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Research Article

Methylenetetrahydrofolate Reductase C677T Gene Mutation as a Risk Factor for Diabetic Nephropathy

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ABSTRACT

Background: Methylenetetrahydrofolate Reductase (MTHFR) is a regularly enzyme of homocysteine metabolism. Point mutation in MTHFR and hyperhomocyteinemia are implicated in the pathogenesis of Diabetic Nephropathy (DN) in many ethnic groups. The aim of this study is to find if MTHFR C677T polymorphism is a risk factor of DN in Type 2 Diabetes Mellitus (T2DM) patients.

Subjects and methods: The MTHFR C677T polymorphism was detected in 122 T2DM patients by PCR-RFLP. They were divided into 3 groups; 75 patients with normoalbuminuria. 33 patients with microalbuminuria and 14 patients with macroalbuminuria. Seum levels of Homocysteine (HCY) were determined by nephelometry.

Results: Presence of MTHFR C677T allele increase the risk of macroalbuminuria 2.6 folds (p=0.009) in T2DM patients. The presence of mutant genotypes CT and TT increase the risk of macroalbuminuria 3.7 folds (p=0.002). Serum levels of Homocysteine (HCY) were not associated with C677T mutation (p=0.22), also there was no significant association between high levels of Homocysteine (HCY) and Diabetic Nephropathy DN (p=0.05).

Conclusion: MTHFR C677T gene mutation was associated with increased risk for overt Diabetic Nephropathy (DN) in type2 diabetic patients and had no effect on serum levels of homocysteine.

Keywords: Diabetic nephropathy; Type 2 diabetes mellitus; Homocysteine

INTRODUCTION

The pathophysiology of Type 2 Diabetes Mellitus (T2DM) is complex and multifactorial. The major cause of diabetic complications such as nephropathy, neuropathy, retinopathy and cardiovascular diseases, has not yet been clarified. Environmental factors contribute to a significant diabetic morbidity and mortality, but genetic background seems to play a vitol role in the development of diabetic complications. Diabetic Nephropathy (DN) is the leading cause of chronic renal disease in patients starting renal replacement therapy [1]. Early stage is characterized by a small increase in Urinary Albumin Excretion (UAE), also called microalbuminuria or incipient DN. More advanced disease is defined by the presence of macroalbuminuria, also called overt DN. The two main risk factors for DN are hyperglycaemia and arterial hypertension. The candidate gene approach is widely used for identifying genes involved in complex human diseases [2]. It relies either on the characterization of the functional polymorphisms which affect some biological phenotypes predisposing to disease, or on linkage disequilibrium existing between observed markers and unidentified functional polymorphisms. Based on its biological function, Methylenetetrahydrofolate Reductase (MTHFR) can be seen as candidate gene that had reasonable prior propability of being involved in vascular complications of diabetes mellitus [3].

MTHFR is a key regulatory enzyme in folate and homocysteine metabolism risk. Deficiency o MTHFR may be associated with an increase in plasma homocysteine, which in turn is associated with an increased risk of vascular diseases. One of most candidate investigated polymorphisms in the MTHFR gene is C677T. The C677T polymorphism converts an alanine residue to a valine, leading to a lower enzymatic activity. Compared with the 677CC wild genotype, the 677TT homozygous and 677CT heterozygous genotypes decrease enzyme activity by approximately 70% and 40% respectively. Homocysteine (HCY) is a sulfur amino acid

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which functions as a precursor for L-methionine synthesis and associated with the metabolism of folic acid and vitamin B6 and B12 [4]. Hperhomocysteinemia could result in different angiopathies through the impairment of endothelial function, oxidative stress, and disorder of lipid metabolism we have evaluated the role of (MTHFR) C677T gene polymorphism and subsequent hyperhomocysteinemia in patients with diabetic nephropathy.

MATERIALS AND METHODS

Subjects

One hundred and twenty two T2DM were recruited from the diabetes to outpatient clinic in Kasr Al-Aini University Hospital and divided into 3 groups based on the urinary albumin excretion, the first group 75 T2DM with normoalbuminuria, the second group 33 T2DM with microalbuminuria the third group 14 T2DM with macroalbuminuria [5]. Diabetes was diagnosed according to the world organization criteria. Hypertension was diagnosed according to the Joint National Committee criteria. BMI was calculated as weight divided by height 2. Nephropathy was defined as the presence of macroalbuminuria (>300 mg/day) and serum urea (>50 mg/dl) and creatinine (>1 mg/dl) levels. History of diabetes was also recorded.

