Study of the Association Between MTHFR Polymorphism (rs1801133) and the Outcome of Methotrexate Treatment in Rheumatoid Arthritis Patients

Original Article

Marium El-Sayed Ahmed Fathi[1], Karim Yehia Shaheen[1], Amani Mohamed Abdel-Ghani[1], Marwa Mohamed Rafat Mohamed[1], Fatma Mohammed Badr Mohammed[2] and Noha Refaat Mohamed[1]

[1]Clinical Pathology, [2]Internal Medicine Rheumatology and Immunology, Faculty of Medicine, Ain Shams University, Cairo, Egypt

ABSTRACT

Background: Rheumatoid arthritis is a long-term autoimmune condition that impairs joint function and drastically lowers quality of life. Methotrexate, one of the disease-modifying anti-rheumatic drugs acts differently depending on the genotypes being examined when used as an anchor medicine to treat rheumatoid arthritis.

Aim of the Study: The present study was aimed to evaluate the impact of methylenetetrahydrofolate reductase gene C677T (rs1801133) polymorphism on the clinical outcome of methotrexate treatment as regards treatment efficacy or toxicity in Egyptian rheumatoid arthritis patients.

Subjects and Methods: This study was carried on 45 patients diagnosed with rheumatoid arthritis receiving methotrexate as a first line of treatment. Determination of methylenetetrahydrofolate reductase gene C677T (rs1801133) polymorphism was carried out using Real-time polymerase chain reaction.

Results: The results of the this study revealed that statistically significant difference was found between the responders to the treatment and non-responders in the disease activity measured by DAS 28 score; however no statistically significant difference was observed between the responders and non-responders in the rate of treatment complications nor the frequencies of the studied genotypes.

Conclusion: Our findings suggested that there is no significant association between the genotypic frequencies of the methylenetetrahydrofolate reductase gene C677T (rs1801133) polymorphism among the responders and the non-responder's group in patients with rheumatoid arthritis.

Key Words: DAS 28, methotrexate, MTHFR, polymorphism, real-time-PCR, rheumatoid arthritis.

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Corresponding Author: Marium El-Sayed Ahmed Fathi, Clinical Pathology, Faculty of Medicine, Ain Shams University, Egypt, **Tel.:** +2 010 0359 8014, **E-mail:** marium87@med.asu.edu.eg

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INTRODUCTION

The most prevalent chronic autoimmune inflammatory arthritis, rheumatoid arthritis affects around 1% of the global population. The primary effects of RA on the articular joints include swelling, stiffness, joint deterioration, loss of joint function, disability, leading to marked decline in quality of life[1]. RA is a multifactorial autoimmune disease presenting at any age and affected by environmental and genetic factors[2].

Long-term medication therapy is necessary for rheumatoid arthritis in order to manage symptoms and enhance prognosis. MTX is one of the medications that plays a major role since it not only treats symptoms but also enhances prognosis. It is categorized as a disease-modifying anti-rheumatic medication (DMARD)[3]. When treating RA, MTX is often the first prescription prescribed because it is the least expensive medication currently available. Still, only 55% of patients use this medication for more than two years because of either the development of adverse effects or appearance of non-responders to MXT treatment[4]. The most frequent adverse effects that individuals with RA encounter are pancytopenia, alopecia, hepatotoxicity, gastrointestinal intolerance, renal toxicity, and occasionally unanticipated pulmonary and bone marrow toxicity. After at least six months of treatment, almost 30% of RA patients receiving modest dosages of MTX reported negative effects[5].

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Genetic variations in the MTX-metabolizing enzyme and transporter can also cause individual variations in MTX efficacy and toxicity, in addition to physiological, environmental, and pathological factors that can affect MTX efficiency and toxicity[1]. Because MTX and folic acid share a structural similarity, it competitively inhibits the enzyme dihydrofolate reductase (DHFR), which converts folic acid to tetrahydro folic acid (THF). This decreases the synthesis of thymidylate and purines, which in turn affects the synthesis of DNA in cells[6]. Methylene tetrahydrofolate reductase (MTHFR) catalyzes the conversion of THF, the precursor of the folate cofactor 5-methyl-THF. One of the most important enzymes, MTHFR is primarily investigated for its regulatory roles in the folate system and MTX medication response[7].

The MTHFR gene is located on the short arm of chromosome 1 (1p36.3), composed of 11 exons and 10 introns. The gene encodes for a 77-kDa protein. MTHFR mediates the conversion of 5, 10-methylene tetrahydrofolate into 5-methyl tetrahydrofolate (5 methyl-THF). 5 methyl-THF acts as a methyl donor for remethylation of homocysteine to methionine[8].

There are several polymorphisms in the MTHFR gene, but only two of them-C677T (rs1801133) and A1298C (rs1801131)-affect the activity of the enzyme. Therefore, they are regarded as the two most significant polymorphisms that could affect how effectively MTX works as a treatment. By substituting alanine for valine at codon 222 as a result of the C to T mutation at nucleotide 677, MTHFR becomes more thermolabile and its enzyme activity is decreased[7].

