Psychoneuroendocrinology 104 (2019) 243–248

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

MIF 173 G > C variation was associated with depressive disorder in type 2 diabetes in an Iranian population

Armita Kakavand Hamidi[a], Seyed Masoud Arzaghi[c,*], Mostafa Qorbani[b], Fatemeh Khatami[g], Mehdi Ebrahimi[d], Fatemeh Bandarian[e], Samaneh Enayati[a], Mahsa M. Amoli[a,f,*]

[a] Metabolic Disorders Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[b] Department of Community Medicine, School of Medicine, Alborz University of Medical Sciences, Karaj, Iran
[c] Elderly Health Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[d] Internal Medicine Department, Sina Hospital, Tehran University of Medical Sciences, Tehran, Iran
[e] Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[f] Endocrinology and Metabolism Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[g] Chronic Diseases Research Center, Endocrinology and Metabolism Population Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Depression
Type 2 diabetes
Macrophage migration inhibitory factor
Single nucleotide polymorphism
Expression

ABSTRACT

Background: Type 2 diabetes mellitus (T2DM) is a continuous metabolic disease linked with increased rate of mortality and morbidity. High levels of glucose can damage organs including kidneys, eyes, and the nervous system. Individuals with T2DM have a high prevalence of major depression. One possible question we aimed to address was the extent of co-occurrence of diabetes and depression resulting from correlated genetic risk factors.
Objectives: The current study aimed to investigate the possible associations between the macrophage migration inhibitory factor (*MIF*) functional variant and the risk of developing depression in T2DM patients.
Patients and methods: The study groups consisted of 120 patients with T2DM and comorbid depression and 120 patients with T2DM, without depression, who were recruited from the same region. Genotyping of the *MIF* -173 G > C (rs755622) variant was performed using Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP). In addition, the level of *MIF* expression was comparatively evaluated in both groups by quantitative real-time PCR.
Result: The data showed that the presence of C allele (GC + CC *vs*. GG) might predispose females to depression in patients with T2DM. In addition, patients with T2DM carrying at least one C allele showed significantly elevated levels of *MIF* RNA expression in comparison to individuals with GG genotype.
Conclusion: MIF variant could be considered as a factor making female patients with T2DM vulnerable to depression. So, this might be an important result for precise diagnosis and/or earlier treatment.

1. Introduction

Depression and type 2 diabetes mellitus (T2DM) are two of the most widespread and devastating diseases worldwide (Stuart and Baune, 2012). There is a strong bidirectional connection between T2DM and depression. Cross-sectional and longitudinal investigations suggest that people with depression are 60% more likely to develop T2DM, while T2DM patients have 15% increased risk for depression (Kan et al., 2016). Inflammation is a key shared factor in the depression and T2DM bidirectional relationship. The role of inflammatory biomarkers such as C-reactive protein (CRP) has been validated in both diseases. Although genetic factors are known as significant factors in both T2DM and

Abbreviations: T2DM, type 2 diabetes mellitus; CRP, C-reactive protein; MIF, macrophage migration inhibitory factor; SNP, single nucleotide polymorphism; RA, rheumatoid arthritis; IBD, inflammatory bowel disease; HPA, hypothalamic-pituitary-adrenal; MDD, major depressive disorder; GENDEP, genome-based therapeutic drugs for depression; EMRI, Endocrine and Metabolism Research Institute; TUMS, Tehran University of Medical Sciences; EMRC, Endocrinology and Metabolism Research Center; HbA1C, hemoglobin A1C; NPDR, non-proliferative diabetic retinopathy; DD, depressive diabetes; NDD, non-depressive diabetes; CES-D, The Center for Epidemiologic Studies Depression Scale; EDTA, ethylene diamine tetra acetic acid; RFLP-PCR, restriction fragment length polymorphism-polymerase chain reaction; OR, odds ratio; CI, confidence interval; BMI, body mass index; BBB, blood–brain barrier; PLA2, phospholipase A2; JNK, Jun N-terminal kinases; IDO, indoleamine 2,3- dioxygenase; NMDA, *N*-methyl-d-aspartate; SSRIs, selective serotonin reuptake inhibitors; SNRIs, serotonin and norepinephrine reuptake inhibitors

* Corresponding authors at: EMRI, Dr. Shariati Hospital, North Karegar St., Tehran 14114, Iran.
E-mail addresses: dr.arzaghi@gmail.com (S.M. Arzaghi), amolimm@tums.ac.ir (M.M. Amoli).

https://doi.org/10.1016/j.psyneuen.2019.03.011
Received 5 August 2018; Received in revised form 12 March 2019; Accepted 14 March 2019

depression etiology, the extent to which their comorbidity involves correlated similar genetic factors is unknown (Kan et al., 2016).

