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# Estimation of miRNA-21, miRNA-422, miRNA-142-3p, and miR-181c Gene Expression as Potential Biomarkers of Osteoporosis in Premenopausal Women

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## Abstract

**Background:** MicroRNAs (miRNAs) regulate several biological pathways in osteoporosis patients. The study aimed to estimate the correlation of miRNA-21, miRNA-422, miRNA-142-3p, and miR-181c with osteoporosis in premenopausal women.

**Patients and Methods:** This study was performed on 60 osteoporosis premenopausal and 30 healthy women. Determination of osteocalcin (OC), deoxypyridinoline (DPD), and bone-specific alkaline phosphatase (BAP) were done by ELISA, and estimation of miRNA gene expression was done by using qRT-PCR.

**Results:** OC, DPD, and BAP levels indicated a significant decrease in osteoporosis women. In addition, our data showed that miRNA-21 (p=0.0001, r=-0.5585) and miRNA-422 (p=0.0035, r=-0.3715) have a high expression and negative correlation with BMD. Meanwhile, the miRNA-142-3p (p=0.0136, r=0.3089) and miRNA-181c (p=0.0401, r=-0.2685) have decreased expression and positive correlation with BMD value.

**Conclusion:** The results of this study indicated a clear association of miRNA-21 and miRNA-422 in osteoporosis thus they may be useful as biomarkers for osteoporosis.

**Keywords:** Osteoporosis, miRNA-21, miRNA-422, miRNA-142-3p, and miR-181c.

## Introduction

Osteoporosis is a systemic disease characterized by bone loss, resulting in deterioration of bone tissue and low bone mineral density (BMD). Thus, the risk of fragility-related fractures increases in women and worsens with age (1). Osteoporosis prevalence is estimated at 23.1% for women and around 11.7% for men (2). Osteoporosis leads to severe complications such as fractures. It was projected that by 2050, the worldwide incidence of hip fracture in women would increase by 240% (3). Osteoporosis can be classified into two types: primary osteoporosis, which is related to menopause and aging, and secondary osteoporosis, which is caused by medical conditions or medications (4). It affects a significant portion of the global human population, with a prevalence rate of 18.3%. women are particularly impacted, experiencing a prevalence of 23.1%. Furthermore, Africa has the highest

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prevalence at an alarming 39.5% (2).

Premenopausal osteoporosis can be attributed to various factors, for example, anorexia nervosa, hyperprolactinemia, and drug-induced amenorrhea resulting from GnRH analog (5). chronic inflammation, lack of physical activity, and insufficient sex steroids (6–7). Genetic conditions, such as osteogenesis imperfecta, can predispose individuals to primary bone fragility disorders (6). Idiopathic osteoporosis is one of the conditions that can affect premenopausal women (7).

Biochemical bone markers, such as osteocalcin (OC), deoxypyridinoline (DPD), and bone-specific alkaline phosphatase (BAP), are increasingly recognized for their critical role as osteoporosis biomarkers (8). Genome-wide association studies (GWAS) have identified numerous susceptibility loci related to osteoporosis and bone mineral density (BMD). BMD and osteoporosis share many common susceptibility loci. The corresponding susceptibility genes are significantly enriched in biological pathways associated with bone health (9).

In silico approaches for small non-coding RNAs, particularly microRNAs, are crucial for predicting their interactions with target molecules and regulatory functions. Therefore, researchers can gain vital insights into the binding mechanisms and biological roles of microRNAs, thereby enhancing our understanding of their impact on post-transcriptional regulation and advancing this important field (10). Research shows that epigenetic changes may link genetic factors and environmental influences, increasing the risk of osteoporosis. Among these changes, certain RNA types, such as microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), play crucial roles in regulating gene expression and are essential to bone metabolism (11). MicroRNAs play a crucial role in osteoporosis by influencing bone metabolism, particularly bone resorption and synthesis, which leads to an understanding of the disease and potential therapies (12). Osteoporosis is a complex disease influenced by multifactorial risk, and its underlying causes can differ based on factors such as race, geographic location, and age. Therefore, this study aimed to estimate the correlation among miRNA-21, miRNA-422, miRNA-142-3p, and miR-181c with osteoporosis in a sample of premenopausal Iraqi women.

## Patients and Methods

**Selection of the study population:** The present study included 60 women with osteoporosis, aged 19 to 50 years, and 30 matched-age healthy women who served as a control group. All subjects were selected from the Tikrit/Iraq population. The inclusion criteria focused on women with osteoporosis who had not yet reached menopause. In contrast, the exclusion criteria ruled out women who exhibited clinical signs of poor general health unrelated to osteoporosis. Bone mineral density (BMD) was assessed using a dual-energy X-ray absorptiometry (DEXA) device. A T score of -2.5 or lower indicates the presence of osteoporosis.

**Analysis of bone biomarkers:** Venous blood samples were drawn from patients and controls in the morning after a 8- to 12-hour fast. To separate the serum from the blood components, the samples were centrifuged at 3000 RPM for 15 minutes. Once the serum was separated, it was divided into three smaller replicates and stored at -80°C to preserve its integrity for biochemical analysis. Finally, Serum levels of OC, DPD, and BAP were measured using enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Sunlong, China).

