ORIGINAL ARTICLE

# Adverse Effects of Low Dose Methotrexate in Rheumatoid Arthritis Patients

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## ABSTRACT

**Objective:** To determine the frequency of adverse effects attributed to Methotrexate (MTX) toxicity and serum minimum toxic concentration with low dose MTX in Rheumatoid Arthritis (RA) patients.

**Study Design:** Cross-sectional observational study.

**Place and Duration of Study:** Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, from March 2010 to March 2011.

**Methodology:** One hundred and forty adult patients of RA receiving low dose MTX (10 mg/week) for at least 3 months, were included by consecutive sampling. Blood samples were collected 2 hours after the oral dose of MTX. Serum alanine transaminase and creatinine were analyzed on Hitachi and blood counts on Sysmex analyzer. Serum MTX concentration was measured on TDX analyzer.

**Results:** Out of one hundred and forty patients; 68 males (49%) and 72 females (51%), 38 developed MTX toxicity (27%), comprising of hepatotoxicity in 12 (8.6%), nephrotoxicity in 3 (2.1%), anaemia in 8 (5.7%), leucopenia in 2 (1.4%), thrombocytopenia in 3 (2.1%), pancytopenia in 2 (1.4%), gastrointestinal adverse effects in 5 (3.6%) and mucocutaneous problems in 3 (2.1%). Receiver operating characteristic curve revealed serum minimum toxic concentration of MTX at cutoff value of 0.71 μmol/l with a sensitivity of 71% and specificity of 76%.

**Conclusion:** Adverse effects of low dose MTX were found in 27% of RA patients, mainly comprising of hepatotoxicity and haematological problems. MTX toxicity can be detected by therapeutic drug monitoring of serum concentration of 0.71 μmol/l with sensitivity of 71% and specificity of 76% in the patients on low dose MTX maintenance therapy.

**Key words:** *Methotrexate. Rheumatoid Arthritis. Toxicity. Minimum toxic concentration.*

## INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterised by a chronic polyarticular synovial inflammation because of increase release of cytokines that may lead to irreversible joint damage.[1] Methotrexate (MTX) in low doses, is commonly used as the anti-rheumatic drug, because of its effectiveness, low toxicity and costs.[2-4] MTX inhibits the formation of polyamines that reduce production of rheumatoid factors and its anti-inflammatory action by increasing adenosine concentration and reducing cytokines.[5] MTX has half life of 7-10 hours, mainly metabolized in liver and excreted by the kidneys.[5,6] MTX may cause adverse effects such as liver damage, nephrotoxicity, myelosuppression, gastrointestinal and mucocutaneous problems.[7-9]

The rates of discontinuation of low dose MTX in RA patients due to toxicity has been reported from 10 to 37%.[1,8] Therapeutic drug monitoring of MTX in rheumatoid arthritis has revealed its toxicity in 9% patients and prevented the adverse effects by decreasing the dose.[2] The raised liver enzymes above two times the upper limit of reference (ULR) has been found in 13% of patients using MTX; 3.7% of patients stopped MTX permanently owing to liver toxicity, a meta-analysis showed incidence of fibrosis in 2.7% after 4 years of MTX administration.[1,10,11] The rate of thrombocytopenia was 4.1% and pancytopenia seems to be less common with an incidence of 0.96-1.4% in a few retrospective studies.[1] Side effects leading to renal abnormalities are also reported in 1-7% of the patients on low dose MTX maintenance therapy.[11,12]

MTX therapy in RA is a dynamic process with a goal of preventing joint damage and other undesirable consequences.[5,13] Inspite of widespread use of MTX over 20 years, considerable variation in serum toxic concentration has been found among patients with its low doses.[5,10] Pharmacokinetic studies have revealed peak serum MTX levels after 2 hours of oral dose.[14] However, minimum toxic concentration (MTC) of MTX varies 0.8 - 1 μmol/l after low dose administration.[7,14] Therapeutic drug monitoring of MTX can prevent its toxicity on different organs in the body much earlier, so minimum toxic concentration needs to be established in order to reduce toxicity.[7,10]

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Methotrexate is often injudiciously used in RA patients without its monitoring in the clinical setup in Pakistan. There is limited data on adverse effects of MTX and its levels causing toxicity in the local RA patients. This study aimed to find out the frequency of adverse effects attributed to MTX toxicity and serum minimum toxic concentration with low dose MTX in RA. This will help in reducing the adverse effects of MTX in RA patients by using our cutoff value of MTC for prediction of toxicity.

