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MTHFR *C677T* and *A1298C* Gene Polymorphisms in Human Kidney Cancer Tissues

İnsan Böbrek Kanseri Dokularında MTHFR *C677T* ve *A1298C* Gen Polimorfizmleri

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ABSTRACT

Introduction: The potential effect of 5,10-methylenetetrahydrofolate reductase (MTHFR) on DNA methylation, DNA repair and DNA synthesis has made MTHFR a cancer-inducing gene. In this study, we aimed to evaluate *C677T* and *A1298C* gene polymorphisms in kidney cancer.

Methods: During the normal treatment procedure, 100 tumor and 100 surrounding healthy kidney tissue samples were obtained from patients after surgery. DNA was isolated from the tissues by DNA Isolation kit. Polymerase chain reaction and restriction fragment length polymorphism methods were used. Genotype and allele distributions were analyzed using SPSS 22 and $p<0.05$ was considered statistically significant.

Results: No significant difference was found between the genotypes and alleles of MTHFR C677T and A1298C polymorphisms in tumor and control groups ($p>0.05$). MTHFR C677T CC genotype (51%) was found to be higher in kidney cancer tissues than CT genotype (34%). Odds ratio of MTHFR C677T CC genotype was found 1.8 compared to CT genotype ($p<0.05$).

Conclusion: Our findings indicate that MTHFR C677T polymorphism may be effective in normal genotype in kidney cancer tissues.

Keywords: MTHFR, kidney cancer, polymorphism, *C677T*, *A1298C*

ÖZ

Amaç: 5,10-metilenetetrahidrofolat redüktaz'ın (MTHFR) DNA metilasyonu, DNA onarımı ve DNA sentezi üzerindeki potansiyel etkisi MTHFR'yi kanseri indükleyen bir gen yapmıştır. Çalışmamızda böbrek kanserinde *C677T* ve *A1298C* gen polimorfizmlerini değerlendirmeyi amaçladık.

Yöntemler: Normal tedavi prosedürü sırasında cerrahi operasyon sonrası hastalardan 100 tümör ve 100 sağlıklı çevre böbrek dokusu örneği alındı. DNA izolasyon kiti ile dokulardan DNA izole edildi. Polimeraz zincir reaksiyonu ve restriksiyon fragman uzunluğu polimorfizmi yöntemi uygulandı. Genotip ve allel dağılımları SPSS 22 ile analiz edildi ve $p<0,05$ istatistiksel olarak anlamlı kabul edildi.

Bulgular: Tümör ve kontrol gruplarında MTHFR C677T ve A1298C polimorfizmlerinin genotipleri ve allelleri arasında anlamlı fark bulunmadı ($p>0,05$). Böbrek kanserli dokularda MTHFR C677T CC genotipinin (%51) CT genotipine (%34) oranla daha fazla olduğu bulundu. MTHFR C677T CC genotipinin CT genotipine oranla elde edilen tahmini rölatif riski 1,8 olarak belirlendi ($p<0,05$).

Sonuç: Elde ettiğimiz bulgular böbrek kanserli dokularda MTHFR C677T polimorfizminin normal genotipte etkili olabileceğini göstermektedir.

Anahtar Kelimeler: MTHFR, böbrek kanseri, polimorfizm, *C677T*, *A1298C*

Introductıon

While kidney cancer is the third most common cancer among urogenital cancers, it ranks seventeen among the most common cancers (1). Although kidney cancer patients usually have good outcomes after surgical intervention, less than 20% of individuals with advanced disease have a 2-year survival. Kidney cancer is seen twice in men than in women (2). Kidney cancer is not a single type of cancer and it has genetically and histologically different types responding to therapy differently due to mutations in various genes (3).

5,10-methylenetetrahydrofolate reductase (MTHFR) is the key enzyme in folate metabolism. MTHFR catalyzes the reduction of MTHF to 5-methyl THF using 5,10 FAD as a cofactor that acts as a regulator of folate coenzymes for purine, pyrimidine and methionine synthesis (4). Five-methyl THF provides the methyl group for the synthesis of methionine (5)

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and homocysteine is the carbon donor in methionine remethylation (6). Because of its role in DNA methylation, DNA repair and DNA synthesis, MTHFR may be categorized as a cancer-inducing gene (7).

The effects of gene variants C677T and A1298C of MTHFR, the most important enzyme involved in homocysteine metabolism and regeneration, has been associated with many diseases such as cancer, coronary heart disease, myocardial infarction, diabetes and renal diseases (8-12). In the fourth exon encoding MTHFR, the homozygous C677T mutation at the folate-binding site (MTHFR TT) reduced the enzyme activity to 35% and lead to predisposition to folate deficiency and associated with hyperhomocysteinemia (13,14). It has been observed that enzyme activity with MTHFR A1298C polymorphism in seventh exon is reduced to 60% of normal value (15).