Macrophage migration inhibitory factor (*MIF*), a T cell derived pro-inflammatory cytokine inhibiting the random migration of macrophages is considered as the primary molecule to appear at the inflammation site and the possible factor that determines the degree of cellular inflammation (Sánchez-Zamora and Rodriguez-Sosa, 2014). Position −173 single nucleotide polymorphism (SNP) (rs755622) is a functional polymorphism located in the promoter region of *MIF*. −173C allele is linked with higher transcription activity of *MIF* gene and increased production of Mif protein. This polymorphism apparently plays an important role in many inflammatory and autoimmune conditions such as rheumatoid arthritis (RA), inflammatory bowel disease (IBD) etc.

The functional prominence of *MIF* in immune-mediated inflammatory diseases is also extended to T2DM and depression (Bloom and Al-Abed, 2014). It has been shown that serum *MIF* level is elevated in T2DM patients. It is also suggested that high blood *MIF* level precede the T2DM onset. Apart from T2DM, patients with impaired glucose tolerance experience significantly higher levels of serum *MIF* (Herder et al., 2006). *MIF* has also indicated an association with insulin resistance through production of some pro-inflammatory cytokines and adipocytokines including resistin and IL-6 as the most significant factors in development of insulin resistance (Sánchez-Zamora and Rodriguez-Sosa, 2014). *MIF*, as a common molecule in the pancreatic cell microenvironment, can induce cell apoptosis following altered homeostasis resulting from inflammation.

MIF is also considered as a significant factor in depression physiology (Bloom and Al-Abed, 2014). Several in vitro and in vivo studies highlighted the critical role of *MIF* in both animal and human model of depression (Pignatiello et al., 1989; Kostowski et al., 1991). Previous findings on involvement of *MIF* in pathophysiology of depression include identifying *MIF* expression in parts of brain controlling behavioral symptoms of depression(Conboy et al., 2011), acknowledging the hypothalamic-pituitary-adrenal (HPA) axis importance in depression, which is in close connection with *MIF* (Edwards et al., 2010), interaction between *MIF* and both lifestyle and pharmacological antidepressant treatments (Cattaneo et al., 2013; Moon et al., 2012), recognizing a close correlation between *MIF* and neurogenesis as central pass of depression (Conboy et al., 2011). The most striking exploration was identification of *MIF* as a potential biomarker of depression (Bloom and Al-Abed, 2014). Hippocampus, whose dysfunction is known as one of the main reasons leading to depression, is known as site with high *MIF* expression (Bacher et al., 1998). Several recent studies indicated to the hippocampal complex (HC) as the key role players in the pathophysiology of major depressive disorder (MDD). (Sheline, 2011; Khan et al., 2015). It is suggested that *MIF* is linked to monoamine production and neurogenesis, which are both involved in the pathobiology of depression (Conboy et al., 2011; Moon et al., 2012). Finally, genetic deletion of *MIF* can result in increased anxiety- and depression-like behaviors (Conboy et al., 2011).

Serum proteomic profiling of patients with major depressive disorder (MDD) has clarified MIF as a strong analyte in patients compared to controls. Examination of serum *MIF* levels in human with depressive disorder also demonstrated higher *MIF* levels in subjects with mild to moderate depression (Edwards et al., 2010). This result has been replicated in pregnant women with depression when elevated serum *MIF* as well as significant association was found between *MIF* and depression (Christian et al., 2010). *MIF* expression in leukocytes from patients with severe depression is 40% greater than that in patients with moderate depression, with depressed patients having reduced glucocorticoid sensitivity during and after stress events (Edwards et al., 2010). It is likely to be related to the up regulation of *MIF* induced by glucocorticoids. *MIF* is also involved in mediating the pharmacological effects of antidepressants. *MIF* inhibition and *MIF* knockout can block neuronal proliferation induced by fluoxetine (Conboy et al., 2011) suggesting the *MIF* role in then eurogenesis induced by antidepressants. Increased *MIF* serum levels even during treatment was observed in studying *MIF* as a biomarker in patients with depression who were treated with celecoxib added to reboxetine (Musil et al., 2011). Cattaneo et al. found higher mRNA levels of *MIF* in the analysis of leukocyte of depressed patients in the Genome-based Therapeutic Drugs for Depression (GENDEP) project in addition to higher relative mRNA levels of *MIF* in non-responders to treatment *vs* patients who did (Cattaneo et al., 2013). The strong association between *MIF* levels and the treatment was repeated by Cattaneo highlighting the predictive power of higher *MIF* mRNA levels (absolute values of mRNA molecules) for non-responsivity to antidepressants.

Based on the above findings, we hypothesized that *MIF* is involved in the genetic pathophysiology of T2DM and depression comorbidity through the same inflammatory mechanism. Also, a genetic variation (single-nucleotide G/C substitution located at position –173) in *MIF* leading to elevated serum *MIF* levels and *MIF* promoter activity may contribute to depression and diabetes. In this study, we investigated whether the *MIF*173 G > C polymorphism genotype distribution and *MIF* expression level are different in both genders of Iranian subjects suffering from T2DM with or without depressive symptoms. This study is, to the best of our knowledge, the first to investigate this hypothesis.