Numerous investigators discovered a variation in the MTHFR gene linked to RA susceptibility as well as the effectiveness and side effects of MTX. But the outcomes were debatable[9].

AIM OF THE WORK

The aim of the present study was to investigate the impact of methylenetetrahydrofolate reductase (MTHFR) gene C677T (rs1801133) polymorphism on the clinical outcome of methotrexate treatment as regards treatment efficacy or toxicity in Egyptian rheumatoid arthritis patients.

MATERIALS AND METHODS

Ethical approval

This study followed the tent of declaration of Helsinki and was approved by the faculty of medicine Ain Shams University research ethics committee (FMASU183/2021).

Subjects in this study were recruited from the in-patient ward and the outpatient clinic of the Internal medicine Rheumatology and Immunology Department and conducted at the Clinical Chemistry Laboratory, Clinical Pathology Department, Ain Shams University Hospitals, in the period from September 2021 till July 2023. The study was approved by the Research Ethics Committee of Ain Shams University.

The included subjects were classified into 45 patients diagnosed with RA who received MTX as a first line of treatment 25 in group I (Responders Group) and 20 in group II (Non-Responders Group). Rheumatoid arthritis patients fulfilling the American College of Rheumatology (ACR)/European League against Rheumatism (EULAR) 2010 classification criteria for RA.

Patients who aged between 18–90 years. All patients received low-dose MTX for at least 3 months any patients comprised any contraindications to MTX therapy, receive biopharmaceutical therapy or another DMARD concomitantly during the 2-month period prior to enrollment in the study, pregnancy, lactation or refusal to provide consent for participation were excluded from the study.

In this study, a comprehensive medical history was obtained from all participants, with particular attention to disease duration, the extent of joint involvement, and details about the treatments administered (including steroids, NSAIDs, and DMARDs) and their duration. Full clinical examination with special emphasis on number of tender joints and number of swollen joints and disease activity. Assessment of disease activity state was done by disease activity score (DAS28-ESR). All participants in this study were subjected to complete blood count, liver function, renal function, CRP and rheumatoid factor were measured using the available laboratory methods. Determination of MTHFR gene (rs1801133) polymorphism was carried out using Real-time polymerase chain reaction.

Ten milliliters of venous blood were collected using strict aseptic techniques and then divided into two distinct tubes:

1. A plain tube containing gel for serum separation to perform AST, ALT, total & direct bilirubin, RF, serum creatinine and urea;

2. A tri-potassium ethylene diamine tetra acetate "k3 EDTA" vacutainer for CBC and RT-PCR;

3. A Na citrate tube for ESR estimation. Blood drawn into plain tubes was allowed to clot for twenty minutes before being centrifuged at 2000–3000 RPM for ten minutes. The sera were separated for assay of Liver profile, total & direct bilirubin, RF, and serum creatinine. The EDTA vacutainer collected for MTHFR polymorphism assay was stored at -70˚C as whole blood until the time of analysis. Repeated freezing and thawing of samples was avoided. Three main steps were involved in the process of detecting the MTHFR gene (rs1801133) polymorphism using Thermo Scientific's TaqMan real-time PCR kit: extracting genomic DNA from peripheral blood leucocytes in an EDTA whole blood sample, amplifying the extracted DNA, and performing allelic discrimination using real-time PCR.

Statistical analysis

Was performed using Statistical soft-ware program IBM SPSS statistics (V. 26.0, IBM Corp., USA, 2019) for data analysis. Data were presented and suitable statistical analysis was done according to the type of data obtained for each parameter. $P < 0.05$ was considered significant, $p < 0.01$ was considered highly significant and $p > 0.05$ was considered non-significant.

RESULTS

Results of the present study are illustrated in (Tables 1-7). Comparison between responders and non-responders in the sociodemographic, clinical, and laboratory data are presented in (Table 1). Statistically significant difference was found between the two groups only in the DAS 28, with a median of 4.1 in the non-responders and 3.6 in the responders ($p<0.001$), and in the ESR, with a median of 47.5 in the non-responders and 20 ($p=0.036$). However, a non- significant difference was observed between both groups regarding other sociodemographic, clinical, and laboratory ($p > 0.05$).

Table 1: Comparison between responders and non-responders in the sociodemographic, clinical, and laboratory data using Mann- Whitney test.

