MiRNA-133a and MiRNA-25 3p and their relationship with some variables in serum of patients with Osteoporosis

MiRNA-133a y MiRNA-25 3p y su relación con algunas variables en suero de pacientes con Osteoporosis

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Abstract

Osteoporosis is a chronic disease characterized by bone fragility that results in fractures and a variety of miRNAs are involved in osteoclast differentiation therefore, the current case control study aimed to estimate miRNA-133a and miRNA-25 3p in osteoporotic patients and evaluate relationship of these miRNAs with some variables including (calcium, vitamin D, BMD, smoking, history of previous fracture and gender, this study conducted on fifty patients suffering from osteoporosis with age range between 50-88 years, other group consist of 45 healthy individuals with an age range between 55-87 years included in this study as a control group. Blood samples used to extraction of miRNA-133a and miRNA-25 3p from the serum of patients and healthy control as a biomarker for osteoporosis were quantitated by using RT-PCR. Results: miR-133a fold change was significantly upregulated in serum of osteoporotic patients and highest in patients group compared with control group, miR-133a highly significant difference in miR-133 among study groups ($P < 0.001$); while no significant difference in miR-25 among study groups ($P = 0.295$); although the level of patients groups was higher than that of control group. Receiver operator characteristic (ROC) curve of miR-133 was carried out and cutoff value was >8.3 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve of 76%, 80%,82.6%, 72.7% and 0.815 (0.723-906), while (ROC) curve analysis and cutoff value of miR-25 was >1.32 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Area under curve of 66%, 45%, 60%, 51.4% and 0.565 (0.444- 685).Conclusion: miRNA-133a is high sensitivity and Specificity in this study which was bushed to using them as a biomarker for osteoporosis diagnosis.

Keywords: miRNA, Osteoporosis, Osteoclastogenesis, Osteoblast, Osteoclast, BMD

Resumen

La osteoporosis es una enfermedad crónica caracterizada por la fragilidad ósea que resulta en fracturas y una variedad de miARN están involucrados en la diferenciación de los osteoclastos, por lo tanto, el presente estudio de casos y controles tuvo como objetivo estimar miARN-133a y miARN-25 3p en pacientes osteoporóticos y evaluar la relación de estos miARN. con algunas variables que incluyen (calcio, vitamina D, DMO, tabaquismo, antecedentes de fracturas previas y sexo, este estudio se realizó en cincuenta pacientes que padecían osteoporosis con rango de edad entre 50-88 años, otro grupo conformado por 45 individuos sanos con rango de edad entre 55-87 años incluidos en este estudio como grupo control. Las muestras de sangre utilizadas para la extracción de miARN-133a y miARN-25 3p del suero de pacientes y control sano como biomarcador de osteoporosis se cuantificaron mediante RT-PCR. Resultados: el cambio de miR-133a se incrementó significativamente en el suero de pacientes osteoporóticos y fue más alto en el grupo de pacientes en comparación con el grupo de control, miR-133a hi diferencia muy significativa en miR-133 entre los grupos de estudio ($P <0,001$); mientras que no hubo diferencias significativas en el miR-25 entre los grupos de estudio ($P = 0,295$); aunque el nivel de los grupos de pacientes fue superior al del grupo de control. Se realizó la curva de características del operador del receptor (ROC) de miR-133 y el valor de corte fue> 8,3 con sensibilidad, especificidad, valor predictivo positivo (VPP), valor predictivo negativo (VPN) y área bajo la curva de 76%, 80%, 82,6 %, 72,7% y 0,815 (0,723-906), mientras que el análisis de la curva (ROC) y el valor de corte de miR-25 fue> 1,32 con sensibilidad, especificidad, valor predictivo positivo (VPP), valor predictivo negativo (VPN) y Área bajo la curva de 66%, 45%, 60%, 51,4% y 0,565 (0,444- 685). Conclusión: el miARN-133a es de alta sensibilidad y especificidad en este estudio que se propuso utilizarlos como biomarcador para el diagnóstico de osteoporosis.

