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Interleukin 33 as A Marker for Early Detection of Osteoporosis in Women

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ABSTRACT

Background: Osteoporosis is a widespread skeletal disorder distinguished by diminished bone mineral density (BMD) and raised possibility of fracture, particularly in postmenopausal females. Objective: This research aimed to evaluate serum Interleukin 33 (IL-33) concentration in postmenopausal females with osteoporosis and investigate its correlation with bone turnover indicators to assess its potential as an early biomarker for osteoporosis. Methods: A case-control investigation has been performed on 100 postmenopausal women, separated into 50 osteoporotic cases and 50 age-matched healthy controls. BMD has been assessed utilizing dual-energy X-ray absorptiometry (DXA). Serum concentrations of IL-33, calcium, alkaline phosphatase (ALP), Vit D, procollagen type I N-terminal propeptide (P1NP), parathyroid hormone (PTH), and C-terminal telopeptide of type 1 collagen (CTX) have been evaluated. IL-33 levels have been determined using ELISA. Results: IL-33 concentrations were significantly reduced in osteoporotic cases (3.32 ± 2.09 pg/mL) in comparison with controls (12.01 ± 5.74 pg/mL; p below 0.001). A positive association has been discovered between IL-33 and PTH (r equal 0.398, p equal 0.02) and P1NP (r equal 0.401, p equal 0.009), while a negative association existed with CTX (r = −0.495, p = 0.0014). Significant variances were also discovered in ALP, CTX, calcium levels, and fracture incidence between groups. Conclusion: Serum IL-33, a marker of bone turnover, is significantly reduced in females with osteoporosis after menopause, suggesting it may have a protective role in bone metabolism and aid in early osteoporosis detection.

KEYWORDS: IL-33, osteoporosis, postmenopausal women, bone turnover markers.

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INTRODUCTION

Osteoporosis is a skeletal illness that primarily affects women with hallmarks that include diminished bone mineral density, disruption of bone microarchitecture, and loss of bone mass. These factors reduce the physical strength of the bone and increase its fragility, making patients more susceptible to fractures from minor trauma **(1)**. More than 200 million individuals globally suffer from osteoporosis, and the fractures it generates are a main source of disability, sickness, and death among the elderly **(2)**. Osteoporosis is a complex condition with overlapping etiopathogenetic processes; it can be classified as primary (postmenopausal and senile) or secondary (induced by numerous medicines and illnesses) **(3-5)**.

The T helper 2 (Th2) cytokine interleukin 33 is mainly released via stromal cells in response to proinflammatory stimulation. It serves as a nuclear factor that might be an alarming, traditional cytokine and a regulator of gene transcription **(6)**. When cells are injured or die, IL-33 is produced as an alarmin, which contributes to stress reactions & triggers the production of other cytokines **(7)**. While some research claims that interleukin 33 promotes matrix mineralization in vitro, others show that it has no effect on the production of bone matrix **(8)**. **Mun et al. (9)** demonstrated that Interleukin 33 directly prompted osteoclast distinction from human monocyte precursors, thereby promoting bone resorption independently by the receptor activator of nuclear factor kappa-B ligand (RANKL) pathway, despite the fact that Interleukin 33 participates in Th 2-mediated processes, which stimulate osteoblast maturation and decrease osteoclastogenesis.

Conversely, **Kiyomiya et al. (10)** illustrated that IL-33 suppressed osteoclast development that was reliant on RANKL. Similar findings were made by **Velickovic et al. (11),** who showed that mice lacking the Interleukin 33 receptor had reduced bone mass and increased osteoclast development, and **Zhu et al. (12)**, who illustrated that IL-33 suppressed osteoclast distinction by deactivating cytoplasmic 1 (NFATc1), Nuclear Factor of Activated T-cells, the primary regulator for receptor activator of nuclear factor kappa-B ligand -induced osteoclast development. Consequently, it is challenging to examine IL-33's total impact on bone homeostasis because of its pleiotropic activity, which may account for the conflicting findings reported in the literature.

This study aimed to evaluate serum IL-33 concentrations in postmenopausal females with osteoporosis and investigate its correlation with bone turnover indicators to assess its potential as an early biomarker for osteoporosis.

PATIENTS AND METHODS

This case-control research,100 subjects had been collected from internal medicine clinics of Banha and Zagazig hospitals in Banha and Zagazig Universities. **Participants had been separated into 2 groups:** the case group (number = 50) & the control group (number = 50).

