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# Assessment of the Relationship between Genetic Polymorphisms in Macrophage Migration Inhibitory Factor Gene and the Risk of Type I Diabetes Mellitus in an Iraqi Patients

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**ABSTRACT**— Type 1 diabetes mellitus (T1DM) is a chronic, immune-mediated disease characterized by the destruction of insulin-producing β cells in the pancreas. This study investigated the influence of single nucleotide polymorphisms (SNP) in gene of macrophage migration inhibitory factor (*MIF*), -173 G > C (rs755622), on T1DM patients. The study was included 75 T1DM patients and 25 healthy subjects. The-173 G / C SNP *MIF* gene was detected by using tetra-primer amplification refractory mutation system (ARMS) in T1DM patients and controls. The genotype distribution results of the -173G/C SNP of *MIF* gene showed significant difference ($p$<0.05) between controls (GG: n= 25, 100%; GC: n=0, 0%; CC: n=0, 0%) and T1DM patients (GG: n= 42, 56%; GC: n=25, 33.33%; CC: n=8, 10.67%). These results showed an increased in GC, CC genotype and C allele of the -173G/C in T1DM patients than controls, and they were significantly more likely than controls to have the mutant allele C (OR=1.881, 95%CI=0.213-16.585, p=0.564). These results indicate a possible role for the C allele in T1DM disease. The present study suggested that the -173G/C SNP in the *MIF* gene statistically association ($p$<0.05) with the risk of T1DM occurrence in Iraqi patients.

**KEYWORDS:** T1DM, *MIF* 173G/C, Polymorphism, tetra-primer ARMS-PCR

## 1. INTRODUCTION

Diabetes mellitus is a group of disorders of carbohydrate metabolism, whose main feature is chronic hyperglycemia that results from defects of insulin secretion, insulin action, or a combination of those. Metabolic abnormalities observed in diabetes can be caused by the low level of insulin production and/or insulin resistance of the target tissues [36].

T1DM can develop at any age but it is the most common metabolic disease in children and youth with incidence increased by 2–5% worldwide (5.3% in North America, 4.0% in Asia and 3.2% in Europe) with the exception of South America and the West India where T1DM is less prevalent and where there is a 3.6% decrease in incidence, If the worldwide trend were to continue at this rate for the next decade, the number of T1DM cases would nearly double [4], [43].

The T1DM association with many genetic factor which play role important in disease.

The MIF is a pro inflammatory cytokine mainly released from Th2 cells and macrophages, which can mediate the host response to infection and stress by activating innate and adaptive immune pathways [16], [3]. In humans, *MIF* is encoded by a single gene located on chromosome 22q11.2m, a 12 kD peptide

comprising 114 amino acids [2].

The *MIF*-173G/C gene polymorphism, a G to C transversion within the *MIF* promoter region at position -173 creates an AP4 transcription factor binding site [26]. Previous studies have shown that *MIF*-173G/C polymorphism was related to the susceptibility of cancer [45].

The *MIF*-173G/C plays a dual effect, which is not only a risk factor for the morbidity of goiter but also a protective role in the development of untreated severe goiter [29]. MIF is constitutively expressed by a variety of immune and nonimmune cells (e.g., eosinophils, neutrophils, monocytes/macrophages, lymphocytes, endocrine, endothelial, and epithelial cells) [33]. Previous research has shown that MIF can participate in the pathophysiology of chronic degenerative and autoimmune diseases by significantly increasing body fuids, T1DM and T2DM [24], [27], metabolic syndrome [34] and oral squamous cell carcinoma [14], among others. Macrophage migration inhibitory factor (Mif) is highly expressed in T1DM. However, there is limited information about how Mif influences the activation of macrophages (Mφ) and dendritic cells (DC) in T1DM. These findings suggest that Mif plays a role in the molecular mechanisms of Mφ and DC activation and drives T cell responses involved in the pathology of T1DM. Therefore, Mif is a potential therapeutic target to reduce the pathology of T1DM [40].

