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COMPARISON OF SOME INTERLEUKINS LEVELS IN POSTMENOPAUSAL OSTEOPOROTIC WOMEN WITH OR WITHOUT DIABETES TYPE II

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Abstract:
Osteoporosis is a disease causes bone damage, it is typically linked to postmenopausal, but can also be caused by diabetes mellitus.
Present study goaled to assess serum levels of Interleukin-6, interleukin-31, and interleukin-33 in postmenopausal Iraqi women with osteoporosis and those with osteoporosis and type II diabetes, 120 postmenopausal women (their ages ranged between 46-68 years) diagnosed biochemically and radiologically with osteoporosis only or osteoporosis and type II diabetes were included in present study and divided into 3 groups (healthy subjects, postmenopausal women with osteoporosis, and osteoporotic postmenopausal women with type II diabetes).
The study revealed that IL-6, IL-31, and IL-33 were increased significantly ($P<0.05$) in both patient's groups compared to healthy subjects, and these increases were highest in osteoporotic postmenopausal women with type II diabetes compared to those with osteoporosis only.
This study concludes that diabetes type II increases inflammation in osteoporotic postmenopausal.

Key words: IL-6, IL-31, IL-33, Osteoporosis, Type II diabetes

Introduction

Osteoporosis is a disease that slowly deteriorates the microstructure of bone, It is typically linked to aging and a lack of estrogen, but can also be caused by diabetes mellitus (DM).
An increase in bone fragility and fracture susceptibility are symptoms of osteoporosis, a systemic skeletal illness marked by low bone mineral disorder (BMD) and micro architectural degeneration of bone tissue. Postmenopausal osteoporosis is the primary osteoporosis that affects menopausal women the most frequently. The significant consequences of osteoporosis, such as low-energy fractures, which raise the risk of morbidity and mortality, especially in the elderly, are what give the condition its clinical significance (Marozik *et al*., 2021; Abdulwahed,*et al*.,2020).
After menopause, estrogen levels significantly decrease, leading to postmenopausal osteoporosis, a systemic illness that is defined by bone mass loss and an increased risk of bone fractures (Fischer & Haffner Luntzer, 2021).
Chronic hyperglycemia and metabolic abnormalities are features of the metabolic illness diabetes. Diabetes patients have lower bone turnover, lower bone density, and a higher fracture risk. Type I diabetes (TID) and type II diabetes (TIID) are also associated with osteoporosis (Hygum& Langdahl, 2019).

There are numerous interleukins that affect bone, including Interleukin-6 (IL-6), IL-31, and IL-33, which play a role in a variety of age-related diseases like osteoporosis and are also indicators of inflammation in the body. An increase in IL-6 level is seen in the natural aging and menopause processes, which is characterized by osteoclast activation (Lazzaro *et al*., 2018).

The pro-inflammatory cytokines IL-33 and IL-31 mediate a number of immunological processes, are involved in a variety of illnesses, and play a critical role in inflammation and bone remodeling (Ginaldi *et al*., 2019). Clinical and experimental data point to an important role for the IL-33/IL-31 axis in osteoporosis and the implications of IL-31 and IL-33 in this disease (Ginaldi et al., 2019).

Locally in Iraq several studies to assess the role of several cytokines in osteoporosis or diabetes. Kashat and Ali (2021) show that both IL-6,and IL-33 is important in osteoporosis patients, while Salih and Salman (2014) show the important role of IL-33 in patients with TIID, there are no study locally in Iraq concerning comparison levels of interleukins between osteoporotic and osteoporotic with TIID patients, so the present study aimed to assess serum levels of IL-6, IL-31, and IL-33 in osteoporotic postmenopausal women and compare to those with osteoporosis and TIID.

Materials and methods

Study population and design

This was a cross sectional study, which was conducted in Dijla for Medical Rehabilitation Hospital, Specialized clinics in Tikrit. The study started from January 2022 to March 2022 on 120 postmenopausal women diagnosed biochemically and radiologically with osteoporosis only or osteoporosis and TIID whose ages are 48 years old and over until 68 years. categorized into three groups:

1-G1: included 30 of the healthy Postmenopausal subjects' women.

