Research Article

Anıl Çağla Özkılıç, Ahmet Çetin, Burcu Bayoğlu*, Huriye Balcı and Müjgan Cengiz*

Association between *MTHFR* C677T polymorphism and folate, vitamin B12, homocysteine, and DNA fragmentation in patients with ovarian cancer

Over kanserli hastalarda MTHFR C677T polimorfizmi ile folat, vitamin B12, homosistein düzeyleri ve DNA kırıkları arasındaki ilişki

DOI 10.1515/tjb-2016-0151
Received July 1, 2015; accepted April 19, 2016; previously published online October 8, 2016

Abstract

Objective: Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme that regulates the metabolism of methionine and folate. *MTHFR* C677T polymorphism was reported to be associated with breast and ovarian cancer. The aim of this study was to evaluate the association between the *MTHFR* C677T (rs1801133) polymorphism and homocysteine, vitamin B12, and folate levels, and DNA fragmentation in patients with ovarian cancer and healthy controls.

Materials and methods: This case-control study was conducted in Istanbul University Cerrahpasa Medical Faculty. We studied 50 ovarian cancer patients and 54 healthy controls. The *MTHFR* C677T polymorphism was determined by PCR followed by restriction fragment length polymorphism (RFLP) and agarose gel electrophoresis. DNA fragmentation was assessed by the comet assay. Homocysteine levels were measured by ELISA, whereas vitamin B_{12} and folate levels were measured by chemiluminescence methods.

Results: We found no correlation between the *MTHFR* C677T polymorphism and ovarian cancer. No significant difference was found in homocysteine, folate, and vitamin B_{12} levels between patient and control groups. Increased DNA fragmentation was detected in patients with ovarian cancer.

Conclusion: Our findings suggest that *MTHFR* C677T polymorphism, as well as homocysteine, folic acid, and vitamin B12 levels, are not associated with an increased risk for ovarian cancer.

Keywords: Ovarian cancer; *MTHFR* gene; C677T polymorphism; Homocysteine; Folic acid; Vitamin B12; Comet assay.

Özet

Amaç: Metilentetrahidrofolat redüktaz (MTHFR), metionin ve folat metabolizmasını düzenleyen önemli bir enzimdir. Spesifik folat metabolitleri; DNA sentezi, tamiri ve metilasyonu için metil grubu vericisi olduğundan, folat metabolizması, DNA sentez ve tamirinde önemli bir role sahiptir ve kanser gelişimini etkileyebilmektedir. *MTHFR* C677T (rs1801133) polimorfizminin meme ve over kanseri ile ilişkili olduğu bildirilmiştir. Çalışmamızda, over kanserli hastalar ile sağlıklı kontrollerde, *MTHFR* geni C677T (rs1801133) polimorfizmi ile homosistein, B12 vitamini, folat düzeyleri ve DNA kırık düzeylerinin belirlenerek karşılaştırılması amaçlanmıştır.

Gereç ve Yöntemler: Çalışmamızda 50 over kanserli hasta ile 54 sağlıklı kontrolde *MTHFR* C677T (rs1801133)

***Corresponding authors: Burcu Bayoğlu and Müjgan Cengiz,** Istanbul University, Cerrahpaşa Medical Faculty, Department of Medical Biology, Istanbul, Turkey,
Phone: +90 0212 414 30 00/22657, Fax: +90 0212 586 15 48,
e-mail: burcu.bayoglu@istanbul.edu.tr (B. Bayoğlu);
mcengiz@istanbul.edu.tr (M. Cengiz)
Anıl Çağla Özkılıç: Refik Saydam National Public Health Agency, Istanbul, Turkey
Ahmet Çetin: Haseki Education and Research Hospital, Department of Obstetrics and Gynecology, Istanbul, Turkey
Huriye Balcı: Istanbul University, Cerrahpaşa Medical Faculty, Fikret Biyal Central Research Laboratory, Istanbul, Turkey

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 1/2/17 1:30 PM

polimorfizmi genotipleri, polimeraz zincir reaksiyonu (PZR) işlemini takiben restriksiyon parça uzunluk polimorfizmi (RFLP) ve agaroz jel elektroforezi yöntemleri ile belirlendi. Over kanserli hastalarda meydana gelen DNA kırıkları Comet deneyi ile gösterildi. Homosistein düzeyleri ELİZA testi, B12 vitamini ve folat düzeyleri ise kemilüminesans yöntemi ile tayin edildi.

