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CORRELATION BETWEEN METHOTREXATE PLASMA CONCENTRATION AND CLINICAL EFFICACY IN RHEUMATOID ARTHRITIS: A 6-MONTH PROSPECTIVE STUDY

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ABSTRACT: Background: Methotrexate (MTX) is the first-line disease-modifying anti-rheumatic drug (DMARD) for rheumatoid arthritis (RA), with therapeutic efficacy often attributed to optimal systemic exposure. However, interindividual variability in MTX plasma concentration can influence treatment response. **Objective:** To evaluate the correlation between plasma MTX concentrations and clinical efficacy over a 6-month period in patients with RA, using ESR, CRP, and pain scores as markers of therapeutic response. **Methods:** A prospective observational study was conducted on 72 newly diagnosed RA patients receiving oral MTX (15 mg/week). Plasma samples were collected at 2 hours post-dose to estimate MTX concentration using validated HPLC. Disease activity was assessed at baseline and after 6 months using ESR, CRP, and visual analogue scale (VAS) pain scores. Pearson's correlation was used to assess associations between MTX levels and clinical parameters. **Results:** The mean MTX plasma concentration at 6 months was $1.29 \pm 0.39 \mu\text{mol/L}$. Reductions in mean ESR (from 34.4 to 19.9 mm/hr), CRP (from 13.2 to 6.0 mg/L), and pain score (from 6.53 to 1.98) were noted. However, weak and statistically non-significant correlations were found between MTX concentration and ESR ($r = +0.16$, $p = 0.17$), CRP ($r = -0.21$, $p = 0.07$), and pain score ($r = -0.08$, $p = 0.47$). **Conclusion:** While MTX therapy led to significant clinical improvement in RA patients over 6 months, plasma concentration alone did not strongly predict therapeutic response. Intracellular MTX metabolites and other pharmacodynamic factors may play a more dominant role, indicating the need for more comprehensive monitoring strategies.

INTRODUCTION: Rheumatoid arthritis (RA) is a chronic, immune-mediated inflammatory disorder that primarily targets synovial joints³. It leads to progressive cartilage degradation, bone erosion, and joint deformity, ultimately resulting in significant impairment of physical function and quality of life.

The disease is systemic in nature and is associated with extra-articular manifestations and an elevated risk of cardiovascular, pulmonary, and metabolic comorbidities. Affecting approximately 0.5% to 1% of the global population, RA is more prevalent among women and typically presents during middle age.

If left untreated, it imposes a considerable clinical and socioeconomic burden due to long-term disability, reduced work productivity, and the need for lifelong pharmacotherapy². Methotrexate (MTX), a structural analog of folic acid, has long been established as the first-line disease-modifying

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anti-rheumatic drug (DMARD) in the management of RA. It is a conventional synthetic agent (csDMARD) with well-documented efficacy in suppressing inflammation, limiting radiological progression, and preserving joints function³. MTX is generally administered once weekly, either orally or parenterally, with doses ranging from 7.5 mg to 25 mg, tailored according to disease activity and individual tolerance. Its relatively favorable risk-benefit profile and cost-effectiveness have made it the cornerstone of RA therapy, often used as monotherapy or in combination with other agents including biologics or targeted synthetic DMARDs⁴.

The mechanism of action of MTX in RA extends beyond its classical antifolate properties. It inhibits dihydrofolate reductase and thymidylate synthase, interfering with DNA synthesis and cellular replication. More importantly in RA, MTX enhances extracellular adenosine signaling, which has broad anti-inflammatory effects⁵. Adenosine modulates neutrophil activity, downregulates proinflammatory cytokines such as TNF- α and IL-6, and promotes a shift toward anti-inflammatory macrophage phenotypes⁶. In addition, MTX influences T-cell apoptosis, adhesion molecule expression, and oxidative stress pathways. These pleiotropic mechanisms collectively contribute to its disease-modifying effects⁷.

Despite its established place in RA treatment algorithms, clinical response to MTX is known to be highly variable. Some patients exhibit rapid symptom relief and disease control, while others demonstrate partial or minimal benefit even after adequate dosing⁸. Such inter-individual differences in efficacy may stem from genetic, metabolic, and immunological variability, but a major contributing factor is pharmacokinetic diversity⁹. The extent of MTX absorption, distribution, metabolism, and excretion varies widely among patients and can influence both therapeutic outcomes and adverse event profiles.