2. Patients and methods

2.1. Patients

The ethics committee of Endocrinology and Metabolism Research Institute (EMRI), Tehran University of Medical Sciences (TUMS), approved the protocol of this study. All participants provided written informed consent of their willingness to be involved. All subjects were of Iranian descent ageing between 18 and 70 years. A total of 240 T2DM patients were recruited from Specialized Clinic for Diabetes and Metabolic Disorders of Endocrinology and Metabolism Research Center (EMRC), TUMS. All subjects were age matched. The following criteria were considered for subjects' selection: 1) poorly controlled Hemoglobin A1C (HbA1C) (HbA1C ≥ 9%); 2) confirmed T2DM: Subjects were diagnosed with T2DM if they had fasting blood sugar of ≥ 126 mg/dL on at least 2 occasions, HbA1C ≥ 6.5%, random blood sugar ≥ 200 mg/dL or glucose tolerance test > 180 (blood test 2 h once the person is given 75 g of glucose) andT2DM confirmed by a physician; 3) having T2DM for more than 1 year; 4) absence of long-term diabetes complications including severe retinopathy, severe nephropathy, and foot ulcer (severe retinopathy was considered as macular edema, proliferative retinopathy, or severe non-proliferative diabetic retinopathy (NPDR) with severe nephropathy considered as blood creatinine > 1.5 mg/dL in men and > 1.4 mg/dL in women); 5) no record of using antidepressants and thiazolidinediones; 6) no records of chronic diseases such as renal insufficiency, liver failure, and rheumatic diseases; 7) no record of smoking and alcohol consumption. Information about age, gender, marital status, level of education, ethnicity, family history of diabetes and depression, weight, and height was collected from each subject. All T2DM subjects were divided into two groups: depressive diabetes group (DD group, n = 120) and non-depressive diabetes group (NDD group, n = 120), depending on cut-off score above 16 and was confirmed by psychiatrist.

The Center for Epidemiologic Studies Depression Scale (CES-D), Iranian edition, was used to assess depressive symptoms (Amiri et al., 2008). CES-D is the most commonly used self-report depressive symptom scales first published by Radloff in 1977 to assess depressive symptoms in a community-based population (Radloff, 1977). The recruited subjects were requested to complete the questionnaire by themselves. Those who had CES-D sum score ≥16were considered as individuals meeting the threshold value of depressive symptoms.

2.2. Method

2.2.1. Sampling

All subjects recruited in this study had referred to the Specialized Clinic for Diabetes and Metabolic Disorders of Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences for regular follow up as T2DM cases. Following receiving written consent from both groups (DD & NDD) for participating in the study, each individual was referred to the blood sampling unit of the mentioned center. Initially, the informed trained laboratorian poured blood into two separate 3.5 ml ethylene diamine tetra acetic acid (EDTA) coated tubes. Then, they were labeled pre-printed with the patient's name and unique patient identification number and put on the rotor to stop possible coagulation until being transported to the genetics laboratory on dried ice to be stored at-20 °C. All blood samplings were done in the morning of weekdays to minimize the circadian variability of MIF levels.

2.2.2. MIF genotyping

Genomic DNA was extracted from peripheral blood of 120 subjects with T2DM and comorbid depression as well as120 patients with T2DM, using the phenol-chloroform method. For *MIF*173 C/G(*rs755622*) genotyping, Restriction Fragment Length Polymorphism-Polymerase Chain Reaction (RFLP-PCR)was used. PCR amplifications were performed in a final volume of 20 µL. Briefly, 6.5 µL of master mix (AMPLIQON Taq 2x master mix, Denmark), 0.8 µL (10 mol/mL) of each primer, 0.5 µL of template DNA, and 11.4 µL of distilled water were used. The *MIF*-173 forward PCR-RFLP primer was 5′-ACT-AAG-AAA-GAC − CCGAGG-C-3′, and the *MIF*-173 reverse PCR-RFLP primer was 5′-GGG-GCA-CGT-TGG-TGT-TTA-C-3′.For SNP rs755622, the cycling conditions were as follows: an initial denaturation step at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 62 °C for 30 s, and 72 °C for 40 s with a final extension at 72 °C for 5 min. Further, 366bp PCR product was visualized using agarose gel 2%electrophoresis and the quality was confirmed. For enzymatic digestion, 1 µL of buffer Tango with BSA 10x, 3 µL of PCR product, 0.5 µL of AluI enzyme (Thermo Scientific, made in Lithuania), and 5.5 µL of H2O were employed. The digested products were resolved on agarose gel 3%stained with cyber and visualized using UV transillumination. The genotype of each individual was determined as follows: *MIF*-173 CC homozygote (205 bp, 98 bp, and 63 bp); *MIF*-173 GC heterozygote (268 bp, 205 bp, 98 bp, and 63 bp); and MIF-173 GG homozygote (268 bp and 98 bp).

