Association of MTHFR C677T Variant Genotype with Serum Folate and Vit B12 in Iranian Patients with Colorectal Cancer or Adenomatous Polyps

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Research article

Keywords: Colorectal cancer, C677T, MTHFR, Folate, Vitamin B12, TaqMan

Posted Date: October 5th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-58792/v1

Abstract

Background: The incidence of colorectal cancer (CRC) has increased vigorously during the last decades in Iran and clinical studies suggest that moderate deficiency of methylenetetrahydrofolate reductase *(MTHFR*) and essential folate dietary intake may reduce the risk of CRC.

The aim of this study was to investigate the clinical significance of C677T polymorphism within *MTHFR* gene and its correlation with the serum folate and Vit B_{12} in Iranian population suffering from CRC.

Methods: Blood samples were taken from 207 Iranian individuals (103 males and 104 females) who were referred for colonoscopy. TaqMan probe assay was performed for C677T *MTHFR* polymorphism. Besides, sera were fractionated from the blood samples of 43 patients and controls and folate and Vit B_{12} concentrations were measured by a monobind kit. Finally, the correlation of *MTHFR* polymorphisms and folate/vitamin-B_{12} with CRC risk were analyzed.

Results: In the current study, the most percentage of the *MTHFR* polymorphisms in healthy individuals belongs to CC genotype (wild type). In patients with adenoma the percentage of CC and CT genotype were approximately similar, but surprisingly in adenocarcinoma the percentage of CC genotype (wild type) was the highest (approximately 50%), and the percentage of CT and TT genotypes were slightly similar. Our study revealed that there was no significant difference between the amount of folate and Vit B_{12} in case and control groups (p value >0.05).

Conclusions: We concluded that there is an association between pivotal concentration of serum folate/vitamin B_{12} and CRC, and this association is altered by *MTHFR* C677T genotype.

Background

The incidence of colorectal cancer (CRC) has rapidly increased globally [1]. It is the second most common cancer and the fourth leading cause of cancer death (about 1,400,000 new cases and about 700,000 deaths) in the world. Because of smoking and Westernized diet (high intake of meat and fat), CRC incidence has also been raised in Iran during the recent decades [2–6]. Immigration studies disclosed that the CRC incidence in immigrants of industrialized countries is in a higher rate than individuals who still live in their native countries. This report was observed among Japanese moving to the United States in 1960s, when the number of CRC cases had increased [7].

This cancer is a multifactorial disease, involving genetic, epigenetic and environmental risk factors [8]. There are several number of studies on the molecular pathway of CRC [9]. A recent study has reported the impact of folate intake on CRC tumorigenesis by transforming the template of gene expression, which indicates an association of polymorphisms among folate methabolizing genes and the establishment of CRC [7]. Folate is an important water-soluble B vitamin that is presented naturally in foods like vegetables, fruits, and whole grains. Folate's function highly depends on Vit B_{12}, which is a coenzyme in methionine synthase in colorectal tumorigenesis. The synthetic form of folate is folic acid that is added

to vitamin supplements and foods like cereals in many countries, which have right food programs [7, 10, 11].

Methylenetetrahydrofolate reductase (*MTHFR*) is an essential enzyme regulating folate-metabolizing pathway, which effects DNA synthesis and methylation. Conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate occurs by *MTHFR*, which is necessary for homocysteine methylation to methionine. Methionine is converted to S-adenosylmethionine (SAM), a common methyl donor in many transmethylation reactions, such as methylation of DNA, RNA, proteins, and other molecules. The *MTHFR* gene polymorphism is betided with single nucleotide variants within codon 677 in exon 4 (C to T or Ala to Val). The result of this variant is to encode a thermoliable enzyme with reduced activity causing a decreased plasma folate level [12–14]. The 677CC is the wild type form of *MTHFR* gene. The 677TT homozygous variant have less than 30% of normal enzyme activity, and heterozygotes CT genotype have 65% of normal enzyme activity [15]. In the current article, we investigated the association of folate/Vit B12 concentrations in serum with *MTHFR* C677T polymorphism in Iranian patients with cancerous and precancerous lesions of colon.

Methods

Study population and samples:

A total of 116 blood samples of cancer patients (98 adenomas and 18 adenomacarcinoma) as well as 91 normal blood samples were collected from Reza Radiotherapy and Oncology Center (RRCO) in Mashhad, Iran with the ethic committee approval of Mashhad University of Medical Sciences, Mashhad, Iran (grant #961906). Informed written consent had been obtained from all participants in this study. Peripheral blood samples were taken using EDTA +/- tubes.

