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The correlation between SMAD3 and MIR 155 in Rheumatoid arthritis patients and athletes

Russul M. Shuku, and Angham J. M. Ali

College of Health & Medical Technology, Pathological Analysis, Al-Furat Al-Awsat Technical University, Iraq.

Corresponding author: Russul M. Shuku, College of Health & Medical Technology, Pathological Analysis, Al-Furat Al-Awsat Technical University, Iraq.
Email: russul.sahip.chm@student.atu.edu.iq

Abstract

Rheumatoid arthritis (RA), an autoimmune and inflammatory condition, occurs when the immune system erroneously targets healthy cells, leading to inflammation in the affected regions. The objective of this study was to evaluate the association of Mir-155 and SMAD3 in patients with RA, athletes with RA, and control groups. The study was carried out during the beginning of January 2024 and the end of June 2024. , The study was conducted at the Medical Rehabilitation Center in AL-Sader medical city. It included 50 patients, both female and male, diagnosed with RA by a specialist physician, along with 50 individuals without the condition and 50 athletes with RA. It comprised individuals of diverse ages. We collected 5 ml of blood from patients to assess the immunological parameter SMAD3 using the Enzyme-Linked Immunosorbent Assay. Additionally, we invested MIR-155 by using the reverse transcription polymerase chain reaction (RT-PCR). There was a significant positive correlation between MIR-155 with SMAD3 ($r = 0.529$, $p < 0.001$) in the RA group, and MIR-155 has a positive correlation with SMAD3 ($r = 0.574$, $p < 0.001$) in athletes, suggesting that it may play a part in boosting signaling pathways linked to fibrosis or repair. In conclusion, there was a statistically significant positive correlation between MIR-155 and SMAD3 among RA patients and athletes compared to healthy controls.

Keywords: Rheumatoid arthritis, SMAD3, MIR-155

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Introduction

Rheumatoid arthritis (RA) is a chronic systematic autoimmune condition characterized by asymmetrical inflammatory synovial inflammation and the formation of pannus. The continuous deterioration of bone and cartilage resulting from these pathological processes can result in joint deformity, stiffness, and disability. In RA, immune pathology can affect extra-articular organs, resulting in pulmonary and cardiac dysfunction.[1] Pannus formation represents a significant pathological mechanism contributing to the damage observed in RA.[2,27] The normal synovium consists of a layer of fibroblast-like synovial cells and macrophages. In RA, hyperplastic fibroblast-like synoviocytes and macrophages, along with additional recruited immune cells, play a role in synovial thickening. Dysregulated angiogenesis results in neovascular dysfunction, marked by a compromised vessel wall. This impairs the ability of cells to transport oxygen, leading to a hypoxic environment. The thickened synovium,

dysregulated neovascularization, and inflammation collectively contribute to pannus formation.[3] The exact cause of RA is not well understood. The prevailing hypothesis centers on genetic-environmental interactions, highlighting the significance of the human leukocyte antigen (HLA)–DRB1 gene, while acknowledging environmental triggers such as smoking, infections, and sexual hormones as risk factors for the development of RA.[4] Epigenetics includes transcriptional factors, DNA methylation, histone modification, and non-coding RNA, serving as a link between environmental stimuli and genetic regulation.[5] Recent studies demonstrated that SMAD3 is essential for T cell activation and the synthesis of inflammatory cytokines.

SMAD3, a transcription factor, may interact with other transcription factors at composite sites within gene promoters, thus influencing the transcriptional regulation of gene expression.[6] SMAD3 signaling pathway is crucial for preserving the integrity of articular cartilage, as it inhibits the terminal hyper-trophic differentiation of chondrocytes and regulates the synthesis of matrix components.[7] SMAD3 signaling is fundamentally linked to both innate and adaptive immunity. SMAD3 is essential for transcriptional activation and regulation in T lymphocytes; however, the impact of SMAD3 polymorphisms on autoimmune diseases, such as RA, remains largely unexplored.[8] MicroRNAs (miRNA-155) are a type of non-coding RNA located on chromosome 21 and consist of one exon.[9] Thus, they have a substantial impact on cell growth, immune system issues, and the organism's physiology. The miRNA-155 gene's role in immune response and cell growth has been extensively researched.[10] The elevation of miRNA-155 expression influences macrophage polarization, leading to the production of cytokines and chemokines, as well as resistance to apoptosis, significantly affecting arthritis.[11,26] miRNA-155 has been identified as a potential biomarker for the diagnosis of RA. The expression of miRNA-155 was examined in the synovial tissue of RA patients, and its influence on the release of inflammatory cytokines was assessed, indicating that it may mitigate the course of RA by regulating the inflammatory response of monocyte and T cells in these patients.[12.28]

Materials and Methods

The study included a cohort of 100 patients who were diagnosed as RA by specialist physicians. They were male and female patients of various ages, all presented with morning stiffness lasting at least one hour, arthritis in more than three swollen joint sites, and a confirmed diagnosis of RA was by a specialist physician.

Exclusion criteria

This study excluded individuals diagnosed with osteoarthritis, rheumatoid arthritis (autoimmune), and rheumatoid arthritis resulting from bacterial infection.