We aimed to evaluate the effects of *MTHFR* gene on kidney cancer formation due to its effects on folate and homocysteine mechanisms.

Materıals and Methods

One hundred patients with kidney cancer, who were admitted to the Urology Department of İstanbul Okmeydanı Training and Research Hospital, were included in the study. Kidney cancer tissues constituted the "tumor group" and surrounding healthy kidney tissues from the same subjects constituted the "control group". The study was approved by the Ethics Committee of İstanbul University Faculty of Medicine, (decision no: 2016/600, date: 13/05/2016) and informed consent were taken. Tissue samples were stored at -80 °C after treated with liquid nitrogen. DNA was obtained from kidney cancer tissues and surrounding healthy kidney tissues by using DNA isolation kit (DNeasy Blood & Tissue Kit, Qiagen, Cat No. 69504) according to the manufacturer's instructions. DNA samples were stored at -20 °C until analysis by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Genotyping

The oligonucleotide primers for amplifying the polymorphism regions of MTHFR C677T and A1298C were: 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' (forward), 5'-AGGACGGTGCGGTGAGAGTG-3' (reverse) and 5'-ATGTGGGGGGAGGAGCTGAC-3' (forward), 5'-GTCTCCCAACTTACCCTTCTCCC-3', respectively.

PCRs were performed in a total volume of 25 µL with 1 µl of 100-200 ng DNA, 1 µl of forward and reverse primers, 1.5 µl of dNTPs, 2.5 µl of buffer, 0.25 µl of DNA polymerase (GeneMark GMbiolab Co., Ltd. Taichung, Taiwan) and 18.75 µl of dH_2O. The PCR mixture was incubated for 5 minutes at 95 °C, followed by 35 cycles of 45 seconds at 94 °C, 45 seconds at 59 °C and 45 seconds at 72 °C and a final step at 72 °C for 7 minutes for MTHFR C677T. For MTHFR A1298C; PCR mixture was incubated for 8 minutes at 95 °C, followed by 40 cycles of 1 minute at 94 °C, 1 minute at 63 °C and 1 minute at 72 °C and a final step at 72 °C for 7 minutes.

The PCR products for MTHFR C677T sites were digested by HinfI (Jena Bioscience, Cat. No. EN-117S) restriction enzyme. The RFLPs were performed in 20 µl reaction volume with 5 µl of PCR product, 2 µl of Buffer, 0.4 µl of restriction enzyme and 12.6 µl of distilled water. The 198 base pair (bp) PCR product was digested with HinfI 10 minutes at 37 °C and 20 minutes at 65 °C. The fragments were separated on a 3% agarose gel stained with ethidium bromide. After digestion, CC homozygotes showed 1 band of 198 bp, while TT homozygotes and CT heterozygotes showed 2 bands (175 and 23 bp) and 3 bands (198, 175 and 23 bp), respectively.

The PCR products for MTHFR A1298C sites were digested by MboII (Jena Bioscience, Cat. No. EN-E2284-01) restriction enzyme. The RFLPs were performed in 20 µl reaction volume with 5 µl of PCR product, 2 µl of Buffer, 0.4 µl of restriction enzyme and 12.6 µl of distilled water. The 240 bp PCR product was digested with MboII 10 minutes at 37 °C and 20 minutes at 65 °C. The fragments were separated on a 3% agarose gel stained with ethidium bromide. After digestion, CC homozygotes showed 1 band of 240 bp, while 2 bands (215 and 25 bp) with AA homozygotes and 3 bands (240, 215 and 25 bp) with AC heterozygotes were observed under ultraviolet light.

Statistical Analysis

Statistical analyses were performed using SPSS 22 statistical software (IBM Corp., Armonk, NY, USA). Pearson chi-square test was used for evaluating the differences between the two groups. Spearman's rho test was performed to analyze the correlation between clinical parameters, genotypes, and alleles in the two groups. A p value of less than 0.05 was considered statistically significant.

Results

One hundred patients with kidney cancer, including 71 men and 29 women, were included in the study. The clinical parameters of only 50 patients were identified in Table 1, as the remaining 50 patients did not have clinical parameters. According to the Fuhrman grading system, 11 of the samples were grade 1, 47 were grade 2, 34 were grade 3 and eight were grade 4. The mean age was 59.21±11.79 years (range: 31-86) and mean tumor diameter was 5.97±2.57 (range: 2.3-14) (These data were not shown in the table).

