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The relationship of Mirna 155 with the target gene SOCS1 in Rheumatoid arthritis in Iraqi patients

Fatimah Ghuraibawi[1*], Haidar J. Muhammed[2]

[1]Biology department/ College Science/ Mustansiriyah University, Baghdad, Iraq
Email: fatima97bio@gmail.com
[2]Biology department/ College Science/ Mustansiriyah University, Baghdad, Iraq

***Correspondence author:** Fatimah Ghuraibaw[1] (fatima97bio@gmail.com)

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Abstract

The organs of the body are affected by the chronic inflammatory disease rheumatoid arthritis (RA) in which related with autoimmune disease (AID). The purpose of this research was to study the miRNA155 molecule and its related with the target SOCS1 gene in RA patients and to determine the range of miRNA155 gene expression in the diagnosis of RA, in order to assess the potential diagnostic significance of the circulating miRNA155 gene as a biomarker in RA patients. The total of study was included 14 healthy people for comparison with 36 samples of RA patients, ranging in age from 20 to 60. The patients with RA were diagnosed and tested clinically and immunologically under supervision of the rheumatologist unit in Baghdad Educational Hospital/Altib city/ Baghdad-Iraq. Relative quantification (RQ) of miRNA155 expression was performed after miRNA extraction from the serum of all study participants, was estimated using RT- qPCR. Significant Findings observed an increased in the expression of the miRNA155 profile based on the folding test (11. 12-fold expression 1.08) in the patients with RA, in comparison to healthy individuals, as well as the increased of the target gene (SOCS1) expression through the control compared to the patients whom were at risk showed a highly significant (P≤0.01) in expression levels in which assessed by ELISA technique. In conclusion, the study discovered increased levels of miRNA155 in the blood and depression in the target gene (SOCS1), which may serve as prognostic indicators as a biomarker for illness.

Keywords

miRNA155gene, SOCS1 , RA.

Rheumatoid arthritis (RA) is a condition brought on by an immune system malfunction that damages not just the rest of the body's organs but also the musculoskeletal system. It is seen as a condition that manifests with a variety of symptoms throughout life [1]. They observed the anti-inflammatory protein anti-citrullin antibodies together with IL-23 and hT-17 cell [2] in the early stages of the disease. People with rheumatoid arthritis (RA), a chronic inflammatory autoimmune disease, frequently have major long-term health effects. Despite major breakthroughs in treatment, the incurable nature of RA and its usually unpredictable course can be burdensome for patients on a psychosocial level [3]. Rheumatoid arthritis (RA), a chronic inflammatory disease that mostly affects the joints and has extra-articular symptoms, has no recognized cause. Due to its complexity and the fact that it is based on a pathophysiological mechanism that is only partially understood, good RA therapy requires a multidisciplinary approach [4]. In the adult population, RA affects between 0.5% and 1.0% of people, and females are (2-4) times more likely to have it than males. The fourth and sixth decades of life are when the condition most frequently manifests itself [5]. RA primarily affects the synovial joint lining, which can lead to progressive disability, early death, and financial difficulties. Clinical symptoms of symmetrical joint involvement include

arthralgia, edema, redness, and even a limitation in range of motion. The major metric of improvement for the desired outcomes is early diagnosis [6]. Fibroblast-like synoviocytes (FLS), which emit inflammatory molecules like IL-1, IL-6, and IL-8 while they are growing, are the main effector cells in the pathophysiology of RA. Chemokines that FLS produces upon activation draw inflammatory cells into synovial tissue. This worsens the synovial milieu and starts a vicious cycle by releasing additional inflammatory chemicals and stimulating synovial cells [7]. MicroRNAs (miRNAs), a subclass of endogenous small non-coding RNAs, regulate both transcriptional repression and mRNA degradation. Due to the dual roles that miRNAs can play as tumor suppressors and promoters, dysregulation of one miRNA or a small fraction of miRNAs can significantly affect the expression of hundreds of mRNAs and cellular processes, leading to the emergence of a wide range of diseases [8]. The miR-155 was first identified on the chromosome 21 B-cell integration cluster non-coding RNA of the human genome [9]. Due to their collective role in regulating the expression of more than one-third of the human genome, miRNAs act as persuading molecules that can predict human development at a genetic level. They might accomplish two goals by acting as biomarkers and therapeutic targets, respectively, should abnormalities arise [10].

It has been revealed that RA patients' plasma contains a significant expression of microRNA (miR) 155, a potential biomarker for the diagnosis of RA. Researchers looked into miR-155 expression and how it affected inflammatory cytokine production in RA patients' synovial tissue [11]. In RA, lymphocytes play a crucial role in the disease's etiology. There are plasma cells and lymphocytes that are activated in the inflammatory synovial tissue of RA patients [12].