2-G2: included 45 Postmenopausal women osteoporotic patients.

3-G3: included 45 Postmenopausal Osteoporotic women and diagnosed with type II Diabetes Mellitus.

Included and excluded criteria

Only Postmenopausal women with confirmed osteoporosis and those with osteoporosis and TIID who agreed to be part of the study were included in this study.

Evaluation of serum concentrations of IL6, IL-31 and IL-33

Commercial kits mybiosource were used to evaluate serum concentration of these interleukins.

Statistical analysis

A program SPSS version 23 was used to summarize the results in present study as Mean±Standard deviation and the differences between all groups assumed by Duncan test.

Results and discussion

In the present study, serum levels of all studies interleukins (IL-6, IL-31, and IL-33) were significantly increased ($P<0.05$) in both patients (osteoporosis only and osteoporosis with TIID) compared to their concentration in healthy subjects.

As shown in figure 1 and table 1 the highest increase in serum level of IL-6 was showed in osteoporotic with TIID postmenopausal women (10.61±1.25) pg/ml compared with its concentration in those with osteoporosis only (9.63±1.31) pg/ml. Also both serum levels of IL-31 and IL-33 showed the same indication, as their concentration in osteoporotic with TIID

postmenopausal women reached (109.46±12.06 and 679.32±106.67) pg/ml, for both interleukins respectively, compared to (63.51±8.17 and 253.11±89.36) pg/ml in osteoporotic postmenopausal women. As shown in figure 1, figure 2, and table 1.

Table (1): Assessed serum levels of IL-6, IL-31, and IL-33 in osteoporotic and osteoporotic with TIID postmenopausal women compared to healthy postmenopausal women.

Parameter	G1 Mean±S.D	G2 Mean±S.D	G3 Mean±S.D	P-value
IL-6 (pg/ml)	8.12±1.04c	9.63±1.31b	10.61±1.25a	0.000
IL-31 (pg/ml)	50.59±4.79c	63.51±8.17b	109.46±12.06a	0.000
IL-33 (pg/ml)	186.65±69.39c	253.11±89.36b	679.32±106.67a	0.000

- Different letters mean that there is a significant difference at P≤0.05.
- G1= Control, G2= Osteoporosis only, G3= Osteoporosis with diabetes type II.

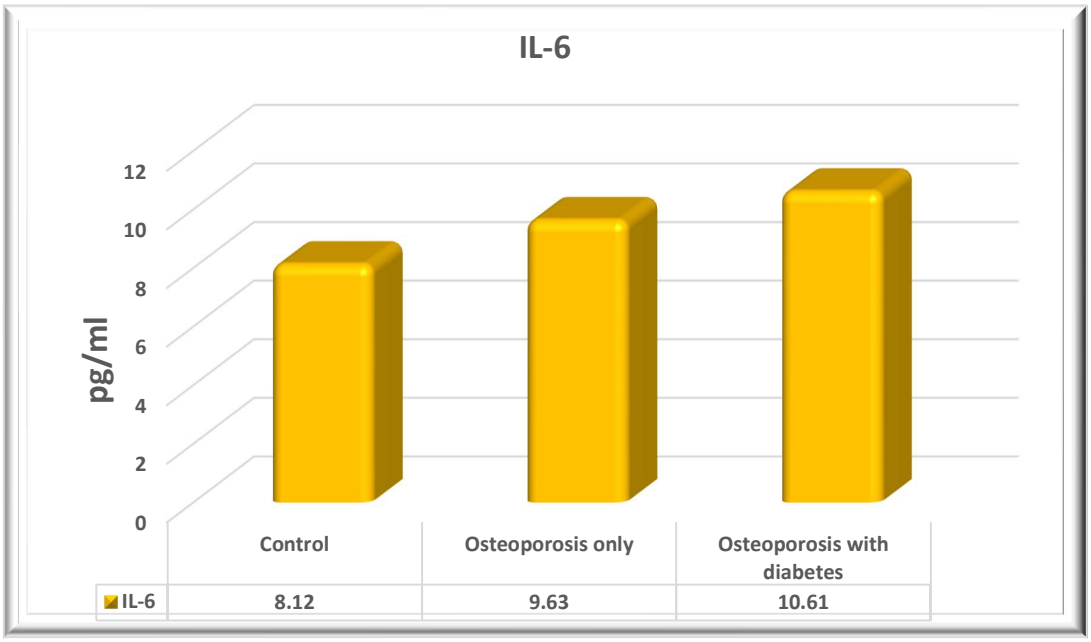

Figure (1): Assessed serum level of IL-6 pg/ml in studied groups.