Table 1. Clinical and demographic characteristics of fifty patients

Group		Height, (cm)	Weight (kg)	Age (year)	BMI (kg/m²)	WC (cm)	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	Lymp (mg/dL)	Neut (mg/dL)
Male	Mean	171.08	80.5	59.6	27.49	104.24	195.95	162.29	42.92	125.16	1.64	5.17
n=38	SD	5.21	9.67	9.91	3.8	6.2	36.25	35.17	3.14	27.28	0.19	0.95
Female	Mean	163.17	73.75	57.58	28.7	95.33	200.58	139.17	49.5	121.33	1.66	4.69
n=12	SD	4.23	6.62	10.67	3.1	7.97	38.44	24.79	5.56	32.91	0.13	0.61

SD: standard deviation, BMI: body mass index, WC: waist Circumstance, TC: total Cholesterol, TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein, Lymp: lymphocyte, Neut: neutrophil

Table 2. The frequency of genotypes and alleles for MTHFR C677T and risk estimation

MTHFR C677T	Group				Distribution relationship		Risk estimation	
	Tumor		Control		Total n (%)	p	OR (95% CI)	p
Allele	n	%	n	%				
C	136	68	126	63	262 (65.5%)	0.29	Reference	Reference
T	64	32	74	37	138 (34.5%)		1.248 (0.826-1.886)	0.293
Genotype	n	%	n	%	Total n (%)	p	OR (95% CI)	p
CC	51	51	39	39	90 (45%)	0.127	Reference	Reference
TT	15	15	13	13	28 (14%)		1.133 (0.484-2.656)	0.773
CT	34	34	48	48	82 (41%)		1.846 (1.007-3.383)	0.046*

OR: odds ratio, CI: confidence interval, *: p<0.05

Table 3. The frequency of genotypes and alleles for MTHFR A1298C and risk estimation

MTHFR A1298C	Group				Distribution relationship		Risk estimation	
	Tumor		Control		Total n (%)	p	OR (%95CI)	p
Allele	n	%	n	%				
A	123	61.5	119	59.5	242 (60.5%)	0.682	Reference	Reference
C	77	38.5	81	40.5	158 (39.5%)		1.087 (0.728-1.624)	0.682
Genotype	n	%	n	%	Total n (%)	p	OR (%95CI)	p
AA	38	38	34	34	72 (36%)	0.825	Reference	Reference
CC	15	15	15	15	30 (15%)		1.118 (0.477-2.621)	0.798
AC	47	47	51	51	98 (49%)		1.085 (0.479-2.459)	0.845

OR: odds ratio, CI: confidence interval

The Hardy-Weinberg equilibrium analysis showed that both polymorphisms were in equilibrium (p>0.05). No significant difference was found between the genotypes and alleles of MTHFR C677T and A1298C polymorphisms in tumor and control groups (p>0.05) (Table 2 and 3).

The genotype and allele distributions of MTHFR C677T in tumor and control groups are defined in Table 2. There was no significant difference between tumor and surrounding healthy tissues in terms of MTHFR C677T genotypes and alleles. Tumors with homozygous normal CC genotype had higher frequency (51%) than the control tissues (39%) (p>0.05). The frequency rate of the mutant T allele was also slightly higher in the surrounding healthy tissues (37%) than tumors (32%) (p>0.05). Compared to CT genotype, odds ratio (OR) of MTHFR C677T CC genotype was found as 1.8 (OR: 1.846 (1.007-3.383), p=0.046). Thus, wild type genotype is higher in kidney cancer due to possible effects on DNA.

The genotype, allele distributions and risk estimation of MTHFR A1298C in tumor and control groups are shown in Table 3. The polymorphism of MTHFR A1298C mutant CC genotype was similar in the control (15%) and tumor (15%) tissues (p>0.05). The frequency of A alleles was not statically different between the tumor (61.5%) and control (59.5%) groups (p>0.05). There was no effect of MTHFR A1298C polymorphism on the risk of kidney cancer formation (p>0.05).

There was no correlation between MTHFR C677T, A1298C, tumor diameter and Fuhrman grade for 100 tumors (p>0.05). Furthermore, no correlation was found between MTHFR polymorphisms and clinical parameters of 50 patients and these results were not given in the table.

Discussion

We aimed to analyze the possible effects of MTHFR C677T and A1298C polymorphisms on kidney cancer. We found that these polymorphisms had no effect on kidney cancer. However, there was no correlation between MTHFR polymorphisms and clinical parameters (p>0.05).