The proliferation of T and B lymphocytes, monocytes, neutrophils, synovial fibroblasts, and neutrophils contributes to joint inflammation. B cells are transformed into plasma cells, which produce RFs and ACPAs [13]. The SOCS family, a downstream target of the JAK/STAT pathway, inhibits JAK/STAT phosphorylation and activation through a negative feedback loop. SOCS1, the most potent member of the SOCS family, negatively regulates the JAK/STAT pathway and is crucial for preventing the secretion of the pro-inflammatory cytokine IFN [14]. An inducible intracellular protein known as SOCS1 plays a significant role in both immunological and nonimmune activities. The immune system's T cells are controlled by SOCS1 in a way that works in concert with one another to regulate T cell development and response [15]. The research focused on the effect of miR-155 gene on the RA through inhibition of the target gene (SOCS1).

Methods

Subject

This study was agreemented by the Ethics Committee of the College of Science/ University of Mastansiriyh, as did by the Ministry of Iraqi Health. Seventy patients (8 male and 62 female) conflicted with RA in addition to 20 healthy individual subjected by Baghdad educational hospital and clinical of private collected for detection of target gene Suppressors Of Cytokine Signaling 1 (SOCS1): from these samples carried out the gene expression in 36 patient compared to 14 healthy control, aged between 20 to 60 years.

Measurement of Suppressors Of Cytokine Signaling 1 (SOCS1):

The microplate included in this kit has been pre-coated with an antibody specific to SOCS1 and is used in the kit's sandwich enzyme immunoassay for in vitro quantitative detection of SOCS1. An SOCS1-specific biotin-conjugated antibody was next added to the relevant microplate wells with standards or samples. Following this, each microplate well was added with Avidin conjugated to Horseradish Peroxidase (HRP), and the microplate is then placed in the incubator. Only the wells containing SOCS1, biotin-conjugated antibody, and enzyme-conjugated Avidin will show a change in color after the TMB substrate solution is applied. The addition of sulphuric acid solution stops the enzyme-substrate reaction, and the color change is detected spectrophotometrically at a wavelength of 450 nm + 10 nm. Then, by contrasting the O.D. the samples to the standard curve. The kit SOCS1 ELISA test system from mybiosource company (USA).

Quantification of the validate expression of miR-155 gene.

Real-time PCR (RTqPCR) was used to determine the expression of miRNA 155 in samples from both sick and healthy individuals. Using serum from both patients and healthy individuals, the first total RNA was produced by Trizol®/Invitrogen, USA. The stages listed below are a concise summary of the manufacturer's methodology: The mixture was rapidly transferred to a micro-centrifuge tube after melting, well mixed by multiple inversions, and then incubated for 5 minutes in reverse. To separate the aqueous phase, the lysate was combined with chloroform.

Samples were incubated at room temperature for 3 min. before being centrifuged at 4 °C for 15 min. at 14,000 rpm. The aqueous layer was placed in a fresh Eppendorf tube to precipitate RNA. The aqueous layer was transferred into a fresh Eppendorf tube, and an equivalent volume of isopropanol was added in order to precipitate RNA. To create ideal binding conditions for all RNA molecules, ethanol was added to the separated aqueous phase. After that, samples were eluted in water devoid of RNase and kept at -20°C for further processing. Reverse transcription was achieved by utilizing a cDNA synthesis kit (NEB®-USA) and the specific primer GCGAGGCGGTGGCAGTGGAA GCGTGATTTAT TCACCGCCTCGCACCCCTAT [16]. In addition to a set of primers, the relative quantitative test was carried out using a Real-Time PCR apparatus and the Luna Universal master mix (NEB®-USA). The forward primer utilized was CTCAGACTCGGTTAAT GCTAATCG TGATAGG [16], and the reverse primer was GCTGTGGCAGTGG AAGCGTGATT [16]. The reference gene was the U6 snRNA gene, and the forward and reverse primers were CGCTTCGGCAGCAC ATATACTAAAATTGGAAC and GCTT CACGAATT TGCGTGTCATCCTTGC, respectively [16]. The Real-time PCR reaction was conducted using a smart cycler Real-time PCR System (Bioner, Germany). The threshold of cycle (CT) in real-time PCR refers to the quantity of cycles required for the fluorescent signal to reach the threshold. The quantitative gene expression was reported and evaluated using the Livak's 2-Ct technique.

Analytical Statistics

The Statistical Analysis System- SAS (2018) application was used to determine the effects of various factors on the research parameters. The T-test significant difference was used to compare the means in a significant way. (0.05 and 0.01 likelihood [17]

Result and Discussion

Determination the level of SOCS1 expression in RA:

Abnormal functioning of immune response is the main underlying cause of rheumatoid arthritis (RA) . To find out the indication of RA injury by SOCS1 expression in the patients, the results by the ELISA technique showed that SOCS1 concentrations were decrease compared to the healthy control and there were highly significant differences between them (P≤0.01) tilted to the group as shown in Table 1.

Table 1: Comparison between patients and control groups in Socs1

Group	Mean ± SE
Patients	1.73 ±0.08
Control	2.40 ±0.07
T-test	0.317 **
P-value	0.0001
** (P≤0.01) .	