Palabras clave: miARN, Osteoporosis, Osteoclastogénesis, Osteoblastos, Osteoclastos, DMO

Introduction

The osteoporosis is a systemic skeleton disease characterized by a low bone density and deterioration of the bone tissue micro architecture, with a following high fragility and susceptibility of fractures[1]. Menopause is the main cause of osteoporosis in women due to declining estrogen levels physiologically or by surgical removal of the ovaries [2].

changing in thyroid hormone level is three times more common in women than men and usually develops in the time of menopause have a role in women with osteoporosis[3].The progressive decrease in growth hormone secretion is considered as one of the contributing factors to the loss bone related to age and postmenopause[4]. Genetic factors, endocrine status, nutrition, physical activity and general health during growth play pivotal role in the occurrence of osteoporosis[5].Osteoporosis has been documented to elicit approximately 8.9 million fractures annually, targeting around 200 million osteoporotic women across the globe[6,7]

Osteoporosis is an age-related growing problem in the world [8], although an awareness about the concerns for osteoporosis is increasing day by day, the diagnosis and prevention methods are still inadequate. Osteoporosis is a skeletal disease that affects the bone strength and makes them susceptible to the fractures. This disease is described by damage of bone mass and the weakening bone microarchitecture[9]. Bone Mineral Density (BMD) is a degree of mineral content present in the bone and is an exceptional pointer for osteoporosis. BMD is typically defined by the Dual Energy X-ray Absorptiometry (DXA)[10]. However, using only BMD is unsatisfactory for detection the fracture risk precisely[11]. It has been verified that evaluating osteoporosis using bone mass is unclear[12].

MicroRNAs (miRNAs) are endogenous small noncoding RNA molecules (of about 15–25 nucleotides), which regulate post-transcriptional gene expression through targeting of mRNA that have partially complementary sequences, inhibiting their translation or enhancing their degradation. MiRNAs play an important role in many physiological and pathological contexts. In physiological processes, miRNAs regulate bone formation and bone resorption, thus contributing to the maintenance of bone homeostasis. Under pathological conditions, a deregulation of miRNA signaling contributes to the onset and progression of skeletal disorders, e.g. osteoporosis[13].

MiRNAs regulate gene expressions by blocking translations and promoting degradations of target transcripts. There are at least 1400 mammalian miRNAs and 45,000 miRNAs target sites in human genome, covering 60% of the genes[14].

MiRNA can be found in human biofluids and in blood as free (mainly protein-associated) and exosome-/microvesicle-/LDL-associated miRNAs. These two distinct subsets are believed to exert different functions: the free fraction is somehow passively released from cells during normal recycling of the subcellular components, whereas the encapsulated fraction is actively released and finely packaged together with other components with specific functions addressed to other target tissues. In these terms, free-miRNAs can be considered classical biomarkers, while encapsulated miRNAs more likely act as endocrine-like factors[15].

MiR-133a was initially considered to be a muscle-specific miRNA involved in the regulation of muscle-cell differentiation and the pathogenesis of myogenic disease and heart disease[16], miR-133a plays a key role in skeletal system regulation. This miRNA was shown to directly target the Runx2 (Runt-Related Transcription Factor 2) gene 3'-UTR when overexpressed in MC3T3, an osteoblast cell line, and suppress ALP (alkaline phosphatase, a marker of osteoblast formation) production and osteoblast differentiation[17]. Overexpression of miR-133a inhibited trans differention of vascular smooth muscle cells into osteoblast-like cell by targeting RUNX2(Runt-Related Transcription Factor 2) directly[18].

On the other hand, miR-25 is a member of miR-106b ~ 25 clusters, which includes miR-106b, miR-93 and miR-25, its role in osteoblasts/ osteoblastic cells has not been extensively studied; miR-25 expression is pro-survival in human osteoblastic cells[19].

Nowadays, there are several diagnostic tools used in clinical practice for fracture risk assessment, such as DEXA, WHO-FRAX score, QCT, and BTMs. However, there are some limitations that hamper their utility in the clinical setting; therefore, there is a great need to find novel easily measurable biomarkers that can assist the clinician in making decisions in this field [20,21].

Therefore this study aimed to estimate miRNA-133a and miRNA-25 3p in osteoporotic patients as a biomarker for this disease in Iraqi patients and evaluate relationship of these miRNAs with some variables including (Calcium, vitamin D, BMD, smoking, history of previous fracture.