Parameters		Non-Responders (*n* =18)	Responders (*n* =27)	Z	*P*
		Median (IQR) / *n* (%) **			
Age (years)		56.5 (48.75 – 66)	55 (45 - 62)	-0.789	0.43
Gender	F	11(61.1%) **	15(55.6%) **		0.712
	M	7(38.9%) **	12(44.4%) **		
Treatment Duration		8 (4 – 11.75)	8 (3 – 13)	-0.023	0.981
Dose of MXT		20(15 – 21.25)	20 (20 – 20)	-0.136	0.891
Other dis eases	No	10(55.6%) **	18(66.7%) **		0.451
	Yes	8(44.4%) **	8(33.3%) **		
DAS28		4.1(3.9- 4.35)	3.6(3.5-3.8)	-4.441	<0.001*
Creatinine (mg/dL)		0.72(0.6-0.98)	0.77(0.63-1.15)	-0.243	0.808
WBCs (10^9/L)		5(3.975-7.25)	5.3(4-9)	-0.545	0.586
Hb (g/dl)		11(10.3-12.125)	11(10-12)	-0.35	0.727
Plt (10^9/L)		352.5 (204.5- 403.75)	270(200-370)	-1.043	0.297
CRP (mg/dL)		1(0.8-2.6)	1.1(0.7-1.7)	-0.743	0.458
RF (IU/mL)		16(8-32)	16(8-32)	-0.121	0.904
Anti. CCP (EU/mL)		28.5(17.25-223.75)	80(30-291)	-1.182	0.237
ESR (mm/hr)		47.5(20-67.5)	20(15-40)	-2.093	0.036*
ALT (IU/L)		21.5(14.75-34.25)	28(17-40)	-0.95	0.342
AST (IU/L)		21.5(18-35.25)	25(20-40)	-0.835	0.403
ALP (mg/dL)		79(71.5-113.25)	80(67-100)	-0.603	0.547
T. Bilirubin(mg/dL)		0.45(0.4-0.625)	0.6(0.4-0.8)	-1.199	0.23
D. Bilirubin (mg/dL)		0.2(0.1-0.225)	0.2(0.1-0.2)	-0.113	0.91

P-value > 0.05: Non-significant; *P-value* < 0.05: Significant; $p < 0.001$: highly significant; Z: Mann-Whitney test; MXT: Methotrexate; DAS28: Disease Activity Score-28; WBCs: White blood cells; Hb: Hemoglobin; Plt: Platelet; CRP: C-reactive protein; RF: Rheumatoid factor; Anti. CCP: Anti–cyclic citrullinated peptide; ESR: Erythrocyte sedimentation rate; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

Comparison between the responders and non-responders in the treatment complications is shown in (Table 2). There were no statistically significant differences between the responders and non-responders in the rates of complications (p>0.05).

Table 2: Comparison between the responders and non-responders in the treatment complications using chi-square test.

Parameters		Non- Responders (n =18)	Responders (n =27)	X^2	P
		n (%)			
Liver toxicity	No	17(94.4%)	23(85.2%)	0.938	0.333
	Yes	1(5.6%)	4(14.8%)		
Kidney toxicity	No	16(88.9%)	25(92.6%)	0.183	0.669
	Yes	2(11.1%)	2(7.4%)		
Hematology toxicity	No	15(83.3%)	23(85.2%)	0.028	0.867
	Yes	3(16.7%)	4(14.8%)		
Pulmonary toxicity	No	16(88.9%)	22(81.5%)	0.451	0.502
	Yes	2(11.1%)	5(18.5%)		

P-value > 0.05: Non-significant; X^2: Chi-square test.

(Table 3) demonstrates comparable distribution of the MTHFR gene polymorphism and allele frequency (p>0.05). The gene analysis showed that CC constituted 33.3% of the non-responders and 40.7% of the responders, CT constituted 61.1% of the non-responders and 51.9% of the responders, and TT constituted 5.65 of the non-responders and 7.4% of the responders (p=0.827). Regarding the allele frequency, C was found in 83.9% of the non-responders and 66.7% of the responders, and T was found in 36.1% of the non-responders and 33.3% of the responders (p=0.786).

Table 3: Comparison between the responders and non-responders in the gene polymorphism and allele frequency using chi-square test.

Parameters		Non- Responders (n =18)	Responders (n =27)	X^2	P
		n (%)			
MTHFR Gene polymorphism	CC	6(33.3%)	11(40.7%)	0.379	0.827
	CT	11(61.1%)	14(51.9%)		
	TT	1(5.6%)	2(7.4%)		
Allele Frequency	C	23(63.9%)	36(66.7%)	0.074	0.786
	T	13(36.1%)	18(33.3%)		

P-value > 0.05: non-significant; X2: Chi-square test.

(Table 4) reveal comparison of the treatment complications in the responders according to the gene polymorphism. There was statistically significant difference among the three types in the rates of hematology toxicity only (36.4% of the CC group, and 0% in the two other groups, p=0.033). A non-significant difference was observed among the three types in the rates of other complications (p> 0.05).

Table 4: Comparison of the treatment complications in the responders according to the gene polymorphism.

