Association of *MTHFR* Gene with Methotrexate Hepatotoxicity in Thai Rheumatoid Arthritis Patients

ความสัมพันธ์ระหว่างยีน *MTHFR* กับการเกิดพิษต่อตับของยาเมโธเทรกเซทในผู้ป่วยโรคข้ออักเสบรูมาตอยด์ชาวไทย

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ABSTRACT

Methotrexate (MTX) is the first drug of choice for the treatment of rheumatoid arthritis (RA) patients. Genetic polymorphism of *MTHFR* C677T and A1298C gene could be involved in reducing enzyme activity leads to MTX-induced hepatotoxicity. This study aimed to determine the strength of the association between *MTHFR* C677T and A1298C and the risk of low-dose MTX induced hepatotoxicity in Thai RA patients. A total of 145 patients with RA were included, the genetic polymorphisms were analyzed by TaqMan® SNP genotyping assays. *MTHFR* 677TT was associated with hepatotoxicity in RA patients (OR 53.25, 95% CI (5.67-500.2), $P<0.001$). However, there was no correlation between MTX toxicity with *MTHFR* A1298C. These data suggest that the presence of the *MTHFR* 677TT SNPs induced hepatotoxicity in Thai RA patients by fifty-three times higher than wild-type.

บทคัดย่อ

ยาเมโธเทรกเซทเป็นยาหลักในการรักษาผู้ป่วยโรคข้ออักเสบรูมาตอยด์ ความหลากหลายทางพันธุกรรมของ *MTHFR* C677T และ A1298C มีผลในการลดการทำงานของเอนไซม์ *MTHFR* ทำให้เพิ่มความเสี่ยงของการเกิดพิษต่อตับจากยาเมโธเทรกเซท การศึกษานี้มีวัตถุประสงค์เพื่อหาความสัมพันธ์ระหว่าง *MTHFR* C677T และ A1298C กับการเกิดพิษต่อตับของยาเมโธเทรกเซทขนาดต่ำในผู้ป่วยโรคข้ออักเสบรูมาตอยด์ จีโนไทป์ของผู้ป่วยโรคข้ออักเสบมาตอยด์ 145 คน วิเคราะห์ด้วย TaqMan® SNP พบว่ายีน *MTHFR* 677TT มีความสัมพันธ์กับการเกิดพิษต่อตับในผู้ป่วยโรคข้ออักเสบ

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รูมาตอยด์ (OR 53.25, 95% CI (5.67-500.2), *P*<0.001) แต่ไม่มีความสัมพันธ์ระหว่างความเป็นพิษต่อตับของยาเมโธเทรกเซทกับ *MTHFR* A1298C ซึ่งข้อมูลเหล่านี้ชี้ให้เห็นว่า *MTHFR* 677TT เพิ่มความเสี่ยงของการเกิดพิษต่อตับของยาเมโธเทรกเซทในผู้ป่วยโรคข้ออักเสบรูมาตอยด์ชาวไทยได้มากกว่าผู้ป่วยที่มียีนปกติ 53 เท่า

Keywords: Methotrexate, Methylenetetrahydrofolate reductase, Hepatotoxicity
คำสำคัญ: ยาเมโธเทรกเซท เมทิลินเททระไฮโดรโฟเลตรีดักเทส ความเป็นพิษต่อตับ

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune of joint diseases, which primarily affects the synovial tissue and leading to joint damage destruction and disability (Smolen *et al*., 2018). The rheumatoid arthritis patient with the presence of autoantibodies as rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPAs), elevated C-reactive protein level (CRP), and erythrocyte sedimentation rate (ESR) suggests a useful early diagnosis of rheumatoid arthritis (Scott *et al.*, 2010; Guo *et al.*, 2018). The therapeutic approach for RA patients includes an early start of conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) is methotrexate (MTX), aimed to a low disease activity or remission. Although MTX is a highly effective csDMARDs for the treatment of RA, it can cause adverse drug reactions e.g., hepatotoxicity, blood dyscrasias, and interstitial lung disease. Methotrexate is contraindicated in patients with hepatic disease, such as hepatitis, and in patients with renal impairment. Monitoring of adverse effects requires pretreatment screening and subsequent safety recording of blood counts and liver function tests (Saag *et al.*, 2008).