Sample Size

Sample size has been measured as regards the subsequent formula:

$$n = 2\left[\frac{(Z_{\alpha/2} + Z_{\beta}) * \sigma}{\mu_1 - \mu_2}\right]^2 \quad \textbf{(13)}$$

Where:

N equal sample size

Z α/2 = 2.58 (the critical value that separates the central 99% of the Z distribution from the 1% in the tail)

Zβ equals 0.84 (the critical value that separates the lower twenty percent of the Z distribution from the upper eighty percent).

σ equal the evaluation of the SD = 7.39

μ1 equal mean IL-33 serum concentration of osteoporotic patients' group = 3.53

μ2 = mean IL-33 serum concentration in the healthy control group = 13.72 **(14)**

According to the previous calculation, the total sample size was 13 participants in each group; after the 20% dropout was calculated, the total sample size was 16 participants in each group.

Inclusion criteria: Post-menopausal women with osteoporosis and age-matched healthy females following menopause as controls was involved in this research following written informed consent.

Exclusion criteria: Women who were taking drugs that influence bone turnover or women who are influenced by illnesses involving secondary osteoporosis have been excluded from the investigation.

METHODS

All cases were subjected to the following:

Bone mineral density has been estimated at the hip and lumbar spine utilizing dual-energy X-ray absorptiometry (DEXA). **Osteoporosis diagnosis followed WHO criteria:** A T-score not above than −2.5 indicated osteoporosis, −1.5 to −2.5 osteopenia, and above −1.5 normal BMD. All participants underwent a physical examination, clinical history review, and assessment of anthropometric measures (weight, height, and BMI). Vertebral morphometry and spine radiography were performed to detect asymptomatic fractures. Laboratory evaluations involved serum calcium, 1α,25-dihydroxyvitamin D_3, alkaline phosphatase (ALP), as well as PTH. Bone turnover indicators—CTX and P1NP were calculated to evaluate osteoclast and osteoblast activity, correspondingly.

Evaluation Variable

Serum IL-33 concentrations was assessed in both osteoporotic cases and healthy controls. Blood samples was collected by venipuncture, centrifuged at 3000 revolutions per minute for five minutes, and sera was stored at −twenty degrees Celsius until analysis. IL-33 concentrations were measured by applying a sandwich Enzyme-Linked Immunosorbent Assay kit, following the manufacturer's instructions. Absorbance reading was at 450 nanometers utilizing a microspectrophotometer (model 340 ATTC, SLT Lab Instruments, Salzburg, Austria), and results have been presented in picograms per milliliter.

Statistical analysis

Information was entered, cleaned, and examined applying the Statistical Package for Social Sciences (IBM® SPSS® Statistics version 25). Categorical parameters have been defined as column percentages and frequencies, whereas continuous parameters were summarized using SD and mean. The correlation among the study group and other categorical parameters was assessed for significance application the Fisher's exact test or chi-square test. The Kolmogorov-Smirnov test determined the normality of the continuous information and indicated that all continuous parameters aren't normally distributed. The t-test has been applied to estimate the significance of the variance between the study groups. Statistical significance has been evaluated when the p-value is below 0.05.

Ethical consideration

The research based on the principles stated in the Declaration of Helsinki for medical ethics for every research including human materials. Each precaution was taken to protect the privacy of investigation subjects & the confidentiality of their personal data. The study was performed after an informed written consent of the patients after approval from the Ethical Committee Banha University Hospital, Banha, Egypt (approval code: RC 18-7-2025).

RESULTS

Table (1): Patients' characteristics between the examined groups.

	Case group Number= fifty	Control group Number= fifty	p-value
Age (yrs) Mean ±SD	**66.4±9.8**	**63.6±7.1**	**0.11**
BMI Mean ±SD	**28.07±4.5**	**29.5±4.3**	**0.1**
BMD (T-score) Mean ±SD	**-3.4±0.76**	**-0.3±1.4**	**<0.001**

P-value under 0.001 is highly significant, P under 0.05 is statistically significant, P above 0.05: Not significant; BMI: body mass index; BMD: Bone mineral density.

There was a statistically insignificant variance among the studied groups according to BMI and age (p above 0.05), while a highly statistically significant variance found among the examined groups as regards BMD (T-score) (p below 0.05). (Table 1)

Table (2): Patients' characteristics among the examined groups.