Bulgular: Çalışmamızda *MTHFR* C677T (rs1801133) polimorfizmi ile over kanseri arasında bir ilişki bulunmadı. Hasta ve kontrol grubunda homosistein, folat ve B12 vitamini düzeyleri karşılaştırıldığında aralarında istatistiksel olarak anlamlı bir fark bulunmadı. Over kanserli hastalarda DNA kırık sayısının hastalık şiddeti ile birlikte arttığı belirlendi.

Sonuçlar: Sonuç olarak, *MTHFR* C677T polimorfizmi ile homosistein, folat ve B12 vitamini düzeylerinin hasta ve kontrol grubu arasında farklı bulunmaması, *MTHFR* C677T polimorfizmi ile homosistein, folat ve B12 vitamini düzeylerinin over kanseri patogenezi ile ilişkili olmadığını düşündürmektedir. Ancak sonuçların anlamlı bulunmaması hasta sayımızın azlığına bağlı olabilir.

Anahtar kelimeler: Over kanseri; *MTHFR* geni; C677T polimorfizmi; Homosistein; Folik asit; B12 vitamini; Comet testi.

Introduction

Ovarian cancer is the most prevalent gynecological cancer, and is the most common type of epithelial cancer. Two-thirds of the cases are diagnosed in a late stage, as they remain asymptomatic until metastasis occurs. Moreover, ovarian cancer has the highest overall mortality rate among all fatal tumors in women [1–3].

In this scenario, folate and methionine play a significant role in DNA synthesis, repair, and methylation, and methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the regulation of both folate and methionine metabolism. MTHFR catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which ultimately donates the methyl group to homocysteine for further DNA methylation. The conversion to 5-methyltetrahydrofolate is, therefore, essential for DNA methylation. Decreased MTHFR activity leads to decreased availability of methyl groups for DNA methylation, and may thereby affect the expression of tumor suppressor genes as well as overall DNA stability [4–6]. Additionally, in vitro studies in human lymphocytes have shown that low folate serum levels are associated with misincorporation of dUMP into DNA and leading to double-strand breakage [7, 8]. It has also been argued that low folate levels, along with gene polymorphism-associated hyperhomocysteinemia, might be associated with neural tube defects [9].

A common *MTHFR* variant C677T (rs1801133), resulting in an alanine to valine amino acid substitution, is associated with reduced MTHFR enzymatic activity. C677T *MTHFR* gene polymorphism was also reported as a risk factor for breast, ovarian, and colorectal cancer [10–12]. Previous studies have shown that this polymorphism was also increased in Polish *BRCA1* mutation carriers [11], and that 677T homozygosity was associated with bilateral mammary cancer and with both breast and ovarian carcinoma among Jewish *BRCA1/2* mutation carriers [12].

Moreover, deficiency of methionine formation from homocysteine, due to reduced MTHFR activity, results in reduced methionine levels and excess of homocysteine with toxic effect for the organism [13].

The degree of the genetic damage in ovarian cancer patients was assessed by single-cell gel electrophoresis, also known as the comet assay [14, 15]. This technique was used to evidence the effects of the oxidative damage induced by ionizing ultraviolet radiation on lymphocytes. In the current study, we investigated the *MTHFR* gene C677T polymorphism in ovarian cancer patients and compared them to healthy controls. We also analyzed homocysteine, folate, and vitamin B12 levels, which play crucial roles in MTHFR metabolism. Furthermore, DNA damage in patients with different stages of ovarian cancer was evidenced by the comet assay, in order to unveil the mechanisms responsible for its pathogenesis.

Materials and methods

The study cohort included 50 ovarian cancer patients (age 52.88 ± 12.83 years old) from the Department of Obstetrics and Gynecology of the Haseki Education and Research Hospital, and 54 healthy subjects (age 50.75 ± 9.64 years old), without cancer or cardiovascular disease history, who underwent a regular check-up from Cerrahpasa Medical Faculty Hospital. All patients and controls provided informed consent before being enrolled in the study. This study was approved by the Ethics Committee of the Cerrahpasa Medical Faculty (#28113). All patients and controls were from the Istanbul district, with middle-income brackets. Patients and controls were not doing regular exercise. All patients who underwent blood sample collection were not subjected to chemotherapy or anticancer therapy.