**Quantitative real-time PCR (qRT-PCR):** In this study, to evaluate the expression levels of microRNA (miRNA) using quantitative qPCR, RNA was first extracted from blood samples

ORIGINAL RESEARCH
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collected from both patient and healthy groups (QIAGEN, Hilden, Germany). The extracted RNA molecules were converted into complementary DNA (cDNA). For the quantitative RT-PCR, we utilized an ABI Prism 7500 system (Applied Biosystems, Foster City, CA, USA) along with SYBR Green QPCR Master Mix, which ensured accurate and sensitive detection of the RNA molecules. The primer sequences used in the current study are listed in Table 1. The $2^{-\Delta\Delta CT}$ method was used to estimate relative expression (13).

**Table 1.** primer sequences of miRNA genes**.**

| Genes | Nucleotides sequence | | Source |
|---|---|---|---|
| miRNA-21-5p | F | 5′-AACACGCTAGCTTATCAGACTGATG-3′ | Current study |
| miRNA-422a-5p | F | 5′-AACACGCACTGGACTTAGGGT -3 | |
| miR-142-3p | F | 5′-AACACGCTGTAGTGTTTCCTACTTT-3′ | |
| Universal | R | 5′-CAGTGCAGGGTCCGAGGT-3′ | |
| miR-181c | F | 5′-GTGTGGGAACATTCAACCTGTCGGTG-3′ | |
| | R | 5′-CCAGTCTCAGGGTCCGAGGTATTC-3′ | |
| U6 | F | GTGCTCGCTTCGGCAGCA | |
| | R | CAAAATATGGAACGCTTC | |

## Statistical analysis

Statistical analyses were performed using GraphPad Prism 10 software. A Student's T-test was strategically employed to reveal significant associations between crucial clinical and pathological osteoporosis-related factors. Furthermore, the Receiver Operating Characteristic (ROC) test was implemented to accurately establish osteoporosis markers risk. An in-depth investigation using Pearson correlation analysis shed light on the correlation between miRNA and BMD degree, as the correlation coefficient (r) effectively measures these associations. A probability value of less than 0.05 was applied to signify the presence of statistically significant differences, reinforcing the validity of our findings.

## Results

**Demographic data:** The mean age of osteoporosis women is 43.45 years, with a standard deviation of 7.303 years. The mean BMI is 30.92 kg/m2, with a standard deviation of 2.96. Their average Body Mass Index (BMI) stands at 30.92 kg/m², accompanied by a standard deviation of 2.96, emphasizing the association between weight and bone health. Furthermore, the mean bone mineral density (BMD) is -2.043 g/cm², with a standard deviation of 0.9241 g/cm², underscoring the critical need for early detection in this demographic age to promote better health outcomes. Table 2 presents more details about demographic data.

**Table (2).** The mean and standard deviation of the demographic in patients and control.

| Categories | Osteoporosis group | | Control group | |
|---|---|---|---|---|
| | Number | Mean ± SD | Number | Mean ± SD |
| Age (years) | 60 | 43.45 ± 7.303 | 30 | 41.93± 6.664 |
| BMI (kg/m$^2$) | 60 | 30.92 ± 2.96 | 30 | 32.56± 3.56 |
| Normal weight | 32 | 22.8 ± 4.28 | 15 | 19.21± 7.11 |
| Overweight | 20 | 28.29 ± 2.53 | 11 | 26.81± 5.23 |
| Obesity | 8 | 34.13 ± 2. 5 | 4 | 35.12± 8.45 |
| BMD | 60 | -2.043 ± 0.9241 | | |

**Biochemical profile:** The osteoporosis group's mean ± standard deviation osteocalcin is 21.81± 2.093 ng/ml, while 13.51± 3.269ng/ml in the control group with p-value = 0.0001 (highly

ORIGINAL RESEARCH
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significant). The mean± standard of DPD was 24.68± 6.143 nmol/l in the osteoporosis group and 22.16± 3.517 nmol/ in healthy women with p-value = 0.0405 (significant). The osteoporosis group's mean ± SD BAP is 37.67± 9.223 U/L, while 33.37± 6.906 U/L in the control group with p-value = 0.0373 (significant). Table 3 shows all the details of biochemical markers.

**Table 3.** The mean, standard deviation (SD), and p-value of biochemical data in patients and control.

| Categories | Osteoporosis | | Control | | P value |
|---|---|---|---|---|---|
| | Median | Mean ± SD | Median | Mean ± SD | |
| Osteocalcin (ng/ml) | 21.70 | 21.81± 2.093 | 14.48 | 13.51 ±3.269 | 0.0001 |
| DPD (nmol/l) | 27.39 | 24.68± 6.143 | 24.83 | 22.16 ±3.517 | 0.0405 |
| BAP (U/L) | 36.38 | 37.67± 9.223 | 40.56 | 33.37 ±6.906 | 0.0373 |

**miRNA expression:** According to the Figure 1A, the osteoporosis group exhibits a considerably greater Mean ± SD of miRNA-121 expression than the control group (1.435±0.5429 vs 0.6415±0.3246, p value= 0.0001). The osteoporosis group's median value is higher than the control group's (1.956 vs0.7849). The ROC curve shows an AUC of 0.8539, indicating a high diagnostic accuracy of miRNA-121. Thus, miRNA-121 may successfully discriminate against osteoporosis cases. There is 76.67% sensitivity and 86.66% specificity. It can therefore detect about 76.67% of osteoporosis cases and 86.66% of healthy cases by miRNA-121 expression (Figure 1B).


