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REVIEW

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Methotrexate intolerance in Rheumatoid Arthritis

Harjit Singh Nalwa[1], Peeyush Prasad[1], Nirmal Kumar Ganguly[1], Ved Chaturvedi[2] and Shivani Arora Mittal[1*]

Abstract

Rheumatoid Arthritis is one of the most common autoimmune diseases, causing irreversible joint damage and disability. Methotrexate (MTX) is the gold standard drug for this. The low cost, easy availability and high efficacy makes it the most important and commonly used DMARD in developing and low income countries. However, long term use of MTX is also associated with intolerance including gastrointestinal effects such as nausea, vomiting, abdominal pain and diarrhoea. In addition, anticipatory, associative and behavioural symptoms such as anxiety and irritability are also observed. These adverse effects arise as a conditioned response and are often inadequately managed, leading to discontinuation of treatment. Understanding and assessing the incidence of MTX intolerance across ethnicities and geographical regions would lead to a better treatment compliance. In this review we present a compilation of the available literature on Methotrexate intolerance in Rheumatoid Arthritis and strategies to mitigate this effect.

Keywords Methotrexate, Rheumatoid Arthritis, Intolerance, Therapy

Introduction

Rheumatoid Arthritis (RA) is one of the most common autoimmune diseases. Approximately 1% of the population is affected, causing irreversible cartilage and joint damage leading to disability [1]. Women are three times more likely to develop RA as compared to men, and a strong association with sex hormones, particularly oestrogen has been demonstrated. Pregnancy itself has been investigated as a risk factor in RA development [1]. Genetic factors also contribute to pathogenesis. It is a chronic inflammatory disease with autoimmune pathogenesis, characterised by joint involvement and multiple systemic manifestations like cardiovascular, pulmonary, psychological and skeletal disorders along with hyperplasia ("swelling"), autoantibody production (rheumatoid factor and anti–citrullinated protein antibody [ACPA]), cartilage and bone destruction ("deformity") [2]. Although many factors including genetic, environmental and infectious agents are known to contribute to disease pathogenesis, the exact pathological mechanism is yet to be fully understood [3]. The most common clinical presentation of the disease is multiple joint involvement, usually bilateral and symmetrical, most commonly affecting the metacarpophalangeal, metatarsophalangeal and proximal interphalangeal joints. Morning joint stiffness is another characteristic feature of the disease [4]. Left untreated, extra articular manifestations such as rheumatic nodules or rheumatic vasculitis may develop [5]. Cardiovascular disease is a common sequelae of chronic inflammation in these patients, and is the primary cause of death in people afflicted by RA [6]. Early diagnosis and maintenance of remission is thus paramount to preventing long term malignant joint damage leading to disability and greatly reduced quality of life. Patients with RA often have an elevated erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP) level, which may indicate presence of inflammatory processes in the body [7]. Currently, the ACR/ EULAR 2010 criteria for diagnosis

*Correspondence:
Shivani Arora Mittal
shivanimittal@icloud.com
[1] Department of Research, Sir Ganga Ram Hospital, New Delhi, India
[2] Department of Clinical Immunology & Rheumatology, Sir Ganga Ram Hospital, New Delhi, India

of RA use the rheumatoid factor (RF) and antibodies against cyclic citrullinated proteins (anti-CCP), and these remain the gold standard diagnostic biomarkers in clinical practice [8]. Besides these, other diagnostic biomarkers that can help with the early diagnosis of RA have been identified. These include anti-citrullinated peptide antibodies (ACPA), antibodies against mutated citrullinated vimentin (Anti-MCV), and antibodies against carbamylated proteins (Anti-Carp) [9].

Treatment of RA involves disease control, remission of symptoms, and maintenance therapy. Modern therapeutic approaches can lead to effective disease control greatly improving the quality of life. The first line and most common treatment is disease modifying anti-rheumatic drugs (DMARDs). These drugs alleviate symptoms of RA, improve physical function, and also inhibit progression of joint damage. This class of drugs, unlike non steroidal anti inflammatory drugs (NSAIDs), are effective in managing not only the symptoms but also preventing irreversible damage to the joints [10]. DMARDs are categorised as either synthetic (small chemical molecules given orally) or biologics (proteins administered parenterally). Among the empirically developed DMARDs, methotrexate (MTX) is the gold standard drug for RA treatment [11]. Other conventional synthetic DMARDs include sulfasalazine, leflunomide, and hydroxychloroquine. Common biologic DMARDs include TNF inhibitors (such as infliximab, adalimumab, and golimumab) or IL-6 inhibitors (such as tocilizumab) or JAK inhibitors (such as baricitinib) [12]. When patients do not respond to 2 or more conventional synthetic DMARDs, they are unlikely to achieve the treatment target. In the presence of poor prognostic markers, insufficient response to synthetic DMARDs or intolerance, EULAR recommends shifting to any biological DMARD.

Methotrexate: Mechanism of action and intolerance

The use of Methotrexate in treating RA was discovered serendipitously. Considering that folic acid is essential for cell proliferation via its role in DNA and RNA synthesis, it was reasoned that starving rapidly dividing cells of folic acid would inhibit their proliferation [13]. As a result, MTX was synthesised as a folic acid antagonist to treat childhood leukaemia with great success, as was proved in the landmark trial reported in 1948 [14]. With this background, Gubener and colleagues in the early 1950s were able to demonstrate a steroid like effect of MTX in several in vitro studies on cell cultures of the components of mesenchymal tissues, describing the action of the drug as 'steroid—sparing'. Using a dose that was several log orders lower than that used for treating leukaemia, they showed its efficacy in the treatment of RA and psoriatic arthritis. Low dose MTX is the standard of care today for RA treatment [15]. MTX is also useful for patients with juvenile idiopathic arthritis [16]. Today, MTX is one of the major chemotherapeutic choices for various types of cancers such as lymphoma [17], carcinoma of the breast [18], small cell carcinoma of the lung [19], carcinoma of the ovary [20], and non-metastatic osteosarcoma [21]. It is also safe and effective for patients with other inflammatory diseases such as psoriasis, systemic lupus erythematosus, inflammatory bowel disease [22], vasculitis, and many other connective tissue diseases. MTX has also found to be effective in patients with organ transplantation because of its anti-inflammatory and immunomodulatory activity.