Methotrexate is transported into the cells via reduced folate carrier 1 *(RFC1),* then is metabolized by folylpolyglutamate synthase *(FPGS)* to the active methotrexate polyglutamate in the liver as well as in the other tissues. Intracellular formation of MTX-polyglutamate also plays a critical role in MTX activity, inhibition of dihydrofolate reductase *(DHFR),* methylenetetrahydrofolate reductase *(MTHFR),* thymidylate synthase *(TYMS),* and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase *(ATIC),* leading to diminished production of purines and pyrimidines (Inoue and Yuasa, 2014). MTX-polyglutamate inhibits aminoimidazole-4-carboxamide ribonucleotide and inhibition of *ATIC* leading to intracellular accumulation of AICAR and increased adenosine release therefore adenosine binds to cell surface receptors and suppresses inflammatory and immune reactions (Chabner *et al.*, 1985).

The *MTHFR* gene located on chromosome 1 (1p36.6), this gene involved in folate metabolism and DNA synthesis, which irreversibly catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Blount *et al.*, 1997). *MTHFR* genetic variations (single-nucleotide polymorphisms, SNPs) may be associated with clinical variation in patients receiving MTX, the two most studied polymorphisms are C677T (rs1801133) and A1298C (rs1801131), which are both nonsynonymous SNPs. The C to T change at nucleotide 677 leads to alanine to valine substitution at codon 222, rendering

MTHFR more thermolabile and reducing its enzyme activity (Rozen, 1997). Similar to the C677T polymorphism, the A to C change at nucleotide 1298 results in glutamine to alanine substitution, also leading to reduced enzyme activity (Weisberg *et al.*, 1998). There have been reported that *MTHFR* C677T associated with an increased risk of discontinuing MTX treatment mainly due to abnormally elevated liver enzymes, indicating T allele may increase liver toxicity (van Ede *et al.*, 2001). However, *MTHFR* A1298C associated with increasing alanine aminotransferase (ALT) within 3 to 6 months of MTX therapy has been recently observed in Swedish RA patients (Karlsson Sundbaum *et al.*, 2019).

Two common non-synonymous variants, the C677T (Ala222Val) and A1298C (Glu429Ala), are mainly described with decreased enzymatic activity and an alteration of intracellular folate distribution. It is reported that the *MTHFR* C677T genotype decreases by 30 percent of the *MTHFR* enzyme activity *in vitro* compared with the wild-type (Goyette and Rozen, 2000). The A1298C causes conformational changes within the *MTHFR* enzyme that alters the activity of the enzyme but with a lower degree compared to C677T (Yang *et al.*, 2008). The decreased enzyme activity of *MTHFR* theoretically could increase the drug action of MTX and at the same time increase side effects and toxicity (De Mattia and Toffoli, 2009). Low-dose MTX use as either monotherapy or in combination with other DMARDs to treat RA, has good efficacy. However, not all patients are responsive to MTX, 30-40% of patients discontinue therapy within a year of starting the treatment, usually because of lack of efficacy or MTX toxicity. The reasons why some patients are not responding to MTX or may occur adverse drug reactions remain unclear. Several studies have been focused on genetic polymorphisms as possible predictors of MTX efficacy and toxicity. MTX may cause a variety of adverse effects over a wide range of severity, the risk of most side effects is influenced by the MTX dose and treatment regimen. Some patient develops severe side effects such as abnormal liver function and hematological toxicity as leucopenia, thrombocytopenia, which may limit using MTX. A single nucleotide polymorphism C677T of *MTHFR* gene, TT, and CT genotype shows a remarkable decrease of the liver enzyme activity and decreasing the levels of 5-CH_3-THF therefore there is less conversion of homocysteine to methionine. The *MTHFR* C677T knockdown in hepatoma (HepG2) cells interact to MTX low-dose treatment has been found an effect on methylation process (Wang *et al.*, 2019). Long-term MTX administration can cause accumulation of MTX-polyglutamates in the liver and decreased folate levels. The depletion of hepatic folate stores by accumulated MTX-polyglutamates is one possible toxic effect of MTX on the liver (Kremer *et al.*, 1986).