**Figure 1. (A)** Box plot of the Relative miRNA-21 expression, T-test, significant at $p \leq 0.05$, **(B)** ROC curve of the Relative miRNA-21 expression, significant at $p \leq 0.05$.

As can be observed in the Figure 2A, the osteoporosis group has a significantly higher miRNA-422 expression than the control group (Mean ± SD=1.767± 0.5599 vs 1.034± 0.2996, p value= 0.0001). Furthermore, the median of the osteoporosis group is higher than that of the control group (2.136 vs 1.270). According to ROC curve analysis in Figure 2B, the miRNA-422 has a strong diagnostic accuracy, as indicated by its area under the curve (AUC) of 0.9033. Therefore, miRNA-422 can correctly detect the majority of osteoporosis cases and exclude the healthiest individuals, with a sensitivity of 73.33% and a specificity of 93.33%.

Diyala Journal of Medicine

ORIGINAL RESEARCH
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**Figure 2. (A)** Box plot of the Relative miRNA-422 expression. T-test, significant at $p \leq 0.05$, **(B)** ROC curve of the Relative miRNA-422 expression, significant at $p \leq 0.05$.

The relative expression of miRNA-142-3p in the osteoporosis group appeared to be considerably lower (1.824±0.5680) than that in the healthy group (3.712±0.8056), with a p-value of less than 0.0001, indicating a statistically significant difference, as shown in Figure 3A. The ROC Curve for the expression of relative miRNA-142-3p is illustrated in Figure 3B. The AUC of 0.9711 indicates excellent discrimination between the two groups. Sensitivity (88.33%) refers to the percentage of all volunteer women who have been correctly diagnosed with osteoporosis.

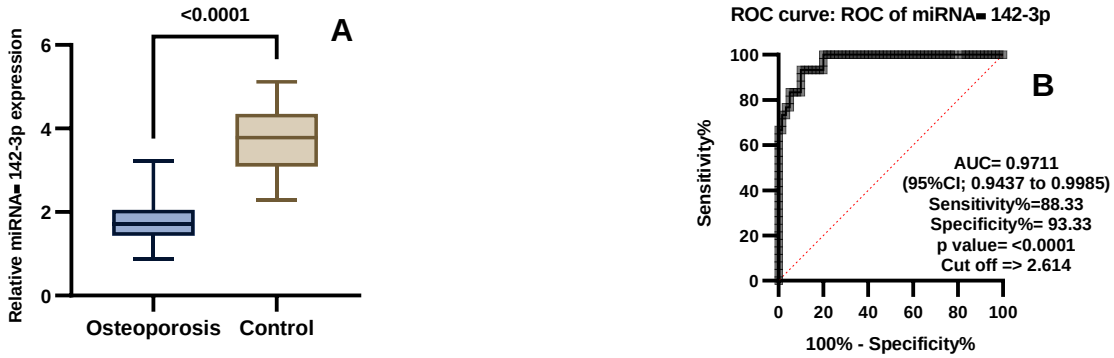


**Figure 3. (A)** Box plot of the Relative miRNA-142-3P expression, T-test, significant at $p \leq 0.05$, **(B)** ROC curve of the Relative miRNA-142-3P expression, significant at $p \leq 0.05$.

At 1.396, the median expression of miRNA-181C level for the osteoporosis women is lower than that of the healthy women at 4.091. Compared to the healthy women, the osteoporosis group's gene expression of miRNA-181C seems to be significantly lower (1.589± 0.6601 vs 4.100± 1.160, p value= 0.0001) as seen in Figure 4A. Figure 4B displays the ROC curve analysis of two study groups, as indicated by the AUC of 0.9728. Sensitivity at 88.33% is the percentage of osteoporosis women who had their condition correctly identified out of all those patients with osteoporosis. Specificity at 96.66% is the percentage of healthy controls that were accurately recognized out of all healthy volunteers. As well as for sensitivity and specificity, the AUC results indicate that miRNA-181C gene expression may be an excellent biomarker for differentiating between healthy and osteoporosis women.

Diyala Journal of Medicine

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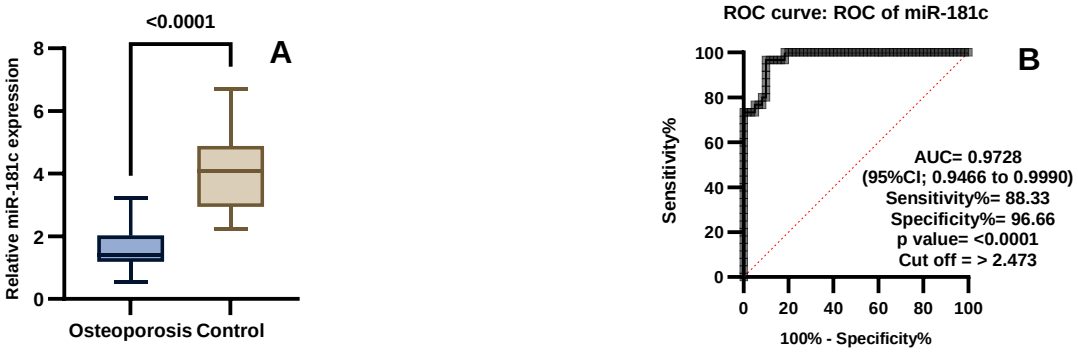
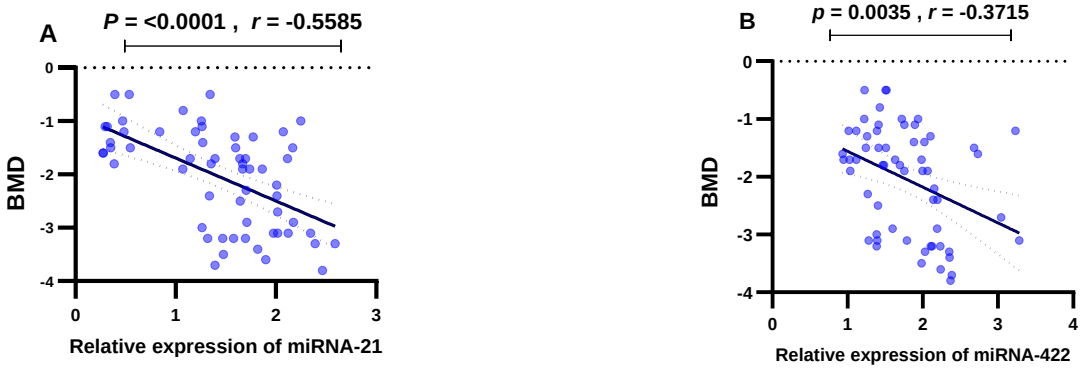