Parameters/ Genotypes	CC	CT	TT	P
Liver toxicity	2 (18.2%)	2 (14.3%)	0 (0%)	0.799
Kidney toxicity	2 (18.2%)	0(0%)	0(0%)	0.208
Hematology toxicity	4 (36.4%)	0(0%)	0(0%)	0.033*
Pulmonary toxicity	3(27.3%)	2(14.3%)	0(0%)	0.554

P-value > 0.05: Non-significant; *P-value* < 0.05: significant

(Table 5) present comparison of the treatment complications in the non- responders according to the gene polymorphism. There was statistically significant difference among the three types in the rates of hematology toxicity only (0% of the CC group, 18.2% in the CT group, and 100% of the TT group, p=0.045). A non-significant difference was observed among the three types in the rates of other complications (p> 0.05).

Table 5: Comparison of the treatment complications in the non-responders according to the gene polymorphism.

Parameters/ Genotypes	CC	CT	TT	*P*
Liver toxicity	1 (16.7%)	0 (0%)	0 (0%)	0.374
Kidney toxicity	2 (33.3%)	0 (0%)	0 (0%)	0.105
Hematology toxicity	0 (0%)	2 (18.2%)	1 (100%)	0.045*
Pulmonary toxicity	1 (16.7%)	1 (9.2%)	0 (0%)	0.836

P-value > 0.05: Non-significant; *P-value* < 0.05: significant

Comparison of the baseline sociodemographic, clinical, and laboratory data in the two gene allele is illustrated in (Table 6). There was no statistically significant difference between the patients according to the gene alleles in the baseline sociodemographic, clinical, and laboratory data in the two gene allele.

Table 6: Comparison of the baseline sociodemographic, clinical, and laboratory data in the two gene allele using chi-square test for qualitative data and Mann- Whitney test for non-parametric data.

Parameters		C (n =59)	T (n =31)	Z/ X^{2}**	*P*
		Median (Q1-Q3) / n (%) *			
Age (years)		55(45-65)	53(46-63)	-0.06	0.953
Gender	F	33(55.9%) *	19(61.3%) *	0.239**	0.625
	M	26(44.1%) *	12(38.7%) *		
ttt.Dur		8 (5 – 14)	7(3 – 13)	-0.792	0.428
Dose.MXT		20(20-20)	20 (20 – 20)	-0.14	0.889
Other diseases	No	36(61.0) *	20(64.5%) *	0.106**	0.745
	Yes	23(39.0%) *	11(35.5%) *		
DAS28		3.8(3.6-4.1)	3.8(3.5-4.1)	-0.818	0.413
Creatinine(mg/dL)		0.79(0.65-1.15)	0.69(0.6-0.97)	-1.682	0.093
WBCs (10^{9}/L)		5(4-8)	5.3(4-8)	-0.119	0.905
Parameters		C (n =59)	T (n =31)	Z/ X^{2}**	*P*
		Median (Q1-Q3) / n (%) *			
Hb (g/dl)		11(10-12)	11.5(10.6-12)	-0.991	0.322
Plt (10^{9}/L)		290(200-370)	300(210-400)	-0.773	0.439
CRP (mg/dL)		1(0.7-2.1)	1(0.8-3.2)	-0.68	0.496
RF(IU/mL)		16(8-32)	16(16-32)	-1.643	0.1
Anti. CCP(EU/mL)		75(21-215)	75(19-324)	-0.093	0.926
ESR (mm/hr)		30(20-60)	30(20-50)	-0.103	0.918
ALT(IU/L)		22(16-37)	26(17-37)	-0.153	0.878
AST(IU/L)		24(20-40))	23(19-34)	-0.519	0.604
ALP (mg/dL)		80(68-100)	80(67-103)	-0.008	0.993
Tot.Bil(mg/dL)		0.5(0.4-0.7)	0.5(0.4-0.7)	-0.155	0.877
D.Bil (mg/dL)		0.2(0.1-0.2)	0.2(0.1-0.2)	-0.469	0.639

P-value > 0.05: Non-significant; X2: Chi-square test; Z: Mann-Whitney test; MXT: Methotrexate; DAS28: Disease Activity Score-28; WBCs: White blood cells; Hb: Hemoglobin; Plt: Platelet; CRP: C-reactive protein; RF: Rheumatoid factor; Anti. CCP: Anti–cyclic citrullinated peptide; ESR: Erythrocyte sedimentation rate; ALT: Alanine transaminase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase.

(Table 7) demonstrate comparison of the treatment complications according to the gene allele. There was statistically significant difference among the three types in the rates of kidney toxicity only (13.6% of the C group, and 0% of the T group, p=0.032). A non-significant difference was observed among the three types in the rates of other complications (p> 0.05).

Table 7: Comparison of the baseline sociodemographic, clinical, and laboratory data in the two gene allele using chi-square test.

