The Role of miR-155 Gene Expression in Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a systemic autoimmune disease that can cause prolonged, inflamed, and progressive disability. MicroRNAs influence the processes of autoimmune disease through systemic consequences, including irritant activities of the immune system. Aim of the study: This research aims to evaluate the range of expression profile of the circulating miRNA155 gene as a potential diagnostic biomarker in RA patients in which specific antibodies with high sensitivity for RA are the anti-CCP antibodies. Method: The overall number comprised 36 RA patients and 14 healthy individuals with ages ranging from 20 to 60 years. All samples from patients and control are suspected of being diagnosed with the anticitrullinated protein antibodies test (ACPA) by the enzyme sorbent enzyme assay (ELISA). Relative quantification (RQ) of miRNA155 expression was performed after extraction of miRNA from serum from all participants and was estimated using RT-qPCR. Result: RA showed a significant increase in miRNA155 profile expression depending on the expression fold (11.12-fold expression ± 1.08), in comparison to healthy individuals, as significant (p ≤ 0.0001) of anti-ccp Abs in RA patients in whom they were at risk and evaluated the change in expression levels. In conclusion: The study identified upregulation in circulated miRNA155, which may serve as a prognostic form as a biomarker for the development of disease.

Keywords: miRNA155 gene, autoimmune disease, anti-ccp, RA.

Introduction

Rheumatoid arthritis (RA) is autoimmune disease defined as chronic inflammatory condition that affects the organs of the body and can lead to early death; recent genetic research on chronic diseases has been confirmed (1). Characterization of RA depends on synovial inflammation and leads to deformity of joints and cartilage; the essential variables affecting RA are age, sex, genetics and environmental exposure, examples of which are tobacco use, air pollution and occupational exposure (2). RA is known to have a high prevalence of infections in the general population. However, it might be challenging to diagnose at the onset of an illness that is quickly progressing and can be fatal when linked to cardiovascular diseases (3). So modification of immune factors is the main cause of the disease such as autoantibodies, The antibody against the cyclic citrullinated peptide (anti-CCP), which concentrates on antibodies that neutralize autoantibodies against citrullinated proteins that were initially present in the synovial fluid, is one of the most crucial tests for illness diagnosis which can be divided into positive and negative antibodies, are all associated with hereditary factors that create these illnesses. Both antibodies were connected to environmental variables and genetic of alleles HLA typing origin (4). These, which can be divided into positive and negative antibodies, are all associated with hereditary factors that create these illnesses. Both antibodies were connected to environmental variables and genetic of alleles HLA typing origin (5). Studies have shown that 76% of RA sera included autoantibodies with a 96% specificity for RA, and these antibodies interacted with linear synthetic peptides containing the amino acid citrulline, and several studies have shown that anti-CCP antibodies can identify early RA (6). Certain HLADRB1 alleles with specific amino acid sequences, or the so-called common epitope, have been associated with RA-positive individuals who do not have a higher frequency of HLA-DRB1 despite representing 1 - 2% of the population. Therefore, a shared epitope may act as a genetic marker to distinguish RA from non-RA
in people who test positive for ACPA (7). Furthermore, proteins and antigens that undergo translational changes after evolving into plasma cells stimulate B cells to produce antibodies, in which the observation of helper T17, IL-23, anti-inflammatory proteins and anti-citrulline antibodies in the early stages of the illness (8). Therefore, promotion of TNF and IL-1β are the only cytokines and chemokines that stimulate inflammation. As a result of these signals, enzymes that damage joint tissues and activate immune and nonimmune cells are released, histocompatibility of MHC class II and leukocyte antigens (9). There are also other factors that have a significant effect on this disease, which are considered epigenetic, including microRNAs (miR-155), the type of gene as noncoding RNA on chromosome 21 and has 1 exon, as cited by (10). Hence, they significantly affect cell growth, immunological problems, and the physiology of the organism (11). Interpretation of the miRNA-155 gene of immune response and cell growth has been the subject of research (12,13). The miR-155 gene was initially recognized as the B-cell integration cluster gene (BIC) in chickens when activated by a viral promoter insertion that increased BIC transcription. The subsequent investigation demonstrated that miR-155 is essential for controlling CD34+ hematopoietic stem-progenitor cells’ erythropoiesis and myelopoiesis (14,15). Elevation of miRNA155 expression has increased by controlling macrophage polarization, causing the generation of cytokines and chemokines and resistance to apoptosis; it has a substantial impact on arthritis (16,17). miRNA155 is a possible biomarker in the diagnosis of RA that has been reported. The expression of miR-155 was investigated in synovial tissue of RA patients and the effect of its expression on the release of inflammatory cytokines, suggesting that it may slow the progression of RA by controlling the inflammatory response of monocytes and T cells in RA patients (18). The miRNA-155 has a cumulative effect on RA development, miRNA 155 expression has been found to increase in a variety of RA-related cell types or tissues, including synovial tissue, CD68+ synovial macrophages, and RA synovial fluids (RAFs), synovial fluid CD14+ cell, PBMCs and whole blood of RA patients, miRNA155 can affect the different types of cytokines in RA for instance, several cells, including monocytes, macrophages, B cells, T cells, and fibroblasts, produce the pleiotropic cytokine tumor necrosis factor a (TNF-a). An elevated level of miRNA-155 has been shown to coincide with overexpression of TNF-a and IL-1 β and downregulation of SOCS in RA. TNF-a is significantly increased in RA and promotes bone distraction, pain, and inflammation (19). as well as a previous study declared that miRNA155 expression is greatly enhanced by toll-like receptor (TLR) agonist stimulation of macrophages and dendritic cells. Immune lineage cells are very abundant, pro-inflammatory ligands such as LPS and TNF can activate the miR-155 gene, which is encoded inside the B cell integration cluster gene (20,21). This study aims to evaluate the range of expression profile of the circulating miRNA155 gene as a potential diagnostic biomarker in RA patients in which specific antibodies with high sensitivity for RA are the anti-CCP antibodies.

