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The Role of Some Hormones and Interleukins and Their Relationship with Vitamin D3 Concentration in Osteoporosis Patients

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***Abstract*— The role of Serum Hormones concentration (estrogen and calcitonin) , Interleukins (IL6, IL 33) , Vitamin-D3 and calcium Is a Major Predictor of Osteoporosis in Women and Men, Osteoporosis affects men and women of all races. But women are more than men especially older women over menopause.**

Collection (55) blood sample from patients (30 female and 25 male) those patients suffering from Osteoporosis in Al-Hussein Teaching Hospital in a province Thi-Qar, Iraq. the period of study from October / 2019 to January /2020. The patients' ages ranged from (22-70 years) , while the control group included (33) included samples (20) a sample of women and (13) a sample of men healthy with age (22-70).

3ml of blood samples were collected into plain centrifuge tubes, at room temperature for clotting. Serum was separated by centrifugation at 3000g for 30 min and analyzed, for determination concentration of Estrogen, Calcitonin, Vitamin –D3, Interleukin 6, Interleukin 33 and Calcium .

The results showed a significant decrease (P≤0.05) in Estrogen, Ca^{+2} and vit- D3 and significant increase (P≤0.05) in IL-6 and IL-33 in the second group (patients) compared with the first group (control). Also, there was non-significant difference of calcitonin in the second group compared with the first group.

Keywords*— *Osteoporosis, Calcitonin, interleukins (6- 33), Ca^{+2}, Vit-D3.

I. INTRODUCTION

The Bone is a mineralized connective tissue with four cell kinds: osteoblasts, bone lining cells, osteocytes, and osteoclasts (Bala *et al*., 2016) . Bone performs significant tasks in the body including locomotion, soft tissue support, protection, calcium and phosphate storage and bone marrow harboring (Buo and Stains, 2014). Several diseases cause various abnormalities or bone deformities such as rickets, osteomalacia , osteogenesis imperfecta, marble bone disease (osteoporosis), bone disease Paget, fibrous dysplasia and osteoporosis (Buo and Stains, 2014) . Clinically speaking, bone disease can lead to bone pain and height loss (due to vertebrae compression) and the patients bones more are predisposed to fractures ,the most common bone disease is osteoporosis (Bala *et al*., 2016).

Osteoporosis is a chronic disease characterised by bone fragility. Bone fragility predisposes to minimal trauma fractures like many common chronic diseases such as diabetes mellitus, hypertension and hyperlipidemia, or Osteoporosis is a systemic disorder characterized by reduced bone mass and micro architectural tissue deterioration resulting in bone fragility and increased susceptibility to hip, spine and neck fractures (Bala *et al*., 2016; Tian *et al*., 2017). More than half of all individuals older than the age of 50 are affected by poor bone health, osteopenia and/or osteoporosis, and the prevalence is expected to rise for many years. Fragility fracture rates along with their inherent morbidity and mortality are likewise predicted to rise (Friedman and Mendelson , 2014; Miller *et al*., 2015). Calcium is one most abundant minerals in the body . About 99% of calcium of human body is found in the bones and teeth and also 1% is in blood, muscles and another body tissues (including nerves, lungs, etc.) This 1 percent plays an essential role in our health in normal contraction and relaxation of muscle, nerve functions , blood pressure, blood clotting as well as immune responses(Silanikove *et al*., 2015). Vitamin D3 is produced in the human skin after exposition to UV (ultraviolet)-B.
This regulates homeostasis of calcium and phosphorus and thus plays important role in the mineralisation of bones (Piotrowska *et al*., 2016).

Hormones are really important to bone strength. Hormones are chemicals made by glands that travel throughout the body and have many effects on growth, maturation, energy, weight, and bone strength. Sex hormones (estrogen) made in the ovary of females reason that bone strength increases in the early teenage years. When teenagers have low estrogen levels, the bone becomes weaker, Estrogen deficiency can lead to excessive bone resorption accompanied by inadequate bone formation. Osteoblasts, osteocytes, and osteoclasts all express estrogen receptors. In addition, estrogen affects bones indirectly through cytokines and local growth factors. (Lobo *et al*,. 2016; Bednarska and Siejka, 2017; Monique *et al.*, 2019) .

