International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 3S, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Association between TYMS gene polymorphism and methotrexate treatment adverse drug reaction a sample of rheumatoid arthritis patients

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Received: 18.09.2024 Revised: 13.10.2024 Accepted: 26.11.2024

ABSTRACT
Methotrexate (MTX) is a popular therapy for MTX for several diseases, including RA, owing to its anti-inflammatory and immunomodulatory effects, but its usage is associated with adverse effects. The current study aims to study the possible relationship between thymidylate synthetase (TYMS) gene polymorphism and MTX's adverse drug reaction (ADR). The study involved 95 RA patients; Sanger sequencing polymerase chain reaction was utilized for the TYMS gene region. The MTX ADR had a high rate (88.4%); however, there was no significant association between genetic polymorphism and the incidence of overall ADR. At the same time, some haplotypes carry an increased risk of ADR, like the $A_{657334}C_{rs2853741}C_{rs2606241}T_{rs2853742}$haplotype, while others reduce the risk of ADR, like $C_{657334}C_{rs2853741}C_{rs2606241}T_{rs2853742}$haplotype, but none of the haplotypes reach statistically significant. In conclusion, there is no association between the genetic polymorphism of the TYMS gene and the development of methotrexate adverse drug reactions.

Keywords: TYMS gene, polymorphism, adverse reaction, methotrexate, rheumatoid arthritis

INTRODUCTION

Methotrexate (MTX) is a popular therapy for several immune-mediated diseases, including RA, owing to its anti-inflammatory and immunomodulatory effects, but its usage is associated with a magnitude of ADR [1-4].
MTX hinders the activity of many enzymes involved in the production of folate and the creation of new nucleotides. TYMS is a crucial enzyme that plays a significant role in de novo pyrimidine production. It is situated on chromosome 18q11.32 and plays a crucial role in controlling the equitable provision of four DNA precursors required for DNA replication [5, 6].
The most common ADR of MTX are gastrointestinal symptoms such as nausea, vomiting, mucosal ulcers, and decreased appetite. These symptoms are commonly observed in most patients and can be readily controlled [7]. Despite these ADRs, MTX is still a popular treatment option in Iraq because of its low cost, good responses, and it can be combined safely with biological agents like infliximab etanercept [8].
There is a lack of studies that address predicting the ADR of MTX, especially those regarding genetic factors. As such, we carried out this study to examine the clinical significance of the TYMS gene polymorphisms (in exon1 and partial regions of promotor and intron 1 [877 bp, 657,220 – 658,096 bp]) and ADR of MTX.

METHODS

Study design and settings

A cross-sectional study examined 95 RA patients, who were further classified based on the presence of ADR [8]. The participants in the present research were selected exclusively from the Diwaniya Teaching Hospital. It was conducted from June 1, 2022, to March 1, 2023.

Inclusion criteria

Patients who are 18 years of age or older and have been diagnosed with RA [9], and all patients receive MTX treatment for a minimum of three months.

Exclusion criteria

Patients with another disease (aside from RA), those on immunosuppressor drugs aside from MTX, pregnancy, and another ethnic group aside from the Arabic population.

Variables

Laboratory analysis

Sufficient venous blood sample obtained from all patients (5 ml); measurement of erythrocyte sedimentation rate (ESR) was conducted using the modified Westergren method, which involves the use of whole blood that has been anticoagulated with EDTA (VISION Pro ESR Analyzer, Shenzhen YHLO Biotech Co., Ltd), while rheumatoid factor **(**RF**)** measured using Chemistry Analyzer, Smart – 150, Geno Lab-TEK Corporation, Canada.

SNP identification and genotyping

Genomic DNA was isolated from venous blood using a solid-phase DNA extraction method [10], The DNA samples were analyzed using agarose gel electrophoresis with a 1% agarose gel [11].

The TYMS gene region [exon1 and partial regions of promotor and intron 1 of TYMS gene] (877 bp, chr18: 657,220 – 658,096 bp, NC_000018.10, GI: 568815580) was amplified by PCR (TProfessional TRIO combi, Biometra GmbH, Germany) from 95 RA patients.

