Association of \textit{MTHFR} C677T variant genotype with serum folate and Vit B12 in Iranian patients with colorectal cancer or adenomatous polyps

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\textbf{Research Article}

\textbf{Keywords:} Colorectal cancer, C677T, MTHFR, Folate, Vitamin B12, TaqMan

\textbf{Posted Date:} April 2nd, 2021

\textbf{DOI:} https://doi.org/10.21203/rs.3.rs-58792/v2

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Abstract

**Background:** The incidence of colorectal cancer (CRC) has increased during recent years in Iran and other developing countries. Clinical studies suggest that essential folate dietary intake and moderate deficiency of methylenetetrahydrofolate reductase (*MTHFR*) may protect and reduce the risk of CRC.

The aim of the present 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 the Iranian population suffering from CRC.

**Methods:** Blood samples were taken from 1017 Iranian individuals (517 cases and 500 controls) who were referred for colonoscopy. TaqMan probe assay was performed for C677T *MTHFR* polymorphism. 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. The correlation of *MTHFR* polymorphisms and folate/vitamin-B$_{12}$ with CRC risk were analyzed.

**Results:** In the current study, we found the frequency of three different genotypes of *MTHFR* polymorphism in the Iranian population i.e., CC, CT and TT, to be 51.31, 26.73, 21.96 and 61, 32.2, 6.8 in case and control groups, respectively. The homozygote genotype of *MTHFR* rs1801133 polymorphism is associated with increased risk of CRC by 3.68, 1.42 and 3.74-fold in codominant, dominant and recessive models respectively (p value< 0.01). 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:** This study revealed that there was no significant difference between the amount of folate and Vit B$_{12}$ in the case and control groups. Furthermore, our results demonstrated a higher risk association for 677TT and 677TT+C677T genotypes of *MTHFR* compared with 677CC carriers among CRC patients.

**Background**

Colorectal cancer (CRC) is the third most diagnosed cancer and the second leading cause of cancer death (about 1,900,000 new cases and about 935,000 deaths) in the world [1]. This cancer is a multifactorial disease, involving genetic, epigenetic and environmental risk factors [2, 3]. Because of smoking and Westernized diet (high intake of meat and fat), the incidence has also been raised in Iran during the recent decades [4-8].

It has been shown that the dietary Vit B$_{12}$ alone or combined with other factors (e.g. folate), could affect different parts of the colon and rectal [9]. While folate is a vitamin B group involved in multiple biochemical processes, it acts as an important modulator of carcinogenesis.

Folic acid, the synthetic form of folate, is a pivotal nutrient in one-carbon cycle, which has major roles in the synthesis of nucleotides and methylation reactions. Besides, methylenetetrahydrofolate reductase (*MTHFR*) enzyme has crucial functions during synthesis, repair and methylation of DNA, as well as a duty in circulating folate levels [10]. Several studies have reported the impact of folate intake on tumorigenesis by transforming the template of gene expression, which indicates an association of polymorphisms among folate metabolizing genes such as *MTHFR* and the establishment of CRC [11].

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 [12-15]. The 677CC is the wild type form of *MTHFR* gene. The 677TT homozygous variant and
the heterozygous CT genotype have less than 30% and 65% of the normal enzyme activity (wild type homozygous CC genotype), respectively [16].

In the current article, we studied the association between the risk of CRC and \textit{MTHFR} C677T polymorphism and also investigated the correlation of the polymorphism with serum folate/Vit B12 concentrations in our patients.

\textbf{Methods}

\textbf{Study population and samples}

A total of 517 blood samples of colon disease patients (98 adenomas and 419 adenomacarcinoma) as well as 500 normal blood samples were collected from Reza Radiotherapy and Oncology Center (RRCO), 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.

\textbf{MTHFR C677T polymorphism analysis}

\textit{MTHFR} C677T polymorphism was detected 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 standard salting out method [17]. Primers and probes were synthesized by Bioneer Company (Bioneer Corporation, South Korea). The sequences of the primer and probe were as follows: primer forward, 5'-TGACCTGAAGCACTTGAAGGAA-3', primer reverse, 5'-GGAAGAATGTGTCAGCCTCAAAGA-3', probe C, 5'-ATGAAATCGGCTCCCG-3' (reporter: FAM), probe T, 5'-ATGAAATCGACTCCCG-3' (reporter Cy5).

