

C677T POLYMORPHISM OF METHYLENETETRAHYDROFOLATE REDUCTASE (MTHFR) GENE IN PATIENTS WITH CHRONIC LYMPHOCYtic LEUKEMIA AND DIFFUSE LARGE B CELL LYMPHOMA

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C677T POLIMORFIZAM GENA METILENTETRAHIDROFOLAT REDUKTAZE (MTHFR) KOD PACIJENATA SA HRONIČNOM LIMFOCITNOM LEUKEMIJOM I DIFUZNIM KRUPNOĆELIJSKIM B LIMFOMOM

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ABSTRACT

Methylenetetrahydrofolate reductase (MTHFR) plays an important role in folate metabolism, contributing to DNA synthesis, methylation and eventually to cancer susceptibility and it has been implicated in cancer risk. In the present study we investigated the association of the common MTHFR C677T polymorphism with B cell chronic lymphocytic leukemia and diffuse B cell large non Hodgkin's lymphoma. Patients were compared with age and sex matched control subjects.

Our results indicate significantly lower distribution of variant allele 677T in patients with chronic lymphocytic leukemia compared with control group (frequency of variant allele 677T 24% vs 33% respectively). The difference in allelic distribution of MTHFR gene among those two groups was statistically significant ($p=0.05$). Results were the same when we compared CLL with DLBCL (frequency of variant allele 677T 24% vs. 34, 5% $p=0.05$). This was accompanied by a significantly higher frequency of homozygote normal genotype (677CC) among the patients with CLL. The difference of allele distribution between DLBCL and control group did not reach statistical significance ($p=0.065$).

Our results suggest that the distribution of polymorphism of MTHFR gene may vary among the different group of lymphoproliferative diseases and that 677CC genotype may represent risk factor for developing of CLL.

Key words: methylenetetrahydrofolate reductase, polymorphism, genetic, leukemia, lymphocytic, chronic, B cell, lymphoma, large B-cell, diffuse

INTRODUCTION

Lymphoproliferative diseases include a heterogeneous group of lymphoid neoplasms characterized by typical morphological, immunophenotypical, genotypic and clinical features (1). In that group, beside characteristic neoplasms which are general for WHO classification, one can distinguish low progressive (follicular lymphoma, chronic lymphocytic leukemia, etc.) and high progressive lymphoid neoplasms (acute leukemia, diffuse large B cell lymphoma, etc.).

Etiology of most cases of lymphoproliferative neoplasms is unknown, although some factors such as immunodeficiency, viral infections, exposure to environmental and chemical factors and genetic factors have been defined (2–4). Certain genetic events during cell differentiation, such as chromosomal translocations, mutations in various genes, genetic polymorphisms and many other chromosomal aber-

SAŽETAK

Metilentetrahidrofolat reduktaza ima bitnu ulogu u metabolizmu folata, sintezi i metilaciji DNK, i samim tim predstavlja važan faktor u mehanizmu kancerogeneze. U prikazanoj studiji smo ispitali povezanost naj~učestalijeg polimorfizma C677T gena za metilentetrahidrofolat reduktazu sa etiologijom hronične limfocitne leukemije i difuznog krupnoćelijskog B limfoma. Pacijenti su upoređivani sa kontrolnom grupom koja je imala istu polnu i starosnu distribuciju kao i grupa pacijenata.

Naši rezultati su pokazali značajno nižu učestalost mutanog 677TT alela kod pacijenata sa hroničnom limfocitnom leukemijom, poredeći sa kontrolnom grupom (frekvencija mutanog alela 24% nasuprot 33%, redom). Razlika u distribuciji alela je statistički značajna ($p=0,05$). Rezultati su isti kada se poredi grupa pacijenata sa HLL i grupa pacijenata sa DLBCL (24% nasuprot 34,5%, $p=0,05$). Takvi rezultati su praćeni značajno većom frekvencijom normalnog 677CC homozigota kod bolesnika sa HLL. Razlika u alelskoj distribuciji između bolesnika sa DLBCL i kontrolne grupe nije pokazala statističku značajnost ($p=0,065$).