Genomic DNA was isolated from peripheral blood using a commercial kit (Roche Diagnostics GmbH,

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 1/2/17 1:30 PM

Mannheim, Germany) according to the manufacturer's instructions. *MTHFR* C677T (rs1801133) polymorphism was determined by PCR followed by restriction fragment length polymorphism (RFLP), as previously described [16]. PCR products digested by *Hinf*I resulted in one 198-bp fragment in wild-type homozygotes (CC), two 175 and 23 bp fragments in mutated homozygotes (TT), and three 198, 175, and 23 bp fragments in heterozygotes (CT).

Homocysteine levels were determined by ELISA [The Axis® Homocysteine Enzyme Immunoassay (EIA), Scotland] and vitamin B12, and folate levels were determined by chemiluminescence method (Access Immunoassay Systems, Beckman Coulter, USA). DNA fragmentation in patients with ovarian cancer was assessed by the comet assay, as previously reported [14]. For the comet assay, 5 mL of peripheral blood were taken and placed into a heparinized tube. Lymphocytes were isolated by the Ficoll–Histopaque method. Briefly, the basic steps of the comet assay included the preparation of microscopic slides layered with cells embedded in agarose gel, lysis of the cells to release the DNA, electrophoresis, neutralization of the alkali, drying, DNA staining by ethidium bromide (20 mg/mL), and scoring [14]. Cells were assessed visually and scores from 0 (undamaged) to 4 (maximally damaged) were assigned according to the tail intensity (size and shape). Statistical comparison of DNA damage between patient and control groups was performed using the method reported by Feng et al. [17]. All experiments were performed by fluorescent microscopy (Olympus).

Statistical analyses (mean, standard deviation, and percentages) were performed using SPSS 21 software. One-way analysis of variance (ANOVA) was used to compare folate, homocysteine, vitamin B12, and comet assay results between the genotypic sub-groups of cases and controls. The Chi-square (χ^2) test was used to compare the association between the genotypes and alleles in cases and controls, and to test for deviation of genotype distribution from the Hardy-Weinberg equilibrium (HWE). A p-value < 0.05 was considered statistically significant.

Results

The number and percentages of patients with different histologic subtypes of ovarian cancer were: n = 40 (80%) serous, n = 3 (6%) mucinous, n = 3 (6%) sex-cord, n = 1 (2%) clear-cell, n = 2 (4%) germ-cell, and n = 1 (2%) müllerian mixed-cell type ovarian cancer.

The *MTHFR* C677T polymorphism genotype distribution is presented in Table 1 and Figure 1. The genotypes were in HWE among patients ($\chi^2 = 2.31$, p = 0.12) and controls ($\chi^2 = 1.97$, p = 0.16). The frequencies of the genotypes were consistent with the HWE in the whole sample ($p > 0.05$).

Table 1: The distribution of the *MTHFR* gene C677T (rs1801133) genotype and allele frequencies between ovarian cancer patients and controls.

MTHFR C677T (rs1801133) genotypes	Ovarian cancer patients n (%) (n = 50)	Controls n (%) (n = 54)	p-Value
CC (Ala/Ala)	18 (36.0)	19 (35.2)	
CT (Ala/Val)	28 (56.0)	30 (55.6)	
TT (Val/Val)	4 (8.0)	5 (9.2)	0.97
C allele frequency	0.64	0.63	
T allele frequency	0.36	0.37	> 0.05

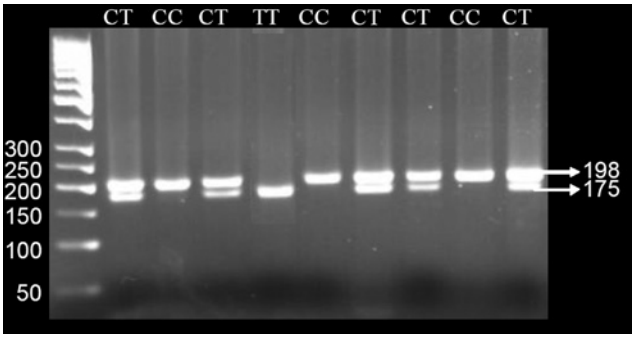


Figure 1: RFLP products of *MTHFR* C677T (rs1801133) polymorphism in agarose gel electrophoresis.

