Hepatic and Hematological Adverse Effects of Low Dose of Methotrexate (≤ 15 mg/week) in Rheumatoid Arthritis Patients Treated with Biological Therapy with or without Steroids

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ABSTRACT

Background: Rheumatoid arthritis is the most common inflammatory arthritis, affecting 0.5-1% of the general population worldwide.

Objectives: To evaluate hepatic and hematological adverse effects of low dose methotrexate ≤ 15 mg/week in rheumatoid arthritis patients treated with biological therapy with or without steroids.

Methods: This cross-sectional descriptive study was done at Rheumatology Unit in Baghdad Teaching Hospital from 1st September 2017 to 1st August 2018. The inclusion criteria included rheumatoid arthritis patients more than 18 years old receiving low dose of methotrexate ≤ 15 mg/week for more than one year and on biological therapy with or without steroid. Eighty patients compliant with the inclusion criteria. Data collected by special questionnaire, clinical examination, and laboratory tests. Verbal consents were taken from patients after explaining the aim of the study.

Results: A total study sample was 80 patients. Females represent 90% of study group. Mean age was 50 ± 9.3 years, 46 patients were moderate score of clinical disease activity index, mean duration of using methotrexate was 5.8 ± 5.5 years, mean of methotrexate dose was 12 ± 3 mg, 38 patients were on steroid, 42 patients were without steroid. aspartate aminotransferase and alanine aminotransferase levels three fold the upper limit of normal were 3.7% and 2.5%, respectively. Severe anemia was present only in two patients (2.5%). There was no significant correlation between the duration of using the steroid, the dose or duration of using the methotrexate, body mass index, duration of smoking and any of hepatic or hematological elements.

Conclusions: There is no significant hepatic and hematological adverse effects of low dose methotrexate ≤15 mg/week in rheumatoid arthritis patients treated with biological therapy.

Keywords: Low dose methotrexate, Rheumatoid arthritis, Biological therapy.

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Rheumatoid arthritis (RA) is the most common inflammatory arthritis, affecting from 0.5 to 1% of the general population worldwide. RA has features of a systemic disease that can involve many organs[1].

Synovial cells can exhibit aggressive behavior and can invade and destroy articular cartilage, subchondral bone, tendons and ligaments[2].

Methotrexate (MTX) is most commonly given as first-line disease-modifying anti rheumatic drug (DMARD) due to its favorable cost-effectiveness profile[3]. Possible risk factors suggested for hepatic and hematological adverse effects are; increased age, female gender[4], smoking, disease duration, dose of MTX[5], associated medications mainly steroidal drugs, other DMARD, biological therapy[6] and genetic factors[7]. Methotrexate is a structural analogue of folic acid[8], it enters cells via a reduced folate carrier[9]. Folic acid enters cells via another group of transmembrane receptors called folate receptors[10]. Folate receptors may be upregulated in cells with increased metabolic activity[11], including synovial macrophages and serve as a second conduit for MTX influx[12].

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Genetic polymorphisms may affect MTX transporter proteins (influx and efflux) and can result in a variable MTX response and toxicity profile[13]. Furthermore, multidrug-resistance proteins have been identified that transport MTX, folic acid and leucovorin out of cells leading to MTX resistance[14].

The risk of significant liver toxicity appears to be low when MTX is given once weekly to patients who abstain from alcohol and are monitored carefully, and occurs in approximately one patient per 1000 after 5 years of use[15]. Elevations in aspartate aminotransferase (AST) and alanine aminotransferase (ALT) greater than one time the upper limit of normal (ULN) occurred in 22% of patients taking MTX, elevations greater than two times ULN occurred in 1 to 2% on monotherapy[16]. Morbid obesity, alpha one anti-trypsin deficiency and concomitant hepatotoxic drugs have all been implicated as possible risk factors for MTX toxicity[17]. Hematologic side effects by bone marrow toxicity in most cases are dose dependent and respond to folic acid administration, pancytopenia, leucopenia, anemia and thrombocytopenia can occur but are rare, pancytopenia was found to develop in 1 to 2% of RA patients on MTX therapy[18].