Oral MTX displays dose-dependent and saturable absorption in the gastrointestinal tract, with bioavailability ranging between 30% and 70%. After administration, peak plasma concentration (C_{max}) is typically achieved within 1 to 2 hours¹⁰. MTX binds moderately to plasma proteins and

distributes into third spaces, including synovial fluid. It is minimally metabolized in the liver to 7-hydroxymethotrexate and is primarily excreted unchanged by the kidneys via glomerular filtration and active tubular secretion. Renal function, hydration status, age, and concurrent medications significantly influence MTX clearance¹¹.

Given this variability, the measurement of plasma MTX concentrations, especially C_{max}, has been explored as a potential marker for therapeutic monitoring. A higher C_{max} may reflect adequate systemic exposure and correlate with better disease control, while subtherapeutic levels could contribute to treatment failure¹². However, the relationship between MTX pharmacokinetics and clinical efficacy remains incompletely understood. Although some studies suggest a positive correlation between MTX levels and disease activity reduction, others have reported weak or inconsistent associations. Additionally, plasma levels may not fully reflect the drug's intracellular activity, where MTX is converted to active polyglutamated metabolites that exert prolonged immunosuppressive effects¹³.

From a clinical standpoint, inflammation in RA is routinely assessed using biomarkers such as erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), both of which reflect systemic inflammatory burden¹⁴. These parameters are commonly used to evaluate treatment response and guide therapeutic decisions. Similarly, subjective measures such as visual analogue scale (VAS) for pain provide insight into patient-perceived symptom relief. Whether MTX plasma concentrations are predictive of improvements in these markers is still under investigation¹⁵.

This study was designed to address this gap in evidence by prospectively evaluating the correlation between MTX plasma concentrations and treatment response in patients with RA over a six-month period. By analyzing ESR, CRP, and VAS pain scores in relation to MTX C_{max} measured at steady state, we aimed to determine whether plasma drug levels could serve as a surrogate marker for clinical efficacy¹⁶. The study also intended to explore the feasibility of integrating therapeutic drug monitoring (TDM) into routine RA management, especially in resource-

limited settings where advanced immunological assays may not be available. Understanding this correlation is crucial in optimizing MTX therapy. If a strong relationship between plasma levels and clinical response can be established, it could pave the way for individualized dosing regimens, early identification of non-responders, and more precise prediction of therapeutic outcomes. On the other hand, if plasma MTX concentrations fail to correlate with clinical improvement, it would reinforce the importance of intracellular pharmacokinetics and pharmaco-genomics in governing MTX's immunomodulatory actions.

MATERIALS AND METHODS:

Study Design and Participants: This was a prospective, observational study conducted at J.L.N. Medical College, Ajmer. Seventy-two adult patients newly diagnosed with RA (per ACR/EULAR 2010 criteria)¹⁷ and new to DMARD therapy were enrolled. Ethical approval was obtained from the institutional review board.

Inclusion Criteria:

- Diagnosed with RA based on ACR/EULAR criteria.
- Willing to initiate oral MTX therapy (15 mg/week)
- With or without comorbid T2DM (diagnosed by HbA1c >6.5%).

Exclusion Criteria:

- ❖ Use of corticosteroids or biologics.
- ❖ GI, hepatic, renal, or autoimmune comorbidities.
- ❖ Pregnancy, lactation, HIV, or refusal to consent.

MTX Administration and Sampling: Patients received oral MTX once weekly after a baseline assessment. Venous blood samples were drawn 2 hours post-dose after 6 months of consistent therapy, assuming steady-state kinetics. Plasma was separated and stored in cryotubes at -20°C until analysis.

HPLC Analysis: MTX plasma levels were quantified using YL9100 HPLC system with a UV detector at 302 nm. The mobile phase consisted of

45% acetonitrile and 55% water, flow rate 1.0 mL/min, with a C18 column. Method validation was performed for accuracy, precision, linearity, and recovery.

Treatment and Follow-up: All patients received oral MTX at a fixed weekly dose of 15 mg. No concomitant steroids or biologics were used. Patients were followed up for 6 months, with clinical and laboratory evaluations at baseline and at study completion.

Sample Collection and Analysis: Venous blood was collected 2 hours after MTX administration at the 6-month mark. Plasma was separated and analyzed using HPLC (YL9100 system) with UV detection at 302 nm. The mobile phase was a mixture of 45% acetonitrile and 55% water, with a flow rate of 1 mL/min.

