Methylenetetrahydrofolate Reductase C677T Gene Polymorphism in Hepatitis C Virus-related Hepatocellular Carcinoma: An Egyptian Perspective Study

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Received: 4 August 2021

Accepted: 5 August 2021

Abstract

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. It constitutes a major health problem in both developed and developing countries. In Egypt, the incidence of HCC is remarkably increasing because of the high prevalence of hepatitis C viral infection. In this study, we investigated whether methylenetetrahydrofolate reductase *(MTHFR) C677T* polymorphism has a role in the development of HCC in Egyptians. We recruited 128 patients diagnosed with HCC from the Hepatology and Gastroenterology Department, Mansoura Specialized Medical Center, MansouraUniversity Hospitals at the Delta region of Egypt. In addition, a total of 130participants who were confirmed as having hepatitis C viral infection constituted the other group in this study. The genotypes and allele distributions in both groups were investigated using PCR-RFLP and were compared. On comparing the frequency of alleles, genotypes in two patient groups revealed statistical significance for *MTHFRC677T* gene polymorphism in HCC patients. The *CT* and *TT* genotype and the *T* allele of *MTHFR C677T* gene polymorphism all showed a significant risk of developing HCC [odds ratio (OR)=1.92; 95% confidence interval (CI)=1.11–3.32; *P*=0.019; (OR=4.91; 95% CI=1.32–18.24; *P*=0.018); (OR=2.113; 95% CI=1.36–3.29; *P*=0.001), respectively].

Keywords: hepatitis C, hepatocellular carcinoma, MTHFR polymorphism

Introduction

Hepatocellular carcinoma (HCC), the main primary liver cancer, is considered as one of the most commonly occurring cancers worldwide. According to the statistics on cancer-related deaths, HCC represents the third main cause of cancer-related deaths in male population and the fourth main cause in female population, with nearly 600 000 deaths per year worldwide [1]. The incidence rate of HCC varies widely as it ranges from 2.1 in Central America to 35.5 in Eastern Asia [2]. 'The burden of HCC has been increasing in Egypt, with a doubling in the incidence rate in the past 10 years' [3], mainly due to the high prevalence of hepatitis C virus (HCV) in Egypt [4,5]. The molecular pathogenesis of HCC is a complex genetic process with changes in the genomic landscape [6]. Nevertheless, there is no clinically available molecular marker for drug response, recurrence, and prognosis [7]. Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in folate metabolism, converting 5,10- methyleneTHF, a carbon donor for nucleotide synthesis, to 5-methyl THF. 5-Methyl THF is then essential for the remethylation process of homocysteine to methionine, which is subsequently used to generate the global methyl donor, SAM. MTHFR gene has been mapped to chromosome 1p36.3, with Between Februar a complex genomic structure [8]. The C677T polymorphism is a point mutation with the substitution of cysteine to thymine nucleotide at the position 677 on MTHFR gene, leading to the substitution of alanine to valine in the MTHFR enzyme, associated with hyperhomo- cysteinemia. This polymorphism is common in the general population, with a wide-range population specificity depending on the ethnic backgrounds [9, 10].

MTHFR C677T polymorphism seems to be involved in the genetic pathogenesis of many cancers [11]. However, studies on the associations of MTHFR genetic polymorphisms with HCC risk had inconsistent results [12–19]. Moreover, there are no available data in the Egyptian population. Therefore, we performed this study to assess whether MTHFR C677T gene polymorphism is associated with the risk for HCC in Egyptian patients.

Patients and methods

Patients

Between February 2012 and January 2015, 128 patients with HCC were recruited from

the Hepatology and Gastroenterology Department, Mansoura Specialized Medical Center, Mansoura University Hospitals at the Delta region of Egypt. HCC was diagnosed using the laboratory data of α-fetoprotein elevation, liver imaging (ultrasonography, computed tomography, MRI, and/or angiography), and/or histologic exam. Exclusion criteria for HCC patients were cardiac or renal diseases, overt diabetes, hepatitis B virus, and noncompliance. In addition, 130 unrelated participants with PCR-proven HCV were recruited from the outpatient clinic. Those participants who had no sign of HCC served as a disease control. An informed consent was obtained from all participants prior to their enrollment in the study, and approval of the local ethics committee of Mansoura University was also obtained.

