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Associations of methylene tetrahydrofolate reductase (MTHFR) polymorphism with hepatocellular carcinoma in Egyptian population

Fatma Abozeid^{1*} , Maysaa Zaki², Wafaa Nagah³, Amany Ragab², Aya fathy⁴, Dina Elhammady⁵, Hayam Ghazy⁶, Ahmed Yassen⁵, Mohamed Serria⁷, Omar Ammar⁸, Maha Ragab⁹ and Manal Nomir¹⁰

Abstract

Liver serves as a hub for key metabolic pathways such as folate cycle that provides one-carbon units for a network of metabolic reactions. Methylene tetrahydrofolate reductase (MTHFR) is a rate limiting enzyme in folate metabolism and thus it is vital for DNA methylation, synthesis and repair [1]. The objective of this study was to evaluate an eventual association between MTHFR polymorphisms C677T (rs1801133) and A1298C (rs1801131) and the susceptibility to hepatocellular carcinoma (HCC) in Egyptian population.

Blood samples from patients and controls from Mansoura university hospital were used after signed consent and approval from Medical ethical committee. The two genetic loci were designed for amplification and genotyped by using PCR-RFLP.

Our results clarify that, the most important predictors for HCC are T/T genotype of variant C677T and C/C genotype of variant (A1298C) with odds ratio 3.28 and 2.99 respectively. Also, MTHFR variant C677T genotype C/C or T/T combined with MTHFR variant A1298C genotype C/C were associated with an increased risk of HCC, with the OR, 2.6 and 7 respectively. CT genotype of MTHFR variant C677T showed significant difference between HCC grades and C allele of variant C677T showed significant difference in BCLC stages of HCC.

Our data indicates that, the two variants (C677T and A1298C) constitute a risk factor for the development of HCC and this could be attributed to the low activities of the enzyme MTHFR that disturb one carbon metabolism and subsequently, DNA synthesis, repair and methylation, thus cellular redox state, growth, and proliferation.

Keywords MTHFR, HCC, Polymorphism, MTHFR C677T, MTHFR A1298C

*Correspondence:

Fatma Abozeid

Profatma2000@mans.edu.eg; Profatma2000@hotmail.com

Full list of author information is available at the end of the article



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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world and the third leading cause of cancer deaths [2]. The etiology of liver cancer remains unclear with varied risk factors such as hepatitis, obesity, type 2 diabetes, nonalcoholic fatty liver disease, the lifestyle of the individuals (consumption of contaminated food with aflatoxin, heavy drinking of alcohol, ingestion of moldy foods, raw water drinking) and genetic predispositions [3].

The liver is the main site for the metabolic detoxification of carcinogens, and for storing vitamins which serve as cofactors for enzymes involved in different metabolic pathways such as one-carbon metabolism [4].

Folate metabolic reaction involves the conversion of homocysteine to methionine which is transformed along with ATP to produce S-adenosylmethionine (SAM). SAM is a key substrate for methyl transferases [5]. Disturbance of folate cycle hinders methionine cycle with intracellular accumulation of S-adenosyl-homocysteine (SAH). Hyperhomocysteinemia (Hcy) may accelerate the progression of liver fibrosis possibly leading to liver cancer. SAH is a potent inhibitor of (SAM) dependent methylation reactions [6]. Homocysteine is a vascular toxin which may cause endothelial injury at various organs; because it is involved in the pathogenesis of oxidative stress [7].

Genetic polymorphisms are risk factors which necessitate some other pathological conditions in order to trigger a chain of biological events that cause cancer [8]. Folate levels and metabolism are necessary for normal and malignant cells [9]. Damage to the liver caused by aflatoxin B1, smoke toxins, ethanol consumption, as well as inflammatory reactions of HBV/HCV infection, may lead to the alteration of folate metabolism [10].

Single nucleotide polymorphisms (SNPs) play an important role in normal human variation [11]. Methyltetrahydrofolate reductase (MTHFR) is a rate limiting enzyme in folate metabolism. MTHFR has two common genetic SNPs, (*C677T* and *A1298C*), encoding two MTHFR variants with a reduced activity and heterogeneous frequency in different regions of the world, about 10% in Caucasians [12]. MTHFR enzyme is coded by the MTHFR gene that is located on the short arm of chromosome 1 (1p36.3) and there are two isoforms in humans [13]. The first isoform is about 77 kDa protein, and the second is 70 kDa [14].