MTX-related hepatic fibrosis may be mediated through adenosine releasing from cultured HepG2 cells and its capacity to interfere with the generation of methionine from homocysteine. Excess of homocysteine induces endoplasmic reticulum stress promoting fat accumulation in the liver. Homocysteine can also activate hepatic stellate cells and proinflammatory cytokines, leading to liver fibrosis (Aithal, 2011; Ortega-Alonso and Andrade, 2018). The reductions in folate-dependent methionine synthesis fluxes as well as in adoMet (S-adenosylmethionine) content were more drastic in HepG2 cells

with stabilized inhibition *MTHFR* gene with low-dose MTX indicated that *MTHFR* gene function affects transmethylation in HepG2 cell models treated with low-dose MTX. When these cells were treated with low-dose MTX, therefore purine and thymidine were inhibited, and the methionine synthesis from serine via folate-dependent remethylation was completely inhibited. Although lymphoblast cell is not the main site for adoMet production or transmethylation reactions, the impacts of low-dose MTX and its interactions with genetic factors such as *MTHFR* genotype on the transmethylation pathway should not be ignored. The reductions in folate-dependent methionine synthesis fluxes as well as in adoMet content were more drastic in both *MTHFR* TT carriers and knockdown *MTHFR* gene in MTX treated HepG2 cell lines (Wang *et al.*, 2019).

To date, the study of the genetic factors involved in the low-dose MTX-induced hepatotoxicity especially in Thai RA patients is undiscovered.

Objective of the study

The aim of this study was to investigate the strength of the association between both *MTHFR* C677T and A1298C polymorphism and the risk of low-dose MTX-induced hepatotoxicity in Thai RA patients.

Materials and methods

Study population

This study was performed as a retrospective study. One hundred and forty-five patients have been diagnosed with rheumatoid arthritis (RA) according to the criteria (American College of Rheumatology (ACR)/Rheumatology (EULAR), between 2017-2018 at Srinagarind and Khon Kaen hospital. Written informed consent was obtained from the subjects after they had been informed both verbally and in writing about the experimental procedures and purposes of the study were enrolled to determine frequencies of *MTHFR* variants related to clinical variables such as *MTHFR* C677T (rs1801133), *MTHFR* A1298C (rs1801131). Ethical approval for this study was obtained from the Ethical Research Committee of Khon Kaen University (HE621219) and Khon Kaen hospital (KEMOU63004).

MTX treatment

All patients received either oral methotrexate monotherapy or combined with the other DMARDs and treated with methotrexate at 5 to 15 mg/week. Patients with RA were given folic acid, and gastric mucosal protective agents during treatment. Folic acid supplementation reduces mucosal and gastrointestinal toxicity, and likely liver toxicity, without reducing MTX efficacy. The combination therapy regimen included sulfasalazine, antimalarial drugs, cyclosporin A and leflunomide tablets combined with appropriate NSAIDs or hormonal drugs according to the specific condition of each patient. The efficacy and ADRs of MTX was evaluated after one year of treatment.

DNA extraction and *MTHFR* C677T and A1298C genotyping

Peripheral blood leukocytes from each patient were separated and gDNA was purified by using a DNA extraction kit (QIAamp DNA Blood mini kit, Germany). gDNA was used for genotyping of *MTHFR C677T* and *MTHFR A1298C* polymorphism using Custom TaqMan® SNP genotyping assays (Applied Biosystems) on an ABI PRISM 7500 Real-Time PCR System (Applied Biosystems) (ID: C___1202883_20 and C____850486_20). Genotyping primers and probes obtained from Thermo Fisher Scientific Inc. The Real-Time PCR was performed by a QuantStudio™ 6 Flex Machine (The Applied Biosystems). The PCR thermal cycling was set as initial denaturing at 95 °C for 10 minutes; 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minute.

Statistical analysis

The demographic data were shown as mean ± standard deviations. Genetic polymorphism *MTHFR* C677T association analysis with MTX adverse drug reactions was calculated by using Pearson Chi-square to test the difference in frequency of abnormal liver function (three-times above the upper normal level of liver enzyme), gastrointestinal disturbance, mucositis, leucopenia, and alopecia with the genotypes, the results were presented as the corresponding *P*-values. The recessive and dominant models were used for genotype distribution analysis, the results were showed as odds ratio (OR) with 95% confidence interval (95% CI) to determine the association between the genetic polymorphism and hepatitis. Logistic regression models were performed to test the association of *MTHFR* C677T polymorphism and the potential confounding factors such as leflunomide, NSAIDs, dyslipidemia, and diabetes mellitus. These multiple comparisons were performed using either the Mann-Whitney or Kruskal-Wallis test. All statistical analyses were interpreted using SPSS (Statistical Package for the Social Science for Windows, version 23.0, IBM Corp., New York). Statistical significance for all tests was set at a *P*-value <0.05.