Calcitonin (CT) is synthesized by C cells located in the thyroid secrete to the circulation, and act as a hormone throughout the body, Calcitonin is an endogenous regulator of calcium homoeostasis,
acting principally on bone. It also has a direct action on the kidneys and gastrointestinal secretory activity (Felsenfeld and Levine , 2015 Apr; Tanaka
et al., 2017May).

There are many of interleukins that effect on bone such as Interleukin-6 is involved in a spectrum of age-associated diseases such as osteoporosis, cytokine with a wide range of biological activities . It is also an indicator of inflammation within the body, It is produced by macrophages and monocytes in response to other inflammatory cytokines containing tumor necrosis factor (TNF) beta and interleukin-11 . Enhancement of IL-6 level is observed in the ongoing processes of aging and menopause which is manifested by osteoclast activation (Khosla *et al.*, 2002; Lazzaro *et al*., 2018).

Interleukin 33 (IL-33) is a protein that in humans is encoded by the IL33 gene. Interleukin 33 is a member of the IL-1 family that potently drives production of T helper-2 (Th2)-associated cytokines . Interleukin 33 (IL-33) is a cytokine belonging to the IL-1 superfamily. IL-33 induces helper T cells, mast cells, eosinophils and basophils to produce type 2 cytokines. This cytokine was previously named NF-HEV 'nuclear factor (NF) in high endothelial venules' (HEVs) since it was originally identified in these specialized cells. IL-33 acts intracellularly as a nuclear factor and extracellularly as a cytokine (Yagami *et al.*, 2010; Mirchandani AS *et al*., (August 2012); Cohen *et al*,. 2015 ; Fu *et al.*, 2016 ; Gorbacheva *et al.*, 2018).

II. Methods and Materials

Collection (55) blood sample from patients (30 female and 25 male) those patients suffering from Osteoporosis in Al-Hussein Teaching Hospital in a province Thi-Qar, Iraq. the period of study from October / 2019 to January /2020. The patients(second group) with age ranged from (22-70 years) , while the control group(first group) included (33) included samples (20) a sample of women and (13) a sample of men healthy with age (22-70).

3ml of blood samples were collected into plain centrifuge tubes, at room temperature for clotting. Serum was separated by centrifugation at 3000g for 30 min and analyzed, for determination concentration of Estrogen, Calcitonin, Vitamin –D3, Interleukin 6 , Interleukin 33 and Calcium .

1- Calcium

Calcium level was measured in serum by (Tietz , 1999) with using equipped kit from company (Biolabo) and extracted calcium concentration by following equation :

Calcium concentration (mg\dl) =Abs (Assay) \Abs (Standard) ×Standard concentration

2- Vitamin d3

Vitamin D3 level was measured in serum by (Holick, 2009) with using kit from company (Monobind Inc .) and extracted vit-d3 concentration by ELISA method.

3-Estrogen

Estrogen level was measured in serum by using kit from company (Monobind Inc) and extracted estrogen concentration by ELISA method (Abraham,1981).

4- Calcitonin

Calcitonin level was measured in serum by using prepared kit from company (Bioassay Technology Laboratory) and extracted calcitonin concentration by ELISA method (Lequin, 2005).

5-Interleukin 6

Interleukin 6 level was measured in serum by using prepared kit from company (Bioassay Technology Laboratory) and extracted Interleukin 6 concentration by ELISA method (Lequin , 2005).

6-Interleukin 33

Interleukin 33 level was measured in serum by using prepared kit from company (Bioassay Technology Laboratory) and extracted Interleukin 33 concentration by ELISA method (Lequin , 2005).

A. Statistical Analysis

A Student's t-test was used. The data are presented as means ± S.D. and statistically analyzed using SPSS (version 16).Significance was set at the level of ($P \leq 0.05$).

III. Results

The results showed a significant decrease ($P \leq 0.05$) in Estrogen, Ca and vit. D_3 and a significant increase in IL-6 and IL-33 in the second group (patients) compared with the first group (control). Also, there was non-significant difference of calcitonin in the second group compared with the first group, (Table1).

