Methotrexate in Rheumatoid Arthritis: Effect on blood indexes, liver and renal parameters in Duhok Governorate, Iraq

Running Title: Methotrexate effect on rheumatoid arthritis patient

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Abstract

Background and objectives: Rheumatoid arthritis (RA) is a long-term autoimmune disorder that primarily affects joints. The disease may also affect other parts of the body, including the skin, eyes, lungs, heart, nerves, and blood. This study Aimed To evaluate the effect of methotrexate on blood, liver, and renal parameters in patients with Rheumatoid arthritis.

Methods: A six-month cross-sectional study was carried out on 60 consecutive patients of aged 19-70 years with diagnosed Rheumatoid arthritis on methotrexate treatment (10 mg) orally per week. A questionnaire form was taken from participants, and laboratory tests were done on renal and liver function and serological tests (complete blood count, erythrocyte sedimentation rate, glutamic pyruvic transaminase, a glutamic-oxaloacetic transaminase, creatinine, C-reactive protein, and rheumatoid factor as follow-up of drug taking).

Results: At the end of sample collection, age 19-70 years, female: male ratio 1.5:1, while the only significant differences in platelet level were between day one and fourteen of treatment with a P value < 0.05, Glutamic Pyruvic Transaminase (GPT) level was between day one and thirty with a P value < 0.05, and rheumatoid factor level was between day one, fourteen, and day one, thirty with P values of (0.01) and (0.04) respectively which were significant.

Conclusion: The recommended medication for all kinds of rheumatoid arthritis patients is methotrexate, which has had a notable impact on blood, liver, and kidney parameters. These characteristics could be used to track how well this medication works, how safe it is, and to follow up with patients.

Keywords: C - reactive protein; Glutamic-Oxaloacetic Transaminase; Glutamic-Pyruvic Transaminase; Hemoglobins; Methotrexate; Rheumatoid

Introduction

Rheumatoid arthritis (RA) is a chronic, symmetrical, inflammatory autoimmune disease that initially affects small joints, progressing to larger joints, and eventually the skin, eyes, heart, kidneys, and lungs. Often, the bone and cartilage of joints are destroyed, and tendons and ligaments weaken (1).

The onset of this disease is usually from the age of 35 to 60 years, with remission and exacerbation. It can also afflict young children even before the age of 16 years, referred to as juvenile RA (JRA), which is similar to RA except that rheumatoid factor is not found (2-4). In the West, the prevalence of RA is believed to be 1–2% (5, 6), and 1% worldwide (7).

One of the most common screening procedures in rheumatology therapy is liver enzyme testing, as recommendations, such as the widely used American College of Rheumatology guidelines, urge monitoring liver enzymes at intervals of at least 8–12 weeks in all RA patients treated with MTX (8). Also, because MTX precipitates in tubules and is mostly eliminated by the kidneys, it might cause acute kidney injury (AKI) when used in large dosages for oncologic purposes (9), and conclude that using MTX, close observation of renal function should be necessary.

Although low dose MTX therapy is regarded as an anchor therapy in RA, full details of its mechanism of action and off target effects are still incompletely understood (10).

Because, MTX is well known to affect folic acid metabolism, MTX treatment can result in alterations of MCV, which may impact on RDW, as MCV levels feed into RDW calculation (11). We thus questioned, whether RDW levels and subsequently its diagnostic utility and potential in RA subjects, as reported before, are influenced by ongoing MTX therapy (10, 12-14).

Method

A cross-sectional study conducted at Duhok Rheumatology Center in a period of 5 months. From October 2023 to March 2024 data collection and, March to April 2024 data analysis were did. Build statistics and a basis to standardize future practice and Hospital protocol.