Methods

Patients and Methods

The current study was carried on 50 patients suffering from osteoporosis according to physician diagnosis in Rheumatology Consultation Clinic of Marjan Teaching Hospital (Babylon province, Iraq) patients' age range between 50-88 years from March to August 2020. Other groups consist of 45 healthy individuals with an age range between 55-87 years without any history of systemic disease were clinically considered as healthy also included in this study as a control group. We excluded patients with renal failure, patients with cancer, kidney failure and patients undergoing treatment for osteoporosis.

Collection of Blood Samples

Three mL of blood for each case and control groups were collected from vein puncture in EDTA free tube and allowed to clot for few minutes at room temperature then serum was separated by centrifugation for 10 minutes at 2500 r.p.m., 600µL of serum was added in Eppendrof tube containing 400 µL of Trizol then stored at -20 to be used for RT-PCR to estimate the Micro RNA 133 a and 25 3p. This study was in agreement with ethics of Marjan Teaching Hospital and verbal informed consent was obtained from all participants.

Total RNA Extraction

The TRIzol® reagent kit (Bioneer, Korea) was used to extract the total RNA as per the instruction of the company.

Molecular detection of miRNA-133 a and miRNA-25 3p

Measurement of miRNA (133 a and 25 3p) by using PCR technique, analysis and calculation of gene expression levels of one or more genes depend on RNA /miRNA concentration after conversion it to cDNA. All processes include total RNA purification, qPCR amplification and data analysis were performed, the RNA Purification purity of DNA and concentration of DNA was estimated by reading the absorbance at 260/280 nm.

miRNA primers design

The design of primers for 133 a miRNA and 25 3p miRNA was done in recent research to select the sequence of miRNA while utilizing miRNA primer design tool by the sanger center miRNA database registry. Macrogen Company, Korea delivered these primers as given in the table below:

Data analysis of qRT-PCR

The data results of q RT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) by using the ɑCT Method Using a Reference the described by [22] according to the equations:

ɑɑCT = ɑCT, target in treated sample- ɑCT, target in calibrator

Statistics analysis: were summarized, presented and analyzed using statistical package for social science (SPSS version 24). Numeric data were presented as mean, standard deviation, median and interquartile range (IQR) while nominal data were expressed as number and percentage. Mann Whitney U test was used to compare median value between two non-parametric groups. Correlation coefficient was estimated by spearman correlation and Pearson correlation.

Table 1. Primers sequences

Primer Name	Seq.	Annealing Temp. (°C)
miR-25-3p-RT	5`-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACTCAGAC-3`	55
miR-25-3p-F	5`-GTGCATTGCACTTGTCTCG-3`	
miR-133a-3p-RT	5`-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAAC CAGCTG-3`	
miR-133a-3p-F	5`-GTGTTTGGTCCCCTTCAAC-3`	
RNU43_RT	5`-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAATCAG-3`	
RNU43_F	5`-GTGAACTTATTGACGGGCG-3`	
Universal miRNA	5`-GTGCAGGGTCCGAGGT-3`	

Results

Demographic characteristics of patients and control subjects

The present study enrolled 50 patients with osteoporosis and 40 apparently healthy subjects. The mean age of patients was 72.5 ± 9.45and that of control subjects was 71.4±8.33 years and there was no significant difference between patients and control subjects in mean age (P=0.561). Again, there was no significant difference in the frequency distribution of patients and control subjects according to age (P=0.699). Patients' group included 8 (16%) males and 42 (84%) females, whereas control group included 10 (25%) males and 30 (75%) females and there was no significant difference in the frequency distribution of patients and control subjects according to gender (P=0.289).

According to BMI, the mean of BMI of patients was 30.07±5.1and that of healthy control subjects was 30.55±4.07 and there was no significant difference between patients and control subjects in mean BMI (P=0.628). The above results have ensured statistical matching between patients' group and control group regarding age and gender which is a prerequisite for such case control study.

Family History of Osteoporosis Patients.

Osteoporotic patients with positive family history of this disease accounted for 19 (38%) whereas, control subjects with positive family history for obesity patients accounted for 0 (0%), therefore, the rate of positive family history of Osteoporosis was higher in patients than control groups in a highly significant manner (P <0.001).

Risk Factors of Osteoporosis Patients.