The single nucleotide polymorphism in MTHFR 677C-to-T results in a subsequent reduction in the enzyme activity and an increase in its thermolability [15]. The activity of the homozygous TT genotype is reduced by 70%, [16] and that the heterozygous CT genotype is reduced by 35% in comparison with the normal CC

genotype [17]. MTHFR is pivotal for one-carbon metabolism that fuels the methionine cycle, de novo purine synthesis, transsulfuration pathway, serine, glycine, glutathione, thymidine production, and NADPH pools, and thereby regulates cellular redox state, growth, and proliferation. Dietary folic acid is the starting material for the folate cycle and folate deficiency may be due to genetic variation in (MTHFR) gene. Folate deficiency is associated with an increase in circulating homocysteine, hypomethylation, and low level of 5-methyl tetrahydro folate (5-MTHF) leading to gene mutation or DNA strand breaks [18], [19]. Excess homocysteinemia in the presence of MTHFR variants C677T (rs1801133) and A1298C (rs1801131) increase pathology risks in general, and especially of, infertility, miscarriage and cancer susceptibility [20].

Excessive cigarette smoking and heavy alcohol consumption are associated with low plasma folate concentration, and hyperhomocysteinemia among patients with chronic hepatitis C infection, which may accelerate the progression of liver fibrosis in hepatic patients and possibly lead to liver cancer [21, 22]. MTHFR variant C677T has also been associated with an increased risk of invasive cervical cancer and premalignant lesions, which also indicate a possible role of MTHFR in viral infection-associated cancers [23]. The purpose of this case-control study is to assess the existence of associations between the MTHFR C677T and A1298C polymorphisms and the risk of HCC.

Material and method

The study is a case-control, including 50 patients with hepatocellular carcinoma on top of chronic HCV and 50 healthy subjects as a control group from Mansoura university hospital. The participants signed written approval consent. The study was approved by the Mansoura Faculty of Medicine ethical committee. Hepatocellular carcinoma was diagnosed by clinical, histopathological laboratory findings and radiological investigations including ultrasound and CT as indicated and reported according to Barcelona clinic liver cancer (BCLC). The patients with HCC had positive HCV antibodies and positive reverse transcriptase-polymerase chain for HCV genotype 4. Exclusion criteria included patients with hepatitis B virus, HIV, and patients with other cancers.

Fifteen-milliliter blood samples will be obtained from each participant and will be divided into three aliquots. One aliquot over EDTA for genomic DNA separation from the blood sample and the other aliquot will be used for sera separation. Sera will be subjected to the routine laboratory study of complete liver functions including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and direct bilirubin by

autoanalyzer (Dialab 450 system), determination of circulatory anti-HCV by Elecys system (Roche-diagnostic) and α fetoprotein (AFP) measurement by enzyme-linked immunosorbent assay (ELISA—DRG International Inc., USA.). The presence of HCV-RNA in patients' sera was detected by real-time polymerase chain reaction. The third aliquot will be used for total blood counts by Sysmex and the determination of INR.

Molecular study of MTHFR C677T and A1298C polymorphisms

DNA Extraction

DNA was extracted from the blood samples with a Qia-gen extraction kit from blood samples (Qiagen-Germany). The extracted DNA was kept frozen at -80°C till amplification.

Multiplex PCR for 677 and 1298 Loci of MTHFR Gene

Molecular study of methyl tetrahydrofolate reductase Polymorphism at 677 C /T and 1298 A / C polymorphisms was done by using PCR/RFLP. Odds ratios (OR) with 95% confidence intervals (CI) were used to assess the strength of association between both MTHFR C677T and A1298C polymorphisms and the risk of HCC.