2.2.3. Real-time PCR

Lymphocytes extracted from 3 ml of peripheral blood were directly homogenized in 1 ml of TRIZOL reagent (Life Technologies). Total cellular RNA was extracted using chloroform method and quantified spectrophotometrically at 260 nm. Further, 250 ng of total RNA was reverse transcribed using Revert Aid first strand cDNA synthesis kit (Thermo Scientific, Lithuania) and thermal cycler (BioRad, PTC-1148, Singapore).The final volume was set at5 µl.The thermocycling parameters were as follows: 25 °C, 5 min.;45 °C, 60 min; and 70 °C, 5 min. cDNA was immediately used for PCR amplification. We evaluated the *MIF* gene expression in both DD and NDD groups via quantitative real-time PCR using Applied Biosystems Step One Real-Time PCR Systems (Thermo Fisher Scientific, USA).The sequences for applied oligonucleotide primers were as follows: forward, 5′AGCGCCTGCGCATCA3′; and reverse, 5′GGCTCTTAGGCGAAGGTGG3′(Sainsbury et al., 2008). The 20µl-volume reaction mixture contained 10 µl of SYBR Green PCR master mix (AMPLIQON), each primer with 0.5 µl, and 1 µl of cDNA. After activation for 10 min at 95 °C, we conducted 40 cycles, with each cycle lasting 5 s at 95 °C, followed by 40 s at 61 °C.

2.3. Statistical analysis

Fisher's exact test was applied to examine the significance of genetic association between *MIF* 173 G > C polymorphism and depression by comparing genotype and allele frequencies between DD and NDD groups (SPSS version 16.0). Odds ratio (OR) and 95% confidence interval (CI) were used for assessing the risk factors. Evaluation results of age, HbA1C, and body mass index (BMI) were expressed as mean ± SD. The means for variables (age, HbA1C, and BMI) were compared between the two groups by a two-tailed unpaired student's t-test. Hardy-Weinberg equilibrium was calculated for the expected genotype distributions. To compare the means of *MIF* expression level between case and controls, nonparametric Mann–Whitney test was employed (compare mean ranks ± Standard deviation). Significance level of 0.05 indicates a 5% risk of concluding that a difference exists when there is no actual difference. If the p-value is less than or equal to the significance level, the decision is to reject the null hypothesis

Table 1
Characteristics of NDD and DD groups.

Variables	NDD group (n = 120)	DD group (n = 120)	*p*-value
Age (year)	56.9 ± 8.3	55 ± 8.2	0.076
BMI (kg/m2)	27.6 ± 5	28 ± 4.25	0.499
HbA1C(%)	7.1 ± 0.9	7 ± 0.1	0.805

Data expressed as mean ± S.D.; *p*-value < 0.05 for statistical significance;
Abbreviations: NDD: non-depressive diabetes group; DD: depressive diabetes group;
BMIbody mass index;HbA1C: glycosylated hemoglobin A1C.

3. Results

In this study, we totally recruited 240 T2DM Iranian patients (gender ratio: M/F 95/145) in two groups: 120 DD and 120 NDD (gender ratio: M/F 33/87 *vs.* 62/58). There were no significant differences in terms of age, HbA1C, and BMI between the two groups (Table 1). To assess whether *MIF*-173 G > C polymorphism contributed to development of depression in T2DM patients, all subjects from both groups were genotyped. The genotype distributions of *MIF*-173 G > C are summarized in Table 2. There was no statistical difference in genotype distributions between DD and NDD groups ($p = 0.43$). There was no association between genotype distribution and family history of diabetes ($p = 0.75$) and that of depression ($p = 0.88$) under the dominant model (GC + CC vs. GG) either. There was one missing genetic data of a female. However, stratification analysis by gender supported the association with T2DM and depression in females when GC and CC genotypes were pooled against GG genotype ($p = 0.04$; OR = 1.25, 95% CI: 1.003–1.57). It seems that C allele (GC + CC *vs.* GG) to predisposeT3DM females to depression (Table 3). No significant association was observed after stratification by family history of diabetes (p-values = 0.4) and family history of depression ($p = 0.5$). The observed genotype frequencies in these subpopulations were in Hardy–Weinberg equilibrium (Table 4). As can be seen in Fig. 1, *MIF* gene expression showed a significant difference between various genotype carriers (CC + GC *vs.* GG) with *MIF* expression being considerably

Table 2
Genotype distributions of MIF-173 G > C.