Results

Characterization of the Studied Population

Demographic and clinical data of the 145 RA patients (124 females and 21 males), mean age of onset and mean RA initiation were 59.7 and 49.1 years. The median disease duration was 10 years. The co-morbidity, most patients were diagnosed with hypertension (33%), osteoporosis (29%), dyslipidemia (22%), and diabetes mellitus (16%). The disease activities score-28 (DAS28) of RA were used to evaluate the treatment response of all patients with the mean value of 3.72 ± 0.88 and the mean of erythrocyte sedimentation rate (ESR) was 56.94 ± 24.30. The other DMARDs were used in combination with MTX in case of nonresponse to MTX such as sulfasalazine (52.4%, n = 76) antimalarial drugs (32.4%, n = 47), and leflunomide (17.9%) (Table 1).

The distribution of *MTHFR* C677T genotype among RA patients showed that the CC wild-type, CT, and TT were 53.1%, 43.4%, and 3.4%. For *MTHFR* A1298C genotype, the AA wild-type, AC and CC were 60.0%, 37.2%, and 2.7% (Table 1).

Table 1 Demographic data and medical history of rheumatoid arthritis patients (N = 145)

Characteristics	Value
Sex	
Male, n (%)	21 (14.48)
Female, n (%)	124 (85.52)
Age, mean ± SD, years	59.76 ± 10.66
Age at RA initiation, mean ± SD, years	49.10 ± 12.62
Disease duration, median (IQR), years	10.00 (5-16)
Co-morbidity	
Hypertension, n (%)	47 (33)
Osteoporosis, n (%)	42 (29)
Dyslipidemia, n (%)	32 (22)
Diabetes Mellitus, n (%)	22 (16)
DAS28, mean ± SD	3.72 ± 0.88
ESR (mm/hr), mean ± SD	56.94 ± 24.30
Treatment-related*	
Other DMARDs	
Sulfasalazine, n (%)	76 (52.41)
Antimalarial drugs, n (%)	47 (32.41)
Leflunomide, n (%)	43 (29.65)
Supplements	
Folic acid**, n (%)	145 (100.00)
Combined synthetic DMARDs (Dual therapy)	
MTX + Sulfasalazine, n (%)	41 (28.28)
MTX + Leflunomide, n (%)	26 (17.93)
MTX + Antimalarial drugs, n (%)	17 (11.72)
Combined synthetic DMARDs (Triple therapy)	
MTX + Sulfasalazine + Antimalarial drugs, n (%)	29 (20.00)
MTX + Sulfasalazine + Leflunomide, n (%)	20 (13.79)
MTX + Antimalarial drugs + Leflunomide, n (%)	7 (4.83)
Distribution of *MTHFR* C677T, rs1801133, n (%)	
CC	77 (53.10)
CT	63 (43.45)
TT	5 (3.45)

Table 1 Demographic data and medical history of rheumatoid arthritis patients (N = 145) (Cont.)

Characteristics	Value
Distribution of *MTHFR* A1298C, rs1801131, n (%)	
AA	87 (60.00)
AC	54 (37.24)
CC	7 (2.76)

**Drugs co-administered with methotrexate in case of non-response to methotrexate*

***All patients in compliance with folic acid supplementation*

RA, rheumatoid arthritis; DAS-28, Disease activity score-28; ESR, erythrocyte sedimentation rate; DMARDs, disease-modifying antirheumatic drugs; MTX, methotrexate; MTHFR, Methylenetetrahydrofolate Reductase.

***MTHFR* C677T genetic polymorphism**

The most common ADRs observed were gastrointestinal (n = 37, 25.5%) and elevations in liver transaminases (n = 31, 21.4%). Alopecia (n = 9, 6.2%), oral mucositis and leucopenia (n = 4, 2.8%) insignificant occurred (Table 2).