**Figure 4. (A)** Box plot of the Relative miRNA-181C expression, T-test, significant at $p \leq 0.05$. **(B)** ROC curve of the Relative miRNA-181C expression, significant at $p \leq 0.05$.

The correlation between bone mineral density (BMD) and the gene expression of four types of microRNAs (miRNAs) miRNA-21, miRNA-422, miRNA-142-3p, and miRNA-181c is shown in scatter plots with regression lines and statistical data in Figure 5.

Figure 5A (miRNA-21) shows a strong negative correlation (r = -0.5585) with high statistical significance ($p < 0.0001$). This implies that higher gene expression of miRNA-21 is correlated with lower BMD value in osteoporosis women. On the other hand, a moderately negative correlation (r = -0.3715) and statistical significance (p = 0.0035) are seen in Figure 5B (miRNA-422). This means that although the correlation between increased gene expression of miRNA-422 and lower value of BMD is smaller than that of miRNA-21, it still exists in osteoporosis women.

The results of the correlational analysis are presented in Figure 5C and show a weak positive correlation (r = 0.3089) with statistical significance (p = 0.0163) between miRNA-142-3p gene expression and BMD. Although the correlation is weak, higher expression of miRNA-142-3p is correlated to higher value of BMD. From Figure 5D, it can be seen that a weak positive correlation (r = 0.2658) and statistical significance (p = 0.0401) are observed for miRNA-181c gene expression with BMD value. This implies that there is a weak association between BMD value and miRNA-181c gene expression in women with osteoporosis.



ORIGINAL RESEARCH
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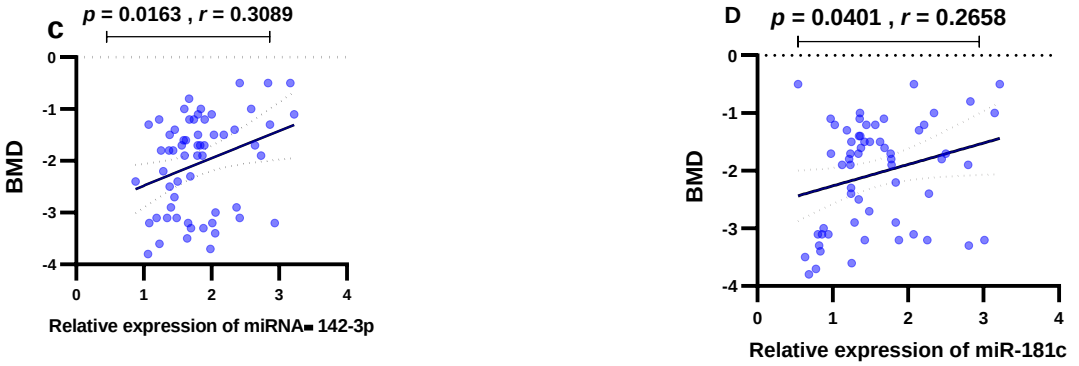


**Figure 5.** Correlation between BMD and relative expression of the following: (A) miRNA-21, (B) miRNA-422 (C) miRNA-142-3P and (D) miRNA-181C , significant at $p \leq 0.05$.