A 60 consecutive patients of aged 19-70 years with diagnosed Rheumatoid arthritis on methotrexate treatment (10 mg) orally per week. A questionnaire form was taken from participants after taking ethical approval paper of research ethic committee of Duhok directorate of health (13122023-11-17), and laboratory tests were done on renal and liver function and serological tests (complete blood count, erythrocyte sedimentation rate, glutamic pyruvic transaminase, a glutamic-oxaloacetic transaminase, creatinine, C-reactive protein, and rheumatoid factor as follow-up of drug taking) after withdrawal of 6 ml of venous blood from each participants (3 ml in EDTA tube) and (3 ml for biochemical tests).Instruments for data collection were (Roche Cobas c 311 biochemical auto analyzer machine with its kits for serum CRP,GOT,GPT, Creatinine level measurement), and Boule Medonic hematological auto analyzer machine with its kits for Hb, WBC,PLT level measurement.

Patients treated by methotrexate treatment included, while patients treated with anti-rheumatoid drug therapy other than methotrexate treatment, blood disease, renal and liver disease patients will be excluded.

Results

Descriptive study of age and hematological changes during 1st, 14th, 30th days of treatment:

Age and hematological changes results were, age (44.13 ±10.31 SD) years, while mean hemoglobin, white blood cells, platelets count and ESR in the first day of treatment were (12.30 ±1.67 SD) gm/dl, (8.85 ± 2.68 SD) x 10^{9}/L ,(287.67 ±68.42 SD) x 1000/L, (31.37±20.19 SD) mmhg/hr respectively, (12.20±1.59 SD),(8.79 ±2.65 SD),(270.38±73.96 SD),(28.12 ±14.97SD) in day fourteen of treatment ,

and (12.20±1.92 SD),(9.06 ±2.98 SD),(278.38±35.96 SD),(26.86±17.03 SD) in day thirty after treatment as shown in Table 1.

Descriptive study of biochemical tests changes during 1st, 14th, 30th days of treatment:

Also biochemical changes results were mean serum creatinine, GOT, and GPT level in the first day of treatment were (0.74 ±0.34 SD), (24.38 ± 16.76 SD) ,(25.58 ±17.26 SD) gm/dl, respectively, (0.71 ±0.32 SD), (20.66 ± 7.62 SD) ,(21.18 ±7.77 SD) gm/dl in day fourteen of treatment , and (0.72 ±0.34 SD), (20.32 ± 8.11 SD) ,(20.53 ±8.07 SD) gm/dl in day thirty after treatment shown in Table 2.

Descriptive study of serological tests changes during 1st, 14th, 30th days of treatment:

While serological tests changes results were mean CRP, and level in the first day of treatment were (21.29 ±53.84 SD) gm/dl, (76.20 ± 77.15 SD) U/ml respectively, (12.39 ±18.97 SD) gm/dl, (44.46 ± 46.52 SD) U/ml in day fourteen of treatment, and (11.82 ±14.04 SD) gm/dl, (43.26 ± 59.41 SD) U/ml in day thirty after treatment shown in Table 3.

Comparison in hematological tests changes during 1st, 14th, 30th days of treatment

There were no significant differences between hemoglobin, white blood cells, ESR in all course treatment days (1^{st}, 14th and 30^{th}) with p- value > 0.05, while the only significance differences in platelets level were between day 1^{st} and 14^{th} with P value < 0.05 shown in Table 4.

Comparison in biochemical tests changes during 1st, 14th, 30th days of treatment

There were no significant differences between creatinine, GOT in all course treatment days (1^{st}, 14th and 30^{th}) with p- value > 0.05, while the only significance differences in GPT level was between day 1^{st} and 30^{th} with P value < 0.05 shown in Table 5.

Comparison in serological tests changes during 1st, 14th, 30th days of treatment:

There were no significant differences between CRP level in all course treatment days (1^{st}, 14th and 30^{th}) with p- value > 0.05, while there were significance differences in RF level was between day 1^{st} ,14^{th} and day 1^{st} ,30^{th} with P value (0.01) and (0.04) respectively shown in Table 6.

Discussion

In accordance with EULAR guidelines, csDMARDs-most notably, MTX in combination with low-dose glucocorticoids-should be used to start RA treatment. Despite being considered an essential therapy for RA, low dose MTX therapy's exact mechanism of action and side effects are yet unknown (10).