The used primers for multiplex PCR are summarized in Table 1 [24]. The PCR reactions were conducted in 50 microns reaction mixture supplied by the Qiagen premix amplification kit. The mixture contains 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 50 pmol 677F, 50 pmol 677R, 100 pmol 1298F, and 100 pmol 1298R, 2.5 units of polymerase and 10 microns of the extracted DNA. The reaction mixture was incubated at 95°C for 8 min for initial activation of the Taq polymerase, followed by 40 cycles of 95°C for 1 min, 63°C for 1 min, and 72°C for 1 min, with a final extension for 7 min at 72°C in the thermal cycler (Biosystem).

Table 1 The primers used for multiplex PCR

Loci	Primers	PCR Product (bp)	Restriction Enzyme	Bands (bp)
MTHFR C677T	5'-TGAAGG	198-bp	Hinfl	175
	AGAAGG		TT	198-175
	TGTCTG		CT	198
	CGGGA-3'		CC	
	5'-AGGACG			
MTHFR A1298C	GTGCGGTGA	128-bp	Mobil	72
	GAGTG-3		AA	72-100
	5'-CAAGGA		AC	100
	GGAGCTGCT		CC	
	GAAGA-3'			
5'-CCACTC				
CAGCATCAC				
TCACT-3'				

bp Base pair

Appropriate amplification of the predicted fragments was confirmed by digestion of the multiplex PCR products with HinfI or MboII and then electrophoresis on a 3% agarose gel for identification of the variants 677 C/T and 1298 A/C polymorphisms. Conditions for RFLP digestion are exactly as described by the manufacturer (Thermo Scientific-USA) [25].

Statistical analysis

Data are expressed as mean \pm standard deviation or median for continuous data and number and % for discrete data. Non-normally distributed variables were analyzed by the Mann-Whitney test, while normally distributed variables were analyzed by t-test; categorical variables were compared using the Chi-square test. The effect of demographic parameters, clinical features, disease characteristics, genotypes, and alleles frequencies on HCC was investigated using univariate analysis in which all studied variables with p -value < 0.05 were considered as potential predictors and entered multivariable regression models. Deviation from Hardy-Weinberg equilibrium (HWE) expectations were determined using the chi-squared test. The statistical analysis was done by SPSS (statistical package for social sciences) version 25. Online SNP Stats software (<http://www.snpstats.net/start.htm>) analysis was applied to estimate the haplotypes and linkage.

Results

Table 2 shows the distribution of age, gender, and tumor characters among cases. More than two-thirds of the cases were male with a mean age of 55 years old. More than half of the cases were a class c Child-Pugh score (58%). The mean size of the mass was 4.8 cm. Two-thirds of the cases had one mass. Most of them were grade 3 and stage A Barcelona classification (42%). Two-thirds of them (60%) were right-sided tumors.

As in Table 3, platelets, albumin, ALT, AST and AFP were higher in cases than in control with a significant difference (P 0.01- 0.001). No significant difference was found regarding WBCs, Hb, and INR levels between the two groups.

Three genetic forms for each polymorphic locus were identified by RFLP. In cases, wild-type MTHFR 677CC was found in 30 (60%), the MTHFR 677CT heterozygous variant was present in 4 (8%), and the MTHFR 677 TT homozygous variant was found in 16 (32%). The MTHFR allele C was 64%, and T was 36%. While, in controls, wild-type MTHFR 677CC was found in 37 (74%), the MTHFR 677CT heterozygous variant was present in 10 (20%), and the MTHFR 677 TT homozygous variant was found in 3 (6%). The MTHFR allele C was 84% and T was 16%. For the allelic model C vs. T, the OR was 0.34 (95% CI:

Table 2 Demographic and clinical data of the studied cases

	No= (50)	%
Gender		
Male	36.0	72.0
Female	14.0	28.0
Age (mean ± SD)	55.42 ± 6.57	
Child–Pugh score		
Class A	1	2.0
Class B	20	40.0
Class C	29	58.0
Size	4.86 ± 1.71	
Number of mass		
1	36.0	72.0
2	14.0	28.0
Grade		
1	11	22.0
2	21	42.0
3	15	30.0
4	3	6.0
Barcelona classification		
Stage 0	15	30.0
Stage A	21	42.0
Stage B	12	24.0
Stage C	2	4.0
Site		
Right	30	60.0
Left	20	40.0

Data are expressed as Mean ± SD for continuous data and or no and % for discrete data