Group	N	Genotype N (%)			Allele N (%)	
		G/G	**G/C**	**C/C**	**G**	**C**
NDD	120	79 (66)	33(27)	8(7)	191(80)	49(20)
DD	119	70(59)	42(35)	7(6)	182(76)	56(24)

*We did not have the genotype of one female from DD group; There was no significant differences between NDD and DD genotype (p = 0.43) and allele distribution, p = 0.4, OR: 1.19; 95%CI: 0.7–1.89.Abbreviations: NDD: non-depressive diabetes group; DD: depressive diabetes group.

Table 3
Genotype distribution under dominant model after gender stratification in case and controls.

Sex			Genotype		Allele	
			GG N(%)	GC + CC N(%)	G	C
Male	Type	NDD(62)	35(56)	27 (44)	70(56)	54(44)
	Total	DD (33)	18 (55)	15 (45)	36(55)	30(45)
		95	53(56)	42(44)	106(56)	84(44)
Female	Type	NDD (58)	44 (76)	14 (24)*	88(76)	28(24)
	Total	DD(86)	52 (60)	34 (40)	104(60)	68(40)
		144	96(67)	48(33)	192(67)	96(33)

* p = 0.04; OR = 1.25, 95% Confidence Interval (CI): 1.003–1.57; Abbreviations: NDD: non-depressive diabetes group; DD: depressive diabetes group.

Table 4
Hardy-Weinberg equilibrium of MIF173 G > C.

Group	N	A(E)			$\chi 2$	P
		G/G	G/C	C/C		
DD	119*	59(58)	35(36)	6(6)	0.02	P > 0.05
NDD	120	66(64)	27(32)	7(4)	1.2	P > 0.05

A is the actual number of genotype; E is the expected number of genotype; * We did not have the genotype of one female from DD group. Abbreviations: DD:depressive diabetes; NDD: non-depressive diabetes.

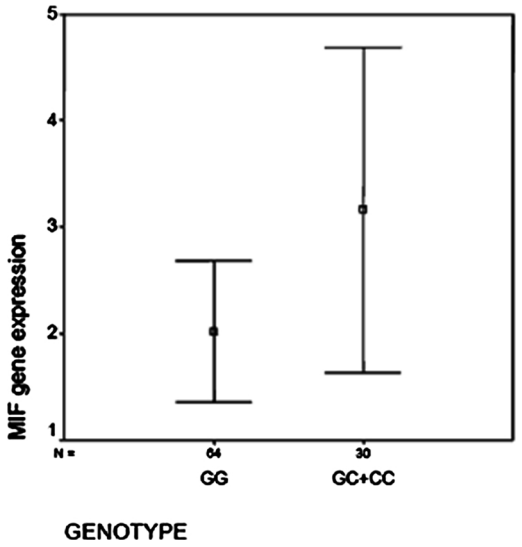

Fig. 1. MIF gene expression difference under dominant model (GG vs. GC + GC); MIF expression is considerably higher in subjects with at least one C allele (p-value = 0.009).

higher in subjects with at least one C allele ($p = 0.009$). After stratification by DD and NDD groups, the association between *MIF* expression and genotype was only significant in the DD group ($p = 0.034$), but the difference for *MIF* expression according to genotype was not significantly different in NDD group ($p = 0.093$). According to Fig. 2, there was no significant difference in terms of MIF expression between male and female groups ($p = 0.048$). The outcome increased odds of depression with exposure to C genotype and females with C genotype are more likely to be depressed. Some risk factors including extreme fat or sugar free diet, overestimation of depression or misdiagnosis of depressed patients as the no depression are taken into the account.

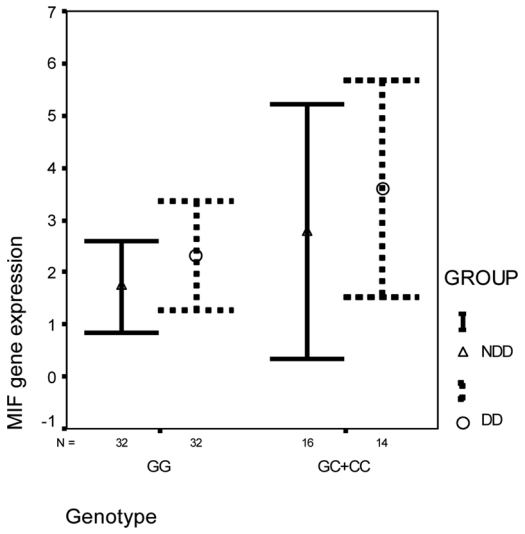

Fig. 2. MIF gene expression association with genotype divided by NDD and DD groups; DD group (GG vs GC + CC), p = 0.034. NDD group (GG vs GC + CC), p = 0.093.
Abbreviations: DD: depressive diabetes; NDD: non-depressive diabetes.

4. Discussion

In the present study, we investigated the association between *MIF*-173 G > C polymorphism and depression in T2DM Iranian subjects. Our results revealed a positive association between *MIF*-173 G > C polymorphism and comorbid depression among female T2DM patients. Comorbid depression in T2DM patients is connected to the increased numbers and severity of diabetic symptoms and complications. In other words, T2DM female patients who carry allele C are more susceptible to show depression symptoms. In addition, all subjects who carried allele C indicated a significantly higher *MIF* expression in comparison to subjects with G allele.

