The Expression pattern of miR-34a-5p in primary knee osteoarthritis patients

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Abstract

Background: MicroRNAs serve a crucial role in the post-transcriptional control of gene expression, as well as in development and cellular activities. Differential miRNA expression patterns between osteoarthritis (OA) patients and healthy persons demonstrate the significance of miRNAs in the pathogenesis of OA. miR-34a-5p affects biological activities such as p53-induced cell cycle arrest, apoptosis, and senescence, and its expression is markedly elevated in the plasma of patients with advanced primary knee osteoarthritis (KOA) Methods: The expression of miR-34a-5p in human plasma (n = 60) was measured using quantitative real-time PCR, all subjects were divided into two groups: Group (A): Forty primary KOA patients who met the American College of Rheumatology (ACR) criteria .Group (B): Twenty healthy individuals. They were of the same age and gender as OA patients. Results: Our research demonstrated that miR-34a-5p expression is considerably elevated in the plasma of patients. Conclusion: Our study illustrates the function of miR-34a-5p in the pathogenesis and joint destruction of primary KOA.

Key words: Osteoarthritis, miR-34a-5p, Quantitative Real Time PCR.

1.Introduction:

Osteoarthritis (OA) is a chronic, degenerative joint disease characterised by increasing articular cartilage loss, joint-space narrowing, subchondral bone remodelling, joint marginal osteophyte production, and synovitis [1]. There have been concerted attempts to comprehend the genetic and epigenetic pathways driving OA [2].

miRNAs are short noncoding RNA segments that bind complementary sequences in the 3′-untranslated region of target messenger RNAs (mRNAs) to inhibit gene expression [3]. In mammalians miR-34 family consists of miR-34a, miR-34b and miR-34c expressing 5p strands (guide strand) and 3p strands or strand* (passenger strand) that are encoded by two different genes, the 5p strands of miR-34a inhibit proliferation, anchorage-independent growth, migration, and invasion, and induce apoptosis in a variety of models but the role of the 3p strand, as well as its targets, is largely unknown [4].

p53-induced cell cycle arrest and senescence are modulated by miR-34a-5p [5]. Rats injected with miR-34a antagomir prior to surgical OA induction exhibit decreased chondrocyte mortality and cartilage degradation, suggesting a preventative benefit [6].

2.Aim of the work:

The purpose of this research is to investigate circulating miR-34a-5p expression in knee osteoarthritis patients (KOA) using quantitative real-time PCR and to link the acquired data with clinical symptoms and indicators.

3.Patients and Methods:

The study was approved by the local ethical committee of Benha Faculty of Medicine. Informed written consents were taken from all participants before to participation in the research, which included 60 people. Subjects were divided into two groups:

Group (A): Forty primary KOA patients who met the American College of

Rheumatology (ACR) criteria [7]. Regarding the Kellgren and Lawrence (KL) scale which based on standard weight-bearing anteroposterior and lateral radiographs of the affected knee, primary KOA patients were subdivided into: group A I: Patients with < grade 3 KOA and group A II: Patients with ≥ grade 3 KOA.

Group (B): Twenty healthy individuals. They were of the same age and gender as OA patients.

Forty primary KOA patients reported knee discomfort, with stiffness lasting between 15 and 20 minutes in the morning.

Criteria for classification of idiopathic knee osteoarthritis (KOA) [7]:

Clinical diagnosis: Knee pain + at least 3 of 6:

1. Age > 50 years,
2. Stiffness < 30 minutes,
3. Crepitus,
4. Bony tenderness,
5. Bony enlargement,
6. No palpable warmth.

All patients were subjected to:

1. ***Full history taking:*** Personal history, Complaint, Present history, Past history, Family history, Gynecological and obstetric history.
2. ***General and local examination:***

➢ General examination.

➢ Musculoskeletal examination:

- All joints of the body were examined by inspection, palpation and movements.
- Local examination of both knees:
  - Check for redness, edoema, deformity, muscle atrophy, and skin abnormalities in both the standing and supine positions.
  - Warmth, discomfort, effusion, popping, grinding, or clicking noises are indicated by palpation.
  - Mobility of motion.
  - Specialized examinations of knee ligaments and menisci.

3. ***Investigations include:***

A- Routine laboratory tests include a complete blood count (CBC), liver function tests, the erythrocyte sedimentation rate (ESR), serum creatinine, and serum uric acid.

B- Quantitative reverse transcription polymerase chain reaction (QRT-PCR) for detecting expression levels of miR-34a-5p:

Total RNA was extracted using MiRNeasy Mini Kit (Qiagen, Germany), according to the manufacturer's protocol. RT-qPCR was performed using the reverse RT-qPCR kit (BioRad) according to the manufacturer's protocol, cDNA was subsequently amplified with **Bio Rad sybr green PCR MM.**

PCR was performed in a 50µ reaction volume

2X SYBR® Green RT-PCR Reaction Mix 25 µl

Forward primer (10 µM) 1.5 µl

Reverse primer (10 µM) 1.5 µl

Nuclease-free H2O x µl

RNA template (1 pg to 100 ng total RNA)

then incubate complete reaction mix in a real-time thermal detection system as follows:

cDNA synthesis:	10 min. at 50°C
iScript Reverse transcriptase inactivation:	5 min. at 95°C
PCR cycling and detection (30 to 45 cycles):	10 sec. at 95°C 30 sec. at 55°C to 60°C (data collection step)

using the iScript One-Step RT-PCR Kit with SYBR® Green.

