MTHFR polymorphisms, C677T and A1298C, are not a genetic risk factor for liver stiffness index in methotrexate-treated rheumatoid arthritis patients

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Methotrexate (MTX) is an effective disease modifying anti-rheumatic drug for the management of rheumatoid arthritis (RA). Hepatotoxicity might be a major concern in cases of long-term, low dose MTX treatment (1). Prevalence of hepatic fibrosis in RA patients receiving MTX was reported to be from 3 to 52%. Mutations in the methylenetetrahydrofolate reductase (MTHFR) gene were introduced to explain the toxicity of MTX in RA patients (2-5). The MTHFR polymorphism was considered an important determinant for liver cirrhosis in chronic liver disease patients (6). Our hypothesis is that MTHFR gene polymorphisms might contribute to the MTX-related liver fibrosis in patients with RA treated with MTX. We investigated whether two MTHFR polymorphisms, C677T and A1298C, might be potential indicators for increased risk of liver fibrosis assessed using transient sonoelastography, a rapid and non-invasive method for measuring liver fibrosis, in RA patients taking low dose MTX.

One hundred and sixty-seven patients (95.2% female) taking MTX (3982.4±1584.1 mg of mean cumulative doses) for RA, fulfilled the 1987 revised criteria for the classification of RA of the American College of Rheumatology, were consecutively enrolled. Detailed clinical data, including age, sex, cumulative MTX doses, duration of MTX use, mean MTX doses, disease duration, cumulative folic acid doses, and body mass index, were collected from review of medical records. Serologic liver function parameters such as aspartate aminotransferase and alanine aminotransferase were measured

Measurement of liver fibrosis index was performed by transient sonoelastography (FibroScan, EchoSens, Paris, France). Total ten measurements were performed on each patient. The results of liver stiffness were described as kilopascal (kPa). MTHFR C677T and A1298C genotypes were identified using Seeplex MTHFR genotyping kit (Seegen, Seoul, Korea). Statistical analysis was performed using using the SPSS 13. 0 program (SPSS Inc., Chicago, IL, USA). Statistical significance was considered at $p<0.05$.

Frequencies of genotypes and haplotypes of the MTHFR polymorphisms, C677T and A1298C, were identified (Table I). The clinical and laboratory parameters, including age, BMI, MTX dosages, folic acid dose, disease duration of the enrolled patients, and serologic liver function parameters, were not different among each genotype ($p>0.05$ of all parameters). There are no statistical differences between liver fibrosis index values and genotypes and haplotypes of C677T and A1298C polymorphisms (Table I).

MTX might induce progression of hepatic fibrosis by increased pericellular or sinusoidal collagen deposition in liver tissues of RA patients (7). Progression of liver fibrosis in MTX-treated RA patients was also proved from serial liver biopsy studies. Meta-analysis for MTX-treated patients with diverse rheumatic diseases revealed that a cumulative dose of MTX, alcohol consumption, and psoriasis could be considered major risk factors for histological progression of liver (8, 9). A prospective double blind study showed a close relationship between MTX dose at biopsy and histological hepatic abnormality (9). However, a potent risk factor associated with liver fibrosis has not been clearly determined.

C677T and A1298C MTHFR polymorphisms are most commonly studied in the field of MTX-related toxicity and efficacy. The C677T mutation of the MTHFR gene significantly increased the risk of developing MTX-related adverse effects, mainly due to worsening liver function tests in RA patients receiving MTX (2, 3). In addition, the second common polymorphism of MTHFR gene, A1298C, was also considered as a risk genetic factor associated with toxicity (4, 5). However, it reported that there was no association between two MTHFR polymorphisms and MTX-related adverse effects in Italian RA patients (10).

Although the role of MTHFR polymorphisms for liver toxicity remains controversial, we found that MTHFR polymorphisms were not associated with MTX-related liver stiffness. It suggests that MTHFR polymorphism is not a genetic risk factor for liver stiffness in RA patients receiving MTX therapy. However, longitudinal studies should be required for confirmation in larger population.

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Table I. Comparison of liver fibrosis index for genotypes and haplotypes of MTHFR polymorphisms*.

	Genotypes (n=167)			*p*-value			
C677T	CC (n=44)	CT (n=91)	TT (n=32)	CC *vs*. CT	CC *vs*. TT	CC *vs*. CT/TT	CC *vs*. CT *vs*. TT
Stiffness index (kPa)	4.57 ± 1.52	4.28 ± 1.24	4.31 ± 1.31	0.277	0.431	0.227	0.482
A1298C	AA (n=116)	AC (n=47)	CC (n=4)	AA *vs*. AC	AA *vs*. CC†	AA *vs*. AC/CC	AA *vs*. AC *vs*. CC††
Stiffness index (kPa)	4.29 ± 1.32	4.55 ± 1.35	4.25 ± 1.56	0.251	0.878	0.283	0.260
	Haplotypes (n=333)‡			*p*-value			
	Haplotype 1 (n=154)	Haplotype 2 (n=125)	Haplotype 3 (n=54)	Haplotype 1 *vs*. 2	Haplotype 1 *vs*. 3	Haplotype 2 *vs*. 3	Haplotype 1 *vs*. 2 *vs*. 3
Stiffness index (kPa)	4.28 ± 1.25	4.40 ± 1.40	4.48 ± 1.35	0.444	0.330	0.740	0.576

*Data were described as mean ± standard deviation. Student t test for genotypic comparison between 2 groups and ANOVA test for genotypic comparison among 3 groups were performed, except for †Mann-Whitney U-test and ††Kruskal-Wallis test used for each comparison.
‡677T/1298C haplotype were excluded at the analysis of haplotypes because its frequency was only one (0.3% of total haplotypes). Haplotype 1: 677T/1298A; Haplotype 2: 677C/1298A; Haplotype 3: 677C/1298C.

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