\textbf{Folate and vitamin B12 measurement}

Serum folate and vitamin B\textsubscript{12} measurements were totally 43 samples in case and control groups. For this reason, 5 mL of blood was collected and the serum was obtained by a centrifugation method. Serum was then stored frozen at \(-80^\circ\text{C}\) 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).

\textbf{Statistical analysis}

Association analyses were performed using SNPassoc [18]. The Hardy-Weinberg equilibrium (HWE) and the p-value for categorical variables were calculated by chi-square test. Mann–Whitney U test was used to establish the difference in levels folate and Vit B12.

\textbf{Results}

The association between the risk of CRC and \textit{MTHFR} C677T polymorphism and the correlation of the polymorphism with serum folate/Vit B12 concentrations in the Iranian patients were investigated. The details of age, gender distribution and demographic characteristics of samples were shown in Tables 1 and 2. Moreover, the percentage of genotypes in different groups; normal, adenoma and adenomacarcinoma has been indicated in Tables 1 and 3. Table 3 shows that the control group follows the Hardy Weinberg equilibrium. Notably, CRC patients and controls demonstrated significantly different frequencies for the rs1801133 alleles. Allele T carriers have an 84% higher risk of CRC than allele C carriers (OR = 1.84, 95%CI = 1.5–2.26, p = 5.31e-09).
Table 1
Distribution and demographic characteristics of adenocarcinoma samples and controls

|                | control (N = 500) | case (N = 419) | p-value |
|----------------|-------------------|----------------|---------|
| **Age**        |                   |                |         |
| < 45           | 229 (45.8%)       | 86 (20.5%)     | < 0.001 |
| >=45           | 271 (54.2%)       | 333 (79.5%)    |         |
| **Sex**        |                   |                |         |
| Female         | 261 (52.2%)       | 200 (47.7%)    | 0.2     |
| Male           | 239 (47.8%)       | 219 (52.3%)    |         |
| **Addiction**  |                   |                |         |
| No             | 387 (77.4%)       | 353 (84.2%)    | 0.0115  |
| Yes            | 113 (22.6%)       | 66 (15.8%)     |         |
| **TL**         |                   |                |         |
| Colon          | 206 (49.2%)       |                |         |
| Rectosigmoid   | 213 (50.8%)       |                |         |
|                    | N (%)                  |
|--------------------|------------------------|
| **Sex**            |                        |
| 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
Allelic distribution and Hardy Weinberg equilibrium

| Genotypes | Whole Population | Controls | Cases  |
|-----------|------------------|----------|-------|
| C/C       | 520 (56.58%)     | 305 (61%)| 215 (51.31%) |
| C/T       | 273 (29.71%)     | 161 (32.2%)| 112 (26.73%) |
| T/T       | 126 (13.71%)     | 34 (6.8%)  | 92 (21.96%)  |

| Alleles | Whole Population | Controls | Cases  |
|---------|------------------|----------|-------|
| C       | 1313 (71.44%)    | 771 (77.1%)| 542 (64.68%) |
| T       | 525 (28.56%)     | 229 (22.9%)| 296 (35.32%) |

| HWE (p value) | Whole Population | Controls | Cases  |
|---------------|------------------|----------|-------|
| 8.07E-16      | 0.0566881        | 2.93E-17 |

Table 4 represents the association analysis for each genetic model. Our data showed that homozygote TT carriers, compared with CC genotype, were associated with an increased risk of CRC both before and after adjustment for sex, age and addiction (OR = 3.68; 95% CI 2.35–5.75, p = 2.516e-09). Besides, the recessive model showed that individuals with TT genotype had a higher risk of CRC than C allele carriers (CC + CT) (OR = 3.74 95%CI 2.42–5.78, p = 3.291e-10). Furthermore, the dominant model indicated that T allele carriers had a 42% higher risk of CRC after adjustment compared with CC homozygotes (OR = 1.42 95%CI 1.08–1.87, p = 1.134e-02).
In subgroup analysis, TT genotype of rs1801133 polymorphism was associated with an increased risk in both colon and recto sigmoid cancer sites compared with CC homozygotes and C allele carriers (Table 5).

