
# The Correlation of MIR-423 gene polymorphism with Diabetes Miletus type 2 among sample of Iraqi population


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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by persistent hyperglycemia and is associated with serious complications. The risk factors for T2DM include both genetic and lifestyle factors. Genome-wide association studies have indicated the association of genetic variations with many diseases, including T2DM. Genetic alterations in the MicroRNAs (miRNA s/miRs) are small non-coding RNA molecules that are involved in the important physiological processes including glucose metabolism. In the present study, the association of MIR-423 rs6505162 C>A gene poly morphism was studied by using of Tetra primer- amplification refractory mutation system-based polymerase chain reaction (T-ARMS-PCR) technique. It was found that demographic features and baseline characteristics it was found that out of the 50 patients, 29 were males and 21 were females. The age range of the patients was 18-79 years. There was no correlation between age and MIR gene polymorphism in both case and control. Regarding of MIR-423 rs6505162 C>A genotypes variation with T2DM. The results indicated that there was nonsignificant difference in genotype distribution of the MIR-423 genotypes polymorphism and T2DM disease after results interpretation

between cases and controls regarding genotypes and allelic frequencies. The frequency of CC, CA and AA genotypes was 16 (32.2%), 20(40%) and 14(285 %) for patients, and 17 (44%), 22 (34%)and 11(22%) for the controls, respectively. On the other hand there was no significant relationship between allelic frequencies and T2DM disease.

**Keywords:** Iraqi population, gene polymorphism, diabetes mellitus

## Introduction

Participating

### *1.1 Polymorphism and human diseases*

In genetics Polymorphism involves one of two or more of a particular DNA sequence. A gene is said to be polymorphic if more than one allele occupies that gene's locus within a population. The most common type of polymorphism involves variation at a single base pair (1). Polymorphism is the occurrence of two or more clearly different phenotypes within the same population of a species. Occurs when there are two alleles in the gene pool and one allele is gradually replacing the other. Polymorphism is a genetic variant that appears in at least 1% of a population. (e.g., the human ABO blood groups, the human Rh factor, and the human major histocompatibility complex). The most common type of polymorphism involves variation at a single base pair (2) . Gene polymorphisms can occur in any region of the genome. The majority of polymorphisms are silent, meaning they do not alter the function or expression of a gene . Some polymorphisms are visible. For example, in animals there is some dogs the E locus can have any of five different alleles, known as E, Em, Eg, Eh, and e. Varying combinations of these alleles contribute to the pigmentation and patterns seen in dog coats. In human type of blood groups, human Rh factor, and the human major histocompatibility complex are the best example of human polymorphism (3). A polymorphic variant of a gene can lead to the abnormal expression or to the production of an abnormal form of the protein; this abnormality may cause or be associated with disease (1,3).

MicroRNAs (miRNAs/miRs), small non-coding RNAmolecules, regulate gene expression and are involved in important physiologic processes (4). miRNA dysfunctions have been implicated in several diseases, such as cancer, cardiovascular disease and diabetes (5). The miR-423 blood levels are significantly decreased in cases with proliferative diabetic retinopathy (6). The inhibition of miR-423-5p decreases gluconeogenesis, reduces insulin resistance and decreases blood glucose (7). In contrast, overexpression of liver miR-423-5p increases gluconeogenesis, elevates blood glucose, and enhances the deposition of fat in mice (8).

### *1.2 Diabetes mellitus*

Diabetes mellitus is a general term for heterogeneous disturbances of metabolism for which the main finding is chronic hyperglycaemia. The cause is either impaired insulin secretion or impairedinsulin action or both (9). Psychological, social and genetic factors have an intimate role in the course of managing the disease, and in some ways may have a role in the cause of the disease. Inappropriate activation of hypothalamo pituitary adrenal axis can lead to the metabolic syndrome(10). Diabetes predicted in early stages can prevent many health issues such as damage of kidneys, eyes, ulcer, heart, lungs, and blood vessels. There are many existing processes to detect diabetes at an early stage, and more work needs to be done to detect and cure diabetes (11).