The polymorphic allele frequencies of *MTHFR* C677T values were 53.1, 43.4, and 3.4% for CC, CT, and TT genotypes. The *MTHFR* 677TT showed a significant association with abnormal liver function and gastrointestinal disturbance during methotrexate therapy were at $P<0.0001$ and $P = 0.039$ compared to CC and CT using the Pearson Chi-square test. No significant differences were observed for oral mucositis, leucopenia, and alopecia between the *MTHFR* C677T genotype.

Table 2 Relationship between *MTHFR* C677T polymorphism and the frequency of occurrence of toxicity (n = 145)

Toxicities	*MTHFR* C677T polymorphism			
	CC, n = 77 (53.1%)	**CT, n = 63 (43.4%)**	**TT, n = 5 (3.4%)**	***P*-value***
Abnormal liver function	7 (9.1)	19 (30.2)	5 (100.0)	**<0.0001***
Gastrointestinal disturbance	13 (16.8)	22 (34.9)	2 (40.0)	**0.039***
Oral mucositis	3 (3.9)	1 (1.6)	0 (0.0)	0.659
Leucopenia	2 (2.6)	2 (3.2)	0 (0.0)	0.909
Alopecia	3 (3.9)	5 (7.9)	1 (20.0)	0.264

***MTHFR* C677T genetic polymorphism and hepatotoxicity**

The allelic and genotypic distribution of genetic polymorphisms associated with MTX-induced hepatotoxicity are presented in Table 3. The C677T (rs1801133) polymorphism analysis showed a strong association with increased MTX toxicity for the TT vs. CC genotype (OR 53.25, 95% CI 5.67–500.02, *P*<0.001). In addition, the associations were detected in both the dominant (CC vs. CT & TT, OR 5.02, *P*<0.001) and recessive models (CT + CC vs. TT, OR 24.86, *P*<0.001).

The homozygous TT and heterozygous CT were associated with an increased risk of MTX-induced hepatotoxicity, these data suggest that the presence of the *MTHFR* 677TT SNPs induced hepatotoxicity in Thai RA patients by fifty-three times higher than wild-type.

Table 3 Genotype distribution and allele frequency of *MTHFR* C677T polymorphism in RA patients according to hepatotoxicity (n = 145)

Polymorphism	Hepatotoxicity[a]			
	Yes n (freq)	No n (freq)	OR [95% CI]	*P*-value*
***MTHFR* C677T**				
CC	7 (0.23)	70 (0.61)	-	
CT	19 (0.61)	44 (0.39)	3.94 (1.60-9.71)	**0.002***
TT	5 (0.16)	0 (0.00)	53.25 (5.67-500.02)	**<0.001***
C	26 (0.52)	114 (0.72)	-	
T	24 (0.48)	44 (0.28)	2.34 (1.24-4.41)	**0.008***
Dominant model				
CC	7 (0.23)	70 (0.61)	-	
CT + TT	24 (0.77)	44 (0.39)	5.02 (2.09-12.03)	**<0.001***
Recessive model				
CT + CC	26 (0.84)	114 (1.00)	-	
TT	5 (0.16)	0 (0.00)	24.86 (2.87-214.86)	**<0.001***

[a]Hepatotoxicity; liver enzyme levels were defined as AST and ALT values >3 times the upper limit of the normal level.

n; number, *freq;* frequency, *OR*; odd ratio, *CI*; confidence interval

***MTHFR* A1298C genetic polymorphism**

Gastrointestinal complications, including nausea and vomiting, were the most common side effects and were occurred in 37 patients (25.5%). There was no significant association found in any of the three genetic models. Besides, no significant association was found between the *MTHFR* A1298C

polymorphism and MTX-induced hepatotoxicity, gastrointestinal disturbance, mucositis, leucopenia, and alopecia in any of the genetic models.