Genomic analysis

From the whole venous blood samples, genomic DNA samples were extracted using GeneJET Whole blood Genomic DNA Purification Mini Kit (Thermo Scientific, Lithuania, US), according to the manufacturer's protocol and then were stored at −20°C. The MTHFR C677T polymorphism was genotyped in each case of both groups using PCR-RFLP method. Forward and reverse primers specified in Table 1 were used (Biosearch Technologies, USA). Amplification was performed in a Thermal Cycler (TC-312; Techne, Cambridge, UK): 5 min initially at 95°C, 40 cycles of 30 s of denaturation at 95°C, followed by 30 s of annealing at 60°C, 60 s of extension at 72°C, and a final extension at 72°C for 7 min. RFLP analysis was performed using the restriction enzyme FastDigest HinfI (Thermo Scientific FastDigest, USA). DNA fragments were resolved in 3% agarose gel electrophoresis. An undigested 198 bp fragment showed the wild-type CC genotype, whereas the two digested fragments of 175 and 23 bp represented the mutant TT genotype, and the three fragments of 198, 175, and 23 bp denoted the heterozygous CT genotype.

Statistical analysis

The statistical analysis of data was performed using Excel (Microsoft Office 2013) and SPSS software (SPSS Inc., Chicago, Illinois, USA) version 20. Qualitative data were presented as frequency and percentage, whereas quantitative data were presented as mean and SD. Allele and genotype frequencies were obtained by direct counting. The $\chi 2$-test was utilized to calculate the genotypic and allelic differences in frequencies between patients and controls.

The prevalence of genotypes was examined for deviation from the Hardy–Weinberg equilibrium using the exact $\chi 2$-test. The $\chi 2$-test and Fisher's exact test were used to compare groups. Odds ratio (OR) and 95% confidence interval (CI) were calculated. P value less than 0.05 was considered to be statistically significant (Bonferroni correction).

Results

The basic demographics and clinical characteristics of the two groups are summarized in Table 2. Both patients with HCC and HCV+ were selected randomly from the population living in Delta in Egypt. All patient groups were of matched distributions for age, sex, and clinical risk factors.

Table 3 demonstrates the distribution of genotypes and alleles of the genotype and alleles of MTHFR C677T gene polymorphism among the HCC and HCV groups.

On comparing the frequency of alleles, genotypes in two patient groups revealed statistical significance for MTHFR C677T gene polymorphism in HCC patients. The CT and TT genotype and the T allele of MTHFR C677T gene polymorphism all showed significant risks for developing HCC [(OR=1.92; 95% CI=1.11–3.32; P=0.019); (OR=4.91; 95% CI=1.32–18.24; P=0.018); (OR=2.113; 95% CI=1.36–3.29; P=0.001), respectively].

Table 4 demonstrates the association between the clinical and laboratory variables and the *MTHFR C677T* polymorphisms in HCC patients. There were no statistically significant relation between the *MTHFR C677T* genotypes and any of the clinical or laboratory variables.

Table 1: Characteristics of used primers and restriction enzyme

Polymorphisms		Primers	Annealing temperature (°C)	PCR product (bp)	Digestion	Bands (bp)
MTHFR C677T	**Forward**	5′-TGA AGGAGA ACG TGT CTG CGG GA-3′	60°C	198	Hinf I	175, 23
	Reverse	5′-AGG ACGGTG CGG TGA GAG TG-3′				

bp, base pair.