Possible effects of risk factors such as (body mass index, history of previous fracture, and smoking on the osteoporosis disease was studied and results revealed that there was higher percentage of Osteoporosis patient associated with body mass index, where 29 (58%) of Osteoporosis patients were obese, although the relation was not significant when compared to the healthy controls (P=0.170).

The frequency distribution of Osteoporosis patients and healthy controls according to History of previous fracture was as following: 20 (40%) patients having previous fracture and 30 (60%)

don't having previous fracture in compared to healthy controls was 0(0%) with previous fracture and 40 (100.0%) without previous fracture. This result indicated the prevalence of Osteoporosis was higher among patients who have history of previous fracture (P<0.001).

The same results indicate 9(18%) from individuals with Osteoporosis are active smokers, these results indicate non-significant differences when compared to healthy controls, 2(5%) with active smokers, (P=0.061).

Subjects Real time PCR Results

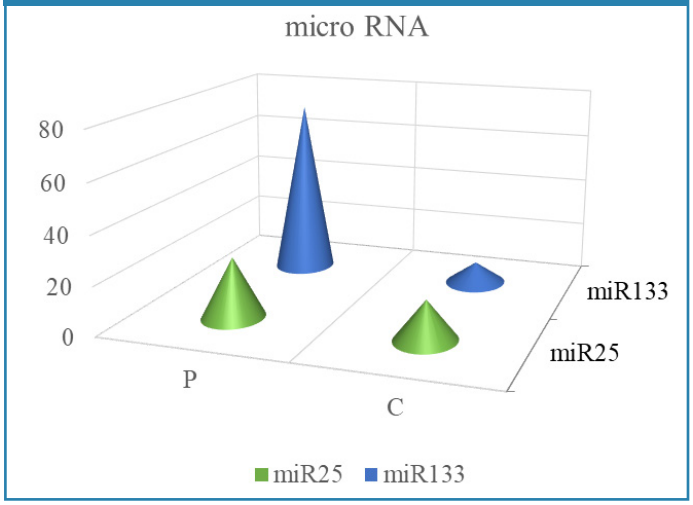
miRNA-133 Levels in Patients and Control Groups.

There was highly significant difference in miR-133 among study groups (P<0.001); the level of patients groups was higher than that of control group with median 59.75 (90.00) in compared to median of control groups1.25 (6.78) (P<0.05).

miRNA-25 Levels in Patients and Control Groups.

There was no significant difference in miR-25 among study groups (P=0.295); the level of patients groups was higher than that of control group with median 4.11(16.00) vs 1.5 (12.26) for control groups (P<0.05). miR-133a and miR-25 3p levels in patient with osteoporosis and control subjects are shown in figure 1.

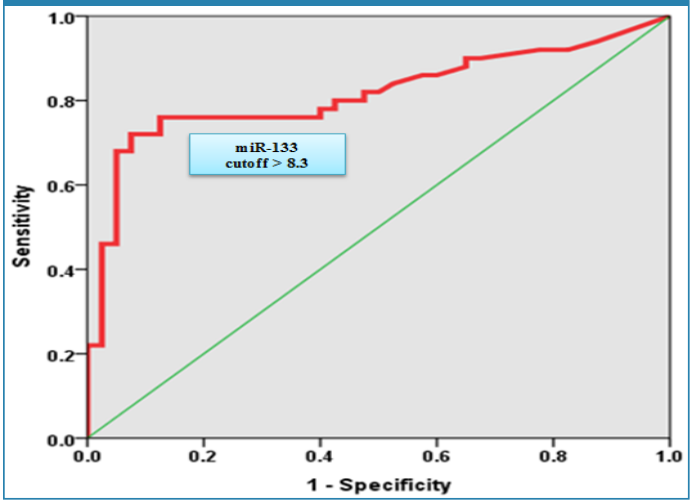
Figure 1. Distribution of patients with Osteoporosis and control subjects according to the level of miR-133 a and miR-25 3p



miRNA-133 a Levels in Patients and Control Groups.

To evaluate the miR-133 cutoff value as well as to predict the Osteoporosis as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out (figure 2). The miR-133 cutoff value was >8.3 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Area under curve of 76%, 80%,82.6%, 72.7% and 0.815 (0.723- 906).

