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miR-199b-5p como potencial biomarcador de fragilidad ósea en diabetes mellitus tipo 2: papel dual en la fisiopatología musculoesquelética

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ABSTRACT

Type 2 diabetes *mellitus* (T2DM) is associated with increased risk of bone fragility and fractures, yet early diagnostic tools for musculoskeletal complications remain lacking. This study aimed to identify microRNAs (miRNAs) differentially expressed in T2DM patients and assess their potential as biomarkers for bone fragility. Serum samples from 8 T2DM patients and 8 matched healthy controls were analyzed using high-throughput sequencing and RT-qPCR. Among the miRNAs examined, hsa-miR-199b-5p was significantly under-expressed in T2DM patients, particularly in those with degraded trabecular bone score (TBS) and lower bone mineral density (BMD).

Statistical analyses revealed strong positive correlations between miR-199b-5p expression and indicators of bone integrity, including cortical and trabecular volumetric BMD, as well as serum periostin levels. Conversely, negative correlations were found with fracture risk (FRAX), TBS-adjusted FRAX, and CTX levels, supporting its role in bone metabolism. Literature suggests miR-199b-5p promotes osteogenesis via the GSK-3β/β-catenin and periostin-mediated Wnt/β-catenin signaling pathways. Thus, its reduced expression may contribute to impaired bone remodeling in T2DM. Interestingly, miR-199b-5p shows contrasting effects in osteoarthritis (OA), where it is upregulated and contributes to cartilage degradation. Periostin, similarly, promotes bone formation in T2DM but may exacerbate inflammation in OA. These findings underscore the context-dependent roles of miR-199b-5p and highlight its potential as a dual biomarker: protective in T2DM bone loss, yet detrimental in OA. Further research is needed to clarify its therapeutic relevance and ensure disease-specific targeting strategies.

Keywords: Type 2 diabetes *mellitus.* microRNAs. Bone fragility. Osteoarthritis. Biomarkers.

RESUMEN

La diabetes *mellitus* tipo 2 (DM2) se asocia con un mayor riesgo de fragilidad ósea y fracturas, aunque aún faltan herramientas de diagnóstico temprano para las complicaciones musculoesqueléticas. Este estudio tuvo como objetivo identificar microRNAs (miRNAs) diferencialmente expresados en pacientes con DM2 y evaluar su potencial como biomarcadores de fragilidad ósea. Se analizaron muestras de suero de 8 pacientes con DM2 y 8 controles sanos emparejados por edad y sexo mediante secuenciación de alto rendimiento y RT-qPCR. Entre los miRNAs examinados, hsa-miR-199b-5p se encontró significativamente subexpresado en los pacientes con DM2, particularmente en aquellos con *trabecular bone score* (TBS) degradado y menor densidad mineral ósea (DMO).

Los análisis estadísticos revelaron fuertes correlaciones positivas entre la expresión de miR-199b-5p y los indicadores de integridad ósea, incluyendo la DMO volumétrica cortical y trabecular, así como los niveles séricos de periostina. Por el contrario, se observaron correlaciones negativas con el riesgo de fractura (FRAX), FRAX ajustado por TBS y niveles de CTX, lo que respalda su papel en el metabolismo óseo. La literatura sugiere que miR-199b-5p promueve la osteogénesis a través de las vías de señalización GSK-3β/β-catenina y Wnt/β-catenina mediada por periostina. Por tanto, su expresión reducida podría contribuir a una remodelación ósea deficiente en la DM2. Curiosamente, miR-199b-5p muestra efectos opuestos en la osteoartritis (OA), donde está sobreexpresado y contribuye a la degradación del cartílago. De manera similar, la periostina promueve la formación ósea en la DM2, pero puede agravar la inflamación en la OA. Estos hallazgos destacan los roles dependientes del contexto de miR-199b-5p y subrayan su potencial como biomarcador dual:

protector en la pérdida ósea por DM2, pero perjudicial en la OA. Se necesita más investigación para esclarecer su relevancia terapéutica y garantizar estrategias de direccionamiento específicas para cada enfermedad.

Palabras clave: Diabetes *mellitus* tipo 2. microRNAs. Fragilidad ósea. Osteoartritis. Biomarcadores.

INTRODUCTION

Diabetes *mellitus* is a leading cause of morbidity and mortality worldwide (1), with type 2 diabetes *mellitus* (T2DM) accounting for 90 % of all diabetes cases (2). The recent surge in the diagnosis rates of these and other lifestyle-related metabolic disorders can largely be attributed to the widespread adoption of sedentary lifestyles and excessive energy consumption in industrialized nations (3). Additional factors such as advanced age, hypertension, and family history further increase the predisposition to develop T2DM (4).

