ORIGINAL RESEARCH

Circulating miR-4739 and IGFBP-4 Levels in Postmenopausal Women with Osteoporosis and Osteoporotic Vertebral Fracture

Yisong Lei, MMed; Li Yu, MMed; Guoying Tang, MMed; Anping Liu, MD

ABSTRACT

Objective • This study aimed to investigate the potential plasma miRNA-mRNA regulatory networks in postmenopausal women with osteoporosis (OP) and osteoporotic vertebral fracture (OVF).

Methods • The study employed a cross-sectional design, and the microarray dataset GSE93883 was acquired from the Gene Expression Omnibus (GEO) to assess plasma miRNA profiles in postmenopausal women with osteoporosis (OP) and osteoporotic vertebral fracture (OVF). Subsequently, plasma microRNA-4739 (miR-4739) and Insulin-like Growth Factor Binding Protein-4 (IGFBP-4) levels were validated in a well-defined cohort comprising 210 postmenopausal women. This cohort consisted of three distinct groups: healthy controls (HC, n = 70), OP patients (n = 70), and OVF patients (n = 70).

Results • Analysis of the GSE93883 dataset revealed a stepwise increase in four miRNAs (hsa-miR-4739, hsa-miR-4505, hsa-miR-4459, hsa-miR-665) in plasma samples from HC to OP patients to OVF patients. Conversely, plasma miR-4666a-3p showed a gradual decrease. We predicted six genes targeted by miR-4739 using six online databases. Plasma miR-4739 levels were significantly higher in OP and OVF patients compared to HC, especially in OVF patients. However, plasma IGFBP-4 exhibited an inverse pattern. Pearson analysis demonstrated a significant negative correlation between plasma miR-4739 and plasma IGFBP-4 in OP and OVF patients. Receiver operating characteristic (ROC) curve analysis of plasma miR-4739 yielded a sensitivity of 35.71% and specificity of 95.71% for predicting the presence of OP and a sensitivity of 71.43% and specificity of 95.71% for predicting OVF, with an AUC of 0.865. Moreover, the area under the curve (AUC) for IGFBP-4 was higher than that for plasma miR-4739 when differentiating OP patients from OVF patients.

Conclusions • Circulating miR-4739 and IGFBP-4 demonstrated a negative correlation in OP and OVF patients, suggesting their potential as diagnostic biomarkers for OP and OVF in the future. (*Altern Ther Health Med.* 2023;29(7):204-209).

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INTRODUCTION

Osteoporosis (OP) is a chronic musculoskeletal disease characterized by low bone mineral density (*T* score <2.5 SDs) and microstructural degeneration of bone tissue.[1] The global prevalence of OP remains challenging to determine due to under-diagnosis; however, it is estimated that 200 million women worldwide are affected by OP, primarily due to the rapid increase in aging populations.[2] The primary cause of OP in postmenopausal women is the loss of sex hormones, particularly estrogen, and the subsequent decrease in bone mineral density.[3] Studies have reported that approximately 50% of women over the age of 50 years are affected by OP.[4] OP significantly increases the risk of fractures in elderly and postmenopausal women, resulting in more than 8.9 million fractures annually and posing substantial burdens on public healthcare and individual costs.[2,5]

Several factors, including low body mass index, glucocorticoid therapy, smoking, excessive alcohol consumption, low physical activity, and family history of fractures, are well-known risk factors associated with osteoporosis-related fractures, especially spinal and hip fractures.[6] Vertebral fractures (VFs), including vertebral compression fractures and burst fractures, are common types of osteoporotic fractures caused by indirect or direct external

forces acting on the lumbar or thoracic vertebrae.[7] Previous evidence has shown that VF is associated with disability and increased mortality, with approximately 28% of female fracture-related deaths attributed to VF.[8,9]

Currently, biochemical markers of bone turnover and bone mineral density testing are commonly used to diagnose OP.[10] However, these methods have limitations in terms of specificity and sensitivity. With advancements in biotechnology, microRNAs (miRNAs), small non-coding RNA molecules, can now be detected in blood serum, plasma, urine, or saliva. MiRNAs have been reported to play a role in bone metabolism and osteoporosis by regulating cell gene expression.[11] They have emerged as promising biomarkers for the diagnosis of OP and assessment of fracture risk.[12] For example, increased miR-21 and miR-125b expression in serum and bone tissue has been observed in OP patients.[13]

Our study aimed to explore differentially expressed miRNAs in the plasma of postmenopausal women with OP and osteoporotic vertebral fractures (OVF) through microarray dataset analysis. Furthermore, we sought to investigate the potential miRNA-mRNA regulatory networks, focusing on miR-4739 and IGFBP4, in the context of OVF in postmenopausal women with OP. This study may contribute to the early diagnosis of OP and the assessment of OVF risk in postmenopausal women.