| Model         | Genotype | Control (%) | Case (%) | OR (CI95%)     | P     | OR<sup>a</sup> (CI95%) | P<sup>a</sup> |
|---------------|----------|-------------|----------|----------------|-------|------------------------|-------------|
| **Codominant**| C/C      | 305 (61%)   | 215 (51.3%) | Ref             | 1.624e-10 | Ref                        | 2.516e-09 |
|               | C/T      | 161 (32.2%) | 112 (26.7%) | 0.99 (0.73–1.33) | 0.95 (0.70–1.29) |
|               | T/T      | 34 (6.8%)   | 92 (22%)   | **3.84 (2.50–5.90)** | 3.68 (2.35–5.75) |
| **Dominant**  | C/C      | 305 (61%)   | 215 (51.3%) | Ref             | 3.165e-03 | Ref                        | 1.134e-02 |
|               | C/T-T/T  | 195 (39%)   | 204 (48.7%) | **1.48 (1.14–1.93)** | 1.42 (1.08–1.87) |
| **Recessive** | C/C-C/T  | 466 (93.2%) | 327 (78%)  | Ref             | 1.897e-11 | Ref                        | 3.291e-10 |
|               | T/T      | 34 (6.8%)   | 92 (22%)   | **3.86 (2.54–5.86)** | 3.74 (2.42–5.78) |
| **Overdominant**| C/C-T/T | 339 (67.8%) | 307 (73.3%) | Ref             | 7.013e-02 | Ref                        | 5.199e-02 |
|               | C/T      | 161 (32.2%) | 112 (26.7%) | 0.77 (0.58–1.02) | 0.74 (0.55-1.00) |
| **log-Additive** | 0,1,2  | 500 (54.4%) | 419 (45.6%) | **1.62 (1.35–1.95)** | 1.842e-07 | 1.58 (1.30–1.91) | 2.353e-06 |

<sup>a</sup> = adjusted for age, sex and addiction

In subgroup analysis, TT genotype of rs1801133 polymorphism was associated with an increased risk in both colon and recto sigmoid cancer sites compared with CC homozygotes and C allele carriers (Table 5).
Table 5
The association analysis stratified for tumor location

| Location       | Model      | Genotype | Control (%) | Case (%) | OR (95%CI)     | P     | ORa  | Pa  |
|----------------|------------|----------|-------------|----------|----------------|-------|------|-----|
| Colon          | Codominant | C/C      | 305 (61%)   | 105 (51%)| Ref 2.17e-05   | Ref   | 5.30e-05 |
|                |            | C/T      | 161 (32.2%) | 62 (30.1%)| 1.12 (0.77–1.62) | 1.1 (0.76–1.6) |
|                |            | T/T      | 34 (6.8%)   | 39 (18.9%)| 3.33 (2.55)    | 3.27 (1.93–5.54) |
|                | Dominant   | C/C      | 305 (61%)   | 105 (51%)| Ref 0.014404   | Ref   | 0.021843 |
|                |            | C/T-T/T  | 195 (39%)   | 101 (49%)| 1.5 (1.08–2.09) | 1.48 (1.06–2.07) |
|                | Recessive  | C/C-C/T  | 466 (93.2%) | 167 (81.1%)| Ref 4.31e-06   | Ref   | 1.04e-05 |
|                |            | T/T      | 34 (6.8%)   | 39 (18.9%)| 3.2 (1.96–5.24) | 3.16 (1.9–5.26) |
|                | Overdominant | C/C-T/T | 339 (67.8%) | 144 (69.9%)| Ref 0.583813   | Ref   | 0.558616 |
|                |            | C/T      | 161 (32.2%) | 62 (30.1%)| 0.91 (0.64–1.29)| 0.9 (0.63–1.29) |
|                | log-Additive | 0,1,2   | 500 (70.8%) | 206 (29.2%)| 1.6 (1.26–2.02) | 9.44e-05 | 1.57 (1.24–2) | 0.000217 |
| Rectosigmoid   | Codominant | C/C      | 305 (61%)   | 110 (51.6%)| Ref 7.24e-10   | Ref   | 5.88e-09 |
|                |            | C/T      | 161 (32.2%) | 50 (23.5%)| 0.86 (0.59–1.27) | 0.79 (0.53–1.18) |
|                |            | T/T      | 34 (6.8%)   | 53 (24.9%)| 4.32 (2.67–7)  | 4.16 (2.48–6.96) |
|                | Dominant   | C/C      | 305 (61%)   | 110 (51.6%)| Ref 0.020807   | Ref   | 0.080438 |
|                |            | C/T-T/T  | 195 (39%)   | 103 (48.4%)| 1.46 (1.06–2.02) | 1.35 (0.96–1.9) |
|                | Recessive  | C/C-C/T  | 466 (93.2%) | 160 (75.1%)| Ref 1.17e-10   | Ref   | 1.49e-09 |
|                |            | T/T      | 34 (6.8%)   | 53 (24.9%)| 4.54 (2.85–7.24) | 4.5 (2.73–7.41) |