The term 'biologic' refers to a group of medications that includes monoclonal antibodies, fusion proteins and decoy receptors, which are used in the treatment of several inflammatory rheumatic diseases[19]. They are targeted towards specific cytokines, receptors and other cell-surface molecules regulating the immune response, the main adverse effect of the biologics used in inflammatory diseases is an increased risk of infections. Biologics are not carcinogenic but patients who develop cancer while on treatment may exhibit accelerated progression of the tumor due to suppression of the immune response[20]. In clinical trials, etanercept, infliximab, adalimumab, golimumab, and certolizumab pegol have generally been well tolerated[21,22].

The main objective of the study was to evaluate hepatic and hematological adverse effects of low dose methotrexate ≤ 15 mg/week in rheumatoid arthritis patients.

Methods

This cross-sectional descriptive study was conducted at Rheumatology Unit in Baghdad Teaching Hospital from 1st September 2017 to 1st August 2018. The total number of cases in this study was 120 patients who were diagnosed as rheumatoid arthritis, according to the criteria of the American College of Rheumatology whom receiving methotrexate and biological therapy with or without steroid. Prior to data collection, a verbal consent from each of the participants was obtained after explaining the purpose of the study and ensuring the privacy of the data. The criteria for inclusion were rheumatoid arthritis patients more than 18 years old receiving low dose of methotrexate ≤ 15 mg/week for more than one year on biological therapy with or without steroid. Exclusion criteria were: Juvenile RA, methotrexate dose more than 15 mg/week and duration of treatment less than one year, diabetic patients and patients receiving other DMARD with methotrexate. Forty patients were excluded from the study because they did not fit into inclusion criteria. A convenience sample of 80 patients was confirmed (72 females, 8 males).

The data were collected through a special questionnaire prepared for this purpose covered sociodemographic data include name, age, gender, weight, height and smoking status; clinical data include disease duration from onset of symptoms, past medical history and current treatment. Reviewing of the patients' files to determine the dose of methotrexate, duration of the treatment, type and dose of biological therapy. Physical examination for all cases to detect clinical disease activity index of rheumatoid arthritis (CDAI). CDAI = Swollen 28-joint count SJC (28) + Tender 28-joint count TJC (28) + Patient global disease activity (PGA) + Evaluator's global disease activity (EGA).

Laboratory tests and blood samples were taken from all patients for AST, ALT, hemoglobin level (Hb), mean corpuscular volume (MCV), red blood cell diameter width (RDW), white blood cell (WBC) and platelets (PLT).

The study protocol was reviewed; approval and official permission were obtained from the Ministry of Higher Education and Scientific Research, Baghdad University, College of Medicine to conduct the present study.

Statistical Package for the Social Sciences (SPSS) version 23 was used for data entry and analysis. Descriptive statistics and Pearson correlation test were used to confirm significance. P value ≤ 0.05 was considered significant.

Results

The study sample was 80 patients. Females represented 90% (72 patients), 10% of study group were males (8 patients), (Figure 1). Mean age of studied group was 50 years ± 9.3. Mean duration of RA disease was 11.2 years ±3.4. Regarding CDAI scores the findings were 23 patients (28.8%) have a low score, 46 patients (57.5%) have moderate score and 11 patients (13.7%) have severe score. Mean duration of using methotrexate was 5.8 years ± 5.5. Mean of methotrexate dose was 12 mg ± 3 mg. Patients on biological therapy Etanercept were 56 (70%), on Infliximab 13 patients (16.3%) and on Rituximab were 11 patients (13.7%). Patients on steroid therapy were 38 (47.5%), others patients without steroid were 42 (52.5%), (Table 1).

The findings demonstrated that the mean values of hepatic and hematological laboratory tests in studying group were: mean value of AST was 21.7 u/l ±10.9, for ALT was 26.0 u/l ±18.8, for Hb was 12.1 g/dl ±1.7 for MCV was 83.5 fl ±8.1, for RDW-CV was 14.5 % ±2.1, for WBC was 7.7×10^9/l ±1.3 and for platelets was 282.1 $x10^9$/l ± 24.8, (Table 2).

