ORIGINAL ARTICLE

Methotrexate in Rheumatoid Arthritis: Effect on Blood, Liver and Renal Laboratory Parameters

Fuad Shaikh[1], Rabia Arshad[2], Fahad Azam[3], Nasim Karim[4]

ABSTRACT:

Objective: To evaluate the effect of Methotrexate on blood, liver and renal laboratory parameters in patients having rheumatoid arthritis.

Materials and Methods : A 24-week, single-blind, interventional study was carried out on 155 consecutive patients, aged 29-70 years, diagnosed with rheumatoid arthritis. They received tablet Methotrexate, 10 mg (2.5 mg, 4 tablets) weekly, orally. Laboratory tests like hemoglobin level, total white cell and platelet counts, erythrocyte sedimentation rate, serum glutamic pyruvic transaminase and serum creatinine levels were recorded at the initial visit as well as at 6, 14 and 24 weeks follow-up visits.

Results: At the end of 24 weeks hemoglobin level increased to 12.43 ± 0.92 grams per deciliter (g/dl) from a baseline of 10.76 ± 1.12 g/dl, white cell count fell to 7,142.46 ± 1332.23 per cubic mm (cmm) from 8,572 ± 1445/cmm, the ESR fell to 40.14 ±15.78 mm of Hg in 1st hour from 81.03 ±17.98 mm of Hg, the platelet count fell to 2, 33,738.10 ± 59,769/ cmm from 2, 90,278 ± 68,813/cmm, the SGPT levels increased to 55.29 ± 21.97 international units per litre (IU/l) from 31.67 ± 7.37 IU/l and the serum creatinine increased to 1.11 ± 0.14 mg/dl from 0.95 ± 0.16 mg/dl, all values being highly significant statistically ($p<0.001$).

Conclusion: Methotrexate exerted significant effects on the blood, liver and renal laboratory parameters. These parameters may be utilized for monitoring the response and safety of methotrexate use in RA.

Keywords: Rheumatoid arthritis, Methotrexate, Laboratory parameters, Monitoring, Response, Drug safety

INTRODUCTION:

Rheumatoid arthritis is a progressive, autoimmune disorder of long duration which involves the entire body and is manifested by symmetric, small joint synovitis. It can be a painful condition in which joint destruction, with subsequently deformed joints and loss of function, leads to disability and worsening in a patient's health status. [1] The natural course of the disease is one of persistent symptoms with varying intensity and progressive joint damage resulting in deformities and disability.

The average annual incidence of RA is around 0.03% with a 1% worldwide prevalence rate.[2] Almost one-sixth of the world population lives in India and Pakistan with prevalence rates of 0.5% and 0.2-1% respectively.[3] A prevalence rate of 0.9 and 1.98 per thousand cases was seen in the poor and affluent districts of Karachi respectively.[4] The exact etiology is unknown. Genetic risk factors have been identified like the presence of HLA-DR4/DR1 (human leukocyte antigen) marker cluster in 90% of RA patients.[5] Similarly, research has shown a strong link between the presence of (EBV) and (HHV-6) and the probability of developing RA, as patients with the disease have shown an abnormal immune response to infection with EBV.[6,7,8,9] Classification of RA depends on the ACR criteria given by the American College of Rheumatology (Table 1).[10] Person fulfilling four of these criteria can be said to be suffering from RA. It is now established that permanent joint damage starts early on in the disease in patients with active polyarticular disease.[11] The available treatment options are DMARDs (disease-modifying, anti-rheumatic drugs), anti-inflammatory agents and analgesics.[12,13] DMARDs have been in use in patients with RA for the last several years[14] Anti-inflammatory drugs and analgesics have no effect on joint damage or rate of disease progression though they may reduce the intensity of pain and joint stiffness. Methotrexate (MTX), a DMARD approved by FDA in 1988 for use in the treatment of RA, is the most widely used DMARD today[15,16,17] The 2006 EULAR recommendations consider it as the anchor, first-line drug in the treatment of RA regardless of the severity of disease.[18]

Methotrexate has a three-fold action in RA. Its anti-inflammatory effect may involve inhibition of production of those cells which cause synovitis, inhibition of products of toxic agents seen in chronic inflammation, reduction of intracellular glutathione levels resulting in reduction of macrophage recruitment and function, release of adenosine or probably a combination of these mechanisms. It also blocks dihydrofolate reductase (DHFR), which converts dihydrofolate to tetrahydro-

✉ **Dr. Fuad Shaikh**
Assistant Professor
Department of Pharmacology & Therapeutics
Dow Medical College
Dow University of Health Sciences
Karachi
E-mail: fuad.shaikh@hotmail.com