a = adjusted for age, sex and addiction
| Location       | Model       | Genotype | Control (%) | Case (%) | OR (95%CI)  | P       | OR⁰ | P⁰      |
|---------------|-------------|----------|-------------|----------|-------------|---------|-----|---------|
| Over          | C/C-T/T     | 339 (67.8%) | 163 (76.5%) | Ref      | 0.017874    | Ref     | 0.006936 |
| dominant      |             |          |             |          |             |         |     |         |
| C/T           | 161 (32.2%) | 50 (23.5%) | 0.65 (0.45–0.93) | 0.59 (0.4–0.87) |          |         |     |         |
| log-Additive  | 0,1,2       | 500 (70.1%) | 213 (29.9%) | 1.71 (1.37–2.14) | 2.54e-06 | 1.64 (1.29–2.07) | 3.75e-05 |

a = adjusted for age, sex and addiction

Tables 6 and 7 show the association of characteristic variables with genotypes. In Table 5, regardless of the genotype, patients with 45 and over 45 years of age had a more pronounced risk effect than patients under 45. In this regard, patients with TT genotype represented the highest risk association (≥ 45 TT vs. < 45 TT: OR = 5.4 95%CI 2.21–13.19). In addition, participants with homozygote TT genotype and addiction were associated with a lower risk of cancer in comparison with TT carriers who did not have an addiction (addicted TT vs. not addicted TT, OR = 0.33 95%CI 0.13–0.81). Stratified analysis (Table 7) showed that within each gender group, the TT genotype was associated with a higher risk of CRC in comparison with CC carriers (female TT vs. female CC: OR = 4.89 95%CI 2.67–8.97; male TT vs. male CC: OR = 3.01 95%CI 1.63–5.57). Similarly, within each age group, homozygotes for the alternate allele showed an increased risk for both groups in comparison with CC genotype (< 45 TT vs. <45 CC: OR = 2.31 95%CI 1.04–5.13; ≥ 45 TT vs. ≥ 45 CC: OR = 4.34 95%CI 2.49–7.56). Finally, in the no addiction group, TT genotype was also associated with increased risk of CRC in comparison with CC genotype of the same group (no addiction TT vs. no addiction CC: OR = 4.61 95%CI 2.76–7.69).
| Genotype | Clinical feature | Characteristic | Control | Case | OR (CI95%) | P     |
|----------|-----------------|----------------|---------|------|------------|-------|
| C/C      | Sex             | Female         | 155     | 95   | Ref        | 0.31145 |
|          |                 | Male           | 150     | 120  | 1.31 (0.92–1.85) |      |
| C/T      |                 | Female         | 89      | 54   | Ref        |       |
|          |                 | Male           | 72      | 58   | 1.33 (0.82–2.15) |      |
| T/T      |                 | Female         | 17      | 51   | Ref        |       |
|          |                 | Male           | 17      | 41   | 0.8 (0.37–1.77) |      |
| C/C      | Age             | < 45           | 142     | 50   | Ref        | 0.25262 |
|          |                 | ≥ 45           | 163     | 165  | 2.87 (1.95–4.24) |      |
| C/T      |                 | < 45           | 71      | 23   | Ref        |       |
|          |                 | ≥ 45           | 90      | 89   | 3.05 (1.75–5.31) |      |
| T/T      |                 | < 45           | 16      | 13   | Ref        |       |
|          |                 | ≥ 45           | 18      | 79   | 5.4 (2.21–13.19) |      |
| C/C      | Addiction       | No             | 234     | 180  | Ref        | 0.55023 |
|          |                 | Yes            | 71      | 35   | 0.64 (0.41-1) |      |
| C/T      |                 | No             | 131     | 95   | Ref        |       |
|          |                 | Yes            | 30      | 17   | 0.78 (0.41–1.5) |      |
| T/T      |                 | No             | 22      | 78   | Ref        |       |
|          |                 | Yes            | 12      | 14   | 0.33 (0.13–0.81) |      |
Table 7
The associations of the genotype within clinical characteristics