Table 2 Demographic and clinical data of the studied groups

Parameters	Groups	P
	HCC (n=128) [n (%)]	HCV (n=130) [n (%)]	
Sex			
Male	99 (77.3)	93 (71.5)	0.319
Female	29 (22.7)	37 (28.5)	
Age (years) (mean±SD)	59.2±3.8	58.3±4.6	0.09
BMI (kg/m2) (mean±SD)	26.9±3.5	27.5±3.1	0.15
DM			
Yes	69 (53.9)	73 (56.2)	0.802
No	59 (46.1)	57 (43.8)	
Cigarette smoking			
Yes	87 (68)	84 (64.6)	0.60
No	41 (32)	46 (35.4)	
Bilharziasis			
Yes	58 (45.3)	51 (39.2)	0.46
No	70 (54.7)	79 (60.8)	
Viral load (IU/ml) (median)	680 217	729 461	0.181
Liver cirrhosis			
Yes	107 (83.6)	98 (75.4)	0.124
No	21 (16.4)	32 (24.6)	
Child–Pugh grade			
A	72 (56.3)	67 (51.5)	0.457
B or C	56 (43.7)	63 (48.5)	

DM, diabetes mellitus; HCC; hepatocellular carcinoma; HCV, hepatitis C virus.

Table 3: The genetic distribution of the genotypes and alleles of MTHFR C677T SNP in HCC cases compared with HCV cases

Polymorphisms	Frequency		HCC (n=128) [n (%)]		HCV (n=130) [n (%)]		OR (95% CI)	P
MTHFR C677T	Genotypes	CC	71 (55.47)		95 (73.08)		R	–
		CT	46 (35.94)		32 (24.62)		1.92 (1.11–3.32)	0.019*
		TT	11 (8.59)		3 (2.3)		4.91 (1.32–18.24)	0.018*
	Alleles	C	188	73.44	222	85.38	R	–
		T	68	26.56	38	14.62	2.113 (1.36–3.29)	0.001*

R=reference category (OR=1.0).CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OR, odds ratio; SNP, single-nucleotide polymorphism. *Significant.

Table 4: The associations between the clinical and laboratory variables and the MTHFR C677T polymorphisms in HCC patients

Variables	Genotypes			P
	CC [n (%)]	*CT* [n (%)]	*TT* [n (%)]	
Serum AFP (ng/ml)				
<400	8 (26.9)	12 (43.3)	10 (29.9)	0.983
≥400	25 (24.6)	41 (37.7)	32 (36.1)	
Viral load (IU/ml)				
<400 000	14 (34.7)	19 (38.8)	13 (26.5)	0.915
≥400 000	23 (25.3)	37 (46.8	22 (27.9)	
Tumor stage				
I or II	12 (21.4)	33 (58.9)	11 (19.6)	0.808
III or IV	19 (26.4)	40 (55.6)	13 (18.1)	
Liver cirrhosis				
Yes	24 (32.4)	21 (28.4)	29 (39.2)	0.578
No	13 (24.1)	18 (33.3)	23 (42.6)	
Child–Pugh grade				
A	28 (33.3)	34 (40.5)	22 (26.2)	0.854
B or C	13 (29.5)	20 (45.5)	11 (25.0)	

AFP, α-fetoprotein; HCC, hepatocellular carcinoma.

Discussion

In the present study, HCC patients with HCV were found to have a higher proportion of *CT* and *TT* genotypes, compared with patients with HCV infection with no HCC. In the literature, contradictory results exist as regards the link between *MTHFR C677T* polymorphism and developing cancers, with some studies favoring its association with an increased risk for esophageal [20], gastric [21], ovarian [22], and lung cancers in Whites [23]. In contrast, *MTHFR C677T* single-nucleotide polymorphism (SNP) was linked to a reduced risk in colorectal carcinoma [24], lung cancer in Chinese population [25], and leukemia [26]. Although one meta-analysis denied an association between the *MTHFR C677T* polymorphism and risk for HCC [27], there are other reports suggesting the increased association of this SNP and HCC in Chinese [28–30], Korean [15], Indian [19], and Italian [28] populations and the overall increase in population susceptibility to HCC in other three meta-analyses [29–31]. Moreover, alcoholic cirrhotic men whose *MTHFR C677T* genotypes are TT were prone to a higher risk of developing HCC [16]. Our results are different from that observed in a previously published three studies, in European [32], Chinese [13], and Indian populations [19]. In the study in the European population, an association was