- Different letters mean that there is a significant difference at P≤0.05.

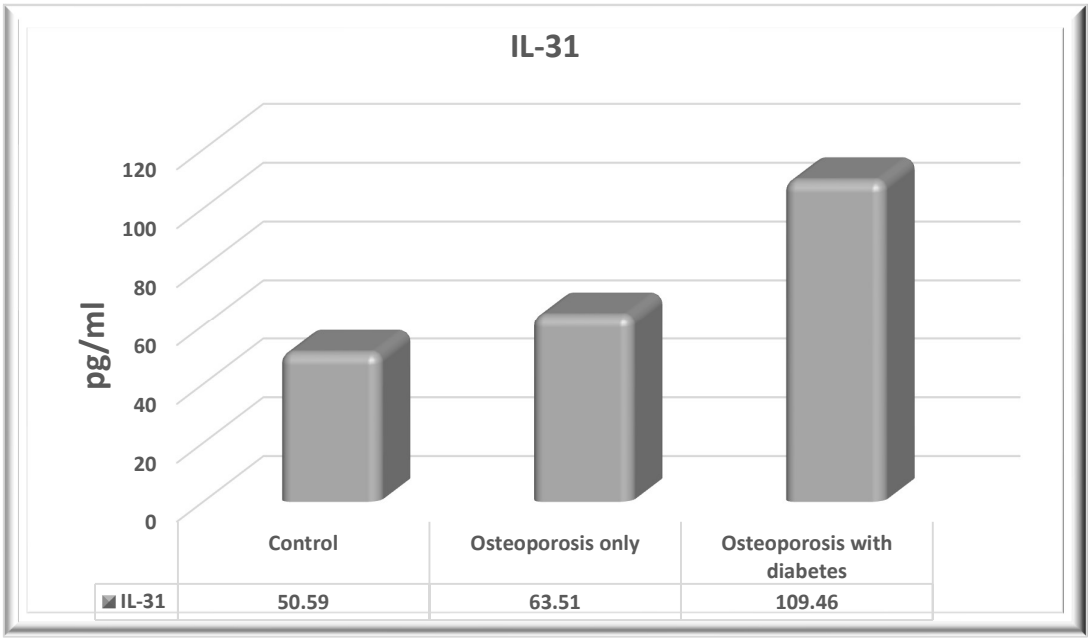

Figure (2): Assessed serum level of IL-31 pg/ml in studied groups.

- Different letters mean that there is a significant difference at P≤0.05.