4.1. MIF, gender difference, and depression-diabetes comorbidity

Previous population-based studies have suggested that prevalence of depression is higher in female patients with diabetes compared to their male counterparts. Sex-limitation genetic modeling also showed a significant genetic link between T2DM and depression, only in Swedish women, where genetic factors accounted for 75% of female's phenotypic correlation between T2DM and depression. This suggests that T2DM and depression comorbidity is due to correlated genetic risk factors but not necessarily the same across males and females (Kan et al., 2016). It was indicated that development of depression in patients with T2DM could be gender dependent and the prevalence of depression is 72.5% in women versus 27.5% in men. Some possible genetic bases of higher prevalence of depression among female patients with T2DM can be attributed to the fact that interactions between the *MIF* 173 G > C polymorphism and environments may differ between men and women. The gender-related influence of the *MIF* 173 G > C polymorphism in depression may be because of sexual dimorphism in brain structures involved in the neurobiology of depression, mainly the hippocampus. Male and female hippocampi have been known to be dissimilar significantly in their anatomical structure, their neurochemical make-up, and their reactivity to stressful situations (Cahill, 2006).

4.2. MIF and T2DM

Studies exploring the relationship between the *MIF* 173-G/C polymorphism and depression/T2DM are sparse. The results of *MIF* studies

indicated that *MIF*−173C allele carriage predicts new onset T2DM in men but not in women. In contrast, MONICA/KORA found increased risk of T2DM in female subjects with *MIF* genotype rs1007888CCstudy. Future analysis for incidence of T2DM also revealed that high *MIF* levels were independently predictive in women (with a twofold hazard ratio), concluding that it was a cause (and not a consequence) of the development of T2DM (Herder et al., 2008). Further, lower *MIF* tertiles were inversely associated with likelihood of T2DM in women from Turkish population (Onat A et al., 2017).These clarify the causal role of *MIF* in the etiology of T2DM.Insulin resistance and development of T2DM are postulated to be led by inflammation-induced inhibition of the insulin signaling pathway (Velloso et al., 2013). *MIF* indirectly promotes the production of pro inflammatory cytokines and adipo-cytokines which are involved in insulin receptor signaling, and in turn insulin resistance (Sánchez-Zamora and Rodriguez-Sosa, 2014). Furthermore, T2DM can be resulted from insulin resistance induced by high levels of inflammatory cytokines interacting with pancreatic β-cells' normal function (Bădescu et al., 2016). However, gender susceptibility to autoimmune activation diverges across different populations.

4.3. MIF and depression

There is not sufficient evidence to prove that *MIF* genotype play a role in development of depression and in depression progress in patients with T2DM. One idea is that immune genetic variants that increase the risk of depression are also likely to intensify the risk of diabetes, and perhaps contribute to the overlaps between these two conditions (Barnes et al., 2017). Depression susceptibility of *MIF* -173C allele in female patients with T2DM, and association of this allele with higher *MIF* expression suggest that *MIF* genetic variation may modify its role in the homeostasis of host immune response and its function as a neuro-immune mediator linking depressive symptoms with inflammation. However, the biological mechanism behind this need to be examined in future.

The main focus of research in the relationship between inflammation and depression has been on cytokines as central intermediary of inflammatory pathways. TNF-α, INF-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, Il-10, and IL-12 are some of these cytokines suggested to be involved in the pathogenesis or pathophysiology of depression in different ways. Increased level of these cytokines should cross blood–brain barrier (BBB) to reach the brain, interacting with a number of mechanisms, which are linked to depression independently and can induce depression (Stuart and Baune, 2012). Thus, *MIF* as a pro inflammatory cytokine which stimulates release of other pro inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8, and IL-12 from macrophages (Kasama et al., 2010) relate to depression indirectly. The possible mechanism is that *MIF* causes up regulation of phospholipase A2 (PLA2), possibly downstream of ERK1/2 signaling pathways. PLA2 also stimulates release of proinflammatory cytokines via Jun N-terminal kinases (JNK). In addition, *MIF* promotes survival of pro-inflammatory cells by inhibiting the tumor suppressor p53 (Bloom and Al-Abed, 2014). Proinflammatory cytokines activate the extra hepatic enzyme indoleamine 2,3- dioxygenase (IDO), which degrades tryptophan, a precursor to serotonin. Tryptophan is channeled increasingly toward production of kynurenine via IDO degradation, competing with the serotonin pathway. Within the microglia, which are preferentially activated over as trocytes during inflammatory states, kynurenineis metabolized into quinolinic acid, which is an agonist of glutamatergic N-methyl-D-aspartate (NMDA) receptors. Therefore, there is a serotonergic deficiency and glutamatergic overdrive in proinflammatory states paving the way for a potential depressive syndrome. In addition, pathophysiological domains involving neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and behavior which typify depression interrelate with pro-inflammatory cytokines (Bădescu et al., 2016).