Changes in Hemoglobin and MCV brought on by MTX treatment may have an effect on RDW because RDW is calculated using MCV levels (11). In addition to biochemical parameter analysis that aids in the prediction of toxicities, clinicians give special emphasis to the management of adverse effects through clinical monitoring. But no study has found that their disturbance is common in the public. This is what motivated the current study's realization, which will allow for a more suitable strategy and better patient care.

As no appreciable elevation in transaminases' levels is observed at an MTX cumulative dose of 1.5 g, this appears to be the case with the current work. This outcome may be explained by the fact that RA-affected patients who were recruited appear to tolerate MTX better than psoriatic patients (15, 16). Even so, there is minimal correlation between elevated transaminases concentration and the likelihood of fibrosis and histological alterations (17, 18). A meta-analysis of randomized controlled trials examined the risk of liver injury in methotrexate users. The study published in Seminars in Arthritis and Rheumatism, 45(2), 156-162, revealed that the frequency of raised liver enzyme concentrations during MTX treatments in patients with psoriasis was more than that of RA patients (14.5% vs. 7.5%) (19).

Although current research on other populations indicates a modest association between cumulative dose and fibrosis, liver enzymes are particularly controlled among the biochemical markers that are examined during a lengthy MTX treatment period (20).

Additionally, a 50% dose reduction in the event that CrCl is less than 45 mL/min may result from a decrease in CrCl, which is another important factor in MTX-based treatment (21).
Effects of moderate renal insufficiency on pharmacokinetics of methotrexate in rheumatoid arthritis patients (22). Kidney illness in patients with RA; results of study indicate that the cumulative dose of MTX appears to have a significant impact on renal function ($p = 0.037$ for creatinine and $p = 0.036$ for CrCl), particularly in female patients. Significantly higher prevalence of CrCl disturbances is also observed when compared to creatinine (13.18% vs. 1.09%, respectively, $p < 0.001$), indicating that CrCl should be prioritized in renal monitoring (23)**.** About the renal function test, we have observed the creatinine level in the (1 day, 14-day, 30 day) and the result against of the results done by (24).
In the evaluation of liver function tests, the level of GOT and GPT in the (1 day, 14 day, 30 day) of taking the treatment is go or related to the result done (25), and this goes with our result as GOT level changed significantly with treatment follow-up, this means that methotrexate sometimes may not be toxic to the liver and renal if given by low dose with a good follow of patient clinically and by investigations.
Regarding serological tests in current study shows no significant difference between (1 day, 14-day, 30 day) among CRP level, this goes with research done by Ismaili, H *et al.* (2019) (26).

Conclusion

Methotrexate is the primary medication recommended for patients with rheumatoid arthritis (RA), demonstrating significant effects on hematological, hepatic, and renal parameters. While the administration of low-dose methotrexate on a weekly basis for autoimmune conditions may result in certain adverse effects, it is generally well tolerated and exhibits considerable efficacy. Proper monitoring is essential to mitigate potential negative outcomes. Given that many of these serious complications can be detected early and potentially avoided, it is imperative for primary care physicians and hematologists to be aware of these issues and the associated guidelines. Prolonged methotrexate therapy may result in elevated liver enzyme levels. Serological assessments, such as C-reactive protein and rheumatoid factor tests, are critical for monitoring disease progression and the side effects of methotrexate. Additionally, conducting bone marrow examinations in relation to methotrexate treatment is advisable, as bone marrow suppression may present valuable insights. Should laboratory test abnormalities arise during treatment, it is recommended to reduce the methotrexate dosage or consider switching to an alternative medication with fewer side effects until the patient's condition stabilizes.

Conflict of interest

Authors declare no conflict of interest.

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Ethical statement

A questionnaire form was taken from participants after taking approval of research ethic committee of Duhok directorate of health (13122023-11-17)

Conflicts of interest

The authors declare that they have no conflicts of interest.

Author contributions

Beri Tawfeq (Data collection, filling questionaire form by asking patients, investigations preparation and working, writing introduction), Bizav Rasheed (Data collectins, arranging data on SPSS, analysis of data, arranging article)

Highlights:

1- Methotrexate have many side effects on patients of Rheumatoid arthritis.
2- Only affect some biochemical tests like GOT, RF after patient's therapy with methotrexate.