METHODS AND MATERIALS

Microarray Dataset Analysis

The microarray dataset analysis was conducted to identify plasma miRNA profiles using the Bone Mineral Density (BMD) and *T*-Score criteria.[14] The dataset GSE93883, available on the GPL18058 platform, was utilized for this analysis. GEO2R was employed to compare the plasma miRNA profiles of three groups: HC with a *T*-Score ≥ -1 (n = 6, mean age: 47.83 ± 9.70 years), OP patients with a T-Score ≤ -2.5 (n = 6, mean age: 68.00 ± 13.86 years), and OVF patients with a *T*-Score ≤ -2.5 (n = 6, mean age: 69.67 ± 8.26 years).

Prediction of Target Genes

To predict the target genes, six online databases, namely miRTarBase,[15] miRWalk,[16] microT-CDS,[17] Targetscan,[18] miRDB,[19] and ENCORI,[20] were utilized. These databases were employed to establish potential miRNA-mRNA regulatory networks.

Participants and Study Design

A total of 210 postmenopausal females aged between 50 and 80 years were retrospectively recruited for this study. The inclusion criteria ensured that participants did not have (1) any diseases affecting bone or calcium metabolism; (2) impaired hepatic function; (3) creatinine clearance <30 mL/min; (4) alcohol or drug abuse; (5) were taking medications that alter bone and mineral metabolism. Menopause was formally defined as the absence of menstrual cycles for 12 consecutive months.[21] The presence of OP was verified using dual-energy X-ray absorptiometry, while OVF was confirmed by radiography showing one or more vertebral fractures.[22] The participant cohort consisted of 70 HC with an average age of 65.04 ± 8.88 years, 70 OP patients with an average age of 66.60 ± 9.21 years, and 70 OVF patients with an average age of 65.66 ± 8.71 years. No significant differences were observed in age among the three groups (ANOVA $F = 0.540$, $P = .584$). All participants provided informed written consent, and the Ethics Committee approved the study design, informed consent process, and protocols.

Plasma Collection

Venous blood samples were collected between 8:00 and 9:00 am after an overnight fast of at least 8 hours. The samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes, followed by centrifugation to separate the plasma. The plasma samples were then stored at -80°C for subsequent analyses.

Total RNA Isolation and RT-qPCR for the Detection of Plasma miR-4739

Total RNA was extracted from the plasma samples, and reverse transcription was performed using the miScript reverse transcription kit (Qiagen). The reverse-transcribed RNA was subjected to RT-qPCR analysis using the TaqMan MicroRNA Assay (4440885, Thermo Fisher Scientific Co., Ltd., Shanghai, China) on a 7500 Fast System (Roche). The relative level of miR-4739 was determined based on the Ct value and normalized with cel-miR-39 using the $2^{-\Delta\Delta CT}$ method.

Enzyme-Linked Immunosorbent Assay (ELISA) for Plasma IGFBP-4

Plasma IGFBP-4 levels were measured using a human solid-phase sandwich ELISA kit (Thermo Fisher Scientific Co., Ltd., Shanghai, China). The minimum detectable dose of human IGFBP-4 is 250 pg/mL, with an intra-assay CV% of <10% and inter-assay CV% of <12%.

Statistical Analysis

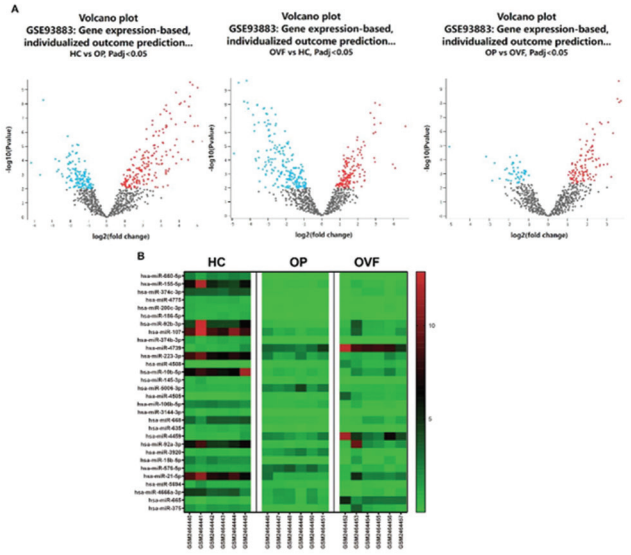
Data were analyzed using GraphPad Prism Software (Version 8.0, La Jolla, CA, USA). A significance level of $P < .05$ was considered statistically significant. Normally distributed data were presented as mean ± SD and analyzed using one-way ANOVA followed by Tukey's test. Enumeration data, expressed as [n (%)], were analyzed using the chi-square test. The Pearson correlation test was used to examine the correlation between plasma miR-483-5p and plasma IGFBP-4. Receiver operating characteristic (ROC) curve analysis was performed to differentiate between HC, OP, and OVF patients based on plasma miR-4739 and IGFBP-4 levels.

