ORIGINAL ARTICLE

Biochemical versus radiological screening for osteoporosis in menopause; will Irisin help?

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Abstract

Objective: To verify the discriminative power of irisin in osteoporosis patients.

Method: The comparative case-control study was conducted at the University Teaching Hospital, Baghdad, Iraq, from March 2020 to June 2021 after approval from the ethics review committee of the College of Medicine, Mustansiriyah University, Baghdad, and comprised post-menopausal women. After being scanned by dual-energy X-ray absorptiometer, the subjects were divided into groups based on T-scores; healthy controls with T-score > −1 in group 1, and osteoporosis patients with T-score ≤−2.5 in group 2. Participants' sera were tested for Irisin, 25-hydroxy vitamin D, and carboxyl-terminal telopeptides of type I collagen levels. T-score and bone mineral density were recorded as radiological markers. Correlation of irisin was determined with T-score and bone mineral density, cut-off value for serum irisin was worked out for osteoporosis prediction. Data was analysed using Medcalic 17.

Result: Of the 142 women, 71(50%) were in group 1 with mean age 58.4±3.5 years, and 71(50%) were in group 2 with mean age 58.7±3.4 years (p=0.87). Levels of irisin, 25-hydroxy vitamin D, carboxyl-terminal telopeptides of type I collagen, bone mineral density and T-scores were significant between the groups (p<0.001). Serum irisin correlated directly with bone mineral density (r=0.97, p<0.001) and inversely with T-score (r= -0.95, p<0.001). The cut-off value of serum irisin was 31.4ng/ml with 84% sensitivity and 100% specificity (p<0.001).

Conclusion: Strong serum irisin correlation to osteoporosis radiological markers and its good discrimination of osteoporosis implied its utility as a good serological marker of osteoporosis.

Key Words: Bone Density, Fibronectins, Collagen, Post menopause, X-Rays, Osteoporosis, Vitamin D

(JPMA 74: S2 (Supple-8); 2024) **DOI: https://doi.org/10.47391/JPMA-BAGH-16-01**

Introduction

Osteoporosis (OP) is a metabolic disease that affects patients' mobility and quality of life (QOL), and worsens bone fragility. OP causes a silent and progressive reduction in bone density and quality, characterised by low bone mineral density (BMD) and deterioration of bone tissue architecture. The global disabilities accredited to osteoporotic fractures have touched the 9 million marks.[1,2] Menopause increases OP risk in several ways. Oestrogen deficiency causes bone loss by increasing bone resorption at the expense of formation. In addition, it lacks triggers for the release of inflammatory markers and cytokines that ultimately promote osteoclast cell differentiation; the latter is responsible for bone resorption.[3] During menopause, age-related alterations begin, where oxidative stress (OS) and inflammation increase alongside decreased regenerative ability of human cells and decreased muscle and bone mass, which lower mechanical loading responsible for new bone formation.[4,5] Aging causes a progressive negative calcium balance. First, there is difficult absorption of calcium as a result of intestinal resistance to endogenous circulating 1, 25 dihydroxy vitamin D. Second, aging systemic inflammatory status mobilises calcium from the bone. Third, secondary hyperparathyroidism triggered by reduced calcium absorption further weakens the bones by increased resorption. The net result is diminished bone strength and OP risk.[6-8] The last two decades witnessed an improved capacity to predict OP risk using fracture prediction tools. The most widely used technique for bone mineral density (BMD) is dual energy X-ray absorptiometry (DEXA). It estimates bone fragility based on the difference between the patient's BMD and the mean BMD of healthy young women. Accordingly, it categorises the population based on their respective T-score values to normal, osteopenia and osteoporosis cases.[9]

Nevertheless, mounting evidence has questioned the T-score optimum diagnostic ability, and many have used serum biomarkers for an earlier and more accurate fracture risk evaluation. Among those biomarkers is serum human carboxyl-terminal telopeptides of type I

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collagen (CTX), a bone resorption marker with promising results. Others relied on biochemical cross-talk of bone and muscle that exceeds conventional mechanical coupling.[10,11] Irisin, a muscle-produced myokine and a cleaved fibronectin type three-domain following exercise, is considered an anabolic mediator in the bone-muscle unit. It has a white adipose tissue browning effect and a muscle effect. Irisin in high doses exhibits thermogenesis effects, while in lower doses it raises the cortical BMD.[12] In athletics, irisin positively correlates to total and hip BMD, and, conversely, it shows an inverse correlation with OP as its levels decline in older women. Several studies have reviewed the indispensable physiological role of irisin in bone metabolism.[10-12] The current study was planned to verify the discriminative power of irisin in OP patients.