The miR-34a-5p expression was calculated with the formula $2^{-\Delta cq}$, and the change ratio of miR-34a-5p in the in vitro experiments was: $(1-1/2^{\Delta\Delta Cq}) \times 100\%$ [8].

4.Statistical analysis:

Data was gathered, coded, and then put into a spreadsheet using Microsoft Excel 2016 for Windows, part of the Microsoft Office 2016 suite; 2016 edition; Microsoft Corporation, United States. IBM Statistical Package for Social Sciences (SPSS), 21st version, IBM, United States, was used to analyse the data. The Kolmogorov-Smirnov test was used to confirm the distribution's normality. Continuous data were reported as mean standard deviation, median, and interquartile range, whereas categorical data were expressed as numbers and percentages. The information was provided as tables and graphs. Results

were deemed statistically significant when the p-value was less than or equal to 0.05 and highly statistically significant when it was less than or equal to 0.01.

5.Results:

From the results, statistically non-significant differences were reported between the studied groups regarding age distribution. There were non-statistical significant differences between the studied osteoarthritic subgroups regarding laboratory parameters (p value > 0.05) table (1).

Table (1): Comparison between the studied OA patients regarding laboratory parameters.

	Group A I (N=20)					**Group A II (N=20)**					**Mann-Whitney U test**	
	Mean	**±SD**	**Median**	**Min.**	**Max.**	**Mean**	**±SD**	**Median**	**Min.**	**Max.**	**Test value (^{Z}MWU)**	**P-value**
HB (g/dl)	11.38	0.34	11.30	11.0	12.0	11.57	0.34	11.60	11.0	12.0	1.876	0.061 (NS)
RBC's (10^6/cmm)	4.49	0.29	4.40	4.2	5.0	4.51	0.28	4.55	4.0	4.9	0.437	0.662 (NS)
WBC's (10^3/cmm)	7.64	0.62	7.70	6.8	8.60	7.08	0.89	7.15	5.50	8.2	1.820	0.069 (NS)
platelet count (10^3/cmm)	316.7	22.78	307.5	290.0	350.0	309.8	12.8	309.0	290.0	330.0	0.489	0.625 (NS)
ESR (mm at 1st hr)	6.00	1.38	6.00	4.0	9.0	6.80	1.94	6.50	4.0	10.0	1.272	0.203 (NS)
CRP (mg/L)	0.49	0.24	0.55	0.10	0.90	0.42	0.16	0.40	0.20	0.70	1.095	0.273(NS)
S. creatinine (mg/dl)	0.84	0.08	0.80	0.70	1.0	0.85	0.11	0.85	0.70	1.00	0.342	0.733 (NS)
S. uric acid (mg/dl)	3.56	0.57	3.35	2.90	4.50	3.79	0.40	3.80	3.0	4.50	1.252	0.211 (NS)
ALT (U/L)	19.00	3.21	19.00	15.0	24.0	17.20	3.21	17.00	13.0	23.0	1.695	0.090 (NS)

p≤0.05 is considered statistically significant, p≤0.01 is considered high statistically significant, SD= standard deviation, -comparison between groups done by Mann Whitney U Test

miR-34a-5p levels were significantly higher in OA patients mainly in subgroup A II than in healthy controls (p = 0.007), and there were highly significant positive correlations between miR-34a-5p expression levels and morning stiffness duration (p 0.001), WOMAC score (p 0.001), KL score (p 0.001), and disease duration (p = 0.016) in subgroup A II patients.

6.Discussion:

OA is a chronic, degenerative, whole-joint disease characterised by progressive articular cartilage degradation, subchondral bone remodelling, ectopic bone production, ligament degeneration, menisci degradation, and synovial inflammation and hypertrophy [9].

Mechanisms contributing to OA include increased expression of extracellular matrix catabolic enzymes, inflammatory cytokines (e.g., interleukin-1, interleukin-6 (IL-6), and tumour necrosis factor [TNF]), and mediators of apoptosis (e.g., caspase 3); decreased expression of anabolic genes and modification of homeostatic processes, including autophagy [10].

Previous studies have identified critical roles for miRNAs in chondrogenesis, extracellular matrix regulation, inflammatory cytokine production, and other biologic processes that govern normal joint function and maintain homeostasis [11].

Using quantitative real-time PCR, we examined the expression of circulating miR-34a-5p in plasma samples from forty primary KOA patients and twenty healthy controls.

Regarding miR-34a-5p level in plasma, our study found a highly statistical significant difference between the studied groups being higher among OA patients mainly subgroup A II than healthy control

group (p value = 0.007). Our results in agreement with [10] results that reported, significantly higher levels of miR-34a-5p were detected in plasma from late-stage radiographic KOA patients compared to healthy controls.

Rats injected with miR-34a-5p antagomir and then subjected to surgical OA induction exhibited decreased chondrocyte mortality and cartilage degradation, suggesting a preventative benefit [6].

Based on these findings, we conclude that elevated levels of miR-34a-5p have joint-destructive effects and may thus be addressed to reduce cartilage degradation in OA.

7.Conclusion:

Our study illustrates the function of miR-34a-5p as a prognostic biomarker in the pathogenesis and joint destruction of primary KOA.

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