RESULTS

Identification of miRNA Profiles in Osteoporotic Patients with and without Vertebral Fractures

A specific miRNA profile (GSE93883) was obtained from the Gene Expression Omnibus (GEO), and the GEO2R method was employed to analyze the differential expression of miRNAs in the plasma (Figure 1). In the comparison

Figure 1. Identification of plasma miRNAs profiles (GSE93883) in osteoporotic patients with and without vertebral fractures



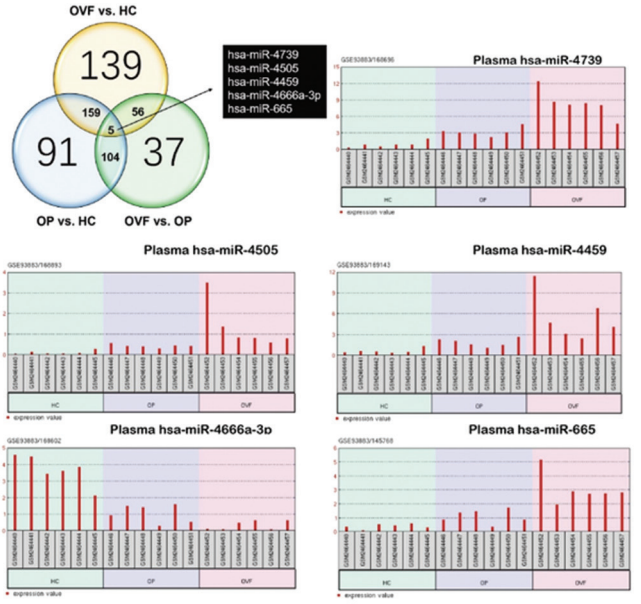
Note: A: Volcano plot of differentially expressed miRNAs in plasma between healthy controls (HC) and osteoporotic (OP) patients, between the HC and osteoporotic vertebral fracture (OVF) patients, and between the OP patients and OVF patients; B: The normalization and cluster heatmaps of the top 30 differentially expressed miRNAs in plasma based on the GSE93883 dataset.

between HC and OP patients, a total of 359 miRNAs showed differential expressions. Similarly, when comparing HC with OVF patients, 359 miRNAs exhibited differential expressions. Furthermore, when comparing OP patients with OVF patients, 202 miRNAs displayed differential expressions (Figure 2). Among these differentially expressed miRNAs, four miRNAs (miR-4739, miR-4505, miR-4459, miR-665) demonstrated a stepwise increase in plasma levels from HC to OP patients and further to OVF patients. Conversely, the plasma levels of miR-4666a-3p showed a gradual decrease.

Target Gene Prediction of miR-4739

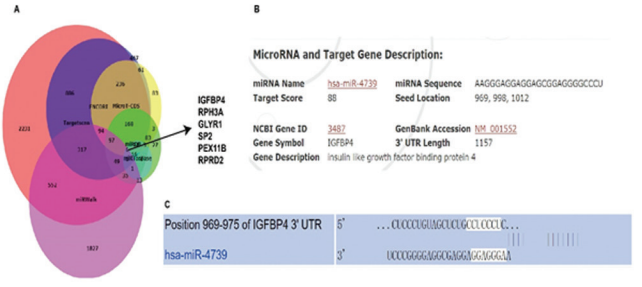
MiR-4739 has previously been reported to have regulatory effects on the osteogenic and adipocytic differentiation of immortalized human bone marrow stromal cells, thereby impacting the progression of osteoporosis.[23,24] However, limited information on the other miRNAs under investigation and their association with osteoporosis is available. Therefore, our focus was specifically directed toward miR-4739 for target gene prediction. As depicted in Figure 3A, Venn diagram analysis revealed that miR-4739 has the potential to target six genes: IGFBP4 (Insulin-like Growth Factor Binding Protein 4), RPH3A (Rabphilin-3A), GLYR1 (Glyoxylate Reductase 1 Homolog), SP2 (Specificity Protein 2), FEX11B (F-box Only Protein 11B), and RPRD2 (Regulation of Nuclear Pre-mRNA Domain Containing 2). This prediction was based on data obtained from six online

Figure 2. Five miRNAs (hsa-miR-4739, hsa-miR-4505, hsa-miR-4459, miR-4666a-3p, hsa-miR-665) were increased or decreased stepwise in plasma from healthy controls (HC) to osteoporosis (OP) patients to osteoporotic vertebral fracture (OVF) patients.