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Tables

Table 1: Descriptive study of age and hematological changes during 1st, 14th, 30th days of treatment

Parameter		Age	HB1	HB2	HB3	WBC1	WBC2	WBC3	PLT1	PLT2	PLT3	ESR1	ESR2	ESR3
N	Valid	60	60	60	60	60	60	60	60	60	60	60	60	60
	Missing	0	0	0	0	0	0	0	0	0	0	0	0	0
Mean		44.13	12.30	12.20	12.20	8.85	8.79	9.06	287.67	270.38	278.35	31.37	28.12	26.86
Median		44.00	12.30	12.40	12.30	8.95	8.55	9.70	281.50	265.00	270.00	27.00	25.00	25.00
Std. Deviation		10.31	1.68	1.59	1.92	2.68	2.65	2.98	68.41	73.96	60.44	20.19	14.97	17.03
Minimum		19	9.3	9.2	8.5	1.3	3.5	3.5	135	118.0	148	4	3.0	1.0
Maximum		70	16.0	15.5	16.0	17.0	16.0	16.1	417	450.0	400	85	69.0	75.0

HB1: Hemoglobin 1st day, **HB2**: Hemoglobin 14th t day, **HB3**: Hemoglobin 30th day, **WBC1**: WBC 1st day, **WBC2**: WBC 14th t day, **WBC3**: WBC 30th day, **PLT1**: PLT 1st day, **PLT 2**: PLT 14th t day, **PLT 3**: PLT 30th day, **ESR1**: ESR 1st day, **ESR 2**: ESR 14th t day, **ESR 3**: ESR 30th day

Table 2: Descriptive study of biochemical tests changes during 1st, 14th, 30th days of treatment

Parameter		Creatinine1	Creatinine2	Creatinine3	GOT1	GOT2	GOT3	GPT1	GPT2	GPT3
N	Valid	60	60	60	60	60	60	60	60	60
	Missing	0	0	0	0	0	0	0	0	0
Mean		0.74	0.71	0.72	24.38	20.66	20.32	25.58	21.18	20.53
Median		0.70	0.65	0.68	20.00	20.00	18.00	22.00	20.00	18.00
Std. Deviation		0.34	0.32	0.34	16.76	7.62	8.11	17.26	7.77	8.07
Minimum		0.10	0.00	0.12	0.9	1	0.2	6.00	2.00	.20
Maximum		1.84	2.09	1.97	135.0	40	40.0	140.00	41.00	40.00

Creatinine 1: creatinine 1st day, **Creatinine 2**: creatinine 14th t day, **Creatinine 3**: creatinine 30th day, **GOT1**: GOT 1st day, **GOT2**: GOT 14th t day, **GOT3**: GOT 30th day, **GPT1**: GPT 1st day, **GPT 2**: GPT 14th t day, **GPT3**: GPT 30th day

Table 3: Descriptive study of serological tests changes during 1st, 14th, 30th days of treatment

Parameter		CRP1	CRP2	CRP3	RF1	RF2	RF3
N	Valid	60	60	60	60	60	60
	Missing	0	0	0	0	0	0
Mean		21.291	12.391	11.829	76.200	44.462	43.268
Median		10.820	9.400	9.400	64.000	32.000	17.500
Std. Deviation		53.848	18.974	14.044	77.151	46.529	59.413
Minimum		.0	.0	.0	.0	.0	.0
Maximum		409.0	130.0	90.0	256.0	232.6	256.0

CRP1: CRP 1st day, **CRP 2**: CRP 14th t day, **CRP 3**: CRP 30th day, **RF1**: RF 1st day, **RF2**: RF 14th t day, **RF3**: RF 30th day

Table 4: Comparison in hematological tests changes during 1st, 14th, 30th days of treatment