### *1.2.1 Diabetes Mellitus Etiology*

DM is broadly classified into three types by etiology and clinical presentation, type 1 diabetes, type 2 diabetes, and gestational diabetes (GDM). Some other less common types of diabetes include monogenic diabetes and secondary diabetes (12).

#### *1. Type 1 Diabetes Mellitus (T1DM)*

Type 1 diabetes mellitus (T1DM) accounts for 5% to 10% of DM and is characterized by autoimmune destruction of insulin-producing beta cells in the islets of the pancreas (13).

#### *2. Type 2 Diabetes Mellitus*

Type 2 diabetes mellitus (T2DM) accounts for around 90% of all cases of diabetes. In T2DM, the response to insulin is diminished, and this is defined as insulin resistance there are many cause of this type included the genetic varaitions (14).

#### *3. Gestational Diabetes Mellitus*

Hyperglycaemia, which is first detected during pregnancy, is classified as gestationaldiabetes mellitus (GDM), also known as hyperglycemia in pregnancy (15).

#### *4. Monogenic Diabetes*

A single genetic mutation in an autosomal dominant gene causes this type of diabetes (16).

#### *5. Secondary Diabetes*

Secondary diabetes is caused due to the complication of other diseases affecting the pancreas (for example, pancreatitis), hormone disturbances (for example, Cushing disease), or drugs (for example, corticosteroids) (17).

### *1.2.2 Diabetes mellitus Pathophysiology*

In T1DM, there is cellular-mediated, autoimmune destruction of pancreatic beta cells. T1DM hasa strong genetic predisposition. The major histocompatibility complex (MHC), also known as human leukocyte antigens (HLA), is reported to account for approximately 40 to 50% of the familial aggregation of T1DM. The significant determinants are polymorphisms of class II HLA genes encoding DQ and DR4-DQ8, with DR3-DQ2, found in 90% of T1DM patients (18). Another form of T1DM is latent autoimmune diabetes of adults (LADA). It occurs in adulthood, often with a slower course of onset.

Over the past decades, an enormous amount of effort has been devoted to understand how microRNAs (miRNAs), a class of small non-coding RNA regulators of gene expression at the post-transcriptional level, are implicated in DM pathology. Growing evidence suggests that the expression signature of a specific set of miRNAs has been altered in the progression of DM. In the present study, we studded the correlation miRNA-423 gene variation at position rs6505162 among sample of Iraqi patients (19,20).

## 3. Materials and methods

### *3.1. Samples collection*

One fifty blood samples were obtained by venipuncture blood collection methods then collected into test tubes containing EDTA anticoagulant. All samples were randomly selected from outpatient clinic at Al-Eayn specialist laboratory. All blood samples were stored at deep freezing (-20 0C). DNA was extracted from frozen whole blood using a QIAamp Blood Kit (QIAGEN Inc,Santa Clarita, CA), using the methodology indicated by the manufacturer.

### *3.2. DNA Isolation*

- **Sample preparation:**

1. Transfer up to 200 µl blood to a 1.5ml micro-centrifuge tube (not provided) .
2. Add 30 ul Proteinase K to the sample and briefly mix . Then incubate for 15 min at 60 C ( 10mg / ml not provided ).

- *Cell Lysis*

3. Add 200 ul FABG Buffer to the sample and mix by overtaxing.
4. Incubate in a 70 ° C water bath for 15 min to lyse the sample. During incubation, invert thesample every 3 min
5. Preheat required Elution Buffer in a 70 ° C water bath for DNA Elution.

- *Column Washing*

6. Add 400 µl of W1 Buffer to the FABG Column and centrifuge for 30 sec at speed 14,000 rpm or 8,000 x g. Discard the flow - through and place the FABG Column back to the Collection Tube.
7. Add 600 µl of Wash Buffer to the FABG Column and centrifuge for 30 sec at speed 14,000 rpmor 18.000 x g. Discard the flow - through and place the FABG Column back to the Collection Tube.

