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# Academia Open



*By Universitas Muhammadiyah Sidoarjo*

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## Circulating MicroRNA-146a as A Biomarker Related To Inflammation in Thalassemia Patients

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### Abstract

This study delves into the intricate realm of microRNAs (miRNAs), non-coding RNA molecules that wield significant influence in the pathogenesis of various disorders. With the advent of advanced technologies, accurate quantification of miRNA abundance across diverse tissues is now attainable. Notably, extracellular circulating microparticles have emerged as robust, stable indicators for blood-based disease diagnostics. This investigation explores the dynamic interplay between circulating microparticles and clinical phenotypes, offering valuable insights into disease intricacies. The research narrows its focus to recent developments in employing circulating microparticles as biomarkers for prevalent inflammatory disorders, including infections endemic to thalassemia patients in the city of Diwaniyah, Iraq. Current findings paint a promising future for the utilization of circulating microparticles in the management of inflammatory diseases. In conclusion, this comprehensive exploration of miRNA's regulatory role in globin gene expression has the potential to revolutionize the landscape of thalassemia, ameliorating clinical manifestations and symptoms of this debilitating hemoglobinopathy. The identification of miRNAs implicated in the disease's pathophysiology not only paves the way for novel diagnostic markers but also opens new horizons for therapeutic interventions.

### Highlights :

- **MiRNA Significance:** MicroRNAs play a pivotal role in the pathogenesis of various disorders, offering potential insights into disease mechanisms.
- **Circulating Microparticles:** Extracellular circulating microparticles are stable blood-based indicators, holding promise for disease diagnostics and management, especially in the context of inflammatory disorders.
- **Thalassemia Insights:** This study highlights the potential impact of miRNAs on globin gene expression, offering new avenues for the diagnosis and treatment of thalassemia, a debilitating hemoglobinopathy.

**Keywords :** MicroRNAs (miRNAs), Circulating Microparticles, Thalassemia, Inflammatory Disorders, Disease Diagnostics

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## Introduction

Hemoglobinopathies are the most prevalent monogenic conditions affecting hemoglobin synthesis or structure. The two most prevalent illnesses are  $\beta$ -thalassemia and sickle cell disease (SCD) [1].  $\beta$ -Thalassemia has a variety of clinical symptoms, ranging from moderate anemia in the thalassemia intermedia group to severe transfusion reliance in the thalassemia major group. It is caused by the lack or diminished production of the globin gene [2].

Thalassemia is a genetic blood condition that leads to chronic hemolytic anemia from faulty hemoglobin synthesis. As a result, the lifespan of red blood cells in thalassemia patients is reduced (less than 120 days) [3]. It has been identified as a threat to global health since it is the most prevalent genetic disease in the entire world. One of the most common symptoms is Cooley's Facies, which occurs when the marrow produces too many red blood cells, causing the marrow cavity to develop and thin the bones as well as protrude the forehead. Other symptoms include anemia, paleness, weariness, a drop in hemoglobin levels, spleen enlargement, and an increase in hemoglobin levels [4].

In order to control the post-transcriptional expression of genes in multicellular animals, microRNAs (miRNAs) are short (20–24 nt) non-coding RNAs that affect the stability and translation of mRNAs [5].

miR-146a is a group of precursors of the microRNA that is found in mammals, including humans. The enzyme Dicer separates the mature miRNA sequence, which is ~22 nucleotides long, from the precursor hairpin [6].

Inflammation is derived from many pathological and physiological processes. Although the pathologic aspects of many types of inflammation are well characterized, less is known about their physiological processes [7]. Two of the most frequent causes of inflammation are infections and tissue damage, both of which cause white blood cells and plasma proteins to be drawn to the location of injured tissue. In this circumstance, tissue stress or dysfunction produces a response capability known as para-inflammation [8]. Even though serum ferritin protein is an acute phase reactant that increases in response to every inflammatory reaction, from infectious disease to chronic conditions, serum Fe levels and the proportion of serum iron binding ability must also be determined in order to distinguish between iron (Fe) overload and inflammation (transferrin), which can both cause high serum ferritin protein [9].

## Method

### Sample Collection

There were 120 participants in the current study, split into two groups: TM sufferers and healthy controls. The participants in the control group were carefully picked to ensure that they had no prior history of diabetes, high blood pressure, or any other illnesses or problems. All patient data, including sex, age, splenectomy, and other illnesses, was also documented.

The study's participants' ages ranged from 2 to 33 on average. The Women and Children Teaching Hospital in the Thalassemia Center is where patients go for routine exams and blood transfusions. The complete blood count (CBC), hemoglobin electrophoresis, ferritin, iron capacity, genetic testing, and reticulocyte count were used to support the diagnosis in all patients in this study who had been referred by a specialist.