This study explored the association of SNP in *MIF*- 173 G > C (rs755622) gene polymorphasim by using tetra-primer ARMS-PCR with the risk of T1DM development in Iraqi population.

## 2. Materials and Methods

a) Study group: This study was included 75 patients. These samples were collected from laboratory of Najaf Center of Diabetes & Endocrine in Al-Sadr Teaching Hospital. All the patients selected for the present study were having T1DM (Blood samples were obtained as part of the routine clinical protocol). Epidemiological information's about patients like age and gender was collected from patients Data sheets from hospital.

b) Control group: It consists from 25 healthy women; all were without any inflammatory disorders or clinical manifestation of any disease.

### *2.1 Blood sample*

The PCR test was performed on 2 ml of venous blood, which was collected tubes with anticoagulant Ethylenediaminetetraacetic acid (EDTA) from patients and controls.

### *2.2 DNA isolation and tetra-primer ARMS-PCR analysis technique*

Genomic DNA was isolated using protocol from Genomic DNA Mini Kit (Geneaid Biotech, Taiwan) protocol procedure, which specially was designed to purifying DNA from frozen blood.

A sequence of SNP in region of promotor in *MIF* -173 G/C gene was amplified using the primer-pairs: Outer forward primer5'CAGTGCGTGCAGTGGAATGAAC-3'and outer reverse 5'-TGGGGAAGTCACCGCCTGCCT-3'and inner forward5'-AGCCGCCAAGTGGAGAACTGG-3'and inner reverse5'-AGCCCGGCGCACCGCTCCTAG-3'. These primers have already been published previously [19], [13].

Primer containers were first centrifuged at 13,000 rpm for 3 minutes, and then reconstituted with appropriate volume of TE buffer for each one (according to the manufacturer) in order to get 100 pmole/µl (stock solutions). Working solution with 10 pmole/µl, was prepared from stock solutions.

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According to the manufacturer's instruction DNA quality extracts were analyzed by electrophoresis. The extracted genomic DNA concentration was estimated by using Nanodrop spectrophotometer (THERMO, USA), which measured DNA concentration (ng/µL) and checked the DNA purity by reading the absorbance at (260 /280 nm) according to [5].

The tetra-primer ARMS-PCR was performed for detection and genotyping *MIF* gene polymorphism 173G/C (rs755622) in blood samples. Based on the amplification products obtained, three possible allele arrangements were identified: genotype GG (298, and 126 bp bands), GC (298, 213, and 126 bp bands), CC (298, and 213 bp). The amplification steps listed in Table (1)

Finally, the gel electrophoresis method, which included preparing the gel loading and running the gel, was done according to [41] as the following:
A 2% agarose gel was made by mixing 2 g agarose with 100 ml 1X TBE buffer.

**Table 1:** Reaction Condition for *Macrophage migration inhibitory* Factor (*MIF*)-173 G/C gene.

| **Gene** | Step Initial Denaturation | Numbers of Cycles | The compositions of each cycle | | | Extension |
|---|---|---|---|---|---|---|
| | | | Denaturation | Annealing | Extension | |
| *MIF* -173 G/C (rs755622) | 95°C for 3 min | 35cycles | 95°C for 0:45 sec | 65°C for 1min | 72°C for 1min | 72°C for 5 min |

***2.3 Statistical analysis***
Statistical analyses of all results were carried out by the help of Statistical Package for the Social Sciences (SPSS) version 23 software statistical package using t-test and Chi-square test (with P value at level of significance less than 0.05) to compare value of results between groups. Result values were expressed as mean ± SE, number of patients, or percentages.

**3. RESULTS AND DISCUSSION**
a) Controls: Among the 25 healthy subjects; 25 (100%) had found as homozygous GG alleles, no healthy subject had found as heterozygous genotype (with the G and C alleles (GC), and no healthy subject had found as homozygous genotype CC alleles; (GG: n= 25, 100%; GC: n=0, 0%; CC: n=0, 0%).