The PCR amplicons were commercially sequenced according to the sequencing company's instruction manuals (Macrogen Inc. Geumchen, Seoul, South Korea). The PCR product was sent for Sanger sequencing using ABI3730XL, an automated DNA sequencer, by Macrogen Corporation. The results were received by email and then analyzed using Geneious Prime software (V2021.1.1) (Biomatters Ltd., Auckland, New Zealand; www.geneious.com). Only clear chromatographs obtained from ABI (Applied Biosystems, Inc.) sequence files were further analyzed.

The identified single nucleotide polymorphisms (SNPs) were cross-referenced with the dbSNP database to determine their uniqueness. Each single nucleotide polymorphism (SNP) position was examined in its matching reference genome to determine if it has been previously recorded in the dbSNP database.

Ethical considerations

The research was authorized by the College of Pharmacy at the University of Baghdad, and all participants provided written consent after being fully informed.

Sample size

Power analysis was used to calculate the sample size, G*Power version (3.1.9.7)[12]. The total sample size was 95, with an alpha probability of 0.05, power of 88%, and one-tailed using one sample case variance.

Statistical analysis

The haplotyping analysis was conducted using the SHEsis online software [13]. The Kolmogorov-Smirnov test is employed to assess the conformity of variables to a normal distribution. Categorical variables were analyzed using chi-square analysis. The statistical analysis was performed using GraphPad Prism version 10.1.0 for Windows. A p-value of ≤0.05 was considered significant when suitable.

RESULTS

By sequencing 95 randomly selected patients in the noncoding region of TYMS, four single nucleotide polymorphisms (SNPs) were identified; three of these polymorphisms were previously documented in the dbSNP of the NCBI database (rs2853741T>C, rs2606241A>C, and rs2853742T>C). One of the documented SNPs is novel (657334C>A), as illustrated by Figures 1 and 2.

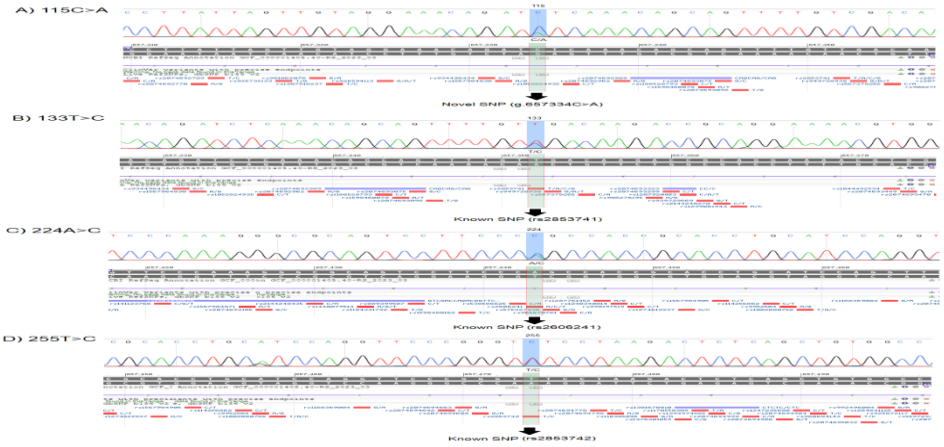

Figure 1: The SNP's novelty checking of TYMS genetic single nucleotide polymorphisms using the dbSNP server

Figure 2: The pattern of the detected SNPs within the DNA chromatogram of the targeted 877 bp amplicons of the TYMS gene.

The study involved 95 patients diagnosed with rheumatoid arthritis (RA), averaging 43.1 years. Most patients were female (85.3%), approximately 35.8% were smokers. The median erythrocyte sedimentation rate (ESR) was 24.0 (with a range of 16.0-39.0), the median rheumatoid factor (RF) was 23.0 (with a range of 16.4-29.0), and the median Disease Activity Score 28 (DAS28) was 3.2 (with a range of 2.8-3.9).
Figure 3 depicts the genetic polymorphism of the four single nucleotide polymorphisms (SNPs), whereas Figure 4 illustrates the distribution of adverse drug reactions (ADR) across different organ systems.

