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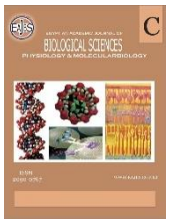
Citation: *Egypt.Acad.J.Biolog.Sci. (C.Physiology and Molecular biology) Vol. 15(2) pp915-921 (2023)*
DOI: 10.21608/EAJBSC.2023.330659

Egypt. Acad. J. Biolog. Sci., 15(2):915-921 (2023)

Egyptian Academic Journal of Biological Sciences
C. Physiology & Molecular Biology
ISSN 2090-0767
www.eajbsc.journals.ekb.eg



Estimation of the Levels of IL-6, IL-8, and CRP in Women Patients of Osteoporosis and Heart diseases in Samarra city

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ARTICLE INFO
Article History
Received:16/11/2023
Accepted:17/12/2023
Available:21/12/2023

Keywords:
Heat diseases, Osteoporosis, inflammation, CRP, IL-6, IL-8.

ABSTRACT

A Cross-Sectional study that includes an assessment of the relation of the physiological and immunological factors among postmenopausal women with osteoporosis and heart diseases who attended Samarra General Hospital in Salah AL-Din Governorate The study started from January 2022 to November 2022 on a study population whose ages ranged from 45-70 years, their total number is 100 women, 25 of whom are healthy women who are considered as a control group, and 75 of them are represented by groups of patients that were distributed into three main groups: 25 women with Heart disease only include myocardial infarction and 25 women with osteoporosis only and 25 women with Heart disease and osteoporosis blood samples were collected from patients and healthy subjects and then serum separated for testing. The present study was designed to obtain more clarification of some Immunological changes in women patients with osteoporosis and heart diseases. and find out the role of some Immunological parameters of pro-inflammatory cytokines like Interleukin – 6 IL-6, Interleukin- 8 IL-8 and C-reactive protein CRP in the serum of women patients with osteoporosis and heart diseases the result of Immunological parameters include: Interleukin-6, Interleukin IL-8 showed had no significant differences ($P \geq 0.05$) in a group of osteoporosis in comparison with the control group and showed significantly increased ($P \leq 0.05$) in groups of Heart disease, and osteoporosis with heart disease in comparison with control group. but (CRP) showed a significant increase ($P \leq 0.05$) in groups of osteoporosis, Heart disease, and osteoporosis with heart disease in comparison with the control group.

To investigate the physiological relationship between Immunological parameters, interleukin 6 and interleukin 8 and high sensitivity C-reactive protein in patients of Osteoporosis and Heart diseases in Samarra city.

Serum interleukin-6 and Interleukin IL-8 showed no significant differences ($P \geq 0.05$) in the group of osteoporosis in comparison with the control group and showed significantly increased ($P \leq 0.05$) in groups of Heart disease, and osteoporosis with heart disease in comparison with the control group , . but (CRP) showed a significant increase ($P \leq 0.05$) in groups of osteoporosis, Heart disease, and osteoporosis with heart disease in comparison with the control group.

INTRODUCTION

Osteoporosis (OP) and cardiovascular diseases are common diseases encountered globally, especially with advancing age. Osteoporosis occurs when there is a loss of bone mineral density leading to increased predisposition to fragility fracture.

The conventional perception of osteoporosis is pure as metabolic bone disease, however, there are mounting reports from a recent study that osteoporosis could be seen as a risk factor for cardiovascular disease just like another traditional factor such as hypertension, dyslipidaemia and diabetes (Yang and Huang , 2023). Parathyroid hormone has many effects, on bone, but higher levels of PTH catabolic effects prevail and impact cortical bones in particular (Goltzman, 2018). Although high levels of PTH have been related to a higher risk of fractures, prospective observational studies have found links between PTH levels and cortical bone degradation (Kužma *et al* ., 2921). Estrogens can be used to prevent common postmenopausal conditions such as osteoporosis and ischemic heart disease and have been shown to decrease the rate of osteoporosis and colorectal cancer, (Philipp *et al* ., 2023).

MATERIALS AND METHODS

A cross-sectional study was done in Samarra General Hospital on patients from Samarra, in Salah-Aladdin governorate. The study started from January to November 2022 on the study population age ranged from (45 to 70) years old. The total subjects were 100 female individuals 25 individuals in the control group and 75 individuals in the patient groups, (Abdullah *et al*.,2019) who volunteered to take part in the research and were recruited and separated into four main groups as follows:

Group 1: Twenty-five subjects, apparently normal and healthy as control.

Group 2: Twenty-five Patients with Heart disease and osteoporosis.

Group 3: Twenty-five patients with Heart disease (MI) only.