Table 4 Relationship between *MTHFR* A1298C polymorphism and the frequency of occurrence of toxicity (n = 145)

Toxicities	*MTHFR* A1298C polymorphism			
	AA, n = 72 (49.7%)	AC, n = 66 (45.5%)	CC, n = 7 (4.8%)	*P*-value
Abnormal liver function	12 (16.6)	14 (21.2)	3 (42.9)	0.241
Gastrointestinal disturbance	34 (47.2)	24 (36.3)	2 (28.6)	0.338
Oral mucositis	8 (11.1)	12 (18.2)	0 (0.0)	0.269
Leucopenia	2 (2.8)	2 (3.0)	0 (0.0)	0.897
Alopecia	6 (8.3)	4 (6.1)	0 (0.0)	0.663

The results of this multivariate analysis are presented in Table 5. The confounding factors, leflunomide, NSAIDs, dyslipidemia, and diabetes mellitus were defined as independent variables and after multiple adjusting by these factors the *MTHFR* C677T polymorphism still detected association with the abnormal liver function (hepatitis) with significant covariates ($P<0.001$) and failed to detect any significant association with those potential confounding factor ($P>0.05$).

Table 5 Multivariate logistic modeling using abnormal liver function as dependent variable (n = 145)

Variables	Multi-adjusted OR (95% CI)[a]	*P*-value*
MTHFR C677T	4.91 (2.13-11.27)	**<0.001***
Leflunomide	0.46 (0.15-1.43)	0.181
NSAIDs	0.49 (0.19-1.22)	0.125
Dyslipidemia	0.63 (0.18-2.29)	0.485
Diabetes mellitus	0.82 (0.23-2.97)	0.762

[a]Adjusted for leflunomide, NSAIDs, dyslipidemia and diabetes mellitus

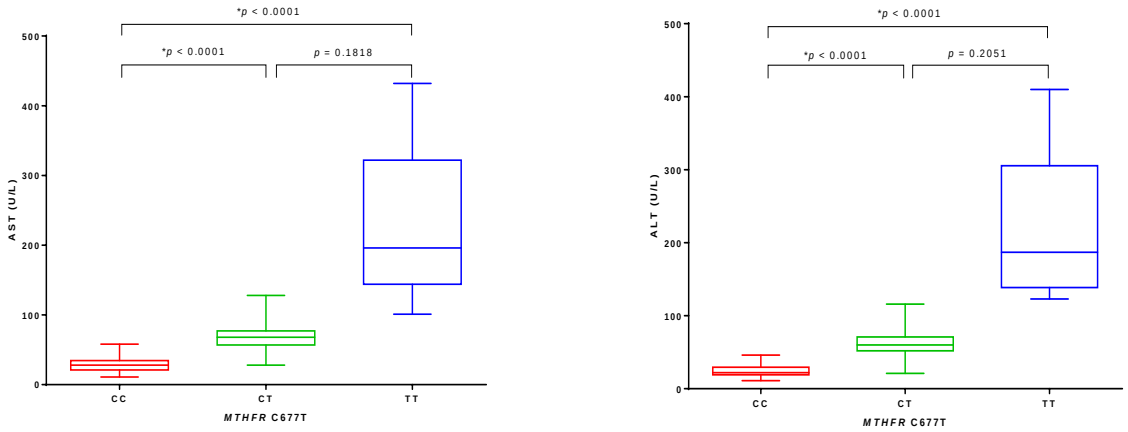
OR; odd ratio*, CI;* confidence interval

***MTHFR* C677T and hepatic enzyme levels**

The result showed higher AST levels among *MTHFR* 677TT genotype than in the CC and CT genotype (Figure 1A). Comparison of CC vs. CT genotype and CC vs. TT genotype were statistically significant ($P<0.0001$ and $P<0.0001$). The mean levels for CC, CT, and TT genotype were 28.88, 67.60, and 225.60 U/L.

A significant difference in ALT level (P<0.0001) was also found between CC vs. TT genotype and CC vs. TT genotype (Figure 1B). The mean ALT levels for CC, CT, and TT genotype were 24.29, 60.89, and 215.00 U/L.

A



B

Figure 1 Box plots of hepatic enzyme level, (A) aspartate aminotransferase (AST) and (B) alanine aminotransferase (ALT) in relation to *MTHFR* polymorphism; red box represents *MTHFR* 677CC group, green box represents *MTHFR* 677CT group and blue box represents *MTHFR* 677TT group. The horizontal line in each box represents the median, the bottom and top of each box show the 25th to 75th percentiles of the group. *P-value of <0.05 was considered statistically significant. The comparison was done by the Kruskal-Wallis test.