Note: The figure compares miRNA expression levels among three groups. Increased expression of miR-4739, miR-4505, miR-4459, and miR-665 is observed stepwise from HC to OP patients and further to OVF patients. Conversely, miR-4666a-3p shows a gradual decrease. The differential expression of these miRNAs suggests their potential role in the pathogenesis of osteoporosis and osteoporotic vertebral fractures.

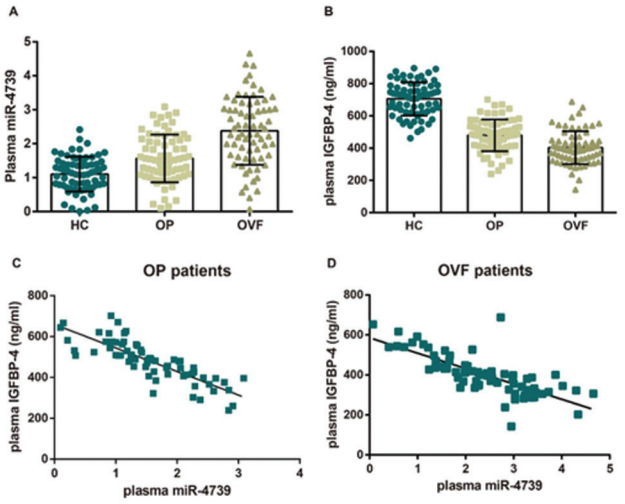
Figure 3. Target gene prediction of miR-4739



Note: A: Target mRNA of hsa-miR-4739 in miRTarBase, miRWalk, microT-CDS, Targetscan, miRDB, and ENCORI. B-C: miRDB; (B) and TargetScan (C) were used to predict the interaction between hsa-miR-4739 and 3'URT region of IGFBP4.

databases, including miRTarBase, miRWalk, microT-CDS, Targetscan, miRDB, and ENCORI. Among these genes, IGFBP4 stands out as a promising candidate due to its involvement in regulating bone mass.[25] A study on female mice with IGFBP4 deficiency demonstrated significant decreases in whole-body areal bone mineral density (BMD)

Figure 4. The plasma levels of miR-4739 and IGFBP4 in osteoporosis (OP) and osteoporotic vertebral fracture (OVF) patients



Note: A-B: Comparison of miR-4739 (A) and IGFBP4 (B) plasma levels among healthy controls (HC), OP patients, and OVF patients; C-D: Peason analysis demonstrated a significant negative correlation between plasma miR-4739 and plasma IGFBP-4 in OP group (C) and OVF group (D).

and bone mineral content (BMC).[26] Recognizing the potential significance of IGFBP4, we further focused on exploring the correlation between miR-4739 and IGFBP4 in both OP and OVF patients.

Figure 3B-C illustrates the complementary binding positions of highly conserved miR-4739 with IGFBP4, as indicated by miRDB (Target score: 88) and TargetScan databases.

The Plasma Levels of miR-4739 and IGFBP4 in OP and OVF Patients

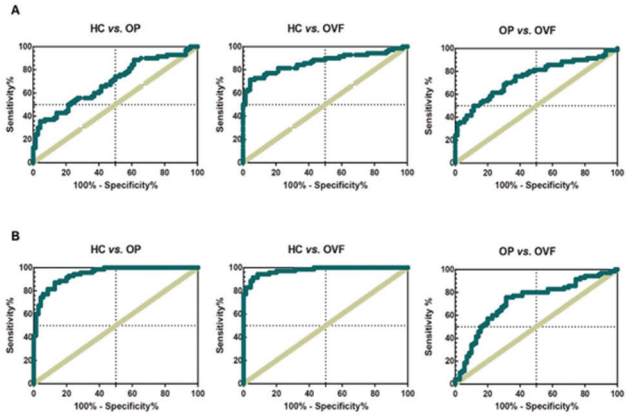
As shown in Figure 4A, the plasma levels of miR-4739 were significantly higher in the OP patients (1.563 ± 0.704) compared to the HC (1.103 ± 0.513, $P < .05$) according to RT-qPCR analysis. Notably, the levels of miR-4739 were even higher in OVF patients (2.378 ± 1.000, $P < .05$). Conversely, plasma IGFBP-4 exhibited an opposite trend, with the highest levels observed in HC (705.6 ± 103.3 ng/ml) compared to both OP patients and OVF patients (479.4 ± 98.13 ng/ml, 402.9 ± 102 ng/ml, respectively, $P < .05$, Figure 4B).

