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EVALUATION THE MIR26 AS A BIO MARKER IN RHEUMATOID ARTHRITIS PATIENTS

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ABSTRACT

Background: *Rheumatoid arthritis, also known as RA, is a systemic inflammatory autoimmune disease that is persistent and affects 1% of people globally.* ***Aim of study:*** *This study was conducted to calculation the expression of miRNA-26 by RT-PCR.* ***Methodology:*** *A case control study has been conducted from 15 October 2023 to 15 of January 2024. This study has been done at the Medical City Hospital, Baghdad Government. And included 45 patients (females and male) which diagnosed with RA disease by specialist physician, and their ages ranges between, 18 to 75 years. Blood was drawn from the patients to investigate the MIR26, by RT- PCR (Real time polymerase chain reaction).* ***Results:*** *The average Ct value for miR-26 in the group of controls is 6.82, with a fold change of around 1.00 while the average Ct value for miR-26 in group of patients is 7.59, with a fold change of around 7.59.* ***Conclusion:*** *There is significance elevation in miRNA26 in patients group other than in control subjects' group. It is possible that miR-26 contributes to the diseases or disorders that these people suffer from it.*

INTRODUCTION

Rheumatoid arthritis (RA) is a common systemic autoimmune disease that results in symptoms, degeneration of bone and cartilage, and persistent inflammation of the joints [1]. Although it is generally accepted that a combination of genetic, immunological, and environmental variables plays a role in the development of RA, the exact molecular pathways responsible for the behavioral changes in RA-FLS are still not fully understood [2]. Rheumatoid arthritis is form of autoimmune disease that occurs when the immune system being compromised attacks the body tissues. The condition that is in contrast to osteoarthritis that is a degenerative disease is rheumatoid arthritis that most commonly impacts the inner lining of the joint which causes painful inflammation that can progress to joint distortion and bone loss [3]. Furthermore, the present inflammation due to rheumatoid arthritis also leads to some body organs to be affected [4]. The role of microRNAs (miRNA) as fundamental players in the immune response continues to gain recognition more so in the recent past [5]. This change showed that one such mRNA called the miRNA-26 alters how

autoimmune diseases begin as it modifies the activation of T cells [6]. Moreover, the finding that "peripheral blood mononuclear cells (PBMCs)" from RA patients had greater levels of miR-26 [7] raises the possibility that miRNAs may be implicated in several pathways impacting the pathogenesis of RA .

Two of "microRNAs (miRNAs)"'s most important functions are RA promotion and transcriptome regulation. MiRNA genetic variants have been linked to an explanation of RA susceptibility [8]. "Fibroblast-like synoviocytes (FLS)" are important cell types that contribute to the development of the illness. Different chemicals that affect the start and course of RA are secreted by these cells [9]. MiR-26 has a role in the control of immunological response, inflammation, differentiation, and proliferation [8]. When overexpressed in RA fibroblast-like synoviocytes, it has been demonstrated to decrease apoptosis rates, but suppression of miR-26 causes apoptosis. It also facilitates cell cycle progression and proliferation in RA. Moreover, in RA fibroblast-like synoviocytes, overexpression of miR-26 promotes cell invasion [2]. In RA fibroblast-like synoviocytes, overexpression of miR-26 has been connected to enhanced invasion, proliferation, and resistance to apoptosis, whilst downregulation of miR-26 has been connected to reduced invasion, proliferation, and resistance to apoptosis [10]. An overexpression of miR-126 in rheumatoid arthritis synovial fluid (RASF) increases synovial hyperplasia by promoting proliferation and decreasing apoptosis. This phenomenon is associated with both the upregulation of PI3K proteins and the downregulation of "phosphatidylinositol 3-kinase regulatory subunit 2 (PIK3R2)" [11]. The lack of regulation in miR-26 influences numerous biological systems present in RA [12]. Moreover, it is important to note that the increased cytokines such as TNF-alpha and IL-17 is caused by the malfunction of miRNAs that may lead to the development of RA and suggests the possibility of its treatment [13]. Moreover, it has been identified that the role of miR-26 is in controlling the T cell differentiation to various T cells for example Th17 and Treg cells. This means that, by employing gene therapy to tackle immune system regulation in RA, there is hope of overcoming it [9]. Therefore, the aim of this study was to establish the level of miRNA-26 in samples of the lung tissue through the RT-PCR method.