When the values of hepatic and hematological parameters analyzed in term of above the upper limit of normal, lower limit of normal and within normal limit in studying patients, the results indicated that AST level was exceeding the upper limit of normal greater than three times in three patients only (3.7%). ALT level was exceeding the upper limit of normal greater than three times were two patients (2.5%), 26 patients (32.5%) have a Hb level below lower limit of normal, the level of white blood cells was a high upper limit of normal in eight patients (10%), PLT level was above the upper limit of normal in 16 patients (20%), (Tables 3 and 4).

AST and ALT levels three fold the upper limit of normal were 3 patients (3.7%) and two patients (2.5%) respectively, mild anaemia was in 20 patients (25%), moderate anaemia was in four patients (5%), while severing anaemia was only in two patients (2.5%), one patient (1.2%) has leucopenia and eight patients (10%) have leucocytosis, patients with thrombocytopenia were two patients (2.5%), (Table 5). The results also showed there was no significant correlation between the duration of using the steroid and any of hepatic or hematological elements ($p \geq 0.05$), (Table 6). There was no significant correlation between the dose or duration of using the methotrexate and any of hepatic or hematological elements ($p \geq 0.05$), (Table 7). The results showed there was no significant correlation between the BMI, duration of smoking and studied parameters, (Table 8).

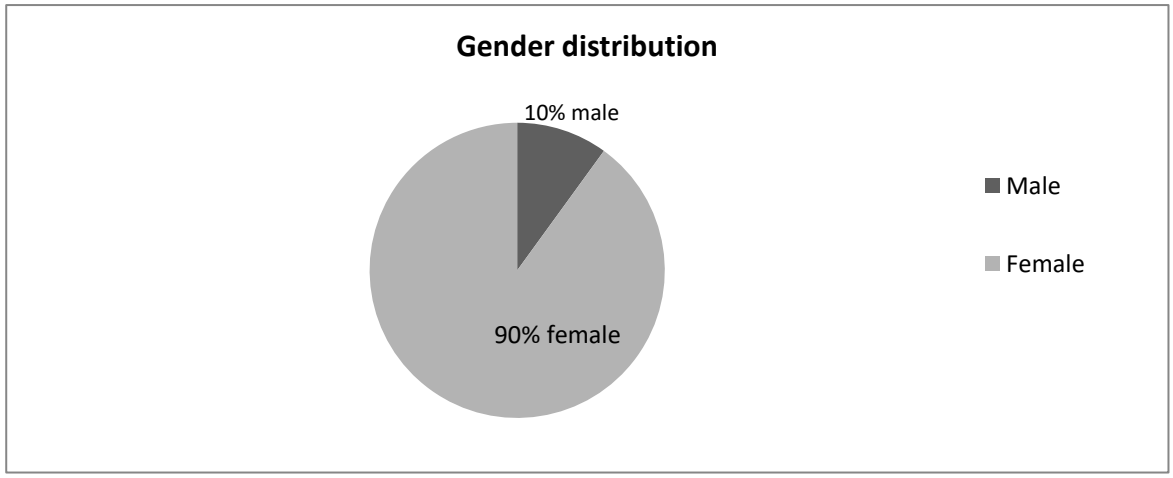

Figure 1: Gender distribution of studied group.

Table 1: Demographic and characteristic of RA patients on low dose MTX < 15 mg/week.

Variables	Descriptive statistics
Mean age (years) ± SD	50 ± 9.3
Female no. (%) Male no. (%)	72 (90) 8 (10)
Mean duration of RA disease (year) ± SD	11.2 ± 3.4
CDAI Low Moderate High	 23 (28.8%) 46 (57.5%) 11 (13.7%)
Mean duration of using MTX (year) ± SD	5.8 ± 5.5
Mean of MTX dose (mg) ± SD	12 ± 3
Biological therapy Etanercept Infliximab Rituximab	 56 (70%) 13 (16.3%) 11 (13.7%)
Steroid intake Yes No	 38 (47.5%) 42 (52.5%)

MTX= Methotrexate, RA = Rheumatoid arthritis, SD = Standard deviation, CDAI = Clinical disease activity index.

Table 2: Mean value of hepatic and hematological elements of studied group.