TABLE1. compare between some hormones, Interleukins and some bone minerals in patients group and control group

Parameter	First group (Control) Mean ±SD	Second group (Patient) Mean ± SD	P-value
E2 (pg\ml)	65.02 ±30.01 [a]	42.39 ±41.78 [b]	**0.008**
CT (mmol\L)	33.15 ±17.77 [a]	34.24 ±20.19 [a]	0.79
IL-6 (ng\L)	39.23 ±17.51 [b]	73.17 ±39.21 [a]	**0.000**
IL-33 (ng\L)	140.97 ±79.82 [b]	259.69 ±251.3 [a]	**0.02**
Ca (mmol\L)	2.34 ±0.16 [a]	2.01 ±0.22 [b]	**0.000**
Vit-D3 (ng\ml)	41.4±5.81[a]	33.06±23.08[b]	**0.01**

Values are means ± S.D.

Different letters refer to a significant difference ($p \leq 0.05$).

Same letters refer to non a significant differences ($p \leq 0.05$).

The results showed a significant decrease ($P \leq 0.05$) in Estrogen, IL-6, IL-33 and Ca in the second group (patients) compared with the first group (control). Also, there was non-significant difference of calcitonin and Vit. D_3 in the second (Patients) group compared with the first group (control), (Table 2).

TABLE2. compare between some hormones, Interleukins and some bone minerals in Male patients group and control group

Parameter	First group (Male Control) Mean ±SD	Second group (Male Patient) Mean ± SD	P- value
E2 (pg\ml)	60.18 ±23.72 [a]	36.67 ±18.41 [b]	**0.002**
CT (mmol\L)	35.16 ±18.52 [a]	31.08 ±18.51 [a]	0.52
IL-6 (ng\L)	38.13 ±16.63 [b]	68.35 ±36.9 [a]	**0.001**
IL-33 (ng\L)	158.26 ±67.004 [b]	301.71 ±304.09 [a]	**0.03**
Ca (mmol\L)	2.37 ±0.19 [a]	2.11 ±0.22 [b]	**0.001**
Vit-D3 (ng\ml)	42.61±6.27[a]	46.69±17.02[a]	0.29

Values are means ± S.D.
Different letters refer to a significant difference ($p \leq 0.05$).
Same letters refer to non a significant differences ($p \leq 0.05$).

The results showed a significant decrease ($P \leq 0.05$) in IL-6, IL-33, Ca and vit. D_3 in the second group (patients) compared with the first group (control). Also, there was non-significant difference of estrogen and calcitonin in the second (Patients) group compared with the first group (control), (Table 3).

TABLE3. compare between some hormones, Interleukins and some bone minerals in Female patients group and control group

Parameter	First group (Female Control) Mean ±SD	Second group (Female Patient) Mean ± SD	P- value
E2 (pg\ml)	68.17 ±33.69 [a]	47.15 ±54.02 [a]	0.12
CT (mmol\L)	31.85 ±17.62 [a]	36.88 ±21.45 [a]	0.38
IL-6 (ng\L)	39.94 ±18.45 [b]	77.18 ±41.22 [a]	**0.000**
IL-33 (ng\L)	129.73 ±86.93 [b]	224.67 ±195.63 [a]	**0.04**
Ca (mmol\L)	2.32 ±0.15 [a]	1.92 ±0.19 [b]	**0.000**
Vit-D3 (ng\ml)	40.62±5.51[a]	21.7±21.42[b]	**0.000**

Values are means ± S.D.
Different letters refer to a significant difference ($p \leq 0.05$).
Same letters refer to non a significant differences ($p \leq 0.05$).

The results showed non-significant difference ($P \leq 0.05$) in estrogen, calcitonin, IL-6 and IL-33 in the second (Patients) group compared with the first group (control), Also, a significant decrease in Ca and vit. D_3 in the second group (patients) compared with the first group (control), (Table 4).