**Table 2:** The results of genotypic frequencies of -173G/C at *MIF* gene in patients and controls.

| **Genotypes** | **Healthy controls (N=25)** | **Diabetes mellitus type 1patients (N=75)** |
|---|---|---|
| **GG** | 25(100%) | 42 (56%) |
| **GC** | 0 | 25 (33.33%) |
| **CC** | 0 | 8 (10.67%) |
| **P-value** | 0.003** | |

| Alleles frequency | N(%) | N(%) |
|---|---|---|
| G allele | 50(100%) | 109(72.67%) |
| C allele | 0 | 41 (27.33%) |
| $X^2$ | 0.334 | |
| P-value | 0.564 | |
| OR (95%CI) | 1.881(0.213-16.585) | |

Data were expressed as number and a percentage (N%). *$p$ <0.05 significant. Abbreviations: $X^2$= chi-square, OR= odds ratio, CI= confidence interval

b) patients: Among the 75 T1DM patients; 42 (56%) had found as homozygous GG alleles, 25 (33.33%) found as heterozygous genotype (with the G and C alleles (GC), and 8 (10.67%) had found as homozygous genotype CC alleles; (GG: n= 42, 56%; GC: n=25, 33.33%; CC: n=8, 10.67%).

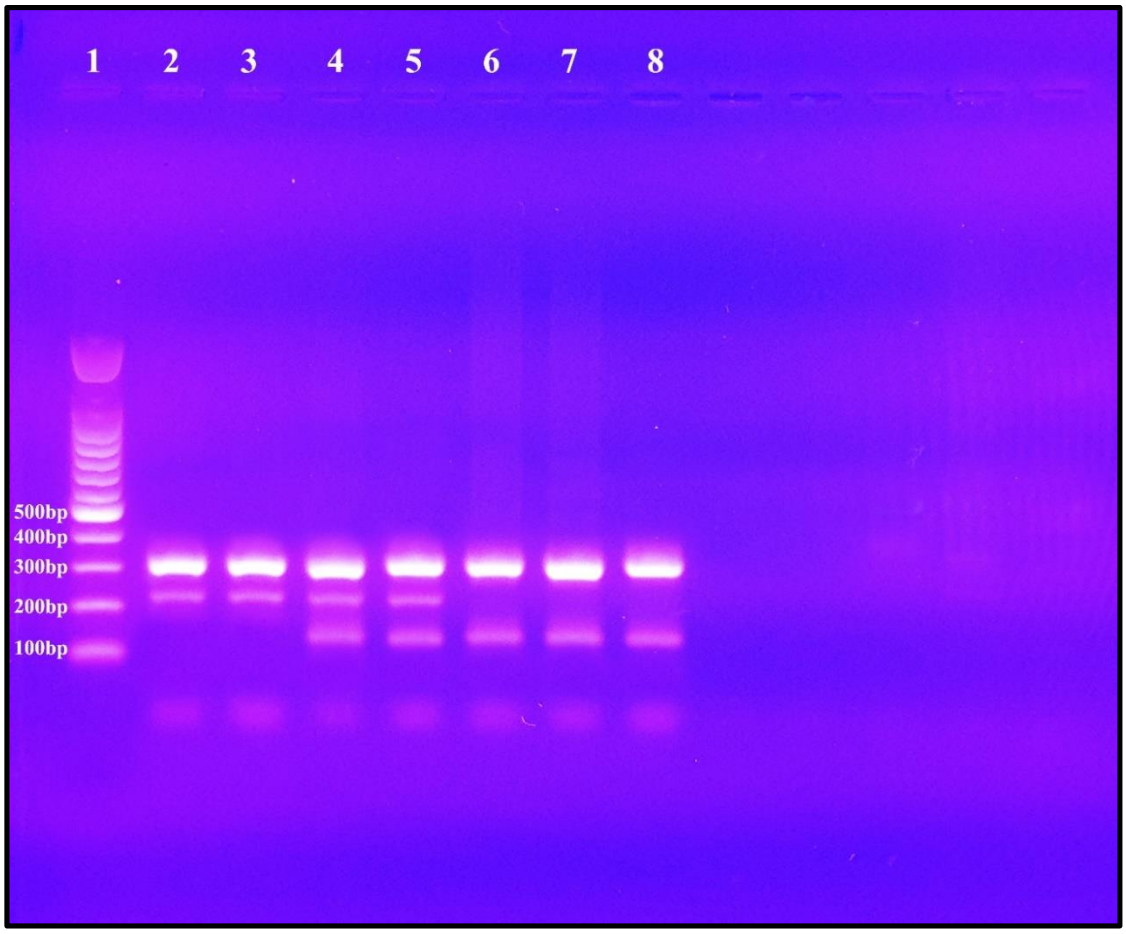


**Figure 1:** The electrophoresis image of tetra-primer ARMS-PCR analysis of -173G/C SNP in the *MIF* gene. Lane 1: 100 bp DNA Ladder. Lane 6,7, and 8: homozygous genotype GG (298, and 126 bp bands); Lane 4 and 5: heterozygous genotype GC (298, 213, and 126 bp bands); Lane 2, and 3: mutant homozygous genotype CC (298, and 213 bp).

That means the frequencies of -173G/C SNP in the *MIF* gene in the 75 Iraqi of T1DM patients in Al-Najaf province were with significant differences with that of the 25 healthy controls group ($p<0.05$).

These results showed an increased in GC, CC genotype and C allele of the -173G/C in T1DM patients than

controls, and they were significantly more likely than controls to have the mutant allele C (OR=1.881, 95%CI=0.213-16.585, p=0.564). These results indicate a possible role for the C allele in T1DM disease. The single nucleotide polymorphism (SNP) was in the located at *MIF* gene promotor region at position -173 consisting of a G to C transversion [15], [9], which highlights on the potential role of -173G/C SNP and its functional influence that cause change in sequence of promoter region in in *MIF* gene that form a new binding site for activator protein transcription factor 4 (TCF4). The −173*C allele has been associated with mRNA expression and circulating MIF levels [32].