A record of the patient's details is also kept, including their age, gender, BMI, family history, and any medications they may be taking. In the current investigation, all laboratory test analyses performed at the Women and Children Teaching Hospital, Baghdad Lab in Diwaniyah, Iraq, and Nabu Scientific Foundation in Baghdad, Iraq, were taken into consideration.

Patients with splenectomy, infections of any kind, persistent bone inflammation, heart failure, liver failure, and other blood disorders were excluded from this study.

### Storage

Each person's blood was drawn into two tubes, each holding five milliliters : four milliliters of gel and one milliliter of blood in a K2EDTA tube for the complete blood count (CBC). Centrifuging the gel tube at 3600 rpm for 10 to 15 minutes helped separate the serum from the blood. Using Eppendorf tubes, the collected serum was split into four parts. For biochemical analysis, the remaining components of the miRNA-146a research were kept at -20°C while one component was preserved at -40°C.

### Methods

Quantitative polymerase chain reaction (qPCR) and the Elabscience® Human IL-6 (Interleukin. 6) ELISA. Kit (BT-Lab, U.S.A) were used to measure the blood expression of miRNA-122. the serum level of IL-6 was determined. 0.3



mL of serum was utilized for RNA extraction using the TRIzol<sup>TM</sup> Reagent from Invitrogen, USA. The miRNA and miR-146-RT-primer were utilized to produce the cDNA using the protoscript<sup>®</sup> first strand cDNA synthesis kit from NEB in the UK. NEB in the UK's Luna Universal qPCR MasterMix was used for the PCR process. cDNA Bright Green master mix and forward and reverse universal primers specific to miR-146 (Table 1) were mixed with the resulting cDNA. Gene U6 was applied as an internal check. A comparative threshold cycle (Ct) was used to calculate the relative levels of MiR-146 and (2(-Ct)), and the results demonstrated the fold change in expression.

Primers	Sequence s	Product Size (bp)
mi.R-146. R . T	GTCGTATCCAGTGCCTGTCTGGAGT CGGCAATTGCACTGGATACGACAAC CCA	0
mi . R-146 . For mi . R-146 . Rev	GGGTGAGAACTGAATTCCA CAGTGCCTGTCTGGAGT	0
U6 . For U6 . Rev	CTCGTTCGGCAGCACAAACGCTTCAC GAATTTGCGT	94

**Table 1.** Primers.used for qPCR. experiments

## Statistical Analysis

All analyses were performed using SPSS version 26. The mean standard deviation (SD) was used to represent values in the t test, which was intended to compare normally distributed continuous data. Categorical variables were compared using the chi-square test, and data were reported as frequency and percentage. To connect numerical data with a normal distribution, the person correlation test was used. The correlation coefficient (r), which measures the relationship between two continuous variables, was used to identify associations with P.value less than 0.5.

## Result and Discussion

### Interleukin-6 (IL-6) level in patients and healthy control.

The level of IL-6 was highly significant higher than in TM patients group in comparison with healthy control (P < 0.001), **Table 2** and **Fig.1** .

### miRNA-146a level in patients and healthy control.

The level of miRNA-146a was highly significant higher than in TM patients group in comparison with healthy control (P < 0.001), **Table 3** and **Fig. 2** .

