Association of MTHFR gene polymorphism (rs1801133) with Type 2 Diabetic Iraqi population

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Abstract

Background: Type 2 Diabetes Mellitus is a complex of endocrine metabolic disorder. It is believed that polymorphism of Methylenetetrahydrofolate reductase (MTHFR) C677T related to type 2 diabetes mellitus. However, results are conflicted from different ethnic and races. This study aimed to evaluate the relationship between MTHFR (rs1801133) gene polymorphism and type 2 diabetes mellitus.

Methods: This case-control study was included 100 patients were diagnosed with type 2 diabetes mellitus (T2DM) cases and 100 healthy control individuals. The blood sample was collected for estimation of biochemical parameters for analysis among the T2DM cases and healthy control groups. DNA extraction from whole blood was done to study the MTHFR gene polymorphism by RFLP-PCR method.

Results: There were significant difference in genotype distribution among Type2 diabetic patients and control group. Compared with CC wild genotype, CT heterozygous genotype (OR= 5.7, 95% Cl= 3.0-11.0 and P=0.0001) and TT homozygous genotype (OR=4.3, CI=1.5-11.9 and P=0.0005). The T allele frequency increased the risk in diabetic patients by three folds when compared with C allele (OR= 3.6, C. I=2.2-5.5, P= 0.0001), suggested that the effect of MTHFR point mutation on type 2 diabetes mellitus implicated with increased risk of disease.

Conclusion; Our results indicate that polymorphism in MTHFR C677T plays significant role in type II diabetes mellitus risk for Iraqi populations

Key words: MTHFR, gene, C677T, polymorphism, T2DM.

Introduction:

Type 2 diabetes mellitus (T2DM), is a polygenic and multifactorial disease that is considered a major life-threatening health problem throughout the world (1)**.** Diabetes is a type of glucose metabolic disorder which is one of the major health related global problems (2)**.** According to WHO, approximately 422 million people affected with diabetic conditions globally. In Iraq more than 13.9% of adults live with diabetes (3,4). The alteration in genetic material such as changes in nucleotide sequences may lead to alteration in protein ultimately which directly affect the signaling process could be the determining factor for complication of diabetic (4) . Evidences suggest that gene polymorphisms involved in folate metabolism play a critical role in the etiology of diabetes and diabetic complications (5)**.** Folate metabolism related disorders can be caused by genetic or environmental factors that include an individual's genetic variability and diet (6). Many of the genes involved in folate metabolism are polymorphic**.**

Methylenetetrahydrofolate reductase (MTHFR) is one of the important enzymes in the first step of folate metabolism and converts dietary folate to 5- methyltetrahydrofolate, the methyl group donor required for the remethylation of homocysteine (Hcy) to methionine (7, 8**).** Methionine is the substrate for S-adenosyl methionine (SAM), a major cofactor and a methyl group donor for numerous methylation reactions (9)**.** MTHFR regulates the metabolism of folate and it is an important factor in DNA methylation and synthesis (10). Low MTHFR activity reduces DNA methylation but may enhance de novo thymidylate biosynthesis **(**11,12)**.**

Patients and methods:

The present case-control study was done in Diabetic Centre of Al- Sadder medical city hospital in Najaf/ Iraq. The study population was composed of one hundred patients with type2 diabetes mellitus (50 males and 50 females) their age range (30-70 years), were chosen compared with age and sex matched 100 healthy control individuals (50 males and 50 females), between the period of 2023 to 2024. In this study the lipid profile parameters were evaluated for each participant. The medical examinations for patients were carried out by experienced physician, they were identified with T2DM based on WHO classification and diagnostic criteria (4).

Anthropometric and clinical parameters, which include age; gender; BMI, blood sugar, glycosylated hemoglobin (HbAIc), homocysteine, blood urea and serum creatinine were

determined by standard enzymatic technique and colorimetric method applied to evaluation of blood urea and serum creatinine by using RANDOX kits (United Kingdom BT 29 4QY) with standard procedures. Total plasma homocysteine was analyzed according to the manufacturer procedure for determination by using microplate enzyme immunoassay ELISA kit method of Biorad laboratories.

From all participants a total of 5 milliliter venous blood samples were collected after obtained informed consent. One ml of blood transferred to EDTA tube for DNA extraction, another 4 ml centrifugated in 2000xg for serum separation, and serum were stored at -17°C until the assayed to be performed. DNA extracted by method that has been published previously (13). The Single nucleotide polymorphism was done by polymerase chain reaction (PCR) using primers mentioned by Alkhafaji S.M. (14). The primers that used for PCR–RFLP were 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ forward and 5′-AGG ACG GTG CGG TGA GAG TG-3′ reverse that resulting of 198bp PCR product. The polymorphism was detected by enzymatic digestion of the initial polymerase chain reaction product with HinfI (Promega.USA) at 37˚C for 4 hrs. The resulting of DNA fragments was separated on 3% agarose gel stained with ethidium bromide, and photographed under UV light. Accordingly, Samples who lack the mutation appeared one 198bp fragment, sample with heterozygous for the mutation revealed both 198bp and 175bp fragments, and homozygous sample revealed one 175bp fragment.

The resulting data were collected and analyzed on SPSS software package (revision 20 Inc., Chicago, USA), the appropriate tests such as Chi-square, t-tests and ANOVA were used. Also diabetic risk was tested and estimated by the use of odds ratios (ORs) and 95% confidence intervals (95% CI) . Values of $P < 0.05$ were considered statistically significant.