### Materials and Methods

#### Subjects:

The Ethics Committee of the Iraqi Ministry of Health and College of Science, Mustansiriyah University approved this study.

Serum samples of seventy patients (8 male and 62 female) who attended Baghdad Educational Hospital and private clinics were collected from August 2022– January 2023 with RA, in addition to 20 healthy individuals that were included in this study; the healthy group was within the criteria based on clinical and laboratory tests, and to be serologically negative for the RF and Anti-ccp tests. Within the age group of the patients, without chronic diseases, non-smokers, and non-alcohol drinkers. The total of the samples studied 36 patients with RA and 14 healthy controls (aged between 20 to 60 years) were used for miR-155 gene expression analysis.

A rheumatologist clinically diagnosed all the patients. Depending on the primary symptoms (more than one joint aching or aching, stiffness in many joints, several joints feeling stiff and swollen) and immunological tests such as the Anti-ccp test.

#### Inclusion criteria: depending on the clinical condition of the patients, which ranges from moderate to severe, and if they have not received any chemotherapy.

#### Exclusion criteria: The excluded patients included: pregnancy, hepatic disease, malignant illness, cardiac failure, renal failure, psoriasis, autoimmune disease (multiple sclerosis, Sjogrens syndrome, psoriatic arthritis, systemic lupus erythematosus, and another autoimmune disease).

#### Measurement of anticyclic citrullinated peptide antibody:

The serum assay was applied to the qualitative enzyme immunoassay technique (indirect). In which the microplate provided in this kit has been precoated with CCP antigen. The kit Anti- CCP ELISA kit (Monocent company, USA).