Hepatic and hematological elements	Mean	SD
AST (10-45)u/l	21.7	±10.9
ALT (10-50)u/l	26.0	±18.8
Hb (11.5 -18) g/dl	12.1	±1.7
MCV (70 - 100) fl	83.5	±8.1
RDW- CV (11.6-14.6%)	14.5	±2.1
WBC (4 - 11 x10*9/l)	7.7	± 1.3
PLT (150 - 350 x10*9/l)	282.1	±24.8

SD = Standard Deviation, AST = Aspartate amino transferase, ALT = Alanine aminotransferase, Hb = Hemoglobin, MCV = Mean Corpuscular Volume, RDW-CV = Red blood cell Diameter Width, WBC = White Blood Cell, PLT = Platelets.

Table 3: Values of hepatic parameters in term of above upper limit of normal, below lower limit of normal and within normal limit in studied group.

Hepatic parameters	Above ULN (3 folds) No. (%)	Below LLN No. (%)	Within normal limit No. (%)
AST (10-45)u/l	3 (3.7)	1(1.2)	76(95)
ALT (10-50)u/l	2(2.5)	1(1.2)	77 (96.3)

ULN = upper limit of normal, LLN = Lower limit of normal, AST = Aspartate amino transferase, ALT = Alanine aminotransferase.

Table 4: Values of hematologic parameters in term of above upper limit of normal, below lower limit of normal and within normal limit in studied group.

Hematologic parameters	Above ULN No. (%)	Below LLN No. (%)	Within normal limit No. (%)
Hb (11.5 -18) g/dl	2(2.5)	26 (32.5)	52(65)
MCV (70 - 100) fl	2(2.5)	3(3.7)	75(93.8)
RDW- CV (11.6-14.6%)	26 (32.5)	1(1.2)	53(66.3)
WBC (4 - 11 x10^9/l)	8(10)	1 (1.2)	71(88.8)
PLT (150 - 350 x10^9/l)	16(20)	2 (2.5)	62(77.5)

ULN = Upper limit of normal, LLN = Lower limit normal, Hb = Hemoglobin, MCV = Mean corpuscular volume, RDW-CV = Red blood cell diameter width, WBC = White blood cell, PLT = Platelets.

Table 5: Values of AST and ALT 3 fold upper limit of normal and severity of anemia in studied group.

Parameters	Number of participants (%)
AST ≥ 3 fold ULN	3 (3.7)
ALT ≥ 3 fold ULN	2(2.5)
Mild anaemia (lower limit of normal -10 g/dl)	20 (25)
Moderate anaemia (10-8 g/dl)	4 (5)
Severe anaemia (≤ 8g/dl)	2(2.5)
Leucopenia (≤ 4000/mm^3)	1(1.2)
Leucocytosis	8(10)
Thrombocytopenia (≤ 150.000 x10^9/l)	2(2.5)

ULN = Upper limit of normal, LLN = Lower limit of normal, AST = Aspartate amino transferase, ALT = Alanine aminotransferase.

Table 6: Correlation between the duration of using steroids and studied parameters.

Variable	Statistical test	AST	ALT	Hb	MCV	RDW	WBC	PLT
Steroid (year)	Pearson Correlation	0.04	0.03	0.16	0.01	0.09	0.04	0.03
	p-value	0.3	0.07	0.3	0.6	0.6	0.9	0.7

AST = Aspartate amino transferase, ALT = Alanine aminotransferase, Hb = Hemoglobin, MCV = Mean corpuscular volume, RDW-CV = Red blood cell diameter width, WBC = White blood cell, PLT = Platelets.

Table 7: Correlation between the dose and duration of using methotrexate and studied parameters.

Variables	Statistical test	AST	ALT	Hb	MCV	RDW	WBC	PLT
MTX dose (mg)	Pearson Correlation	0.05	0.02	0.17	0.09	0.07	0.02	0.02
	p-value	0.6	0.8	0.1	0.4	0.5	0.8	0.8
Duration of MTX (year)	Pearson Correlation	0.07	0.09	0.11	0.19	0.04	0.12	0.15
	p-value	0.9	0.3	0.3	0.1	0.7	0.2	0.1

MTX= Methotrexate, AST = Aspartate amino transferase, ALT = Alanine aminotransferase, Hb = Hemoglobin, MCV = Mean corpuscular volume, RDW-CV = Red blood cell diameter width, WBC = White blood cell, PLT = Platelets.