Parameters		C (n =59)	T (n =31)	X^2	*P*
		n (%)			
Liver toxicity	No	51(86.4%)	29(93.5%)	1.040	0.308
	Yes	8(13.6%)	2(6.5%)		
Kidney toxicity	No	51(86.4%)	31(100.0%)	4.613	0.032*
	Yes	8(13.6%)	0(0.0%)		
Hematology toxicity	No	49(83.1%)	27(87.1%)	0. 253	0. .615
	Yes	10(16.9%)	4(12.9%)		
Pulmonary toxicity	No	48(81.4%)	28(90.3%)	1.244	0.265
	Yes	11(18.6%)	3(9.7%)		

P-value > 0.05: Non-significant; *P-value* < 0.05; X^2: Chi-square test

DISCUSSION

The introduction of new medications, especially DMARDs, has improved the prognosis for RA patients. Owing to its affordable price and shown effectiveness, MTX is one of the DMARDs that is most often used in clinical practice. However, 40–60% of RA patients do not achieve a satisfactory response to DMARDs and about 15–30% develop adverse effects. Thus, the outcome of MTX treatment varies unpredictably between RA patients[10].

The antifolate medication methotrexate inhibits a number of important enzymes involved in the pathways leading to the production of folate, methionine, adenosine, and de novo nucleotides, which has antiproliferative and anti-inflammatory properties[11].

The folic acid pathway's key regulatory enzyme, methylene tetrahydrofolate reductase (MTHFR), is indirectly inhibited by MTX and this enzyme is necessary for the regeneration of reduced folate. It is regulated by the MTHFR gene and alteration of enzyme activity as a result of the gene mutation remains controversial[12,13].

Therefore, the present study aimed to evaluate the impact of MTHFR gene C677T (rs1801133) polymorphism on the clinical outcome of MTX treatment as regards treatment efficacy or toxicity in Egyptian rheumatoid arthritis patients.

This study showed that statistically significant difference was found between the responders to the treatment and non-responders in the DAS 28, with a median of 4.1 in the non-responders and 3.6 in the responders. Our study agrees with the recent USA study of[14] who found that DAS28 was among the top predictors of lack of response to MTX. Similarly, DAS28 was found to be among the top predictors of insufficient response to MTX treatment in a recent study from The Netherlands[15].

This finding can be attributed to that patients who do not respond to methotrexate may have a higher baseline inflammatory burden, leading to more persistent symptoms and joint damage. Also, Non-response to methotrexate may be associated with specific pathogenic mechanisms that contribute to disease progression and increased disease activity. These mechanisms might involve different cytokine profiles or autoantibody production, leading to a more aggressive disease course[16]. Since methotrexate is an anti-metabolic medication, methotrexate therapy can have side effects. However, because RA patients require lower dosages of methotrexate than cancer patients, these side effects are less prevalent[17].

In the present study the treatment complications were hepatic, renal hematologic, and lung toxicity. This is supported by the previously described data that the most common adverse effects include liver damage, myelosuppression, and lung fibrosis[18].

No statistically significant difference was found in this study between the responders and non-responders in the rate of treatment complications. The precise mechanisms behind methotrexate-induced side effects remain unclear. While cytopenia, mucositis, and gastrointestinal disturbances are linked to folic acid deficiency, other side effects like lung fibrosis, liver damage, and renal impairment occur independently of folate levels. This suggests that additional mechanisms may contribute to these adverse effects[19]. This partially explain why non-response to treatment, which could indicate

non-affection of the folate synthesis, resulted in a comparable rates of treatment complications.

In the current study, the MTHFR gene polymorphism analysis showed that CC genotype constituted 33.3% of the non-responders and 40.7% of the responders, CT genotype constituted 61.1% of the non-responders and 51.9% of the responders, and TT genotype constituted 5.65 of the non-responders and 7.4% of the responders. Regarding the allele frequency, C allele was found in 83.9% of the non-responders and 66.7% of the responders, and T allele was found in 36.1% of the non-responders and 33.3% of the responders. Our study displayed that genetic polymorphisms and alleles frequencies in MTHFR C677T were not associated with clinical efficacy response of MTX, and appeared consistent with the studies of[20] from Poland,[9,13] from China who found no significant difference in the gene polymorphism according to the response to treatment.

It has been established through study[21] that C677T polymorphism is not able to predict clinical response in the population of Western Algeria. In the relevant genetic statistical models, research conducted by[22] in the East Bohemian population has not revealed any association between the genotypes of C677T and the ineffectiveness of MTX therapy. Nevertheless,[11] compared to MTHFR 677CC and 677C carriers, 677TT genotype was linked to a roughly three-fold higher incidence of non-response to MTX in a Portuguese sample of RA patients.

This discrepancy could be related to various factors. Genetic variations, including polymorphisms like MTHFR C677T, can exhibit population-specific differences. The prevalence of specific genotypes may vary between different ethnic or geographical groups. It is possible that the genetic distribution of MTHFR C677T is different in the populations studied, leading to varying associations with MTX response. Also, variability in patient characteristics, disease severity, and treatment regimens across different studies can impact treatment response. Other genetic or environmental factors potentially influence MTX response and affect the observed associations.

Whether the MTHFR C677T polymorphism is related to toxicities is controversial. In this study, comparison of the treatment complications in the responders and non-responders according to the gene polymorphism revealed that there were statistically significant higher rates of hematologic toxicity in the CC genotype subgroup of the responders and in the TT genotype group in the non-responders. Comparison according to the gene allele revealed that the C allele subgroup showed statistically significant higher rates of kidney toxicity than the T allele subgroup. A non-significant difference was observed in the rates of other complications.