Group 4: Twenty-five patients with osteoporosis only

Serum Samples Treatment: Approximately 5 ml of fasting human blood was collected from each subject (patients and control) and transferred into sterilized test tubes and allowed for 30 minutes to clot at room temperature. The sample was centrifuged for 15 minutes at 3000 rotations per minute and the serum was immediately separated and stored at (- 20 ^{0} C) till used for Calcitonin Estrogen and Parathyroid. (Abdulbaqi *et al.*,2018).

Determination of Calcitonin (CT) in Serum:

Calcitonin concentration in the serum of osteoporosis with heart disease patients was estimated depending on the kit procedure is an Enzyme-Linked Immunosorbent Assay (ELISA) from Bioassay Technology, China.

Determination of Estrogen (E_2) in Serum:

Estrogen concentration in the serum of osteoporosis with heart disease patients was estimated depending on the kit procedure is an Enzyme-Linked Immunosorbent Assay (ELISA) from Bioassay Technology, China. (Al-Tekreeti *et al.*,2017).

Determination of Parathyroid (PTH) in Serum:

Parathyroid concentration in the serum of osteoporosis with heart disease patients was estimated depending on the kit procedure is an Enzyme-Linked Immunosorbent Assay (ELISA) from Bioassay Technology, China.

Statistical Analysis:

All data were presented as a mean ± standard deviation by ANOVA test and to compare the mean of different variables used Duncan multiple range test. The significant level is P value ≤ 0.05. (M.T. *et al.*,2019).

RESULTS AND DISCUSSION

1- Levels of Calcitonin (CT) in Patients and Control Groups:

The results in Table (1), showed a significant decrease (P≤0.05) in groups of osteoporosis, Heart disease, and osteoporosis with heart disease in comparison with control group.

Table 1: Calcitonin (ng/ml) in control and Patient groups**.**

Groups	No. of Individuals	Calcitonin (ng/ml)
Control	25	50.20±7.20 a
Osteoporosis	25	19.30±2.40 d
Heart disease	25	38.50±5.10 b
Osteoporosis with Heart disease	25	24.50±3.60 c

In the present study, Serum levels of Calcitonin (CT) were significantly decreased (P≤0.05) in postmenopausal osteoporosis women when compared with the control group. (Hussain *et al.*,2018).

These results agreed with (Shamsulddin, 2020; Al-Samarrai, 2022) and the present study agrees with a previous study which found a significant reduction in serum calcitonin in women patients with osteopoprosis and or coronary heart diseases complicated by chronic heart failure, (Marushchak, and Krynytska, 2019).

Thyroidal C cells release a calcium-lowering factor, which has an influence on bone remodeling. CT has been proven to work via interacting with the CT receptor (CTR). CT links to osteoclasts, which have the highest CTR density and inhibits their action in bone. PTH, which triggers bone resorption by changing gene expression in osteoblasts, was thought to be its functional equivalent (Davey and Findlay, 2013; Gosink ., 2015). Calcitonin hormone helps to maintain calcium homeostasis by inhibiting osteoclast-mediated bone resorption and increasing calcium outflow via high-affinity calcitonin receptors in the kidney (Davey and Findlay, 2013).

Calcitonin has been shown in several publications to enhance bone mineral density (BMD) and the higher rate of vertebral fractures in osteoporosis patients. (Mahmood Z. F. *et al.*,2023). Although low BMD has been linked to an increased risk of fracture in several studies, improvements in BMD alone cannot explain the anti-fracture effectiveness of antiresorptive medications like calcitonin. By slowing bone turnover and maintaining the integrity of the trabecular architecture, therapies that moderately improve BMD might minimize fracture risk in osteoporotic patients, preserving bone strength and quality. CT has been shown to help patients with pain from a variety of causes, including osteoporosis-related acute vertebral fractures, Paget's disease, bone cancer, and other musculoskeletal diseases. (Keller *et al* ., 2014) .

While calcium levels in the circulation rise, osteoclasts destroy bone tissue, therefore CT lowers calcium levels in the blood by preventing bone degeneration and hormone imbalance. It also lowers the amount of calcium reabsorption by the kidneys, lowering calcium levels, (Hussein *et al*.,2019).Calcium levels in the blood directly control the release of this hormone. When the rates start to climb, the body responds by producing more CT. Low levels of CT may be attributable to reduced serum calcium in post-menopausal women, as well as elevated levels of P.T.H., which is the inverse of calcitonin levels. (Al-Samarrai , 2022) .

Previous studies suggested that patients with osteoporosis have a higher risk of CHD than those without osteoporosis, (Li *et al* ., 2014). Patients who have osteoporosis and have received treatment with Calcitonin have a significantly lower risk for CHD than those without treatment, (Farhat and Cauley, 2008; Marushchak, and Krynytska, 2019).