4.4. Translation and clinical application

The early diagnosis of T2DM and depression is important as the treatment strategy might have adverse effect and may raise the complications. If depression awareness grows in patients with diabetes, the outcomes will be far more satisfactory. A simple method for screening depression is the primary step to achieve this goal. It is proposed that diabetic patients with a better mood follow their diabetes treatment more efficiently (Bădescu et al., 2016; Petrak et al., 2015). This is more significant in middle aged and elderly population who experience high prevalence of autoimmune processes (Onat et al., 2016).Therefore, studies like the present study, which provide a genetic understanding about vulnerability of T2DM patients to depression, would be beneficial especially for public health and prevention purposes. Further, identification of a biomarker that predicts depression in T2DM patients is crucial in reducing the social and economic burden of depression and improving quality of life of patients. If *MIF* is well established to promote depression, then *MIF* inhibitors (Dziedzic et al., 2015) could be used as an antidepressant as with its counterpart for controlling the systemic inflammation and some symptoms of diabetes(Sanchez-Zamora Y1 et al., 2010). One possible clinical implication of investigating *MIF* genotype and level would be identifying candidate patients for receiving anti-inflammatory agents as an adjunctive treatment in attenuating depressive symptoms. The reasons is that those with high inflammatory cytokine levels seem to be less responsive to selective serotonin reuptake inhibitors (SSRIs)and serotonin and norepinephrine reuptake inhibitors (SNRIs) (Audet and Anisman, 2013).

4.5. Limitations and future research

Limitations of the present study include rather small sample size and lack of healthy normal population to study the *MIF* polymorphism and compare its distributions. Unmatched gender population, which might be the reason for significant association between depression and *MIF* genotype only in female and lack of further interventions on our patients have been among other limitations. Another constraint was not replicating the results in independent materials. Correlation of functionality of polymorphism with sex-specific changes could be clarified by *MIF* serum concentration, which is not performed either. This study is cross-sectional and individuals already exhibit depression symptoms so unfortunately new symptoms and there is a bi-directional relationship between diabetes and depression. In the current study design we were not able to determine which of these disorders developed first. A comprehensive study will be necessary to confirm our results and validate the therapeutic response in T2DM patients with depression who carry C allele. It is also noteworthy that immune function genes are greatly variable, thus, other genes might also be implicated in diverse population groups (Wong et al., 2008).

5. Conclusion

In conclusion, our study suggested that the presence of the functional *MIF*-173 G > C polymorphism C allele was associated with susceptibility to depression in female T2DM patients, with C carriers harboring higher levels of *MIF* gene expression in patients with T2DM. Although further research is definitely warranted, undoubtedly *MIF* has a great potential in studying the genetic basis of depression and T2DM comorbidity and possible sex differences involved. This would contribute to emerging more suitable and effective interventions such as immune-targeted pharmacotherapy and improvement in the outcome of both of these common conditions.

Conflict of interest

None to declare.

CRediT authorship contribution statement

Armita Kakavand Hamidi: Conceptualization, Data curation, Investigation, Methodology, Writing - original draft. **Seyed Masoud Arzaghi:** Data curation, Investigation, Methodology. **Mostafa Qorbani:** Software, Formal analysis. **Mehdi Ebrahimi:** Data curation. **Fatemeh Bandarian:** Data curation. **Mahsa M. Amoli:** Funding acquisition, Resources, Supervision, Validation, Investigation, Methodology, Writing - review & editing.

Acknowledgment

The work has been funded by Diabetes Research Center, Endocrinology and Metabolism Clinical Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran, Grant No. 1393-03-106-1845.

References

Amiri, M., Mohammadi, A., Forghani, A., 2008. The Center for Epidemiologic Studies Depression Scale (CES-D) validation in order to depression screening among students. Proceedings of the 4th Students' Mental Health Seminar 43–45. http://www.ensani.ir/storage/Files/20101228162124.

Bacher, M., Meinhardt, A., Lan, H.Y., Dhabhar, F.S., Mu, W., Metz, C.N., Chesney, J.A., Gemsa, D., Donnelly, T., Atkins, R.C., Bucala, R., 1998. MIF expression in the rat brain: implications for neuronal function. Mol. Med. 4 (4) p.217.

Bădescu, S.V., Tătaru, C., Kobylinska, L., Georgescu, E.L., Zahiu, D.M., Zăgrean, A.M., Zăgrean, L., 2016. The association between diabetes mellitus and depression. J. Med. Life 9, 120–125.

Bloom, J., Al-Abed, Y., 2014. MIF: mood improving/inhibiting factor? J. Neuroinflamm. 11, 11.