### Correlations between IL-6 and miRNA-146a

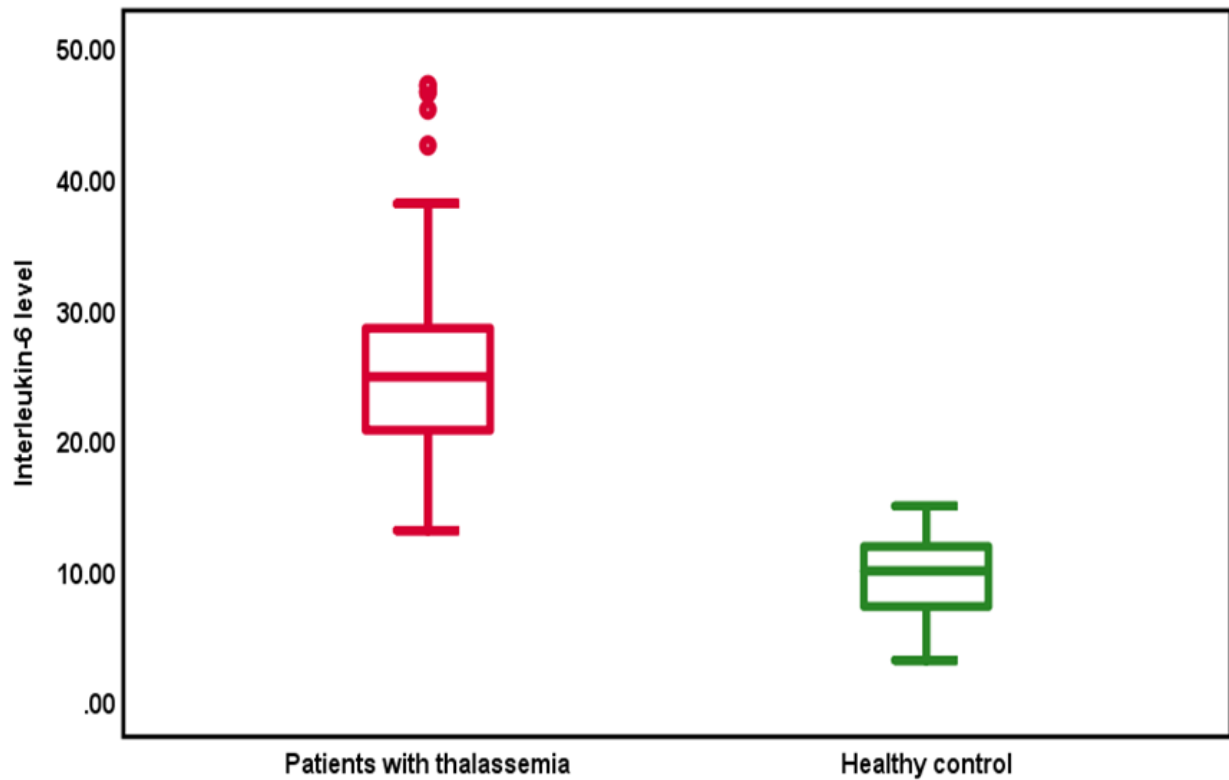
The correlations between IL-6 and miRNA-146a in patients with thalassemia were shown in table (4). The present results show there was highly significant correlation between all IL-6 and miRNA-146a.

### Table ( 2 ): Interleukin-6 (IL-6) level in patients and healthy control.

	Cases -control comparison		P
	Patients n = 80	Healthy control n = 40	
Interleukin-6 (IL-6) level			
c	28.81 ± 11.32	9.59 ± 3.03	< 0.001 † HS
Range	15.49 - 46.32	3.08- 14.86	< 0.001 † HS

**Table 2.**

**n**: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: Highly significant at P ≤ 0.001.



**Figure 1. Interleukin-6 (IL-6) level of patients and healthy controls**

	Cases -control comparison		P
	Patients n = 80	Healthy control n = 40	
miRNA-146a level			
Mean± SD	4.16 ± 1.96	1.0	< 0.001 † HS
Range	1.15 - 9.16	1.0- 1.0	< 0.001 † HS

