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MTHFR 677C/T and 1298A/C mutations and non-alcoholic fatty liver disease

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ABSTRACT

Common genetic mutations encountered in folate metabolism may result in increased homocysteine (Hcy) levels. It has been reported that increased serum Hcy levels may affect the intracellular fat metabolism and may cause enhanced fatty infiltration in the liver resulting in non-alcoholic fatty liver disease (NAFLD). In total, 150 patients diagnosed with FLD by ultrasound examination and 136 healthy control patients that do not have any fatty infiltration in the liver were included in the study. Patients were grouped as mild (n=88), moderate (n=38) or severe (n=24) according to the stage of fatty liver in ultrasound. Serum liver function tests, Hcy, folic acid and vitamin B12 levels of the patients were studied. The genetic *MTHFR* C677T and A1298C polymorphisms of the patients were also evaluated. Although there was no significant difference in vitamin B12 and folic acid levels, in the severe group, Hcy levels were significantly higher than that of control and mild groups ($p<0.001$). By contrast, there was no significant difference in heterozygote *MTHFR* 677C/T and 1298A/C mutations, both *MTHFR* 677C/T and *MTHFR* 1298A/C mutations were more common in NAFLD groups compared with the control patients ($p<0.001$). We have determined increased Hcy levels and increased prevalence of homozygote *MTHFR* 677C/T and *MTHFR* 1298A/C mutations in patients with NAFLD compared with healthy controls. Larger studies are warranted to clarify the etiological role of the *MTHFR* mutations and Hcy levels in FLD.

KEYWORDS: *MTHFR* mutations, homocysteine, fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of histological abnormalities, ranging from simple fatty infiltration of the liver to non-alcoholic steatohepatitis (NASH), in patients without significant alcohol consumption.[1] NAFLD is the most common cause of abnormal liver function tests worldwide.[2] Nowadays, NAFLD is regarded as the hepatic manifestation of metabolic disorders and insulin resistance is the mainstay of these disorders.[3] Although the exact pathogenesis is not clear, insulin resistance is considered to initiate fatty infiltration of the liver, with a role for oxidative stress in the development of steatohepatitis from simple fatty infiltration of the liver.[4] Considering the increasing incidence of NAFLD in developed and developing countries, understanding the pathophysiology of FLD is an important approach to determine new treatment modalities.

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that plays an important role in folate metabolism. MTHFR catalyses the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-MTHF.

5-MTHF functions as a co-substrate of the methionine synthase enzyme during methylation of homocysteine (Hcy) to methionine in the presence of vitamin B12 as a co-factor.[5] The *MTHFR* gene is localised on the short arm of chromosome 1.[6] There are two common mutations in the *MTHFR* gene described. The C677T polymorphism is the substitution of cytosine (C) to thymine (T) at nucleotide 677, resulting in an alanine to valine transition, and the A1298C polymorphism is an adenine (A) to C transition at nucleotide 1298, leading to a glutamate to alanine substitution.[7] Decreased activity of the *MTHFR* gene due to the homozygous or heterozygous mutations, results in the decreased concentration of folate which promotes higher serum Hcy levels.[8]

Increased Hcy concentrations are associated with vascular damage and generation of formation of superoxide free radical and hydrogen peroxide.[9] In that aspect, hyperhomocysteinemia, is considered as an independent risk factor for liver diseases by way of oxidative stress and activation of proinflammatory factors.[10] In this study we aimed to determine the association of FLD with hyperhomocysteinemia and the *MTHFR* C677T and A1298C polymorphisms, which are the common genetic causes for increased Hcy levels.