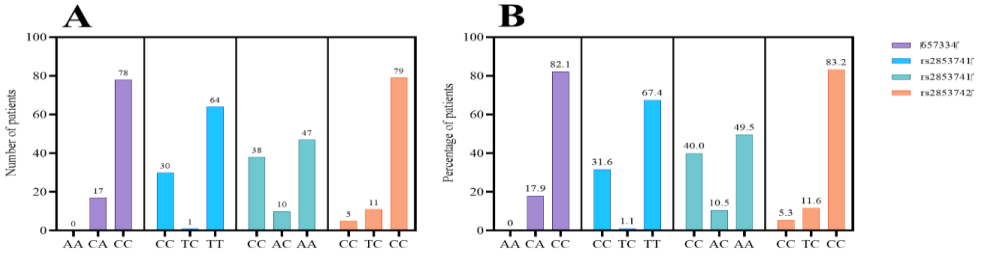

Figure 3: genetic distribution of TYMS gene

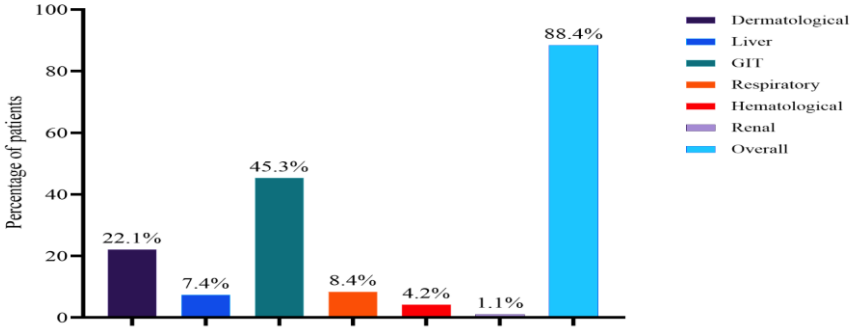

Figure 4: Evaluation of the ADRs

Table 1 demonstrates that there is no substantial correlation between the overall adverse drug reaction (ADR) and single nucleotide polymorphism (SNP) polymorphism.

Table 1: the comprehensive adverse drug reaction (ADR) data and its correlation with genetic polymorphism.

Genotype	Adverse drug reaction	No adverse drug reaction	p-value
657334			
CA	1(5.9%)	16(94.1%)	0.418
CC	10(12.8%)	68(87.2%)	
rs2853741			
CC	4(13.3%)	26(86.7%)	0.767
TC	0(0.0%)	1(100.0%)	
TT	7(10.9%)	57(89.1%)	
rs2606241			
CC	5(13.2%)	33(86.8%)	0.801
CA	0(0.0%)	10(100.0%)	
AA	6(12.8%)	41(87.2%)	
rs2853742			
CC	1(20.0%)	4(80.0%)	0.388
TC	0(0.0%)	11(100.0%)	
TT	10(12.7%)	69(87.3%)	

There was no significant association between various haplotypes with overall ADRs of notice; the $A_{657334}C_{rs2853741}C_{rs2606241}T_{rs2853742}$haplotype was slightly associated with increased ADR odds (OR = 1.636), but it did not reach statistical significance, as illustrated by table 2.

Table 2: presents the results of the haplotype analysis concerning ADRs

Haplotyping	Adverse drug reaction	No adverse drug reaction	OR [95%CI]	p-value
$A_{657334}C_{rs2853741}C_{rs2606241}T_{rs2853742}$	11.53(0.069)	1.00(0.045)	1.636 [0.202~13.275]	0.641
$C_{657334}C_{rs2853741}C_{rs2606241}C_{rs2853742}$	10.53(0.063)	2.00(0.091)	0.707[0.145~3.438]	0.665
$C_{657334}C_{rs2853741}C_{rs2606241}T_{rs2853742}$	26.47(0.158)	5.00(0.227)	0.676[0.230~1.994]	0.476
$C_{657334}T_{rs2853741}A_{rs2606241}T_{rs2853742}$	92.00(0.548)	12.00(0.545	1.135[0.463~2.781]	0.781
$C_{657334}T_{rs2853741}C_{rs2606241}T_{rs2853742}$	19.00(0.113)	2.00(0.091)	1.352[0.293~6.247]	0.698
$A_{657334}C_{rs2853741}C_{rs2606241}C_{rs2853742}$	4.47(0.027)	0.00(0.000)	-	-
$C_{657334}T_{rs2853741}C_{rs2606241}C_{rs2853742}$	4.00(0.024)	0.00(0.000)	-	-

Fisher's p-value is 0.916, and frequency<0.03 has been dropped.