The results agreed with [8] results which demonstrated that the acute pancreatitis (AP) associated with *MIF* -173 G/C polymorphism. They recorded that 33(53.2%) in patients had GG genotype, 4(6.5%) in patients had GC genotype and 25(40.3%) patients with CC genotype. The frequency of G allele was 70(56.4%) in patients, while C allele was 54(43.6%) in patients.

These results were consistent with results of [12] which clarified the relationship between Crohn's disease (CD) patients and *MIF* -173 G/C, where the results showed that 52(26.3%) in patients had GG genotype and 51(32.1%) in controls, 45(22.7%)in patients had GC genotype and 45(28.3%) in controls, while 7(3.5%)in patients had CC genotype and 6(3.8%) in controls.

These results were similar to [35] which clarified the relationship between rheumatoid arthritis (RA) and *MIF* -173 G/C, where the results showed that 209 (71%) patients had GG genotype and 398 (76%) in controls, 76 (26%) patients had GC genotype and 121 (23%) in controls, while 8 (3%) patients had CC genotype and 7 (1%) in controls. The frequency of G allele was 494 (84%) in patients and 917 (87%), while C allele was 92 (16%) patients and135 (13%) in controls.

These results agreed with study by [31] which showed that 43 (98%) Rheumatoid arthritis (RA)patients had GG genotype versus 110(53%) among controls, 111(49%) patients had GC genotype versus 42 (89%) among controls, while 8 (17%) patients had CC genotype versus 8 (17%) among controls.

These results were similar to [22] study which recorded that the frequency of *MIF*-173 G/C alleles were GG 34.09% and GC 65.91% in Rheumatoid arthritis (RA)patients. *MIF*-173 G/C genotype was associated significantly (P=0.040, P=0.044) with disease. *MIF* gene creates an activating enhancer binding protein 4 transcription factor binding site and it has been associated with RA (Martinez et al., 2007).