0.17–0.66, *p* 0.001) (Table 4). In cases, wild-type MTHFR 1298AA was observed in 21 (42%) while MTHFR 1298AC heterozygous variant was noted in 15 (30%) and the MTHFR 1298CC homozygous variant was seen in

14 (28%) OR (0.23) (95% CI: 0.07–0.81, *p* 0.02). While, in controls, wild-type MTHFR 1298AA was observed in 26 (52%) while MTHFR 1298AC heterozygous variant was noted in 20 (40%) and the MTHFR 1298CC homozygous variant was seen in 4 (8%). For the allelic model A vs. C, the OR was 1.09 (95% CI: 0.62–1.9, *p* 0.67).

MTHFR (C677T and MTHFR A1298C) polymorphisms and the risk of HCC

Using the C/C genotype as the reference for MTHFR C677T, ORs were 2.02 (95% CI: 0.58–7.11) and 0.15 (95% CI: 0.04–0.57 *P*<0.005) for the C/T and T/T genotypes, respectively with significant difference. OR for the T allele was 0.34 (95% CI: 0.17–0.66 *P* 0.001) when compared with the C alleles. Using the A/A genotype as the reference group for MTHFR A1298C, ORs were 1.08 (95% CI: 0.45–2.6) for the A/C without significant difference and 0.23 (95% CI: 0.62–1.9 *P* 0.02) for the C/C genotypes, with significant difference. Applying Hardy Weinberg equation (HWE), revealed that MTHFR C677T and in MTHFR A1298C in control subjects was in HWE while cases of both genotypes weren't in HWE. We evaluated the combined effect of different exposures on the risk of HCC. MTHFR 677 CC or TT genotype combined with MTHFR 1298 CC genotype were associated with an increased risk of HCC, with the OR, 2.6 and 7 respectively.

CT genotype of MTHFR variant C677T showed significant difference in different grade of HCC. The C allele of variant C677T showed significant difference BCLC stages. No significant difference in genotypes and alleles distribution exists between classes of Child–Pugh score or according to No or site of tumor mass (Table 5).

Regarding Multivariate analysis for HCC risk predictors, the most important predictors for HCC are T/T genotype of variant (C677T) and C/C genotype of

Table 3 Distributions of laboratory findings in HCC patients and control subjects

	Case	Control	t-test	P value
WBCs (mean ± SD) *10 ³ /ml	7.22 ± 3.96	7.55 ± 2.22	t-test = -0.52	0.603
Hb (mean ± SD) gm/dl	13.122 ± 2.48	13.11 ± 1.79	t-test = 0.03	0.97
Platelets (mean ± SD) *10 ³ /ml	156.98 ± 83.61	221.44 ± 61.04	t-test = -4.4	<0.001*
INR (mean ± SD)	1.31 ± 0.49	1.19 ± 0.15	t-test = 1.6	0.09
Albumin (mean ± SD) gm/dl	3.72 ± 0.53	3.99 ± 0.53	t-test = -2.5	0.01*
ALT (IU/L) Median (min–max)	45 (20.06–85.0)	28 (20.0–37.0)	Mann Whitney Z = -5.9	<0.001*
AST (IU/L) Median (min–max)	56 (20.0–96.0)	27 (21–34)	Mann Whitney Z = -6.9	<0.001*
AFP (IU/L) (mean ± SD)	137.71 ± 110.3	5.94 ± 2.21	t-test = 8.4	<0.001*

WBCs White blood cells, Hb Hemoglobin, INR International normalized ratio, ALT Alanine aminotransferase, AST Aspartate aminotransferase, AFP Alpha-fetoprotein

* Significant *p* ≤ 0.05

Table 4 The joint genotypes of the MTHFR677 and MTHFR1298 polymorphisms among cases and controls and risk of HCC

