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miR3-22-p as a Novel Biomarker in Rheumatoid Arthritis

Maryam Hassanpoor[1*], Bahareh Abbasi[2]

[1]Department of Cell and Molecular Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran.
[2]National Institute of Genetic Engineering and Biotechnology.

**Corresponding author: Maryam Hassanpoor, Department of Cell and Molecular Biology, Faculty of BIological Sciences, Kharazmi University,Tehran, Iran. Email: Maryamhassanpoor92@gmail.com*

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Abstract:

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune. Early diagnosis of RA remains challenging. A significant portion of RA patients also experience unremitting symptoms despite treatment. miRNA are involved in the regulation of autoimmunity- and inflammation-related processes. In this study, we evaluated the expression of miR-22-3p in serum of RA patients as a novel biomarker. Expression level of this gene in the blood serum of 30 people with RA compared with 30 healthy individuals by the qRT-PCR method. Results showed levels of miR22-3p were significantly higher in the serum of patients with RA in comparison with healthy control ($p<0.0001$). We suggest that miR 22-3p can be used as a biomarker in early detection and screening.

INTRODUCTION:

Rheumatoid arthritis (RA) is a chronic, progressive inflammatory disorder that manifests as asymmetric polyarthritis of small and large joints that may lead to joint and per articular structural damage and the consequences of systemic inflammation. Recent advances have resulted in better diagnostic criteria, improved serologic testing, novel new drugs, and better guidelines to manage patients with RA (1). The disorder is most typical in women and occurs at any age. It affects about 0.5 ~ 1.0% of the population worldwide (2). The aetiopathogenesis of rheumatoid arthritis is thought to result from a multistep process, where environmental factors induce a pathological activation of the immune system in susceptible individuals (3). MicroRNAs (miRNAs) are small non-coding RNAs that have been implicated as potential biomarkers or therapeutic targets in autoimmune diseases (4). MicroRNAs (miRNAs) are small non-coding RNAs that play an important role in numerous biological processes such as cell differentiation and homeostasis, through the regulation of gene expression (5). Since their discovery, they have been implicated in cancer, viral, neurodegenerative, and autoimmune diseases. Binding to complementary sequences on messenger RNA (mRNA), miRNAs generally function to suppress the translation of target proteins, however, they have also been shown to control the rate of transcription. Furthermore, under certain conditions and in specific cell types, they can, in fact, induce gene expression (6). A number of studies have reported that dysregulated miRNA expression influences immune regulation, enhances pro-inflammatory signaling pathways, and leads to the overproduction of pro-inflammatory cytokines in RA (7-8). Among the miRNAs, miR-22-3p is a 22-nucleotide noncoding RNA that was originally identified as a tumor suppressor in HeLa cells. miR-22-3p is located at a fragile cancer-relevant genomic region in chromosome 17 (17p13.3), close to p53. miR-22-3p might induce complex changes and extensive cooperation with p53 (9). Its expression has since been detected in a variety of tissues, including the liver, breast, lung, skin, and gastric cancer. Several studies have also shown that miR-22-3p is associated with many important biological processes, including neuroprotection, tumorigenesis, and various other tumor progressions (10). However, the roles of miR-22-3p in the progression of various tumors are inconsistent. In some studies, miR-22-3p was reported to act as an oncogene, promoting malignancy in breast cancer, lung cancer, and multiple myeloma (11-12). While several reports have also shown that it may act as a tumor suppressor in gastric cancer and esophageal squamous cell carcinoma (13). Many

miRNAs discovered in several cells, tissues, and body fluids have been confirmed that are involved in the pathogenesis of RA (14). A study demonstrated that miR-22-3p promoted fibroblast-like synoviocyte (FLS) proliferation and interleukin (IL)-6 production by targeting Cyr61 (15).In this study, we evaluated the expression of miR-22-3p in serum of RA patients as a novel biomarker. For this purpose, we evaluated the expression level of this gene in the blood serum of 30 people with RA compared with 30 healthy individuals by the qRT-PCR method.

MATERIALS AND METHODS:

The samples used in this experiment included 30 people with rheumatoid arthritis and 30 healthy people as a control group which was received from Shariati Hospital in Tehran. All RA patients fulfilled the 2010 American College of Rheumatology/ European League Against Rheumatism (ACR/EULAR) criteria. All subjects gave informed consent and the study protocol was approved by local medical ethics committees. For miRNA extraction, RNA from freshly sera samples was isolated using Plasma/Serum RNA Purification Mini Kit (Norgenbiotek Cat. 55000, Canada) according to the manufacturers. According to the kit protocol, the cDNA was synthesized using BONmiR High Sensitivity MicroRNA 1st Strand cDNA Synthesis kit (STEMCELL Technology, Iran). qPCR reaction was performed using a BON microRNA QPCR Master mix kit (STEMCELL Technology, Iran), a universal reverse primer (CGAGGAAGAAGACGGAAGAAT), and a specific design primer (AAGCTGCCAGTTGAAGAACTGTA). U6 was used as an internal reference, and the relative expression of RNAs was calculated by the $2^{-\Delta\Delta Ct}$ method. All statistical differences analysis and correlation analysis were performed using GraphPad Prism 8 statistical software (GraphPad Software Inc., San Diego, USA). Differences between two groups were utilised by the Mann-Whitney U-test to compare quantitative variables. All tests were two-tailed, and a p-value <0.05 was considered statistically significant.