Naši rezultati sugerišu da distribucija polimorfizma C677T MTHFR gena može da varira u okviru različitih grupa limfoproliferativnih oboljenja, kao i da 677CC genotip može da predstavlja faktor rizika za razvoj hronične limfocitne leukemije.

Ključne reči: metilentetrahidrofolat reduktaza, polimorfizam gena, B-ćelijska hronična limfocitna leukemija, krupnoćelijski difuzni B-limfom

rations play an important role in genesis of lymphoid malignancies. Also, methylation status of various oncogenes or tumor suppressor genes may induce selective growth of cells or its inhibition (5).

Folate is an important nutrient required for DNA synthesis, repair or methylation; it donates a methyl group to uracil and converts it to thymine. Low folate could increase risk of malignancy by following mechanisms: 1) DNA hypomethylation and inappropriate activation of oncogenes or 2) uracil misincorporation during DNA repair and synthesis, leading to DNA strand breaks, chromosome damages and eventually malignant transformation (6–8). Folate metabolism requires the optimal activity of multiple enzymes including 5, 10- methylenetetrahydrofolate reductase (MTHFR) which catalyses the irreversible conversion



of 5, 10- methylenetetrahydrofolate to 5, 10- methyltetrahydrofolate, the methyl donor for the conversion of homocysteine to methionine, which is converted to S-adenosylmethionine (SAM). SAM methylates cytosine residues in DNA (figure 1.) The consequence of inappropriate activity of MTHFR is hypomethylation of critical genes and this makes MTHFR cancer predisposing gene (1).

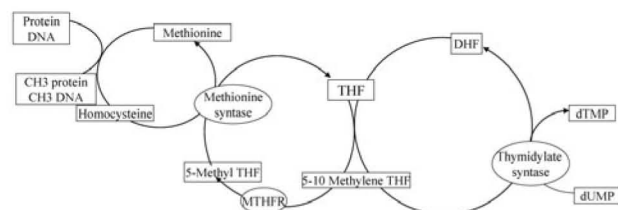


Figure 1. Methionine metabolizing and folate pathways. MTHFR catalyzes the reduction of 5,10-methylene THF to 5-methyl THF. Reduced activity of MTHFR results in the accumulation of 5,10-methylene THF, which accelerates methylation of dUMP to dTMP.

Several single nucleotide polymorphisms within the MTHFR gene have been described, resulting in variant enzyme activity. Most frequent MTHFR polymorphism is base exchange at nucleotide position 677 (C→T; alanin→valin). This polymorphism leads to the expression of thermo labile form of MTHFR and its reducing enzyme activity (9). Homozygosity for the MTHFR 677 T allele is associated with many diseases such as cardiovascular disease, neural tube defect and with many cancers such as colorectal, ovarian, oropharyngeal, breast, endometrial (7,10–15). Due to role in cancerogenesis of different solid tumors, this polymorphism is also of interest in the pathogenesis of lymphoid malignancies.

Data on the association of MTHFR 677 polymorphism with risk of CLL and DLBCL are controversial. Great number of studies described no association of risk of CLL and this polymorphism (16). Although some authors described that MTHFR 677CT polymorphism is associated with risk of CLL progression (17, 18). In a group of non Hodgkin's lymphomas, including DLBCL, results are also conflicting. While some of them show decreased risk for DLBCL in patients with 677TT genotype (16, 19), the other authors described controversial results (20), or do not show any connection (21).

Controversial data of influence of MTHFR 677TT genotype in pathogenesis of lymphoproliferative disease could be explained with fact that increased activity of MTHFR enzyme increase availability of methylenetetrahydrofolate and reduce the frequency of misincorporation of uracil into DNA, reducing the risk of DNA double strand breaks. On the other hand, reduced MTHFR activity might result in hypomethylation of DNA promoter regions, leading to increased expression of protooncogene (22–24).

The aim of the present study was to investigate the allele frequency of MTHFR C677T polymorphism in group of patients with CLL, and in the group of patients with DLBCL. Whereas we considered low progressive disease (CLL) and high progressive disease (DLBCL), we investigated difference of MTHFR 677 polymorphism among these two groups.