MTX inhibits dihydrofolate reductase enzyme, which is essential for de novo purine and pyrimidine synthesis, thereby inhibiting proliferation of rapidly dividing malignant cells [23]. By inhibiting dihydrofolate reductase, MTX also prevents the reduction of dihydrobiopterin (BH2) to tetrahydrobiopterin (BH4), leading to nitric oxide synthase uncoupling and increased sensitivity of T cells to apoptosis [24, 25] Other postulated mechanisms by which MTX suppresses inflammation include enhanced adenosine release [26], inhibition of transmethylation reactions required for cellular functions, diminished accumulation of polyamines [27]. Inhibition of trans-methylation reactions result in subsequent reduction in polyamide production, leading to diminished production of ammonia and H_2O_2. Reduced production of such toxic metabolites leads to diminished anti-inflammatory response and synovial damage. MTX also leads to enhanced adenosine release by inhibiting aminoimidazole-4- carboxamide ribonucleotide (AICAR) transformylase (ATIC), leading to intracellular accumulation of AICAR and increased adenosine release [28]. MTX also inhibits adenosine deaminase, again leading to extracellular release of adenosine nucleotides. Adenosine nucleotides are converted into adenosine by the action of the cell surface enzymes such as ectonucleoside triphosphate dephosphorylase 1 (CD39) and ecto-5'-nucleotidase (CD73). Adenosine, thus produced, is a potent stimulus for the adenosine receptors, all of which has potent inhibitory effects on nearly all inflammatory cell types. Adenosine causes inhibition of adhesion and recruitment of neutrophils [29]. It also inhibits cytokine expression in macrophages. In addition, adenosine inhibits T cell receptor triggered activation and FAS-FASL mediated cell death in T cells. In endothelial cells, it causes increased barrier integrity and inhibits oedema formation. Thus, MTX exerts anti-inflammatory effects by directly and indirectly regulating the function of most cell types involved in inflammation including neutrophils, monocytes, T cells, B cells, endothelial cells and fibroblast like synoviocytes [30]. Figure 1 shows

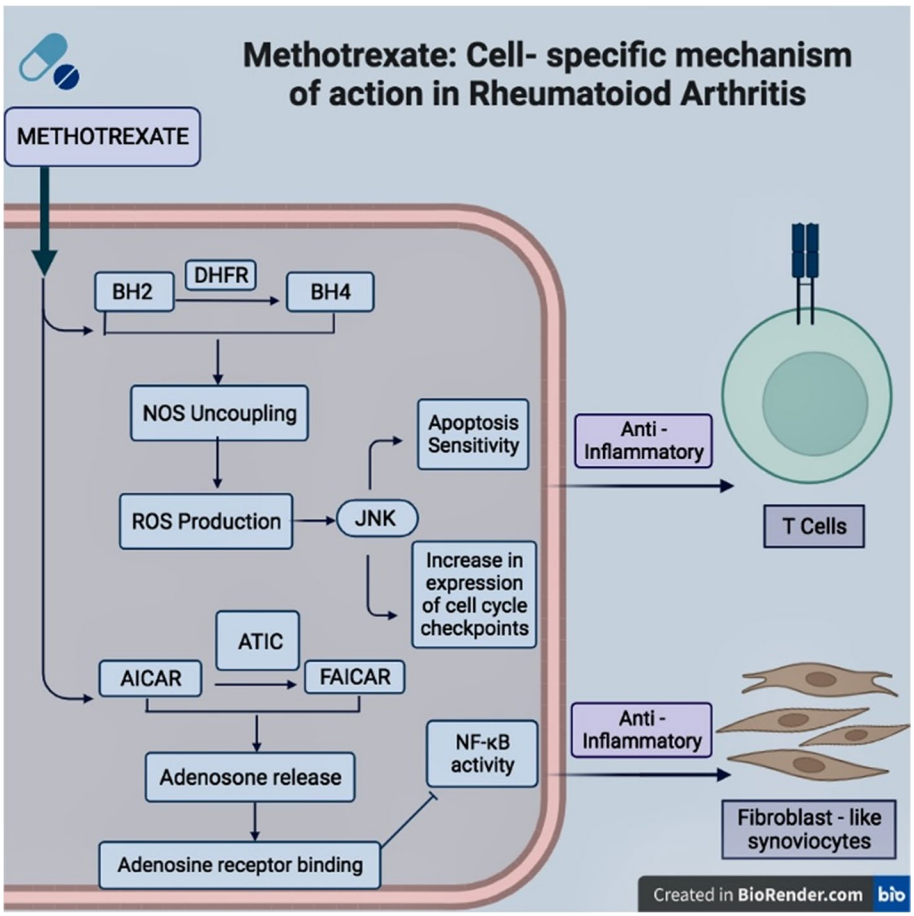

Fig. 1 Methotrexate: Cell—specific mechanism of action in Rheumatoid Arthritis. With respect to T cells, methotrexate causes the inhibition of the dihydrofolate reductase (DHFR)-mediated reduction of dihydrobiopterin (BH2) to tetrahydrobiopterin (BH4). This results in nitric oxide synthase (NOS) uncoupling and increased production of reactive oxygen species (ROS). The ROS activate JUN N-terminal kinase (JNK). In turn, the activated JNK induces genes encoding proteins that regulate sensitivity to apoptosis and cell cycle progression. In the case of fibroblast—like synoviocytes, methotrexate causes inhibition of of 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) transformylase (ATIC). This causes increased release of adenosine and activation of adenosine receptors, resulting in the inhibition of NF-κB with subsequent anti-inflammatory effects [30]

the schematic representation of mechanism of action of methotrexate in rheumatoid arthritis.