## Discussion

Osteoporosis is typically common in postmenopausal women with low estrogen levels decreases dramatically with progress age and bone mineral density (BMD) typically decreases. There is very important in initiating discussions and encouraging people to take proactive steps to increase bone mass and lower fracture risk far earlier in life, especially when bones are still growing (14). Numerous biochemical markers have been confirmed to help evaluate bone metabolism, bone loss, and osteoporosis. These include deoxypyridinoline (DPD), which is associated with the process of bone resorption, and osteocalcin (OC) and bone-specific alkaline phosphatase (BAP), which is correlated with bone growth (15, 16, 17, 18, 19, 20). However, the specificity of these indicators can differ and cannot fully represent bone metabolism. Therefore, more research is needed to standardize them (20, 21).The diagnosis of osteoporosis, the individual prognosis of bone loss, fracture, or the choice of pharmacological treatment are areas in which bone turnover markers are not helpful. In clinical trials, the turnover markers have helped clarify the pharmacodynamics and efficacy of osteoporosis medicines. Bone turnover markers could help track the stages of osteoporosis treatments as an alternative to BMD testing (23). The common cause of low bone mineral density (BMD) is increased bone resorption by osteoclasts, as opposed to bone synthesis, which is carried out by osteoblasts (24). Therefore, Bone mineral density (BMD) is an established and active measure to diagnose osteoporosis in patients (25-27). MicroRNAs (miRNAs) have grown in significance for the research of osteoporosis pathogenesis because they are the main regulators of gene expression and can alter processes associated with bone homeostasis (27-30). This study's results observed an increase in miRNA-21 expression and miRNA-422 expression in women with osteoporosis compared to healthy women. Some studies reported higher expression of miR-21 in osteoporotic fractures (31), whereas others noted a decrease in miR-21 expression (32). Some authors found exogenous miR 21 has demonstrated the ability to speed up the formation of new bone, indicating that it may find use in osteoporosis bone regeneration therapy (33). On the other hand, increased fragility fracture risk is linked to upregulation of miR-21, suggesting that miR-21 may be used as a biomarker to predict fractures caused by osteoporosis (34). Also, in previous meta-analysis which included and examined 27 trials with 2,263 individuals in total. According to the findings, miR-21-5p was significantly upregulated (WMD 0.88, 95% CI: 0.22 to 1.55) and may be a useful biomarker for

ORIGINAL RESEARCH
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osteoporosis diagnosis (35). Previous studies found a negative correlation between miR-422a levels and T- and Z-scores in patients with osteoporosis. These results indicate that miR-422a contributes to hBMSC adipogenesis through downregulating MeCP2 and that the loss of bone density in primary osteoporosis is associated with its circulating levels (36). MiR-422a was significantly upregulated in the low BMD group compared to the high BMD group. Nevertheless, it is still unknown how miR-422 a lowers BMD (37,38). Both miRNA-21 and miRNA-422 a showed significant inverse correlations with the BMD-lumbar spine, and miRNA-21 and miRNA-422 at showed inverse correlation with the BMD-femoral neck (38).

According to the current study, women with osteoporosis have lower levels of miRNA-142-3P and miRNA-181C than women in good health. A comparison of the findings with those of other studies confirms that miRNA-142-3P was significantly downregulated in osteoporosis and showed a strong correlation with bone mineral density (15,39). miR-142-3p inhibits Human periodontal ligament stem cells (hPDLSC) osteogenic development by downregulating SGK1 expression (40). A previous study indicated that miR-142-3p gene expression was down-regulated in osteoprotegerin through animal experiments and analysis of blood miRNA validation in osteoporosis cases (41). On other hand, bone Marrow-Derived Mesenchymal Stem Cells' miR-142-5p Inhibits Cell Migration and Targets the Adhesion Molecule VCAM-1 to Promote Osteoporosis (42). MiR-181c-5p has an important role in bone metabolism and is associated with progressive bone loss in osteoporosis patients (43). On other hand, the MiR-181c-5p inhibits Foxo1 from adversely controlling the osteogenic development of bone marrow mesenchymal stem cells (BMMSCs) in osteoporosis (44). MiR-181c-5p has a crucial role in bone formation and mineralization by upregulating Runx2 expression and downregulating Notch2 to improve osteogenic differentiation and mineralization of osteoblastic cells generated from the human jawbone (45). MiR-181c-5p contributes to bone loss by encouraging cell cycle arrest, mainly through the downregulation of cyclin B1 expression (46).

These data suggest that miRNA-21 and miRNA-422 may play a role in bone mineral density, with higher gene expression leading to lower BMD. The correlation with miRNA-142-3p is less clear and may require further research. The expression of miRNA-181c does not appear to be significantly correlated with BMD value. When the correlation value is moderate to weak, it indicates that other factors may also contribute to BMD value. It is important to remember that correlation does not always imply causation. In addition, according to the available data on miRDB, there are 469 predicted target genes for hsa-miR-21-5p, 418 predicted for hsa-miR-142-3p, and 1409 predicted for hsa-miR-181c-5p. This indicates the multiple functions expected for each of the study miRNA genes. Although there have been global studies on the role of miRNA-21, miRNA-422, miRNA-142-3p, and miR-181c genes in patients with postmenopausal osteoporosis, this is the first study to investigate these genes in premenopausal osteoporosis patients. It is the first study conducted on osteoporosis patients in the Iraqi population. The findings of this study have numerous essential implications for future practice, particularly in the development of early diagnosis methods and the treatment of osteoporosis.

## Conclusions

The present study aimed to investigate the expression of miRNA in osteoporosis. This study has identified increased expression of miRNA-21 and miR-422a, along with decreased gene expression of hsa-miR-

ORIGINAL RESEARCH
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142-3p and hsa-miR-181c-5p, in women withosteoporosis. The evidence from this study suggests that miRNA plays a role in determining bone mineral density (BMD) values. Although this current study is limited by a relatively small sample from one ethnic group, our data may be considered possible markers for diagnosis and future therapy for osteoporosis. Additionally, it is recommended to evaluate the role of microRNAs *in vivo* and silico, alongside other forms of epigenetics. This may help identify potential diagnostic or therapeutic targets for women patients with osteoporosis.

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**Ethical clearance:** Before conducting the study, ethical approval was obtained from the College of Education for Women at Tikrit University in Iraq (No. 3\7\4768, 7\11\2023), in accordance with the ethical guidelines outlined in the Declaration of Helsinki (1975).