Material and methods

This study was carried out in Turgut Ozal University Hospital, Ankara between January 2013 and January 2014. In total, 286 patients, 136 control and 150 with FLD, admitted to the gastroenterology department were enrolled in the study. The

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study was an unmatched case control study performed in a tertiary center. The control patients were selected among the patients with the diagnosis of gastritis or gastroosephageal reflux disease. Exclusion criteria were as follows: a significant history of alcohol use (>30 g for males and >20 g for females), positive results for HBsAg or anti HCV, autoimmune hepatitis, Wilson's disease, hemochromatosis, any chronic liver disease, malignancies and diabetes mellitus. Prior to subject recruitment, the study protocol was reviewed and approved by the university ethics committee. Informed consent was obtained from all participants. Height and weight of all participants were measured and the body mass index was calculated by dividing the weight (kg) by the square of the height (m^2).

Laboratory tests

Liver function tests (including alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase and alkaline phosphatase levels), lipid profiles (including total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein levels), fasting blood glucose, insulin, Hcy, uric acid, C-reactive protein, sedimentation, vitamin B12, folate and ferritin levels were studied after overnight fasting of 8 hours.

Insulin resistance was measured using the homeostatic model of the assessment of insulin resistance (HOMA-IR) and was obtained by applying the following formula where FI is fasting insulin and FGB is fasting blood glucose:

$$\text{HOMA} = \text{FI (IU/ml)} \times \text{FBG (mmol/l)}/22.5^{11}$$

In addition, all patients underwent liver ultrasonography, and the presence and stage of fatty liver were graded by an experienced radiologist who was blind to the laboratory values of the patients. Liver steatosis was scored on a scale of 0–3: 0, absent; 1, mild; 2, moderate; 3, severe. Steatosis was graded according to Saverymuttu *et al* on the basis of abnormally intense, high-level echoes arising from the hepatic parenchyma, liver-kidney difference in echo amplitude, echo penetration into the deep portion of the liver and clarity of liver blood vessel structure.[12]

MTHFR polymorphisms

Genomic DNA was isolated from peripheral blood samples of all participants as previously described.[5]

Statistical analysis

Continuous variables are reported as ranges, while categorical variables are presented as number and %. Comparisons of demographic features of groups were performed with Pearson chi-squared tests. Differences in continuous variables between the cases and control groups were determined by ANOVA. A logistic regression model was used to determine the independent predictors of elevated serum Hcy levels and results are presented as odds ratios and 95% confidence intervals. All analyses were performed using SPSS for Windows 17.0. Any missing value was not present in statistical analysis.

Results

In total, 286 patients were included in the study (87 male and 199 (69.6%) female). General characteristics and laboratory data are presented in Table 1. The large number of females participating in the study was due to the admission profile in our hospital.

In Table 1, since many comparisons were performed without Bonferroni correction, the significance level was regarded as $p \leq 0.0005$. Although there were no significant differences in vitamin B12 and folic acid levels, in the severe NAFLD group, Hcy levels were significantly higher than that of control and mild NAFLD groups ($p < 0.0005$).

The results of *MTHFR* gene polymorphism evaluations are summarised in Table 2.

On the other hand, although there was no significant difference in heterozygote *MTHFR* 677C/T and 1298A/C mutations; both *MTHFR* 677C/T and *MTHFR* 1298A/C mutations were more common in NAFLD groups compared with the control patients ($p < 0.001$). In logistic regression analysis the degree of FLD in ultrasound was determined to significantly correlate with elevated Hcy levels (Table 3).

Discussion

In this study we have determined an increased prevalence of both homozygote *MTHFR* 677C/T and *MTHFR* 1298A/C mutations in patients with NAFLD. Moreover, especially in severe FLD, serum Hcy levels were significantly higher than the control group, although vitamin B12 and folic acid levels were similar between groups.