MTX is the mainstay drug for RA management and has played an important role in changing the approach as well as outcome of the therapy [16]. Its use is indicated as first-line therapy and occupies a prominent position in many guidelines and recommendations for treatment of rheumatic diseases [31–33]. Irrespective of the mode of administration, oral or parenteral, MTX has a short half-life of approximately 6 h, and is undetectable in the serum after 18 h. The bioavailability of orally administered MTX is highly variable owing to limited gut absorption. It is now known that polyglutamated MTX is the active form of the drug and the maximally absorbed dose is < 25 mg [30]. 25–40% of RA patients report a significant improvement with MTX therapy alone, making it the most significant and commonly used DMARD. Another advantage is that most of the adverse effects are well documented, and can be prevented/managed with supplementary prophylactic folate therapy. Due to low cost, easy availability and high efficacy, MTX is the most important and commonly used DMARD in developing and low income countries.

Despite its high efficacy and low cost, MTX is far from an ideal drug and is associated with adverse events that could limit its use. Few studies indicate discontinuation of therapy in less than 5% of patients due to intolerance [34]. High prevalence of MTX intolerance is also observed in other diseases such as juvenile idiopathic arthritis, psoriatic arthritis, inflammatory bowel disease and cancer chemotherapy [35]. The complaints and side effects can present in many ways, most commonly,

gastrointestinal effects such as nausea, vomiting, abdominal pain and diarrhoea. Other symptoms include hair loss, stomatitis and hepatotoxicity. Although folic acid supplementation during MTX treatment may reduce such effects, many patients discontinue treatment, something that negatively impacts disease control and quality of life [36–38]. In addition, patients may develop anticipatory symptoms, which occur prior to MTX intake, and associative symptoms, when patients think about taking the drug, as well as behavioural symptoms such as anxiety and irritability. These adverse effects arise as a conditioned response to previous symptoms experienced by patients on MTX and are not often clinically evident; therefore, they are often inadequately managed and can severely impact the quality of life and adherence to MTX therapy [39]. Fewer than 10 percent of patients experiencing MTX intolerance may show signs of improvement with a lower dose.

Two mechanisms involved in MTX related intolerance are known. The epithelial cells located in the oral cavity and intestines are rapidly renewing cells and are sensitive to MTX irrespective of folate deficiency. With time, the gastrointestinal epithelium becomes more sensitive due to increased accumulation of MTX causing nausea, vomiting and bone marrow suppression leading to cytopenia [39, 40]. Another mechanism of intolerance would be through binding to adenosine receptors in the central nervous system [41, 42]. The third mechanism of MTX intolerance is through stimulation of the chemoreceptor trigger zone (CTZ). The CTZ contains receptors that detect emetic agents in the blood and relays that information to the vomiting centre, which is responsible for inducing the vomiting reflex. Hepatic and central nervous system (CNS) toxicity are more complex and include elevation of liver enzymes, headaches and behavioural changes [43, 44]. Serious adverse effects such as hepatotoxicity and bone marrow suppression are infrequent and usually transient if MTX is stopped [45]. Understanding the pharmacology and anti-inflammatory mechanism of actions of MTX will go a long way in increased efficacy of RA treatment [30]. Further understanding into its side effects, could lead to appropriate strategies to improve drug tolerance.

Some studies have determined genetic polymorphisms, use of corticosteroids, gender or psychosocial factors to be associated with MTX intolerance [46]. Genetic polymorphisms of GGH and ABCC2 are associated with MTX intolerance in RA patients. GGH (Gamma-Glutamyl Hydrolase) is a protein coding gene, which catalyses the hydrolysis of folylpoly-gamma-glutamates and antifolylpoly-gamma-glutamates by the removal of gamma-linked polyglutamates and glutamate, to yield folic acid and free glutamate. The ABCC2 gene provides instructions for producing a protein called multidrug resistance protein 2 (MRP2), involved in the transport of substances out of cells. The MRP2 clears certain drugs from organs and tissues, playing a part in drug metabolism. MTX intolerance was found significantly associated with AA/AG genotype of the GGH-T401C polymorphism compared with presence of the GG genotype in a cohort of RA patients. Also, higher MTX tolerance was seen in carriers of TT/TC genotype of the ABCC2-C24T polymorphism compared with CC carriers [46]. Another study found FPGS rs10106 and BMI score to be associated with MTX intolerance. Significant correlation was observed between MTX intolerance and lower BMI score. This study proposed that a clinico-genetic model including BMI and SNP FPGS rs10101 was found to have a modest prediction ability for MTX intolerance, with an accuracy of 66.3% [47]. A cross- sectional interview based study conducted in Saudi Arabia among 117 adult RA patients concluded that female gender was associated with higher incidence of MTX intolerance. The Arabic MISS (Methotrexate Intolerance Severity Score) was used in this survey and statistical analyses were performed to understand associations between MTX intolerant and MTX tolerant patients. The study found that MTX intolerance was associated with the female gender (adjusted odds ratio (AOR) 6.724; 95% CI 1.420, 31.843, $P=0.016$) [48]. Thus, in addition to disease activity, the patient characteristics may significantly affect MTX intolerance. Renal excretion is the main MTX elimination route, and since females exhibit a lower average glomerular filtration rate than males, this could result in higher intolerance in them. This study also observed that the Disease Activity Score (DAS 28) score, which correlates with disease severity, was also significantly associated with MTX intolerance [48, 49]. Interestingly, another study analysed psychosocial variables to predict MTX response in 1050 RA patients, revealed that patient anxiety is a strong predictor of MTX intolerance. Thus, mood of the patient, along with conditions such as depression or anxiety could be predisposing factors for MTX intolerance [50].