✉ **Dr. Rabia Arshad**
Assistant Professor & Head
Department of Pharmacology
Altamash Institute of Dental Medicine
Karachi

✉ **Dr. Fahad Azam**
Assistant Professor
Department of Pharmacology & Therapeutics
Shifa College of Medicine
Islamabad

✉ **Dr. Nasim Karim**
Professor & Head
Department of Pharmacology
Bahria University Medical & Dental College
Karachi

Received: 03-11-2015
Revised: 08-12-2015
Accepted: 09-12-2015

folate, thereby preventing the production of chemicals and proteins needed for the production of cells involved in the inflammatory process. Its anti-metabolic effect is due to the inhibition of growth of rapidly proliferating cells by acting on the S-phase of the cell cycle.[19,20,21,22] Thus methotrexate acts by inhibiting amino-imidazole-carboxamide ribonucleotide (AICAR) transformylase and thymidylate synthetase. AICAR produces accumulation of adenosine monophosphate (AMP) which in turn is converted to adenosine and inhibits inflammation.[23] It has demonstrated effects on blood parameters, renal and liver function.[24]

These very effects of Methotrexate could be used to monitor response to therapy in controlling the disease process and to determine its safety of use in RA patients. With this background the present study was designed to evaluate the effect of Methotrexate on blood, liver and renal laboratory parameters in patients having rheumatoid arthritis.

MATERIALS & METHODS:

This was a 24-week, prospective, single-blind, interventional study conducted between October, 2009 to March, 2011, on patients of either sex suffering from rheumatoid arthritis who visited the out-patient department of a private teaching hospital and a private consultant's clinic in Korangi, Karachi. Approval was obtained from and granted by the Dow University of Health Sciences (DUHS) Institutional Review Board (IRB). 155 consecutive patients, fulfilling the ACR criteria, were chosen and put on 10 mg of Methotrexate (4 tablets of 2.5 mg) orally, weekly. They were given drugs and directed to return for follow up with laboratory tests (hemoglobin level, total white cell and platelet counts, erythrocyte sedimentation rate, serum glutamic pyruvic transaminase and serum creatinine levels,) at which time they were given drugs for further use. Follow ups were conducted at 6, 14 & 24 weeks. Statistical analysis was done by SPSS version 18.0 with paired t-test. The results are shown as mean along with standard deviation. The cut off P value was taken to be less than 0.05.

RESULTS:

Of the 155 recruited patients 29 were lost to follow up. Of the remaining 126 patients 89 (70.6 %) were female and 77 (61.1%) were positive for Rheumatoid factor. They had a mean age of 45.57 ± 10.32 years, ranging between 29 to 70 years.

The mean baseline hemoglobin was 10.76 ± 1.12 grams per deciliter (g/dl), mean white cell count was 8,572 ± 1445 per cubic mm (/cmm), ESR was 81.03 ±17.98 mm in 1st hour, mean platelet count was 2, 90,278 ± 68,813/cmm, mean SGPT value was 31.67 ± 7.37 international units per litre (IU/l) and mean serum creatinine was 0.95 ± 0.16 mg/dl (Table 2). At the end of the study at 24 weeks the hemoglobin increased to 12.43 ± 0.92 g/dl, white cell count fell to 7,142.46 ± 1332.23 /cmm, the ESR fell to 40.14 ± 15.78 mm of Hg, the platelet count fell to 2, 33,738.10 ± 59,769 /cmm, the SGPT levels increased to 55.29 ± 21.97 IU/l and the serum creatinine increased to 1.11 ± 0.14 mg/dl, all values being statistically significant (P<0.001, Table 3). A total of 19 patients (15.1%, 14 female & 5 male) showed elevations of SGPT which were greater than twice the upper limit of normal (Table 4).

Table: 1
The American College of Rheumatology classification criteria

S.#	Parameter	Features
1	Morning stiffness	>1 hour most mornings
2	Arthritis and soft-tissue swelling	of >3 of 14 joints/
3	Arthritis of hand joints	Inflammation of joints of the hands
4	Symmetric arthritis	Arthritis of the same joints on both sides of the body.
5	Subcutaneous nodules	Usually found over bony prominences that sustain repeated mechanical stress e.g olecranon, calcaneum
6	Rheumatoid factor	Present
7	Radiological changes	Suggestive of joint erosion

Criteria 1-4 should have been present for at least 6 weeks.
At least 4 criteria have to be met for classification as Rheumatoid arthritis