These results agreed with study by [38] which clarified the relationship between ulcerative colitis (UC)patients and *MIF* -173 G/C, where the results showed that 38(0.66%) patients had GG genotype versus 99(0.80%) among controls, 19(0.33%) patients had GC genotype versus 23(0.19%) among controls, while 1(0.02%) patient had CC genotype versus 1(0.01%) among controls. The frequency of G allele was 95(0.82%) in patients versus 221(0.90%) among controls, while C allele was 21(0.18%) in patients and 25(0.10%) in controls.

The results this study agreed with [38] which found that C allele of *MIF*- 173 G/C polymorphasim was associated with the increased risk of the development of IBD. In the same manner, study by Shen et al. (2013) which found a relationship between the *MIF*-173G/C polymorphism and (IBD). They recorded that the homozygotic CC genotype may be a risk factor for IBD The CC homozygotic carriers at higher risk of IBD than GC and GG homozygotic individuals.

These results agreed with study by [11] which clarified the relationship between Cardiomyopathy (CMP)

and MIF -173 G/C, where the results showed that 17 (56.7%) patients had GG genotype and 10(50%) in controls, 13(43.3%) patients had GC genotype and 6(30%) in controls, while 4(20%) patients had CC genotype and no healthy subjects. The frequency of G allele was 47(78.3) in patients and 26(65%) in controls, while C allele was 13(21.7%) in patients and 14(35%) in controls.

These results were in accordance with those of [44] results, which showed that 59(59%) Meniere's Disease (MD) patients had GG genotype while 54(75%) in control, 31(31%) patients had GC genotype and 16(22.2%) control, 10(10%) patients had CC genotype while 2(2.8%) control. The frequency of G allele was 149(74%) in patients and 124(86%) in controls, while C allele was 51(26%) in patients and 20(14%) in controls.

These results agreed with [37] study which found that there were an association between Necrotizing enterocolitis (NEC) and *MIF* gene -173 G/C polymorphasim. The prevalence of the CC genotype among 21 patients with severe NEC was 4.76% versus 1.51% among 66 controls ($p = 0.37$). Overall, genotyping showed no significant differences in the allele frequency of the polymorphic *MIF*-173 G/C variation between cases and controls.

These results were similar to [14] which indicated that 43 (80%) Systemic lupus erythematosus (SLE) had GG genotype and 53 (106%) in controls, 49 (91%) patients had GC genotype and 43 (85%) in controls, while 8 (15%) patients had CC genotype and 4 (9%) in controls.

These results were consistent with results of [20] which showed that 341 (64%) Alzheimer's disease (AD) had GG genotype versus 497 (69.7%) among controls, 163 (30.6%) patients had GC genotype versus 188 (26.4%) among controls, while 29 (5.4%) patients had CC genotype versus 28 (3.9%) among controls.

These results were consistent with the results of Coban et al. (2015) which showed that 65(77.4%) DM had GG genotype, 667 (66.2%) patients had GC genotype, while 14(16.6%) patients had CC genotype. The frequency of G allele was 85.7% in patients, while the frequency of C allele was 14.3%in patients.

Present results agreed with study by [22] which clarified the relationship between Tuberculosis (TB) and *MIF* -173 G/C, where the results recorded those 99(61.5%) patients had GG genotype versus 105(74%) among controls, 53(32.9%) patients had GC genotype versus 32(22.5%) among controls, while 9(5.6%) patients had CC genotype versus 5(3.5%) among controls. The frequency of G allele was 251(78%) in patients, while C allele was 71(22%) in patients. As well as another study by [30] confirmed the relation between *MIF* gene -173 G/C (TB), the GC + CC vs. GG genotype variants were compared between total cases of TB and controls. They observed a moderate difference of *MIF* −173 C allele in TB cases compared with healthy controls. A statistically significant difference of frequencies for the *MIF* gene polymorphism (GC + CC vs. GG) were identified between cases of TB and controls. The frequencies of *MIF* -173 genotypes GC + CC were 60% also significantly higher in cases of TB than in controls.

These results agreed with study by [25] which suggested that CC genotype was a risk factor for coronary heart disease (CHD) compared with GG genotype. No significant difference was found for both GG and GC frequencies between CHD patients and controls. *MIF*-173 C allele may increase susceptibility to CHD.