MTHFR C677T polymorphism analysis

In this study, we demonstrate the successful detection of *MTHFR* C677T polymorphism in DNA extracted from whole blood by the use of real-time PCR (TaqMan® assay).

The DNA was extracted from 300 ul of blood using the salting out method [16]. Primer and probe were synthesized by Bioneer company (Bioneer Corporation, South Korea). The sequences of the primer and probe were as follows: primer forward, 5′-TGACCTGAAGCACTTGAAGGAGAA-3′, primer reverse, 5′-GGAAGAATGTGTCAGCCTCAAAGA-3′, probe C, 5′-ATGAAATCGGCTCCCG-3′ (reporter: FAM), probe T, 5′-ATGAAATCGACTCCCG-3′ (reporter Cy5).

Folate and vitamin B12 measurement

Serum folate and vitamin B_{12} measurements were limited to 43 case and control samples. For this reason, 5 mL of blood was collected and the serum was obtained by a centrifugation method. Serum was then stored frozen at − 80 °C until the time of usage. The determination of serum folate/vitamin B12 was

done using ACCUBIND ELISA folate/Vit B12 test system kits (Monobind Inc., Lake Forest, CA 92630, USA).

Statistical analysis

Fisher's exact test was used to compare characteristics between case and control groups. The association of *MTHFR* polymorphisms, folate/Vit B_{12} dietary, and CRC risk were evaluated. To calculate odds ratio (OR), 95% confidence intervals (95% CI), and logistic regression was applied.

Results

In this study, we investigated the association of *MTHFR* polymorphisms, folate and vitamin B_{12} levels in Iranian patients with CRC and adenoma lesions. The details of age, gender distribution and demographic characteristics of samples were shown in Tables 1, 2 and 3.

Table 1
MTHFR 677T polymorphism in normal samples

NORMAL SAMPLES		
Sex		Number (%)
	Female	49 (53.84%)
	Male	42 (46.15%)
Age		57[36,76]
Genotype		
	Normal / CC	55 (60.43%)
	Normal / CT	30 (32.96%)
	Normal / TT	6 (6.59%)

Table 2
MTHFR 677T polymorphism in adenoma samples

ADENOMA SAMPLES		
Sex		Number (%)
	Female	48 (48.97%)
	Male	50 (51.02%)
Age		57 [27,80]
Genotype		
	Tubular adenoma / CC	41 (41.83%)
	Tubular adenoma / CT	30 (30.61%)
	Tubular adenoma / TT	3 (3.061%)
	Tubulavillous adenoma / CC	4 (4.081%)
	Tubulavillous adenoma / CT	11 (11.22%)
	Tubulovillous adenoma / TT	5 (5.1%)
	Serrated adenoma / CC	1 (1.02%)
	Traditional serrated adenoma / CT	2 (2.04%)
	Villous / CC	1 (1.02%)
Location		
	Anal	1 (1.02%)
	Rectum	28 (28.57%)
	Sigmoid	46 (46.93%)
	Transvers colon	0
	Descending colon	8 (8.16%)
	Ascending colon	12 (12.24%)
	Cecum	3 (3.06%)
Dysplasia		
	Low grade	80 (81.63%)
	High grade	18 (18.36%)

Table 3
MTHFR 677T polymorphism in adenocarcinoma samples

ADENOCARCINOMA SAMPLES		
Sex		Number (%)
	Female	7 (38.8%)
	Male	11 (61.11%)
Age		59 [27–80]
Genotype		
	Adenocarcinoma / CC	9 (50%)
	Adenocarcinoma / CT	5 (27.77%)
	Adenocarcinoma / TT	4 (22.22%)
Location		
	Anal	0
	Rectum	5 (27.77%)
	Sigmoid	6 (33.33%)
	Transverse colon	3 (16.66%)
	Descending colon	0
	Ascending colon	2 (11.11%)
	Cecum	2 (11.11%)

The median age was 57, 57 and 59 in normal, adenoma and adenocarcinoma groups respectively. In normal samples, 53.84% and 48.15% were female, and male, respectively and the percentage of genotypes were; 60.43% CC, 32.96% CT, 6.59% TT (Table 1). In adenoma samples 48.97% were females and 51.02% were males. Different types of adenoma samples had different percentages of polymorphisms as 47.95%, 43.87% and 8.16% belonged to CC, CT and CT genotypes, respectively. The majority and minority of polymorphisms in the adenoma group were detected in sigmoid and anal, respectively. Approximately 81.63% was low grade and 18.36% was high grade adenoma (Table 2).