Pearson correlation analysis revealed a significant negative correlation between plasma miR-4739 and plasma IGFBP-4 in the OP group (r = -0.826, $P < .05$, Figure 4C) and the OVF group (r = -0.754, $P < .05$, Figure 4D).

Diagnostic Value of Plasma miR-4739 and IGFBP-4 in OP and OVF Patients

ROC curves were constructed to evaluate the diagnostic value of plasma miR-4739 (Figure 5A) and IGFBP-4 (Figure 5B) in differentiating between HC, OP patients, and OVF patients.

Figure 5. ROC curve was draw for differentiating healthy control (HC), osteoporosis (OP) patients and osteoporotic vertebral fracture (OVF) patients on the basis of the plasma miR-4739 (A) and IGFBP-4 (B).



Note: The ROC curves depict the diagnostic performance of plasma miR-4739 and IGFBP-4 in differentiating between the three groups. The area under the curve (AUC) provides a measure of the accuracy of the test, with higher values indicating better discriminatory ability. The sensitivity and specificity values are also presented, representing the proportion of true positive and true negative results, respectively. These results suggest that plasma miR-4739 and IGFBP-4 have the potential as biomarkers for distinguishing OP and OVF in postmenopausal women.

Table 1. Diagnostic value of plasma miR-4739 and IGFBP-4 in osteoporosis (OP) patients with or without vertebral fracture (VF)

	AUC	95%CI	*P* value	Sensitivity %	Specificity %
Plasma miR-4739					
HC vs. OP	0.693	0.606~0.780	<.001	35.71	95.71
HC vs. OVF	0.865	0.801~0.929	<.001	71.43	95.71
OP vs. OVF	0.746	0.664~0.828	<.001	75.71	62.86
Plasma IGFBP-4					
HC vs. OP	0.945	0.911~0.978	<.001	87.14	87.14
HC vs. OVF	0.977	0.957~0.997	<.001	91.43	94.29
OP vs. OVF	0.721	0.635~0.807	<.001	75.71	68.57

Abbreviations: AUC, Area Under the Curve; CI: Confidence Interval; HC, Healthy Controls; OP, Osteoporosis; OVF, Osteoporotic Vertebral Fracture.

Table 1 summarizes the diagnostic performance of miR-4739 and IGFBP-4 in predicting the presence of OP and OVF. For plasma miR-4739, the ROC curve analysis showed a sensitivity of 35.71% and specificity of 95.71% for predicting the presence of OP, with an AUC of 0.693. Furthermore, it exhibited a sensitivity of 71.43% and specificity of 95.71% for predicting the presence of OVF, with an AUC of 0.865. In contrast, IGFBP-4 demonstrated higher diagnostic accuracy compared to plasma miR-4739. The AUC for IGFBP-4 was 0.945 when differentiating

OP patients, with a sensitivity of 84.14% and specificity of 87.14%. Similarly, in distinguishing OVF patients, the AUC for IGFBP-4 was 0.945, with a sensitivity of 91.43% and specificity of 94.29%. These results suggest that IGFBP-4 shows superior diagnostic potential compared to plasma miR-4739 in distinguishing both OP and OVF patients.

DISCUSSION

The incidence of osteoporosis increases with age, particularly in postmenopausal women. While the diagnosis of this disease relies on the quantitative evaluation of bone mineral density, its clinical significance lies in the occurrence of fractures. Fragility fractures, including VF, contribute to physical disability, impaired quality of life, increased mortality, and substantial medical costs.[27] Consequently, early diagnosis and fracture prevention in OP patients have emerged as crucial public health challenges.

In this study, we analyzed the microarray dataset using a specific miRNA profile and consulted six online databases to identify differentially expressed miRNAs in postmenopausal women with OP and OVF. Among the five differentially expressed miRNAs identified, four miRNAs (miR-4739, miR-4505, miR-4459, and miR-665) exhibited increased expression in plasma, while miR-4666a-3p displayed decreased levels.

Furthermore, we predicted that six genes (IGFBP4, RPH3A, GLYR1, SP2, FEX11B, and RPRD2) were targeted by hsa-miR-4739. Subsequently, we explored the role of the regulatory network involving hsa-miR-4739 and IGFBP4 in postmenopausal OP patients and OVF patients in our hospital. Wang et al.[28] demonstrated that the up-regulation of miR-4739 and the decreased level of bone morphogenetic protein 7 contributed to pleural fibrosis in mice. The bone morphogenetic protein belongs to the TGF-β family and has been shown to have therapeutic effects on fractures, bone defects, and OP.[29] These findings indirectly suggest the potential role of miR-4739 in the pathogenesis of OP and OVF.