Loci	Genotypes and alleles		Case (n=50)		Control (n=50)		OR	95% CI	P
			N	%	N	%			
MTHFR C677T	Genotypes	CC	30	60	37	74	1.9	0.81–4.43	0.1
		CT	4	8	10	20	2.02	0.58–7.11	0.27
		TT	16	32	3	6	0.15	0.04–0.57	<0.005*
	Alleles	C	64	64	84	84	0.34	0.17–0.66	0.001*
		T	36	36	16	16			
Hardy–Weinberg	×2	34.1		3.27					
	P	<0.001*		0.07					
MTHFR A1298C	Genotypes	AA	21	42	26	52	1.4	0.6–3.2	0.2
		AC	15	30	20	40	1.5	0.68–3.56	0.3
		CC	14	28	4	8	0.23	0.07–0.81	0.02*
	Alleles	A	55	55	52	52	1.09	0.62–1.9	0.67
		C	45	45	48	48			
Hardy–Weinberg	×2	7.01		0.003					
	P	0.008*		0.9					
Combined analysis	C677T A1298C		Case		Control				
	CC	AA	11	52.4	19	73.1	0.58	0.23–1.44	0.19
	CC	AC	11	78.6	15	75	0.73	0.28–1.9	0.48
	CC	CC	8	53.3	3	75	2.6	0.59–13.56	0.15
	CT	AA	3	14.3	7	26.9	0.43	0.08–1.99	0.22
	CT	AC	1	7.1	3	15	0.33	0.01–3.8	0.32
	CT	CC	0	0	0	0	-	-	-
	TT	AA	7	33.3	0	0	-	-	0.01*
	TT	AC	2	14.3	2	10	1	0.1–10.44	1
	TT	CC	7	46.7	1	25	7	0.81–156.8	0.04*

OR Odds ratio, CI Confidence interval, r Reference category (OR = 1.0)

* Significant $p \leq 0.05$

variant (A1298C) with odds ratio 3.28 and 2.99 respectively (Table 6).

Discussion

HCC is a complex disease that involves multistep with multiple genes and gene-environment interactions. MTHFR is a critical enzyme in both DNA synthesis and methylation, thereby affecting DNA stability and gene expression; thus playing an important role in tumor progression. The frequency of the variant genotype is heterogeneous in different regions of the world.

The association of MTHFR C677T (rs1801133) and A1298C (rs1801131) polymorphism with HCC was more conflicting. Molecular epidemiologic studies have revealed that MTHFR polymorphisms are associated with an increased risk of esophageal cancer, breast cancer [26], gastric cancer [27], hepatocellular carcinoma [28], cervical neoplasia [29], [30], bladder cancer [31], and indolent prostate cancer [32]. Conversely, MTHFR polymorphisms have also been associated with a reduced risk of colon cancer [33], leukemia and lymphoma [34]

and highly aggressive prostate cancer [35]. In addition, no association has been found between the C677T variant and colon adenoma and lung cancer [36].

Our study was a case–control, including 50 patients with hepatocellular carcinoma on top of chronic HCV and 50 healthy subjects, we evaluated the association between MTHFR polymorphisms C677T (rs1801133) and A1298C (rs1801131) and the susceptibility to hepatocellular carcinoma (HCC) in tertiary medical Egyptian center. More than two-thirds of the cases were male with a mean age of 55 years old. This goes with literature according to the demographic distribution of HCC [37]. Platelets count, serum albumin, ALT, AST and AFP showed a significant difference in cases than control as more than half of the cases were a class C Child–Pugh score (58%).

Three genetic forms for each polymorphic locus were identified by RFLP. Our study revealed that both cases of HCC and controls have more mutant alleles (i.e., homozygous mutant at either the 677 or 1298 site or heterozygous mutant at both loci) but without significant