established between *MTHFR C677T* polymorphism and the risk for HCC only in patients with end-stage liver disease due to alcoholism, but not in HCC patients with viral cirrhosis, as in our study. Moreover, although our results demonstrated about two-fold and five-fold increases in the risk of developing HCC in association with the *CT* and *TT* genotypes of *MTHFR C677T* polymorphism, respectively, Saffroy et al. [32] found that the wild *MTHFR CC* genotype was associated with the risk for HCC, whereas the *TT* genotype was related to a reduced risk for HCC (protective). Further, Mu et al. [13] found HCC to be more related to the *CT* genotype; however, they did not report the etiology of the liver diseases. As regards the Indian population, both the *TT* genotype and T allele were associated with about two- fold increase in the susceptibility to HCC. However, in contrast to our study, all HCC patients enrolled in that study were proved to be positive for hepatitis B virus but negative for HCV infection. Moreover, in the same study, the statistical analysis of the genotypic distribution was established between HBV-related HCC patients and healthy controls, but not with HBV-positive individuals with no HCC [19]. Importantly, the thrombo and genetic mechanisms of *MTHFR 677TT* were recently suggested to have a role in the pathogenesis of liver cirrhosis in patients without HCV and HBV infections [33].Given that MTHFRR enzyme is directly involved in folate metabolism and folate deficiency, it may be associated with cancer risk in two ways [34]. First, the low levels of 5,10-methylenetetrahydrofolate *(MTHF)* would increase the ratio of deoxyuridylate monophosphate to deoxythy- midylate monophosphate, leading to increased thymidine depletion and increased uracil incorporation into DNA instead of thymine, resulting in an increased susceptibility to point mutations with DNA/chromosome breakage and failure of DNA repair mechanisms. The DNA destabilization may lead to chromosome aberrations and thus malignant transformation [35,36]. Second, the alteration of the S-adenosyl-l-methionine (SAM) levels results in changes in methylation and modification of DNA conformation and the gene expression. A less active form of *MTHFR* results in decreased SAM levels and, consequently, in hypomethylation [37], which predisposes to cancer. Thus, the *MTHFR T* allele may either increase the risk for HCC from the theory of DNA methylation or decrease it according to the DNA synthesis point of view [38,39]. The above- mentioned data may explain the discrepancies between the different studies as regards the association of *MTHFR C677T* SNP with HCC risk.

This study was limited by the absence of dietary folate measurement to determine any potential interaction between folate levels and *MTHFR* genotypes on risk for HCC. A second limitation may be the lack of the possible effect of other polymorphisms related to *MTHFR* gene and folate pathway on HCC. Third, because of high mortality rate in HCC patients in this single-center study, our sample may be not fully representative of the actual sample of HCC patients in our locality. However, this study has been strengthened by excluding the effect of HCV virus on the association of *MTHFR C677T* polymorphism with the risk for HCC, as we compared HCC patients with HCV-positive individuals. According to our knowledge, this study is the first one to demonstrate that HCV-related HCC.

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To cite this article: Wael Alkhiary, Tarek Shata, Ola Ali El-Emam. Methylenetetrahydrofolate Reductase C677T Gene Polymorphism in Hepatitis C Virus-related Hepatocellular Carcinoma: An Egyptian Perspective Study. BMFJ 2021;38(2):784-793. DOI: 10.21608/bmfj.2021.89248.1452