## METHODOLOGY

A cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP) in collaboration with Rheumatology Outpatient Department of Military Hospital (MH), Rawalpindi, Pakistan, from March 2010 to March 2011 after approval of the institutional review committee. One hundred and forty patients of RA aged 16 - 70 years of either gender, reporting to the Rheumatology OPD, MH, Rawalpindi for follow-up were included after informed consent. History of illness, findings of physical examination, demographic data and baseline routine investigations were carried out at the start of the study. Patients with known liver disease, renal insufficiency, leucopenia, thrombo-cytopenia, anaemia (Haemoglobin < 120 g/L for female and < 130 g/L for male) and pregnant ladies were excluded.

A total of 140 patients receiving MTX at dose of 10 mg/week for at least 3 months were included in the study. All samples were collected in vacutainer tubes (BD, NJ USA) after 2 hours of MTX dose to get peak drug levels.[11,15] Blood samples were allowed to clot and then centrifuged for 10 minutes at 1,000 g. The serum was separated and stored at - 8°C until assayed. Blood samples (2.5 ml) were collected in EDTA tubes for blood counts. All the routine investigations including MTX levels were carried out in the Department of Chemical Pathology and Endocrinology, AFIP, Rawalpindi. Complete blood counts including RBC, WBC, platelets and haemoglobin were carried out on a haematology Analyser, SYSMEX KX-21 (Japan). Serum alanine transaminase (ALT) and creatinine were analysed by Roche assay on Hitachi chemistry auto-analyzer. Serum MTX levels were measured by Fluorescence Polarization Immunoassay on TDX analyzer (Abbott Laboratories, Diagnostic Division, Abbott Park, USA). The coefficient of variation of MTX assay was 4.7%.

Diagnostic criteria of MTX toxicity was established if any one of the following was found; serum ALT more than 2 times upper limit of reference (ULR) for liver toxicity,[9,10,16] serum creatinine more than 1.5 times ULR for renal toxicity,[12,16] anaemia at haemoglobin less than 120 g/L for females and less than 130 g/L for males,[1] leucopenia at TLC less than $4x10^9$/L,[1] thrombocytopenia at platelets less than 150 x $10^9$/L[1]; gastrointestinal adverse effects included nausea, vomiting and diarrhoea and others were mucocutaneous problems.

Data was entered in Statistical Package for Social Sciences Version 17 (SPSS Inc, Chicago, IL, USA) and analyzed by one-sample Kolmogorov-Smirnov test. Data were non-Gaussian, so presented as median (25th-75th percentiles) and Mann Whitney U test was applied for comparison. Frequencies and percentages were calculated for qualitative variables like gender and toxic effects. Quantitative variables including age, duration of drug intake, serum MTX levels, serum ALT levels, serum creatinine levels and blood counts were compared between the groups by using Mann-Whitney U test. Medcalc was used for receiver operating characteristic (ROC) curve between cases that develop toxicity and controls for peak MTX levels to establish cut-off value for MTC of MTX with optimal sensitivity and specificity. A p-value of < 0.05 in two tails was considered significant.

## RESULTS

A total of 140 RA patients comprising of 68 males (49%) and 72 females (51%) with mean age of 45 years, ranging from 16 to 70 years, participated in the study. Baseline clinical characteristics of the study patients are shown in Table I. Serum ALT, and MTX concentrations at 2 hours (h) were significantly high in the patients who had adverse MTX effects as compared to the other group of patients. MTX concentrations ranged between 0.01 – 1.87 μmol/l at 2 hours after the dose of MTX. MTX levels in 6 patients did not reach the threshold of 0.02 μmol/l. Blood counts were reduced in 15 patients (10.7%).