Similar with our results, Sazci *et al* previously reported that the *MTHFR* A1298C allele significantly correlates with NASH.[13] By contrast, Brochado *et al* reported that the *MTHFR* C677T and A1298C polymorphisms were not genetic risk factors for the development of NAFLD in their study on 268 cases. The authors also identified higher Hcy levels in NAFLD subjects, however, a correlation between this increase and liver disease severity was not identified.[14] In the same way, Serin *et al* did not identify the *MTHFR* C677T mutation as a risk factor for the progression of NAFLD to NASH in their study on 34 patients with NAFLD diagnosed by histological analysis and 282 healthy controls.[15]

The association of increased plasma Hcy levels with FLD has been determined in some studies.[16,17] Frelut *et al* determined that an increase in ALT and ALT/AST ratio was associated with the mutation in their study on 57 obese girls, 23 of whom were heterozygote (CT) for the *MTHFR* 677 mutation and 5 were homozygote (TT).[18] Hyperhomocysteinemia has been demonstrated to activate hepatic nicotinamide adenine dinucleotide phosphate oxidase leading to augmented superoxide anion production and peroxynitrite formation in the liver in a rat model.[16] Moreover Hyc has an auto-oxidation effect by stimulation of the endothelial nitric oxide production and leads to increases in endothelium-damaging hydrogen peroxide levels.[19]

Hyperhomocysteinemia may be the result of older age, low intake or deficiencies of B vitamins, genetic defects, polymorphisms of enzymes involved in Hcy metabolism and lifestyle factors, such as smoking and lack of exercise.[20] In our study, age, gender and vitamin B12 levels were not different

Table 1. Clinical and biochemical characteristics of study population.

Parameter	Control (n=136)	NAFLD stage 1 (n=88)	NAFLD stage 2 (n=38)	NAFLD stage 3 (n=24)	p value
Age, years	45.8±14.9	47.5±15.1	50.5±10.4	50.3±8.3	0.65
Gender, female/male (%)	94/42 (69.1)	61/27 (69.3)	27/11 (71.0)	17/7 (70.8)	0.82
BMI, kg/m^2	28.4±3.1	28.7±3.9	29.2±3.1	29.6±3.7	0.12
Hcy	15.7±8.4	15.5±6.4	19.9±9.5	22.3±8.3	0.04
HOMA-IR	1.5±1.4	2.6±0.9	3.6±1.1	3.7±1.0	0.11
ALT, IU/l	19.8±9.9	20.6±9.8	25.9±12.5	24.4±13.7	0.54
AST, IU/l	19.2±7.7	20.3±9.7	22.6±10.5	21.2±11.4	0.61
GGT, IU/l	27.7±15.2	36.0±17.3	38.6±19.4	38.2±17.0	0.43
ALP, IU/l	76.6±19.4	80.5±21.2	81.6±18.4	84.4±22.7	0.51
Total cholesterol, mg/dl	185.8±36.4	214.6±44.2	207.2±43.5	217.5±58.2	0.62
HDL cholesterol, mg/dl	49.6±13.1	48.0±11.2	47.2±14.9	45.2±3.4	0.61
LDL cholesterol, mg/dl	113.8±33.1	111.6±31.7	125.5±40.0	135.8±47.8	0.44
Triglyceride, mg/dl	115.9±62.8	114.2±68.4	146.0±64.1	141.2±79.8	0.46
CRP, mg/l	4.6±2.6	4.4±3.1	6.5±3.7	6.4±3.9	0.51
Uric acid, mg/dl	5.0±1.8	4.7±1.9	7.0±1.9	6.9±2.1	0.03
Ferritin, ng/ml	41.6±12.7	59.6±21.8	61.8±22.8	83.2±37.1	0.21
Vitamin B12, pg/ml	382.5±182.2	402.6±192.1	390.6±154.1	316.6±135.1	0.43
Folic acid, ng/ml	9.2±4.9	9.3±4.9	10.5±3.9	10.4±2.9	0.52

ALP = alkaline phosphatase; ALT = alanine amino transferase; AST = aspartate amino transferase; BMI = body mass index; CRP = C-reactive protein; GGT = gamma glutamyl transferase; Hcy = homocysteine; HDL = high-density lipoprotein; HOMA-IR = homeostatic model of the assessment of insulin resistance; LDL = low-density lipoprotein levels; NAFLD = non-alcoholic fatty liver disease.

between the groups, however smoking and daily exercise performance of the subjects were not asked, which may also alter Hcy levels.