Our findings demonstrated higher levels of plasma miR-4739 in OP and OVF patients compared to the HC. Specifically, OVF patients exhibited the highest level of miR-4739. These results are consistent with a study on osteogenic differentiation,[24] which reported increased expression of miR-4739 in bone marrow-derived mesenchymal stem cells from OP patients. The study also found that overexpression of miR-4739 was detrimental to cell viability. These findings support the involvement miR-4739 in osteogenic differentiation and suggest its potential role in the pathogenesis of OP and OVF.

The regulatory mechanism underlying the complex interplay between miRNAs and mRNAs has been well-established. Multiple pieces of evidence have shown that miR-4739 is involved in osteogenic differentiation by targeting specific genes such as Notch2,[30] LRP3,[23] and DLX3.[31] These findings further support the notion that miR-4739 plays a significant role in the regulation of bone metabolism and may contribute to the development of OP and OVF.

The data from our study revealed decreased levels of plasma IGFBP-4 in both OP and OVF patients, and a significant negative correlation was observed between the expression of miR-4739 and IGFBP-4. Age-associated diseases, including OP, are characterized by an imbalance between bone formation and resorption rates, and insulin-like growth factors play a crucial role in bone development. However, our findings contradict some studies conducted by Jehle et al.[32] and Mohan et al.[33], which reported increased circulating serum levels of IGFBP-4 with age and 1.3-fold higher levels in OP patients compared to age- and sex-matched control subjects. In the study of postmenopausal women by Kudo et al.[34], different doses of growth hormone were found to increase serum IGFBP-4 levels. On the other hand, our results are supported by an in vivo study,[26] which demonstrated that IGFBP-4 knockdown in adult female and male mice resulted in decreased bone formation and growth retardation. Systemic administration of IGFBP-4 led to increased osteocalcin and alkaline phosphatase levels in mice.[35] These findings highlight the complex and multifactorial nature of IGFBP-4 regulation and its role in bone metabolism.

We conducted a ROC curve analysis to confirm the diagnostic value of plasma miR-4739 and IGFBP-4 in OP and OVF patients. The results showed that miR-4739 exhibited a specificity of 95.71% for predicting both OP and OVF, with corresponding AUCs of 0.693 and 0.865, respectively. In the case of IGFBP-4, the AUC for predicting both OP and OVF was 0.945. These findings suggest that plasma miR-4739 and IGFBP-4 have the potential to serve as biomarkers for distinguishing OP and OVF in postmenopausal women.

Limitations and Future Directions

It is important to acknowledge a limitation of our study, which is the exclusive focus on miR-4739 while neglecting the other four miRNAs (hsa-miR-4505, hsa-miR-4459, hsa-miR-665, and miR-4666a-3p) that showed different expression patterns in the plasma of individuals with OP and OVF. Although miR-4739 exhibited a stepwise increase and has been implicated in regulating osteogenic and adipocytic differentiation of human bone marrow stromal cells, the lack of prior reports or studies associating the other four miRNAs with osteoporosis limits our understanding of their potential role in the disease. Further investigations considering these additional miRNAs may provide a more comprehensive understanding of their diagnostic value and contribution to the pathogenesis of OP and OVF. Due to limitations in terms of time and funding, we were unable to conduct a comprehensive investigation of all the identified miRNAs in our study. Therefore, our findings provide only a limited perspective on the complex miRNA landscape associated with OP.

Future studies must delve into the roles of these miRNAs and their interactions within regulatory networks to obtain a more comprehensive understanding of their involvement in the pathogenesis of OP. Further research is needed to gain valuable insights into the intricate mechanisms underlying OP and potentially uncover novel therapeutic targets or diagnostic markers.

CONCLUSION

In conclusion, our study found a significant association between postmenopausal OP and OVF with increased plasma levels of miR-4739 and decreased plasma levels of IGFBP-4. It is important to note that our findings regarding IGFBP-4 contradict some previous studies on OP. Therefore, further investigation is warranted to explore the correlation between IGFBP-4 and bone turnover markers in postmenopausal women, which could provide valuable insights into the underlying mechanisms. Additionally, conducting research with larger clinical sample sizes will enable a more in-depth examination of the pathological mechanisms involved. By addressing these areas, we can enhance our understanding of OP and pave the way for potential advancements in diagnosis, treatment, and prevention strategies.

DATA AVAILABILITY

The data used to support this study are available from the corresponding author upon request.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS

All authors read and approved the final manuscript. Yi-song Lei and Li Yu conceived the study and contributed to the writing and revision of the manuscript. Guo-ying Tang performed data analysis and visualization. An-ping Liu assisted in the manuscript revision. All authors made contributions to data collection. Yi-song Lei and Li Yu contributed equally to this work.