**Table I:** Baseline characteristics of rheumatoid arthritis patients on low dose MTX with and without MTX toxicity after 3 months treatment.

| Parameter | No toxicity (n=102) median (IQR) | Toxicity ( n=38) median (IQR) | p-value |
|---|---|---|---|
| Age (years) | 45 (35-45) | 46 (35-55) | 0.82 |
| Duration of MTX (months) | 07 (3-36) | 24 (5-47) | 0.90 |
| MTX after 2 hours (μmol/L) | 0.5 (0.3-0.7) | 0.8 (0.6-1.01) | 0.00 |
| ALT (U/L) | 26 (20-36) | 40 (22-90) | 0.00 |
| Creatinine (μmol/L) | 69 (61-77) | 73 (65-84) | 0.97 |
| Haemoglobin (g/L) | 135 (129-141) | 108 (102-130) | 0.00 |
| TLC x$10^9$/L | 8.2 (7.0-10.2) | 7.9 (6.8-10.3) | 0.71 |
| Platelets x$10^9$/L | 320 (278-356) | 307 (267-336) | 0.19 |

*IQR = Inter quartile range*

Out of 140 RA patients on low dose MTX maintenance therapy, 38 patients (27%) developed adverse effect due to high serum MTX levels intoxication (Figure 1). Liver toxicity was found in 12 patients (8.6%) in which increased liver enzyme values based on ALT more than 2 times ULR were found in 8 (5.7%) and 3 times ULR in

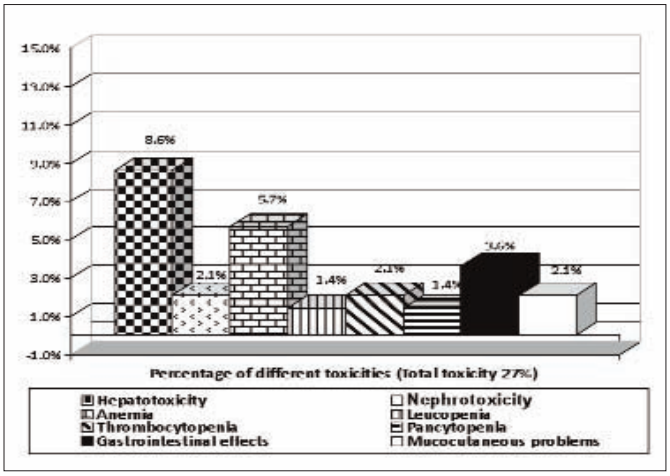


**Figure 1:** Frequency of common adverse effects attributed to MTX toxicity in RA patients.

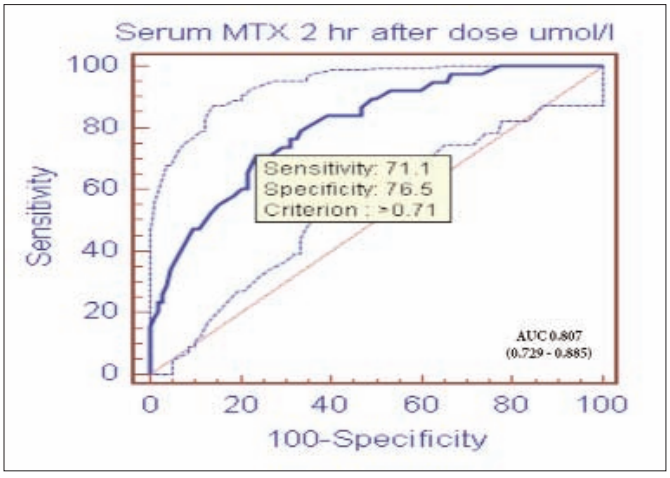


**Figure 2:** Receiver operating characteristic (ROC) curve of serum MTX levels after 2 hours of dose in RA patients who developed MTX toxicity and controls.