Results:

Table (1): General and clinical characteristics of diabetic and non diabetic groups

Variables	*DM Patients No. =100 Mean ±SD*	*NDM Patients No. =100 Mean ±SD*	*P – value*
Gender:Male/Female	50/50	50 /50	
Age at study (years)	47±4	46 ±3.4	**0.058**
Blood glucose(mg/dl)	183± 12	92 ± 8	**0.0001**
HbA1C (%)	9 ± 1.9	5.5 ± 0.7	**0.0001**
BMI (kg/m^2)	29 ± 5.0	21 ± 2.7	**0.0001**
Blood Urea(mg/dl)	37±1.0	34 ± 1.6	**0.073**
Creatinine(mg/dl)	0.8± 0.3	0.5± 0.2	**0.085**
Homocysteine (µM)	**19.7±2.1**	**6.0±0.9**	**<0.0001**

P<0.05 statistically significant; BMI: body mass index; HbA1c: glycated hemoglobin.

Table 2: Genotype frequency of MTHFR (C→ A) gene polymorphism in diabetic patients and control groups.

Genotype and allele frequency	*DM No.=100*	*NDM No.=100*	*OR*	*(95% C.I)*	*P- Value*
CC	30 (30%)	70(70%)	1	Ref.	-
CT	57 (57%)	23 (23%)	5.7	3.0-11.0	0.0001
TT	13 (13%)	7 (7%)	4.3	1.5-11.9	0.0005
C	117(38.6%)	186 (61.4%)	1	Ref.	-
T	83(69.2%)	37 (30.8%)	3.6	2.2-5.5	0.0001

No: number; OR: odds ratio, 95%C.I, confidence interval, P<0.05 statistically significant

Results.

Characteristic feature of patients and control groups.

The general and clinical features of type 2 diabetic patients with normal control groups are showed in Table1. The studied groups were matched for gender and age. Blood urea, serum creatinine showed no significant differences, while blood sugar, HbA1c, body mass index (BMI), and the levels homocysteine were considerably increased in diabetics patients when compared with healthy control group as revealed in table (1). The alleles frequency of MTHFR gene is studied using PCR-RFLP technique. The distributions of genotype and allele frequencies were compared between type2 diabetic patients with normal control group (Table 2). The allele frequency and genotype of SNP of the MTHFR gene in DM patients 30% for CC, 57% for CT and 13.0% for TT respectively, whereas in control group individuals 70%, 23% and 7% respectively. The allele frequency obtained in the DM patients for C was (38.6%) and for T was (69.2%), whereas in the control group for C was (61.4%) and for T was (30.8%). The genotypes frequency of C677T in TT variant 13 (13.0%) which was significantly increased the risk of diabetic's patients by four folds in homozygous genotype of DM patients when compared with wild genotype (OR 4.03, 95% CI= 1.5-11.9, p=0.0005). The T allele frequency increased the risk in diabetic patients by four folds when compared with C allele (OR= 3.6, C.I=2.2-5.5, P= 0.0001)

Discussion

Diabetes mellitus type II is a complex metabolic and endocrine interaction between multiple genetic and environmental factors cause a progressive, various disorder and dysfunction of pancreatic beta cells and is associated with changes in biochemical, physiological and pathological liver diseases (15). The biochemical characteristics of diabetic patients and control groups are revealed that T2DM patients showed significantly higher HbA1C, blood sugar and homosystiene, same finding was also observed in a study by Sherwani SI et al which were presented the higher HbA1C in T2DM patients (16-18). In our study we tried to evaluated a possible role of the point mutation C677T on gene coding for MTHFR enzyme as a risk factor that increasing in diabetic patients so we summarized possible association of mutation polymorphism of MTHFR (677 C>T) gene in diabetic patients in contrast to healthy individuals in Iraqi population.

According to the relation between T2DM and C677T point mutation on gene coding for MTHFR enzyme, our results showed that homozygous mutated TT genotypes of C677T and T allele was higher in study group of DM patients compared to Control group NDM. Indeed, homozygous mutation for the C677T in MTHFR gene, causing decrease production of 5-methyltetrahydrofolate, the main source of methyl donor in alteration of homocysteine to methionine which lead to rise of homocysteine in plasma Di et al.[19]. A 677 C→ T mutation is responsible for reduced MTHFR activity, and it is found significantly effective only in recessive homozygous state Paul and Sreyoshi (20).

Furthermore, the association between recessive homozygous 677C→T in MTHFR gene and DM, and the presence of higher 677C→T mutations in MTHFR gene among patients with DM compared to normal individuals, the TT genotype and T allele frequencies were increased and they were significantly increased in diabetic patients than in those without DM since our normal individuals had a higher frequency of the C allele than those diabetic patients. Several investigations supported our findings, including one by Abd Raboh et al., who used RFLP techniques to investigate the effects of A1298C and C677T polymorphisms in Egyptian patients with type II diabetes mellitus. According to their findings, polymorphisms in the MTHFR gene increase the incidence of type II diabetes (OR: 2.2, 95% CI = 0.7-6.9, P = 0.004) Abd Raboh et al. (21). The MTHFR C677T polymorphism also suggests that the T allele confers a considerable genetic risk in subacute combined degeneration illness, according to Zhang X et al. in 2019 (22), the MTHFR C677T mutation was proposed as a reliable biomarker for type 2 diabetes in the Chinese population. MTHFR C677T mutation in Chinese population was suggested to the predictable biomarker among T2DM as found by Sun et al. (23). MTHFR polymorphism C677T, CC genotype suggested to have protective role in T2DM while TT genotype increases the risk of diabetics (24).

We concluded from this study that the polymorphism in MTHFR C677T plays significant role in type II diabetes risk and MTHFR C677T gene polymorphism may confers toT2DM, especially in Iraqi populations.

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