The distribution of *MTHFR* C667T genotypes among ovarian cancer patients was as follows: CC genotype n = 18 (36%), CT genotype n = 28 (56%), and TT genotype n = 4 (8%). The allelic distribution was: C allele n = 64 (64%) and T allele n = 36 (36%). The *MTHFR* C667T genotype frequencies in the control group were: CC genotype n = 19 (35.2%), CT genotype n = 30 (55.6%), and TT genotype n = 5 (9.2%). The allelic frequencies were: C allele n = 68 (63%) and T allele n = 40 (37%). There were no significant differences in *MTHFR* C667T genotype frequencies between the ovarian cancer patients and the control group (p = 0.97) (Table 1).

Homocysteine, folate, vitamin B12 levels, and comet assay scores for ovarian cancer patients and healthy controls are shown in Table 2. We found no significant differences regarding homocysteine (p = 0.6), folic acid (p = 0.92), and vitamin B12 plasma levels between ovarian cancer patients and controls (p = 0.14). We compared the genotypes of patients and controls according to homocysteine, folate, vitamin B12 levels and comet assay

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 1/2/17 1:30 PM

Table 2: Biochemical and demographic characteristics of ovarian cancer patient and control groups.

Parameters	Patients (n=50)	Controls (n=54)	p-Value
Age (years)	52.88±12.83	50.75±9.64	t=1.94, p=0.054
Homocysteine (mmol/L)	8.26±4.37	8.73±3.88	t=0.53, p=0.60
Folate (ng/mL)	6.62±4.03	6.55±2.19	t=0.10, p=0.92
Vitamin B12 (pg/mL)	268.48±124.71	302.22±96.95	t=1.48, p=0.14
Comet assay scores	8.82±2.31	3.45±1.13	t=14.82, [a]p<0.01

[a]$p < 0.05$ was considered as statistically significant.

Table 3: Comparison of homocysteine, folic acid, vitamine B12 levels, and comet assay scores with *MTHFR* C677T (rs1801133) genotypes of ovarian cancer patient and control groups.

Parameters	Ovarian cancer patients		Controls		p-Value
	CT+TT (n=32)	CC (n=18)	CT+TT (n=35)	CC (n=19)	
Homocysteine (mmol/L)	8.7±4.9	7.40±3.11	8.79± 4.13	8.62±3.50	p=0.76
Folic acid (ng/mL)	6.3±4.1	7.14±3.97	6.28±1.99	7.03±2.48	p=0.72
Vitamin B12 (pg/mL)	260.8±135.7	281.73±105.9	301.17±90.0	304.1±110.5	p=0.47
Comet assay scores	9.26±2.24	8.03±2.28	3.44±1.25	3.47±0.89	[a]p<0.001
	CC+TT (n=22)	CT (n=28)	CC+TT (n=24)	CT (n=30)	
Homocysteine (mmol/L)	7.50±2.90	8.79±5.14	8.94±3.88	8.55±3.94	p=0.73
Folic acid (ng/mL)	6.72±3.91	6.56±4.19	6.83±2.59	6.31±1.79	p=0.94
Vitamin B12 (pg/mL)	279.8±99.3	260.4±141.4	295.4±112.3	307.6±84.2	p=0.45
Comet assay scores	8.52±2.51	9.05±2.16	3.46±1.00	3.45±1.20	[a]p<0.001

[a]$p < 0.05$ was considered as statistically significant.

scores. The comet assay scores of CC and CT genotypes showed a significant difference between patients and controls (Table 3). The comet assay scores also showed a significant increase in DNA fragmentation among ovarian cancer patients compared to controls ($p < 0.01$) (Figure 2).

Homocysteine, folic acid, vitamin B12 levels, and comet assay scores in association with the tumor grades of ovarian cancer in our cohort of patients are shown in Table 4. Comet assay scores were significantly higher for grade 3 than for grade 1 and 2 tumors ($p = 0.0007$) (Table 4).

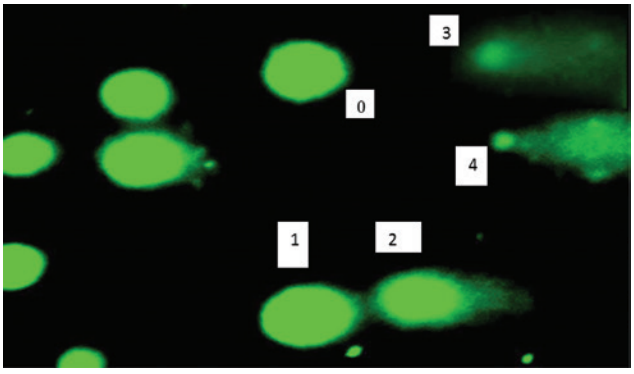


Figure 2: DNA fragmentation in patients with ovarian cancer.