Currently, 13.8 % of the Spanish population suffers from T2DM, with an estimated 6 % of individuals unaware of their condition. Furthermore, approximately 12.6 % of the Spanish population is at risk of developing T2DM due to impaired glucose tolerance or abnormal fasting glucose levels (5). According to the di@bet.es study, 5.98 million Spaniards have issues with glucose metabolism (5). The International Diabetes Federation (IDF) projects that the number of diabetic patients globally will reach 643 million by 2030, making diabetes one of the “epidemics of the 21st century” (6,7).

In the early stages of T2DM, metabolic disturbances such as hyperglycemia, insulin resistance, dyslipidemia, and hyperinsulinemia contribute to cellular and organ-level damage, resulting in both microvascular complications (e.g., retinopathy, nephropathy, neuropathy) and macrovascular complications (e.g., coronary artery disease, cerebrovascular disease, peripheral artery disease). These vascular issues account for approximately 70–80 % of diabetes-related mortality (8,9). In addition to these vascular issues, growing evidence has highlighted that bone fragility is also prevalent in T2DM (10). The risk of fractures in patients with T2DM has significantly increased due to alterations in bone remodeling and microarchitecture. Several studies have demonstrated a higher risk of fractures, particularly at the hip and vertebrae (11,12). This fragility is driven by chronic hyperglycemia, insulin resistance, and the accumulation of advanced glycation end products (AGEs), which impair bone quality and disrupt osteoblast-osteoclast balance (10). Indeed, multiple meta-analyses have confirmed an increased risk of incident hip, vertebral, and non-vertebral fractures in individuals with T2DM, with major consequences for quality of life (QoL) and long-term disability (13,14). On the other hand, recent studies have also drawn a connection between osteoarthritis (OA) and metabolic syndrome, a cluster including insulin resistance, dyslipidemia, and hypertension, further linking diabetes to musculoskeletal deterioration (15,16). The presence of diabetes is believed to accelerate the progression of OA and complicate its management, leading to the proposed diabetes-induced osteoarthritis (DM-OA) phenotype (17). This phenotype suggests that systemic inflammation and oxidative stress in diabetes predispose patients to OA, with chronic hyperglycemia promoting cartilage degradation, joint inflammation, AGEs accumulation, and matrix stiffening, all of which impair joint cushioning and function (18). Recognizing the musculoskeletal complications of T2DM as clinically significant outcomes, on par with traditional vascular complications, highlights the urgent need for early detection strategies and targeted therapies aimed at preserving bone and joint health in this growing patient population.

However, there is currently no tool available for the early diagnosis of these musculoskeletal complications. In this context, evaluating the differential expression of molecules such as microRNAs (miRNAs) may offer valuable insights into the development of diagnostic techniques for this population. The potential of miRNAs as biomarkers for musculoskeletal complications is supported by a growing body of scientific literature, which reports differential miRNA responses to various diseases (19,20), further highlighting the importance of investigating their role in T2DM-associated musculoskeletal deterioration.

In addition to miRNAs, proteins such as periostin have also emerged as important biomarkers in T2DM-related bone fragility. Periostin, a protein originally identified as osteoblast-specific factor-2, is involved in bone formation and remodeling but also plays a role in other complications of T2DM, such as cardiovascular disease (21). Periostin's interaction with pathways like Wnt/β-catenin, which regulate osteoblast differentiation and bone metabolism, makes it a potential therapeutic target for improving bone health in diabetes (22). Its levels are altered in various musculoskeletal disorders, including those seen in T2DM, making it a promising biomarker for osteoporosis and bone fragility in these patients.

MicroRNAs (miRNAs) are small endogenous RNA molecules that post-transcriptionally regulate gene expression. They have been shown to play key roles in a wide range of biological processes. Recent advancements in transcriptomic technologies, especially next-generation sequencing and advanced bioinformatics tools, enable more in-depth exploration of messenger RNAs (mRNAs) and non-coding RNAs (ncRNAs), including miRNAs (23). Over 2,000 miRNAs have currently been identified, and it is estimated that they regulate approximately 30 % of all human genes. miRNAs are present in circulating blood, representing an opportunity to use these disease-related circulating miRNAs as potential biomarkers (24). In this study, the miRNome of a group of healthy individuals and patients with type 2 diabetes *mellitus* (T2DM) was analyzed to identify differentially expressed miRNAs between the two groups. The differential expression of selected miRNAs was subsequently validated using RT-qPCR. Following validation, the potential role of these miRNAs, particularly miR-199b-5p, as biomarkers in T2DM was further investigated through statistical analysis of their correlation with determinants of bone fragility and fracture risk.

MATERIALS AND METHODS

Study population

This study included 16 participants, 8 T2DM patients (males with a mean age of 60 ± 5 years) and 8 control subjects, sex and aged matched. T2DM was diagnosed according to the American Diabetes Association criteria from 2017. The recruitment of T2DM patients was during 2015 in the Endocrinology and Nutrition Unit of the Hospital Universitario Clínico San Cecilio in Granada. Samples from healthy controls were managed and provided by the Andalusian Biobank.