It has been stated that the substitution of the C nucleotide by the T nucleotide in the C677T variant can lead to an amino acid change from alanine to valine[23] has been reported to be associated with decreased activity of MTHFR, elevated plasma homocysteine levels and altered distribution of folate. This could explain the difference in hematologic toxicity among the different genotypes.

Studies examining the relationship between the two MTHFR gene polymorphisms and MTX toxicity revealed significant variation between nations, even within the same one. In this regard, it was hypothesized by[24] that individuals with the TT genotype were more susceptible to possible MTX-induced toxicity because of the possibility that decreased MTHFR enzyme activity would result in delayed cell repair and slower folate metabolism.

The association between MTHFR gene polymorphisms and methotrexate (MTX)-related adverse events in patients with rheumatoid arthritis (RA) has been the subject of discordant meta-analyses in the past[25] found that the 677C>T polymorphism was linked to increased toxicity. Similarly,[26] identified an association between the 677C>T polymorphism and MTX toxicity in RA patients, including both the general and Asian populations[27] also reported that the 677C>T polymorphism was connected to MTX-induced toxicity in East Asian RA patients[2] demonstrated a substantial correlation between the 677C>T polymorphism and MTX-related toxicity.

The observed differences in hematology toxicity among the three MTHFR C677T genotypes in responders and non-responders MTX in the current work can be attributed to the complex interactions between genetic factors, drug metabolism, and individual patient characteristics. It's important to acknowledge that the relationship between MTHFR C677T genotypes, MTX response, and toxicity is complex and multifactorial. Further research, including functional studies and larger, well-controlled clinical trials, is needed to better understand the underlying mechanisms driving these observed differences and to inform personalized treatment strategies for RA patients receiving MTX therapy.

CONCLUSION

The present study did not confirm the presence of significant association between the genotypic frequencies of the MTHFR gene C677T (rs1801133) polymorphism among the responders and the non- responders group in patients with RA. The MTHFR gene C677T (rs1801133) polymorphism did not show significant difference between disease complications among the responders and the

non-responders group in patients with RA. Our findings suggest that statistically significant difference was found between the responders to the treatment and non-responders in disease activity (DAS 28) in patients with RA. There were statistically significant higher rates of hematologic toxicity in the CC subgroup of the responders and in the TT group in the non-responders. Regarding C allele, showed statistically significant kidney toxicity in all patients groups. An increased sample size is required to replicate the MTX treatment response. Furthermore, our research may open the door to MTX treatment strategies tailored specifically for RA patients.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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AUTHORS CONTRIBUTION STATEMENT

Marium El Sayed and Marwa Raafat wrote the manuscript and contributed to data collection and interpretation. Marwa Rafat and Fatma Mohamed contributed to patients' recruitment, sample, and data collection. Noha Refaat contributed to samples collection, laboratory processing of samples, data interpretation, and drafted the paper. Karim Shahin planned and designed the study. Amani Abdel Ghani contributed to sample collection, laboratory processing of samples, data interpretation, and reviewed the manuscript. The authors read and approved the final manuscript.

REFERENCES

1. **Singh, A.; Gangadharan, H.; Gupta, V.; Patro, P.S.; Misra, R. and Aggarwal, A. (2021):** Polymorphism of genes involved in methotrexate pathway: Predictors of response to methotrexate therapy in Indian rheumatoid arthritis patients. International Journal of Rheumatic Diseases, 24(5): 654-662.

2. **Huang, J.; Fan, H.; Qiu, Q.; Liu, K.; Lv, S.; Li, J.; Yang, H.; Shu, X.; Xu, Y.; Lu, X.; Lu, C.; Zhang, Y. and Xiao, C. (2020):** Are gene polymorphisms related to adverse events of methotrexate in patients with rheumatoid arthritis? A retrospective cohort study based on an updated meta-analysis. Therapeutic Advances in Chronic Disease, 11: 1-17.

3. **Kurzawski, M.; Malinowski, D.; Szarmach, N.; Nowak, A.; Goryniak, A.; Pawlik, A. and Droździk, M. (2016):** ATIC missense variant affects response to methotrexate treatment in rheumatoid arthritis patients. Pharmacogenomics, 17(18): 1971-1978.

4. **D'Cruz, L.G.; McEleney, K.G.; Tan, K.B.; Shukla, P.; Gardiner, P.V.; Connolly, P.; Conway, C.; Cobice, D. and Gibson, D.S. (2020):** Clinical and laboratory associations with methotrexate metabolism gene polymorphisms in rheumatoid arthritis. Journal of Personalized Medicine, 10(4): 1-14.