Discussion

Ovarian cancer is the fourth leading cause of cancer-related deaths worldwide, following lung, breast, and colon cancer [1]. Several studies proved that the risk of having ovarian cancer at any stage of a woman's life is 1.42% [18]. The disease is usually rare before the age of 40, increasing steeply and showing a peak after 65 years of age [19].

Sazci et al. studied the allelic frequencies of C677T and A1298C polymorphisms in the *MTHFR* gene in 1684 randomized individuals from the Turkish population [20]. They reported the frequencies of C677T genotypes as 42.9% CT, 47.4% CC, and 9.6% TT. In our cohort, we found the CT genotype frequency to be 55.8%, CC genotype 35.6%, and TT genotype 8.6%, in accordance to the previously mentioned findings.

Previous studies on the effects of *MTHFR* C677T polymorphism showed an increased breast cancer risk in premenopausal women associated with the TT genotype [21]. In Korean population, the *MTHFR* C677T polymorphism has been reported as a risk factor for cervical cancer [22]. Additional studies indicate that *MTHFR* C677T polymorphism frequencies are higher in *BRCA1* mutation carriers, with higher rates of TT homozygotes in women with

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 1/2/17 1:30 PM

Table 4: Comparison of homocysteine, folic acid and vitamin B12 levels, and comet assay scores with the grades of ovarian cancer patients.

Parameters	Grade 1	Grade 2	Grade 3	p-Value
Homocysteine (mmol/L)	8.52 ± 2.69 (n = 7)	8.77 ± 5.50 (n = 16)	7.63 ± 3.79 (n = 16)	F = 0.27 p = 0.75
Folic acid (ng/mL)	5.83 ± 2.34 (n = 7)	6.14 ± 3.25 (n = 17)	7.43 ± 5.17 (n=17)	F = 0.58 p = 0.56
Vitamin B12 (pg/mL)	340.00 ± 107.22 (n = 7)	253.29 ± 130.19 (n = 17)	254.23 ± 122.13 (n = 17)	F = 1.41 p = 0.25
Comet assay scores	6.87 ± 2.35 (n = 10)	8.51 ± 1.68 (n = 19)	10.02 ± 2.14 (n = 21)	F = 8.56 [a]p = 0.0007

[a]$p < 0.05$ was considered as statistically significant.

bilateral breast and ovarian cancer [11]. Another study demonstrated that 677T homozygosity is associated with bilateral mammary and joint breast and ovarian carcinoma among Jewish *BRCA1/2* carriers [12]. In a review by Pu et al. an association between the *MTHFR* C677T polymorphism and susceptibility of ovarian cancer in Asian population suggests that the TT genotype may act as a risk factor for ovarian cancer among Asians but not among Caucasians [23]. Moreover, a meta-analysis including 3379 ovarian cancer cases and 4078 controls evidenced that *MTHFR* C677T polymorphism is not associated with ovarian cancer in Caucasians, whereas the T variant may contribute to increase the risk in East Asians [24]. Finally, it was suggested that the *MTHFR* T allele increases the risk for ovarian carcinoma development in three times [25]. In our study, there were no significant differences in *MTHFR* C667T genotype frequencies between the ovarian cancer patients and the control group. Our findings were consistent with the studies of Pu et al. and Ding et al. since our patient and control groups were composed of Turkish Caucasians [23, 24].

Wu et al. reported an association between folic acid deficiency and *MTHFR* C677T polymorphism in human peripheral blood lymphocyte cytotoxicity [26]. DNA methylation defects may result in carcinogenesis by causing genomic instability and mutations [27]. Chen et al. found that DNA hypomethylation leads to an increase in mutation rates [28]. The present study was unable to confirm the role of the TT genotype in the development of ovarian cancer, given its small sample size (50 ovarian cancer patients and 54 controls) as we only selected patients who were not receiving chemotherapeutic medication before enrollment. The homocysteine levels were not significantly different from the control group between the different C677T genotypes. Our findings regarding homocysteine levels could be explained by an improved quality of life and an increased folic acid intake.