Table 5 Genotypes and alleles distribution in different HCC presentation

Child–Pugh score		Class A		Class B		Class C		P value	
(677 C-T)	CC	1(3.3%)	14(46.7%)	15(50.0%)				0.312	
	CT	0	1(25.0%)	3(75.0%)				0.76	
	TT	0	5(31.2%)	11(68.8%)				0.49	
	C	2(3.1%)	29(45.3%)	33(51.6%)				0.16	
	T	0	11(30.6%)	25(69.4%)					
(1298 A-C)	AA	1(4.8%)	6(28.6%)	14(66.7%)				0.2	
	AC	0	7(46.7%)	8(53.3%)				0.68	
	CC	0	7(50.0%)	7(50.0%)				0.58	
	A	2(3.6%)	19(34.5%)	34(61.8%)				0.24	
	C	0	21(46.7%)	24(53.3%)					
No of mass		One mass		Two masses					
(677 C-T)	CC	20(66.7%)	10(33.3%)					0.24	
	CT	4(100.0%)	0					0.25	
	TT	12(75.0%)	4(25.0%)					0.5	
	C	44(68.8%)	20(31.2%)					0.23	
	T	28(77.8%)	8(22.2%)						
(1298 A-C)	AA	16(76.2%)	5(23.8%)					0.4	
	AC	9(60.0%)	6(40.0%)					0.16	
	CC	11(78.6%)	3(21.4%)					0.39	
	A	39(70.9%)	16(29.1%)					0.48	
	C	33(73.3%)	12(26.7%)						
Grade		1		2		3		4	
(677 C-T)	CC	8(26.7%)	12(40.0%)	10(33.3%)	0			0.13	
	CT	0	1(25.0%)	1(25%)	2(50%)			0.002*	
	TT	3(18.8%)	8(50.0%)	4(25.0%)	1(6.2%)			0.88	
	C	16(25.0%)	25(39.1%)	21(32.8%)	2(3.1%)			0.26	
	T	6(16.7%)	17(47.2%)	9(25.0%)	4(11.1%)				
(1298 A-C)	AA	4(19.0%)	9(42.9%)	5(23.8%)	3(14.3%)			0.19	
	AC	4(26.7%)	5(33.3%)	6(40.0%)	0			0.45	
	CC	3(21.4%)	7(50.0%)	4(28.6%)	0			0.86	
	A	11(29.1%)	22(40.0%)	16(20.0%)	6(10.9%)			0.15	
	C	11(24.4%)	20(44.4%)	14(31.1%)	0				
BCLC		Zero		1		2		3	
(677 C-T)	CC	11(36.7%)	14(46.7%)	5(16.7%)	0			0.1	
	CT	0	1(25.0%)	3(75.0%)	0			0.09	
	TT	4(25.0%)	6(37.5%)	4(0.25%)	2(12.5%)			0.2	
	C	22(34.4%)	29(45.3%)	13(20.3%)	0			0.02*	
	T	8(22.2%)	13(36.1%)	11(30.6%)	4(11.1%)				
(1298 A-C)	AA	6(28.6%)	10(47.6%)	4(19.0%)	1(4.8%)			0.88	
	AC	3(20.0%)	8(53.3%)	4(26.7%)	0			0.51	
	CC	6(42.9%)	3(21.4%)	4(28.6%)	1(7.1%)			0.29	
	A	15(27.3%)	26(47.3%)	12(21.8%)	2(3.6%)			0.7	
	C	15(33.3%)	16(35.6%)	12(26.7%)	2 (4.4%)				
Site		Right		Left					

Table 5 (continued)

(677 C-T)	CC	17(56.7%)	13(43.3%)	0.38
	CT	2(50.0%)	2(50.0%)	0.52
	TT	11(68.8%)	5(31.2%)	0.29
	C	36(56.2%)	28(43.8%)	0.21
	T	24(66.7%)	12(33.3%)	
(1298 A-C)	AA	13(61.9%)	8(38.1%)	0.52
	AC	8(53.3%)	7(46.7%)	0.37
	CC	9(64.3%)	5(35.7%)	0.47
	A	32(58.2%)	23(41.8%)	0.41
	C	28(62.2%)	17(37.8%)	0.17

BCLC Barcelona clinic liver cancer

* Significant $p \leq 0.05$ **Table 6** Multivariate analysis for HCC predictors

	B	P value	Exp(B)	95.0% C.I for EXP(B)	
				Lower	Upper
Platelets	0.01	0.4	1.01	0.99	1.02
Albumin	0.62	0.49	0.54	0.09	3.11
ALT	0.17	0.00	0.84	0.77	0.92
AST	0.004	0.58	1.0	0.99	1.02
AFP	0.34	0.00	0.71	0.59	0.86
TT (C677T)	3.68	0.1	3.28	0.48	3.28
CC (A1298C)	1.09	0.2	2.99	0.57	15.68

CI confidence interval, ALT alanine aminotransferase, AST aspartate aminotransferase, AFP alpha-fetoprotein

difference. Most of cases and control were of wild type MTHFR. Wild-type MTHFR 677CC was found in 30 (60%) of cases and in 37 (74%) of control. While wild-type MTHFR 1298AA was observed in 21 (42%) of cases and in 26 (52%) of controls. For the allelic model C vs. T, the OR was 0.34 (95% CI: 0.17–0.66, p 0.001) while for the allelic model A vs. C, the OR was 1.09 (95% CI: 0.62–1.9, p 0.67).