Prevalence of Methotrexate Intolerance

A quantitative questionnaire based scoring method to determine Methotrexate Intolerance in patients has been developed and termed as MISS (Methotrexate Intolerance Severity Score) [35]. This self administered questionnaire (Fig. 2) was first designed and validated to evaluate MTX intolerance in juvenile idiopathic arthritis patients in a cross-sectional study that was performed across four university medical centres in the Netherlands. Currently, this is the only validated questionnaire available that evaluates MTX intolerance and accounts for the most frequent side effects as well as anticipatory,

	No Complaints 0	Mild Complaints 1	Moderate Complaints 2	Severe Complaints 3
Stomach Ache				
Has a Stomach Ache after taking MTX				
Has a Stomach Ache several hours to 1 day before taking MTX				
Has a Stomach Ache when thinking of MTX				
Nausea				
Is nauseous after taking MTX				
Is nauseous several hours to 1 day before taking MTX				
Is nauseous when thinking of MTX				
Vomiting				
Vomits after taking MTX				
Vomits hours to 1 day before taking MTX				
Behavioural Complaints				
Is restless when taking MTX				
Cries when taking MTX				
Is irritable when taking MTX				
Refuses to take MTX				

Fig. 2 Methotrexate Intolerance Severity Score (MISS) questionnaire

associative and behavioural symptoms – which are very important and often missed for identification of intolerance. Using the MISS, MTX intolerance was considered positive with a score of 6 points or greater [35]. The MISS questionnaire has now been validated for use in multiple languages including French, Arabic and Portuguese [51].

Various studies have assessed prevalence of MTX intolerance in rheumatoid arthritis. In the first study where MISS score was validated in JIA patients, a significantly high prevalence of MTX intolerance was observed [35]. Here, all patients had been receiving either oral or parenteral MTX for at least 3 months. Patients receiving parenteral MTX received a higher median MTX dosage (13.5 mg/ m2/ week) than those patients receiving oral MTX dosage (9.6 mg/m2/ week). Very high prevalence of MTX intolerance, 50.5%, was observed in this cohort of 297 JIA patients. Amongst these, percentages of intolerant patients who had nausea and behavioural symptoms were 91.3% and 88.7% respectively, whereas only 18.7% of the MTX intolerant patients experienced anticipatory vomiting. 56.1% of the 41 patients who were taking antiemetics were still intolerant to MTX. A slightly higher MTX dosage was associated with a greater incidence of intolerance, probably due to an increased drug concentration in the blood, triggering the CTZ. Furthermore, 23% higher prevalence of MTX intolerance was observed in patients receiving parenteral MTX as compared to those on oral MTX. The findings of this study do not indicate which clinical variables (i.e., MTX dose, age) are associated with the development of MTX intolerance.

It also does not indicate the percentage of patients which discontinue MTX due to intolerance. The MISS questionnaire used here has been used as a tool to determine MTX intolerance in all subsequent studies [35].

In a cross-sectional study, adult patients of rheumatoid arthritis and psoriatic arthritis were studied for MTX intolerance. 11% prevalence of MTX intolerance was found here in a sample size of 291 patients. All patients included had been receiving treatment for MTX for at least 3 months and were on weekly folic acid supplementation. 42.3% of the patients still experienced at least one gastrointestinal side effects. MTX intolerance prevalence was again found higher in patients on parenteral (20.6%) than oral MTX (6.2%). Besides MTX induced gastrointestinal symptoms, these patients also experienced anticipatory and associative gastrointestinal and behavioural symptoms before drug administration. Pre-treatment nausea was the most prevalent, 8.6% had anticipatory and 11.0% associative nausea. Behavioural symptoms, overall, affected 16.5% of patients, with restlessness being the most prominent symptom in 13.1% of patients. This finding was significantly lower, compared to 88.7% of behavioural symptoms seen in JIA patients in the previous study [52].

Another single institutional study conducted on 150 patients from Pakistan found much higher prevalence of MTX intolerance, around 33.3%. All the patients were taking oral MTX and folic acid supplementation. The results revealed that 44% of the subjects had behavioural symptoms, which were significantly higher than other symptoms of intolerance. Only 11.33% of subjects complained of vomiting. This study clearly shows that the most recurring symptom was behavioural, which is often clinically missed and could be a leading cause of non-compliance. Also, the study observed that the use of other DMARDs had no effect on MTX intolerance. However, the major limitation of this study was that of a small sample size of 150 patients, predominantly females [38].