Table 8: Correlation between the BMI, duration of smoking and studied parameters.

Variables	Statistical test	AST	ALT	Hb	MCV	RDW	WBC	PLT
BMI	Pearson Correlation	0.04	0.06	0.17	0.02	0.01	0.03	0.05
	p-value	0.5	0.09	0.2	0.7	0.7	0.8	0.9
Duration of smoking / year	Pearson Correlation	0.04	0.4	0.2	0.2	0.07	0.19	0.2
	p-value	0.2	0.1	0.4	0.3	0.6	0.3	0.2

BMI= Body mass index, AST=Aspartate amino transferase, ALT = Alanine aminotransferase, Hb = Hemoglobin, MCV = Mean corpuscular volume, RDW-CV = Red blood cell diameter width, WBC = White blood cell, PLT = Platelets.

Discussion

Low-dose of methotrexate serves as the cornerstone treatment and first-line medication rheumatoid arthritis, used by millions of people worldwide[23]. Dosages used for rheumatoid arthritis are typically 10-25 mg/week[24]. Low-dose methotrexate has been used in rheumatoid arthritis for over 30 years with studies demonstrating reduced symptoms, less joint damage, synergistic benefits with biologic treatments, and possible mortality benefits[25-27].

In the present study, females represent 90% of the study group (72 patients), only 10% of these were males (8 patients), this result consistent with study conducted in Iraq by Nizar A Jassim (2013) in which females represented 83.2% of the patients participating in that study[28], also compatible with other previous studies regarding gender (in all countries, prevalence was higher in females compared to males)[29].

In the current study, we found that 57.5% of the study group (46 patients) had a moderate CDAI score, this observation may reflect the improvement in the disease

process after using MTX with biological therapy. This result agreed with study conducted in Iraq by Niaz Al-Barzinji 2016 which showed that the majority of patients with long standing rheumatoid arthritis who had continued to receive the standard treatment of infliximab plus methotrexate for up to 5 years experienced significant sustained improvement in sign and symptoms, disease activity, and functional status[30].

Etanercept was used more than other biological agents due to it is more available, cheaper and easier method of use. This result compatible with study conducted in 2003 by Kremer et al[31].

Mean values of hepatic and hematological parameters were within normal limits in our study, but there was exceeding the upper limit of normal greater than three times AST level in three patients (3.7%) and ALT level in two patients (2.5%) only. These results found in this study are in concordance with studies done in Srinagar (India) in 2006 showed that the prevalence of ≥ 2 ULN ALT levels among RA patients on low dose of MTX therapy was 1.2%[32]. In other hand, the results of present study disagreed with studies conducted in the USA, which showed that the incidence of raised liver enzymes was 14.1%[33]. Haematological side effects of low dose of methotrexate ≤ 15 mg/week seemed to be less common in the present study, only two patients (2.5%) had severe anemia one of them had a history of bleeding hemorrhoids before starting the treatment, one patient (1.2%) has leucopenia, ten percent of participants had leukocytosis. This could be due to their use of biological therapy because the main adverse effect of the biologics used in inflammatory diseases is an increased risk of infections[20,34]. These findings agreed with a study done by Ali Buhroo in India 2006 which found that mild side effects of low dose of methotrexate which are mostly reversed with folic acid supplementation and other drugs[32]. On other hand, these findings inconsistent with study conducted in Pakistan from march 2010 to march 2011 in which found that adverse effects of low dose of methotrexate were 27% of RA patients[27].

There is no significant correlation between the low dose of methotrexate ≤ 15 mg/week, duration of using methotrexate, duration of using steroids, BMI and duration of smoking with hepatic or hematological elements. These results are consistent with a study conducted in Saudi Arabia by Susan M Attar in 2010 from which it was concluded that methotrexate has a very few clinically significant side effects[33]. Another study conducted at India 2016 by Lily Dubey et al concluded that long term low dose of methotrexate is safe in rheumatoid arthritis patients in the Indian population[35].

In conclusion, there is no significant hepatic and hematological adverse effect of low dose of methotrexate in rheumatoid patients treated with biological therapy.

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