Original article

Lower Serum Irisin Levels Are Associated with Increased Osteoporosis and Oxidative Stress in Postmenopausal

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Abstract

Background: Irisin as an exercise-induced myokine was proposed to improve bone health. This study investigated the role of serum irisin (s-irisin) in patients with osteoporosis (OP) through correlating to most biological bone markers and oxidative stress.

Methods: A cross-sectional study recruited an eligible 175 postmenopausal women at Al-Hussien Teaching Hospital, Iraq. They were scanned by DEXA and stratified into two groups based on T-score; the first 95 patients as control group (GI) with $-1 \leq$ T-score and the second 80 patients as cases group (GII) with T-score ≤ -2.5. Demographic criteria were age, bone mineral density (BMD, g/cm^2) and T-score. Serum irisin, total serum calcium (s-calcium), serum inorganic phosphate (s-phosphate), serum alkaline phosphatase (s-ALP), serum 25 [OH] vitamin D, the serum parathyroid hormone (s-PTH), serum Carboxy terminal collagen crosslinks (CTx), serum procollagen type I C-termidnal peptide (s-PICP), serum malondialdehyde (s-MDA) and serum superoxide dismutase (s-SOD) were collected from blood samples.

Results: Serum irisin were 31.84 ± 2.65 vs. 20.88 ± 2.71 ng/mL for control and trial groups, respectively. Lower levels of BMD, T-score, 25 [OH] vitamin D, and s-irisin along with a higher serum levels of PTH, CTx, PICP, MDA and SOD were observed in patients with osteoporosis. All parameters were statistically meaningful upon correlation ($p < 0.0001$), except age and s-calcium ($p = 0.0088$ and $p = 0.187$, respectively).

Conclusions: The results showed that, a significantly lower serum irisin levels among osteoporosis women, was intimately correlated to most bone turnover markers and it can be considered as encouraging results for clinical application in prediction and treatment of osteoporosis.

Keywords: Bone turnover markers, DEXA scan, Irisin, T-score, BMD, osteoporosis, post-menopause.

Introduction

Osteoporosis (OP) is characterized structurally by bone mineral density (BMD) T-score -2.5 or less. OP is associated with an aging population particularly for women during postmenopausal years. Menopause is a major etiological factor for OP; a daunting period when females are most vulnerable to climate change and hormone degradation (1-3). BMD assessed with dual-energy X-ray absorptiometry (DXA) rigorously predicts substantial fractures, especially those involving the hip. Predictions strengthened when BMD is paired with additional diagnostic risk factors in predictive modules (4,5).

Numerous risk prediction methods have been suggested, both radiological and biological modules, aiming for the detection of individuals at high risk of fracture that are mostly like to get the maximum benefit from treatment (6). Dynamic changes in bone metabolism especially turn-over markers may be best estimated by assessing

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different bone biological markers in the blood and urine, whereas changes in BMD evolve very slow and do not reflect the actual magnitude of bone loss. The latest studies have highlighted the possible use of bone turnover markers (BTMs) to classify patients with early bone loss, to make proper clinical choices, and to track therapy outcomes. Harmonization of bone turnover markers can promote bone turnover markers for the prediction of fracture risk (7).

None of the prediction modules cover all established risk factors each have their strengths and weaknesses in routine clinical practice (7,8). Irisin is a novel endocrine myokine marker produced in an exercise that works by inducing browning of the adipose tissue. The latter is a positive indicator of femoral bone architecture and is directly related to thigh muscle. Interestingly; irisin increased osteoblast differentiation, and its levels were lower among women with a history of osteoporotic fractures. Irisin is also secreted by fatty tissue, in the experimental animals, highlighting its action in the muscle–fat–bone cross-talk (9-11). A growing body of evidence correlates s-irisin and osteoporotic fractures still the earlier study did not specify the irisin role in that relationship. The current study was designed to explore irisin correlation in OP as a possible predictor, our knowledge for its role among Iraqi women is scrutinized.

Materials and Methods

This study was a prospective cross-sectional study involving 175 postmenopausal Iraqi women PMIW at Al-Hussien Teaching Hospital in Thi-Qar City over one year time; from January 2020 to March 2020.

Ethics approval
Ethical approval was made by the local ethics committee together with verbal approvals by all participants. PMIW were those who experienced 12 months of amenorrhea consecutively and 40 mIU/ml of follicle-stimulating hormone providing there is no medical reason. Exclusion criteria included rheumatic disease, or urinary, gout, drug abuse, alcohol use, smoking history. History of blood disorders, pathological fracture, diabetes hypertension, and irregular (renal, liver, or thyroid function test). We also removed volunteers with drugs and supplements that affect bone metabolism, vitamin D, calcium supplements, bisphosphonates, and fluoride glucocorticoids.