| Clinical feature | Characteristic | Genotype | Control | Case | OR (CI95%) | P     |
|------------------|----------------|----------|---------|------|------------|-------|
|                  |                | C/C      | 155     | 95   | Ref        | 0.51725 |
|                  |                | C/T      | 89      | 54   | 0.99 (0.65–1.51) |     |
|                  |                | T/T      | 17      | 51   | 4.89 (2.67–8.97) |     |
|                  |                | Male     |         |      |            |       |
|                  |                | C/C      | 150     | 120  | Ref        |       |
|                  |                | C/T      | 72      | 58   | 1.01 (0.66–1.53) |     |
|                  |                | T/T      | 17      | 41   | 3.01 (1.63–5.57) |     |
|                  |                | <45      |         |      |            |       |
|                  |                | C/C      | 142     | 50   | Ref        | 0.43875 |
|                  |                | C/T      | 71      | 23   | 0.92 (0.52–1.63) |     |
|                  |                | T/T      | 16      | 13   | 2.31 (1.04–5.13) |     |
|                  |                | >=45     |         |      |            |       |
|                  |                | C/C      | 163     | 165  | Ref        |       |
|                  |                | C/T      | 90      | 89   | 0.98 (0.68–1.41) |     |
|                  |                | T/T      | 18      | 79   | 4.34 (2.49–7.56) |     |
|                  |                | No       |         |      |            |       |
|                  |                | C/C      | 234     | 180  | Ref        | 0.30624 |
|                  |                | C/T      | 131     | 95   | 0.94 (0.68–1.31) |     |
|                  |                | T/T      | 22      | 78   | 4.61 (2.76–7.69) |     |
|                  |                | Yes      |         |      |            |       |
|                  |                | C/C      | 71      | 35   | Ref        |       |
|                  |                | C/T      | 30      | 17   | 1.15 (0.56–2.36) |     |
|                  |                | T/T      | 12      | 14   | 2.37 (0.99–5.65) |     |

The median concentration of serum Vit B12 was 298 and 293 pg/ml in cancer and normal groups, respectively. The median concentration of folic acid in cancer and normal groups was calculated 11.4 and 9.2, respectively (Table 8). As illustrated in Fig. 1 and Table 8, there was no significant difference between the amount of folic acid and Vit B12 in cancer and normal groups (P = 0.202 and 0.951, respectively).
Table 8
Statistical analysis of folate and Vit B12 in adenocarcinoma and normal samples

|                  | Case (N = 19) | Control (N = 23) | Case (N = 17) | Control (N = 22) |
|------------------|--------------|------------------|--------------|------------------|
| High (> 982 pg/ml) | 4            | 2                | 17           | 19               |
| Low (< 193 pg/ml) | 2            | 4                | 0            | 3                |
| Normal (193–982 pg/ml) | 13         | 17               | 11.4         | 9.2              |
| $X^2$            | 1.4993       |                  | 0.95805      |                  |
| $P$              | 0.4725       |                  | 0.3277       |                  |
| Median (mad*)    | 298 (130.47) | 293 (127.5)      | 11.4 (8.01)  | 9.2 (5.56)       |

*Median Absolute Deviation

Discussion

In the present study, among CRC patients an increase in 677TT MTHFR polymorphisms has been indicated.

Previous studies on the association of C677T polymorphism and susceptibility to CRC revealed no constant results. Some studies suggested a protective effect of TT genotype for colon cancer because, a reduced risk of CRC progress was observed in TT individuals with a sufficient folate intake [19]. Chen et al. performed the first study to investigate at the relation between the MTHFR C677T polymorphism and CRC. According to their results, the MTHFR C677T polymorphism affected enzyme activity and was involved in aberrant methylation and DNA synthesis, resulting in colorectal tumorigenesis [20]. Similar findings were subsequently reported by Slattery et al [21], Ma et al [22] and Le Marchand et al [23]. However, due to statistically non-significant findings, several other published studies failed to support an impact of MTHFR gene polymorphisms on CRC risk [24–26].