Inclusion criteria for T2DM patients were had no cardiovascular disease (CVD), no history of CV events, renal, hepatic, gastrointestinal, or thyroid disease. All patients were receiving diabetes medications, such as metformin, sulfonylureas, insulin, or a combination of these medications.

Venous blood samples were obtained at the Clinical Analysis Unit of the Hospital Universitario Clínico San Cecilio.

The study was performed with the approval of the ethics committee of Jaén (Andalucía) with ID 1630-M1-18/PI-0514-2018 approved on 20 December 2018 and conformed to the relevant ethical guidelines for human and animal research. Written informed consent was obtained from all subjects.

Clinical evaluation, biochemical and bone parameters of the study population

Anthropometric and biochemical measurements

Height, weight, and waist circumference were recorded following standard protocols. BMI was calculated as weight (kg) divided by height squared (m^2). Fasting venous blood samples were collected in the morning, with serum stored at 80 °C until analysis. Biochemical parameters measured included HbA1c, total cholesterol, HDL-c, LDL-c, triglycerides, creatinine, calcemia, phosphorus, and vitamin D, using routine automated methods. Serum bioactive periostin was quantified in duplicate by ELISA (Biomedica Medizinprodukte GmbH), with detection ranges of 20-4,000 pmol/L and intra-/inter-assay variation ≤ 6 % and ≤ 3 %, respectively. Average periostin levels in healthy individuals are ~864 pmol/L.

Hypertension and Dyslipidemia Assessment: Blood pressure was measured using an automated sphygmomanometer after 5 minutes of rest, with two readings 1-2 minutes apart; additional readings were taken if differences exceeded 10 mmHg. The average of the last two readings was used. Hypertension was defined as systolic/diastolic ≥ 140/90 mmHg or antihypertensive treatment. Dyslipidemia was defined by HDL-c < 50 mg/dL, LDL-c > 100 mg/dL, triglycerides > 150 mg/dL, and/or lipid-lowering medication use.

Areal bone mineral density (aBMD)

aBMD of the left hip was measured using a Hologic QDR 4500 densitometer. The shaft region was located 2 cm distal to the midpoint of the lesser trochanter along the shaft axis. Osteoporosis and osteopenia were classified based on World Health Organization (WHO) criteria. Scans were performed by an experienced operator following ISCD guidelines; the device was calibrated daily with a spine phantom. Laboratory coefficients of variation were 1.8 % (femoral neck) and 1.5 % (total hip), with prior studies reporting 2.13 % and 3.14 % for shaft and trochanter, respectively.

Trabecular Bone Score (TBS)

TBS was measured at the LS using the latest version of TBS iNsight (version 3.0.2.0, Medimaps, Merignac, France). TBS was calculated as the mean value of the individual measurements for vertebrae L1-L4, based on a grey-level analysis of the DXA images. The TBS precision error (percentage of the coefficient of variation) was 1.82 %. Diagnosis of preserved and degraded microarchitecture was based on the following TBS ranges: patients with TBS values ≥ 1.31 were classified as preserved TBS and patients with TBS values < 1.31 were classified as degraded TBS (25).

3D-DXA modeling

Trabecular macrostructure, cortical thickness, and femoral shape were assessed using 3D-Shaper software (v2.2), which generates participant-specific QCT-like models by registering a statistical shape and density model from QCT scans onto DXA images. Cortical thickness and density were derived from fitted mathematical functions along the femur surface. Measures included volumetric BMD (vBMD) of trabecular, cortical, and integral compartments, and cortical surface BMD (sBMD). Strong correlations with Quantitative Computed Tomography (QCT) (r = 0.86-0.95) and low coefficients of variation demonstrated high accuracy and precision.

Fracture Risk Assessment (FRAX)

FRAX estimates the 10-year probability of hip and major osteoporotic fractures by integrating clinical risk factors such as age, sex, BMI, fracture history, parental hip fracture, smoking, glucocorticoid use, rheumatoid arthritis, secondary osteoporosis, alcohol intake adjusted by BMD and TBS values.

RNA extraction

miRNeasy Serum/Plasma kit (QIAGEN) was used to extract total RNA from 200 µL of serum samples following the protocol indicated by the manufacturer.

UniSp2, UniSp4 and UniSp5 sequences were added using RNA Spike-in mix (vial 1 of the miRNeasy Serum/Plasma kit) to be used as a control for this step. The extracted RNA was used to carry out the sequencing and validation steps.

Sequencing

Library development and sequencing

The extracted total RNA samples were sequenced through Illumina technology. miRNA libraries development was performed using the QIAseq miRNA Library Kit (QIAGEN). Adapters were ligated with molecular unique identifiers to subsequently back-transcribe the samples to cDNA, which was amplified by PCR. To guarantee the quality of the amplified samples, these were purified by capillary electrophoresis analysis (Alignent DNA 1000 Chip). Libraries were pooled equimolar and quantified by qPCR. Libraries were sequenced using a NextSeq sequencer (Illumina Inc). FASTAQ files of the sequences were created using bcl2fastq software.