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All authors contributed to the study and agreed to be listed as authors.

REFERENCES

1. Aspray TJ, Hill TR. Osteoporosis and the Ageing Skeleton. *Subcell Biochem*. 2019;91:453-476. doi:10.1007/978-981-13-3681-2_16
2. Johnston CB, Dagar M. Osteoporosis in Older Adults. *Med Clin North Am*. 2020;104(5):873-884. doi:10.1016/j.mcna.2020.06.004
3. Wu D, Cline-Smith A, Shashkova E, Perla A, Katyal A, Aurora R. T-Cell Mediated Inflammation in Postmenopausal Osteoporosis. *Front Immunol*. 2021;12:687551. doi:10.3389/fimmu.2021.687551
4. Pfeifer M, Kohlwey L, Begerow B, Minne HW. Effects of two newly developed spinal orthoses on trunk muscle strength, posture, and quality-of-life in women with postmenopausal osteoporosis: a randomized trial. *Am J Phys Med Rehabil*. 2011;90(10):805-815. doi:10.1097/PHM.0b013e31821f6df3
5. Coughlan T, Dockery F. Osteoporosis and fracture risk in older people. *Clin Med (Lond)*. 2014;14(2):187-191. doi:10.7861/clinmedicine.14-2-187
6. Lane NE. Epidemiology, etiology, and diagnosis of osteoporosis. *Am J Obstet Gynecol*. 2006;194(2)(suppl):S3-S11. doi:10.1016/j.ajog.2005.08.047
7. Schousboe JT. Epidemiology of Vertebral Fractures. *J Clin Densitom* 2016;19(1):8-22,Griffith JF, Guglielmi G. Vertebral fracture. *Radiol Clin North Am*. 2010;48(3):519-529.
8. Goldstein CL, Chutkan NB, Choma TJ, Orr RD. Management of the Elderly With Vertebral Compression Fractures. *Neurosurgery*. 2015;77(suppl 4):S33-S45. doi:10.1227/NEU.0000000000000947
9. Kanis JA, Cooper C, Rizzoli R, Reginster JY; Scientific Advisory Board of the European Society for Clinical and Economic Aspects of Osteoporosis (ESCEO) and the Committees of Scientific Advisors and National Societies of the International Osteoporosis Foundation (IOF). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporos Int*. 2019;30(1):3-44. doi:10.1007/s00198-018-4704-5
10. Camacho PM, Petak SM, Binkley N, et al. American Association of Clinical Endocrinologists/American College of Endocrinology Clinical Practice Guidelines for the Diagnosis and Treatment of Postmenopausal Osteoporosis-2020 Update. *Endocr Pract*. 2020;26(suppl 1):1-46. doi:10.4158/GL-2020-0524SUPPL
11. Donati S, Ciuffi S, Palmini G, Brandi ML. Circulating miRNAs: A New Opportunity in Bone Fragility. *Biomolecules*. 2020;10(6):927. doi:10.3390/biom10060927
12. Xu J, Li M, Pei W, et al. Reduced Circulating Levels of miR-491-5p and miR-485-3p Are Associated with the Occurrence of Vertebral Fractures in Postmenopausal Women with Osteoporosis. *Genet Res*. 2022;2022:3838126. doi:10.1155/2022/3838126
13. Seeliger C, Karpinski K, Haug AT, et al. Five freely circulating miRNAs and bone tissue miRNAs are associated with osteoporotic fractures. *J Bone Miner Res*. 2014;29(8):1718-1728. doi:10.1002/jbmr.2175
14. Kanis JA. Diagnosis of osteoporosis and assessment of fracture risk. *Lancet*. 2002;359(9321):1929-1936. doi:10.1016/S0140-6736(02)08761-5
15. Huang HY, Lin YC, Cui S, et al. miRTarBase update 2022: an informative resource for experimentally validated miRNA-target interactions. *Nucleic Acids Res*. 2022;50(D1):D222-D230. doi:10.1093/nar/gkab1079
16. Sticht C, De La Torre C, Parveen A, Gretz N. miRWalk: an online resource for prediction of microRNA binding sites. *PLoS One*. 2018;13(10):e0206239. doi:10.1371/journal.pone.0206239
17. Paraskevopoulou MD, Georgakilas G, Kostoulas N, et al. DIANA-microT web server v5.0: service integration into miRNA functional analysis workflows. *Nucleic Acids Res* 2013;41(Web Server issue):W169-73.
18. McGeary SE, Lin KS, Shi CY, et al. The biochemical basis of microRNA targeting efficacy. *Science*. 2019;366(6472):eaav1741. doi:10.1126/science.aav1741