This study supported earlier research that found highly significant differences in SOCS1 expression included serum levels in RA patients, the significantly came for the control group. Suppressor of Cytokine Signaling (SOCS 1) proteins, which are generated at lower levels in RA [18,19].Regulates JAK/STAT signaling negatively The SOCS1 expression is reduced by miR-155 because of its control on SOCS1 expression in the PBMC model. MiR-155 targets the 3′ UTR region of the SOCS1 gene and reduces its expression. In addition, the higher level of miR-155 causes SOCS1 to express less, which may help explain why RA patients produce more TNF and IL-1.[20]. The phosphorylated activation loop of JAK2 is a target for SOCS1, which is very selective, Although SOCS1 has a negative regulatory effect on cytokine signaling through its association with JAK, SOCS proteins may inhibit JAK activity by attaching to either the signaling receptor or the JAK activation protein [21].

In addition to the fact that SOCS1 expression is linked to CD4+ T-cell resistance to IL-10 immunosuppressive effect, which is discovered prior to the cells migrating to synovial tissue, PBMCs from RA patients have been found to express miR-155, a microRNA that targets SOCS1 and is associated with the elevation of TNF and IL-1. The previous studies mentioned that in individuals with HLA-B27+ spondylitis disease, in which discovered the correlation between the DNA methylation of SOCS1 and the levels of inflammation related cytokines was increased by TNF and IL-6 levels [22].

The role of miR-155 gene expression in RA

As a chronic inflammatory disease, rheumatoid arthritis (RA) can be influenced by both genetic and environmental factors. The outcomes of this investigation revealed an extremely important. MiRNA155 was expressed more frequently in the patient's serum when compared to the control group, as determined by the equation for folding expression, as shown clearly by the Tables 2 and Figure 1.

Table 2- Comparison between patients and control in miRNA 155 gene expression

Fold expression	2^- ΔΔct	ΔΔct	Δct	Mean of ct HKG	Mean of ct 155	Study of group
11.12 ±1.08	12.12	-3.6	-6.96	29.8	22.84	Serum group of patients
1.00 ±000	1	0	-3.26	27.65	24.39	Serum group of control
0.0001 **	--	--	--	--	--	P value

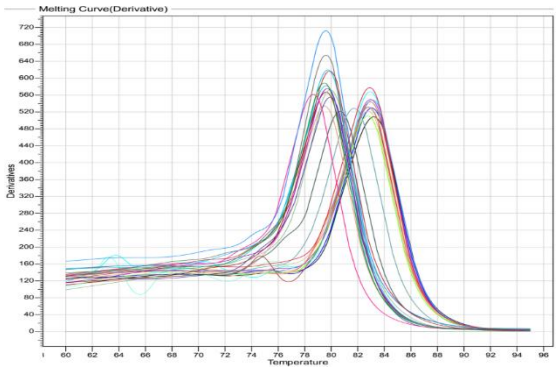

Figure 1: The relationship between miR-155 expression and melting curve.

The study supported previous research showing miR-155 is elevated in serum of RA pathogenesis that has been activated [23, 24]. The causes were increased because of the genes of the synthesis of miRNA-155 in RA is connected to increase a number of cytokines, including the activation of TNF-, IL-1, and IL-6 in synovial tissue and the buildup of immune cells in an inflammatory state. Numerous studies have demonstrated that RA patients' heightened pathogenic synovial tissues have high concentrations of miRNA-155 in their mononuclear cells. Research suggests that miR155 expression, which causes the release of cytokines, is regulated by mononuclear cells. By controlling the inflammatory response of monocytes and T cells, the disease's course can be slowed increased miR155 expression decreased FOXO3 expression because it regulated FLS proliferation [25]. In contrast to their counterparts from peripheral blood, CD14+ cells from RA patients' synovial fluid may be able to spot a rise in the expression of the miR155 gene in mononuclear cells, which is associated with the severity of the condition. a specific gene A potent inflammatory pathway inhibitor called SHIP-1, which is encoded by INPP5D, the IFN signaling type I negative regulator SOCS1, and which inhibits BCL6's NF-B signaling and increases inflammation, are all part of the miR-155 family[26, 27].The pro-inflammatory response is facilitated by these genes working in concert. When miR-155 was overexpressed in CD14+ monocytes, it was discovered that TNF, IL-6, IL-8, and IL-1 were generated alongside CCL3, CCL4, CCL5, and CCL8 chemokines and that resistance to spontaneous apoptosis was also increased. miR-155's role in antigen presentation has been shown to be implied by the observation that miR-155 expression increases during DC maturation [28].

While other studies found no statistically significant difference between patient and healthy groups, they also suggested that miR-155 may not be a reliable predictor of disease pathogenicity [29].

These conditions could be brought on by patients undergoing many treatments, including biological or chemical drugs that interfere with gene expression [30].

Conclusion

The study showed highly expression of miRNA155 gene by Suppressor of Cytokine Signaling (SOCS1) in the patients with auto immune RA compared to healthy, likely to explain its effect in RA disease.

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