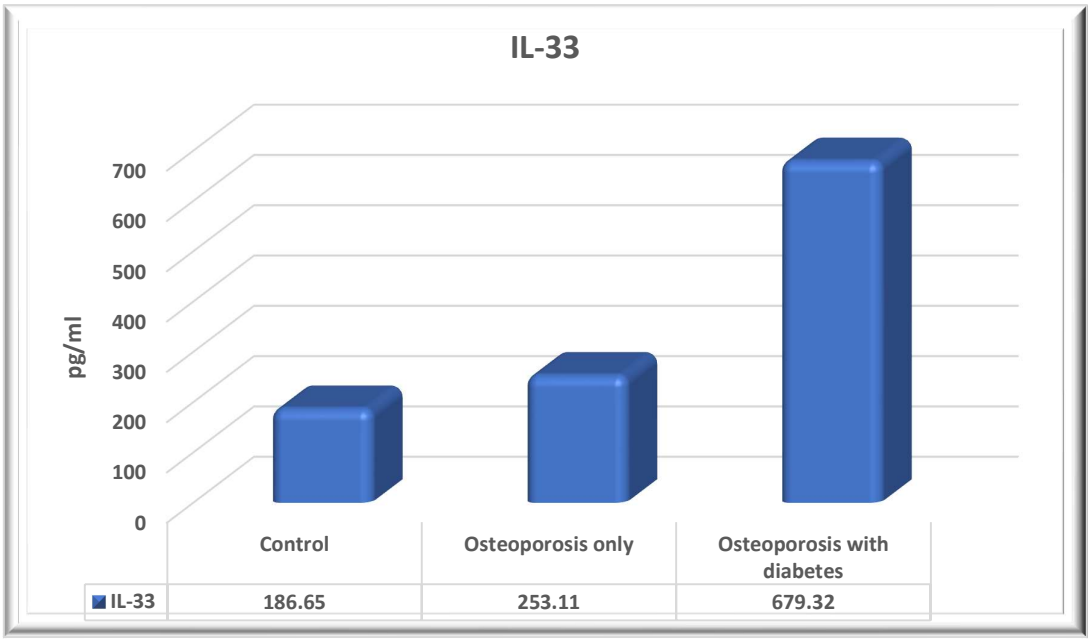

Figure (3): Assessed serum level of IL-33 pg/ml in studied groups.

- Different letters mean that there is a significant difference at P≤0.05.

After menopause, which is a stage of change in the female reproductive cycle that affects bone health and mineral metabolism, there is an increase in the production of cytokines and markers of bone turnover. Pro-inflammatory cytokines increase as ovarian function declines with menopause, causing postmenopausal bone loss and osteoporosis. Hormonal imbalances also cause physiological and biochemical changes that increase bone turnover in these women, which is evident by decreased BMD in our research findings (Rehman *et al*., 2021; Alkanaani,

et al.,2020). So, this study aimed to assess the serum concentrations of IL-6, IL-31, and IL-33 in postmenopausal women with osteoporosis and those with osteoporosis and TID.

The statistical analysis of the present study reveals a significant increase ($P<0.05$) in serum level of IL-6 compared to healthy subjects, and this agrees with recent study by Kashat and Ali (2021) which showed that IL-6 in aged women was significantly increased.

Increased IL-6 in post-menopausal women contributes to the development of osteoporosis, and it is also a major factor in inflammation and insulin resistance by impairing the internal signal that cells use to take up glucose (Akbari and Hassan-Zadeh, 2018), and this reflects the highest increase in IL-6 in the present study as showed in table (1).

Jeedigunta *et al* (2020) demonstrate that poor BMD is related to higher serum levels of hs CRP, IL-6, and TNF in both pre- and postmenopausal women. Therefore, focused anti-inflammatory therapy may have a potential role in the prevention of fast bone loss, and inflammation alone may be a significant risk factor in the development of the disease (Jeedigunta *et al*., 2020).

The findings of certain investigations support the hypothesis that inflammation significantly contributes to the etiopathogenesis of osteoporosis by influencing variables related to bone physiology and remodeling (Arron and Choi, 2000; Lorenzo, 2000). Since chronic, low-grade, systemic inflammation manifests with advancing age, the term "Inflammaging" was created to combine the processes of inflammation (within the normalrange) and aging (Fischer *et al*., 2020).

Due to a lack of estrogen, postmenopausal females experience chronic inflammation and have higher levels of circulating pro-inflammatory cytokines (Fischer *et al*., 2018). Estrogen prevents the release of IL-6, and IL-6 is a factor in the osteoclast recruitment from the monocyte cell line, a factor in osteoporosis. In females, the effects of low estrogen levels include decreased bone density and muscle mass (GUPTA and SOOD, 2022).

Understanding these cytokines can help understand normal calcium homeostasis and conditions of bone turnover like osteoporosis. Certain cytokine levels in the bone homeostasis play a crucial role in the regulation of osteoclast activity (Chen *et al*., 2018).