Differential gene expression

Differential expression of miRNAs was analyzed using the Empirical Analysis of Differential Gene Expression (DGE) algorithm implemented in CLC Genomics Workbench v20.0.4. The NormFinder program, developed by Andersen et al. (26), was used to identify endogenous control miRNAs and to generate the volcano plot. Visualization of the volcano plot was performed using the ggplot2 and ggrepel packages in RStudio. For miRNAs selected based on the volcano plot criteria, Mann-Whitney tests were conducted on variance stabilizing transformation (VST)-normalized data, retaining those with p-values < 0.05.

Validation

Polyadenylation and reverse transcription

miRNA molecules were reverse transcript to cDNA with a Poly(A) tail at the 3' end using the miRCURY LNA RT kit, following the protocol indicated by the manufacturer. UniSp6 RNA spike-in was used as a control for this step.

Quality control

RNA sequences added in the total RNA extraction step (UniSp2, UniSp4 and UniSp5) and in reverse transcription step (UniSp6) were amplified to ensure that the process had been carried out correctly. In addition, hemolysis-related sequences (has-miR-451a and has-miR-23a-3p) as well as endogenous miRNA sequences (hsa-miR-26a-5p and has-miR-30d-5p) also were amplified to determine the quality of starting samples. All reactions were carried out in triplicate. miRCURY LNA SYBR Green PCR kit was used to perform RT-qPCR following the protocol indicated by the manufacturer. According to the miRCURY LNA miRNA PCR assay protocol, the PCR conditions consisted of two steps: first, a denaturation step at 95 °C for 10 seconds, followed by a combined annealing/extension step at 56 °C for 60 seconds. The reaction was carried out for a total of 40 cycles.

RT-qPCR

First, miRCURY LNA miRNA Custom PCR Panels were designed (Fig. 1). These plates were designed to amplify: 1) miRNAs identified in the sequencing; 2) 3 endogenous miRNAs; and 3) 2 control miRNAs (UniSp3 and UniSp6) in triplicate. miRCURY LNA SYBR Green PCR kit as well as in the quality control step was used.

Fig. 1. miRCURY LNA miRNA Custom PCR Panels scheme.

miRNA expression analysis

The ΔΔCt relative quantification method was employed to evaluate the differential expression of miRNAs in the study groups. miRNA expression data are presented as the mean ± standard deviation (SD).

$\Delta CT(\text{patients}) = CT(\text{miRNA study}) – CT(\text{miRNA problem})$

$\Delta CT(\text{controls}) = CT(\text{miRNA study}) – CT(\text{miRNA problem})$

$\Delta\Delta CT = \Delta CT(\text{patients}) – \Delta CT(\text{controls})$

$FC = 2\text{-}\Delta\Delta CT$

Statistical analysis

t Student analysis was performed, considering as significant differences those with a value $p \leq 0.05$. Binary logistic regression was performed, using the Wald forward stepwise method. Based on the results obtained in the regression, Receiver Operating Characteristic (ROC) curves were developed. SPSS v.25 was used to perform the statistical analysis.

RESULTS

Study population

Tables I and II summarize the clinical, biochemical, and bone-related parameters of T2DM patients stratified by preserved versus degraded TBS. Patients with degraded TBS tended to have higher BMI and waist circumference, along with lower levels of total cholesterol, LDL, and HDL. Regarding bone parameters, this group showed lower values of BMD (both volumetric and areal), reduced levels of periostin and CTX, and higher PTHi concentrations, suggesting a trend toward impaired bone quality in individuals with degraded TBS.

Table I. Biochemical characteristics of the T2DM patients

	Preserved TBS	**Degraded TBS**
Age (years)	61.75 ± 2.75	60.50 ± 2.08
Weigh (kg)	85.83 ± 10.	98.33 ± 14.

	74	87
Height(cm)	169.75 ± 9.00	170.25 ± 10.34
BMI (kg/m^2)	29.72 ± 2.28	33.96 ± 4.54
Waist (cm)	102.25 ± 6.59	112.25 ± 10.90
HbA1c (%)	7.95 ± 1.42	7.68 ± 1.89
Uric acid (mg/dL)	5.98 ± 1.54	5.93 ± 0.97
Cholesterol (mg/dL)	215 ± 29,92	148 ± 35,42
LDL (mg/dL)	158 ± 29.07	80.25 ± 30.89
HDL (mg/dL)	53.75 ± 9.64	45.50 ± 11.82
TGs (mg/dL)	207 ± 123.94	113 ± 38.03
Creatinine (mg/dL)	0.72 ± 0.12	0.84 ± 0.1
AHT (%)	50 %	50 %
Dyslipidemia (%)	100 %	75 %

BMI: body mass index; HBA1c: glycated hemoglobin; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TGs: triglycerides; AHT: arterial hypertension; T2DM: type 2 diabetes *mellitus*; TBS: trabecular bone score.