Experimental design
The recruited volunteers who were eligible for inclusion criteria were 175 participants. They were scanned by the DEXA scan and further subdivided into two groups; the control group (95/175) referred to as G I, and the cases group (80/175) referred to as G II based on their DEXA scan. The Control group had ($-1 \leq$ T-score), Cases; OP group had (T-score $\leq$ -2.5). Demographic parameters for participants include age, from the radiological analysis, we estimated BMD (g/cm2) and T-score. From blood samples, we collected s-irisin, s-calcium, s-phosphate, s-ALP, serum 25 [OH] vitamin D, s-PTH, serum CTx (s-CTx), serum PICP (s-PICP), serum MDA, and serum SOD.

BMD measurement
Dual-energy X-ray absorptiometry [DEXA, Ltd., Korea/ Osteosys Co.] was used to calculate BMD and T-score at the lumbar spine (L2-L4). A procedure used for the DEXA test, the patient is placed in a supine position below the radiation source indicated by a red laser pointer, which steadily travels from the umbilical to the middle thigh known as the Ward triangle, over a test;

DEXA scans the femur neck, the commonest location for fractures. Height and body weight were also assessed when scanning for bone density was in progress.

T-score values estimated by DEXA scan are the standard for OP diagnosis. According to WHO criteria for the diagnosis of osteoporosis. Participants are sub- divided into three: regular (T-score value > 1.0 S.D), osteopenia (1.0 S.D T-score value > 2.5 S.D), and osteoporosis (T-score value 2.5 S.D).

Laboratory measurement

Samples of blood were obtained in all volunteers between 8:30 and 12:00 a.m. They were collected, frozen for chemical processing in the enzyme-linked immunosorbent assay (ELISA) assay. Serum irisin, s-CTx, s-PICP, serum 25 [OH] vitamin D, and s-PTH, were measured using the ELISA kit provided by Demeditec diagnostic testing GmbH Germany. S-calcium, s-phosphate, s-ALP calculated by (Biolabs), (France, man). The serum MDA amount was spectrophotometrically calculated using the Muslih et al. method in 2002 (12). Serum levels of SOD were assessed using the Superoxide Dismutase Microplate Assay Kit provided by Cohesion Biosciences USA. All samples were examined in one set.

Statistical analysis

The unpaired T-test was used to compare two mean ± SDs for age, s-irisin, s-calcium, s-phosphate, s-ALP, serum 25 [OH] vitamin D, s-PTH, s-CTx, s-PICP, serum MDA, and serum SOD all obtained from blood analysis. We calculated BMD and T-score by radiological tests. For both calculations, the relevant P-value was < 0.05. To clarify the correlation between irisin and other parameters we used Linear regression. Both statistical analyzes were carried out using Statistical Package Social Sciences version 24 (SPSS v.24).

Results

A total of 175 postmenopausal women volunteered to study. Their demographic criteria were summarized in Table 1, including, age, s-irisin, s-calcium, s-phosphate, s-ALP, serum 25 [OH] vitamin D, the s-PTH, s-CTx, s-PICP, serum MDA, and serum SOD all yielded via blood analysis; the radiological exam estimated BMD (g/cm2) and T-score.

The mean for the age was 58.41 ± 3.51 versus 59.73 ± 3.44 for GI and GII respectively, s-irisin (ng/mL) had a mean of 31.84 ± 2.65 versus 20.88 ± 2.71 for GI and GII respectively. All the variables taken by the study were significant upon comparison among the 2 groups as the p-value was < 0.0001 except for age and s-calcium with a p-value of 0.0088 and 0.187 respectively.

Table 2 demonstrated the correlations of s-irisin versus all parameters under study. A significant positive correlation was reached between irisin versus the BMD, s-phosphate, serum 25 [OH] vitamin D, and s-PICP in the GI and GII groups. While, a significant negative correlation was proved between s-irisin with T-score, s-ALP, s-PTH, s-CTx, serum MDA, and serum SOD in the G1 and GII groups. Irisin showed a non-significant negative correlation with s-calcium.

Table 1. Basic criteria for study volunteer-involving healthy controls and cases.