**Conflict of interest:** None.

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## References

1. Bartl R. Osteoporosis in Clinical Practice. Springer; 2023 Mar 3. https://doi.org/10.1007/978-3-031-14652-7
2. Salari N, Ghasemi H, Mohammadi L, Behzadi MH, Rabieenia E, Shohaimi S, Mohammadi M. The global prevalence of osteoporosis in the world: a comprehensive systematic review and meta-analysis. Journal of orthopaedic surgery and research. 2021 Dec;16:1-20. https://doi.org/ 10.1186/s13018-021-02772-0.
3. Gullberg B, Johnell O, Kanis J. World-wide projections for hip fracture. Osteoporosis international. 1997 Sep;7:407-13. https://doi.org/10.1007/PL00004148
4. Liu S, Ge L, Hong Y. Causes, diagnose and treatment of osteoporosis. Highlights in Science. 2023. https://doi.org/10.54097/hset.v36i.6109
5. Pepe J, Body JJ, Hadji P, McCloskey E, Meier C, Obermayer-Pietsch B, Palermo A, Tsourdi E, Zillikens MC, Langdahl B, Ferrari S. Osteoporosis in premenopausal women: a clinical narrative review by the ECTS and the IOF. The Journal of Clinical Endocrinology & Metabolism. 2020 Aug;105(8):2487-506. https://doi.org/10.1210/clinem/dgaa306
6. Formosa MM, Christou MA, Mäkitie O. Bone fragility and osteoporosis in children and young adults. Journal of Endocrinological Investigation. 2024Feb;47(2):285-98. https://doi.org/10.1007/s40618-023-02179-0
7. Shane E, Shiau S, Recker RR, Lappe JM, Agarwal S, Kamanda-Kosseh M, Bucovsky M, Stubby J, Cohen A. Denosumab after teriparatide in premenopausal women with idiopathic osteoporosis. The Journal of Clinical Endocrinology & Metabolism. 2022 Apr 1;107(4):e1528-40. https://doi.org/10.1210/clinem/dgab850
8. Konukoglu D. Bone markers. Int J Med Biochem.2019;2(2):65-78. https://doi.org/10.14744/ijmb.2019.60362
9. Dong H, Zhou W, Wang P, Zuo E, Ying X, Chai S, Fei T, Jin L, Chen C, Ma G, Liu H. Comprehensive analysis of the genetic and epigenetic mechanisms of osteoporosis and bone mineral density. Frontiers in Cell and Developmental Biology. 2020 Mar 25;8:194. https://doi.org/10.3389/fcell.2020.00194
10. Grešová K, Vaculík O, Alexiou P. Using attribution sequence alignment to interpret deep learning models for miRNA binding site prediction. Biology. 2023 Feb 26;12(3):369. https://doi.org/10.3390/biology12030369
11. Baniasadi M, Talebi S, Mokhtari K, Zabolian

ORIGINAL RESEARCH
Published: 25 June 2025
DOI: 10.26505/djm.v28i2.1295

AH, Khosroshahi EM, Entezari M, Dehkhoda F, Nabavi N, Hashemi M. Role of non-coding RNAs in osteoporosis. Pathology-Research and Practice. 2024 Jan1;253:155036. https://doi.org/10.1016/j.prp.2023.155036

12. Shi H, Jiang X, Xu C, Cheng Q. MicroRNAs in serum exosomes as circulating biomarkers for postmenopausal osteoporosis. Frontiers in Endocrinology. 2022 Mar 10;13:819056. https://doi.org/10.3389/fendo.2022.819056

13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. methods. 2001 Dec 1;25(4):402-8. https://doi.org/10.1006/meth.2001.1262

14. McPhee C, Aninye IO, Horan L, Society for Women's Health Research Bone Health Working Group. Recommendations for Improving Women's Bone Health Throughout the Lifespan. Journal of Women's Health. 2022 Dec 1;31(12):1671-6. https://doi.org/10.1089/jwh.2022.036

15. Al-Rawaf HA, Gabr SA, Iqbal A, Alghadir AH. MicroRNAs as potential biopredictors for premenopausal osteoporosis: a biochemical and molecular study. BMC Women's Health. 2023 Sep 9;23(1):481. https://doi.org/10.1186/s12905-023-02626-3

16. Singh S, Kumar D, Lal AK. Serum osteocalcin as a diagnostic biomarker for primary osteoporosis in women. Journal of clinical and diagnostic research: JCDR. 2015 Aug1;9(8):RC04. https://doi.org/10.7860/JCDR/2015/14857.6318

17. Kalaiselvi VS, Prabhu K, Ramesh M, Venkatesan V. The association of serum osteocalcin with the bone mineral density in post menopausal women. Journal of clinical and diagnostic research: JCDR. 2013 Mar 20;7(5):814. https://doi.org/10.7860/JCDR/2013/5370.294

18. Abbass SA, Ali SH. The beneficial role of some bone markers in evaluating women with osteoporosis under different therapeutic regimens. Iraqi J Pharm Sci.2011;20(1):1-7. https://doi.org/10.31351/vol20iss1pp1-7

19. Jassim HI, Hussein AL. The Effect of Osteocalcin in Middle-Age Women With and Without Type2 Diabetes Mellitus. https://doi.org/10.26505/djm.v27i2.1224

20. Ibrahem IH. Biochemical Risk Determinants of Osteoporosis in Overweight and Obese Postmenopausal Women with Type 2 Diabetes Mellitus. Al-Kindy College Medical Journal. 2018 Oct21;14(1):33-6. https://doi.org/10.47723/kcmj.v14i1.15

21. Naeem ST, Hussain R, Raheem A, Siddiqui I, Ghani F, Khan AH. Bone turnover markers for osteoporosis status assessment at baseline in postmenopausal Pakistani females. J Coll Physicians Surg Pak. 2016 May 1;26(5):408-12.