It has been shown that the effect of 677TT MTHFR polymorphism, the phenotype of valine amino acid, significantly depends on folate intake. An in-vitro study on HCT116 colon carcinoma cells reported 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 suggested folate as a genotype modifier. In fact, biochemical changes in the valine-containing enzyme is important, which shows the enzyme stabilization by the addition of 5-methyltetrahydrofolate (5-MTHF) to the culture medium. Therefore, folate might modify correlation between SNPs and the CRC risk [27]. According to Kennedy et al., MTHFR 677TT genotype was linked to a lower incidence of CRC. Furthermore, the associated risk of CRC was decreased for both the MTHFR 677 CC and TT genotypes when total folate intake was high [28].
Migration and proliferation of cancer cells are two important events in cancer development. The main cause of death for cancer patients is metastasis, migration of cancerous cells from organ to other organs. It has been demonstrated that about 10 µM folic acid reduced the migration and proliferation of human cell lines (COLO-205, LoVo and HT-29) [29].

It has also been reported that MTHFR 677TT genotype is one strong reason to lower the 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 of colon are K-ras and P53 mutations but microsatellite instability (MSI) is more frequent in the proximal site in CRC [15, 30, 31]. Totally, decreased risk of distal colon cancer, rectal cancer and proximal colon cancer have been reported to be associated with 677TT genotype [16]. However, in the present study, we found an increase of CRC risk in MTHFR TT and TT + CT genotypes compared to CC genotype (OR = 3.68 95%CI 2.35–5.75; OR = 1.42 95%CI 1.08–1.87, respectively). We also found that regardless of tumor site individuals with MTHFR 677TT genotype were associated with higher risk than C allele carriers (Colon: OR = 3.16 95%CI 1.9–5.26; Rectosigmoid: OR = 4.5 95%CI 2.73–7.41). Similarly, two studies on Iranian population on 406 (175 cases, 231 controls) and 491 (234 cases, 257 controls) subjects, respectively reported that CC genotype has a protective effect on CRC [5, 32]. Although a more recent combined case-control study and meta-analysis on 2421 subjects has shown no significant association between MTHFR C677T polymorphism and the risk of CRC in Iranian population suggesting the need for a bigger sample size for MTHFR association studies [33]. There are also some reports indicating the increased risk of TT genotype in other populations [34, 35].

Some studies have reported a reduced risk of developing CRC with only TT genotype with a sufficient folate intake, suggesting a protective effect against CRC [21, 22]. In high folate intake cases, the risk of CRC risk is reduced for both MTHFR 677CC and 677TT genotypes [36]. Similarly, others have also reported the association of high-methyl diets like high folate dietary intake and low alcohol consumption with the protective effect of MTHFR 677TT genotype [16, 20, 23, 37, 38], although not everyone were able to demonstrate the protective effect of the MTHFR 677TT genotype even in high folate dietary intake [39, 40]. However, it seems to be an association between the MTHFR polymorphism and dietary methyl supply, although the relation remains inconsistent.

Even though we genotyped the MTHFR polymorphism in 1017 blood samples, there was still a limited sample size for folate and Vit B12 measurement that hindered us from incorporating this part of data into a comprehensive association study. Regard to the pilot study based on the limited number of samples for folate and Vit B12 measurement, further investigations is needed to support our findings. Furthermore, due to the heterogeneity of the Iranian population, there is an inevitable need for more multicentric studies in the Iranian population.

**Conclusions**

In summary, our study revealed that there was no significant difference between the amount of folate and Vit B12 in the case and control groups. Furthermore, our results demonstrated a higher risk association for 677TT and 677TT + C677T genotypes of MTHFR compared with 677CC carriers among CRC patients in Iran.

**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 and MA were responsible for the investigation and conducting the experiments. RK prepared figure and statistical analysis of data. All authors were responsible for reviewing and editing the final version of the paper.

Acknowledgements

We thank Mashhad University of Medical Sciences and Reza Radiotherapy and Oncology Center for supporting this study.

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Figures

![Figure 1](image)

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