osteoporosis (sometimes called *senile osteoporosis*) (Smith and Shoukri 2014). Postmenopausal osteoporosis, It is the most common osteoporosis condition mediated by decreased levels, or lack of estrogen manufacture during the postmenopausal (Riggs., *et al* 2012). While age-related osteoporosis in women includes two stages, a rapid stage that starts at menopause and occupy 4–8 years followed by a slower persistent stage that continues throughout life (Riggs., *et al* 2012).

Secondary osteoporosis

The bone loss can occur secondary to a number of chronic conditions such as, cancer, endocrine disturbances, gastrointestinal diseases, inflammatory diseases and renal failure, also the condition could be produce with long term treatment with glucocorticoids, Therefore, secondary osteoporosis can happen at any age and involve men and women similarly (Malik, 2013).

Patients and methods

This prospective study was conducted between December 2016 to October 2017 in Basra province to include a 65 women patients referring from urban and rural housing aging between (50-69) years old and weighting between (55-75 kg) while the length of this patient about (155-169 cm). This patients was visit al zahraa clinic in Ibnalbitar private hospital to determine the percentage of bone density by measuring the bone mineral density of lumber spine and hip by a dual energy x-ray absorptiometry (DEXA) from Lunar Prodigy (version 16) (USA). The main outcome measure is low bone mineral density (T-score) and according to the WHO criteria the patients are divided into normal, osteopenia and osteoporosis.

Human models

The patients are divided into four groups according to age and residing of housing status:

1. Patients age between (50-59) years old residing in urban.
2. Patients age between (50-59) years old residing in rural.
3. Patients age between (60-69) years old residing in urban.
4. Patients age between (60-69) years old residing in rural.

Every category of these are subdivided into two categories:

1. Patients age between (50-59) years old residing in urban

a. Healthy patients age between (50-59) years old residing in urban.
b. Osteoporosis patients age between (50-59) years old residing in urban.

2. Patients age between (50-59) years old residing in rural

a. Healthy patients age between (50-59) years old residing in rural.
b. Osteoporosis patients age between (50-59) years old residing in rural.

3. Patients age between (60-69) years old residing in urban

a. Healthy patients age between (60-69) years old residing in urban.
b. Osteoporosis patients age between (60-69) years old residing in urban.

4. Patients age between (60-69) years old residing in rural

a. Healthy patients age between (60-69) years old residing in rural.
b. Osteoporosis patients age between (60-69) years old residing in rural.

Information of patients and healthy women are represented in forma included many of information's related to search subject.

Dual Energy X-ray Absorptiometry

The World Health Organization (WHO) has defined criteria for diagnosing osteoporosis and assessing risk of fracture using DXA screening, a BMD value at the spine or hip that is more than 2.5 *SD*s below the optimal mean for healthy young individuals of the same race and gender defines an individual as having osteoporosis (T-score $\leq$ -2.5) **(Vondracek and Linnebur 2009)**.

Sample collection

Investigation of the effects of osteoporosis on physiological parameters in women by drawing five milliliters of venous blood sample by 5 ml disposable

syringe from each patient after diagnosing of disease by using DEXA machine. Blood specimens were collected from patients and healthy persons. The sample (2 ml of blood sample) was transferred into a clean anticoagulant tube (provided with EDTA) to be used in assaying of blood parameters. While the remainder blood was transfused into disposable plain tube, left at room temperature for at least 30 minutes for clotting, centrifuged (3500 r/m for 10 minutes) then the produced serum was divided into five parts then removal into eppendorf tube and stored at (– 20 C^0) unless used directly, this serum was then used in hormonal tests.

Assay of hormones levels

For the determination of human sexual hormones, it has been used VIDAS automated quantitative test that assay serum or plasma by using the ELFA technique (Enzyme Linked Fluorescent Assay).

The data has been analyzed by the use of a statistical package SPSS ver. 16 description statistics were used to summarized the data and to study background of participants with osteoporosis. One way ANOVA test was used to study association of duration of sex hormones and development of osteoporosis. P-value was considered statistically significant if <0.05.