**Table 3. miRNA-146a level in patients and healthy control**

**n**: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: Highly significant at P ≤ 0.001.

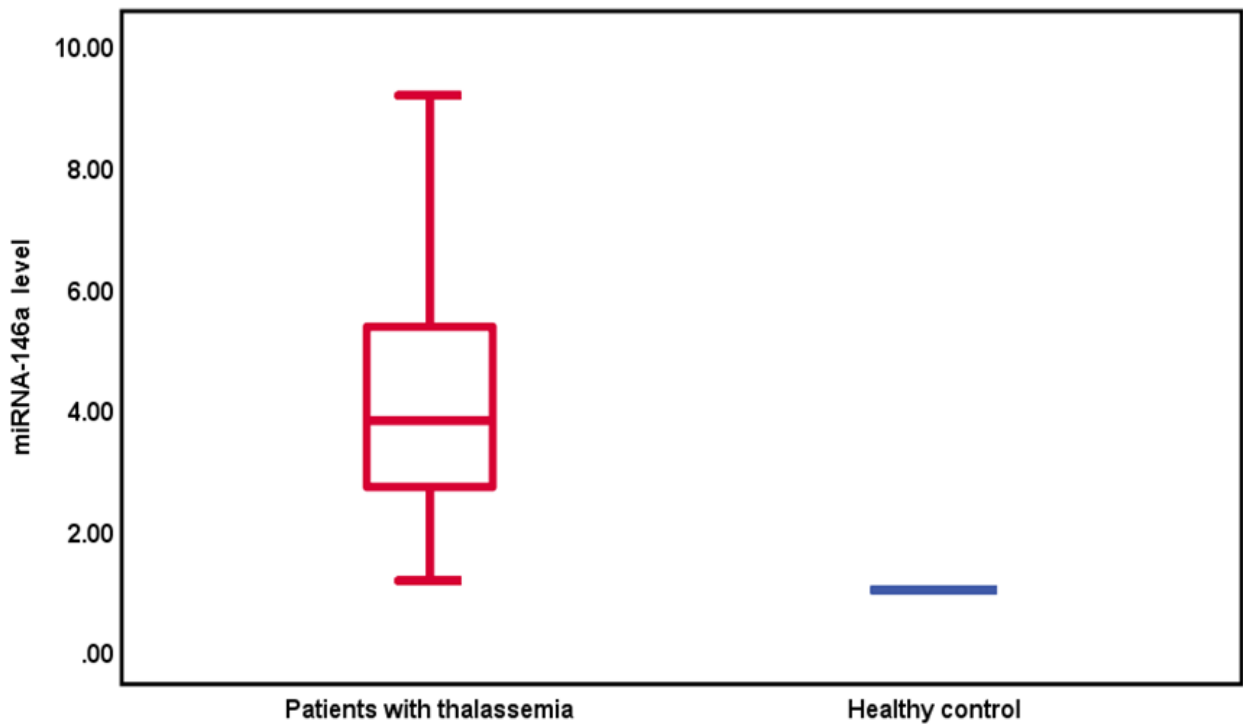


Figure 2. miRNA-146a level of patients and healthy controls

Characteristics		IL-16	miRNA-146a
IL-16	r	1	0.439*
	p	1	0.005
miRNA-146a	r	0.439*	1
	p	0.005	1

Table 4. between IL-6 and miRNA-146a in patients with thalassemia

r. correlation coefficient

Through statistical analysis, it was found that both suffering and healthy individuals had significant variations in the levels of the cytokine IL-6, a type of soluble immune mediator[10]. This finding suggests that changes in the particular immune cell subsets involved in their synthesis may be caused by the administration of numerous transfusions. Notably, our study showed that only within the cohort of patients with transfusion-dependent thalassemia with IL-6 was the decrease in levels of these molecules statistically significant. The cytokine IL-6 is critically important for the inflammatory response[11]. The differences in medication regimens and patient demographics are probably to blame for the variability in IL-6 blood levels among thalassemia patients. These results differ from those that were noted in earlier research that was done in this field (12). thalassemia. major. patients who had undetectable or normal levels of IL-6 showed an increase in the levels of this particular cytokine in the blood that was circulating[13].

84.8% of those with thalassemia major showed signs of zinc deficiency, which is a substantial percentage. noticed that thalassemia patients' serum levels significantly dropped as compared to the control group [14].

To determine the "accuracy" and "precision" of the targeted miRNAs, it is crucial to conduct studies on gene-targeting miRNAs and their expression in both in vivo and in vitro contexts [15]. Prior research has shown that miRNAs exhibit unusual stability in the bloodstream, and certain human diseases have been shown to manifest in the circulating miRNA profile[16]. It is known that a large number of miRNAs have important roles in controlling erythropoiesis. When miR-146a expression was examined in the context of typical erythropoiesis, it was discovered that its levels rose during the process' latter phases. Plasma miR-146a levels in those with thalassemia were higher and above normal in these people. The severity of the thalassemia condition is also linked with the expression levels of this particular microRNA[17]. To the best of our knowledge, our research is the first to show how plasma miR-146a can be used as a biomarker to measure erythroid cell mortality in thalassemia patients. The results of earlier investigations were supported by the miR-146a levels found in erythrocytes from both healthy controls and thalassemia patients[18]. The hypothesis put forth by the authors contends that individual differences in intravascular hemolysis may account for the relationship between plasma miR-146a levels and the severity of the

disease[19]. Splenectomy surgery is used in cases of severe thalassemia to reduce the amount of blood lost as a result of extravascular hemolysis induced by the spleen. Additionally, it has been noted that those who have had splenectomy surgery may experience intravascular hemolysis [20].

## Study limitations

problems with study samples and sample selection, a dearth of relevant prior studies on miRNA-146a, a lack of adequate sample size, a lack of funding, and time and expense restrictions.

## Conclusion

In summary, a thorough investigation of the miRNAs that regulate the expression of the globin gene may alter the status of thalassemia and ameliorate the clinical signs and symptoms of such fatal hemoglobinopathies. Developing new diagnostic indicators and treatment targets for thalassemia is undoubtedly aided by the identification of miRNAs implicated in the pathophysiology of the disease.

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