Clinical Assessment: Pain intensity was assessed using the Visual Analogue Scale (VAS), a validated tool where patients rated their pain on a scale from 0 to 10, with 0 indicating no pain and 10 representing the worst imaginable pain. Inflammatory status was evaluated through objective laboratory markers erythrocyte sedimentation rate (ESR), measured in millimeters per hour (mm/hr), and C-reactive protein (CRP), measured in milligrams per liter (mg/L). Both ESR and CRP were recorded at baseline and after six months of methotrexate therapy to monitor the treatment's impact on systemic inflammation.

Statistical Analysis: Mean values were compared using paired t-tests. Pearson correlation coefficient was used to assess relationships between MTX plasma concentration and clinical parameters.

RESULTS:

MTX Concentration and Clinical Improvement:

- Mean MTX plasma concentration at 6 months: 1.29 ± 0.39 μ mol/L.
- ESR: reduced from 34.4 ± 8.75 to 19.9 ± 6.17 mm/hr.
- CRP: reduced from 13.2 ± 4.54 to 6.0 ± 2.17 mg/L.
- Pain Score: reduced from 6.53 ± 1.61 to 1.98 ± 1.42 (all $p < 0.001$).

TABLE 1:

Parameter	Baseline (Mean $\pm$ SD)	6 Months (Mean $\pm$ SD)	p-value
MTX Conc. (μ mol/L)	—	1.29 $\pm$ 0.39	—
ESR (mm/hr)	34.4 $\pm$ 8.75	19.9 $\pm$ 6.17	< 0.001
CRP (mg/L)	13.2 $\pm$ 4.54	6.0 $\pm$ 2.17	< 0.001
Pain Score (VAS 0–10)	6.53 $\pm$ 1.61	1.98 $\pm$ 1.42	< 0.001

DISCUSSION: This study highlights an important observation in the clinical pharmacology of methotrexate (MTX) in rheumatoid arthritis (RA). Over a six-month treatment period, patients receiving fixed-dose oral MTX experienced significant reductions in inflammatory biomarkers erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) as well as marked improvement in patient-reported pain scores. These findings affirm MTX's well-established anti-inflammatory and disease-modifying properties in RA. However, our central objective was to evaluate whether plasma MTX concentrations, specifically peak levels (C_{max}), could serve as a predictive marker of clinical efficacy. The data revealed that while clinical outcomes improved significantly, there was only a weak and statistically non-significant correlation between MTX plasma levels and reductions in ESR, CRP, and VAS pain scores. These findings suggest that plasma MTX concentration, measured at a single post-dose time point, may not reliably predict therapeutic response in RA. A likely explanation lies in MTX's complex pharmacodynamic profile. MTX undergoes intracellular polyglutamation, forming methotrexate polyglutamates (MTX-PGs), which accumulate inside cells and persist longer than the parent compound in plasma. These polyglutamated forms are believed to be the primary mediators of MTX's immunosuppressive effects, influencing purine metabolism, adenosine signaling, and T-cell activation. Therefore, therapeutic efficacy may depend more on intracellular drug retention and action than on transient plasma levels. Since our study did not measure MTX-PG concentrations, this critical pharmacodynamic component remains unaccounted for¹⁸.

Interindividual variability in MTX response is well documented and may be influenced by a host of factors beyond drug levels. These include differences in drug absorption, hepatic metabolism, renal clearance, and expression of folate transporters. Additionally, genetic polymorphisms in genes encoding dihydrofolate reductase, reduced

folate carrier (RFC-1), and enzymes like methylenetetrahydrofolate reductase (MTHFR) can significantly impact both pharmacokinetics and pharmacodynamics. These factors were not assessed in the current study but may explain the variability in response despite similar plasma concentrations¹⁹.

Another limitation is the reliance on a single time-point blood draw to measure MTX C_{max}. A more accurate assessment of systemic exposure would involve area under the concentration-time curve (AUC) or trough levels. Serial measurements across time could better reflect individual pharmacokinetic profiles and enable robust PK-PD modelling²⁰.

Overall, while MTX plasma concentration may provide some insight into systemic drug exposure, it is insufficient alone to predict clinical response. Our findings support the need for a multidimensional approach, incorporating intracellular drug monitoring, genetic profiling, and dynamic disease activity assessment to personalize MTX therapy in RA.

CONCLUSION: Although methotrexate therapy resulted in clinically meaningful reductions in inflammation and pain in RA patients, the peak plasma concentration of the drug did not significantly correlate with these improvements. This suggests that factors beyond systemic drug exposure such as intracellular metabolism and immune response variability play a critical role in therapeutic outcomes. Future studies should consider longitudinal and multi-point MTX level monitoring, along with pharmacogenomic profiling, to optimize individualized treatment strategies.

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CONFLICT OF INTEREST: None declared

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