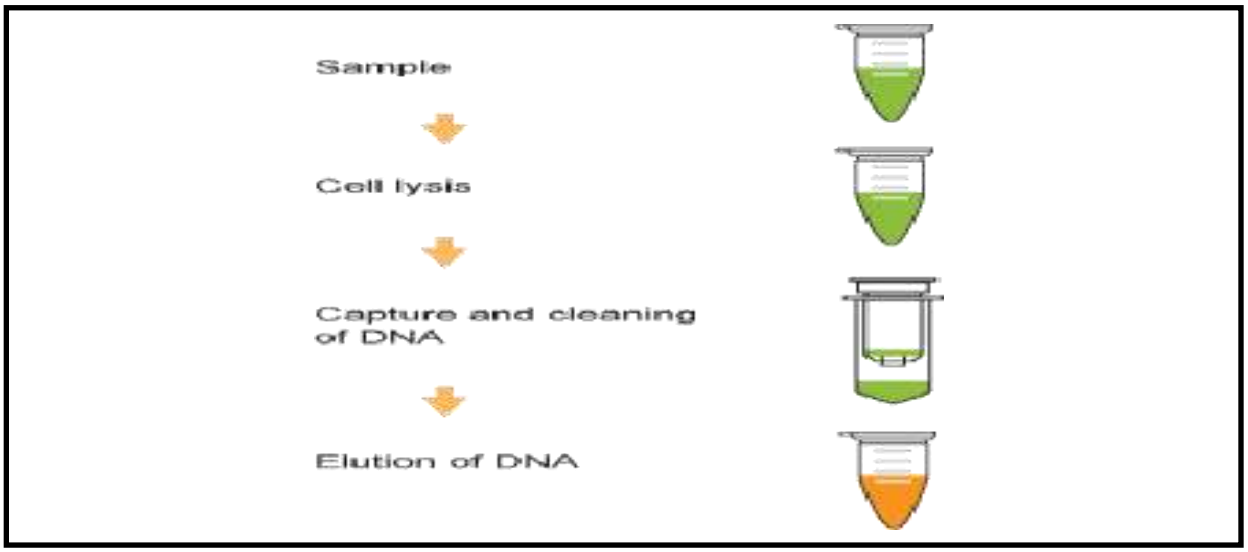
   Make sure that ethanol has been added to Wash Buffer when first open.

8. Centrifuge for an additional 3 min at speed 14,000 rpm or 18.000 xg to dry the column. This step will avoid the subsequent enzymatic reactions from being inhibited by residual liquid.

- *Elution*

10. Place the dry FABG Column to a new 1.5 ml microcentrifuge tube.
11. Add 100 µl of Preheated Elution Butter or TE to the membrane center of FABG Column. Important Step For effective elution, make sure that the elution solution is dispensed onto themembrane center absorbed completely.
12. Incubate the FAGB Column at 37 ° C for 10 min in an incubator.
13. Centrifuge for 1 minute at full speed 14,000 rpm or 18,000 xg to elute the DNA. Standard volume for elution is 100 µl. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total volume could be 200 µl. (see figure 2)
14. Store the DNA fragment at 4 ° C or -20 ° C.