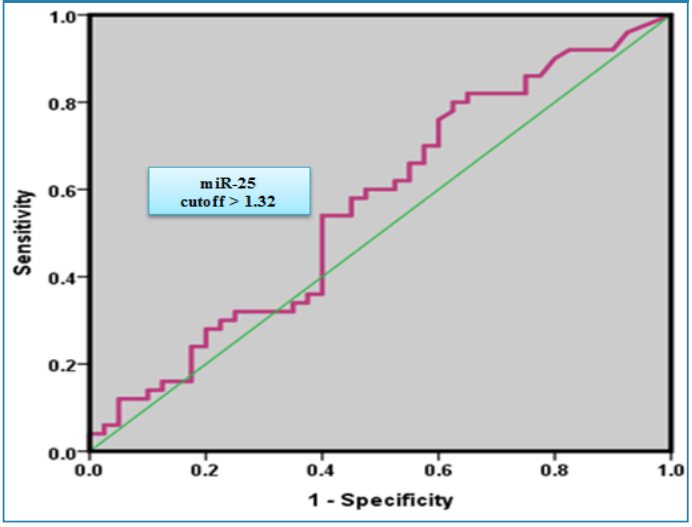
Figure 2. Receiver Operator Characteristic Curve Analysis for the Calculation of miRNA-133 A Possible Diagnostic Cutoff Value.



miRNA-25 Levels in Patients and Control Groups.

To evaluate the miR-24 cutoff value as well as to predict the Osteoporosis as diagnostic tests or adjuvant diagnostic tests, receiver operator characteristic (ROC) curve analysis was carried out (Figure 3). The miR-25 cutoff value was >1.32 with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and Area under curve of 66%, 45%, 60%, 51.4% and 0.565 (0.444- 685).

Figure 3. Receiver Operator Characteristic Curve Analysis for the Calculation of miRNA-25 Possible Diagnostic Cutoff Value.



Correlations of miRNA-25 and miRNA-133 Levels to Some Clinic-Pathological Characteristics and Immunological Parameters in Patients with Osteoporosis.

Results indicated no significant correlation between level of miRNA-25 and age (r= -0.133, P= 0.357), BMI (r = 0.022, P= 0.880), calcium (r = -0.264, P= 0.065), vitamin D (r = -0.207,P= 0.150), but highly significant correlation between miR-25 levels and gender (r = 0.412, P< 0.001), family history (r = 0.422, P< 0.001), smoking (r = 0.450, P< 0.001) and history of previous fracture (r = 0.394, P= 0.001).

The same table showed no significant correlation between level of miRNA-133 and age (r= -0.046, P= 0.752), BMI (r = 0.091, P= 0.357), vitamin D (r = -0.363,P= 0.090) and history of previous fracture (r = 0.213,P= 0.072), but highly significant correlation between miRNA-25 levels and gender (r = 0.387, P = 0.001), family history (r = 0.256, P= 0.030), calcium (r = -0.350, P= 0.013) and smoking (r = 0.418, P< 0.001).

Discussion

The WHO named this disease a "silent epidemic of the 21st century" resulting in more than 8.9 million osteoporotic fractures annually. In fact, osteoporosis is often primarily diagnosed in patients that are admitted to the hospital for fracture treatment. Unfortunately, this diagnosis is mostly performed in a quite late phase of the disease. At that time, osteoporosis is usually already established or severe. The lifetime risk of osteoporotic fractures is relatively high. For example, the risk of hip fractures in osteoporotic patients is as high as 40%, thus in a comparable range as the lifetime risk of coronary heart disease[23].

It is reported that osteoporosis targets every third woman and every fifth man beyond 50 years of their ages[24]. The occurrence ratio in female to male for the osteoporosis is 1:6 with 61% of fractures befalling in the women[25].

The role of miRNAs as biomarkers for bone diseases has drawn much attention recently and have potential use, miRNAs are involved in the osteoclast proliferation, differentiation, cell-fusion, apoptosis, cytoskeleton formation and bone resorption, osteoclast differentiation is regulated by transcriptional, post-transcriptional, and post-translational mechanisms. miRNAs are fundamental post-transcriptional regulators of gene expression and play a key role in the normal bone development[26].

Kocijan et al. (2016)[27] assessed circulating miRNA signatures in male and female subjects with idiopathic or postmenopausal osteoporotic fractures and found that eight miRNAs were confirmed to be excellent discriminators of fractures regardless of age and gender.