Table II. Bone and fracture risk parameters in T2DM patients

	Preserved TBS	**Degraded TBS**
TBS	1.28 ± 0.05	1.02 ± 0.10
Hip FRAX	0.30 ± 0.18	0.20 ± 0.22
Hip FRAX_TBS	0.38 ± 0.28	0.5 ± 0.68
Trabecular_vBMD (g/cm^3)	193.29 ± 40.75	170.15 ± 91.13
Cortical_vBMD (g/cm^3)	869.13 ± 72	860.24 ± 84

	.35	.04
Cortical_sBMD (g/cm³)	186.42 ± 35.52	174.69 ± 21.79
Integral_vBMD (g/cm³)	339.17 ± 67.44	315.58 ± 107.29
TH_BMD (g/cm²)	1.205 ± 0.17	1.11 ± 0.25
Periostin (pmol/L)	1799.99 ± 346.31	1339.66 ± 245.23
PTHi (pg/mL)	25.35 ± 1.34	33.83 ± 9.77
VitD (ng/mL)	20.63 ± 5.98	19.08 ± 6.69
Calcemia (mg/dL)	9.98 ± 0.26	9.85 ± 0.24
Phosphorus (mg/dL)	3.20 ± 0.22	3.53 ± 0.50
CTX (ng/L)	0.46 ± 0.31	0.26 ± 0,12

FRAX: BMD-adjusted (with or without TBS) hip Fracture Risk Assessment Tool; vBMD: volumetric bone mineral density; sBMD: subchondral bone mineral density; TH_BMD: total hip bone mineral density; PTHi: intact parathyroid hormone; CTX: C-terminal telopeptide of type I collagen.

Differential expression analysis and validation of sequencing data by RT-qPCR

Differential expression analysis was performed to identify miRNAs with significant and biologically relevant changes between T2DM patients and healthy control. A volcano plot was generated to visualize differential expression, with cut-off thresholds set at a false discovery rate (FDR) < 0.05 and an absolute log2 fold change |Log2FC| > 1. Based on these criteria, hsa-miR-199b-5p and hsa-miR-491-5p were found to be significantly downregulated in T2DM, while hsa-miR-483-5p, hsa-miR-122-5p, hsa-miR-5010-5p, hsa-miR-193b-5p, and hsa-miR-320c were upregulated (Fig. 2A).

To confirm these findings, raw count data were normalized using the variance stabilizing transformation (VST), and a non-parametric hypothesis test was applied to compare miRNA expression levels between groups. Significant differences were observed for all selected miRNAs except hsa-miR-193b-5p (Fig. 2B).

These miRNAs were subsequently selected for validation by RT-qPCR. Among them, only hsa-miR-199b-5p was successfully validated. Consistent with sequencing data, hsa-miR-199b-5p was found to be significantly downregulated in T2DM patients compared to healthy controls (0.496 ± 0.043-fold, $p < 0.05$).