In the adenocarcinoma group, 38.8% and 61.11% were female and male, respectively. The percentage of genotypes were: 50%, 27.77% and 22.22% for CC, CT and TT, respectively. The most polymorphism of adenocarcinoma group was detected in the sigmoid and the least was in the anal and descending colon (Table 3).

There was no significant difference between adenoma and controls (P > 0.05) (Table 4). On the other hand, we found a significance difference between adenocarcinoma and normal cases in the CC (P = 0.0039) and TT (P = 0.0001) genotypes (Table 5). The OR for TT and CT genotypes between two groups was 14.17, 95%CI = 94.09–48.98, and 1.59, 95%CI = 90.88–2.88, respectively.

Table 4
Statistical analysis of *MTHFR* polymorphism in adenoma and normal controls

Genotype	Adenoma	Normal	P value
CC	47 (47.95%)	55 (60.43%)	0.1081
CT	43 (43.87%)	30 (32.96%)	0.1368
TT	8 (8.16%)	6 (6.593%)	0.7846
Total	98	91	-

Table 5
Statistical analysis of *MTHFR* polymorphisms in adenocarcinoma and normal samples

Genotype	Adenocarcinoma	Normal	P value
CC	9 (50%)	55 (60.43%)	0.0001
CT	5 (27.77%)	30 (32.96%)	0.7865
TT	4 (22.22%)	6 (6.593%)	0.0039
Total	18	91	-

The mean concentration of serum folic acid was 13.4 and 6.7 ng/ml in cancer and normal groups, respectively. The maximum (and minimum) amount of folic acid in cancer patients and normal individuals was 25 (5.8) ng/ml and 58 (3.9) ng/ml, respectively. The median concentration of folic acid in cancer and normal group was 13.42 and 13.54, respectively (Table 6).

Table 6
Amount of folate in case and control samples

	Adenocarcinoma		**Normal**	
	Folic Acid	***Vit B12***	***Folic Acid***	***Vit B12***
Mean	13.42	420.91	6.7	290
Max.	25	1001	13.54	283
Min.	5.8	8.2	20	869
Median	12.56	287	13.55	413.09

The mean concentration of serum Vit B_{12} in cancer and normal groups was respectively 420 ng/ml and 413 ng/ml. Besides, the maximum (and minimum) amount of Vit B_{12} in cancer and normal was 1001 (8.2) ng/ml and 1001(149) ng/ml, respectively. As a result, there was not a significant difference between the amount of folic acid and Vit B_{12} in cancer and normal groups (P = 1.00 in both groups) (Table 7).

Table 7
Statistical analysis of folate and Vit B_{12} in adenocarcinoma and normal samples

	No.	Folate (ng/mL) mean (range)	Low folate[1] /High folate[2] *n* (%)	P value	Vit B_{12} (pg/mL) mean (range)	Low Vit B_{12}[3]/High Vit B_{12}[4] *n* (%)	P value
Adeno-carcinoma	20	13.42 (5.8–25)	10 (50%), 10 (50%)	1	420 (8.2–1001)	10 (50%), 10 (50%)	1
Normal	23	13.54 (3.9–58)	11 (47%), 12 (53%)	-	413 (149–1001)	11 (47%), 12 (53%)	-

[1]Low folate= [Min. (Folate)- Median (Folate)] [2]High folate= [Median (Folate)-Max. (Folate)]

[3]Low Vit B12= [Min. (Vit B12)- Median (Vit B12)] [4]High Vit B12= [Median (Vit B12)-Max. (Vit B12)]

Discussion

Several studies have showed the correlation of *MTHFR* gene polymorphisms with the risk of CRC [17–19]. Some previous studies suggested that the concentration of Vit B_{12} separately or in combination with other factors (e.g. folate), may have effect on different parts of the colon and rectal [20]. While folate is a vitamin B group involved in multiple biochemical processes, it acts as an important modulator of carcinogenesis. Folic acid is a pivotal nutrient in one-carbon cycle, which has a role in the synthesis of nucleotides and methylation reactions. Besides, *MTHFR* enzyme has an important role during synthesis, repair and methylation of DNA, as well as a role in circulating folate levels [21].