Discussion

MTHFR is an essential enzyme in DNA methylation and adenosine signaling pathway. The genetic polymorphism of *MTHFR* has been suspected to involve in MTX-induced toxicity. The increasing risk of hepatic toxicity in patients treated with MTX is the most common ADR and may lead to fibrosis in some cases, and an elevation of ALT is used as an initial marker for hepatic injury. The prediction of MTX-related hepatotoxicity is however very complicated, both at the initiation of therapy and long-term treatment. Several clinical factors can affect the risk of MTX toxicity, such as underlying disease, combined drug therapy, and the high doses of MTX (Karlsson Sundbaum *et al.*, 2019). Furthermore, genetic factors may influence the risk, and genotyping might be a way to identify patients who received MTX at higher risk for liver complications. The present study determined the association between *MTHFR* C677T and A1298C polymorphism with the risk of MTX-induced hepatotoxicity in Thai RA patients. This data provide evidence that the RA patients with *MTHFR* 677TT genotype were associated with increased risk of hepatotoxicity of MTX therapy, but no significant association was found for the *MTHFR* A1298C.

In vitro observations concerning *MTHFR* enzyme activity, such that T allele at C677T was associated with reduced enzyme activity (Weisberg et al., 1998).

The C677T polymorphism leads to alanine to valine amino acid change at codon 222 result in a reduction of enzyme activity (Hider et al., 2007). The C677T polymorphism was first described as the cause of reducing *MTHFR* enzyme activity. Heterozygous (CT) retain 60% of the *MTHFR* enzyme activity and represent approximately 40% of the Caucasian population. It has been suggested that a reduced enzyme activity could lead to an increased risk for MTX-related toxicity (Weisberg *et al.*, 1998). The homozygous variant TT genotype represents 10% of Caucasians and remains only 30% of normal activity (Ranganathan and McLeod, 2006). The C677T mutation of the *MTHFR* gene has previously been published that the relationship between the presence of the C677T mutation and the occurrence of hyperhomocysteinemia during MTX treatment (Haagsma *et al.*, 1999). Those investigators also identified a relationship between the hyperhomocysteine levels and gastrointestinal toxicity. Andersen et al. found a relationship between hyperhomocysteinemia and liver enzyme elevations during MTX treatment (Andersen *et al.*, 1997). Similarly, Weisman et al. reported that there was a significant correlation between MTX-related AEs and 677CT gene polymorphisms, and the mutation of this SNP increased the MTX toxicity (Weisman *et al.*, 2006). In our study found that RA patients with single nucleotide polymorphism (SNP) C677T of *MTHFR* TT and CT genotype showed a remarkable increase of the liver enzyme activity both AST and ALT and results from the multiple regression analysis indicated that 677TT could be risk factors of elevation of AST and ALT these findings suggest that the presence of the C677T mutation and subsequent decreased conversion of intracellular folates into 5-methyltetrahydrofolate (5mTHF) leads to increased toxicity probably via homocysteine metabolism.

The *MTHFR* A1298C polymorphism is a glutamic acid to alanine substitution at codon 429 and leads to reduced enzyme activity (Weisberg *et al.*, 1998) but did not affect the homocysteine level (Wang *et al.*, 2019). Recently, the report in 296 Chinese Han RA patients was not related to *MTHFR* A1298C and MTX toxicity (Wang *et al.*, 2020). Consistency, the A1298C polymorphism was not associated with increased toxicity (Fisher and Cronstein, 2009). The meta-analysis had shown no association between the *MTHFR* A1298C polymorphism and MTX toxicity (Lee and Song, 2010). This similarity to our results confirms no association between A1298C and any adverse drug reactions.

In summary, this study presented the strong association between *MTHFR* 677TT and the risk of MTX-induced hepatotoxicity in Thai RA patients but not *MTHFR* A1298C polymorphism. The 677TT genotype is associated with adverse effects despite a low-dose of MTX, suggests that Thai RA patients with *MTHFR* 677TT carrier obviously increased risk of MTX-induced hepatotoxicity by fifty-three times higher than wild-type. However, further prospective studies with larger sample sizes are required to support a significant impact of the *MTHFR* variant during MTX therapy to avoid hepatotoxicity.

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