Variable	GI: Control Group (n= 95)	GII: Study Group (n= 80)	p-value
Age	58.41 ± 3.51	59.73 ± 3.44	**0.0088**
s-irisin (ng/mL)	31.84 ± 2.65	20.88 ± 2.71	<0.0001*
BMD (g/cm^2)	1.16 ± 0.14	0.55 ± 0.14	<0.0001*
T-score	-0.91 ± 0.13	-4.32 ± 0.79	<0.0001*
s-calcium (mmol/L)	2.24 ± 0.16	2.28 ± 0.22	0.186 ns
s-phosphate (mmol/L)	1.86 ± 0.13	1.59 ± 0.19	<0.0001*
s-ALP (IU/dL)	78.93 ± 4.78	85.83 ± 4.79	<0.0001*
25 [OH] vitamin D (ng/mL)	26.56 ± 2.42	9.64 ± 2.02	<0.0001*
s-PTH (ng/ml)	30.50 ± 3.23	38.17 ± 3.66	<0.0001*
s-CTx (ng/mL)	0.39 ± 0.08	2.11 ± 0.58	<0.0001*
s-PICP (ng/mL)	18.54 ± 0.36	18.07 ± 0.41	<0.0001*
MDA (mmol/l)	4.83 ± 0.27	10.01 ± 1.02	<0.0001*
SOD (U/ml)	2.88 ± 0.37	4.52 ± 0.61	<0.0001*

s-irisin, serum irisin; BMD, bone mineral density; s-calcium, serum total calcium; s-phosphate, serum inorganic phosphate: s-ALP, alkaline phosphatase; s-PTH, parathyroid hormone; s-CTx, Serum human C terminal telopeptides of types I collagen; s-PICP, Serum human carboxy-terminal propeptide of type I procollagen; MDA, Malondialdehyde; SOD, Superoxide dismutase. ns non-significant; * p value< 0.001.

Table 3, illustrated person correlations between BMD versus all other variables. Correlations were significant except for s-calcium as p< 0.158, the coefficient of correlation was most significant for MDA, -0.870.

Table 2. The correlations between s-irisin versus all other variables.

Variable	R	P-value
BMD (g/cm^2)	0.828	<0.0001*
T-score	- 0.575	<0.0001*
s-calcium (mmol/L)	-0.032	0.652 [ns]
s-phosphate (mmol/L)	0.551	<0.0001*
s-ALP (IU/dL)	-0.525	<0.0001*
25 [OH] vitamin D (ng/mL)	0.877	<0.0001*
s-PTH (ng/ml)	-0.636	<0.0001*
s-CTx (ng/mL)	-0.809	<0.0001*
s-PICP (ng/mL)	0.403	<0.0001*
MDA (mmol/l)	-0.847	<0.0001*
SOD (U/ml)	-0.774	<0.0001*

Ns: non-significant; * p value< 0.001.

Table 3. The correlations between BMD versus all other variables.

Variable	R	p-value
s-irisin (ng/mL)	0.828	<0.0001*
T-score	-0.612	<0.0001*
s-calcium (mmol/L)	-0.100	0.158 [ns]
s-phosphate (mmol/L)	0.496	<0.0001*
s-ALP (IU/dL)	-0.512	<0.0001*
25 [OH] vitamin D (ng/mL)	0.859	<0.0001*
s-PTH (ng/ml)	-0.608	<0.0001*
s-CTx (ng/mL)	-0.820	<0.0001*
s-PICP (ng/mL)	0.433	<0.0001*
MDA (mmol/l)	-0.870	<0.0001*
SOD (U/ml)	-0.771	<0.0001*

Ns: non-significant; * p value< 0.001.

Discussion

Despite impressive advances in understanding how estrogen deficiency causes OP, the underlying pathogenic pathways are diverse and multifaceted. One of the most intriguing theories is the capacity of bone turnover markers to predict OP in the affected bone as the changes precede the radiological changes Harmonization of bone markers, can promote prediction of OP and fracture risk. The newly discovered irisin, an exercise-induced myokine, originating from fibronectin type III domain-containing protein 5 (FNDC5) and released into the serum as a soluble form (known as irisin) was discussed in an attempt to verify its role in OP (13, 14).

The most recent guidelines, recommend the use of vitamin D with a daily dosage of 800 IU for postmenopausal women and men > 50 years old as for higher intermittent weekly doses; they were not advised (15). According to the present study's results, BMD and T-score were significantly lower in comparison to control. These results were succinct in the Roomi et al. study, where BMD (g/cm^2) and T-score were substantially lower in OP patients as compared to stable controls (16).