METHODOLOGY

Patients Group

A case control study has been conducted from 15 October 2023 to 15 of January 2024. This study included 45 adult females and male which diagnosed with RA disease by specialist physician, and their ages ranges between, 18 to 75 years. Blood was drawn from the patients in order to investigate the MIR26, by RT- PCR (Real time polymerase chain reaction).

Control Group

The control group were 45 healthy Iraqi people. The control group was used only, for comparing parameters. The control samples were approximately similar with the patient samples in terms, of number, age ratio, and the place of living also urban and rural.

Inclusion Criteria to 45 (case) Patients Diagnosed by Specialist Physicians is, Rheumatoid Arthritis

The study includes both male and female patients and age of patients between 18-75 years, suffered from morning stiffness (at least one hour), arthritis in more than three joints' areas (swollen), and confirmed diagnosis of RA by physician specialist.

Exclusion Criteria Sample were Excluded based on the following criteria.

This study excluded the patients aged less than 18 years or more than 75 years, also excluded the patients suffered from diabetic mellitus, proteinuria, pregnancy females.

Blood Collection

The blood samples (5ml) withdrawn in this study. From each. patient diagnosed with RA patients and controls. The patient's hand was sterilized three times using 70% alcohol, and the venipuncture site was then cleansed with 2% iodine. Approximately 5 ml of venous blood was drawn, and the blood samples were put in an EDTA tube for genetic study. And then stored on a deep freezer at (-20 C°) to determined MIR,26 by RT-PCR stored in deep freezer at (-80 C°).

Culturing identification: **Two-step RT-qPCR is used to determine the activity of the miRNA-26 gene in samples.**

RNA Isolation

We isolated total RNA from patient's serum using RiboEX LS (Gene All, Seoul). Specifically, 600 µL of RiboEX LS Reagent was combined with 200 µL of serum, followed by a 5-minute incubation at 25°C. Following this, 160 µL of chloroform was introduced and energetically agitated before undergoing centrifugation at 12000 g for 15 minutes at four degrees Celsius. The resulting supernatant was combined with 400 microliters of isopropanol and placed for overnight incubation at minus twenty degrees Celsius. Afterward, centrifugation at 12000 g for 1 hour at four degrees Celsius was carried out, and the pellets obtained were washed 75% ethanol, then centrifuged at 7500g for 5 minutes at four degrees Celsius. Ethanol was removed entirely, the pellets were subsequently dissolved in twenty-five microliters of "diethylenepyrocarbonate-treated water (DEPC-treated water)" from "SinaClon", Iran. The quality, quantity, and purity of RNA were assessed by measuring optical density using Nanodrop.

Complementary DNA (cDNA) Synthesis.

The "Easy Script®" First-Strand cDNA Synthesis Super Mix kit is a comprehensive solution designed for the effective synthesis of initial strand miRNA from total RNA templates. To prepare cDNA, the manufacturer's guidelines were followed accordingly:

The RNA template and all accompanying reagents were thawed on ice, gently vortexed to ensure thorough mixing, and briefly centrifuged to gather any remaining liquid from the tube walls.

The mixture was prepared in a tube on ice, mixed gently and centrifuged briefly to collect the contents and added component as table (3-17).

Table 3-17: Reaction components of cDNA synthesis mixture

Components	Volume (microliters)	Final Concentration
RNA template	5	0.1 ng-5 µ
"Anchored oligo(dT)18 primer"	1	0.5 µg/µl
2x "ES Reaction Mix"	10	1X
"Easy Script® RT/RI Enzyme Mix"	1	20X
RNase-free H2O	up to 20	-

The next step is incubation at 42 oC for 15 minutes, followed by 85oC for five seconds to inactivate enzymes.

Determination of expression in samples by qPCR

According to the kit manufacturer instructions:

The GoTaq® qPCR Master Mix, alongside the template cDNA, primers, and Nuclease-free water, were thawed on ice and thoroughly blended prior to utilization.

The master-mix reaction was prepared according to the specifications outlined in table (3-18).

The cycling program for the qPCR reactions was programmed according to a 3-step cycling program, as detailed in table (3-19).