19. Chen Y, Wang X. miRDB: an online database for prediction of functional microRNA targets *Nucleic Acids Res*. 2020;48(D1):D127-D131. doi:10.1093/nar/gkz757
20. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res*. 2014;42(Database issue):D92-D97. doi:10.1093/nar/gkt1248
21. Regmi R. Gurung NJJoH, Sciences A. *Menopausal Symptom Experience Among Middle Aged Women in Pokhara*. 2019;9(2):6-9.
22. Marini S, Leoni E, Raggi A, et al. Proposal of an Adapted Physical Activity Exercise Protocol for Women with Osteoporosis-Related Vertebral Fractures: A Pilot Study to Evaluate Feasibility, Safety, and Effectiveness. *Int J Environ Res Public Health*. 2019;16(14):2562. doi:10.3390/ijerph16142562
23. Elsafadi M, Manikandan M, Alajez NM, et al. MicroRNA-4739 regulates osteogenic and adipocytic differentiation of immortalized human bone marrow stromal cells via targeting LRP3. *Stem Cell Res (Amst)*. 2017;20:94-104. doi:10.1016/j.scr.2017.03.001
24. Zhou Y, Xu B, Wu S, Liu Y. Prognostic Immune-Related Genes of Patients With Ewing's Sarcoma. *Front Genet*. 2021;12:669549. doi:10.3389/fgene.2021.669549
25. Hanson MK, Karkache IY, Molstad DHH, Norton AA, Mansky KC, Bradley EW. Phlpp1 is induced by estrogen in osteoclasts and its loss in Ctsk-expressing cells does not protect against ovariectomy-induced bone loss. *PLoS One*. 2021;16(6):e0251732. doi:10.1371/journal.pone.0251732
26. Maridas DE, DeMambro VE, Le PT, et al. IGFBP-4 regulates adult skeletal growth in a sex-specific manner. *J Endocrinol*. 2017;233(1):131-144. doi:10.1530/JOE-16-0673
27. Sànchez-Riera L, Wilson N. Fragility Fractures & Their Impact on Older People. *Best Pract Res Clin Rheumatol*. 2017;31(2):169-191. doi:10.1016/j.berh.2017.10.001
28. Wang M, Xiong L, Jiang LJ, et al. miR-4739 mediates pleural fibrosis by targeting bone morphogenetic protein 7. *EBioMedicine*. 2019;41:670-682. doi:10.1016/j.ebiom.2019.02.057
29. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. *Growth Factors*. 2004;22(4):233-241. doi:10.1080/08977190412331279890
30. Guo Z, Xie M, Zou Y, et al. Circular RNA Hsa_circ_0006766 targets microRNA miR-4739 to regulate osteogenic differentiation of human bone marrow mesenchymal stem cells. *Bioengineered*. 2021;12(1):5679-5687. doi:10.1080/21655979.2021.1967712
31. Li D, Yuan Q, Xiong L, Li A, Xia Y. The miR-4739/DLX3 Axis Modulates Bone Marrow-Derived Mesenchymal Stem Cell (BMSC) Osteogenesis Affecting Osteoporosis Progression. *Front Endocrinol (Lausanne)*. 2021;12:703167. doi:10.3389/fendo.2021.703167
32. Jehle PM, Schulten K, Schulz W, et al. Serum levels of insulin-like growth factor (IGF)-I and IGF binding protein (IGFBP)-1 to -6 and their relationship to bone metabolism in osteoporosis patients. *Eur J Intern Med*. 2003;14(1):32-38. doi:10.1016/S0953-6205(02)00183-8
33. Mohan S, Farley JR, Baylink DJ. Age-related changes in IGFBP-4 and IGFBP-5 levels in human serum and bone: implications for bone loss with aging. *Prog Growth Factor Res*. 1995;6(2-4):465-473. doi:10.1016/0955-2235(95)00027-5
34. Kudo Y, Itatsu S, Iwashita M, Iguchi T, Takeda Y. Effects of estrogen and parathyroid hormone on osteoblastic activity via regulating the binding activity of insulin-like growth factor binding protein-4 in SaOS-2 cells: implications for the pathogenesis of postmenopausal osteoporosis. *Biochim Biophys Acta*. 1995;1245(3):402-406. doi:10.1016/0304-4165(95)00115-8
35. Miyakoshi N, Qin X, Kasukawa Y, et al. Systemic administration of insulin-like growth factor (IGF)-binding protein-4 (IGFBP-4) increases bone formation parameters in mice by increasing IGF bioavailability via an IGFBP-4 protease-dependent mechanism. *Endocrinology*. 2001;142(6):2641-2648. doi:10.1210/endo.142.6.8192