	Case group Number = fifty	Control group Number = fifty	p-value
PTH (picograms per milliliter)	64.93 ± 22.69	67.97 ± 16.26	0.124
ALP (Units per Liter)	123.26 ± 62.36	103.57 ± 25.76	**0.006**
CTX (picograms per milliliter)	429.01 ± 273.58	290.93 ± 180.57	**<0.001**
P1NP (Nanograms per milliliter)	21.10 ± 11.82	24.37 ± 14.35	0.112
1,25(OH)2-D3 (picograms per milliliter)	19.12 ± 8.98	22.37 ± 5.89	0.429
Ca^{++} (Milliliters per deciliter)	9.16 ± 0.77	7.14 ± 3.58	**<0.001**
Fractured subjects	13 (26%)	0 (0%)	**<0.001**

There was a statistically insignificant variance among the examined groups regarding PTH, P1NP, and 1,25(OH)2-D3 P above 0.05, while a statistically significant variance found between the examined groups as regards ALP, CTX, Ca, and fractured subjects' P < 0.05. (Table 2)

Table (3): IL-33 between the examined groups.

	Case group Number =fifty	Control group Number = fifty	p-value
IL-33 Mean ±SD	3.32 ± 2.09	12.01 ± 5.74	**≤0.001**

There was a greatly statistically significant diminution in the case group in comparison with the control group regarding IL-33 P, not more than 0.001. (Table 3)

Table (4): Correlation among IL-33 and Other Parameters in Osteoporotic cases

IL-33

	Spearman coefficient	p-value
P1NP	0.401	0.009
PTH	0.398	0.02
CTX	−0.495	0.0014

IL-33 levels correlated positively with P1NP and PTH and negatively with CTX in osteoporotic patients, indicating its role in bone metabolism. (Table 4)