**Figure 1**. Blood Kit Procedure



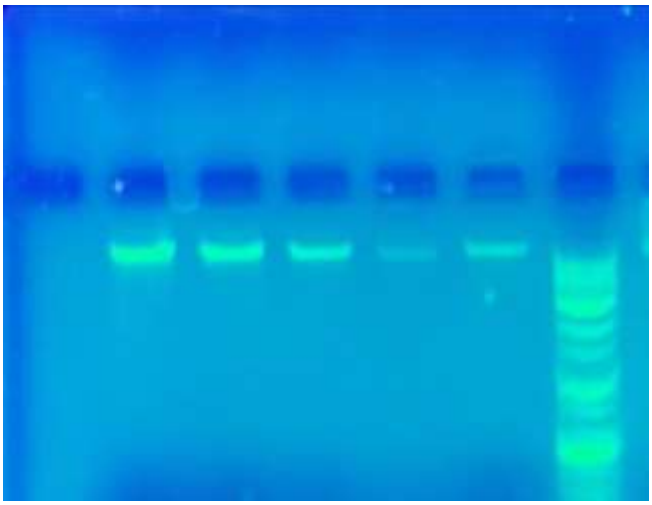



**Figure 2.** Show DNA isolation

### *3.3. MIR gene genotyping*

Single nucleotide polymorphisms (SNPs) in MIR gene was conducted using Tetra primer- amplification refractory mutation system-based polymerase chain reaction (T-ARMS-PCR) toevaluate the potential clinical association of MIR-423 rs6505162 In the present study, MIR-423 genotyping was conducted using Tetra primer-amplification refractory mutation system-based polymerase chain reaction (T-ARMS-PCR) to evaluate the potential clinical association of MIR- 423 rs6505162 C>A. SNP alleles allows selective amplification which can be easily analyzed after electrophoresis. It utilizes four primers ( Table 1) forward outer, reverse outer, forward normal and forward mutant. Outer forward and revers outer give us internal control additional to genotypes. T-ARMS PCR is a flexible, rapid and economical SNP detection tool compared tocontemporary genotyping tools such as allele-specific PCR (20).

**Table 1.** Primers sequence of MIR gene

| Gene | Primers | | Amplicon size (bp) | Tem. |
|---|---|---|---|---|
| MIR-423rs6505162 | Forward outer | 5'-TTTTCCCGGATGGAAGCCCGAAGTTTGA-3' | 336 | 62 C$^0$ |
| | Reverse outer | 5'-TTTTGCGGCAACGTATACCCCAATTTCC-3' | | |
| | Forward normal | 5'-TGAGGCCCCTCAGTCTTGCTTCCCAA-3' | 228 | |
| | Forward mutant | 5'-CAAGCGGGGAGAAACTCAAGCGCGAGG-3' | 160 | |

### *3.4. PCR condition*

T-ARMS-PCR was performed in a reaction volume of 25 µl containing template DNA (50 ng),

0.25 µl primers stock solution containing 5 pmol of each primer and 10 µl from Go Taq®Green Master Mix (cat no M7122; Promega Corp) The final volume of 25 µl was adjusted by adding nuclease-free ddH2O. Finally, 2 µl of DNA was added from each subject. Thermocycling conditions: The conditions used were at 95˚C for 10 min followed by 40 cycles of 95˚C for 35 sec,annealing temperature MIR-423rs6505162 C>A genes (62ºC), extension for 72˚C for 45 msec and final extension at 72˚C for 10 min.

## 4. Results

Demographic features and baseline characteristics it was found that out of the 50 patients, 29 weremales and 21 were females. The age range of the patients was 18-79 years. There was nocorrelation between age and MIR gene polymorphism in both case and control.

Interpretation of MIR genotypes is easy interpretable due to that the assay dose not rely on enzymatic cleavage. The primer that used in this study produce PCR products of either 160 bp or 228 bp additional of 336 bp as internal control (figure 1).

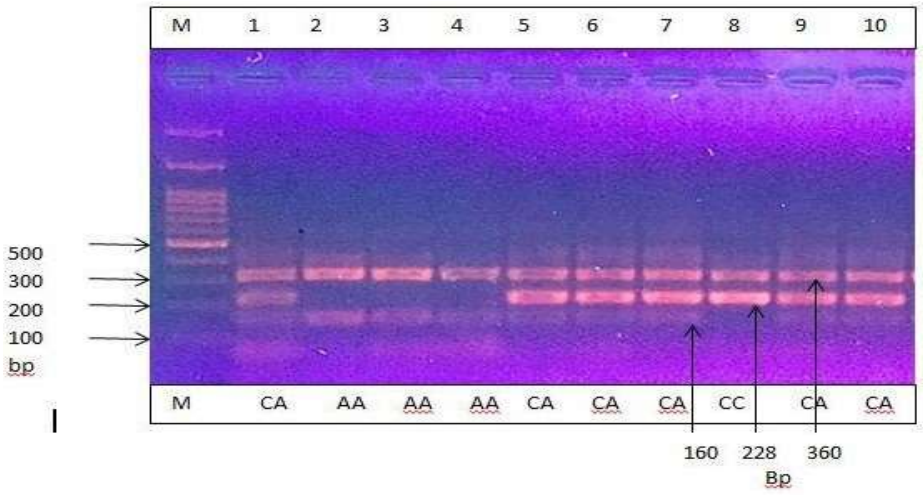



**Figure 1.** Genotypes of MIR gene : line M ( DNA marker), Line ( 1,5,6,7, 9, and 10) representedHeterozygous (CA), while line ( 2,3, and 4) are mutant homozygous (AA) and line 8 represented wile type homozygous (CC). Agarose concentration was 2%.