Cattaneo, A., Gennarelli, M., Uher, R., Breen, G., Farmer, A., Aitchison, K.J., Craig, I.W., Anacker, C., Zunsztain, P.A., McGuffin, P., Pariante, C.M., 2013. Candidate genes expression profile associated with antidepressants response in the GENDEP study: differentiating between baseline 'Predictors' and longitudinal 'Targets'. Neuropsychopharmacology 38, 377–385.

Christian, L.M., Franco, A., Iams, J.D., Sheridan, J., Glaser, R., 2010. Depressive symptoms predict exaggerated inflammatory responses to an in vivo immune challenge among pregnant women. Brain Behav. Immun. 24, 49–53.

Conboy, L., Varea, E., Castro, J.E., Sakouhi-Ouertatani, H., Calandra, T., Lashuel, H.A., Sandi, C., 2011. Macrophage migration inhibitory factor is critically involved in basal and fluoxetine-stimulated adult hippocampal cell proliferation and in anxiety, depression, and memory-related behaviors. Mol. Psychiatry 16, 533–547.

Edwards, K.M., Bosch, J.A., Engeland, C.G., Cacioppo, J.T., Marucha, P.T., 2010. Elevated Macrophage Migration Inhibitory Factor (MIF) is associated with depressive symptoms, blunted cortisol reactivity to acute stress, and lowered morning cortisol. Brain Behav. Immun. 24, 1202–1208.

Kan, C., Pedersen, N.L., Christensen, K., Bornstein, S.R., Licinio, J., MacCabe, J.H., Ismail, K., Rijsdijk, F., 2016. Genetic overlap between type 2 diabetes and depression in Swedish and Danish twin registries. Mol. Psychiatry 21, 903–909.

Khan, S.A., Ryali, V.S.S.R., Bhat, P.S., Prakash, J., Srivastava, K., Khanam, S., 2015. The hippocampus and executive functions in depression. Ind. Psychiatry J. 24 (1) p.18.

Kostowski, W., Danysz, W., Dyr, W., Jankowska, E., Krazaścik, P., Paklejko, W., Stefański, R., Pklaźnik, A., 1991. MIF-1 potentiates the action of tricyclic antidepressants in an animal model of depression. Peptides 12 (5), 915–918.

Moon, H.Y., Kim, S.H., Yang, Y.R., Song, P., Yu, H.S., Park, H.G., Hwang, O., Lee-Kwon, W., Seo, J.K., Hwang, D., Choi, J.H., Bucala, R., Ryu, S.H., Kim, Y.S., Suh, P.-G., 2012. Macrophage migration inhibitory factor mediates the antidepressant actions of voluntary exercise. Proc. Natl. Acad. Sci. U. S. A. 109, 13094–13099.

Musil, R., Schwarz, M., Riedel, M., Dehning, S., Cerovecki, A., Spellmann, I., Arolt, V., Müller, N., 2011. Elevated macrophage migration inhibitory factor and decreased transforming growth factor-beta levels in major depression—no influence of celecoxib treatment. J. Affect. Disord. 134, 217–225.

Petrak, F., Baumeister, H., Skinner, T.C., Brown, A., Holt, R.I.G., 2015. Depression and diabetes: treatment and health-care delivery. Lancet Diabetes Endocrinol. 3, 472–485.

Pignatiello, Michael F., Gayle, A., Olson, Abba J., Kastin, Rudolph, Ehrensing, H., McLean, Jack H., Olson, Richard D., 1989. MIF-1 is active in a chronic stress animal model of depression. Pharmacol. Biochem. Behav. 32 (3), 737–742.

Radloff, L.S., 1977. The CES-D scale:a self-report depression scale for research in the general population. Appl. Psychol. Meas. 1, 385–401.

Sainsbury, A., Goodlad, R.A., Perry, S.L., Pollard, S.G., Robins, G.G., Hull, M.A., 2008. Increased colorectal epithelial cell proliferation and crypt fission associated with obesity and roux-en-Y gastric bypass. Cancer Epidemiol. Biomarkers Prev. 17, 1401–1410.

Sánchez-Zamora, Y.I., Rodriguez-Sosa, M., 2014. The role of MIF in type 1 and type 2 diabetes mellitus. J. Diabetes Res. 2014.

Sheline, Y.I., 2011. Depression and the hippocampus: cause or effect? Biol. Psychiatry 70 (4), 308–309.

Stuart, M.J., Baune, B.T., 2012. Depression and type 2 diabetes: inflammatory mechanisms of a psychoneuroendocrine co-morbidity. Neurosci. Biobehav. Rev. 36, 658–676.

Wong, M.L., Dong, C., Maestre-Mesa, J., Licinio, J., 2008. Polymorphisms in inflammation-related genes are associated with susceptibility to major depression and antidepressant response. Mol. Psychiatry 13, 800–812.