PATIENTS AND METHODS

Patients

This study included 26 patients with DLBCL obtained from Oncology and Radiology Institute, Belgrade and 23 patients with CLL obtained from Clinic of Hematology, Mil-

itary Medical Academy, Belgrade. The patients groups were compared with a control group of healthy individuals (n=35). The control subjects were randomly selected from participants without any sign of a malignant disease.

Methods

Peripheral blood was placed into EDTA containing tubes and lymphocytes were separated by Ficoll gradient centrifugation. Genomic DNA was isolated from peripheral lymphocytes by standard salting out procedure which consist of red cell and mononuclear cell lysis, cell lysis by proteinase K and SDS, salting out by NaCl, DNA precipitation by ethanol and resuspension (25)

Polymerase chain reaction

Genotyping of the MTHFR C677T polymorphism was performed using conventional polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) analysis. A 198 bp region of exon 4 of the MTHFR gene was amplified using the primer and reaction condition (26). The success of amplification was controlled by 2% agarose gel electrophoresis and visualized by ethidium bromide staining.

Digestion

Amplified 198 bp PCR product were digested with Hinf I (Fermentas) according to the manufacture's conditions. Hinf I digest normal 198 bp product into a 175 bp and 23 bp fragments.

Polyacrylamide electrophoresis (PAGE)

Samples of amplificats of MTHFR gene after digestion were electrophoresed in 10% polyacrylamide gel. Gel was run in 0.5X TBE at 150 V and 15 W for 120 min and was silver stained (Serva, Germany). The C allele produced 198 bp band, and the T allele produced 175 and 23 bp fragments. Heterozygote produced bands for each allele.

Statistical analysis

The Fisher exact test was used to determine the difference between the allele and genotype frequencies among the groups. A two sided alpha level of 0.05 was considered statistically significant.

RESULTS

The characteristics of study subject are given in table 1.

Table 1. Characteristics of study participants.

	Number	Gender		Age (years)	
		Female	Male	Median	Range
Controls	35	10	25	43	32–65
DLBCL	26	8	18	42	31–80
CLL	23	7	16	54	30–78

DLBCL→Diffuse large B cell lymphoma, CLL→Chronic lymphocytic leukemia

The characteristic pattern of npolyacrylamide gel electrophoresis for C677T polymorphism of MTHFR gene was shown in figure 2.

The frequency of variant allele was 33% in the control group and 24% in the patients group with CLL, indicating that the variant allele occurred less frequently in patients with CLL compared to control group. That was result of higher frequency of normal homozygote 677CC in patients group with CLL than in control group (56,5 % vs. 45,7 % respectively). The difference of allele distribution among this two groups was statistically significant ($p=0,05$).

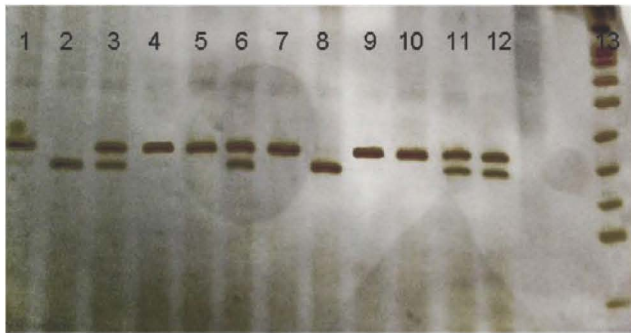


Figure 2. Polyacrylamide gel electrophoresis for C677T polymorphism of MTHFR gene. After digestion with Hinf I, the C allele produced 198 bp band and the T allele produced 175 and 23 bp bands. Homozygote 677CC→lines 1,4,5,7,9,10; homozygote 677TT→lines 2, 8; heterozygote 677CT→lines 3,6,11,12; PCR marker→line 13.

In the group of patients with DLBCL, difference of frequency of variant allele 677T and difference of allele distribution among this group of patients and control group was not statistically significant (34,5% vs. 33% ; $p=0,065$). Our data indicate that there is no considerable difference in the prevalence of the MTHFR C677T polymorphism between control group and DLBCL patients group (table 2).