RESULTS AND DISCUSSION

Hormones levels in women with osteoporosis aged between (50- 59) years and habitant of the urban

The results presented in the table (2) showed significant differences (p<o.o5) including decreased in Estrogen, Progesterone and increased (p<o.o5) in FSH, LH values for women with osteoporosis, while no significant difference in Testosterone of urbanization women age (50-59).

Table (2): Hormones levels in healthy and osteoporosis women at the age (50-59) years in urban habitant. (mean ± SD)

Hormones	Healthy women	Osteoporosis women
FSH (mic. IU/ml)	40.14 ± 4.93	**54.86 ± 7.45
LH (mic. IU/ml)	23.19 ± 1.87	**36.51 ± 7.74
Estrogen (pg/ml)	49.71 ± 6.97	*35.34 ± 4.81
Progesterone (ng/ml)	3.25 ± 4.61	*2.91 ± 0.65
Testosterone (ng/ml)	42.34 ± 9.78	42.59 ± 6.14

Hormones levels in women with osteoporosis aged between (50- 59) years and habitant of the Rural.

The decrease (p<o.o5) in Estrogen, Progesterone, and increased (p<o.o5) in FSH, LH values exhibited in table (3) was significance in women with osteoporosis aged (50-59) compared to their healthy counterparts women living in the same rural areas. Also no change observed in testosterone hormone.

Table (3): Hormones levels in osteoporosis and healthy women at the age (50-59) years in rural habitant. (mean ± SD).

Hormones	Healthy women	Osteoporosis women
FSH (mic. IU/ml)	41.53 ± 8.92	**55.37 ± 6.89
LH (mic. IU/ml)	24.74 ± 5.06	**38.69 ± 528
Estrogen (pg/ml)	52.27 ± 5.55	*37.02 ± 6.25
Progesterone (ng/ml)	3.83 ± 5.48	*2.94 ± 0.50
Testosterone (ng/ml)	42.68 ± 8.15	42.49 ± 6.67

Hormones levels in women with osteoporosis aged between (60- 69) years and habitant of the urban

Table (4) represent that women aged between (60-69) years with osteoporosis disease have decreased (p<o.o5) in their Estrogen, Progesterone, and increased (p<o.o5) in FSH, LH values with no difference in testosterone compared with healthy women of the same age who live in the urban area.

Table 4: Hormones levels in osteoporosis and healthy women at the age (60-69) years in urban habitant. (mean ± SD).

Hormones	Healthy women	Osteoporosis women
FSH (mic. IU/ml)	28.81 ± 5.20	**46.12 ± 9.55
LH (mic. IU/ml)	21.44 ± 2.09	**32.84 ± 3.14
Estrogen (pg/ml)	39.77 ± 9.45	*25.86 ± 4.68
Progesterone (ng/ml)	2.77 ± 4.65	*1.31 ± 0.65
Testosterone (ng/ml)	45.20 ± 6.33	42.50 ± 5.89

Hormones levels in women with osteoporosis aged between (60- 69) years and habitant of the Rural.

A significant changes (p<o.o5) was observed in table (5) of the hormones levels (except Testosterone with no changes) for women with osteoporosis disease who are live in a rural area compared to healthy women living in the same area with the same age.

Table (5): Hormones levels in osteoporosis and healthy women at the age (60-69) years in rural habitant. (mean ± SD).

Hormones	Healthy women	Osteoporosis women
FSH (mic. IU/ml)	22.24 ± 9.30	**42.65 ± 9.79
LH (mic. IU/ml)	15.08 ± 5.52	**27.06 ± 6.09
Estrogen (pg/ml)	33.29 ± 8.95	*19.86 ± 6.91
Progesterone (ng/ml)	1.97 ± 4.99	*0.35 ± 0.53
Testosterone (ng/ml)	44.31 ± 6.45	45.09 ± 7.36

In this study the result was indicate a significant changes in hormonal level in osteoporosis women compared with women of the same age with no osteoporosis. Testosterone hormone have no significant changes in all age in osteoporosis women compared with healthy women in the same age.

Normal value of testosterone in osteoporosis women's may be due to the fact that the hormone has little effect to maintaining bone density in women, which depends mainly on estrogen, that plays an important role in maintaining bone health **(Goldstat., *et al* 2003).**

The circulating concentration of FSH may play an important role in the acceleration of bone loss in osteoporosis women**, (Jie Wang., *et al* 2015)** pointed that FSH, LH serum concentrations in women with osteoporosis increased by effect on cell differentiation into mature osteoclasts. While **(Sowers., *et al* 2013)** add that mRNA expression of genes involved in osteoclast phenotypes and function increase FSH and LH hormone in osteoporosis women.