Patients Group

The study included 100 patients with RA recruited between the beginning of January 2024 and the end of June 2024. The medical rehabilitation center in Al-Sader Medical City selected 50 patients with RA and 50 without it, all of varying ages. Blood samples were taken from patients to test the immunological marker SMAD3 using an enzyme-linked immunosorbent assay (ELISA), and the genetic marker MIR-155.

In addition, 50 apparently healthy individuals of varying ages were randomly selected to form the control group. This control group underwent an RF and anti-cyclic citrullinated peptide test (ACCP) test examination. They did not have a history of autoimmune disease or other illness in their families, and all were asked to complete a questionnaire.

A venous blood sample (5 ml) was drawn from each patient and the control person. The blood samples were split into two parts. One part was immediately placed into an anticoagulant tube for molecular study and then kept at -80°C for the reverse transcription polymerase chain reaction (RT-PCR). The other portion was transferred to a Gel tube for serum separation. Serum samples were kept frozen at a temperature of -20°C for the subsequent determination of SMAD3 by ELISA.

Quantification of SMAD3 by ELISA

The ELISA technique was employed to quantify the immunological indicator SMAD3, using commercial kits (Code Number: ED14460, from the Chinese business Melsin) following the guidelines provided by the manufacturer.

Total RNA extraction from serum

Serum samples: samples retrieved from liquid nitrogen and melted at room temperature, after complete melting, the samples were mixed well by vortex and the RNA was extracted as shown in table 1 and as follows:

I. Direct addition of 1 ml of TRIzol reagent was made to the liquid samples.

II. Using a micro-pipette, the lysate was mixed up and down until no discernible precipitate was present.

III. Then 5 mls were incubated at room temperature.

IV. Then 200 µl of chloroform was added, vigorously shaken by hand for 30 seconds, then allowed to sit at ambient temperature for three minutes.

V. Then the samples were centrifuged for 15 minutes at a speed of 10,000xg at a temperature range of 2-8°C. The centrifugation process separated each sample into 3 distinct layers: the pale white middle layer, which included DNA; the colorless top layer, which contained complete RNA; and the bottom organic pink layer, which contained proteins.

VI. After transferring the transparent top layer to a fresh tube, 500 µl of isopropanol was added and well mixed manually. The tube was thereafter incubated for 10 minutes at ambient temperature.

Primer

The primers (Table 2) used in the PCR for the detection of miR-155 were bought from a Korean company (Macrogen company, Korea).

Table 1. Total RNA extraction and amplification kits for the reverse transcription polymerase chain reaction (RT-PCR).

No.	Kits	Components	Volume or Concentration	Company	Origin
1-	TransZol Up	Trizol reagent	100 ml	TRANs	China
		RNA dissolving Solution	15 ml		
2-	GoTaq® 1- Step RT-qPCR System	GoTaq qPCR Master Mix 2X	2x	Promega	USA
		Go Scrip RT Mix for 1 Step RT qPCR 50X 25mM 30Mm	50x		
		MgCl2	25µm		
		CXR Reference Dye	30 µm		
		Nuclease Free Water	1 ml		

Table 2. primer sequence used in the present study.

Primer	Direction	Sequence	Method
miR-155	Forward	CTCAGACTCGGTTAATGCTAATCGTGATAGG	RT PCR
	Reverse	GCTGTGGCAGTGGAAGCGTGATTTATT	RT PCR

Determination of miR-155 Reference gene level

The first Step RT qPCR GoTaq system was a real-time amplification method that integrated GoScript Reverse Transcriptase with GoTaq qPCR Master Mix in a unified protocol. RT qPCR-optimized Sybr Green Dye, a unique fluorescent DNA-binding agent, was integrated into the system. The technology may ascertain RNA expression levels by a one-step RT-qPCR method:

1. All of the components of the GoTaq 1-Step RTqPCR, including total RNA, primers, and Nuclease-free water, were well mixed after thawing on ice.

2. According to the information in the Table 3, a GoTaq 1-Step RTqPCR reaction

3. Table 3 shows the cycle program that was used to conduct the RT-qPCR experiments.

Table 3. Steps of the reverse transcription polymerase chain reaction (RT qPCR) and settings.

Step	Temperature	Duration	Cycle
Reverse transcription	37°C	15 min	1
RT inactivation hot start activation	95°C	10 min	1
Denaturation	95°C	10 sec	50
Annealing	58°C	30 sec	
Extension	72°C	30 sec	

Table 4. GOTag q- RTPCR Master mix content.

Content	Final volume	Concentration
GoTaq qPCR Master Mix, 2X	10 µl	1X
GoScrip RT Mix for 1Step RTqPCR (50X)	0.4 µl	1X
Forward primer (20 X)	0.6 µl	300 ng
Reverse primer (20 X)	0.6µl	300 ng
$MgCl_2$	1.6 µl	25ng
RNA template	5µ	100 ng
Nuclease-free water	1.8µl	-

Results

According to the current study, SMAD3 expression was considerably higher in the groups with RA and athletes than in the sedentary controls. Athletes' SMAD3 levels were intermediate (3.90 ± 1.84), lower than the RA group (5.08 ± 1.60, $p < 0.001$) but significantly higher than controls (2.10 ± 1.22, $p < 0.001$), Table 5.