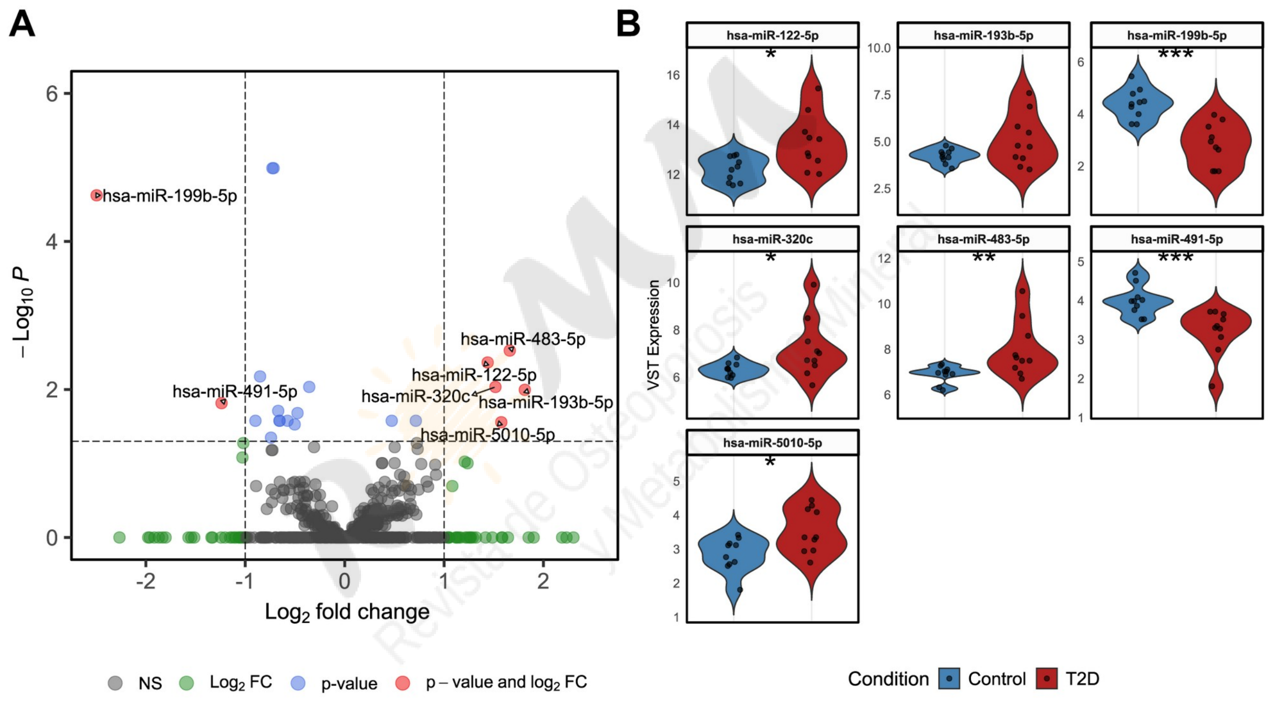

Fig. 2. Differential expression of miRNAs. A. Volcano plot showing Log2 fold change (x-axis) vs. –Log10 *p*-value (y-axis). Dashed lines indicate cut-offs for significance (FDR < 0.05) and expression change (|Log2FC| > 1). Labeled miRNAs passed both thresholds.

B. Violin plots of selected miRNAs showing expression levels after variance stabilizing transformation (VST).

Correlation between hsa-miR-199b-5p expression and determinants of bone fragility and increased risk of fracture in T2DM patients (Fig. 3)

Within the T2DM group, the expression of hsa-miR-199b-5p was correlated with several clinical markers of bone fragility and fracture risk. Spearman correlation analysis revealed significant negative correlations between hsa-miR-199b-5p expression and A) FRAX hip fracture risk (p = -0.74, 95 % CI {-0.95, -0.05}, p = 0.036); B) FRAX hip fracture risk (TBS adjust) (p = -0.862, 95 % CI {-0.98, -0.38}, p = 0.005); C) CTX (pg/ml) (p = -0.719, 95 % CI {-0.95, 0}, p = 0.0446). In contrast, positive correlations were observed between hsa-miR-199b-5p expression and D) serum levels of periostin (pmol/L) (p = 0.786, 95 % CI {0.16, 0.96}, p = 0.0208); E) Cortical vBMD (g/cm^3) (p = 0.893, 95 % CI {0.4, 0.99}, p = 0.0068); F) Cortical sBMD (g/cm^3) (p = 0.857, 95 % CI {0.27, 0.98}, p = 0.0137); G) Trabecular vBMD (g/cm^3) (p = 0.893, 95 % CI {0.4, 0.99}, p = 0.0068), E) Integral vBMD (g/cm3) (p = 0.893, 95 % CI {0.4, 0.99}, p = 0.0068).