Biochemical measurements

Blood samples were drawn, following overnight fasting, into tubes containing EDTA and plasma was immediately separated by centrifugation. Biochemical measurements were carried out according to validated methods. Plasma glucose concentration was evaluated using an enzymatic kit by a total cholesterol and triglycerids by enzymatic methods using randox reagents and LDL and HDL cholesterol determined. Serum homocysteine was determined as described by urinary microalbumin was determined [6].

MTHFR genotype determination

Genomic DNA was extracted using QIAamp DNA blood mini kit-Qiagen (from Hoffmann-La roche AG). Genotyping of kit by MTHFR C677T was performed using DNA amplification, restriction with endonuclease Hinfl as described.

Statistical analysis

Data were statistically described in terms of mean ± standard deviation (± SD), median and range. Odds ratio and its 95% confidence interval (95% CI) was calculated for mutation in relation to albuminuria groups. Comparison of numerical variables between the study groups was done using Student t test for independent samples in comparing 2 groups when normally distributed and Mann Whitney U test for independent samples when not normally distributed [7]. Comparison of numerical variables between more than two groups was done using one way Analysis Of Variance (ANOVA) test with posthoc multiple 2-group comparisons when data were normally distributed and Kruskal Wallis test with posthoc multiple 2-group comparisons when data were not normal. For comparing categorical data, Chi square test was performed. Correlation between various variables was done using Pearson moment correlation equation for linear relation in normally distributed variables and Spearman rank correlation equation for non-normal variables. All statistical calculations were done using computer programs SPSS version 15 for microsoft windows.

RESULTS

General characteristics of the 3 studied groups are presented in Table 1. Hypertension showed higher incidence in group III (92.9%) than group I (18.7%) and group II (57.6%). On comparing laboratory investigations in the three studied groups there was statistically significant difference in creatinine (p=0.000), and A/C ratio (p=0.000) [8]. There was no statistically significant difference in the other laboratory investigations.

	Group I n=75	Group II n=33	Group III n=14	P value
Age (years)	53.6 ± 7	54.3 ± 7.2	51.5 ± 7.8	0.475
Hypertension (%)	14 (18.7%)	19 (57.6%)	13 (92.9%)	0
FPG (mg/dl)	198.6 ± 72.9	214.2 ± 84	203.4 ± 1.8	0.645
HbA1c (%)	8.4 ± 1.6	9.1 ± 2.1	9.6 ± 2.9	0.063
Creatinine (mg/dl)	0.7 ± 0.2	1 ± 0.9	1.8 ± 1.8	0
Total cholesterol (mg/dl)	200.3 ± 43.3	187.6 ± 45.1	212.4 ± 73.8	0.23
HDL (mg/dl)	42.5 ± 11.5	44 ± 10.7	44.2 ± 13.2	0.771
LDL (mg/dl)	124.9 ± 38.5	115.4 ± 38.8	132.8 ± 68.9	0.393
TG (mg/dl)	154.3 ± 75.8	144.26 ± 67.8	196.5 ± 10.4	0.105
A/C (µgm/mg)	10.7 ± 7.6	104.4 ± 70.5	290.9 ± 418	0

Table 1: Clinical and biochemical features of the three groups of the study.

		Creatinine	Cholesterol	HDL	LDL	TG	A/C	Hcy
HbA1c	r	0.146	0.212	-0.08	0.194	0.287	0.038	-0.06
(%)	P	0.11	0.019	0.361	0.032	0.001	0.675	0.5
Creatinine	r		0.247	0.11	0.249	0.022	0.573	0.289
(mg/dl)	P		0.006	0.229	0.006	0.81	0	0.001
Cholesterol	r			0.112	0.361	0.361	0.21	0.018

(mg/dl)	P	0.221	0	0	0.019	0.848
HDL	r		-0.038	-0.321	0.079	0.041
(mg/dl)	P		0.682	0	0.386	0.657
LDL	r			0.146	0.204	0.078
(mg/dl)	P			0.109	0.024	0.394
TG	r				0.06	-0.069
(mg/dl)	P				0.511	0.45
A/C	r					0.127
µgm/mg	P					0.164

Table 2: Correlation between biochemical features of the studied groups.