MTHFR enzyme consists of 656 amino acids and is encoded by the *MTHFR* gene (16). This enzyme converts 5,10 MTHFR irreversibly into 5-methyl THF (16,17). 5-methyl THF provides a methyl group for DNA methylation and methionine synthesis (17). MTHFR polymorphisms have been associated with renal diseases (18,19). Since MTHFR polymorphisms are also associated with chemotherapy response, they may be indicative of the identification of individualized treatment protocols (20). There have been few studies in the literature describing the relationship between kidney cancer and MTHFR polymorphisms. One of these studies was conducted by Sakano et al. (21) in Japanese population. They found that MTHFR C677T and A1298C polymorphisms might be predictive factors in the clear cell renal cell carcinoma with a gender-specific manner and associated with aggressiveness or prognosis.

The C677T polymorphism results in a change from cytosine to thymine, the 677th nucleotide in exon 4, which affects the N-terminal catalytic domain of the MTHFR protein, resulting in decreased MTHFR activity (22). Different results were obtained for C677T polymorphism in various cancer types. Cetintas and colleagues found MTHFR C677T polymorphism TT genotype as a potential biomarker for cancer in the Turkish population via meta-analysis. However, none of these cancer cases were kidney cancer (23). MTHFR C677T polymorphisms were found

to be associated with increased clear cell renal cell carcinoma risk in men (24). MTHFR T allele was identified as a risk factor for the development of ovarian carcinoma (25), but not in breast tumors (26). MTHFR C677T TT genotype was also found to reduce risk of colorectal cancer in the Japanese population. It has been suggested that MTHFR TT genotype has a protective role of folate by ensuring a sufficient thymidylate pool for DNA synthesis (27). Another study showed that MTHFR CC genotype was associated with a higher prevalence of p16 hypermethylation and might contribute to the pathogenesis of multiple myeloma (28). Cancer protective associations of MTHFR C677T TT genotype were identified in the red cell folate by changing a metabolic phenotype (29). In a meta-analysis, *C677T* of the *MTHFR* gene was found to be a low-penetrance susceptibility gene for prostate cancer via protective effects (30). The protective effects of T allele were found also in gastric cancer, colorectal cancer (31) and lung squamous cell carcinoma (32). In our study, C677T mutant TT genotype and T allele were not statically different between tumor and control tissues ($p>0.05$). The OR of the MTHFR C677T polymorphism CC genotype was 1.8 compared to CT genotype (OR: 1.846 (1.007-3.383), $p=0.046$). In our study, we could not observe the protective effects of T allele or TT genotype, but cancer tissues were mostly found in normal genotype (CC, 51%) than heterozygous or mutant genotype (CT, 34% or TT, 15%). Given that the MTHFR C677T TT genotype reduces enzyme activity to almost 1/3 (13,14), the MTHFR C677T CC genotype may be present or mutated as a wild type in cancerous tissues for cancer-inducing effects such as p16 hypermethylation (28).

MTHFR activity is reduced as a result of the change from Adenine (A) → Cytosine (C) nucleotide 1298 in the 7th exon of the *MTHFR* gene (22). The higher risk of breast cancer and/or ovarian cancer with MTHFR A1298C polymorphism was detected in a study by Liu et al. (33). Dixit et al. (34) found an association between A1298C polymorphism and increased risk of gallbladder cancer. They also found correlation between this variation, grade and histopathology. The A1298C AA genotype was significantly higher in patients with oral squamous cancer than in controls. Ferlazzo et al. (35) also found hypermethylation of cancer-related genes such as p16 and O^6-methylguanine-DNA MGMT and suggested that these genes might be affected by MTHFR polymorphisms in oral squamous cancer. Wang et al. (36) suggested A1298C AC+CC genotypes might be a risk factor for the development of breast cancer in Chinese population via meta-analysis. The A1298C CC genotype was found to be lower in prostate cancer tissues than control tissues (37-38). The CC genotype was found to slightly reduce prostate cancer risk in Europeans, whereas increase prostate cancer risk in Asians. In a study by Skibola et al. (39), the frequency of CC genotypes was higher in the controls than in patients with acute lymphoid leukemia (39). However, no association was found between the MTHFR A1298C polymorphism and stomach cancer in another study. Similarly to the results in stomach cancer by Kim et al. (40), we did not find any association between the alleles and genotypes of MTHFR A1298C polymorphism and kidney cancer. The mutant CC genotype and C allele were not different between the groups (p0.05). There was no significant relationship between MTHFR A1298C polymorphism and kidney cancer (p0.05).

Conclusion

We evaluated MTHFR polymorphisms due to their possible contribution to kidney cancer formation. It was found that wild type genotype of MTHFR C677T polymorphism was higher in kidney cancer tissues. However, our results should be verified with larger study groups.

Ethics Committee Approval: Ethics committee approval was obtained for the study (İstanbul University Training and Research Hospital Ethics Committee (decision no: 2016/600, date: 13/05/2016).

Informed Consent: Informed verbal and written informed consent was obtained from the teachers.

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