In same time, this study agreed with a study done by [1] which clarified the relationship between Bladder cancer and *MIF* -173 G/C, where the results showed GG, GC, and CC genotype frequencies were 63.4%, 30.5%, and 6.1%, respectively, among the cases and 50.7%, 43.2%, and 6.1%, respectively, among the

controls.

Present results were similar to [28] which showed that 46(64.8%) Adolescenc patients had GG genotype, 22 (31%) patients had GC genotype, while 3 (4.2%) patients had CC genotype.

These results agreed with study by [17] which showed that 10(33.3%) Dilated cardiomyopathy (DCM) patients had GG genotype and 9(45%) in controls, 12(40%) patients had GC genotype and 11(55%) in controls, while 8(26.7%) patients had CC genotype and no healthy subjects had CC genotype.

These results agreed with study by [39] which clarified the relationship between Nephrotic syndrome (NS) and *MIF* -173 G/C, where the results showed GG, GC and CC genotype frequencies were 59.7%,36.6% and 3.7%, among the patients and 73.8%,24.8% and 1.4%, among the control.

These results were consistent with the results of Çoban *et al.* (2019) which showed that the distributions of *MIF* -173 G/C for GG, and GC+CC genotypes were found to be 65.8% (n=25) and 34.2% (n=13) in the CAD-DM group, respectively. The frequency of C allele was determined as 16.9% in the CAD-T2DM group. They mentioned that the MIF protein plays an indirect role in the development of T2DM by promoting the production of proinflammatory cytokines and adipocytokines that have a role in insulin receptor signaling which leads to the development of insulin resistance.

These results agreed with study by [21] which showed that 79 (66%) T2DM patients had GG genotype, 33(27%) patients had GC genotype, while 8(7%) patients had CC genotype. The frequency of G allele was 191(80%), while C allele was 49(20%) in patients. They recorded that *MIF* -173C allele is linked with higher transcription activity of *MIF* gene and increased production of Mif protein.

The results of [42] study registered that the CC genotype had significantly lower fibrosis stages (median fibrosis stage) compared to hepatitis C virus (HCV) with the GC or GG genotype. only 3% patients were identified to be homozygous for the “C” allele in the *MIF*-173 G/C SNP position of the *MIF* promoter as expected compared to other European populations. Approximately two thirds of patients (69%) were identified to be “GG.

The results this study agreed with of [6] study which showed the frequency of *MIF*-173 G/C genotype variants were 77(50.6%) Breast cancer (BC) patients were GG, 72(47.4%) patients were GC, 3 (2%) patients were CC.

These results agreed with study by [7] which clarified the relationship between Acute kidney injury (AKI) and *MIF* -173 G/C, where the results showed that 99 (12.9%) patients had GG genotype versus 71 (20.1%) among controls, 67 (20.4%) patients had GC genotype versus 103 (13.1%) among controls, while 4 (15.4%) patients had CC genotype versus 166 (15.2%) among controls.

These results were similar to [10] study results which found that 144(54.3%) of Psoriasis patients had GG genotype, 113(46.6) patients had GC genotype while 8(3%) patients had CC genotype, while disagreed with present results in the frequencies of these genotypes in controls and (98(38.9%), 15(5.9%), respectively.

These results were similar with [18] study which clarified the relationship between Heart failure (HF) and *MIF* -173 G/C polymorphism where the results showed GG, GC and CC genotype variants was 56.7%, 40% and 3.3% among HF patients, respectively, and 24%, 30%, and 6%, respectively in the control group. The

risk of HF of GG carriers is 4.25 times greater than that of CC carriers. The frequency of *MIF* G and C alleles were 96.7%, and 43.3%, respectively between HF patients and 54% and 36% among controls. The minor C allele frequency of *MIF* gene was significantly higher in HF subjects than controls.

## 4. CONCLUSIONS

The present study suggested that the -173G/C SNP in the *MIF* gene statistically association ($p<0.05$) with the risk of T1DM occurrence in Iraqi patients.

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