#### Quantification of validated expression of the miR-155 gene:

The expression of miRNA155 was assessed using real-time PCR (RT-PCR for patients and healthy control samples). Total RNA was extracted from both healthy and patients by using serum in Trizol® (Invitrogen, USA). The following are the brief steps mentioned by the manufacturer’s methodology: Serum was properly mixed by inverting several times with trizol reagent and then incubated at 25 °C for 5 min. Chloroform was added to Lysate and incubated on ice for 10 min. After that, centrifuge 14000 rpm for 15 min. To precipitate RNA, the aqueous layer was moved into a new Epipendorf tube, and an equivalent volume of isopropanol was added. The separated aqueous phase was added to ethanol to provide suitable binding conditions for all RNA molecules. Subsequently, the samples were eluted in RNase-free water and stored at -20°C pending further processing using a cDNA synthesis kit (NEB®-USA) for miR-155gene, the specific primers were miR155RT (5'-GCGAGGCGTGTCGAGTG-3').
GAAGCGTGATTATTCACCGCCTCGCACCCCTAT-3’ (22) in which the reverse transcription step was obtained. The relative quantitative assay performed by the Real-Time PCR instrument and Luna Universal master mix (NEB®-USA) in addition to the set of primers, the forward primer: 5’CT-CAGACTCGTTAATGCTAATCGTGATAGG-3’ (22), while the reverse primer

5’GCTGTGGCAGTGGAAGCGTGATTTATT3’ (22). The reference gene was the U6 snRNA gene and the forward primer: CGCTTCGGCAGCACATATACTAAATTGG-GAAC, and the reverse primer GCTTCACGAATTTGG- GTGTCATCCTTGC (22). The real-time PCR reaction was performed using an intelligent cycler real-time PCR system (Bioer, Germany). In real-time PCR, the cycle threshold (CT) is defined as the number of cycles needed for the fluorescent signal to pass the threshold. Livak’s 2−ΔΔCT method was used to report and assess quantitative gene expression, as explained below:

\[
\Delta C_t A = C_t \text{GOI}_A - C_t \text{Ref}_A \\
\Delta C_t B = C_t \text{GOI}_B - C_t \text{Ref}_B \\
\Delta \Delta C_t = \Delta C_t A - \Delta C_t B \\
\text{Normalized expression} = 2^{-\Delta \Delta C_t}
\]

**Statistical analysis:** The Statistical Analysis System- SAS (2018) program was utilized to identify the impact of various factors on study parameters. To significantly compare the means, the T-test significant difference was utilized. (0.05 and 0.01 probability (23).

**Results**

**Determination of anti-ccp levels in RA:** The result of the Anti-ccp assay was highly significant in RA patients compared to the healthy control (Table 1).

**The role of miR-155 gene expression in RA:** Rheumatoid arthritis, being a chronic inflammatory disease, can be affected by both genetic and environmental factors. This study showed a highly significantly increased expression of miRNA155 in the patient’s serum according to the equation compared to the healthy people group, as shown in (Table 2) and (Fig. 3).

**Table 1. Comparison between patients and control groups according to the Anti-CCP mean levels.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE of anti-CCP (QTA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>33.19 ±4.63</td>
</tr>
<tr>
<td>Control</td>
<td>9.82 ±0.75</td>
</tr>
<tr>
<td>T-test</td>
<td>17.085 **</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0079</td>
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<tr>
<td>** (P≤0.01)**</td>
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**Table 2. Comparison between patients and control in gene expression.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fold expression of patients</td>
<td>11.12 ± 1.08</td>
</tr>
<tr>
<td>Fold Expression of Control</td>
<td>1.00±000</td>
</tr>
<tr>
<td>P-value</td>
<td>0.0001 **</td>
</tr>
<tr>
<td>** (P≤0.01)**</td>
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Discussion

This research was consistent with previous studies, which showed that there were highly significant differences in serum anti-CCP levels in patients with RA (24), indicating that the blood biomarker used to diagnose RA has enhanced sensitivity and specificity. It is the anticyclic citrullinated peptide (anti-CCP) antibody, and most patients with RA have specific positive anti-CCP antibodies (25) and suggests that immunological tolerance may have broken down in patients with RA and active immune reactions have been taken, resulting in the generation of anti-CCP antibodies (26, 27).