### *4.2 MIR genotypes distribution.*

Regarding of MIR-423 rs6505162 C>A genotypes variation with T2DM. The results indicated that there was nonsignificant difference in genotype distribution of the MIR-423 genotypes polymorphism and T2DM disease after results interpretation between cases and controls regardinggenotypes and allelic frequencies**.** (Table 2). The frequency of CC, CA and AA genotypes waswas 16 (32.2%), 20(40%) and 14(285 %) for patients, and 17 (44%), 22 (34%)and 11(22%) forthe controls, respectively. On the other hand there was no significant relationship between allelic frequencies and T2DM disease.

**Table 2.** Distribution of MIR genotypes and frequency of the alleles in present study.

| Genotypes | Case (n=50) | Control (n=50) | OR( 95%CI) | P-value |
|---|---|---|---|---|
| CC | 16 (32.0%) | 17(34%) | ref. | 1.0 |
| C/A | 20 (40.0%) | 22(44.0%) | 1.9 ( 1.01 -3.3) | 0.83 N.S |
| CC | 14( 28.o%) | 11(22.0% | 3.1(1.6-4.7) | 1.17 N.S |
| Alleles | | | | |
| C | 52(52.0%) | 45 (45.0%) | Ref. | 1.0 |
| A | 48(48.0%) | 55(55.0%) | 1.43(1.8- 1.9) | 0.96 N.S |

**N** = Number, **N.S** = non significant.

Interpretation of MIR genotypes is easy interpretable due to that the assay dose not rely on enzymatic cleavage. The primer that used in this study produce PCR products of either 160 bp for AA genotype or 228 bp for CC genotypes additional of 336 bp as internal control.

## *5. Discussion*

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by hyperglycemia resulting from impaired insulin action caused by insulin resistance in the liver, muscles and adipose tissues . Insulin resistance leads to hyperinsulinemia and pancreatic β cell dysfunction. In the present study, the impact of *hsa-miR-423* rs6505162 polymorphism was investigated on 50 diabetes mellitus types 2 cases and equal number as a control healthy individuals. The results indicated that *hsa-mirR-423* rs6505162 polymorphism was statistically non-significant with susceptibility to increased risk of diabetes mellitus disease. In this regard, recently genetic susceptibility due to SNP has been one of the major focuses of daibetes molecular biologyresearch (21).

MIR-423 that expresses two microRNA s, MIR-423-3P and MIR-423-5P (22,23,24). The current results showed that there was a no significant difference in MIR-423 rs6505162 C>A genotype distribution between T2DM patients and controls, and that the CA genotype of the MIR

-423 rs6505162 C>A was the highest frequency ( 44%) among MIR genotypes with T2DM (24,).

This study is in line with the study conducted in 2020 by Mohammed Muzaffer and his team ,they reported that the frequency of MIR genotypes was 35 %, 48%, and 17% for wile, hetero and mutant genotypes respectively (25). While the genotypic of the same gene in other study was different compared with our results it was reported that the frequency of MIR gene was 67 ( 43.8%),63 (41.2%), and 23 (15%) for AA,CA, and CC genotypes respectively (26). Whereas in present study was 16 (32.0%), 20 (40.0%), and 14( 28.o%). If we compare the results of the current study with other studies, we find that some of the results are consistent and others are different. Such a difference can be explained due to the ethnic and environmental differences of the samples that were studied. Therefore, further studies will give a clearer highlight of the relationship between T2DM diseases and the genetic background of individuals.

Conclusion

1. The age range of the patients was 18-79 years. There was no correlationbetween age and MIR gene polymorphism in comparison between case and control concerning age effects.

2. There was no significant correlation between MIR-423 rs6505162 C>A genotypes variation with type 2 diabetes millitusT2DM.

Recommendations

1. Study of MIR gene expression to insure the results.

2. Study more genes associated with diabetes mellitus type 2 to see the interaction effects of all those genes with diabetes mellitus disease

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