5. **Muralidharan, N.; Mariaselvam, C.M.; Mithun, C.B. and Negi, V.S. (2016):** Reduced folate carrier-1 80G> A gene polymorphism is not associated with methotrexate treatment response in South Indian Tamils with rheumatoid arthritis. Clinical Rheumatology, 35(4): 879-885.

6. **Romero, M.Z.; Martínez, F.; Montoro, M.M.; Ramírez, C.; Hernández, M.C.; Pete, N.; Martín, S.; Martínez, F.M.; Hernández, M.A.C.; Tortosa, M. R. and Morales, A.J. (2020):** Association of genetic polymorphisms on methotrexate toxicity in patients with rheumatoid arthritis. Archives of Medical Science, 16(1): 1-9.

7. **Dwivedi, A.; Jha, A. K. and Gupta, V. (2020):** Impact of MTHFR C677T and A1298C gene polymorphisms on MTX drug toxicity and efficacy profile of RA patients in North India. Meta Gene, 24(100705): 1-7.

8. **Sharaki, O.A.; Elgerby, A.H.; Nassar, E.S. and Khalil, S.S.E. (2018):** Impact of methylenetetrahydrofolate reductase (MTHFR) A1298C gene polymorphism on the outcome of methotrexate treatment in a sample of Egyptian rheumatoid arthritis patients. Alexandria Journal of Medicine, 54(4): 633-638.

9. **Wang, S.; Zuo, S.; Liu, Z.; Ji, X.; Yao, Z. and Wang, X. (2020):** Association of MTHFR and RFC1 gene polymorphisms with methotrexate efficacy and toxicity in Chinese Han patients with rheumatoid arthritis. Journal of International Medical Research, 48(2): 1-11.

10. **Shao, W.; Yuan, Y. and Li, Y. (2017):** Association Between MTHFR C677T Polymorphism and Methotrexate Treatment Outcome in Rheumatoid Arthritis Patients: A Systematic Review and Meta-Analysis. Genetic testing and molecular biomarker; 21(5): 275–285.

11. **Lima, A.; Monteiro, J.; Bernardes, M.; Sousa, H.; Azevedo, R**. ***et al.*** **(2014):** Prediction of methotrexate clinical response in Portuguese rheumatoid arthritis patients: implication of MTHFR rs1801133 and ATIC rs4673993 polymorphisms. BioMed research international; 2014: 368681.

12. **Froese, D. S.; Huemer, M.; Suormala, T.; Burda, P.; Coelho, D.** ***et al.*** **(2016):** Mutation Update and Review of Severe Methylenetetrahydrofolate Reductase Deficiency. Human mutation; 37(5): 427–438.

13. **Zhang, Q.; Fu, P.; Cao, Z.; Huang, H.; Wen, Q.** ***et al.*** **(2023):** MTHFR and MTRR Genetic Polymorphism of Methotrexate Therapy Outcomes in Early Rheumatoid Arthritis. Pharmacogenomics and personalized medicine; 16: 407–423.

14. **Duong, S. Q.; Crowson, C. S.; Athreya, A.; Atkinson, E. J.; Davis, J. M.** ***et al.*** **(2022):** Clinical predictors of response to methotrexate in patients with rheumatoid arthritis: a machine learning approach using clinical trial data. Arthritis research & therapy; 24(1): 1- 11.

15. **Gosselt, H. R.; Verhoeven, M. M. A.; Bulatović-Ćalasan, M.; Welsing, P. M.; de Rotte, M. C. F. J.** ***et al.*** **(2021):** Complex Machine-Learning Algorithms and Multivariable Logistic Regression on Par in the Prediction of Insufficient Clinical Response to Methotrexate in Rheumatoid Arthritis. Journal of personalized medicine, 11(1): 44.

16. **Sergeant, J. C.; Hyrich, K. L.; Anderson, J.; Kopec-Harding, K.; Hope, H. F.** ***et al***. **(2018):** Prediction of primary non-response to methotrexate therapy using demographic, clinical and psychosocial variables: results from the UK Rheumatoid Arthritis Medication Study (RAMS). Arthritis research & therapy; 20: 1-11.

17. **Smolen, J. S.; Landewé, R. B.; Bijlsma, J. W.; Burmester, G. R.; Dougados, M.** ***et al***. **(2020):** EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease- modifying antirheumatic drugs: 2019 update. Annals of the rheumatic diseases; 79(6): 685-699.

18. **Mustafa, S. H.; Ahmad, T.; Balouch, M.; Iqbal, F. and Durrani, T. (2022):** Safety Profile of Methotrexate Therapy in Patients With Rheumatoid Arthritis. Cureus; 14(7): e27047.

19. **Tian, H. and Cronstein, B. N. (2007):** Understanding the mechanisms of action of methotrexate: implications for the treatment of rheumatoid arthritis. Bulletin of the NYU hospital for joint diseases; 65(3): 168–173.