In addition to genetic factors, behaviors (such as low level of physical activity, cigarette smoking, and caffeine intake) together with nutrients (including dietary calcium intake and vitamin D deficiency) are critical determinants of osteoporosis and bone fracture[28]. Recently, emerging evidence suggests that epigenetic modifications may be the underlying mechanisms that link genetic and environmental factors with an altered risk of osteoporosis[29,30].

The BMD value is currently a gold standard tool, both for the diagnosis and evaluation of the response of the medical treatment in osteoporosis patients[31].

In meta-analysis on a huge cohort of postmenopausal osteoporotic women versus controls, Pala and Denkceken (2019)[32] identified a relevant upregulation of miR-133a involved in osteoclast differentiation regulatory pathway.

Results of a study done by Li et al., (2018)[33] showed that miR-133a was significantly upregulated and negatively correlated with lumbar spine BMD in serum of postmenopausal osteoporotic women. MiR-133a also plays an important role in bone loss by altering the serum levels of osteoclastogenesis-related factors, decreasing lumbar spine BMD and changing bone histomorphology in vivo.

Another evidence was provided by a recent study reporting substantial changes of several miRNAs that regulate osteoblast and osteoclast differentiation and function following treatment with anti-osteoporotic agents[34].

In particular, a decrease expression of miR-133a was found in the serum of osteoporotic postmenopausal women at 12 months of treatment with teriparatide, a bone anabolic drug. This finding is in line with two previous reports demonstrating an increase in miR-133a expression in the serum of osteoporotic patients. In addition, in human clinical studies identifying miRNAs involved in the pathogenesis of osteoporosis the miR-133a one of miRNAs that upregulated in expression in patients with osteoporosis in Plasma samples from postmenopausal normal (n=40) and osteoporotic women (n=40)[35].

Among Circulating miRNA molecules that associated with osteoporosis, Li et al. (2014)[36] had validated upregulation of miR-133a-3p in the plasma from osteoporosis versus normal Chinese post-menopausal women. Additionally, the circulating expression levels of miR-133a-3p was found to be correlated with the BMD.

MiR-133 directly targets Runx2 and therefore affects osteoblastogenesis, it is thus coherent that its expression was decreased following a treatment that stimulates bone formation. Because of ethical and technical limitations, the number of studies investigating miRNAs expression in osteoporotic bone tissue is still limited, besides these few cell-specific studies, a higher number of reports investigated the differences in miRNAs signatures in human whole blood, serum, plasma or peripheral mononuclear cells between osteoporotic and non-osteoporotic subjects, miRNAs are potentially involved in the regulation of osteoblastogenesis, osteoclastogenesis or both but not all miRNA targets are biologically validated[37].

miR-133a-3p was upregulated in human circulating monocytes from the lower BMD post-menopausal Caucasian women groups[38,39], therefore, miR-133a-3p, and some others have potential acting as biomarkers for post-menopausal osteoporosis.

This result was further confirmed by other clinical study on miRNA from serum and bone tissue of patients with osteoporotic fractures which was investigated regarding dysregulated miRNA expression. Five miRNAs were identified that were overexpressed in both bone and serum among them miR-25, in addition, disease-associated miRNAs in bone tissue such as miRNA-25 overexpressed in serum and bone of recent hip fractured patients, affect osteogenic differentiation of MSCs[40] and among miRNAs associated with diseases in bone tissue there is miRNA-25 which overexpressed in serum and bone of recent hip fractured patients affect osteogenic differentiation of MSCs (Mesenchymal Stem Cells)[41].

Human clinical studies identifying miRNAs involved in the pathogenesis of osteoporosis and from them hsa-miR-25-3p

that present in serum and bone samples from osteoporotic fractured patients and non-osteoporotic fractured controls with results of (n=30 per group/serum), increased in bone samples of osteoporosis patients, the same study concluded that among the most abundant miRNAs in the serum from osteoporosis patients, 11 miRNAs, miR25c3p one of these miRNAs, it was significantly upregulated compared with the nonosteoporotic patients' samples[42].