DISCUSSION

The genetic role in predisposition, development, severity, and response for therapeutic intervention of RA is well documented. MTX is an intriguing subject for pharmacogenomics research [14-16]. Pharmacogenomics studies on RA have focused on identifying genetic markers that can predict patients' response to treatment or ADRs [17-19].

In the present study, ADR was very high, with a peak of 88.4% among the sample. The presence of genetic variation did not have an impact on the occurrence of adverse drug reactions (ADRs) in general. At the same time, some haplotypes carry an increased risk of ADR, like the $A_{657334}C_{rs2853741}C_{rs2606241}T_{rs2853742}$haplotype, while others reduce the risk of ADR, like $C_{657334}C_{rs2853741}C_{rs2606241}T_{rs2853742}$haplotype, but none of the haplotypes reach statistically significant, which could be attributed to a reduction in power of association because of small number of patients without ADR (11.6%).

Others examined the TYMS gene polymorphisms in breast cancer women; the authors found that rs2606241 and rs2853741 polymorphisms were associated with hand foot syndrome (HFS). For rs2606241, it followed co-dominant and recessive models and G carrier genotypes associated with HFS, while rs2853741 followed co-dominant, dominant models and C carrier genotypes associated with HFS [20]. Zhao et al. examined TYMS gene polymorphisms in the Chinese population and their association with Congenital Cardiac Septal Defects [CCSD]; the authors identified 15 SNP polymorphisms; neither rs2853741 nor rs2606241 was associated with CCSD [21]. The Blanton et al. study examined the association between the Folate Pathway and Nonsyndromic Cleft Lip and Palate [NSCLP], with 14 genes identified and 97 SNPs identified, 7 of them identified from the TYMS gene. For rs2853741 polymorphism, no significant association with NSCLP was reported [22]. Pellicer et al. examined the risk of chemotherapy-induced toxicity by capecitabine treatment in cancer patients, rs2853741 polymorphism (CT/TT vs CC) associated with diarrhea [23]. These previous studies showed that TYMS gene

polymorphism is some disease associated with side effect prediction; in the current study, we did not report an association between TYMS gene polymorphism and MTX ADR in Iraqi RA patients; this could be attributed to the effect of TYMS gene is responsible on the therapeutic effect of MTX in RA patients rather than the predicting the side effect, additionally, the high number of ADR reported in the study may mask the potential effect of TYMS gene on predicting the ADR, thus large numbers of patients is required.

Thymidylate synthase (TS) is an essential enzyme in DNA synthesis and repair [24]. It is known to be inhibited by methotrexate polyglutamate (MTXPGs), which in turn contributes to the antiproliferative and anti-inflammatory effects of methotrexate [25]. Indeed, previous studies have demonstrated that TS levels can be a reliable predictor of the therapeutic response to MTX treatment [26]. Previous studies have established a correlation between genetic polymorphism in the TYMS gene and levels of TS [27].

Reproducing results in genetic association research is usually complex, and ensuring that different studies can be compared presents difficulties. The strongest evidence of a correlation lies in reproducing this correlation in a separate group that has the same genetic makeup and shows the same effect in the same direction. Many genetic association studies suffer from insufficient statistical power since there are only a limited number of individuals who have the homozygous mutant genotype.

CONCLUSION

Four single nucleotide polymorphisms (SNPs) were identified; three of these polymorphisms were previously documented in the dbSNP of the NCBI database (rs2853741, rs2606241, and rs2853742), and one of the documented SNPs is novel (657334); no association between genetic polymorphism of TYMS gene with methotrexate ADR.

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