Correlations indicate meaningful relations for s-CTx (ng/mL) and s-PICP (ng/mL) as p-value< 0.0001, amongst cases versus controls. These are biological markers for bone turnover, elevated after menopause. Since bone loss velocity differs by marker value; laboratory medicine (IFCC) has suggested two markers as discriminated

analyses for prediction; serum procollagen type I N propeptide (s-PINP) and s-CTx as markers for bone formation and bone resorption, respectively. Researchers explored the relationship between PINP and the likelihood of fracture. Risk ratio raises in s-PINP; 1.23 (95% CI 1.09–1.39) for males and females paired uncorrected for BMD. A substantial correlation was also identified for s-CTx and fracture risk GR=1.18 (95% CI 1.05–1.34) uncorrected for BMD. The correlation between s-CTx and fracture risk was marginally higher for the consequence of the hip fracture, 1.23 (95 percent CI 1.04–1.47) There is also a minor but important association between such markers and the fracture risk (17).

Low intake Ca results in lower serum levels correlates to low BMD and postmenopausal OP; sufficient bone mineralization is obtained by calcium and vitamin D enriched dietary consumption. Calcium increases the mechanical and structural bone qualities of PMW bones. These results became the foundation for promoting an improved dietary fiber intake that could help enhance its absorption via the intestines. These undigested fibers will be ferment by the intestinal bacteria that lower intestinal PTH and promote Ca absorption to emphasize its protective action (18,19).

Based on the present results, PTH serum levels were meaningfully higher in cases versus healthy controls. PTH is prescribed for treating postmenopausal women with osteoporosis, male osteoporosis, and corticosteroid-induced osteoporosis, which induces substantially elevated BMD of the lumbar spine (20).

Chawla et al illustrated a negative correlation between PTH and BMD. Interestingly; upon histomorphometry of iliac crest biopsies; increased BMD shows a compromised bone with increased cortical and trabecular width and reduced cortical porosity consequently, microarchitecture changes on skeletal health and silent vertebral fractures were reported (21).

In the animal model experiment, the concentrations of bone Calcium and p in treated ovariectomized mice were significantly lower than those in the control group; in line with El Wakf et al, which reported that ovariectomized mice have impaired Ca balance ascribed to ovariectomy-induced OP (22). Kharode (23) indicated that menopause causes an increase in bone resorption; and a net loss of bone. The cessation of ovarian hormones and the decreased secretion of estrogen was the trigger (24).

In the present study, analyses have shown significant differences in irisin levels in cases vs. controls (p< 0.0001). Table 2 showed a significant correlation with all variables. Irisin positively correlates with BMD, s-phosphate, 25 [OH] vitamin D, s-CTx, negatively correlate to s-ALP, s-PTH, s-CTx, MDA, and SOD. Table 3 illustrates person correlations between BMD vs. s-irisin and all other variables. Correlations were significant except for s-calcium (p< 0.158).

Radiological screening for OP has its disadvantages, inadequate diet in the elderly affects bone density expressed as osteomalacia, and reduced bone mineralization can lower the overall bone matrix. On the other hand, bone biomarkers will increase throughout menopause each with a different OP-related rate, combined modules will display higher identification and improved clinical outcomes (25).

Irisin's, positive influence on bone is defined as enhancing bone quality by increasing the rate of mineral deposition, bone synthesis, periosteal diameter, and polar bending momentum (26). Irisin was recently reported to impair osteoclast activity and stimulates osteoblast differentiation in bone cell lines it regulates bone anabolism indirectly via browning of the WAT [white adipose tissue] (26). Moreover, irisin reduces body fat, prevents and treats obesity by increasing energy expenditure. Moreover, proinflammatory cytokines caused by accumulated fat (27) can be reduced by irisin especially induced by exercise ultimately so that reduces osteoclast activity and increases osteoblast differentiation (28).

Results in rats' models have declared that irisin is involved in the bone-muscle talk. Experiments in humans aimed to investigate the irisin effect on the skeleton, postmenopausal women showed a negative relation in irisin levels and vertebral fragility fractures. Conversely, its levels were directly correlated with bone strength and density, among athletes

confirming the irisin protective role in bone health (29).

However, the irisin effect extends to include the central nervous system. Irisin attracted the attention for a possible application in neurodegenerative diseases, such as Alzheimer's and Parkinson's (30,31).

In summary, Irisin has favorable effects on bone health due to its promising relation to bone turn over markers and negative relation to other bone-oxidative stress markers. Irisin's effect on bone prosperity is mediated via multiple pathways affecting bone cells, increasing osteoblast differentiation, and inhibiting osteoclasts. Furthermore, it affects adipose tissues, muscle cells, and the neuronal cells that innervate them. It gives a shiny window in the diagnosis, prevention, and treatment of menopause osteoporosis.

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