Table 5. Compression of the immunological marker (SMAD3) among the study groups.

	RA	Athletes	Control	*p value		
	N=50	N=50	N=50	RA vs Athletes	RA vs Control	Athletes vs Control
SMAD3	5.08 ± 1.60 a	3.90 ± 1.84 b	2.10 ± 1.22 c	<0.001	<0.001	<0.001

*$p \leq 0.05$ is significant.

Significant positive correlation between MIR 155 and SMAD3 (r = 0.529, $p < 0.001$) was seen in the RA group. MIR155 had positive SMAD3 (r = 0.574, $p < 0.001$) in the athlete group, Table 6.

Table 6. Correlation between immunological markers and gene expression among the study groups.

Groups			miR155
RA	SMAD3 (pg/ml)	r	0.529
		p value	<0.001
Athletes	SMAD3 (pg/ml)	r	0.574
		p value	<0.001
Control	SMAD3 (pg/ml)	r	-0.538
		p value	<0.001

$p \leq 0.05$ is significant.

Discussion

RA is becoming recognized as a condition that includes various clinical presentations, diverse therapeutic responses, and differing prognoses.[13] The etiology and pathogenesis of RA are complex. The development and evolution of this disease can be a result of interaction between sex, age, immunity (humoral and cellular; innate and adaptive immunity), genetic variation, epigenetics, microbiome dysbiosis, infection and/or environmental factors.[14]

SMAD3 is a critical regulator of inflammatory cytokine production and T cell activation. SMAD3 plays a crucial role in the down regulation of T cells by enhancing the expression of forkhead box protein 3 (FOXP3), which is essential for the differentiation of T regulatory cells (Tregs).[15] MicroRNAs are involved in both innate and adaptive immunity as well as inflammation, which are recognized to influence the onset and progression of RA. MiR-155 targets critical molecules that regulate the immune system. Multiple studies indicated that SMAD3 functions as a mediator in chronic inflammation and joint degeneration associated with autoimmune diseases, which is consistent with the higher SMAD3 levels. For example, they discovered that SMAD3 expression was elevated in RA patients' synovial tissues and that this expression was correlated with fibrosis and the severity of the disease.[17] It is interesting to note that while athletes' SMAD3 levels were still lower than those of RA patients, they were much higher than those of the controls. This may be a reflection of SMAD3's function in anti-inflammatory signaling and tissue remodeling brought on by exercise. It has been demonstrated that exercise alters the TGF-β/SMAD pathways, encouraging tendon adaptability and muscle growth.[18] The increase of miR-155 was documented in various cell types and tissues associated with RA, including CD68+ synovial macrophages in synovial tissue, RA synovial fluids (RASFs), CD14+ cells in synovial fluid, peripheral blood mono-nuclear cells (PBMCs), and whole blood from RA patients.[19] Remarkably athletes had the greatest levels of miR-155, indicating a clear physiological adaptation as opposed to pathological inflammation.[20.28] The immune system's acute reaction to muscle stress and repair is known to temporarily upregulate miR-155 during exercise, especially high-intensity or resistance training.[21.]Athletes' elevated miR-155 seems to be a temporary, regulated physiological reaction. It aids in tissue integrity preservation, immunological balance regulation, and the avoidance of immunosuppression brought on by excessive training.[22] The study by *Kmiolek et al.,* 2020, investigated transcription factors essential for maintaining the balance between Th17 and Treg cells, specifically SOCS1, SMAD3, SMAD4, STAT3, and STAT5, to examine their correlation with miR-155 and other selected miRNAs in RA, osteoarthritis, and healthy individuals. The findings indicated correlations between miR-155 and SMAD3, as well as SMAD4, in Treg cells in RA.[23]

A separate study indicated elevated miR-155 expression in both parenchymal and non-parenchymal liver cells. They proposed that miR-155 influences fibrosis through various mechanisms, involving both direct and indirect targets, such as SMAD3.[24.] n inverse association is observed between SMAD3 expression and primary-miR-155, suggesting that the inhibition

of tumor-suppressing TGF-β signals may contribute to miR-155 oncogenesis. As a result, they examined miR-155 binding locations throughout all SMAD genes.[25.29]

In conclusion, there is a significant elevation of SMAD3 and MIR155 in the rheumatoid arthritis and athlete groups compared to the control group. A significant positive correlation exists between SMAD3 and MIR155.

Author Contributions

RMS, collected the data and wrote the draft of the manuscript. AJMA, proposed the topic of this research and designed the study, and revised draft of the manuscript.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The Ethics Committee of the the Collage of Health and Medical Techniques/Kufa reviewed and approved the protocol of the study (approval dated July 13, 2025).

Informed consent

The patients gave their verbal consent before sample collection. Before obtaining information from patients, we informed them that their blood would be utilized for research purposes, and the majority of them were cooperative and helpful.

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