In a study conducted in Saudi Arabia on 185 patients, 39.5% of cases were observed to be MTX intolerant. Patients with confirmed diagnosis of RA and actively receiving MTX therapy for 3 months or more were included. Using the Arabic version of MISS, 39.5% of the patients were found to be MTX intolerant. Of these patients, 75.3% and 24.7% were using the oral and subcutaneous forms of MTX, respectively. The most frequently occurring complaints were behavioural (58.37%), followed by nausea (37.84%). Vomiting was again the least frequent complaint (8.64%). It is interesting to note that this study showed a higher percentage of behavioural intolerance as compared to gastrointestinal intolerance, which is also consistent with the study from Pakistan. The authors proposed that the major reason for behavioural intolerance could be due to chemotherapy label on the product packaging of the medication dispensed at the outpatient clinic. This study effectively compared the prevalence of MTX intolerance observed between oral (29.7%) and parenteral modes (9.7%) of drug administration. Again, small sample size and female predominant population were the major limitations of this study [49].

A study from Brazil assessed MTX intolerance in 120 long-standing cases of RA, assessed possible associations between certain predisposing factors and MTX intolerance. These predicting factors of MTX intolerance included age as continuous variable ($p=0.089$), female sex ($p=0.022$), MTX dose above 17.5 mg/ week ($p=0.126$), use of non-steroidal anti-inflammatory drugs ($p=0.203$), proton-pump inhibitors ($p=0.164$), use of corticosteroids ($p=0.054$), and antiemetics ($p=0.217$). These were deemed possibly associated with the presence of MTX intolerance, due to a p-value <0.25. Out of these, use of corticosteroids was found significantly associated with MTX intolerance (OR = 2.73; 95% confidence interval [CI], 1.06 to 7.06; $p=0.038$), and highlighted that its use increased the risk of MTX intolerance. It also suggested a trend for decreasing risk of intolerance with increasing age [53]. No association of MTX intolerance with other variables such as subcutaneous or oral formulation, or folic acid dosing was found here. The frequency of MTX intolerance among RA patients was found to be 21.6%. Again, a small sample size and single institutional study were the major limitations. Further multi-centric studies with a large sample size, are required to elucidate association between age and corticosteroid use with MTX intolerance [53].

Another study reported in the Indian population demonstrated 14% prevalence of MTX intolerance in a total of 150 patients studied. Out of the 150 patients of RA on MTX, 21 (14%) were found to have MISS ≥ 6. Of the 21 patients showing MTX intolerance, 18 were on oral MTX and 3 were on parental MTX.This study showed that 14.4% patients on oral MTX and 11.1% patients on parenteral MTX were intolerant [54]. Table 1 mentions the important studies regarding prevalence of MTX intolerance in RA patients.

Discussion

Data from various studies across ethnicities reveals high frequency of MTX intolerance among RA patients. The MISS questionnaire serves as an invaluable tool to assess MTX intolerance in RA patients and is easy to administer. Use of corticosteroid therapy and female sex are found predisposing factors for increased risk of MTX intolerance. Moreover, besides well-known MTX-induced gastrointestinal symptoms upon MTX administration, RA patients also experienced anticipatory and

Table 1 Studies on prevalence of MTX intolerance in RA patients

S. No	Country	Sample Size (Females/ Males)	MTX Intolerance (Percentage)	Most Frequently Occurring Complaints	Major Limitations
1	Netherlands [52]	291 patients (Female- 181, 62.19%)	11%	Nausea (32.0%), Abdominal Pain (11.3%) and Behavioural Symptoms (16.5%)	Percentage of patients which discontinue MTX due to intolerance not indicated
2	Pakistan [38]	150 patients (Female- 150, 100%)	33.3%	Behavioural Symptoms (44%), Abdominal Pain (34.66%), Nausea (34%) and Fatigue (31.1%)	Female predominant small sample size
3	Saudi Arabia [49]	185 patients (Female- 158, 85.4%)	39.5%	Behavioural Complaints and Refusal to take MTX (58.37%), Nausea (37.84%)	Female predominant small sample size, mostly receiving oral MTX
4	Brazil [53]	120 patients (Female- 103, 85.83%)	21.6%	Nausea (92.3%), Abdominal Pain (46.1%) and Behavioural Symptoms (96.1%)	Small sample size and single institutional study
5	India [54]	150 patients (Female- 120, 80%)	14%	Nausea (31.7%), Abdominal Pain (14.6%), Nausea as an Anticipatory Symptom (12.2%) and Vomiting (12.2%)	• Inclusion criteria not been clearly stated • Small sample size

associative gastrointestinal and behavioural symptoms before MTX administration, which develop as a classical conditioning response to physical symptoms after taking MTX.

Studies on JIA patients demonstrated that EMDR (eye movement desensitisation and reprocessing) and pharmacological conditioning may help relieve MTX intolerance. EMDR enables the processing of dysfunctional and traumatic memories using an intensive recall combination with bilateral stimulation usually evoked by eye movement to dissolve the memories by reprocessing them. As a result, affective distress is relieved, reformulating negative beliefs and reducing physiological arousal. In a study which assumed that MTX intolerance is based on dysfunctional or incomplete information processing evoked by strong negative feelings or adverse anticipation of side effects, significant benefit was observed. Eighteen patients with MTX intolerance (median MISS 16.5) were included. Post treatment, intolerance symptoms were significantly reduced (median MISS 1). Thus, MTX intolerance in children with JIA can be effectively reduced using EMDR protocol, with lasting effect over a period of 4 months [55]. Another study demonstrated pharmacological conditioning for JIA as a potential solution to reduce MTX intolerance [56]. Pharmacological conditioning occurs when administration of an active pharmacological component (unconditioned stimulus) is repeatedly paired with the occurrence of another stimulus (conditioned stimulus). In pharmacological conditioning designs, learned positive associations from drug therapies (conditioning effects) are integrated in regular treatment regimens to maximize treatment outcomes. Based on previous experimental and clinical findings of pharmacological conditioning with immune responses, this particular study found that the JIA patient group is particularly suited to benefit from a pharmacological conditioning design, in order to reduce MTX intolerance.

