SHORT COMMUNICATION

Lack of association between *MTHFR* gene polymorphisms and response to methotrexate treatment in Pakistani patients with rheumatoid arthritis

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Abstract: Methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms have been reported to be associated with response to methotrexate (MTX) in certain populations of patients with rheumatoid arthritis (RA). This study aims at investigating any relationship of two single nucleotide polymorphisms (SNPs) in *MTHFR* gene, C677T and A1298C with response to therapy with MTX in Pakistani RA patients. Allelic frequencies of the two polymorphisms (C677T and A1298C) were determined in 67 RA patients (9 males and 58 females; mean age 42.87±13.5 years) who had previously participated in a prospective clinical trial. Fifty-one patients had received MTX and were followed up for response up to 6 months. Genotyping of the two *MTHFR* polymorphisms was carried out using PCR-RFLP, while fasting concentration of plasma homocysteine was determined using a kit method. Twenty-eight patients were found to be "good responders", while twenty-three were "poor responders". *MTHFR* 1298C and *MTHFR* 677T alleles' frequencies in "good responders" were not different from frequencies in "poor responders" (0.574 vs. 0.521; p=0.6 and 0.197 vs. 0.196; p=0.75, respectively). Plasma homocysteine levels in female RA patients were significantly higher compared to general population in Karachi (13.1±6.7 µmol/l vs. 11.4±5.3 µmol/l; p<0.001). *MTHFR* C677T and A1298C polymorphisms are not associated with response to MTX in a population of Pakistani RA patients.

Keywords: Gene polymorphism; Methylenetetrahydrofolate reductase; *MTHFR* C677T; *MTHFR* A1298C; Methotrexate; Pharmacogenomics; Rheumatoid arthritis; Response to methotrexate

INTRODUCTION

Methotrexate (MTX) is a commonly used disease modifying antirheumatic drug in treating rheumatoid arthritis (RA) patients. However, the efficacy of this drug in RA varies from one population to another. It has also been reported that nearly one-third of RA patients discontinue this drug because of its adverse reactions (Hider *et al*., 2007). Studies carried out mostly in the developed countries have shown two single nucleotide polymorphisms (SNPs) in methylenetetrahydrofolate reductase (MTHFR) to be pharmacogenomic markers of MTX toxicity (Urano *et al.,* 2002; Berkun *et al.,* 2004). A meta-analysis of *MTHFR* polymorphisms influencing MTX toxicity showed an association of 677C>T polymorphism with adverse reactions of MTX (Fisher *et al.,* 2009). However, another recent meta-analysis showed no association of both C677T and A1298C polymorphisms with MTX toxicity (Owen *et al.,* 2013). This indicates that response to MTX therapy in RA patients varies from one population to another. Since no study has been carried out to investigate the association of *MTHFR* gene polymorphisms and response to MTX in Pakistani RA patients, we embarked on carrying out such a study. Therefore, the objective was to find out any relationship of these two SNPs of *MTHFR* with response to MTX therapy in a population of RA patients visiting a tertiary-care hospital in Karachi.

PATIENTS AND METHODS

Allele frequencies of *MTHFR* C677T and *MTHFR* A1298C polymorphisms were determined in sixty-seven adult patients (58 females; 9 males; mean age 42.87±13.5 years) visiting the Rheumatology Clinic of the Aga Khan University Hospital who had participated in a prospective study for assessing the response to MTX therapy. These patients were diagnosed to have RA on the basis of criteria described by the American Rheumatism Association for RA. Fifty-one patients received MTX, 15-20 mg/week and were clinically assessed every month for a period of six months (Ali *et al.,* 2006). Assessment of the clinical response to MTX therapy was based on criteria adopted by O' Dell *et al.,* (1998). After 6 months, those patients having fifty percent or more reduction in erythrocyte sedimentation rate, Richie index, number of swollen joints and duration of morning stiffness as compared to these parameters at the start of the therapy were classified as "good responders", while those showing slight improvement in these indices were classified as "poor responders".

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Table 1: Demographic and clinical characteristics of RA patients on MTX therapy with response to the drug

Variable*	Good responders (n=28)	Poor responders (n=23)	P value**
Gender			
Males	5(17.9)	3(13.0)	0.93
Females	23(82.1)	20(87.0)	
Age (Years)	41.3±10.7	42.4±8.8	0.69
Duration of RA (Years)	6.2±4.8	7.0±4.0	0.53
Homocysteine (µmol/l)	14.1±5.1	14.2±9.0	0.82

*Values of gender are N (%), while all other values are expressed as mean ±SD.
**P value compares the percentages in two groups by using Chi-square analysis; while mean values are compared using Independent sample t test. P value<0.05 was considered significant.