TABLE4. compare between some hormones, Interleukins and some bone minerals in patients groups

Parameter	Patient (Male) Mean ±SD	Patient (Female) Mean ± SD	P- value
E2 (pg\ml)	36.67 ± 18.41 [a]	47.15 ± 54.02 [a]	0.35
CT (mmol\L)	31.08 ± 18.51 [a]	36.88 ± 21.45 [a]	0.29
IL-6 (ng\L)	68.35 ± 36.9 [a]	77.18 ± 41.22 [a]	0.41
IL-33 (ng\L)	301.71 ± 304.09 [a]	224.67 ± 195.63 [a]	0.28
Ca (mmol\L)	2.11 ± 0.22[a]	1.92 ± 0.19 [b]	**0.001**
Vit-D3 (ng\ml)	46.69 ± 17.02[a]	21.07 ± 21.42[b]	**0.000**

Values are means ± S.D.
Different letters refer to a significant difference ($p \leq 0.05$).
Same letters refer to non a significant differences ($p \leq 0.05$).

IV. Discussion

The study population consisted of 88 individuals including 55 patients (30 female and 25 male) and 33 control groups (20 female and 13 male) according to ages (22-70) years.

The results of the current study show a significant decrease in the level of estrogen in male patients and thus agree with a study (Lan T Ho-Pham *et al.*, 2013) which showed that a decrease in the level of estrogen associated with an increase in the marker of bone resorption in men and women, can affect the deficiency of the hormone Estrogen is applied directly to the bone by increasing bone resorption and also indirectly via interleukins and local growth factors.

The aim of this review was to examine the evidence regarding the influence of hormones on bone metabolism, followed by clinical data of hormonal changes in the elderly, in the attempt to provide possible poorly explored diagnostic and therapeutic candidate targets for the management of primary osteoporosis.

Calcitonin hormone contributes to calcium homeostasis by direct inhibition of osteoclast-mediated bone resorption and by enhancing calcium excretion by the kidney mediated by high affinity calcitonin receptors (CTRs) (Rachel and David, 2013). When calcium enters the bloodstream, osteoclasts break down bone tissue, so calcitonin reduces the amount of calcium in the blood by avoiding bone deterioration as well as the hormone tipping It also reduces the level of calcium that the kidneys reabsorb, thus reducing its levels. Release of this hormone is directly regulated by the calcium levels in the blood. When the rates begin to rise, the body responds with increased levels of calcitonin.

The results of the current study showed that there was non-significant difference in the level of calcitonin between of patients groups and the control group, as well as between male and female patients groups , and this contradicts the results of J.J.Reynolds, (1968) in him study where he showed that calcitonin reduces calcium in the blood and also showed that the bone is the first site for the action of this hormone.

Cytokine mediates the effect of the immune and inflammatory response on the bones. Not all cytokines involved in bone remodeling have an osteoclast genic effect, however: some cytokines contribute to bone resorption, others play protective roles against osteoporosis, while others still have multi-influencing effects. The results of our study showed an increase in the levels of IL-6 in the patient groups compared to the control group, and this is consistent with a previous study by Nasir Muhammad *et al.,* (2017) This study showed that osteoporosis is a pro-inflammatory condition with higher serum interleukin levels. These results indicate inflammation development is a significant component of osteoporosis . Also we found elevated rates of interleukin-33 in patients which may be due to type-2 immunity and allergic diseases of airway , this against clinical results to Lia Ginaldi *et al.,* (2019) that explained presence low levels of IL-33 in osteoporotic patients and showed that interleukin -33has anti-osteoclastic influence that inhibit differentiation of osteoclasts .

As well as, the results of the current study also showed a significant decrease in calcium Ca^{+2} levels in female patients group, and this consistent with study (P. Modagan *et al.,* 2018). which showed a significant decrease in the level of calcium due to hormonal imbalance and malabsorption, and this may lead to bone disorders, and also Lia Ginaldi *et al*., (2019) study indicated that vitamin D (25 OH) deficiency was associated with decreased density this agree with our work that show deficiency in female patients group that may be due to inadequate sunlight exposure ,enough vitamin D3 in food ,and pathogenic disorders including gastrointestinal ,renal and liver diseases .

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