4 patients (2.9%). Renal impairment was found in 3 patients (2.1%). Anaemia was diagnosed in 8 patients (5.7%), leucopenia in 2 (1.4%), thrombocytopenia in 3 (2.1%) and pancytopenia in 2 (1.4%) cases. Gastrointestinal adverse effects were seen in 5 (3.6%) and 3 showed mucocutaneous problems (2.1%).

Receiver operating characteristic (ROC) curve were drawn between cases that developed toxicity and controls that did not develop toxicity for peak MTX levels (Figure 2). It revealed the area under curve (AUC) as 0.807 (0.729 - 0.885; $p < 0.0001$). ROC curve also established 0.71 μmol/l as the cutoff value for minimum toxic concentration of MTX with sensitivity of 71% and specificity of 76%.

## DISCUSSION

MTX is the treatment of choice in rheumatoid arthritis patients. Comparison of different parameters of RA patients who developed toxicity due to MTX and those who did not develop toxicity showed that there was no significance found for age and duration of drug use. In this study 27% of the patients showed MTX intoxication. The low dose MTX in RA patients due to toxicity presented in various studies are 10-37%.[1,8] The percentage of decreasing the dose due to toxicity is 9%.[2]

The most common adverse effect in this study was on liver with increased ALT more than two times the upper limit of reference. While the prevalence of raised liver enzymes (above two times the ULR) is approximately 13% of patients using MTX; 3.7% of patients stopped MTX permanently owing to liver toxicity, a meta-analysis showed incidence of fibrosis in 2.7% after 4 years of MTX administration.[1,10,11] Increased serum creatinine with MTX therapy was found in 2.1% patients. While the side effects leading to renal abnormalities are reported in 1-7%.[11,12] The rate of haematological side effects seemed to be less common in other studies with an incidence of 0.96-1.4%.[1,11,17] Almost 51% included patients were females and maximum were in reproductive age group, might be having tendency of anaemia but as the already anaemic patients were excluded before the start of MTX therapy, so anaemia presented in this study is likely to be due to MTX toxicity.

A variety of factors can contribute to the bone marrow toxicity of MTX. Edelman *et al*. reported about age as a risk factor for the development of MTX toxicity through an unknown mechanism.[18] However, in this study no patient was above 70 years, so age did not correlate significantly with thrombocytopenia. According to MacKinnon,[19] impaired renal function seems to play a very important role in MTX-induced pancytopenia. Gastrointestinal adverse effects including nausea, vomiting and diarrhea were found in 3.6% of RA patients after MTX therapy while in literature these are documented in 30% patients.[2] Mucocutaneous problems were found to be much less in these patients while in other studies it was found in 8.9% of RA patients after MTX therapy.[2]

Receiver operating characteristic (ROC) curve were drawn between cases that developed toxicity and controls that did not develop toxicity for peak MTX levels. Minimum toxic concentration of MTX in these RA patients was 0.71 μmol/l while other researchers reported 0.8 – 1 μmol/l after 2 hours of oral administration of MTX.[7,14] The pulse dosage schedule of MTX (followed in this study) has been reported to induce lower and shorter lasting blood levels than repeated oral dosages.[11] Several studies have demonstrated interindividual variability of the oral kinetics of low dose MTX in RA.[20] However, this study is illustrating only moderate intraindividual variability of the oral MTX. Therefore, the oral administration with adjustment of individual dose appears justified. Our demonstration of plasma concentrations 2 hours after MTX intake in ROC curve suggests a simplified

estimation of drug monitoring. This can be useful in the clinical practice as it uses limited sampling, allows management and predicts toxic events.

The main limitation of this study was that patients were using other drugs as well, that might have effect on different organs along with MTX in RA patients. Further studies will be required to establish relationships between dose of MTX and plasma concentrations along with plasma concentrations and therapeutic effects.

## CONCLUSION

In conclusion, adverse effects of low dose MTX were found in 27% of RA patients. The effects mainly presenting in our clinical practice comprise hepato-toxicity and haematological problems. MTX toxicity can be detected by therapeutic drug monitoring of serum concentration at 0.71 µmol/l with a sensitivity of 71% and specificity of 76% in the patients on low dose MTX maintenance therapy.

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