Using the C/C genotype as the reference for MTHFR C677T, ORs were 2.02 (95% CI: 0.58–7.11) and 0.15 (95% CI: 0.04–0.57 $P < 0.005$) for the C/T and T/T genotypes, respectively with significant difference. Saffroy et al. [38] found that the wild MTHFR CC genotype was associated with the risk for HCC especially in patients with high alcohol consumption, whereas the TT genotype was related to a reduced risk for HCC (protective). MTHFR CC genotype was significantly higher in patients who had developed HCC on alcoholic cirrhosis rather than on viral cirrhosis ($P = 0.002$) or on non-cirrhotic livers ($P = 0.02$). The relative risk was 2.03. Further, Mu et al. [39] found that the MTHFR 677 C/T

genotype was associated with an increased risk of primary liver cancer in a Chinese population.

CT genotype of MTHFR variant C677T showed significant difference in different grade of HCC. The C allele of variant C677T showed significant difference in BCLC stages. This may highlight the genetic susceptibility and role of gene polymorphism in HCC. Regarding Multivariate analysis for HCC risk predictors, the most important predictors for HCC are T/T genotype of variant (C677T) and C/C genotype of variant (A1298C) with odds ratio 3.282, 2.01 and 2.99 respectively.

Small sample size which represents a single center study may be a limiting factor. Also, serum folic acid was not measured among patients enrolled in this study despite that most of them have malnutrition status and mostly had low folate intake which is an independent factor affecting methylation of DNA.

Our results conclude that MTHFR polymorphism is a risk factor for the development of HCC but additional studies to further define their influence in the different stages of hepatocarcinogenesis are required.

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Not applicable.

Conflicts of interests

Nothing to declare by the authors.

Confirmation

*All authors have confirmed the manuscript for submission in this journal.

*The content of this manuscript has not been published, or submitted for publication elsewhere.

Authors' contributions

All authors contributed to the study conception and design. Data collection and analysis were performed by Fatma Abozeid, Dina Elhammady, Ahmed yassen. Material preparation by Amany Ragab, Aya fathy, Mohamed Serria, Omar Ammar. The manuscript was written by Maysaa Zaki, Fatma Abozeid. All authors read and approved the final manuscript.

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Availability of data and materials

Data were collected from the registered medical records following the institutional ethics committee and agreement with the Helsinki Declaration of 1975, revised in 2008.

Declarations**Ethics approval and consent to participate**

The Ethical Committee of the Faculty of Medicine of Mansoura University approved the study and the patients to participate. The participants signed written approval consent.

Consent for publication

All authors have confirmed the manuscript for submission and publication in this journal.

Competing interests

Nothing to declare.

Author details

¹Gastroenterology and Hepatology, Internal Medicine Department, Mansoura Specialized Medical Hospital, Mansoura University, Faculty of Medicine, Mansoura, Egypt. ²Clinical Pathology, Mansoura University, Faculty of Medicine, Mansoura, Egypt. ³Clinical Oncology & Nuclear Medicine, Mansoura University, Faculty of Medicine, Mansoura, Egypt. ⁴Department of Community Medicine, Mansoura University, Faculty of Medicine, Mansoura, Egypt. ⁵Tropical Medicine, Mansoura University, Faculty of Medicine, Mansoura, Egypt. ⁶Medical Oncology - Faculty of Medicine - Mansoura University, Mansoura, Egypt. ⁷Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Mansoura University, Mansoura, Egypt. ⁸Basic Science Department, Delta University for Science and Technology, Gamasa, Egypt. ⁹Internal Medicine Department, Mansoura University, Faculty of Medicine, Mansoura, Egypt. ¹⁰Clinical Pathology, Mansoura University, Faculty of Medicine, Mansoura, Egypt.

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