A study by **(Rendina., *et al* 2010)** reported that FSHR gene polymorphisms are associated with bone mineral density and bone turnover in postmenopausal women. The FSHR inhibited the osteoclast formation induced by FSH to an extent similar to that noted in FSHR knockout cells.

(Tourkoval., *et al* 2012) concluded that high concentration of FSH binds to the FSHR and inhibits the α subunit of G-protein to activate the signaling proteins associated with cell proliferation, ultimately stimulating the formation of osteoclasts and bone resorption. Whereas, bone mass is significantly decreased in patients with amenorrhea and increased serum FSH and LH levels due to this hormones is an independent predictor of bone loss, The increase during per-menopause, in which serum estrogen does not decline, may cause the loss of bone mass in women with osteoporosis disease **(Anasti., *et al* 1998).**

Furthermore, the decrease of estrogen hormone in osteoporosis patients due to increase the proliferation of osteoblasts and the expression of different genes that encode enzymes, bone matrix proteins, transcription factors, hormone receptors, growth factors and cytokines **(Manolagas, 2000).** In addition, (**Almeida., *et al* 2013**) reported that estrogen primarily acts on bone as an anti resorption agent by reducing osteoclast numbers and osteoclast function, but estrogen receptors have been demonstrated on both osteoblasts and osteoclasts. **(Villiers, 2015)** reported that estrogen deficiency in osteoporosis patients causes an increase in active osteoclasts with increased bone resorption and loss of bone mineral density may be by effect in cytokines and the receptor activator of nuclear factor (RANKL) system. Binding of different cytokines to their receptors in osteoblasts is hypothesized by **(Lorenzo., *et al* 2017)** to result in the release of soluble factors that act directly on osteoclasts to modulate their recruitment or activity and inhibit the release of osteoclast stimulatory factors or could enhance the release of osteoclast inhibitory factors.

(Gowen., *et al* 2003) add that a number of cytokines and growth factors appear to modulate bone resorption and could play roles in the coupling of bone formation to bone resorption. This Cytokines have been reported by **(Ishimi., *et al* 2010 and Hughes., *et al* 2016)** that increase bone activity which include colony stimulating factor (GM-CSF) macrophage-colong stimulating factor

(M-CSF) tumor necrosis factor-a (TNF-a), interleukin-1 (IL-1) and interleukin-6 (IL-6).

The inhibition of estrogen in osteoporosis women may be by cytokines, the role of these cytokines in the pathogenesis of estrogen-deficiency–related bone loss are enhancers of osteoclast function, IL-6 probably acting mainly at the levels of osteoclast generation and IL-1 and TNFs acting more (but not exclusively) at the level of the mature osteoclast **(Ernst., *et al* 2009)**.

(Ottoson, 2013) showed that progesterone play an important role in bone health, it is made in the ovaries of menstruating women and considered a precursor to most steroid hormones and performs a myriad of different functions.

The decrease in progesterone level in women with osteoporosis disease was introduced by **(Bergkvist., *et al* 2011)** progesterone rebuilds bone by stimulating the osteoblast cells that re-mineralize and restore bone mass, so supplementing with natural progesterone has proved useful to prevent and heal osteoporosis.

(Lee, 1991) add that osteoporosis becomes most severe following menopause when women bodies stop producing progesterone because progesterone consider a key to maintaining healthy bones. In addition, (**Popat., *et al* 2014)** clarify when a woman reaches to mid-thirties she may fail to ovulate every period (an ovulatory cycle), leading to a decline in progesterone production that will mean increased risk factor of osteoporosis, which causes a decrease in new bone formation. Other researcher (**Biason., *et al* 2015**) suggests that decrease progesterone level in osteoporosis women probably by progesterone have receptors are present in osteoblasts play a role in bone formation, therefore when progesterone decrease the receptor cannot combine enough with hormone but when adding progesterone will actively increase bone mass and density and can reverse osteoporosis.

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