Paired Samples T-Test									
Parameter		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	HB1 - HB2	0.103	0.780	0.100	-0.098	0.305	1.025	59	0.310
Pair 2	HB1 - HB3	0.100	1.078	0.139	-0.178	0.378	0.721	59	0.474
Pair 3	HB2 - HB3	-0.003	0.997	0.128	-0.260	0.254	-0.023	59	0.981
Pair 4	WBC1 - WBC2	0.054	2.189	0.282	-0.511	0.620	0.193	59	0.848
Pair 5	WBC1 - WBC3	-0.211	2.766	0.357	-0.926	0.503	-0.593	59	0.556
Pair 6	WBC2 - WBC3	-0.266	2.445	0.315	-0.897	0.365	-0.843	59	0.403
Pair 7	PLT1 - PLT2	17.283	64.356	8.308	0.658	33.908	2.080	59	0.042
Pair 8	PLT1 - PLT3	9.317	40.449	5.222	-1.133	19.766	1.784	59	0.080
Pair 9	PLT2 - PLT3	-7.966	59.585	7.692	-23.359	7.426	-1.036	59	0.305
Pair 10	ESR1 - ESR2	3.245	14.726	1.901	-0.559	7.049	1.707	59	0.093
Pair 11	ESR1 - ESR3	4.503	18.831	2.431	-0.361	9.368	1.852	59	0.069
Pair 12	ESR2 - ESR3	1.258	9.967	1.286	-1.316	3.833	.978	59	0.332

Table 5: Comparison in biochemical tests changes during 1st, 14th, 30th days of treatment

Paired Samples T-Test									
Parameter		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Creatinine1- Creatinine 2	0.026	0.224	0.029	-0.031	0.084	0.913	59	0.365
Pair 2	Creatinine1- Creatinine 3	0.021	0.332	0.042	-0.064	0.10707	0.491	59	0.626
Pair 3	Creatinine2- Creatinine 3	-0.005	0.313	0.040	-0.086	0.075	-0.133	59	0.894
Pair 4	GOT1 - GOT2	3.719	16.679	2.153	-0.589	8.028	1.727	59	0.089
Pair 5	GOT1 - GOT3	4.057	16.293	2.103	-0.151	8.266	1.929	59	0.059
Pair 6	GOT2 - GOT3	0.338	5.482	0.707	-1.078	1.754	0.478	59	0.635
Pair 7	GPT1 - GPT2	4.400	17.108	2.208	-0.0194	8.819	1.992	59	0.051
Pair 8	GPT1 - GPT3	5.048	16.870	2.177	0.690	9.406	2.318	59	0.024
Pair 9	GPT2 - GPT3	0.648	5.527	0.713	-0.779	2.076	0.909	59	0.367

Table 6: Comparison in serological tests changes during 1st, 14th, 30th days of treatment

Paired Samples T-Test									
Parameter		Paired Differences					t	Df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	CRP1 - CRP2	8.900	49.537	6.395	-3.896	21.697	1.392	59	0.169
Pair 2	CRP1 - CRP3	9.462	50.557	6.527	-3.598	22.523	1.450	59	0.152
Pair 3	CRP2 - CRP3	0.562	11.184	1.444	-2.327	3.451	0.389	59	0.699
Pair 4	RF1 - RF2	31.737	67.822	8.755	14.217	49.258	3.625	59	0.001
Pair 5	RF1 - RF3	32.931	84.154	10.864	11.191	54.670	3.031	59	0.004
Pair 6	RF2 - RF3	1.193	33.173	4.282	-7.376	9.763	0.279	59	0.781

Abbreviations

CrCl	**Creatinine clearance**
CRP	**C-reactive protein**
csDMARDs	**conventional synthetic Disease-Modifying AntiRheumatic Drugs**
EDTA	**Ethylenediaminetetraacetic acid**
ESR	**Erythrocyte Sedimentation Rate**
EULAR	**European Alliance of Association for Rheumatology**
GOT	**Glutamic Oxaloacetic Transaminase**
GPT	**Glutamic Pyruvic Transaminase**
Hb	**Hemoglobin**
JRA	**Juvenile Rheumatoid Arthritis**
MCV	**Mean Corpuscular Volume**
Mg	**Milligram**
Ml	**Milliliter**
MTX	**Methotrexate**
PLT	**Platelet**
RA	**Rheumatoid arthritis**
RDW	**Red cell Distribution Width**
RF	**Rheumatoid Factor**
SD	**Standard deviation**
WBC	**White blood cell**