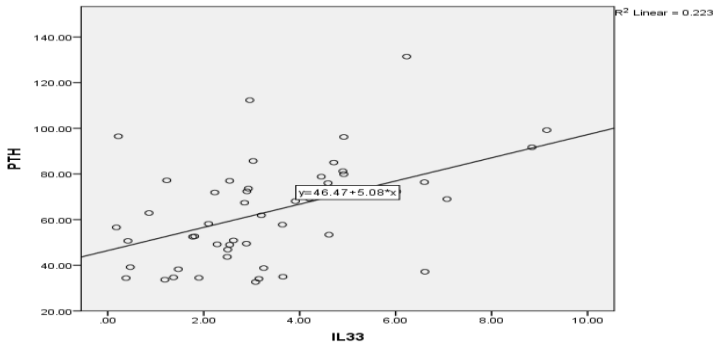

Figure (1): Correlation between PTH and IL-33

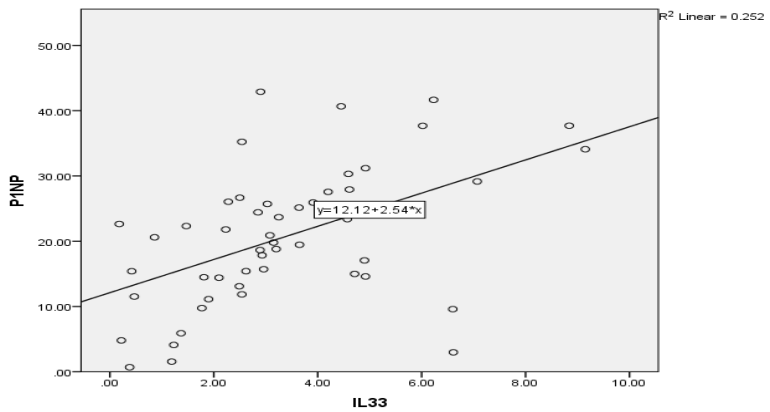

Figure (2): Association among IL-33 and P1NP

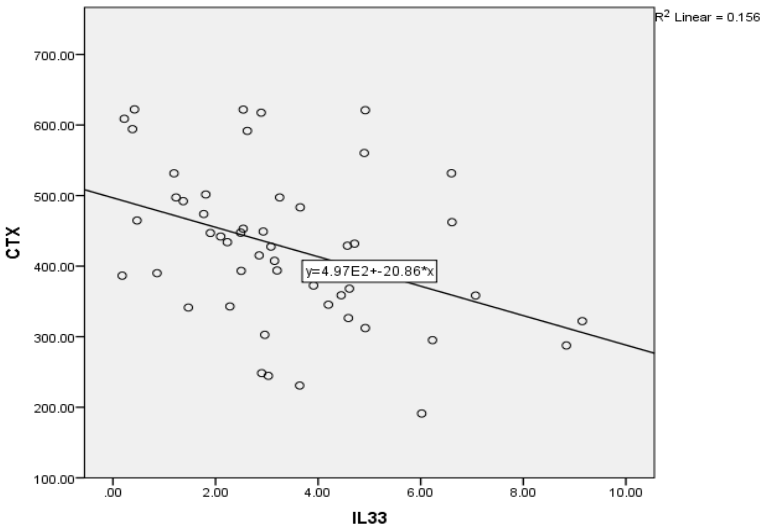

Figure (3): Association among Interleukin 33 and CTX

DISCUSSION

Our results showed that there was a statistically insignificant variance among the examined groups according to age and BMI, whereas a highly statistically significant variance was observed among the examined groups according to BMD (T-score).
This research demonstrated that there was a statistically insignificant variance among the examined groups as regards PTH, P1NP, and 1,25(OH)2-D3, whereas a statistically significant variance has been observed among the examined groups according to ALP, CTX, Ca, and fractured subjects.

In agreement with our findings, **Wei et al., (14)** discovered the correlation of *β* cross-linked C-telopeptide of type 1 collagen (*β*-CTX) and P1NP with bone mineral density in postmenopausal females. They revealed that the validated osteoporosis (OP) population became the older age group & had a decreased body mineral index & extended menopausal period (*P-value* below 0.01). The level of *β*-CTX and P1NP in the non-osteoporotic group (normal group & osteopenia group) was significantly reduced in comparison with that of the validated OP group (*P-value* below 0.01).

Similarly, our outcomes were in accordance with **Abdu Allah et al., (15)** who assessed the association between BMD and CTX1 in postmenopausal Egyptian females. As regards bone mineral density, they have been separated into three groups: osteoporosis, osteopenia, and control groups. They stated that a highly statistically significant variance found among the examined groups as regards BMD. Also, CTX1 concentrations were significantly elevated in osteopenia and osteoporosis compared to the control group (P below 0.001).

Our results revealed that there was a greater statistically significant reduction in the case group in comparison with the control group regarding IL-33. We observed that IL-33 concentrations correlated positively with P1NP and PTH and negatively with CTX in osteoporotic patients, indicating its role in bone metabolism.

In line with our results, **Ginaldi et al., (16)** examined the serum IL-33 concentrations in cases that had osteoporosis following menopause. Serum levels of Interleukin 33 were significantly reduced in osteoporotic cases in comparison with controls. A significant positive association among all cases had osteoporosis among concentration of serum Interleukin 33 & parathyroid hormone, in addition to among serum concentrations of IL-33 and procollagen type I N-terminal propeptide, while a significant negative association has been found among serum concentrations of CTX and IL-33. Their findings recommended that IL-33 is a significant bone-protecting cytokine, that could provide therapeutic aid in the treatment of bone resorption.

Even though a receptor activator of nuclear factor kappa-B ligand -like action of IL-33 in human osteoclast development was recommended **(17)**, the Interleukin 33 pro-resorptive influence on bone is weak relative to the consistent & potent influence of RANKL and greater variable, with certain kinds of osteoclast progenitors capable of distinguishing into functional resorbing cells after Interleukin 33 triggers & other osteoclast progenitor-containing populations illustrating unresponsiveness **(18)**. This might partially clarify the apparently contrasting outcomes of some in vitro investigations that recommend a pro-resorptive & consequently pro-osteoporotic role of Interleukin 33.

A T1 profile & a high level of pro-inflammatory cytokines, primarily Interleukin 6, Interleukin 1, Interleukin 17, & TNF-α, influence the advancement & appearance of primitive and 2^{ry} osteoporosis. Conversely, regulatory cytokines like Interleukin 10 and T2 cytokines like Interleukin 4 demonstrate a frequent bone anabolic action, hence being deemed protective against osteoporosis. Interleukin 33, which potently increases the T2 immune response, might illustrate a frequent protective role in osteoporosis, an inflammatory illness mediated by T1/T17 cells **(19, 20)**. Our outcomes in osteoporotic cases are in accordance with this hypothesis, recommending a frequent protective role of this cytokine in postmenopausal osteoporosis.

CONCLUSION

Serum IL-33concentrations were significantly decreased in postmenopausal osteoporotic cases in comparison with healthy controls, recommending a probable role for Interleukin 33 in the pathophysiology of osteoporosis. Osteoporotic patients exhibited significantly altered levels of ALP, CTX, and calcium and a higher incidence of fractures. Notably, IL-33 levels illustrated a positive relation to PTH and P1NP and a negative correlation with CTX, highlighting its possible involvement in bone formation and resorption dynamics. These outcomes support the notion that IL-33 may serve as a biomarker of bone metabolism and could contribute to the understanding and management of osteoporosis after menopause.

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