Table: 2
Laboratory parameters: baseline and follow up visits
N = 126

Parameter	Baseline Mean ± SD	6 weeks Mean ± SD	14 weeks Mean ± SD	24 weeks Mean ± SD
Hemoglobin (g/dl)	10.76 ± 1.12	11.729 ± 1.1389	11.913 ± 1.6673	12.43 ± 0.92
Total leucocyte count (per cubic mm)	8572.1 ± 1445.1	8,018.25 ± 1,334.310	7,430.95 ± 1,244.281	7,142.46 ± 1,332.23
Erythrocyte Sedimentation Rate (mm of Hg in 1st hour)	81.03 ± 17.98	65.09 ± 17.441	52.02 ± 15.838	40.14 ± 15.78
Platelet count (per cubic mm)	2,90,277.8 ± 68,813.7	260,452.38 ± 63,342.069	256,666.67 ± 131,073.048	2,33,738.10 ± 59,769.58
Serum glutamic pyruvic transaminase (SGPT, IU/l)	31.67 ± 7.37	33.01 ± 10.244	42.54 ± 14.110	55.29 ± 21.97
Serum creatinine (mg/dl)	0.95 ± 0.16	.886 ± 0 .1511	.986 ± 0.1164	1.11 ± 0.14

SD : Standard deviation, g/dl: grams per deciliter, mm of Hg: millimeters of mercury,
IU/l: international units per litre, mg/dl: milligrams per deciliter

Table: 3
Methotrexate
Baseline v/s 24 weeks
N = 126

Parameter	Mean ± SD	P value
Hemoglobin, (g/dl)	10.76 ± 1.12	<0.001 ***
Hemoglobin, (g/dl), 24 weeks	12.43 ± 0.92	
White cell count (per cubic mm)	8,572.06 ± 1445.08	<0.001 ***
White cell count (per cubic mm), 24 weeks	7,142.46 ±1332.23	
Erythrocyte sedimentation rate, (mm of Hg in 1st hour)	81.03 ± 17.98	<0.001 ***
Erythrocyte sedimentation rate, (mm of Hg in 1st hour), 24 weeks	40.14 ± 15.78	
Platelet count (per cubic mm)	2,90,277.78 ± 68,813.68	<0.001 ***
Platelet count (per cubic mm), 24 weeks	2,33,738.10 ± 59,769.58	<0.001 ***
Serum glutamic pyruvic transaminase (SGPT, IU/l)	31.67 ± 7.37	
Serum glutamic pyruvic transaminase (SGPT, IU/l), 24 weeks	55.29 ± 21.97	
Serum creatinine (mg/dl)	0.95 ± 0.17	<0.001 ***
Serum creatinine, (mg/dl), 24 weeks	1.11 ± 0.14	

*** = very highly significant, Paired T-test utilized, SD : Standard deviation, g/dl: grams per deciliter
mm of Hg: millimeters of mercury, IU/l: international units per litre, mg/dl: milligrams per deciliter

Table: 4
Methotrexate
Serum glutamic pyruvic transaminase (SGPT)
N=126

	6 weeks		14 weeks		24 weeks	
Sex	No of Patients	%	No of Patients	%	No of Patients	%
Male	0	00	0	00	5	13.5
Female	0	00	3	3.4	14	15.7
Total	0	00	3	2.4	19	15.1

No of Patients = Number of patients with > 2 x upper limit of normal (> 68 IU/L in females & > 90 IU/L in males)

DISCUSSION:

DMARDs, having the ability to slow down joint destruction, are regarded as the drugs of first choice in treating RA. Since permanent joint damage starts early in patients with active, polyarticular RA initiating therapy with a DMARD shows promising results. The current treatment options can adequately control the acute symptoms and hold the promise of a good prognosis in the long run.[25]

Folic acid deficiency, a known risk factor for Methotrexate-induced cytopenias, affects the rapid multiplication of the cells in the bone marrow and persons treated with Methotrexate show a fall in their cellular folic acid content.[26] The depressant effect of Methotrexate, especially on hemoglobin levels, is transient and the latter is seen to rise gradually about one month after starting the drug. Emery et al showed an improvement in the hemoglobin level accompanied with a fall in the leucocyte and platelet counts.[27] In our study the improvement in the hemoglobin level was statistically very highly significant when comparing end values with its baseline value; 10.76 g/dl to 12.43 g/dl ($p< 0.001$) Similarly the mean leucocyte count fell from a mean baseline value of 8,572 /cmm to 7,142 /cmm, ($p<0.001$) when comparing baseline and 24-week values. Changes in the platelet count too were statistically very highly significant ($p<0.001$). Ishaq has documented a fall of ESR from 52.5 mm of Hg to 24.3 mm of Hg ($p = 0.0001$).[28] This is coinciding with our results as ESR values in our study fell from a mean baseline level of 81.03 mm in 1st hour to 40.14 mm in 1st hour ($p<0.001$). These features indicate that our patients responded favorably to therapy, had tolerated the drugs well and the blood indices had not deteriorated to the extent that any dose alteration was needed. This may have been due to the fact that they belonged to a younger age group (mean ~ 46 years) in comparison to the studies mentioned above.