Some studies speculate that changes in folic acid metabolism might contribute to the oncogenic development of both ovarian and colorectal cancer [10, 29]. Given its essential role in DNA synthesis, repair, and methylation, it has been hypothesized that dietary folate consumption may influence cancer risk [30]. A decrease in folic acid levels may increase DNA instability. However, in our study, no significant differences were observed in the folic acid levels between ovarian cancer patients and controls. Breast cancer has been also reported as a manifestation of abnormal genetic and epigenetic changes. A disruption in folate metabolism might affect genetic and epigenetic changes influencing gene expression, through DNA methylation, and genome integrity, through DNA synthesis and repair [27]. The present study showed decreased folic acid levels in ovarian cancer patients carrying the TT genotype compared to controls, however, this difference could not reach statistical significance given the limited sample size.

In our study, the level of genetic damage in cancer patients was assessed by the comet assay. Lymphocytes circulate through different organs, including the lung, before they return to peripheral blood. They can circulate for years or even decades, accumulating DNA mutations produced by the exposure to different mutagenic agents. The comet assay has previously been used to measure DNA damage by irradiating the peripheral lymphocytes from bladder cancer patients and showing a higher degree of DNA damage in patients compared to controls [31]. Baltaci et al. used the comet assay to show the degree of genetic damage in ovarian cancer patients evidencing higher DNA fragmentation in correlation with increasing histopathological grades of cancer [15]. In our study, we found that DNA fragmentation in the ovarian cancer group was significantly higher than that in the control group, in accordance with previous findings [15]. Furthermore, the comparison of the histopathological grades among ovarian cancer patients, by the comet assay, showed that DNA fragmentation was higher in patients with grade 3 cancer compared to those with grade 1 or 2. Therefore, we suggest that DNA fragmentation increases with the level of malignancy of the tumor.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 1/2/17 1:30 PM

In conclusion, our results disprove the correlation between the *MTHFR* C677T polymorphism and ovarian cancer. Furthermore, no significant differences were found in homocysteine, folic acid, and vitamin B12 levels between ovarian cancer patients and controls. We showed that patients with the TT genotype exhibit low folate levels. In addition, increased DNA fragmentation was detected in the ovarian cancer patient group. DNA fragmentation degree was the highest in grade 3 ovarian cancer suggesting an association between DNA fragmentation and carcinogenesis progression. Larger patient cohorts will be necessary to confirm the association between homocysteine, folic acid, vitamin B12, and the *MTHFR* C677T polymorphism in ovarian cancer.

Acknowledgments: This study was supported by The Research Support Unit of the Istanbul University. Project no: T-309/03112003.

Conflict of interest statement: Authors have no conflict of interest regarding this study.