22. Tournis S, Makris K. Clinical Use of Bone Turnover Markers in Osteoporosis. https://doi.org/10.1016/b978-0-12-801238-3.99538-2

23. Schini M, Vilaca T, Gossiel F, Salam S, Eastell R. Bone turnover markers: basic biology to clinical applications. Endocrine Reviews. 2023 Jun;44(3):417-73. https://doi.org/10.1210/endrev/bnac031

24. Manolagas SC. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocrine reviews. 2000 Apr 1;21(2):115-37. https://doi.org/10.1210/edrv.21.2.0395

25. Banefelt J, Timoshanko J, Söreskog E, Ortsäter G, Moayyeri A, Åkesson KE, Spångéus A, Libanati C. Total hip Bone mineral density as an indicator of fracture risk in bisphosphonate-treated patients in a real-world setting. Journal of Bone and Mineral Research. 2020 Dec 1;37(1):52-8. https://doi.org/10.1002/jbmr.4448

26. Lewiecki EM. Clinical vignettes: using non-BMD measurements in clinical practice. Clinical

ORIGINAL RESEARCH
Published: 25 June 2025
DOI: 10.26505/djm.v28i2.1295

Reviews in Bone and Mineral Metabolism. 2016Mar;14:50-4. https://doi.org/10.1007/s12018-015-9200-2

27. Sakai Y, Koike G, Numata M, Taneda K, Jingu S. Is whole body bone mineral density measured by the dual energy X-ray absorptiometry applied to evaluate risk of osteoporosis among Japanese adult females?. Fukuoka Igaku Zasshi= Hukuoka Acta Medica. 2010 Apr 1;101(4):69-74.

28. Hackl M, Heilmeier U, Weilner S, Grillari J. Circulating microRNAs as novel biomarkers for bone diseases- Complex signatures for multifactorial diseases. Mol Cell Endocrinol. 2016 Sep 5;432(83-95):10-16. https://doi.org/10.1016/j.mce.2015.10.015

29. Sun Y, Chen P, Zhao B. Role of extracellular vesicles associated with microRNAs and their interplay with cuproptosis in osteoporosis. Non-coding RNA Research. 2024 Mar 13. https://doi.org/10.1016/j.ncrna.2024.03.002

30. Huang C, Li Y, Li B, Liu X, Luo D, Liu Y, Wei M, Yang Z, Xu Y. Identifying potential ferroptosis key genes for diagnosis and treatment of postmenopausal osteoporosis through competitive endogenous RNA network analysis. Heliyon. 2024 Jan 15;10(1). https://doi.org/10.1016/j.heliyon.2023.e23672

31. Sun X, Li X, Qi H, Hou X, Zhao J, Yuan X, Ma X. MiR-21 nanocapsules promote early bone repair of osteoporotic fractures by stimulating the osteogenic differentiation of bone marrow mesenchymal stem cells. Journal of orthopaedic translation. 2020 Sep 1;24:76-87. https://doi.org/10.1016/j.jot.2020.04.007

32. Ritter A, Han J, Bianconi S, Henrich D, Marzi I, Leppik L, Weber B. The Ambivalent Role of miRNA-21 in Trauma and Acute Organ Injury. International Journal of Molecular Sciences. 2024 Oct20;25(20):11282. https://doi.org/10.3390/ijms252011282

33. Subramaniam R, Vijakumaran U, Shanmuganantha L, Law JX, Alias E, Ng MH. The role and mechanism of MicroRNA 21 in osteogenesis: an update. International Journal of Molecular Sciences. 2023 Jul 11;24(14):11330. https://doi.org/10.3390/ijms241411330

34. Si ZX, Zhou SF, Shen ZL, Yan JL. Increased circulating microRNA-21 level as a potential indicator for predicting a higher risk of incident fragility fractures. Journal of Osteopathic Medicine. 2024 Feb 23;124(3):121-5. https://doi.org/ 10.1515/jom-2023-0174

35. Gao J, Zhang X, Ding J, Zhang H, Zhang X, Jiang J, Chen W. The characteristic expression of circulating MicroRNAs in osteoporosis: a systematic review and meta-analysis. Frontiers in Endocrinology. 2024 Dec 16;15:1481649. https://doi.org/10.3389/fendo.2024.1481649

36. Giuliani A, Sabbatinelli J, Amatori S, Graciotti L, Silvestrini A, Matacchione G, Ramini D, Mensà E, Prattichizzo F, Babini L, Mattiucci D. MiR-422a promotes adipogenesis via MeCP2 downregulation in human bone marrow mesenchymal stem cells. Cellular and Molecular Life Sciences. 2023 Mar;80(3):75. https://doi.org/10.1007/s00018-023-04719-6

37. Cao Z, Moore BT, Wang Y, Peng XH, Lappe JM, Recker RR, Xiao P. MiR-422a as a potential cellular microRNA biomarker for postmenopausal osteoporosis. PloS one. 2014 May 12;9(5):e97098. https://doi.org/10.1371/journal.pone.0097098

38. Mohammadisima N, Farshbaf-Khalili A, Ostadrahimi A. Up-regulation of plasma miRNA-21 and miRNA-422a in postmenopausal osteoporosis. Plos one. 2023 Oct118(10):e0287458. https://doi.org/10.1371/journal.pone.0287458

39. Chen J, Li K, Pang Q, Yang C, Zhang H, Wu F, Cao H, Liu H, Wan Y, Xia W, Wang J. Identification of suitable reference gene and biomarkers of serum miRNAs for osteoporosis. Scientific Reports. 2016 Nov8;6(1):36347.