In the current study, we investigated the association of folate/vitamin B12 concentrations with *MTHFR* C677T polymorphisms in cancerous and precancerous colon lesions among Iranian patients. In this study, we did not observe an increase in C677T and 677TT *MTHFR* polymorphisms in CRC patients.

Previous studies on the association of C677T polymorphism and susceptibility to CRC, reported no constant consequences. Some studies suggested a protective effect of TT genotype for colon cancer, because during experiment, reduced risk of CRC progress was observed in TT individuals with a sufficient folate intake.

Several other studies suggested that 677TT MTHFR, the phenotype of valine amino acid, significantly depended on folate approachability. A recent in-vitro study on HCT116 colon carcinoma cells showed the association of valine amino acid (TT genotype) with increased genomic DNA methylation in an adequate folate level and a significantly lower DNA genomic methylation in folate deficiency. Thus, folate is a modifier of genotype effect. In fact, biochemical changes in the valine-containing enzyme is important, which shows the enzyme stabilization by the addition of 5-MTHF to the culture medium. Thus, folate might modify correlation between other SNPs and the CRC risk [22]. Migration and proliferation of cancer cell are two important events in cancer development. The main cause of death for cancer patients is metastasis, migration of cancerous cell from organ to other organs. A previous study showed that about 10 μM folic acid reduced the migration and proliferation of human cell lines (COLO-205, LoVo and HT-29) in CRC [23].

It has been shown that MTHFR 677TT genotype is one strong reason of decreased risk of proximal colon cancer. The site-specific analysis indicated the role of different molecular alterations in carcinogenesis in proximal and distal of colon and rectum. The more frequent genetic alterations in distal site are *K-ras* and *P53* mutations as well microsatellite instability (MSI) is particularly in proximal site of colon in cancer [24, 25]. Totally, decreased risk of distal colon cancer, rectal cancer and proximal colon cancer have been reported to be associated with 677TT genotype. In the present study we did not find any increase of CRC risk in MTHFR 677TT genotype[15].

Some studies have shown a reduced risk of developing CRC with a TT genotype with a sufficient folate intake suggesting a protective effect [26, 27] although some other studies were not able to display a protective effect of the MTHFR 677TT genotype against CRC, even in high folate dietary intake [28, 29].

Some results have reported that the 677TT genotype could cause a considerable risk reduction of CRC. In high folate intake cases, the risk of CRC risk is reduced for both MTHFR 677CC and 677TT genotypes [30].

A meta-analysis study managed in twenty-five different countries, reported that different countries intake different sources of folate. Its results indicated that in Asia the 677TT genotype was admitted to a considerable CRC risk reduction [31].

A recent study observed a lower CRC risk through MTHFR 677TT genotype carriers of MTHFR C677T. There is an association between the MTHFR polymorphism and dietary methyl supply, but the relation remains inconsistent. Several studies reported the association of high-methyl diets like high folate dietary intake and low alcohol consumption with the protective effect of MTHFR 677TT genotype of the MTHFR C677T polymorphism [15, 32–35]. In contrast, another study reported that there is no association of folate status with the protective effect of MTHFR 677TT genotype compared with the CC/CT genotype. [19].

Conclusions

In summary, our study revealed that there was no significant difference between the amount of folate and Vit B_{12} in case and control groups. Furthermore, our results demonstrated the association between folate/vitamin B12 and heterozygous/homozygous C677T MTHFR genotype.

Abbreviations

MTHFR: Methylenetetrahydrofolate reductase; CRC: Colorectal cancer; SNP: single nucleotide polymorphism.

Declarations

Ethics approval and consent to participate

This study was ethically approved by ethic committee approval of Mashhad University of Medical Sciences, Mashhad, Iran (grant #961906). Informed written consent had been obtained from all participants in this study.

Consent to publish

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare no conflict of interest with respect to this research.

Funding

This study was supported financially by Mashhad University of Medical Sciences (Grant number: 961906).

Authors' Contributions

MAK and JB were responsible for writing the paper and the original draft. MAK was responsible for the study conception. MG was responsible for the investigation and conducting the experiments. All authors were responsible for reviewing and editing the paper.

Acknowledgements

We thank Mashhad University of Medical Sciences and Reza Radiotherapy and Oncology Center for supporting this study and special thanks to Ms Maryam Yassi and Ms Marjan Azghandi for conducting the statistical analysis and collecting data, respectively.

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