References

1. Look KY. Epidemiology, etiology abd screening of ovarian cancer. In: Rubin SC, Sutton GF, editors. Ovarian cancer. USA: McGraw Hill Inc, 1993:175–86.
2. Greenle RT, Murray T, Bolden S, Wingo PA. Cancer, statistics 2000. Cancer J Clin 2000;50:7–33.
3. Silverberg E, Borings CS, Squires TS. Cancer, statics 1990. CA Cancer J Clin 1990;40:9–26.
4. Shane B, Stokstad EL. Vitamin B12-folate interrelationships. Annu Rev Nutr 1985;5:115–41.
5. Ueland PM, Husted S, Schneee J. Biological and clinical implications of the MTHFR C677T polymorphism. Trends Pharmocol Sci 2001;22:195–201.
6. Brattstrom L, Wilcken DE, Ohrvik J, Brudin L. Common MTHFR gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta analysis. Circulation 1998;98:2520–6.
7. Fenech M. The role of folic acid and vitamin B12 in genomic stability of human cells. Mutat Res 2001;475:56–67.
8. James SJ, Miller BJ, Mc Garrity LJ, Morris SM. The effect of folk acid deficiency on deoxyribonucleotide pools and cell cycle distribution in mitogen stimulated lymphocytes. Cell Prolif 1994;27:395–406.
9. Shield DC, Kirke PN, Mills JL. The termolabile variant of MTHFR and neural tube defects: an evaluation of genetic risk and the relative importance of the genotypes of the embryo and the mother. Am J Hum Genet 1999;64:1045–55.
10. Ma J, Stampfer MJ, Giovannucci E, Artigas C, Hunter DJ, Fuchs C, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–102.
11. Jakubowska A, Gronwald J, Menkiszak J, Górski B, Huzarski T, Byrski T, et al. Methylenetetrahydrofolate reductase polymorphisms modify BRCA1-associated breast and ovarian cancer risks. Breast Cancer Res Treat 2007;104:299–308.
12. Gershoni-Baruch R, Dagan E, Israeli D, Kasinetz L, Kadouri E, Friedman E. Association of the C677T polymorphism in the *MTHFR* gene with breast and/or ovarian cancer risk in Jewish women. Eur J Cancer 2000;36:2313–6.
13. Bailey LB, Duhaney RL, Maneval DR, Kauwell GP, Quinlivan EP, Davis SR. Vitamin B_{12} status is inversely associated with plasma homocysteine in young women with C677T and/or A1298C MTHFR polymorphisms. J Nutr 2002;132:24665–709.
14. Olive PL, Banath JP, Durand RE. Heterogenity in radiation-induced DNA damage and repair tumuor and normal cells measured using the Comet assay. Radiat Res 1990;122:86–94.
15. Baltacı V, Kayıkçıoğlu F, Alpas I, Zeyneloğlu H, Haberal A. Sister cromatid exchange rate and alkaline comet assay scores in patients with ovarian cancer. Gynecol Oncol 2002;84:62–6.
16. Frosst P, Blom HJ, Milos R. A candidate genetic risk factor for vascular disease: a common mutation in MTHFR. Nat Genet 1995;10:111–3.
17. Feng S, Kong Z, Wang X, Peng P, Zeng EY. Assessing the genotoxicity of imidacloprid and RH-5849 in human peripheral blood lymphocytes in vitro with comet assay and cytogenetic tests. Ecotoxicol Environ Saf 2005;61:239–46.
18. Whittemore AS, Harris R, Intyre J. Characteristics relating to ovarian cancer risk. Collaborative analysis of 12 US case control studies. Invasive epithelial ovarian cancers in white woman. Collaborotive Ovarian Cancer Group. Am J Epidemiol 1992;136:1184.
19. Tortolero-Luna G, Mitchell MF. The epidemiology of ovarian cancer. J Cell Biochem Suppl 1995;23:200–7.
20. Sazci A, Ergul E, Kaya G, Kara I. Genotype and allele frequencies of the polymorphic methylenetetrahydrofolate reductase gene in Turkey. Cell Biochem Funct 2005;23:51–4.
21. Ergül E, Sazcı A, Utkan Z, Cantürk Z. Polymorphisms in the MTHFR gene are associated with breast cancer. Tumor Biol 2003;24:286–90.
22. Sull JW, Jee SH, Yi S, Lee JE, Park JS, Kim S, et al. The effect of methylenetetrahydrofolate reductase polymorphism C677T on cervical cancer in Korean women. Gynecol Oncol 2004;95:557–63.
23. Pu D, Jiang SW, Wu J. Association between MTHFR gene polymorphism and the risk of ovarian cancer: a meta-analysis of the literature. Curr Pharm Des 2014;20:1632–8.
24. Ding XP, Feng L, Ma L. MTHFR C677T polymorphism and ovarian cancer risk: a meta-analysis. Asian Pac J Cancer Prev 2012;13:3937–42.
25. Singh A, Pandey S, Pandey LK, Saxena AK. In human alleles specific variation of MTHFR C677T and A1298C associated "risk factor" for the development of ovarian cancer. J Exp Ther Oncol 2015;11:67–70.
26. Wu X, Liang Z, Zou T, Wang X. Effects of folic acid deficiency and *MTHFR* C677T polymorphisms on cytotoxicity in human peripheral blood lymphocytes. Biochem Biophys Res Commun 2009;379:732–7.
27. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr 2000;130:129–32.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 1/2/17 1:30 PM

28. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. Nature 1998;395:89–93.
29. Viel A, Dall'Agnese L, Simone F, Canzonieri V, Capozzi E, Visentin MC, et al. Loss of heterozygosity at the 5,10-methylenetetrahydrofolate reductase locus in human ovarian carcinomas. Br J Cancer 1997;75:1105–10.
30. Navarro Silvera SA, Jain M, Howe GR, Miller AB, Rohan TE. Dietary folate consumption and risk of ovarian cancer: a prospective cohort study. Eur J Cancer Prev 2006;15:511–5.
31. Schabath MB, Spitz MR, Grossman HB, Zhang K, Dinney CP, Zheng PJ, et al. Genetic instability in bladder cancer assessed by the comet assay. J Natl Cancer Inst 2003;95:540–7.

Brought to you by | Turkish Biochemical Society (TBD)
Authenticated
Download Date | 1/2/17 1:30 PM