ORIGINAL RESEARCH
Published: 25 June 2025
DOI: 10.26505/djm.v28i2.1295

https://doi.org/10.1038/srep36347

40. Sun Y, Shi J, Luo X, Xu X. microRNA-142–3p regulates osteogenic differentiation of human periodontal ligament stem cells via mediating SGK1. Journal of stomatology, oral and maxillofacial surgery. 2023 Feb 1;124(1):101369. https://doi.org/10.1016/j.jormas.2022.101369

41. Cheng Q, Tang W, Sheu TJ, Du Y, Gan J, Li H, Hong W, Zhu X, Xue S, Zhang X. Circulating TGF-β1 levels are negatively correlated with sclerostin levels in early postmenopausal women. Clinica Chimica Acta. 2016 Apr 1;455:87-92. https://doi.org/10.1016/j.cca.2016.01.025.

42. Teng Z, Xie X, Zhu Y, Liu J, Hu X, Na Q, Zhang X, Wei G, Xu S, Liu Y, Zhang X. miR-142-5p in bone marrow-derived mesenchymal stem cells promotes osteoporosis involving targeting adhesion molecule VCAM-1 and inhibiting cell migration. BioMed Research International. 2018;2018(1):3274641. https://doi.org/10.1155/2018/3274641

43. Ma J, Lin X, Chen C, Li S, Zhang S, Chen Z, Li D, Zhao F, Yang C, Yin C, Qiu W. Circulating miR-181c-5p and miR-497-5p are potential biomarkers for prognosis and diagnosis of osteoporosis. The Journal of Clinical Endocrinology & Metabolism. 2020 May;105(5):1445-60. https://doi.org/10.1210/clinem/dgz300

44. Yang C, Shi K, Wang J, Yan L. The regulatory effect of miR-181c-5p on the differentiation function of bone marrow mesenchymal stem cells in postmenopausalosteoporotic mice. https://doi.org/10.21203/rs.3.rs-1453818/v1

45. Murodumi H, Shigeishi H, Kato H, Yokoyama S, Sakuma M, Tada M, Ono S, Rahman MZ, Ohta K, Takechi M. Melatonin-induced miR-181c-5p enhances osteogenic differentiation and mineralization of human jawbone-derived osteoblastic cells. Molecular Medicine Reports. 2020Oct;22(4):3549-58. https://doi.org/10.3892/mmr.2020.11401

46. Sun Z, Li Y, Wang H, Cai M, Gao S, Liu J, Tong L, Hu Z, Wang Y, Wang K, Zhang L. miR-181c-5p mediates simulated microgravity-induced impaired osteoblast proliferation by promoting cell cycle arrested in the G2 phase. Journal of Cellular and Molecular Medicine. 2019 May;23(5):3302-16. https://doi.org/10.1111/jcmm.14220

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# تقدير التعبير الجيني لـ miRNA-21 و miRNA-422 و miRNA-142-3p و miR-181c كعلامات حيوية محتملة لهشاشة العظام لدى الاناث قبل انقطاع الطمث

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## الملخص

**الخلفية:** تنظم microRNAs (miRNAs) العديد من المسارات البيولوجية لدى مرضى هشاشة العظام.

**الأهداف:** تهدف الدراسة إلى تقدير ارتباط miRNA-21 وmiRNA-422 وmiRNA-142-3p وmiR-181c بهشاشة العظام قبل انقطاع الطمث.

**المرضى والطرق**أجريت هذه الدراسة على ٦٠ امرأة مصابة بهشاشة العظام قبل انقطاع الطمث و ٣٠ امرأة سليمة. تم بواسطة فحص إليزا بتحديد أوستيوكالسين (OC) وديوكسي بيريدينولين (DPD) والفوسفاتيز القلوي الخاص بالعظام (BAP). قدر التعبير الجيني لـ miRNA باستخدام تقنية الـ qRT-PCR.

**النتائج:** أشارت مستويات OC وDPD وBAP إلى انخفاض كبير لدى النساء المصابات بهشاشة العظام. كما تظهر بياناتنا أن miRNA-21 (p=0.0001, r=-0.5585) و miRNA-422 (p=0.0035, r=-0.3715) لهما تعبير عالي وارتباط سلبي مع كثافة المعادن في العظام. التعبير المنخفض والارتباط الإيجابي مع miRNA-142-3p (p=0.0136, r=0.3089) و miRNA-181c (p=0.0401, r=-0.2685) مع قيمة كثافة المعادن في العظام.

**الاستنتاج:** أشارت نتائج هذه الدراسة إلى وجود تأثير واضح لـ miRNA-21 وmiRNA-422 في هشاشة العظام وبالتالي قد يكونان مفيدين كعلامات حيوية لهشاشة العظام.

**الكلمات المفتاحية:** هشاشة العظام، **miRNA-21، miRNA-422، miRNA-142-3p، وmiR-181c**.

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