20. **Swierkot, J. and Slęzak, R. (2011):** Znaczenie badań farmakogenetycznych w efektywności leczenia metotreksatem chorych na reumatoidalne zapalenie stawów (część 2) [The importance of pharmacogenetic tests in evaluation of the effectiveness of methotrexate treatment in rheumatoid arthritis (part 2)]. Postepy higieny i medycyny doswiadczalnej (Online); 65: 207–215.

21. **Boughrara, W.; Benzaoui, A.; Aberkane, M.; Moghtit, F. Z.; Dorgham, S.** ***et al***. **(2017):** No correlation between MTHFR c.677 C > T, MTHFR c.1298 A > C, and ABCB1 c.3435 C > T polymorphisms and methotrexate therapeutic outcome of rheumatoid arthritis in West Algerian population. Inflammation research: official journal of the European Histamine Research Society; 66(6): 505–513.

22. **Soukup, T.; Dosedel, M.; Pavek, P.; Nekvindova, J.; Barvik, I.** ***et al***. **(2015):** The impact of C677T and A1298C MTHFR polymorphisms on methotrexate therapeutic response in East Bohemian region rheumatoid arthritis patients. Rheumatology international; 35(7): 1149–1161.

23. **Hider, S. L.; Bruce, I. N. and Thomson, W. (2007):** The pharmacogenetics of methotrexate. Rheumatology (Oxford, England); 46(10): 1520–1524.

24. **Lambrecht, L.; Sleurs, C.; Labarque, V.; Dhooge, C.; Laenen, A.** ***et al***. **(2017):** The role of the MTHFR C677T polymorphism in methotrexate-induced toxicity in pediatric osteosarcoma patients. Pharmacogenomics; 18(8): 787–795.

25. **Fisher, M. C. and Cronstein, B. N. (2009):** Metaanalysis of methylenetetrahydrofolate reductase (MTHFR) polymorphisms affecting methotrexate toxicity. The Journal of rheumatology; 36(3): 539–545.

26. **Lee, Y. H. and Song, G. G. (2010):** Associations between the C677T and A1298C polymorphisms of MTHFR and the efficacy and toxicity of methotrexate in rheumatoid arthritis: a meta-analysis. Clinical drug investigation; 30(2): 101–108.

27. **Song, G. G.; Bae, S. C. and Lee, Y. H. (2014):** Association of the MTHFR C677T and A1298C polymorphisms with methotrexate toxicity in rheumatoid arthritis: a meta-analysis. Clinical rheumatology; 33(12): 1715–1724.

العلاقه بين التعدد الشكلي الجيني(MTHFR (rs١٨٠١١٣٣ ونتائج علاج الميثوتريكسات في مرضي التهاب المفاصل الروماتويدي

[١]مريم السيد أحمد فتحي، [١]كريم يحي شاهين، [١]أماني محمد عبد الغني، [١]مروه محمد رأفت محمد عبد العزيز، [٢]فاطمه محمد بدر محمد و [١]نها رفعت محمد

[١]قسم الباثولوجيا الإكلينيكية، [٢]قسم الباطنه عامه وأمراض روماتيزمية، كليه الطب، جامعه عين شمس، القاهره، مصر

الخلفية: يعد التهاب المفاصل الروماتويدي هو أحد أمراض المناعة الذاتية المزمنة التي تسبب فقدان وظيفة المفاصل وتقلل بشكل كبير من جودة الحياة. يستخدم الميثوتريكسات، أحد الأدوية المضادة للروماتيزم المعدلة للمرض، كدواء أساسي لعلاج التهاب المفاصل الروماتويدي وقد تكون هناك اختلافات في عمل الدواء بين الأنماط الجينية المدروسة.

الهدف من الدراسة: هدفت الدراسة الحالية إلى تقييم تأثير تعدد الأشكال لجين اختزال الميثيلين تتراهيدروفولات.

على النتيجة السريرية للعلاج بالميثوتريكسات فيما يتعلق بفعالية العلاج أو السمية في مرضى التهاب المفاصل الروماتويدي في مصر.

النتائج: أجريت هذه الدراسة على ٤٥ مريضا تم تشخيص إصابتهم بالتهاب المفاصل الروماتويدي الذين يتلقون الميثوتريكسات كخط أول للعلاج. تم تحديد تعدد الأشكال لجين اختزال الميثيلين تتراهيدروفولات(rs١٨٠١١٣٣) C٦٧٧T باستخدام تفاعل البلمرة المتسلسل في الوقت الفعلي.

الاستنتاجات: كشفت نتائج الدراسة الحالية أنه تم العثور على فرق ذو دلالة إحصائية بين المستجيبين للعلاج وغير المستجيبين في نشاط المرض. ومع ذلك، لم يلاحظ وجود فرق ذو دلالة إحصائية بين المستجيبين وغير المستجيبين في معدل مضاعفات العلاج ولا ترددات الأنماط الجينية المدروسة. أشارت النتائج التي توصلنا إليها إلى أنه لا يوجد ارتباط بين الترددات الجينية المدروسة بين المستجيبين ومجموعة غير المستجيبين في مرضى التهاب المفاصل الروماتويدي.