Identifying incidence of MTX intolerance could help in early steps to mitigate the effects and lead to better drug compliance. Other timely interventions like change of drug route, folic acid supplementation, antiemetics and behavioural therapy can prevent MTX intolerance and improve compliance, providing a smooth path for an otherwise effective DMARD for RA. In addition, use of caffeine, eye movement desensitisation and reprocessing (EMDR) and pharmacological conditioning may be additional methods to reduce the prevalence of MTX intolerance. MTX is known to activate adenosine receptors in CNS, which may cause behavioural and anticipatory intolerance. Hence, caffeine being an antagonist of adenosine receptors, may cause alleviation of symptoms by working through this mechanism. In one study, adding Caffeine to the diet completely relieved MTX intolerance symptoms in 55% of patients. Additionally, another 13% of patients had partial relief, enough for them to continue MTX treatment [57].

Additionally, it is likely that other unexplored factors like age, diet, ethnicity, lifestyle, psychological factors like stress and anxiety can affect intolerance. Further studies are required to address these. In developing countries, such as India, most RA patients have access to oral MTX drug therapy only. Regular evaluation of MTX intolerance should be included in daily clinical practice by means of the MISS questionnaire. Early identification of MTX intolerance may impact treatment, so that changes

in medications may occur at the right time, promoting patient compliance and, consequently, disease control.

Conclusion

This review article comprehensively discusses the sample size, percentage of MTX intolerance reported, frequently occurring complaints, and the major limitations of each study. A very high frequency of MTX Intolerance was found across the studies, ranging from 11% to 50.5%. In particular, a high occurrence of behavioural symptoms were reported, the frequency ranging from 16.5% to 88.7%. In addition, use of corticosteroid therapy and female sex are found predisposing factors for increased risk of MTX intolerance.

Clinical studies on JIA patients demonstrated that EMDR (eye movement desensitisation and reprocessing) and pharmacological conditioning may help relieve MTX intolerance. Another study found that adding caffeine to the diet completely relieved MTX intolerance symptoms in 55% of patients. Additionally, it is likely that other unexplored factors like age, diet, ethnicity, lifestyle, psychological factors like stress and anxiety can affect intolerance. Further studies are required to address these. Regular evaluation of MTX intolerance should be included in daily clinical practice by means of the MISS questionnaire for early identification of MTX intolerance, so that changes in medications may occur at the right time, promoting patient compliance and, consequently, disease control.

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References

1. Silman AJ, Pearson JE, Pincus T, et al. Epidemiology and genetics of rheumatoid arthritis. Arthritis Res 2002; 4 (suppl 3): S265–72
2. Scott DL, Wolfe F, Huizinga TWJ. Rheumatoid arthritis. Lancet. 2010;376(9746):1094–108. https://doi.org/10.1016/S0140-6736(10)60826-4.
3. Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature. 2007;447(7145):661–78. https://doi.org/10.1038/nature05911.
4. Grassi W, De Angelis R, Lamanna G, Cervini C. The clinical features of rheumatoid arthritis. Eur J Radiol. 1998;27(Suppl 1):S18-24. https://doi.org/10.1016/s0720-048x(98)00038-2.
5. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular Manifestations in Rheumatoid Arthritis. Maedica (Bucur). 2010;5(4):286–91.
6. Dhawan SS, Quyyumi AA. Rheumatoid arthritis and cardiovascular disease. Curr Atheroscler Rep. 2008;10(2):128–33. https://doi.org/10.1007/s11883-008-0019-x.
7. Litao MK, Kamat D. Erythrocyte sedimentation rate and C-reactive protein: how best to use them in clinical practice. Pediatr Ann. 2014;43(10):417–20. https://doi.org/10.3928/00904481-20140924-10.
8. Kay J, Upchurch KS. ACR/EULAR 2010 rheumatoid arthritis classification criteria. Rheumatology (Oxford). 2012;51 Suppl 6:vi5–9. https://doi.org/10.1093/rheumatology/kes279.
9. Nakabo S. Clinical and etiological meaning of anti-carbamylated protein antibodies in rheumatoid arthritis. Immunol Med. 2018;41(4):147–53. https://doi.org/10.1080/25785826.2018.1556017.
10. Benjamin O, Goyal A, Lappin SL. Disease Modifying Anti-Rheumatic Drugs (DMARD). In: StatPearls [Internet]. Treasure Island: StatPearls Publishing; 2022 .
11. Swierkot J, Szechiński J. Methotrexate in rheumatoid arthritis. Pharmacol Rep. 2006;58(4):473–92.
12. Prasad P, Verma S, Surbhi, Ganguly NK, Chaturvedi V, Mittal SA. Rheumatoid arthritis: advances in treatment strategies. Mol Cell Biochem. 2023;478(1):69–88. https://doi.org/10.1007/s11010-022-04492-3.
13. Yang H, Qin D, Xu S, He C, Sun J, Hua J, Peng S. Folic acid promotes proliferation and differentiation of porcine pancreatic stem cells into insulin-secreting cells through canonical Wnt and ERK signaling pathway. J Steroid Biochem Mol Biol. 2021;205:105772. https://doi.org/10.1016/j.jsbmb.2020.
14. Farber S, Diamond LK, Mercer RD, Sylvester RF Jr, Wolff JA. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl- glutamic acid (aminopterin). N Engl J Med. 1948;238:787–93.
15. Malaviya AN. Landmark papers on the discovery of methotrexate for the treatment of rheumatoid arthritis and other systemic inflammatory rheumatic diseases: a fascinating story.
16. Takken T, Van der Net J, Helders PJ. Methotrexate for treating juvenile idiopathic arthritis. Cochrane Database Syst Rev. 2001;(3):CD003129. https://doi.org/10.1002/14651858.CD003129.
17. Sakura T, Hayakawa F, Sugiura I, Murayama T, Imai K, Usui N, Fujisawa S, Yamauchi T, Yujiri T, Kakihana K, Ito Y, Kanamori H, Ueda Y, Miyata Y, Kurokawa M, Asou N, Ohnishi K, Ohtake S, Kobayashi Y, Matsuo K, Kiyoi H, Miyazaki Y, Naoe T. High-dose methotrexate therapy significantly improved survival of adult acute lymphoblastic leukemia: a phase III study by JALSG. Leukemia. 2018;32(3):626–32. https://doi.org/10.1038/leu.2017.283.
18. Yap HY, Blumenschein GR, Yap BS, Hortobagyi GN, Tashima CK, Wang AY, Benjamin RS, Bodey GP. High-dose methotrexate for advanced breast cancer. Cancer Treat Rep. 1979;63(5):757–61.
19. Smyth JF, Ford HT. Methotrexate in the chemotherapy of lung cancer. Cancer Treat Rep. 1981;65(Suppl 1):161–3.
20. Parker LM, Griffiths CT, Yankee RA, Knapp RC, Canellos GP. High-dose methotrexate with leucovorin rescue in ovarian cancer: a phase II study. Cancer Treat Rep. 1979;63(2):275–9]

