The Egyptian Journal of Hospital Medicine (April 2025) Vol. 99, Page 2185-2193

Fibroscan in Rheumatoid Arthritis Patients on Long-Term Methotrexate Therapy

Marwa E. Ibrahim*[1], Tamer O. Elsaid[1], Hatem A. Elalfy[2], Ola M. Gharbia[1]
Departments of Physical Medicine, Rheumatology and Rehabilitation[1],
Tropical Medicine[2], Faculty of Medicine, Mansoura University, Egypt
***Corresponding Author**: Marwa E. Ibrahim, **Mobile**: 01092646182, **Email**: marwaelsaeed927@gmail.com

ABSTRACT

Background: Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory illness, which mainly influences joints; however, it may also affect other organs, involving the heart, lungs, and kidney. Methotrexate is commonly utilized for the management of variety of chronic inflammatory rheumatic illnesses, like rheumatoid arthritis. The liver fibrosis is mostly asymptomatic, however it could be complicated by hepatocellular carcinoma or portal hypertension and the serum enzymes are sometimes poorly predictive to present the importance of the fibroscan.

Objectives: To assess liver fibrosis by fibroscan in Egyptian patients with RA on long-term methotrexate therapy and its correlation with cumulative dose of methotrexate.

Patients and Methods: The current cross-sectional research has been performed on 105 cases with rheumatoid arthritis with uninterrupted weekly oral or injectable methotrexate for ≥ 3 years and their FIB-4 index was more than 1.45.

Results: There was a statistically significant elevation of the median methotrexate cumulative dose, the period of methotrexate utilize and increase of FIB-4 index in cases with significant fibrosis. A statistically significant positive association has been observed between methotrexate dose with liver fibroscan and FIB-4 index. The liver stiffness score and the FIB-4 index demonstrated a significant elevation with an elevation in the period of methotrexate dose.

Conclusion: Long-term usage of methotrexate was related to a higher possibility of fibrosis of the liver as revealed through FIB-4 index and fibroscan. In addition, such association is believed to have a positive correlation with both methotrexate dose and duration.

Keyword: Rheumatoid arthritis, Fibroscan, Methotrexate, Fibrosis-4 index, Hepatotoxicity.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory illness with unidentified cause, which mainly influences joints, resulting in their destruction, however, it may also affect other organs, involving the heart, lungs, and kidneys. The liver is infrequently impacted, but anomalies in hepatic tests may be observed in five percent to seventy-seven percent of cases with rheumatoid arthritis [1].

Methotrexate is commonly utilized for the management of variety of chronic inflammatory rheumatic illnesses, like rheumatoid arthritis, vasculitides and spondyloarthritis [2].

FDA permitted methotrexate as a treatment for rheumatoid arthritis in 1988. It has become the standard of care and the 1st-line treatment for cases with rheumatoid arthritis and it has a relatively good safety profile, but most prevalent side effect is gastrointestinal intolerance like nausea, diarrhea or stomatitis, furthermore extended methotrexate treatment might elevate the possibility of hepatotoxicity resulting in fatty liver illness, cirrhosis and fibrosis [3].

The cumulative methotrexate dose and period of management have an essential role in the progression of methotrexate-induced hepatotoxicity [4].

A cumulative dose above 1.5 grams and a period of treatment above two years in rheumatoid arthritis cases are regarded risk factors for hepatotoxicity [5].

Therefore, usual hepatic biopsy following a cumulative dose of one, three and eight grams may be regarded, but protocols from various societies differ on this problem [6].

The possible hepatotoxicity of extended management with methotrexate has been widely investigated for over twenty years, mostly in retrospective researches. It was recognized that methotrexate might trigger fibrosis of the liver in a small proportion of cases following prolonged management [7].

Current multi-national recommendations propose regular following up (every one to three months) of serum aminotransferases (ALT and AST), whereas medication cessation is suggested in the existence of an elevation in transaminases higher than three folds the upper restrict of usual values, and hepatic biopsy is recommended for cases of continuously increased aminotransferase following medication cessation [8].

Previous protocols suggested serial hepatic biopsies to follow up fibrosis of the liver [9]. Although deemed the reference standard for evaluation of fibrosis of the liver, hepatic biopsy is an invasive process and has a possibility of some morbidity and numerous other restrictions, like sampling errors and interobserver differences [10].

Moreover, the process is poorly accepted and challenging to recurrence in asymptomatic individuals [11]. So, liver biopsy isn't an appropriate screening test for the recognition of fibrosis of the liver in a large population [12]. Consequently, there is a requirement for precise, non-invasive and easy-to-recurrence

Received: 25/12/2024
Accepted: 25/02/2025

techniques of evaluating fibrosis of the liver as FIB-4 index and fibroscan. Fibrosis-4 index is a utilized equation for assessing fibrosis of the liver, depending on age, alanine transaminase, platelet count and aspartate transaminase [13]. Fibroscan is a recently developed, non-invasive technique for evaluating hepatic cirrhosis and fibrosis in different hepatic illnesses, involving chronic hepatitis B and C, alcoholic liver illness and non-alcoholic fatty liver disease (NAFLD) [14].

To the best of our knowledge, no other researchers investigated the role of Fibrosis-4 index and fibroscan in detecting liver affection induced by prolonged usage of methotrexate management in Egyptian rheumatoid arthritis cases. So, the goal of this research was to assess fibrosis of the liver by FIB-4 index and fibroscan in prolonged methotrexate treated Egyptian rheumatoid arthritis cases.

PATIENTS AND METHODS

This cross-sectional research was carried out on 105 cases with rheumatoid arthritis The cases have been selected from outpatient clinics of Rheumatology and Rehabilitation Department at Mansoura University hospitals in the duration from December 2021 to November 2022.