These discrepancies among studies are likely due to the different nature of patients' population (osteoporotic with or without fractures) and selected controls. Moreover, given that fracture significantly affects bone homeostasis, one can assume that this has an impact on miRNA expression and can bias the results. Interestingly, a link between bone tissue and serum/blood expression patterns was found for some miRNAs[43,44].

miRNAs serve as biomarkers and potential therapeutic targets for diseases[45].

Interestingly, the stability of these circulating miRNAs is high because exosomes are impermeable for RNases and AGO-proteins are protecting the miRNAs from enzymatic disruption. Kept on –70ç miRNAs remain stable for at least one year[46,47]. Since circulating miRNAs are stable for a certain time and easily accessible from body fluids, they are ideal candidates for their use as novel biomarkers.

Our results indicated that miRNA-133 a significant more than miRNA-25 3P, however, miRNA-25 3P although not significant but upregulated in some patients and that may related to small size of specimens which may affect our results. Several more miRNAs have been reported to play a role in bone fracture and osteoporosis in humans. In the absence of significant association, the concern of inadequate sample size might be raised[48].

Moreover, some parameters may influence the levels of circulating miRNAs were not controlled: for example, tobacco use just before blood collection[49], diet[50], amount of physical activity and especially just before blood collection[51-52], the use of some medications such as aspirin[53], and lipoproteins levels[54].

Both the overexpression and inhibition of miRNA can be exploited for the development of potential therapeutics. miRNA sponges, anti-miRNAs and miRNA masks are few strategies for the suppression of intracellular miRNAs[55].

As an emerging treatment strategy, miRNAs have exhibited remarkable potential to treat different diseases [56], such as a study on miR-25-3p that suggests that this miRNA is a promising target therapeutic target demonstrating these results in a larger population in the future would identify miR-25-3p as a promising clinical therapeutic target. Importantly, miR-25-3p is easily measured in serum samples; these findings may provide a non-invasive methodology for the diagnosis of clinical osteoporosis same work also demonstrates that miR-25-3p is a key posttranscriptional regulator of osteoclast differentiation and negatively regulates osteoclast function through nuclear factor I X (NFIX), overexpression of NFIX promoted osteoclast proliferation and increased the expression of the osteoclast differentiation, suggesting that it may have clinical relevance and therapeutic value in osteoporosis[57].

Despite some differences in the published data, taken all together, these studies suggest that miRNAs could be useful to investigate alterations of bone metabolism and promisingly, a link between bone and serum/blood expression patterns was found for some miRNAs, providing evidence that circulating miRNA levels may be directly correlated to altered bone metabolism. This finding together with the fact that miRNAs are stable in body fluids makes them potential tools to detect early manifestations of OP. Given that epigenetics is recognized as a significant contributor to pathological conditions, efforts to investigate the epigenetic marks in OP could pave the way for new therapeutic advances[58].

In the OFELY Cohort, researcher not find evidence of an association between the serum levels of the 32 preselected miRNAs and BMD and fractures although the role of these miRNAs may be important in the cellular context, the detectable levels in serum do seem to be useful for determining bone mass and bone turnover in postmenopausal women[59].

Unfortunately, to date, there is still too much conflicting information in the literature concerning the use of circulatory miRNAs (c-miRNAs) as potential biomarkers for OP, due to differences in selection of patients and comparison group (controls), type of samples, analytical methods and the reliability of results/evidence. Moreover, alterations in miRNA expression patterns in osteoporotic patients do not provide enough functional information to assess a direct link between the causality of miRNAs alteration and osteoporosis pathogenesis[60].

However, one of the most important limitation in the current study was finding other studies (for comparison) on osteoporosis that focused on miRNA (exactly miRNA-133 a and miRNA-25 3p) and relationship with variables such as calcium, vitamin D, BMD, smoking, history of previous fracture and gender (few studies on osteoporosis in men) whether in worldwide in general or in Iraq in particular.

Conclusion

miRNAs are key players in various aspects of bone metabolism. Several studies demonstrated the importance of miRNA regulation for osteoblast and osteoclast differentiation. Since significant amounts of miRNAs can be found in the circulation, they provide unique potential for novel biomarkers: they show specificity for certain diseases and are accessible and stable molecules. Therefore, investigating miRNA-patterns in liquid biopsies represents a promising new avenue to early detection of osteoporosis and other bone diseases. Future work may involve the continued examination of how miRNAs control osteoclast differentiation using biological, cytological, and molecular biological principles.

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