21. High dose methotrexate containing regimen in pediatric non-metastatic extremity osteosarcoma patients: experience from a tertiary cancer center in India - PubMed (nih.gov)
22. Schröder O, Stein J. Low dose methotrexate in inflammatory bowel disease: current status and future directions. Am J Gastroenterol. 2003;98(3):530–7. https://doi.org/10.1111/j.1572-0241.2003.07305.x.
23. Rushworth D, Mathews A, Alpert A, Cooper LJ. Dihydrofolate Reductase and Thymidylate Synthase Transgenes Resistant to Methotrexate Interact to Permit Novel Transgene Regulation. J Biol Chem. 2015;290(38):22970–6. https://doi.org/10.1074/jbc.C115.671123.
24. Channon KM. Critical role for tetrahydrobiopterin recycling by dihydrofolate reductase in regulation of endothelial nitric-oxide synthase coupling – relative importance of the de novo biopterin synthesis versus salvage pathways. J Biol Chem. 2009;284:28128–36.
25. Sugiyama T, Levy BD, Michel T. Tetrahydrobiopterin recycling, a key determinant of endothelial nitric-oxide synthase-dependent signaling pathways in cultured vascular endothelial cells. J Biol Chem. 2009;284:12691–700.
26. The antiinflammatory mechanism of methotrexate. increased adenosine release at inflamed sites diminishes leukocyte accumulation in an in vivo model of inflammation. J Clin Invest. 1993;92:2675–82.
27. Yukioka K, et al. Polyamine levels in synovial tissues and synovial fluids of patients with rheumatoid arthritis. J Rheumatol. 1992;19:689–92.
28. Allegra CJ, Drake JC, Jolivet J, Chabner BA. Inhibition of phosphoribosylaminoimidazolecarboxamide transformylase by methotrexate and dihydrofolic acid polyglutamates. Proc Natl Acad Sci USA. 1985;82:4881–5.
29. Cronstein BN, Levin RI, Belanoff J, Weissmann G, Hirschhorn R. Adenosine: an endogenous inhibitor of neutrophil-mediated injury to endothelial cells. J Clin Invest. 1986;78:760–70.
30. Cronstein BN, Aune TM. Methotrexate and its mechanisms of action in inflammatory arthritis. Nat Rev Rheumatol. 2020;16(3):145–54. https://doi.org/10.1038/s41584-020-0373-9.
31. Pincus T, Gibson KA, Castrejon I. Update on methotrexate as the anchor drug for rheumatoid arthritis. Bull Hosp Jt Dis. 2013;2013(71 Suppl 1):S9-19.
32. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016;388(10055):2023–38.
33. Mota L, Kakehasi AM, Gomides APM, Duarte A, Cruz BA, Brenol CV, et al. 2017 recommendations of the Brazilian Society of Rheumatology for the pharmacological treatment of rheumatoid arthritis. Adv Rheumatol. 2018;58(1):2.
34. Singh JA, Saag KG, Bridges SL Jr, Akl EA, Bannuru RR, Sullivan MC, et al. 2015 American College of Rheumatology Guideline for the treatment of rheumatoid arthritis. Arthritis Rheumatol. 2016;68(1):1–26.
35. Bulatovic M, Heijstek MW, Verkaaik M, van Dijkhuizen EH, Armbrust W, Hoppenreijs EP, et al. High prevalence of methotrexate intolerance in juvenile idiopathic arthritis: development and validation of a methotrexate intolerance severity score. Arthritis Rheum. 2011;63(7):2007–13.
36. Aletaha D, Smolen JS. Diagnosis and Management of Rheumatoid Arthritis: A Review. JAMA. 2018;320(13):1360–72. https://doi.org/10.1001/jama.2018.13103.
37. Hannoodee M, Mittal M. Methotrexate. [Updated 2021 Mar 7]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021. Available from: https://www.ncbi.nlm.nih.gov/books/NBK556114/
38. Fatimah N, Salim B, Nasim A, Hussain K, Gul H, Niazi S. Frequency of methotrexate intolerance in rheumatoid arthritis patients using methotrexate intolerance severity score (MISS questionnaire). Clin Rheumatol. 2016;35(5):1341–5.
39. Brunner HI, Johnson AL, Barron AC, Passo MH, Griffin TA, Graham TB, et al. Gastrointestinal symptoms and their association with health-related quality of life of children with juvenile rheumatoid arthritis: validation of a gastrointestinal symptom questionnaire. J Clin Rheumatol. 2005;11(4):194–204.
40. Chan ES, Cronstein BN. Mechanisms of action of methotrexate. Bull Hosp Jt Dis. 2013;71(Suppl 1):S5–8.
41. Chen JF, Eltzschig HK, Fredholm BB. Adenosine receptors as drug targets—what are the challenges? Nat Rev Drug Discov. 2013;12:265–86.