Patients and Methods

The comparative case-control study was conducted at the University Teaching Hospital, Baghdad, Iraq, from March 2020 to June 2021. After approval from the ethics review committee of the College of Medicine, Mustansiriyah University, Baghdad, the sample size was calculated using the formula [13] [r+1/r* (SD)2 (Zβ+ Zα/2)2]/d2. In the formula, r was the ratio of control to cases, 1 for an equal number of cases and control; SD was the standard deviation of the variable which was taken from a previous study[13]; Zβ was the standard normal variant for power, and for 80% power, it was 0.84, and for 90%, it was 1.28; Zα/2 was standard normal variant for level of significance which was 1.96 at 5% type 1 error and 2.58 at 1% type 1 error; and d was the expected mean difference between cases and controls.

The sample was raised using a stratified probability sampling technique from among post-menopausal women presenting to the hospital for regular check-ups. The participants were recruited sequentially.

Those included were post-menopausal women who had amenorrhea for at least 12 months and a serum follicle-stimulating hormone (FSH) level 40mIU/ml for no medical reason. Those with rheumatic disease, gout or urinary stones, smokers, pathological fracture, history of blood diseases, hypertension, diabetes and abnormal liver, renal, or thyroid function were excluded. Also excluded were women on drugs or supplements affecting bone metabolism.

After taking written informed consent from the participant's, detailed history was taken and they were subjected to a general examination. After the DEXA scan, the subjects were divided into groups based on T-scores; healthy controls with T-score >−1 in group 1, and OP patients with T-score ≤−2.5 in group 2.

In addition to the participant's age, serum levels of irisin (ng/mL), 25-hydroxy vitamin D (ng/mL) and CTX (ng/mL) were noted. Through radiological scanning, BMD (g/cm2) and respective T-scores were calculated.

After one night of fasting, 5cc blood samples were aspirated, centrifuged and frozen at -20°C for biochemical assessment using an enzyme-linked immunosorbent assay (ELIZA) kit (Demed itec Diagnostics GmbH, Germany).

BMD and T-score measurement at lumbar spine L2-L4 was done using DEXA (Osteosys Co., Ltd., N. Korea) in which the participants were asked to stay in supine position under the source of radiation marked with a red laser pointer. This point slowly moved from the umbilicus down to the mid-thigh in the triangle of Ward. DEXA scans were also used to check the femoral neck, which is the most common site of fractures. The World Health Organisation (WHO) guideline[9] was used for OP classification into normal, osteopenia and OP.

Data was analysed using Medcalic.[17] Unpaired t-test was used to compare the mean values between the groups. Shapiro test was used to identify data normality. Pearsons's correlation was used to determine the relationship of irisin with T-scores and BMD. Odds ratio (OR) for women in both groups with serum irisin levels <25h percentile were compared with those >25th percentile as the reference value. Logistic regression was used to calculate OR with 95% confidence interval (CI). Receiver operating characteristic (ROC) curve analysis was used to determine the cut-off value for serum irisin most correlated with the highest sensitivity and specificity for OP prediction. P<0.05 was considered significant.

Results

Of the 142 women, 71(50%) were in group 1 with mean age 58.4±3.5 years, and 71(50%) were in group 2 with mean age 58.7±3.4 years (p=0.87). Levels of irisin, vitamin D, CTX, BMD and T-scores were significantly different

Table-1: Inter-group comparison.

Parameters	Control Group (Gr.1) (No.=71)	Study Group (Gr.2) (No.=71)	*P*-value
Age (years)	58.4± 3.5	58.7±3.4	P = 0.87
Serum irisin (ng/mL)	29.3± 2.43	21.7±2.61	P < 0.0001
Serum 25 (OH) vitamin D (ng/mL)	24.5 ±2.4	8.3± 2.2	P < 0.0001
Serum CTX (ng/mL)	0.43±0.06	2.62± 0.4	P < 0.0001
BMD (g/cm2)	1.45±0.2	0.7± 0.2	P < 0.0001
T-Score	-0.87±0.2	-4.63 ±0.7	P < 0.0001

OH: Hydroxy, CTX: Carboxyl-terminal telopeptides of type I collagen, BMD: Bone mineral density..

Table-2: Correlation of serum irisin with T-score and BMD.

Parameters N=142	Coefficient of correlation r	P-value
Irisin vs T-score	-0.95	<0.001*
Irisin vs BMD	0.97	<0.001*

BMD: Bone mineral density.

between the groups (p<0.001) (Table 1).

Serum irisin correlated directly with BMD (r=0.97, p<0.001) and inversely with T-score (r= -0.95, p<0.001) (Table 2).

The cut-off value of serum irisin was 31.4ng/ml with 84% sensitivity and 100% specificity (p<0.001) (Figure).