Table 3-18: Component's volume of qPCR mixture

Components	Volume/Reaction (microliters)	Final Concentration
GoTaqR qPCR Master Mix	10	(2X)
Forward Primer	0.6	300 nM
Reverse Primer	0.6	300 nM
Nuclease-free water	3.8	-
Template cDNA	10	-
Total Volume	0.6	-

Table 3-19: qPCR programs

Step	Temperature	Duration	Cycles
Enzyme activation	95C°	1 min	1
Denaturation	95C°	15 sec	50
Annealing, extension and data collection	60C°	1 min	

RESULTS AND DISCUSSION

Calculating the expression of miRNA-26 with Rheumatoid Arthritis by RT-PCR

As explained in (Table 4-2), The average Ct value for miR-26 in the group of controls is 6.82, with a fold change of around 1.00 while the average Ct value for miR-26 in group of patients is 7.59, with a fold change of around 7.59. Thus, this refers to significance elevation in miRNA26 in patients group other than in control subjects' group. It is possible that miR-26 contributes to the diseases or disorders that these people suffer from it.

Table (4-2): Calculating the gene expression of miRNA-26 with Rheumatoid Arthritis by RT-PCR

control	u6 average Ct	miR-26b average Ct	dCT	ddCT	fold change	averag F change
1	23.77	30.56	6.79	-0.030	1.021	1.00
2	22.36	29.34	6.98	0.160	0.895	
3	19.45	26.9	7.45	0.630	0.646	
4	22.14	28.24	6.1	-0.720	1.647	
5	19.54	26.39	6.85	0.030	0.979	
6	22.58	29.33	6.75	-0.070	1.050	
pateints			6.82			7.59
1	27.56	31.58	4.02	-2.800	6.964	
2	31.01	35.35	4.34	-2.480	5.579	

3	30.54	34.49	3.95	-2.870	7.311	
4	23.14	26.59	3.45	-3.370	10.339	
5	28.12	31.89	3.77	-3.050	8.282	
6	22.01	25.85	3.84	-2.980	7.890	

RA is a long-term autoimmune condition that increases the risk of cardiovascular disease and causes inflammation and joint deterioration. RA synovial fibroblasts, emit cytokines that promote inflammation and are implicated in the development of RA [12].

It has recently been shown that microRNAs are involved in regulating immunological and non-immune cells' inflammatory responses. In certain malignancies, miR-126, which is found inside the seventh intron of epidermal growth factor-like domain 7, promotes angiogenesis, proliferation, cell survival, migration, and invasion. It may also have a role in the onset and progression of RA by preventing the synthesis of interferon [13].

Study of Paradowska-Gorycka et al. [14], recorded that in RA patients, there appeared a favorable correlation between the expression of miR-26 and SMAD3.

Study of Jiang & Cao [15], which mentioned that increasing the expression of miR-26 might reduce cartilage damage, promote chondrocyte proliferation, and prevent apoptosis in RA.

Romo-García et al. [16] referred to that miRNA-26 can be used as potential biomarker for diagnosis of Rheomatoid Arthritis.

Jansen et al. [17] mentioned that elevated plasma levels of gene expression for miRNA26 by RT-PCR was recorded in patients with artery-coronary disease.

Acharya et al. [18], mentioned that miRNA-26 expression systemically elevated in RA patients in contrast to group of controls. This concurred with our result.

Qu et al. [13] findings suggest that miR126 overexpression in RA patients suppresses Subunit of phosphatidylinositol 3-kinase regulating PIK3R2 expression, encourages cell division, and prevents apoptosis. This implies that blocking miR-126 might have therapeutic advantages for the management of RA.

Also our results concur with study of Mattheos [19], which explained that RA patients had systemic elevations in miRNA-26 expression compared to a control group.

While our results conflicted with the results of Cheng et al. [20] which said that miRNA-26 expression was significantly declined in RA patients compared to controls.

Also, our results did not agree with the results Ciechomska et al. [21] which explained that there was decreased level of miRNA26 gene expression in RA patients.

CONCLUSION

There is significance elevation in miRNA26 in patients group other than in control subjects group. It is possible that miR-26 contributes to the diseases or disorders that these people suffer from it.

Ethical Standards

The Department of Medical Laboratories/College of Health and Medical Technologies in Kufa, Baghdad Health Department/Russafa, and the Training and Development Center all provided their approval for the current study. Additionally, all participants in both groups provided their informed written consent.

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