Present study showed increased levels of both IL-31 and IL-33 in both patient's groups with osteoporosis and osteoporotic with TIID compared to healthy women subjects and this agree with the study of ABID *et al* (2018) that show serum level IL-31 level was significantly higher among postmenopausal patients with OP compared to healthy controls.

The increased release of IL-31 by senescent inflammatory immune cells may be the cause of the elevated serum levels of IL-31 that we observed in postmenopausal osteoporotic patients, which may, in turn, contribute to the onset of full-blown OP. Through the production of chemokines and pro-inflammatory osteoclastogenic cytokines, which in turn cause the recruitment, differentiation, and activation of osteoclast precursors from the bone marrow, higher IL-31 expression may promote bone resorption (Ginaldi *et al*., 2015).

The IL-33/IL-31 axis appears to have a significant role in osteoporosis, according to clinical and experimental studies. In order to stop bone loss, IL-33 stimulates Th2 cells to secrete IL-31 and blocks RANKL-dependent osteoclast formation. As a result of limiting Th2 osteoprotective processes and influencing Th1/Th17osteoclastogenetic inflammation, IL31 favors osteoporosis (De Martinis *et al*., 2020).

The complex bone remodeling process that regulates the health of our skeleton is controlled by a number of factors, including the interaction of IL-31 and IL-33 and ultimately the combined

action of the other cytokines and suppressor and/or stimulatory factors involved in the various phases of this process (Ginaldi *et al*., 2015).

Furthermore, the elevated levels of IL-31 that we observed in elderly osteoporotic patients, which are likely due to the enhanced production of this cytokine by senescent inflammatory immune cells, may therefore aid in the onset of full-blown osteoporosis. Age-related osteoporotic patients' elevated serum IL-31 levels point to a link between bone resorption and this cytokine's overexpression (Ginaldi *et al*., 2015).

After menopause, estrogen levels drop noticeably, leading to postmenopausal osteoporosis, a systemic condition that is defined by bone mass loss and an elevated risk of bone fractures. In addition to the direct detrimental effects of estrogen shortage on bone, postmenopausal women frequently have a chronic low-grade inflammatory phenotype with altered cytokine expression and immune cell composition, which may indirectly result in continued bone loss (Fisscher and Haffner-Luntzer, 2021).

Our results agreed with the study of Ginaldi *et al* (2019) which that serum level of IL-33 increased in postmenopausal women with osteoporosis (Ginaldi *et al*., 2019).

Caner *et al* (2014) show that IL-33 level of diabetic patients was higher than the healthy group He confirmed that increased IL-33 in diabetic patients may be a result of inflammation and microvascular complications of diabetes. An increased level of IL-33 may be due to diabetes (Caner *et al*., 2014).

Ohori *et al* (2020) indicates that IL-33 plays an important role on the inhibitory effect in TNF-α-induced osteoclast genesis and bone resorption.

IL-31 limits Th2 osteoprotective processes and effected on Th1/Th17 osteoclast genetic inflammation this led to osteoporosis (De Martinis *et al*., 2020).

Nazzal *et al* (2022) found a significant association between Pruritus and diabetes mellitus, phosphate levels, level ofIL-31 high compare with control.

In chronic inflammatory illnesses, the IL-33/IL-31 axis is a hypothesized inflammatory mechanism. One of them has the capacity to promote the other's production when expressed, which results in the creation of an amplification cycle and the ensuing development of the disease (Imai, 2019).

Because pro-inflammatory cytokines are effective glycemic markers associated with type II DM, it is possible that osteoporotic postmenopausal women with TIID in this study had the highest increase in serum levels of interleukins. A significant global health issue, diabetes mellitus (DM) causes a sharp rise in mortality and serious morbidity (Al-Dahhan and Al-Dahhan, 2015), due to the action of End Products (AGEs), advanced glycation end product receptors are stimulated by AGEs (RAGE). enhanced oxidative stress as well as an increase in the production of reactive oxygen species in macrophages. When AGEs attach to AGE receptors on various cell types, cytokines are released (Kautzky-Willer *et al*., 2016).

Conclusion: IL-6, IL-31, and IL-33 are high correlated with osteoporosis in postmenopausal women, and type 1 diabetes increases in their levels.

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