The adaptive immune system, which consists of T and B of lymphocytes, was created by internal infections, so antibodies to citrullinated proteins are highly specific for RA since they mainly target extracellular citrullinated protein antigens produced in response to inflammation in human tissues and organs (28), mainly through binding to Fc receptors expressed in myeloid immune cells and activating the complement system through both traditional and nonconventional mechanisms. As a result of the combined activation of TLR-4 and FcR, it has been shown that complexes of citrullinated fibrinogen and ACPA (CitFibr-ACPA) present in the RA synovial fluid can excite macrophages, stimulating the synergistic generation of TNF. This raises the possibility that citrullination plays a role in enhancing the effectiveness of innate immune ligands produced by the body (29). While another study explained that anti-CCP antibodies are produced in lymph nodes or ectopic lymphoid structures in synovial tissues, not in circulation, and that treatment affects the peripheral B-cell subpopulation and its ability to produce anti-CCP antibodies in patients with rheumatoid arthritis (RA), a transient increase in post-switch memory B cells after treatment may indicate that this is the case (30). This study by Julien and colleagues showed that miRNA155 is increased in patients with RA (31). Studies on related disorders have revealed that miRNA155 highly expressed may encourage the development of synoviocytes that resemble fibroblasts and secrete cytokines, which can impact the pathophysiology of RA. From the preclinical to the terminal phases of RA, numerous studies have documented the abnormal expression of miRNAs. These dynamic alterations may be used to create a biomarker for RA risk assessment, diagnosis, and clinical care of the mechanisms and roles of miRNAs in the development of RA. As a result, miRNA offers potential avenues for clinical diagnosis and treatment of RA in the future, supported by recently developed theory (32).

The rise is due to miRNA155 in rheumatoid arthritis, plasma cells’ T-helper cells, and antibodies going through the circulation to the joints. To attract more inflammatory monocytes, particularly macrophages, T cells improve the production of cytokines such as interferon-gamma and interleukin-17 in the synovial fluids around the joints. Synovial membranous cells are encouraged to grow by the inflammatory cytokines TNF-alpha and interleukin-6 produced by macrophages (33). This study is consistent with previous studies that have shown that miRNA155 is considerably elevated in serum samples from RA patients compared to healthy individuals, suggesting that it may be a useful marker to assess the severity of disease activity in RA patients with established disease (34). It stimulates the production of miR-155-controlled chemokines and the expression of pro-inflammatory chemokine receptors (35, 36). The current study agrees with a previous study, which indicated that miRNA155 is not regulated in the serum of RA patients, miRNA155 acts as a standalone diagnostic marker for RA since its expression was higher in RA than in controls (37, 38). The result of this increase in miRNA155 may be due to inhibition of the target gene known as Suppressor of Cytokine Signaling (SOCS 1), and down-regulation of SOCS1 may lead to up-regulation of TNF-α and IL (39, 40). Also, a recent study indicates that Mut_P53 may cause a rise of miRNA155 (41, 42). While there were some studies showed that the miRNA155 non-significant for diagnosis RA (43,44).

Conclusion: The study identified upregulation in circulated miRNA155, which may serve as a prognostic form as a biomarker of disease development. Anti-ccp assay is essential for a specific diagnosis in RA patients. There were some limitations in this study, such as the samples taken from patients were without drugs to avoid effects on miRNA155 gene expression, also the samples must be placed in the preservative material so that the concentration of miRNA155 is not affected, and it was supposed to take samples from synovial fluid, as it gives an excellent result, but it is difficult to obtain it in a short time. Further studies on cytokines that contribute to this disease and knowing the effect of drugs, biological or chemotherapy, on gene expression before and after drugs, as well as studying other types of miRNAs effects on RA.

Authors’ Contributions
All researchers contributed to writing this research.

Acknowledgments
None.

Conflicts
No conflicts of interest.
References:


