Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://dx.doi.org/10.21608/zumj.2020.10042.1072 Volume 28, Issue 6, November 2022(272-279) Supplement Issue

Manuscript ID ZUMJ-1902-1072 (R5)
DOI **10.21608/zumj.2020.10042.1072**

ORIGINAL ARTICLE

Assessment of Methylenetetrahydrofolate Reductase Gene Polymorphism in Diabetic Peripheral Neuropathy.

Azza Hassan Abd El-Fatah [(1)], Amal Fawzy [(2)] and Abdullah Ali Abdulhamid[(3)]

(1) Lecturer of Internal Medicine, Faculty of Medicine, Zagazig University
(2) Lecturer of Medical Biochemistry, Faculty of Medicine, Zagazig University
(3) M.B.B.Ch, Internal Medicine Department, Faculty of Medicine, Omar Almokhtar University, Libya

Corresponding author
Abdullah Ali Abdulhamid
M.B.B.Ch., Faculty of Medicine,
Omar almokhtar university,
Libya
E.mail :
abdullahaltayil49@gmail.com

Submit Date 2019-11-24
Revise Date 2020-05-16
Accept Date 2020-05-21

ABSTRACT

Background: Among patients with diabetic polyneuropathy, the status of folic acid, homocysteine, and nerve conduction studies (NCS) variations has been associated with methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms. The current study was aimed to assess the effects of methylenetetrahydrofolate reductase (MTHFR) gene polymorphism in development of Diabetic Peripheral Neuropathy.

Subjects and Methods: This case control study was carried out in outpatient clinic of endocrinology and Medical Biochemistry department, Zagazig University Hospital during a period from February to December 2018. The study included (129) participants were classified into three groups: Group (1): Included 43 apparently healthy peoples who served as a control group. Group (2): Included 43 patients diagnosed with type 2 DM according ADA criteria for diabetes. Group (3): Included 43 diabetic patients with diabetic peripheral neuropathy according to the standard Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) criteria for diabetic neuropathy diagnosis.

Results: the frequency of C677T MTHFR gene polymorphism was significantly higher in the patients with DPN compared with the patients without neuropathy.

Conclusion: MTHFR gene polymorphism was associated with increased risk for DPN development.

Keywords; Methylenetetrahydrofolate Reductase, Gene Polymorphism, Diabetic Peripheral Neuropathy.

INTRODUCTION

Diabetic peripheral neuropathy (DPN) considered a symmetrical length-dependent sensorimotor polyneuropathy related to metabolic and microvessel changes which innervate all systems and organs which affect pain fibers, motor neurons and autonomic nervous system [1]. DPN is the most form of diabetic neuropathy and affects about 50 % of type 2 diabetic mellitus (T2DM) cases and causes health and economic losses for individuals and society [2].

MTHFR, is an enzyme which catalyzes the conversion from homocysteine to methionine via re-methylation. It was proposed that the reduction in the MTHFR encoding enzyme activity could lead to hyperhomocysteinemia. MTHFR gene located in long arm of chromosome 1 p36.3, and the most MTHFR variance was the transformation point of C to T at nucleotide 677 [3].The C677T substitution of the MTHFR gene, via an amino acid conversion from alanine to valine, which increase the enzyme thermo-ability and reduce activity of the enzyme. Plus to this, the mutation was the common genetic reason for the increase in homocysteine levels, which may be a contributor factor for DPN [4].

Genetic mutation in MTHFR was the common inherited risks factor for increasing the levels of homocysteine [5].

Many studies showed that the hyperhomocysteinemia was related with the high risk of diabetic neuropathy in diabetic cases independently[6]

The current study was aimed to assess the effects of methylenetetrahydrofolate reductase (MTHFR) gene polymorphism in development of Diabetic Peripheral Neuropathy

PATIENTS AND METHODS

This case control study was conducted on 129 participants in Outpatient Clinic of Endocrinology and Medical Biochemistry Department, Zagazig University Hospital, during the period from February to December 2018.

Written informed consent was obtained from all patients and the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving human.

All participants were categorized into 3 groups:

Group (1): Included 43 apparently healthy peoples who served as a control group.

Group (2): Included 43 patients diagnosed with type 2 DM according ADA criteria for diabetes.

Group (3): Included 43 diabetic patients with diabetic peripheral neuropathy according to the standard Neuropathy Symptom Score (NSS) and Neuropathy Disability Score (NDS) criteria for diabetic neuropathy diagnosis.

Inclusion criteria:

Both sex (male & female) included. Age more than 18 yrs. Type 2 diabetic patients.

Exclusion criteria:

Patients < 18 years. Patients have other causes of neuropathy.Patient refuses to give consent and lack of cooperation.

All participants were submitted to the following:

Full personal history (including age, sex, smoking, hypertension and Medications, General clinical examination, Anthropometric measurements**:** Weight (kilograms), height (meters) and Body Mass Index (BMI). BMI = weight (kg) / height (m^2).Circumferences of waist and hip were measured by a plastic tape meter at the greater trochanters and level of the umbilicus, so waist-to-hip ratio (WHR) was obtained.

Laboratory investigations:

Complete blood count. Fasting blood glucose. Glycosylated haemoglobin (HbA1c). Lipid profile. Serum creatinine. Determination of Methylenetetrahydrofolate reductase MTHFR (C667T) gene polymorphism by Restriction fragment length polymerase chain reaction (RFLP_PCR).

- Role of sensory conduction study : vibration sense by Tunning fork

DNA extraction and storage:

All the reagents were highly purified analytical PCR-materials. All the tubes, tips pipettes used for DNA extraction were DNAse, RNAse free tubes to avoid contamination purchased from Gentra (Minnapolis. USA). The Genomic DNA extracted from blood using the commercially available G-spin TM Total DNA Extraction Kit (iNtron bio-technology, Seongnam-Si, Korea).

The protocol used for DNA extraction from whole blood

200 µl EDTA blood was added to 1.5 ml micro-centrifuge tube contain 20 µl proteinase K and 5 µl of RNase A solutions. The tube was mixed gently. 200 µl of Buffer BL were added into the tube and mixed gently**.** The mixture was incubated at 56°c for 10 min. Mixing 3- or 4-times during incubation by inverting tube was performed after which the red color of lysate became dark green. The tube was centrifuged for removing drops from the rim. 200 µl ethanol (96-100%) was added and mixed gently 5-6 times and the tube was centrifuged for removing drops from the lid. The mixture was putted in the spin column (in a 2 ml collected tube) without wetting the rim. The cap was closed and the column was centrifuged at 13000 rounds per minute (rpm) for 1 minute. The filtrate was removed and the spin column was placed in the collected tube. 700 µl of buffer WA (washing buffer) were added to the spin column without wetting the rim. The column was centrifuged at 13000 rpm for 1 minute. The flow –through was discarded and the collection tube was reused. 700 µl of buffer WB were added to the spin column without wetting the rim. The column was centrifuged at 13000 rpm for 1 minute. The filtrate was removed and the collection tube was reused. The spin column was centrifuged at 13000 rpm for 1 minute for drying the membrane then the filtrate and collection tube were removed. The spin column was placed in a tube 1.5 ml, 100 µl of buffer CE (elusion buffer) were added to the membrane, incubated for 1 minute at room temperature and centrifuged for 1 minute at 13000 rpm to elute the DNA. The column was discarded and the micro-centrifuge tube which contain the DNA sample was stored at -20 °C till further analysis.

***Determination of Methylenetetrahydrofolate reductase MTHFR (C667T) gene polymorphism by Restriction fragment length polymerase chain reaction (RFLP_PCR):**

PCR-RFLP for detection **C667T** polymorphism of MTHFR gene was done using the following primers.

Forward primer: 5_GCACTTGAAGGAGAAGGTGTC-3

Reverse primer 5_-AGGACGGTGCGGTGAGAGTG -3'.

The PCR was carried on 20 µl volume contain 10 µl of 2x i-TaqTM

PCR Master Mix (iNtRON Biotechnology, Seongnam-Si, Korea),1 ul of each primer (Biolegio, Nijmegen, Netherland), 4 µl of genomic DNA and 4ul of deionised water.

PCR Protocol:

The amplification was carried by using DNA thermal cycler 480, PERKIN ELMER (Norwalk, CT 06856, USA), Serial No. P 16462. Cycling conditions were denaturated at 94°C for 5 minutes, and for 35 cycles at 94°C for 30 seconds, 51°C for 30 seconds and 72°C for 30 seconds; terminal elongation was carried at 72°C for 5min.

Restriction Digest Reaction:

The PCR products were digested using Taq I (New England Biolabs) restriction endonuclease. The digestion was performed in a total volume 25 µl containing 15 ul of PCR Product, 2 ul of enzyme Hha1, 2.5 ul of 10X buffer and 5.5 ul of nuclease-free water.

The digested PCR product samples were incubated at 37°C .Samples were loaded on agarose gel and electrophoresis was performed. The variant allele was created by a C-to-T change at nucleotide 677, which introduces a Taq I site; homozygous individuals (TT) showed 2 fragments of 173 and 30 bp; heterozygotes (CT) showed 3 fragments of 203, 173 and 30 bp; and wild-types (CC) showed only 1 band of 203 bp.

Statistical analysis

Data were collected, tabulated and analyzed by SPSS 20 software. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance;. difference and association of qualitative variable by Chi square test (X^2) . Differences between quantitative independent groups by t test. The significance level was considered at $P < 0.05$.[7]

RESULTS

The early Diabetic neuropathy was found in 25 patients (11 males and 14 females) while late Diabetic neuropathy was found in 18 patients (6 males and 12 females) **(table 1)**. Neuropathy Symptom Score (NSS) for studied patients was reported in **(table 2)**. Neuropathy Deficit Score (NDS) for studied patients was reported in **(table 3)**. There were 10 patients with mild DN (NSS=3-4, NDS=3-5); 15 patients with moderate DN (NSS=5-6, NDS=6-8) and 18 patients with severe DN (NSS=7-10, NDS=9-10) **(table 4)**. There was an important difference between the studied group as regard serum creatinine. There was an important difference between the studied group as regard serum triglycerides, total cholesterol, HDL and LDL cholesterol. There was an important difference between the studied group as regard serum homocysteine. There was an important difference between uncomplicated and complicated diabetics groups as regard disease duration **(table 5)**. There was an important difference between the three groups as regard MTHFR gene polymorphism and alleles **(table 6)**. There was an important difference between different MTHFR gene polymorphism regarding glycemic profile in uncomplicated and complicated diabetic group **(table 7)**. There was a no important difference between different MTHFR gene polymorphism regarding diastolic and systolic blood pressure values in each group **(table 8)**. There was an important difference between different MTHFR gene polymorphism regarding lipid profile in uncomplicated and complicated diabetic patients, while there was no important difference between MTHFR gene polymorphism in the control group, regarding lipid profile **(table 9)**. There was no important difference between different MTHFR gene polymorphism regarding waist circumference, hip circumference and waist to hip ratio values in each group **(table 10)**.

Table (1): Classification of complicated groups according to the duration of diabetes in group 2 (DM) and group 3(DM WITH DPN) into early and late DN:

Complicated group (early Diabetic neuropathy)	complicated group (late Diabetic neuropathy)
Male (11)	**Male (6)**
Female (14)	**Female (12)**
TOTAL = 25	**TOTAL= 18**

Table (2):Neuropathy Symptom Score (NSS) for studied patients

Symptoms	YES	NO
Burning sensation	**2**	**0**
Numbness	**2**	**0**
Paraesthesia	**2**	**0**
Feeling of weakness (fatigue, exhaustion)	**1**	**0**
Cramps	**1**	**0**
Pain	**1**	**0**

Symptoms	YES	NO
Localization		
Feet	**2**	
lower leg	**1**	
Elsewhere	**0**	
Exacerbation		
Present at night	**2**	
Present at Day and Night	**1**	
Only presented during Day	**0**	
Symptoms improvement when		
Walking	**2**	
Standing	**1**	
Sitting or lying down	**0**	

3-4 = mild symptoms 5-6 = moderate symptoms 7-10 = severe neuropathic symptoms

Table (3). Neuropathy Deficit Score (NDS) for studied patients

Ankle jerk		Side	Right	Left
Reflex	**normal**		**0**	**0**
	diminished		**1**	**1**
	absent		**2**	**2**
Vibratory sensibility				
Measurement dorsal on big toe joint				
	normal		**0**	**0**
	diminished absent		**1**	**1**
Pain sensation				
Measurement on the dorsum of the foot				
	normal		**0**	**0**
	diminished absent		**1**	**1**
Temperature perception				
	normal		**0**	**0**
	diminished absent		**1**	**1**

-5 = mild neuropathic deficits
6-8 = moderate neuropathic deficits
9-10 = severe neuropathic deficits

Table (4): Classification of DN according to NSS and NDS score in complicated group

Mild DN (NSS=3-4) (NDS=3-5)	Moderate DN (NSS =5-6) (NDS =6-8)	Severe DN (NSS = 7-10) (NDS =9-10)
10	**15**	**18**

Table (5) comparison between the studied groups regarding demographic criteria:

	Control group	Non-complicated diabetic group	Complicated diabetic group		P
	N (%)	**N (%)**	**N (%)**	**X^2**	
Gender: Male Female	 23 (53.5) 20 (46.5)	 24 (55.8) 19 (44.2)	 26 (56.6) 17 (39.5)	0.442	0.802
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**F**	
Age	58.84±6.52	51.05±6.64	53 ± 5.98	3.78	0.025*
Waist circumference	89.09±6.56$^{\infty}$	105.74±7.85$^{\infty}$	115.12±8.26$^{\infty}$	129.67	<0.001**
Hip circumference	99.93 ± 5.09$^{\infty}$	109.35±8.96$^{\infty}$	124.05±10.49$^{\infty}$	88.101	<0.001**
Waist to hip ratio	0.89 ± 0.04$^{\infty}$	0.96 ± 0.05$^{\infty}$	0.93 ± 0.05$^{\infty}$	32.22	<0.001**

	Control group	Non-complicated diabetic group	Complicated diabetic group		P
Systolic blood pressure(mmHg)	127.79±5.64∞	142.05±9.15	144.86±11.16	45	<0.001**
Diastolic blood pressure(mmHg)	83.02±5.89∞	87.21±4.92	87.44±4.41	10.18	<0.001**
Fasting blood glucose (mg/dl)	91.65±7.23∞	197.14±38.78	210.63±48.38	140.49	<0.001**
Fasting insulin(mIU/L)	6.14 ± 1.23∞	11.14 ± 0.64∞	17.85 ± 5.12∞	493.91	<0.001**
HOMA IR	1.39 ± 0.29∞	5.44 ± 1.16∞	9.2 ± 2.27∞	299.85	<0.001**
Serum creatinine	0.98 ± 0.13∞	1.47 ± 0.21	1.45 ± 0.18	107.26	<0.001**
Triglycerides)	159.17 ± 27.22∞	185.49 ± 30.01	202 ± 31.62	22.79	<0.001**
Total cholesterol(mg/dl)	158.74 ± 22.71∞	233.05 ± 39.12	229.26 ± 38.21	64.44	<0.001**
HDL cholesterol (mg/dl)	42.91 ± 8.33∞	38.33 ± 3.18	39.67 ± 3.14	8.01	0.001**
LDL cholesterol (mg/dl)	115.33 ± 9.44∞	163.91 ± 16.28	158.88 ± 20.55	118.54	<0.001**
Homocystin level	6.98 ± 1.6∞	10.84 ± 1.79	11.33± 2.12	67.96	<0.001**
Duration		7.09 ± 3.22	9.23 ± 2.8	Z -3.14	0.002*

HOMA IR: Homeostatic Model Assessment of Insulin Resistant
HDL: high-density lipoprotein
LDL : Low Density Lipoprotein
*p<0.05 is important difference.

Table (6) comparison between the studied groups regarding MTHFR gene polymorphism:

	Control group	Non-complicated diabetic group	Complicated diabetic group	X2	P	OR	CI
	N (%)	**N (%)**	**N (%)**				
MTHFR polymorphism:							
CC	18 (41.9)	9 (20.9)	5 (11.6)	25.79	<0.001**	3.7	1.6 – 8.52
CT	19 (44.2)	23 (53.5)	14 (32.6)			1.85	0.88 –3.78
TT	6 (13.9)	11 (25.6)	24 (55.8)			5.13	2.3 –11.44
Alleles							
C	55 (127)	41 (95.3)	24 (55.8)	22.53	<0.001**	0.31	0.18-0.54
T	31 (72)	45 (102.3)	62 (144.3)			4	2.26 –7.07

MTHFR: methylenetetrahydrofolate reductase
OR odds ratio CI confidence interval
**p≤0.001 is high important difference

Table (7) relation between MTHFR gene polymorphism and glycemic profile in studied groups:

		Control group	Uncomplicated DM	Complicated DM	F	P
		Mean ± SD	Mean ± SD	Mean ± SD		
FBS	**CC**	92.17±6.2	169.4±38.16	192.77± 10.37	0.697	0.504
	CT	90.37 ± 8.4	190.56±30.2	206.93±40.29	3.259	0.049*
	TT	94.17±6.08	205.9±20.5	227.5±25.94	3.864	0.029*
Fasting insulin	**CC**	5.79 ± 1.22	10.56±0.96	12.25±2.72	1.216	0.307
	CT	6.35 ± 1.18	11.33±0.43	16.14±1.74	3.762	0.032*
	TT	6.47± 1.38	11.04±0.7	18.83±2.69	17.16	<0.001**

		Control group	Uncomplicated DM	Complicated DM	F	P
		Mean ± SD	Mean ± SD	Mean ± SD		
HOMA-IR	**CC**	1.32±0.28	4.16±1.01	8.02±1.6	1.12	0.336
	CT	1.42 ± 0.29	5.18±1.28	8.35±2.43	3.297	0.047*
	TT	1.5±0.31	5.57±1.26	9.99±1.8	4.088	0.024*

FBS: fasting plasma glucose
HOMA-IR: Homeostatic Model Assessment of Insulin Resistant
*p<0.05 = important difference
**p ≤0.001 = high important difference

Table (8) relation between MTHFR gene polymorphism and blood pressure measures in studied groups:

		Control group	Uncomplicated DM	Complicated DM		
		Mean ± SD	Mean ± SD	Mean ± SD	F	P
Systolic	**CC**	126.61±5.28	134 ±5.48	141±11.94	2.487	0.096
	CT	129.79 ± 5.34	143.13 ± 8.7	146.43±12.92	2.328	0.111
	TT	125 ±6.32	143.07±9.95	144.75±10.18	0.426	0.565
Diastolic	**CC**	83.89± 5.3	86±6.52	88±45.71	0.384	0.684
	CT	82.63 ± 6.95	87.29±4.66	88.57±4.13	0.172	0.843
	TT	81.67 ± 4.08	87.5±5.1	86.67±4.34	0.862	0.430

Table (9) relation between MTHFR gene polymorphism and lipid profile in studied groups:

		Control group	Uncomplicated DM	Complicated DM	F	P
		Mean ± SD	Mean ± SD	Mean ± SD		
Triglycerides	**CC**	158.72±24.53	180.23±15.9	190.47±9.2	1.8	0.179
	CT	153.79 ± 23	190.56±20.1	205.07±21.24	4.071	0.025*
	TT	177.5 ±41.96	205.9±20.5	215.08±19.67	3.735	0.033*
Total cholesterol	**CC**	152.67±24.61	202,5±23.67	210.94±8.99	1.31	0.281
	CT	161.58 ±20.17	210.34 ± 20.05	233.14±18.84	4.264	0.021*
	TT	168 ± 23.26	227.42 ± 19.12	250.58±29.38	6.102	0.005*
HDL cholesterol	**CC**	47.78±6.69	34.8±2.16	39.41±1.99	2.525	0.093
	CT	43.26 ± 7.72	38.33±3.24	34.5±3.11	4.94	0.012*
	TT	41.56±7.53	39.57±2.47	30.25±9.98	3.409	0.043*
LDL cholesterol	**CC**	115.28±10.3	142.45±12.85	150.41±9.71	0.351	0.706
	CT	116.26 ± 7.79	155.87±21.13	155.93±18.19	3.459	0.041*
	TT	112.5±12.53	166.78±15	168.98±25.03	3.392	0.044*

HDL: High-density lipoprotein,
LDL: low-density lipoprotein
*p<0.05 = important difference
**p ≤0.001 = high important difference

Table (10): relation between MTHFR gene polymorphism and anthropometic measures in studied groups:

		Control group	Uncomplicated DM	Complicated DM
		Mean± SD	Mean± SD	Mean± SD
Waist circumference	**CC**	87.72±7.51	105±7.91	115.6± 10.57
	CT	89.16 ± 5.71	106.58±7.98	112.64 ± 10.27
	TT	93±5.18	104.57 ± 8.02	116.46±6.31
	F	1.494	0.305	0.951
	P	0.237	0.739	0.359
Hip circumference	**CC**	99±6.47	106±10.39	124.6±13.89
	CT	99 .84±3.24	109.79±8.28	122.43±11.69
	TT	103±4.82	109.79±10	124.88 ± 9.37
	F	1.422	0.383	0.239
	P	0.253	0.684	0.788

		Control group	Uncomplicated DM	Complicated DM
Waist to hip ratio	**CC**	0.88±0.04	0.99±0.04	0.92±0.03
	CT	0.89 ± 0.4	0.97±0.05	0.92±0.03
	TT	0.9±0.02	0.95±0.04	0.93±0.05
	F	0.457	1.394	0.363
	P	0.636	0.260	0.698

DISCUSSION

Diabetes considered a major problem which represent the primary health care challenge of the recent century. The prevalence of diabetes with its complications was raised rapidly. recently, diabetes mellitus of type 2 (DM)was an epidemic disease overall the world. [8].

Diabetic neuropathies were the most chronic complications of diabetes, where the disease affects the nervous system and causes different clinical manifestations [9].

Most common form of neuropathy in diabetic patient was chronic DSPN, which represent about 75% of diabetic neuropathies[10].

This study was designed to evaluate the role of MTHFR gene polymorphism in DPN

To achieve our aim we conducted this study on 129 subjects, 43 participants healthy as control group, 43 patients with T2DM, 43 patients with DPN.

Our study shows significant difference between the three groups as regard MTHFR gene polymorphism and alleles as MTHFR C677T gene polymorphism could be a risk factor in T2DM patients.

In agreement with our results, study done by **Wang et al.** [11] who perform a study on 651 patients with Met. S. (Metabolic Syndrome group) and 727 healthy subjects as control group, they found that waist circumference, Body mass index, the waist-hip-ratio, diastolic and systolic blood pressure, fasting blood glucose, fasting insulin, triglyceride, low-density lipoprotein-cholesterol, total cholesterol, homocysteine level and the homeostasis model evaluation of insulin resistance were higher in the Met. S. group than in the control group, so MTHFR C677T gene polymorphism could be a risk factor for Met. S. including T2DM.

Our study shows non-significant difference between different MTHFR gene polymorphism regarding systolic and diastolic blood pressure values in each group, this was supported by **Bahadır et al.** [12] who studied the relationship between biochemical and cardiac risk variables with the methylenetetrahydrofolate reductase (MTHFR) C677T genotype in T2DM patients, results showed that 29.0% of T2DM patients had CC, 57.9% had CT and 13.1% had TT genotypes. There was no important difference between patients with wild-type (677CC) and mutant (677CT+677TT) alleles in the diabetes duration, systolic and diastolic blood pressure.

In contrast to our results, study done of **Ghogomu et al.** [13] who studied the relation between the MTHFR C677T polymorphism and hypertension, they found the genotype variants were 7.3% CC, 58.5% CT, and 34.1% TT for hypertensive patients compared to 90.0% CC, 10.0% CT, and 0.0% TT for normotensives. Allele genotype variants were 36.6% C and 63.4% T for hypertensive patients and 95.0% C and 5.0% T for normotensive cases. The results showed that the T allele predisposes individuals to hypertension. So, there was an association between variants of the MTHFR gene and hypertension in studied groups.

Our study shows significant difference between different MTHFR gene polymorphism regarding lipid profile in uncomplicated and complicated diabetic patients in control group, as the MTHFR gene polymorphism could be implicated with the development of dyslipidemia in T2DM cases.

A study done by **Raza et al.** [14] was aimed to investigate the association of MTHFR genes polymorphism in T2DM patients with dyslipidemia, they found that MTHFR genes could be associated with the development of dyslipidemia in T2DM patients.

Our study shows polymorphism in the MTHFR gene could be linked to the DPN development, these results were supported by the study of **Wu et al.** [15] who perform a meta-analysis study on 3619 subjects, including 1720 patients and 1899 as control, they assed the relation between the 677 C>T polymorphism in the MTHFR gene with DPN, they demonstrate that polymorphism in the MTHFR gene could be linked to the DPN development.

In contrast to our results the study of **Russo et al.** [16] who perform a study included 263 type 2 diabetic subjects who underwent a specific investigation for DPN, MTHFR C677T polymorphisms were examined, and they found that there was no association between MTHFR C677T mutation and DPN.

Many studies reported the role of genetic factors in development of DPN, current study evaluated the role of ACE and MTHFR genes polymorphisms in diabetic peripheral neuroathy, and confirmed the role of both genes as a risk factor for DPN development. The current study

showed that MTHFR C677T gene polymorphism may be a risk factor for T2DM, the gene polymorphism might be implicated with the development of dyslipidemia in T2DM cases, may be implicated in diabetic nephropathy, and polymorphism in the MTHFR gene might be closely linked to the development of DPN, and MTHFR C677T gene polymorphism was not associated with the risk of hypertension.

Limitations of the Study: the sample size was relatively limited and the lack of objective criteria for assessing DPN. So, further studies are needed to support these results and understand the role of both ACE and MTHFR genes polymorphisms in diabetic peripheral neuropathy

CONCLUSION

MTHFR gene polymorphism was associated with increased risk for DPN development

REFERENCES

1. Tesfaye S, Boulton AJ, Dyck PJ, Freeman R, Horowitz M, Kempler P. Diabetic neuropathies: update on definitions, diagnostic criteria, estimation of severity, and treatments. Diab. Care 2010; (33) : 2285–2293.

2. Yan JE, Yuan W, Lou X, Zhu T. Streptozotocin-induced diabetic hyperalgesia in rats is associated with upregulation of Toll-like receptor 4 expression. Neurosci. Lett. 2012; (526):54–58.

3. Wu S, Han Y, Hu Q, Zhang X, Cui G, Li Z. Effects of Common Polymorphisms in the MTHFR and ACE Genes on Diabetic Peripheral Neuropathy Progression: a Meta-Analysis. Mol. Neurobiol. 2017; (54):2435–44.

4. Yigit S, Karakus N, Inanir A. Association of MTHFR gene C677T mutation with diabetic peripheral neuropathy and diabetic retinopathy. Mol. Vis. 2013; (19) :1626.

5- Varga EA, Sturm AC, Misita CP, Moll S. "Homocysteine and MTHFR mutations: relation to thrombosis and coronary artery disease. Circulation 2005; 111 (19): e289-e293.

6-Molina M, Gonzalez R, Folgado J, Real JT, Martinez-Hervas S, Priego A. Correlation between plasma concentrations of homocysteine and diabetic polyneuropathy evaluated with the Semmes-Weinstein monofilament test in patients with type 2 diabetes mellitus. Med Clin (Barc) 2013 ; 141 (9) : 382-6.

7. Pallant Julie. SPSS survival manual: a step by step guide to data analysis using SPSS for Windows, Open Univ. Press Milt Key UK, USA, 2007.

8. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. Diabetes Res Clin Pract. 2017 ; (128):40-50.

9. **Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik, RA.** Diabetic neuropathy: a position statement by the American Diabetes Association. Diab. Care, 2017;(1):136-154**.**

10. **Albers JW, Pop-Busui R.** Diabetic neuropathy: mechanisms, emerging treatments, and subtypes. Curr Neurol Neurosci Rep. 2014; (8):473.

11. **Wang J, XuL, Xia H, Li Y, Tang S.** Association of MTHFR C677T gene polymorphism with metabolic syndrome in a Chinese population: a case–control study. J Int Med Res. 2018; (7):2658-69.

12. **Bahadır A, Eroz R, Türker Y**. Does the MTHFR C677T gene polymorphism indicate cardiovascular disease risk in type 2 diabetes mellitus patients? Anatol J Cardiol.2015; 15 (7):524.

13. **Ghogomu SM, Ngolle NE, Mouliom RN, Asa BF.** Association between the MTHFR C677T gene polymorphism and essential hypertension in South West Cameroon. Genet. Mol Res 2016; (1):28.

14. **Raza S.T, Abbas S, Siddiqi Z, Mahdi F.** Association between ACE (rs4646994), FABP2 (rs1799883), MTHFR (rs1801133), FTO (rs9939609) genes polymorphism and type 2 diabetes with dyslipidemia. Int J Mol Cell Med. 2017; 6 (2):121.

15. **Wu S, Han Y, Hu Q, Zhang X, Cui G, Li Z.** Effects of Common Polymorphisms in the MTHFR and ACE Genes on Diabetic Peripheral Neuropathy Progression: a Meta-Analysis. Mol Neurobiol 2017; 54 (4):2435–44.

16. **Russo GT, Giandalia A, Romeo EL.** Diabetic neuropathy is not associated with homocysteine, folate, vitamin B12 levels, and MTHFR C677T mutation in type 2 diabetic outpatients taking metformin. J Endocrinol Invest. 2016;(3):305-314

How to cite

Abdulhamid, A., Abd El-Fatah, A., Fawzy, A. Assessment of Methylenetetrahydrofolate Reductase Gene Polymorphism in Diabetic Peripheral Neuropathy. Zagazig University Medical Journal, 2022; (272-279): -. doi: 10.21608/zumj.2020.10042.1072