42. Paul S, Elsinga PH, Ishiwata K, Dierckx RA, van Waarde A, Adenosine A. (1) receptors in the central nervous system: their functions in health and disease, and possible elucidation by PET imaging. Curr Med Chem. 2011;18:4820–35.
43. Tian H, Cronstein BN. Understanding the mechanisms of action of methotrexate: implications for the treatment of rheumatoid arthritis. Bull NYU Hosp Joint Dis. 2007;65:168–73.
44. Becker ML, Rose CD, Cron RQ, Sherry DD, Bilker WB, Lautenbach E. Effectiveness and toxicity of methotrexate in juvenile idiopathic arthritis: comparison of two initial dosing regimens. J Rheumatol. 2010;37(4):870–5.
45. Ortiz-Alvarez O, Morishita K, Avery G, Green J, Petty RE, Tucker LB, et al. Guidelines for blood test monitoring of metho- trexate toxicity in juvenile idiopathic arthritis. J Rheumatol. 2004;31:2501–6.
46. Escudero-Contreras A, López-Medina C, Collantes-Estévez E, Ortega-Castro R, Calvo-Gutiérrez J, Mena-Vázquez N, Panero-Lamothe B, Manzanares-Martín B, Cáliz-Cáliz R, Jiménez-Morales A, Ruiz-Jiménez M, Font-Ugalde P. Genetic Polymorphisms of GGH and ABCC2 Are Associated with Methotrexate Intolerance in Patients with Rheumatoid Arthritis. J Clin Med. 2021;10(18):4070. https://doi.org/10.3390/jcm10184070.PMID:34575187;PMCID:PMC8472669.
47. Sandhu A, Dhir V, Ahmad S, Dhawan V, Kaur J, Bhatnagar A. Clinico-genetic model to predict methotrexate intolerance in rheumatoid arthritis. Clin Rheumatol. 2020;39(1):201–6. https://doi.org/10.1007/s10067-019-04770-4.
48. Almalag H, Abouzaid HH, Alnaim L, Albaqami J, Al Shalhoub R, Almaghlouth I, Dessougi M, Al Harthi A, Bedaiwi M, Alfi E, Omair MA. Risk Factors Associated with Methotrexate Intolerance in Rheumatoid Arthritis Patients. Open Access Rheumatol. 2020;12:193–202. https://doi.org/10.2147/OARRR.S263287.PMID:32982497;PMCID:PMC7498496.
49. Albaqami J, Alshalhoub R, Almalag H, Dessougi M, Harthi AA, Bedaiwi MK, Oma MA. Prevalence of methotrexate intolerance among patients with rheumatoid arthritis using the Arabic version of the methotrexate intolerance severity scoreInt J Rheum Dis. 2019;00:1–6.
50. Sergeant JC 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 Res Ther 20:147 10.1186/ s13075–018–1645–5
51. Amaral JM, Brito MJM, Kakehasi AM. Cultural adaptation and validation of the methotrexate intolerance severity score in Brazilian Portuguese for adults with rheumatoid arthritis. J Clin Rheumatol. 2021;27(6S):S168–72.
52. Maja Bulatović Ćalasan, Oscar FC van den Bosch, Marjonne CW Creemers , Martijn Custers, Antonius HM Heurkens , Jan Maarten van Woerkom and Nico M Wulffraat. Prevalence of methotrexate intolerance in rheumatoid arthritis and psoriatic arthritis. Arthritis Res Ther 2013, 15:R217.
53. Amaral JM, Brito MJM, Kakehasi AM. High frequency of methotrexate intolerance in longstanding rheumatoid arthritis: using the methotrexate intolerance severity score (MISS). Advances in Rheumatology. 2020;60:43.
54. Virdi P, Singh B, Singh R. Role of methotrexate intolerance severity score (miss) in rheumatoid arthritis to know methotrexate intolerance: a 2-year prospective study. Int J Adv Res. 2017;5:2542–8.
55. Höfel L, Eppler B, Storf M, Schnöbel-Müller E, Haas JP, Hügle B. Successful treatment of methotrexate intolerance in juvenile idiopathic arthritis using eye movement desensitization and reprocessing - treatment protocol and preliminary results. Pediatr Rheumatol Online J. 2018;16(1):11. https://doi.org/10.1186/s12969-018-0228-y.PMID:29433504;PMCID:PMC5809965.
56. Smits RM, Veldhuijzen DS, van Middendorp H, Hissink Muller PCE, Armbrust W, Legger E, Wulffraat NM, Evers AWM. Pharmacological conditioning for juvenile idiopathic arthritis: a potential solution to reduce methotrexate intolerance. Pediatr Rheumatol Online J. 2020;18(1):12. https://doi.org/10.1186/s12969-020-0407-5.PMID:32033577;PMCID:PMC7006148.
57. Malaviya AN. Methotrexate intolerance in the treatment of rheumatoid arthritis (RA): effect of adding caffeine to the management regimen. Clin Rheumatol. 2017;36(2):279–85. https://doi.org/10.1007/s10067-016-3398-3.

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