Table 2. Allele and genotype frequencies in the patients and control groups.

	Number	677CC n (%)	677CT n (%)	677TT n (%)	T freq. (%)
Controls	35	16 (45,7)	15 (42,8)	4 (11,5)	33
CLL	23	13 (56,5)	9 (39,2)	1 (4,3)	24
DLBCL	26	11 (42,3)	12 (46,2)	3 (11,5)	34,5

Given the fact that diffuse large B cell lymphoma and chronic lymphocytic lymphoma have different clinical feature we investigated the difference of allele distribution of MTHFR gene among patients group with DLBCL and patients group with CLL. The frequency of variant allele 677T was significantly lower in patients with CLL (24% vs. 34.5%). The difference of allele distribution among those two patients groups was statistically significant ($p=0,05$).

DISCUSSION

Our study investigated possible role of the common MTHFR gene polymorphism as a risk factor for two groups of lymphoproliferative diseases: chronic lymphocytic leukemia and diffuse large B cell lymphoma. Both groups were compared with sex and age matched control group, and groups of patients were compared to each other. The polymorphism was investigated in patients and controls by PCR-RFLP analysis.

Our findings show that the MTHFR C677T polymorphism occurs less frequently in patients with CLL, compared with the distribution and frequency of variant allele in control group and group of patients with DLBCL. Distribution and frequency of variant allele of MTHFR among control and patients group with DLBCL was not statistically significant. These results suggest that distribution of polymorphic allele 677T may vary among different groups of lymphoproliferative diseases.

Actually, conflicting result has been reported about C677T polymorphism in lymphoproliferative disease. Regarding the potential association of MTHFR genotype with diffuse

large B cell lymphoma, only a few studies about non Hodgkin's lymphoma subentities, including DLBCL, have been published (19–21, 23, 26, 27). While same authors do not find association (21, 27), other describe a protective effect of the MTHFR 677TT genotype (19, 23, 26). One large population based study on 1593 patients found an increased risk of diffuse large cell lymphoma in adult patients being homozygous for the mutated allele (20).

The reports concerning the role of the MTHFR polymorphism in chronic lymphocytic leukemia pathogenesis are also inconsistent (18, 28, 29–31). Most of the results does not show association between C677T polymorphism and risk of CLL (16, 29, 30). Some authors describe significantly more aggressive clinical course in patients with 677CT or TT genotype (18), while some showed association of MTHFR 677CC genotype with high relapse rate in patients with CLL (30, 31).

Association of the normal genotype 677CC with increased cancer risk in patients with CLL, which we have found in our study, may indicate protective effect of MTHFR 677TT genotype.

Protective effect of 677TT genotype in pathogenesis of CLL could be explained with the fact that TT homozygote reduce MTHFR activity and result in the accumulation of 5, 10-methylenetetrahydrofolate. This, in turn, reduces the chances for misincorporation of uracil into DNA, which lead to double - strand breaks during uracil excision repair (8,32). Double-strand breaks and deletion in CLL have been reported at specific sites within chromosome 11q where folate sensitive CCG repeats are located (30).

In most cancer types increased cancer risk conferred by MTHFR polymorphism has been associated with homozygote variant of genotype (677TT) (7,12, 13–15). That could be explained by lower MTHFR enzyme activity, hypomethylation promoter region of oncogenes and their higher expression. Interestingly, these examples show that opposite effects may result from identical causes.

Opposite to our expectation we didn't find statistical significance in distribution of variant allele among patients in DLBCL group and control group, although, when we consider strictly defined clinical parameters such as progression free interval, survival time, and treatment free interval, DLBCL is more progressive lymphoproliferative disease and have the same B cell origin like CLL. Reason for this is probably small sample size, heterogeneity of DLBCL, and complicated signal pathways which are the base of the different carcinogenesis mechanisms (33).

In conclusion, our study provide evidence that homozygote normal genotype 677CC of MTHFR is observed at higher frequency than heterozygote 677CT or variant homozygote 677TT in CLL, representing risk factor in pathogenesis of CLL. These results need to be confirmed in further studies with larger sample size.



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