Londono studying the efficacy of Methotrexate found elevations of SGPT levels in 26% of their patients while Attar in a study seeing the adverse effects of Methotrexate found SGPT elevations in 14% of patients. [29,30] In another study Alves showed abnormal levels of liver enzymesÊin 11.5% of patients using Methotrexate.[31] In our patients changes in SGPT levels were seen in 15% of patients, a figure corresponding to that of the above authors, with the levels rising from a baseline value of 31.67 IU/l to 55.29 IU/l ($p <0.001$). Methotrexate-associated hepatic toxicity may be due to interference with homocysteine metabolism leading to increased homocysteine levels in the blood. Administration of folic acid has been seen to normalize the MTX-induced increase in plasma homocysteine.[23] Lack of folate supplementation may have contributed to the incidence of liver toxicity seen in our patients. [32] Furthermore, the difference seen in the number of patients affected is most probably due to the fact that our patients were younger and our values were obtained at the end of 24 weeks as opposed to the other studies which were of a longer duration that is 52 weeks.

Tousson, using Methotrexate, demonstrated an increase in the serum creatinine levels to 1.12 ± 0.159mg/dl from a baseline of 0.97 ± 0.089 mg/dl ($p< 0.01$) whereas the serum creatinine level in our study rose from a baseline level of 0.95 mg/dl to 1.11 mg/dl ($p< 0.001$).[33] The reason for this increment could not be ascertained in our study participants.

CONCLUSION:

Methotrexate, the drug of first choice in all types of RA patients, has exerted significant effects on various parameters of blood, liver and kidney. These parameters could be utilized for monitoring the response and safety of use of Methotrexate in patients having rheumatoid arthritis.

REFERENCES:

1. Allaire SH, Prashker MJ, Meenan RF. The costs of rheumatoid arthritis. Pharmaco Economics. 1994; 6:513–22
2. Del Puente A, Knowler WC, Pettit DJ, Bennett PH. High incidence and prevalence of rheumatoid arthritis in Pima Indians. Am J Epidemiol. 1989; 129:1170-8
3. Akhter E, Bilal S, Kiani A, Haque U. Prevalence of arthritis in India and Pakistan: a review. Rheumatol Int. 2011; 31(7):849-55
4. Hameed K, Gibson G, Kadir M. The prevalence of rheumatoid arthritis in affluent and poor urban communities of Pakistan. Br J Rheumatol. 1995; 34:252-6
5. Pisetsky DS. Laboratory testing in rheumatic diseases. Ch 278 in Cecil's Medicine, eds. Goldman L, Aesiello D. Saunders, Elsevier. 2007; 23rd ed: 1967
6. Álvarez-Lafuente R, Fernández-Gutiérrez B, Miguel S de, Jover JA, Rollin R, Loza E et al. Annals of the Rheumatic Diseases. 2005; 64:1357-9
7. Ferrell PB, Aitcheson CT, Pearson GR, Tan E.M. Sero-epidemiological study of relationships between Epstein-Barr virus and rheumatoid arthritis. J Clin Invest. 1981; 67(3): 681-7
8. Catalano MA, Carson DA, Slovin SF, Richman DD, Vaughan JH. . Proc. Natl. Acad. Sci. USA, Immunology. 1979; 76 (11):5825-8
9. Balandraud N, Roudier J. Epstein–Barr virus and rheumatoid arthritis. Autoimmunity Reviews. July 2004; 3 (5):362-7
10. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. Arthritis Rheum. 1988; 31 (3): 315-24
11. Plant MJ, Saklatvala J, Borg AA, Jones PW, Dawes PT. Measurement and prediction of radiological progression in early rheumatoid arthritis. J Rheumatol. 1994; 10:1808-13
12. O'Dell J. Therapeutic strategies for rheumatoid arthritis. N Engl J Med. 2004; 350 (25): 2591-602
13. Hasler P. Biological therapies directed against cells in autoimmune disease. Springer Semin Immunopathol. 2006; 27 (4): 443-56
14. Ward MM , Fries JF. Trends in anti-rheumatic medication use among patients with rheumatoid arthritis,1981-1996. J Rheumatol. 1998; 25:408-16
15. Eustice C. The facts of Methotrexate. Updated May, 17, 2007, about.com guide
16. Kaltsonoudis E, Papagoras C, Drosos AA. Current and future role of methotrexate in the therapeutic armamentarium for rheumatoid arthritis; Int J Clin Rheumatol. 2012;7(2):179-89
17. Lopez-Olivo MA, Siddhanamatha HR, Shea B, Tugwell P, Wells GA, Suarez-Almazor ME. Methotrexate for treating rheumatoid arthritis. Cochrane Database Syst Rev. Jun 10, 2014
18. Combe B, Landewe R, Lukas C. EULAR recommendations for the management of early arthritis: report of a task force of the European Standing Committee for International Clinical Studies Including Therapeutics (ESCISIT). Ann Rheum Dis. 2007; 66:34-45
19. Quemeneur L, Gerland LM, Flacher M, French M, Revillard JP, Genestier L. Differential control of cell cycle, proliferation and survival of primary T lymphocytes by purine and pyrimidine nucleotides. J Immunol. 2003; 170: 4986-95
20. Nesher G, Osborn TG, Moore TL. In vitro effects of Methotrexate on polyamine levels in lymphocytes from rheumatoid arthritis patients. Clin Exp Rheumatol. 1996; 14: 395-9
21. Phillips DC, Woollard KJ, Griffiths HR. The anti-inflammatory actions of Methotrexate are critically dependent upon the production of reactive oxygen species. Br J Pharmacol. 2003; 138: 501-11
22. Sung JY, Hong JH, Kang HS, Choi I, Lim SD, Lee JK et al. Methotrexate suppresses the interleukin-6 induced generation of reactive oxygen species in the synoviocytes of rheumatoid arthritis. Immunopharmacology. 2000; 47: 35-44
23. Whittle SL, Hughes RA. Folate supplementation and Methotrexate treatment in rheumatoid arthritis: a review; Rheumatology, 2004, 43 (3): 267-71
24. Hazra SC, Choudhury AM, Khondker L, Khan MSI, Ahmed N. Hematological and Biochemical Parameter Changes Related to Methotrexate Therapy; Bangladesh Medical Journal 2011; 40(3):40-3
25. Nemazee D, Hogquist KA. Antigen receptor selection by editing or down regulation of V (D) J recombination. Curr. Opin. Immunol. 2003; 15: 182-9
26. Ortiz Z, Shea B, Suarez-Almazor ME, Moher D, Wells GA, Tugwell P. The efficacy of folic acid and folinic acid in reducing methotrexate gastrointestinal toxicity in rheumatoid arthritis. A meta-analysis of randomized controlled trials. J Rheumatol. 1998; 1:36-43
27. Emery P, Breedveld FC, Lemmel EM, JP, B et al. A comparison of the efficacy & safety of Leflunomide and Methotrexate for the treatment of rheumatoid arthritis. . 2000; 39(6)655-65
28. Ishaq M, Muhammad JS, Hameed K, Mirza AI. Leflunomide or methotrexate? Comparison of clinical efficacy and safety in low socio-economic rheumatoid arthritis patients, Mod Rheumatol 2011; 21(4): 375-80
29. Londono J, Santos AM, Santos PI, Cubidez MF, Guzman C, Valle-Oñate R. Therapeutic efficacy and safety of methotrexate + leflunomide in Colombian patients with active rheumatoid arthritis refractory to conventional treatment, Rev. Bras. Reumatol.2012; 52 (6):837-45
30. Attar SM, Adverse effects of low dose methotrexate in rheumatoid arthritis patients. A hospital-based study. 2010; 31(8):909-15
31. Alves JANR, Fialho SCS, Morato EF, de Castro GRW, Ribeiro GG, Zimmermann AF et al. Liver toxicity is rare in rheumatoid arthritis patients using combination therapy with leflunomide and methotrexate. São Paulo. Rev. Bras. Reumatol. 2011; 51(2): 141-4
32. Morgan SL, Baggott JE, Vaughn WH. Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. Ann Intern Med. 1994; 121:833-41
33. Tousson E, Zaki ZT, Abu-Shaeir W A, Hassan H. Methotrexate-induced Hepatic and Renal Toxicity: Role of L-carnitine in Treatment. Biomedicine and Biotechnology, 2014, 2(4), 85-92