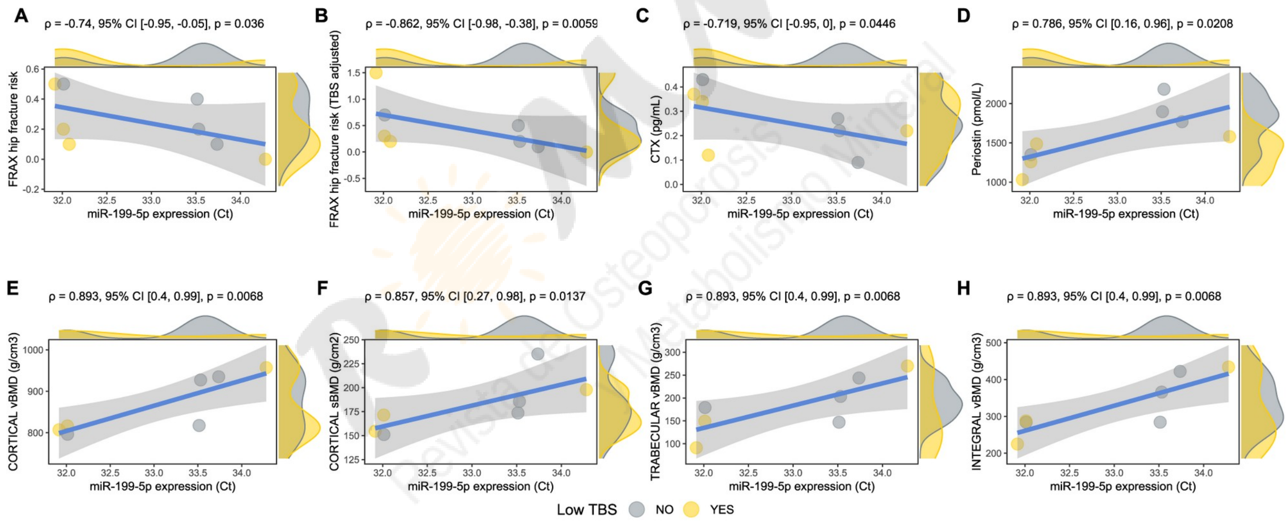

Fig. 3. Scatter plots showing the correlation between hsa-miR-199b-5p expression and FRAX hip fracture risk (A); FRAX hip fracture risk (TBS adjust) (B); CTX (pg/mL) (C); serum levels of periostin (pmol/L) (D); Cortical vBMD (g/cm^3) (E); Cortical sBMD (g/cm^3) (F); Trabecular vBMD (g/cm^3) (G); Integral vBMD (g/cm^3) (H), in T2DM patients (n = 8). The p-values between the different associations were performed by Spearman´s correlation coefficients (showing $p < 0.05$ in each scatter plot).

DISCUSSION

Our results obtained from sequencing and validation steps suggest differential expression of hsa-miR-199b-5p in T2DM patients compared to the healthy group. With the aim of exploring the potential of miR-199b-5p as a novel therapeutic target for the prevention and management of bone fragility associated with T2DM, we conducted multiple correlation analyses in T2DM patients. Our work reveals a positive correlation between miR-199b-5p expression and bone mineral density parameters, including cortical vBMD (mg/cm^3), cortical sBMD (mg/cm^3), trabecular vBMD (mg/cm^3), and integral vBMD (mg/cm^3). Consistently, we also observed a negative correlation between hsa-miR-199b-5p expression and FRAX hip fracture risk, FRAX hip fracture risk adjusted for trabecular bone score (TBS), and serum C-terminal telopeptide of type I collagen (CTX) levels (pg/mL), all of which are established indicators of demineralization and bone fragility. These clinical findings are in line with emerging molecular evidence supporting the involvement of miRNA, particularly miR-199b-5p, in osteogenesis and bone metabolism. It has been demonstrated that primary mesenchymal stem cells (MSCs) secrete small RNAs via exosomes, which are increasingly recognized for their role in intercellular communication (27). Bioinformatics analysis using DIANA-mirPath revealed that the expression of exosomal miR-199b-5p, among others miRNAs, is altered during osteogenic differentiation, suggesting that exosomal miR-199b-5p may play a key role in regulating osteoblast differentiation (28). The effects of knockdown and overexpression of miR-199b-5p on osteoblast differentiation have been studied by measuring alkaline phosphatase (ALP) expression and activity, as well as the expression of the osteogenic marker gene Runx2 (29). The findings indicated that miR-199b-5p enhanced the osteogenic potential of bone marrow-derived MSCs (BMSCs) by modulating the GSK-3β/β-catenin signaling pathway, suggesting that miR-199b-5p and its analogs could serve as promising therapeutic candidates for bone and musculoskeletal disorders. Furthermore, the screening and validation of miRNAs associated with osteoporosis, using high-throughput sequencing, revealed a reduced expression of miR-199b-5p (20). Based on all this evidence, our results suggest that reduced expression of miR-199b-5p in T2DM patients may disrupt the regulation of osteogenesis, potentially resulting in diminished bone mineralization and increased skeletal fragility.

Additionally, our results also demonstrated a positive correlation between miR-199b-5p expression and serum periostin levels (pmol/L). Periostin is a ubiquitous protein originally known as osteoblast-specific factor-2 and belongs to a group of nonstructural extracellular matrix (ECM) proteins. This protein not only plays a role in adverse cardiac remodeling, being associated with cardiovascular disease in T2DM according to the SCORE2-Diabetes algorithm (21), but also promotes bone formation, regeneration, and repair (22). An experimental study on bone loss prevention demonstrated that periostin-induced downregulation of sclerostin can activate the Wnt/β-catenin signaling pathway, thereby inhibiting bone loss (30). This mechanism likely involves the interaction of Wnt proteins with the FZD and LRP6 receptors, leading to the inactivation of the cytoplasmic GSK-3β/Axin2/APC complex. As a result, β-catenin accumulates in the cytoplasm and translocates into the nucleus, where it promotes the transcription of target genes involved in bone formation, ultimately facilitating osteoblast differentiation (31). In this line, our findings suggest that reduced expression of miR-199b-5p in T2DM patients may contribute to impaired osteogenesis through its regulatory effect on periostin. The observed positive correlation between miR-199b-5p expression and serum periostin levels supports the hypothesis that miR-199b-5p may modulate bone metabolism, at least in part, via the periostin–Wnt/β-catenin signaling pathway.

Therefore, taken together, our results suggest that miR-199b (with osteoprotective role in T2DM) could serve as a predictive biomarker, potentially enabling the early identification and monitoring of osteoporosis progression associated with T2DM.