Discussion

The current study confirmed a lower serum irisin, vitamin D level in OP women versus healthy controls. Bone degradation biomarker CTX was significantly higher in OP cases compared to the controls. A strong positive correlation was found for serum irisin with T-score and BMD. The findings agreed with earlier studies.[14,15] The hallmark of OP is the imbalance between bone remodelling and resorption. Thus, balanced bone formation and resorption are of paramount significance in terms of preserving healthy bone.[16]

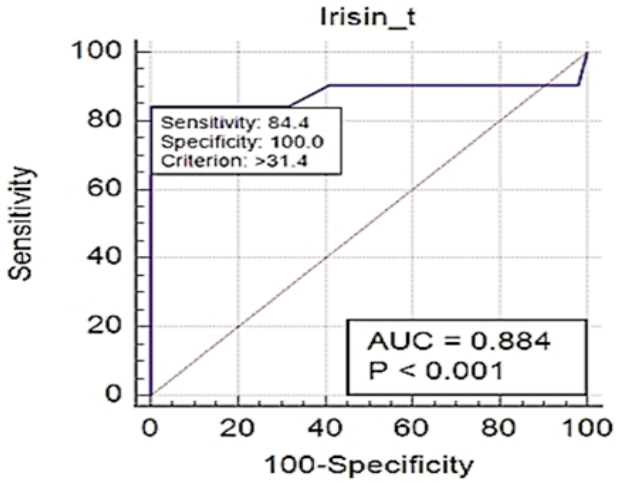

Figure: Receiver operating characteristic (ROC) curve showing irisin cut-off value associated with highest sensitivity and specificity along with area under the curve (AUC).

Palermo et al. included two groups of overweight post-menopausal women; one with previous vertebral osteoporotic fractures, and the other without.[12] They described a significantly low serum irisin in OP patients with previous fracture than in the controls, and reported an inverse correlation between vertebral fragility fractures and irisin level. However, they failed to correlate serum irisin to BMD, contradicting the current results. Small sampling bias may be the cause as the earlier study included 36 patients in each group. They concluded that the protective influence of irisin on bone health was independent of BMD.[12]

Yan J et al. recruited 160 post-menopausal women with hip fracture and 160 matched controls, and evaluated serum irisin as a marker of bone turnover and BMD 2 days after the hip fracture. They confirmed an independent association of low serum irisin with a high risk of hip fractures after adjustment for BMD and Fracture Risk Assessment Tool results. Serum irisin was significantly low in fractures group compared to the controls (p<0.001).[24] Correlation of serum CTX with risk of fractures uncorrected for BMD has also been discussed.[17] Wu et al. explored irisin's correlation to BMD in elderly population in a sample of post-menopausal women with age-matched males.[18]

A study measured serum irisin, procollagen type 1 amino-terminal propeptide, and CTX alongside lipid profile, and confirmed that higher irisin levels in post-menopausal women were significantly different compared to males (p<0.002). The study explained that this represented a compensatory response for increased energy demand generated by decreased sex hormones.[19] Furthermore, in males, irisin correlated positively to BMD and negatively to triglyceride.[19]

Park et al. reported the utility of serum irisin as a biomarker for sarcopenia in post-menopausal women as its level was significantly low among in the sarcopenic group than in the pre-sarcopenia or control groups.[20]

Zhou K et al. conducted a meta-analysis of middle age and elderly adults. Two out of seven studies enrolled women and men, and five studies enrolled post-menopausal women. The meta-analysis showed a low irisin level in osteoporotic women and patients with a positive fracture history. Nevertheless, they declared a weak correlation between femoral neck or lumbar spine BMD versus irisin, contradicting the current results. Their meta-analysis had differences in heterogeneity, wide age range, and genders, which reflected publication bias.[11]

Irisin prevents progressive bone-loss by stimulating osteogenesis, and preventing osteoclast genesis. In addition, irisin has antioxidative and inflammatory actions. Both factors are accredited for microfractures in bony tissues that trigger a vicious circle of bone destruction. Besides, irisin has a role in preserving muscle mass in aging and metabolic diseases.[21]

Although many cytokines and biomarkers have been studied.[22,23], irisin's role in the longevity of the bone-muscle unit remains unique; it outstood other markers with its promising therapeutic application in restoring the bone and wasted muscle mass following long immobility in animal and human studies.[24,25]]

Irisin's role in OP has been investigated among cases with previous osteoporotic fractures or medical diseases.[15,17,20], but the current study offered a new perspective, addressing irisin's role in an average community sample free of co-morbidities.

Limitation: The current study has limitations as it was done at a single centre, and had a relatively small sample size.

Conclusion

Strong serum irisin correlation to OP radiological markers and its good discrimination of OP implied its utility as a good serological marker of OP.

Acknowledgement: We are grateful to Al Mustansiriyah University administration for continuous support.

Disclaimer: None

Conflict of Interest: None.

Source of Funding: None.

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