RESULTS:

We first assessed the expression levels of circulating miR-22-3p in patients with RA and healthy control. As shown in Figure 1, levels of miR22-3p were significantly higher in the serum of patients with RA in comparison with healthy control ($p<0.0001$). The other clinical characteristics of all participants are summarized in Table I. Moreover, further analysis demonstrated that the level of circulating miR22-3p was not associated with age or gender. Also, no significant relationship was observed between the expression of this gene and clinical parameters.

DISCUSSION:

In this study, we evaluated the expression of miR22-3p in the blood serum of people with rheumatoid arthritis as a diagnostic biomarker. Results showed levels of miR22-3p were significantly higher in the serum of patients with RA in comparison with healthy control

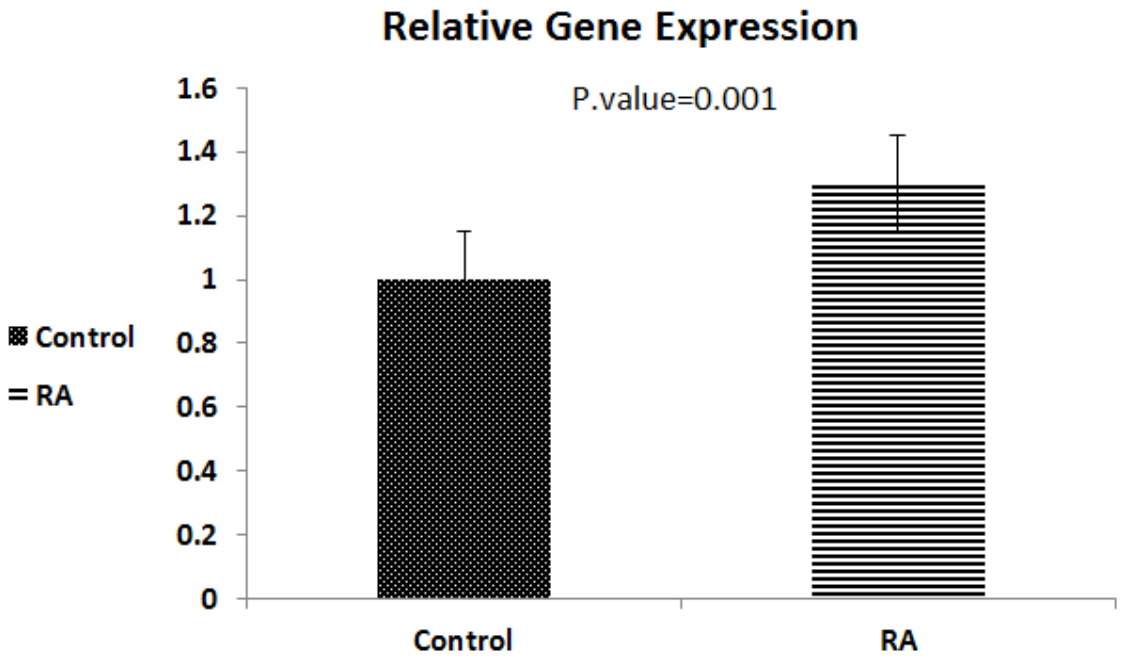

Fig1. Relative miR22-3p expression between RA and healthy control group, circulating miR22-3p in serum RA patient 1.34 fold more than healthy control group.

Table1. Compared and measured clinical parameters.

parameter	RA	Control	sig
Number	30	30	-
age	58±8.6	55±12.7	-
sex (male/female)	11/19	20/10	-
RF(IU/ml)	33±5.3	-	-
anti-CCP (RU/ml)	31±1.1	-	-
Relative expression miR22-3p	1.94	1.1	0.001

($p<0.001$). Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune condition that induces inflammation, stiffness, rigidity, and lack of mobility in the joints and affects the peripheral joint synovial membrane1. It has been characterized by erosive synovitis, penetration of inflammatory cells into the synovium or membrane existing in the synovial joints that line the joint capsules and produce synovial fluid for the joints in the hands and feet is the first structure affected (16). The pathogenesis of RA is complex and involves an intricate interplay between host factors (genetic susceptibilities, aberrant immune response, abnormal metabolic enzymes and sex hormones) and environmental triggers (bacterial or viral infection). Clinically, early diagnosis of RA remains challenging. A significant portion of RA patients also experience unremitting symptoms despite treatment. It is therefore crucial to explore the molecular mechanisms to identify novel diagnostic markers and mechanism-driven therapeutics for RA (17-18). Accumulating studies have shown that miRNA are involved in the regulation of autoimmunity- and inflammation-related processes, including nuclear factor κ-B and Toll-like receptor signaling, cytokine expression, and immune cell proliferation and differentiation (19). several reports have demonstrated that miRNA play an important role in the pathogenesis of a variety of autoimmune diseases, such as multiple sclerosis, systemic lupus erythematosus, type I diabetes and RA (20-22). miR-22-3p is located at a fragile cancer-relevant genomic region in chromosome 17 (17p13.3), close to p53. miR-22-3p might induce complex changes and extensive cooperation with p53 (9). Its expression has since been detected in a variety of tissues, including the liver, breast, lung, skin, and gastric cancer. In this study, it was shown that miR 22-3p can be used as a diagnostic biomarker, although it is suggested that this study be performed on more samples.

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