	Group I	Group II	Group III	Statistics	
CC (%)	38 (50.7%)	21 (63.6%)	3 (21.4%)		
CT and TT (%)	37 (49.3%)	12 (36.4%)	11 (78.6%)	OR=2.4	p=0.02
C (%)	44 (69.3%)	53 (80.3%)	13 (46.4%)		
T (%)	46 (30.7%)	13 (19.7%)	15 (53.6%)	OR=3.7	p=0.009

Table 3: Genotype, allele frequencies of MTHFR C677T in the three studied groups.

	Wild (CC)	Heterozygous (CT)	Homozygoua (T)	P value
Homocysteine µMol/L	8.8 ± 2.3	9.2 ± 4	10.5 ± 4.6	0.22

Table 4: Homocysteine levels in different MTHFR genotypes.

Correlation between the different laboratory investigations was shown in Table 2. There was positive significant correlation between HbA1c and the following; total cholesterol (r=0.212, p=0.019), LDL (r=0.194, p=0.03), triglycerides (r=0.287, p=0.01). There was positive significant correlation between A/C ratio and the following; creatinine (r=0.573, p=0.001) total cholesterol (r=0.212, p=0.019), LDL (r=0.204, p=0.024), There was positive significant correlation between homocysteine and creatinine (r=0.289, p=0.001), while no significant correlation between other parameters.

Furthermore, genotype distribution of MTHFR polymorphism was different between the three studied groups showing that there was an association between C677T polymorphism and diabetic nephropathy (p=0.044). Frequency distribution of mutant genotype (CT+TT) was significantly higher in patients with macroalbuminuria and those with normoalbuminuria (Table 3). On performing odds ratioon the different groups, TT and CT genotypes showed 2.4 fold increased risk for nephropathy (microalbuminuria and macroalbuminuria. Furthermore, the T allele frequency was significantly higher in macroalbuminuria group compared to normoalbuminuria group [9]. On the other hand, there was no statistically significant difference between the mean values of homocysteine in the three studied groups, and on comparing these values among different genotypes; there was no statistically significant association between them and the presence of the mutation (Table 4).

DISCUSSION

In this study, on comparing MTHFR C677T genotypes (CC, CT, TT) among the studied groups, it was found that the mutation was associated with nephropathy (p=0.044), this was in agreement with a study done in Tunisia. They found that allele mutant genotypes CT and TT were more frequent in patients with nephropathy with p<0.001 and 0.002 respectively [10]. The frequency of mutant genotypes (CT+TT) was significantly higher in patients with macroalbuminuria when compared to those with normoalbuminuria (78%, 49% respectively p=0.02, OR=2.4), this was consistent with a study the frequency of (CT +TT) was significantly higher in patients with macroalbuminuria when compared to those with normoalbuminuria. On the other hand, in Japan study were done and proved that there was no risk associated between MTHFR C677T mutation and DN. Regarding the allele frequency, the mutant allele MTHFR677T was higher in macroalbuminuria group when compared to normoalbuminuria group with (p=0.009, OR=3.7), this agreed with the study who found that 677T allele was higher in patients with nephropathy (p<0.001, OR=5.9).

CONCLUSION

Homocysteine (HCY) was assayed in this study as its metabolism is regulated by MTHFR enzyme, although the mean values of (HCY) were higher in patients with micro and macroalbuninuria than those with normoalbuminuria but this wasn't statistically significant (p=0.05). Also on comparing the mean values of (HCY) among different genotypes of (CC, CT, TT), there was no significant association between the mean values of (HCY) and the presence of the mutation (p=0.22), this was consistent with the study where they found no relation between MTHFR C677T polymorphism levels. In conclusion, MTHFR C677T gene mutation may be associated with increased risk for overt diabetic nephropathy in type 2 diabetic patients and had no effect on serum levels of homocysteine.

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