Interestingly, while our findings support a protective role for miR-199b-5p in bone metabolism and osteogenesis, particularly in the context of osteoporosis associated with T2DM, emerging evidence suggests that this microRNA may exert deleterious effects in osteoarthritis (OA). Multiple studies have highlighted the role of miR-199b-5p in chondrogenesis. A longitudinal bioinformatics analysis identified miR-199b-5p as a key pro-chondrogenic regulator (32). Experimental data from the same study showed that inhibition of miR-199b-5p during the early stages of chondrogenesis led to downregulation of chondrogenic markers. A deeper understanding of the regulatory mechanisms involved in chondrogenesis may offer valuable insights into OA pathogenesis. The mechanisms of differentiation differ between growth plate cartilage and articular cartilage. While the growth plate drives longitudinal bone growth via cartilage-to-bone substitution, articular cartilage maintenance depends on delayed chondrocyte maturation and hypertrophy (33). It is plausible that, in OA, the cartilage maturation process becomes aberrantly activated, leading to a loss of biomechanical integrity. In this context, serum exosomal small RNA sequencing from clinical OA patients, along with gene expression data from serum and cartilage samples retrieved from the GEO database, revealed that miR-199b-5p is consistently upregulated (19). In vitro studies further demonstrated that miR-199b-5p negatively affects chondrocyte viability and promotes extracellular matrix degradation. Conversely, inhibition of miR-199b-5p under inflammatory conditions exerted protective effects against tissue damage. Additionally, in an OA model, blocking miR-199b-5p, alleviated disease progression, suggesting that, in contrast to its osteoprotective role in T2DM, miR-199b-5p may contribute to cartilage degeneration in OA. Importantly, none of the patients included in our study had a diagnosis of osteoarthritis (OA), supporting the interpretation that the observed downregulation of miR-199b-5p is specifically linked to diabetic bone fragility rather than joint-related pathology.

Similarly, periostin, which was positively correlated with miR-199b-5p expression in our T2DM cohort, has been shown to be overexpressed in OA cartilage (34). Immunohistochemical analysis localized periostin to chondrocytes and their surrounding matrix, particularly near erosive regions. In vitro, periostin stimulation of chondrocytes induced the upregulation of MMPs, IL-6, and IL-8, supporting its involvement in OA-associated inflammatory responses.

Taken together, these findings underscore the context-dependent nature of miR-199b-5p activity. While it may confer protection against bone fragility in T2DM by promoting osteogenesis through periostin-mediated Wnt/β-catenin signaling, its upregulation in joint tissues under inflammatory conditions may exacerbate OA pathology. Therefore, the development of miR-199b-5p-based therapies requires a nuanced understanding of its distinct effects in bone versus cartilage to ensure both efficacy and safety.

Our study has several strengths and limitations. One of the main limitations is its cross-sectional design, which does not allow us to establish causal relationships between miR-199b-5p expression and bone fragility parameters in

T2DM. While our findings are consistent with the biological role of this microRNA in osteogenesis, longitudinal studies are required to determine whether its downregulation precedes bone loss or results from it. In addition, the sample size of our population was small, although it is acceptable for a pilot study exploring novel molecular markers in a well-defined clinical context. However, caution is needed when extrapolating these findings to broader populations. Moreover, the study cohort included only male participants, which limits the assessment of potential sex-specific differences, so further studies including both, larger populations and women, especially postmenopausal who are at increased risk of osteoporosis, are needed to confirm these results and to enhance generalizability.

Despite these limitations, our study has notable strengths. To our knowledge, this is the first study linking reduced serum miR-199b-5p expression to diabetic bone fragility offering novel insights into its potential role as a biomarker. The associations were robust and supported by correlations with multiple clinical parameters, including BMD, TBS-adjusted FRAX, serum periostin and CTX levels. Additionally, the study excluded patients with osteoarthritis, reducing the risk of confounding from inflammatory joint disease and reinforcing the specificity of the observed associations with bone metabolism. The integration of bioinformatic analysis and molecular validation strengthens the biological plausibility of our findings.

While our study highlights the potential of miR-199b-5p as a biomarker for bone fragility in T2DM, we acknowledge that the use of circulating microRNAs in clinical practice remains limited. Nevertheless, significant progress has been made in recent years toward the development of robust and standardized assays, and circulating miRNAs are increasingly recognized as promising candidates for non-invasive diagnostics. In this context, our findings contribute to the growing foundation for the future incorporation of miRNA profiling into clinical decision-making as a complementary tool for fracture risk assessment

CONCLUSIONS

This study identifies hsa-miR-199b-5p as a promising biomarker for bone fragility in patients with type 2 diabetes *mellitus* (T2DM). Its reduced expression is significantly associated with lower bone mineral density and increased fracture risk, suggesting a potential role in impaired osteogenesis.

Furthermore, the strong correlation between miR-199b-5p and periostin levels highlights its involvement in the Wnt/β-catenin signaling pathway. However, its contrasting role in OA underscores the need for context-specific interpretation. These findings support the potential of miR-199b-5p as both a diagnostic tool and a therapeutic target for skeletal complications in T2DM.

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