2- Levels of Estrogen (E_2) hormone in Patients and Control Groups:-

The results in Table (2), showed a significant decrease (P≤0.05) in groups of osteoporosis, Heart disease, and osteoporosis with heart disease in comparison with control group.

Table 2: Estrogen (E2) (ng/ml) in control and Patient groups.

Groups	No. of Individuals	Estrogen (ng/ml)
Control	25	19.50±1.60 a
Osteoporosis	25	18.40±1.40 b
Heart disease	25	16.30±0.80 c
Osteoporosis with Heart disease	25	14.20±0.40 d

These results agree with previous work which found that the serum level of Estradiol in young and middle-aged healthy women was negatively correlated with age, (Venkat *et al* ., 2009). Estrogen plays a role in women before menopause by preventing the activation of RAAS (the renin-angiotensin-aldosterone system). (Nouri *et al.*,2015).If menopause is associated with ovarian estrogen loss, the pathogenesis of diastolic dysfunction will occur, resulting in an increase in angiotensin II and NOS (nitric oxidase synthase), and ROS (reactive oxygen species) that contribute to hypertension (Sabbatini and Kararigas, 2020).

Estrogen promotes the apoptosis of osteoclasts and inhibits osteoclastogenesis via several pathways. Estrogen not only stimulates the production of OPG, but also reduces the differentiation of osteoclasts by suppressing IL-1 and TNF, therefore inhibiting the release of M-CSF, RANKL, and IL-6. Estrogen promotes the apoptosis of osteoclasts via the effect of TGF-β. Estrogen deficiency leads to the uncoupling of bone resorption and formation, which means an increased osteoclastic resorption without a corresponding osteoblastic activity (Nemat J. A. *et al.*,2015). The osteoblastic activity fails to catch up with increased osteoclastic resorption, therefore resulting in greater bone loss. RANK ligand (RANKL) appears to be the critical uncoupling factor that enhances osteoclastogenesis (Wang *et al* ., 2023). During estrogen deficiency, both the production of TNF and the sensitivity to IL-1 of stromal cells increase, stimulating stromal cells to release IL-6, M-CSF, IL-11, GM-CSF, and TGF. The cascade leads to the secretion of RANKL from osteoblasts, binding to RANK on osteoclasts, and promoting osteoclast development. (Seddiq *et al.*,2022).

On the other hand, osteoprotegerin (OPG) is an antagonist against RANKL secreted by osteoblast lineage cells, and it contributes to the anti-resorptive actions of estrogen (ofer *et al* ., 2019).

3. Levels of Parathyroid hormone (PTH) in Patients and Control Groups:-

The results in Table (3) showed a significant increase (P≤0.05) in groups of osteoporosis, Heart disease, and osteoporosis with heart disease in comparison with the control group.

Table 3: PTH (ng/ml) in control and Patient groups.

Groups	No. of Individuals	Parathyroid (ng/ml)
Control	25	5.70±0.90 d
Osteoporosis	25	15.10±3.70 c
Heart disease	25	18.13±4.20 b
Osteoporosis with Heart disease	25	23.14±6.50 a

The present study found that serum levels of parathyroid hormone (PTH) were significantly increased (P≤0.05) in postmenopausal osteoporosis and Heart disease women when compared with the control group, Table (3).

The present study agrees with previous findings which found a highly significant elevation in serum PTH in women with osteoporosis (Hagstrom, 2006; Nawer *et al*, 2022).

Parathyroid hormone regulates calcium homeostasis by operating on numerous organ systems in order to preserve normocalcemia. PTH also stimulates the release of calcium from bone by distributing calcium from a readily accessible pool in the bones into the blood, whereas the revealed levels of PTH result in increased bone resorption (Lombardi *et al*.,2020).

Moreover, in previous studies, although younger women have shown an inverse relationship between PTH and 25OHD, The concentration of 25OHD required for the achievement of optimum peak bone mass is unknown, (Mendes *et al* ., 2019).

Parathyroid hormone has many effects, on bone, but higher levels of PTH catabolic effects prevail and impact cortical bones in particular (Osagie-Clouard *et al.*,2017; Goltzman,2018).

Also, another previous study found that Serum vitamin D level and parathyroid hormone concentration had a negative association ($p<0.01$, $r= 0.26$). (Lips *et al.,* 2006).

Furthermore, numerous studies have linked excessive PTH levels to enhanced bone loss and fracture risk and an association between PTH and 25(OH)D levels was already postulated as a negative impact of high PTH levels on BMD (Mendes *et al*.,2019; Bover *et al.*, 2020).

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