Table 2: Distribution of genotypes and allele frequencies of single nucleotide polymorphisms (SNPs) of *MTHFR* in "Good responders" and "Poor responders" to MTX therapy

Polymorphism	Good responders (n=28)	Poor responders (n=23)	P value*
	N (%)	N (%)	
MTHFR C677T			
Genotypes			
CC	19(67.9)	16(69.6)	0.89
CT	7(25.0)	5(21.7)	0.78
TT	2(7.1)	2(8.7)	0.75
Allele			
C	45(80.3)	37(80.4)	0.75
T	11(19.7)	9(19.6)	
MTHFR A1298C**			
Genotypes			
AA	2(7.1)	5(21.7)	0.27
AC	19(67.9)	11(47.8)	0.15
CC	6(21.4)	7(30.4)	0.46
Alleles			
A	23(42.6)	21(45.7)	0.72
C	31(57.4)	25(54.3)	

*P value compares the genotypes and allele frequencies between the two groups using Chi-square test. P value<0.05 was considered significant.
**DNA fragment comprising of this polymorphism could not be amplified in one of the samples among "good responders"

Plasma/serum was analyzed for homocysteine using a kit method (Abbott Laboratories Ltd; Pakistan), while DNA was isolated from the leukocytes using standard procedure as described previously (Ali *et al.*, 2006). Genotyping protocol for the *MTHFR* C677T and A1298C polymorphisms was based on polymerase chain reaction (PCR) followed by restriction fragment length polymorphism (RFLP) as described in a previous report (Yakub *et al.*, 2012). The study had been approved by the Ethics Review Committee of the Aga Khan University.

RESULTS

Mean concentrations of plasma homocysteine in males and females were found to be 16.2±3.0 and 13.1±6.7 µmol/l, respectively. However, mean homocysteine concentration in female RA patients was significantly higher compared to mean homocysteine concentration in general population in Karachi (13.1±6.7 µmol/l vs. 11.4±5.3 µmol/l; p<0.001; Yakub *et al.*, 2010). Twenty eight RA patients were found to be "good responders" to MTX, while 23 were identified as "poor responders". No significant differences were observed between "good responders" and "poor responders" in terms of gender, age, duration of RA and plasma levels of homocysteine (table 1). Frequencies of *MTHFR* 1298C and *MTHFR* 677T alleles in RA patients were not significantly different from their frequencies in general population in Karachi (0.553 vs. 0.55, p=0.977; and 0.196 vs. 0.15; p=0.338, respectively), (Yakub *et al.*, 2012). Genotypes and allele frequencies of the two polymorphisms were not significantly different between "good responders" and "poor responders" (table 2).

DISCUSSION

The advent of pharmacogenomics has opened new vistas in the treatment of diseases by employing efficacious drugs according to the genetic make-up of the patient (Ranganathan and McLeod, 2006). MTX in low-dosages is a drug of choice in the treatment of RA because it exerts its anti-inflammatory effect by inhibiting the enzymes in cellular folate and adenosine pathways (Ranganathan, 2013). However, long-term use of this drug is not without toxic side effects. While quite a few studies have shown that efficacy and toxicity of MTX in RA patients is influenced by two SNPs (C677T and A1298C) in the gene of *MTHFR*, a key enzyme in folate metabolism, several others did not find any association of these polymorphisms and MTX toxicity in their populations. For example, an association between C677T/A1298C polymorphism(s) and MTX toxicity has been reported in Spanish, Caucasian American, African-American and Korean RA patients (Ranganathan *et al.,* 2008; Plaza-Plaza *et al.,* 2012; Choe *et al.,* 2012). However, no association of these polymorphisms with increased MTX adverse effects was observed in Indian and Briton RA patients (Aggarwal *et al.,* 2006; Owen *et al.,* 2012). Lee *et al.,* 2010 carried out a meta-analysis of 8 studies and found no association of C677T or A1298C polymorphisms with MTX toxicity in Asian RA patients. The results of these studies conform well to our findings that these polymorphisms are not associated with MTX toxicity in Pakistani RA patients. Two studies showed paradoxical results and reported association of these polymorphisms with increased rate of RA remission in patients treated with MTX (Berkun *et al.,* 2004; Kurzawski *et al.,* 2007). All these reports point towards variable response to MTX in RA patients from different populations. Frequency of 1298CC genotype in RA patients of certain populations has been found to be higher than the expected frequency of this genotype in general population (Berkun *et al.,* 2004). Moreover, this genotype was found to be associated with reduced toxicity to MTX (Berkun *et al.,* 2004). However, in Pakistani RA patients, the frequency of 1298CC genotype was not significantly different from healthy controls (Yakub *et al.,* 2012). This could be one of the reasons for lack of association of A1298C polymorphism in Pakistani RA patients.

The mechanism by which *MTHFR* polymorphisms could be causing MTX toxicity in some RA patients is unclear. However, the role of increased concentrations of plasma homocysteine in RA patients with *MTHFR* 677CT and *MTHFR* 677TT genotypes cannot be discounted (van Ede *et al.,* 2002). Increased levels of homocysteine are known to cause endothelial dysfunction, low density lipoprotein oxidation and prothrombotic changes (Hernanz *et al.,* 1999). All these changes can lead to an increase in adverse effects of MTX. Our results should be viewed in the light of certain limitations of the study. Patients enrolled in this study were in a modest number. Moreover, follow-up of these patients was for 6 months only. The possibility that some of the patients classified under "good responders" might develop MTX toxicity on a long term follow-up cannot be ignored. In spite of these limitations, our data did not show any association of *MTHFR* polymorphisms with efficacy and/or toxicity of MTX.

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