Interestingly, in rat models, insulin has been determined to change the activity of metabolic enzymes involved in the turnover of Hcy.[21] The presence of the *MTHFR*-CT mutation was previously studied on 113 obese adolescents in which 59 subjects were heterozygote (CT, 52.2%) and 8 were homozygote for the mutation (TT, 7.0%). The authors also reported that insulin levels were significantly higher in TT than in CC or CT subjects, as well as HOMA-IR.[22] Movva *et al* determined that the *MTHFR* C677T allele confered a four-fold risk of developing type-2 diabetes mellitus in their study on 236 diabetic patients and 100 control individuals.[23] An increase in mean serum Hcy concentrations in women with polycystic ovarian syndrome, another clinical condition associated with insulin resistance, has also reported.[24] In addition, Schachter *et al* previously reported that insulin resistance and hyperinsulinaemia in patients with PCOS is associated with elevated plasma Hcy levels regardless of body weight.[25] In that aspect, insulin resistance may also play a role in the correlation between FLD with hyperhomocysteinemia.

There are some limitations of this study that should be mentioned. The diagnosis of NAFLD was based on ultrasound

Table 2. Evaluation of *MTHFR* gene polymorphisms in all patients.

Genotype	Control (n=136) (%)	NAFLD stage 1 (n=88) (%)	NAFLD stage 2 (n=38) (%)	NAFLD stage 3 (n=24) (%)
No mutations	40 (29.4)	12 (13.6)	4 (10.4)	3 (12.5)
MTHFR 677C/T heterozygote	56 (41.2)	34 (38.6)	12 (31.6)	8 (33.3)
MTHFR 677C/T homozygote	12 (8.8)	16 (18.1)	8 (21.1)	5 (20.8)
MTHFR 1298A/C heterozygote	24 (17.6)	18 (20.4)	8 (21.1)	5 (20.8)
MTHFR 1298A/C homozygote	4 (2.9)	8 (9.0)	6 (15.8)	3 (12.5)

MTHFR = methylenetetrahydrofolate reductase; NAFLD = non-alcoholic fatty liver disease.

Table 3. Results of logistic regression analysis in prediction of elevated Hcy levels.

Parameter	OR	95% CI	p value
Age, years	0.89	0.77–2.01	0.21
Gender, male	0.26	0.16–0.86	0.18
Vitamin B12 levels, pg/ml	0.54	0.32–0.84	0.19
Folic acid levels, ng/ml	0.65	0.42–0.96	0.54
FLD in ultrasound (reference: control cases)	1.26	0.62–2.52	0.03
Uric acid levels, mg/dl	0.22	0.17–0.54	0.32
CRP, mg/l	0.78	0.11–1.67	0.48
HOMA-IR	0.69	0.34–0.97	0.41

CI = confidence interval; CRP = C-reactive protein; FLD = fatty liver disease; Hcy = homocysteine; HOMA-IR = homeostatic model of the assessment of insulin resistance; OR = odds ratio.

observations, however the ultrasounds were performed by an expert radiologist. Secondly, due to the cross-sectional design of this study, we cannot discuss a cause and outcome relationship. Selection bias can easily arise in case-control studies with hospital controls, however, our control patients were included among patients with diseases which are not associated with liver. Another important point is that we did not adjust the cases for known risk factors which also can affect the results.

In the present study, increased Hcy levels and increased prevalence of homozygote *MTHFR* 677C/T and *MTHFR* 1298A/C mutations were identified in patients with NAFLD compared with healthy controls. Identification of these common genetic mutations is easily available in many laboratories and is not expensive. Moreover, the number of patients with FLD is growing rapidly and measures to prevent further increases must be identified. In this aspect, where the etiological factors can be elucidated; healthy but at-risk patients may be advised to make lifestyle changes earlier. Larger studies are warranted to clarify the etiological role of the *MTHFR* mutations and Hcy levels in FLD. ■

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