This study involved cases over 18 years of age from both genders and fulfilling ACR/EULAR 2010 criteria for rheumatoid arthritis [15] and were undergoing uninterrupted weekly oral or injectable methotrexate for ≥ 3 years and their FIB-4 index was more than 1.45 according to the AIDS Pegasys Ribavirin International Coinfection Trial (Apricot research) 2006 [13].

But we excluded patients on concomitant hepatotoxic drugs (e.g. leflunamide, isoniazid), patients with type II diabetes, Body Mass Index (BMI) >30 kg/m^2, evidence of chronic hepatic illness, hepatitis C virus (HCV), hepatitis B virus (HBV), human immunodeficiency virus (HIV) infection, bilharziasis, chronic cholestatic illness (1ry sclerosing cholangitis, 1ry biliary cirrhosis) and patient cannot undergo fibroscan (e.g. pregnant women and patients with active implantable medical device).

All patients were subjected to medical history and physical examination.

All patients had their venous blood drawn to do laboratory investigations: rheumatoid factor (RF), complete blood count (CBC), erythrocyte sedimentation rate (ESR), anti-cyclic citrullinated peptide (Anti-CCP), C-reactive protein (CRP), antinuclear antibody (ANA), liver function tests (involving serum alanine transaminase [ALT], aspartate transaminase [AST] and s. albumin), HCV antibody test, HIV antibody test, HbsAg test, serum creatinine, and fasting blood glucose.

Calculation of FIB-4 score

FIB-4 score has been estimated utilizing Sterling's formula [13], Fibrosis-4 score = Age (years) x aspartate transaminase concentration (U/L)/ [platelet count (10^9/L) x √ alanine transaminase concentration (U/L)] [16].

Radiological Investigations

Abdominal ultrasonography was performed for all the patients to rule out chronic liver diseases in addition to observing complications like portal hypertension and hepatocellular carcinoma.

All cases have been exposed to fibroscan following overnight fasting, utilizing FibroScan® 530 compact (Echosens, France)[17].

Examination Procedure

Fibroscan investigation was done in Tropical Department. During the investigation, the case was lying in dorsal decubitus position and the right arm in maximal abduction to increase the intercostal space where the probe has been positioned. The probe was positioned between the rib bones opposite to the right lobe of the liver in the middle of the parenchyma away from the liver border.

After all of these steps, the operator triggered a measurement. The median of all valid stiffness measurements within the recent investigation was the outcome of the investigation. Additionally, the success rate and the Interquartile Range (IQR) of all valid measurements were determined. A total of eight to ten valid measurements were obtained. The median of eight to ten valid measurements represent in kPa has been obtained as representative of hepatic stiffness [17,18]. The cutoff values for different phases of fibrosis through fibroscan were greater than or equal to 7.1 kPa for significant fibrosis, 9. 5 kPa for severe fibrosis, and 12.5 kPa for cirrhosis of the liver [8].

Ethics consideration

All procedures were in accordance with the ethical standards of the institutional and/or national research committee and in agreement with the Helsinki Declaration. Our institutional IRB Proposal Code Number: MS.21.11.1733, Faculty of Medicine, Mansoura University. A written informed consent has been attained from all participants prior to enrolment in the research, explaining the value of the research, and the processes which have been carried out. Confidentiality and personal privacy have been respected in all levels of the research.

Statistical Analysis

Outcomes were statistically examined by utilizing statistical package for the social sciences (SPSS 25, IBM/SPSS Inc., Chicago, IL). Descriptive statistics involved calculations for summarizing the continuous information as mean and standard deviation (SD) for usually distributed information or median and range for skewed information.

Incidence with percentage (%) has been utilized for presenting qualitative information. Fisher's exact test

was used to investigate the association among 2 groups with qualitative parameters. Independent Student T Test was used to evaluate the statistical significance of the variance among 2 independent research group with parametric information.

Mann Whitney Test (U test) was used to evaluate the statistical significance of the variance among 2 independent research group with non-parametric information.

One-way ANOVA test was used for continuous information to examine for significant variance between more than 2 usually distributed groups. Spearman's correlation coefficient (rs) has been estimated to show direction and strength of correlation among 2 numerical parameters. P-values below 0.05 were significant, and P-values below 0.01 were greatly significant.

RESULTS

This research involved 105 cases with RA who were diagnosed depending on ACR/EULAR (2010) categorization criteria for rheumatoid arthritis.

Table **[1]** shows that the mean age of the cases group was 50.23 ± 9.35. The mean body mass index was 26.4 ± 1.7 kg/m^2. There were 89 females (84.8%).

The mean MTX cumulative dose was 8.95 ± 5.02 gm. The duration of MTX intake was 555.94 ± 230.53 weeks. The mean Fibrosis-4 index was 1.57 ± 0.08. The mean liver fibroscan was 4.89 ± 1.3 Kpa. According to the liver fibroscan score, there were 12 cases (11.4%) with significant fibrosis (≥ 7.1 kPa).

Table [1]: Demographic data, criteria and methotrexate dose and liver fibrosis in the study cases

	Cases group (N=105)	
	Number	**Percentage**
Sex **Male** **Female**	 16 89	 15.2% 84.8%
	Mean ± SD	**Median (Min- Max)**
Age **(Years)**	50.23 ± 9.35	50 (30- 75)
BMI **(Kg/m2)**	26.4 ± 1.7	26 (23 - 29)
Methotrexate cumulative dose **(gm)**	8.95 ± 5.02	8.1 (2.3 – 22.7)
Duration of methotrexate **(Weeks)**	555.94 ± 230.53	523 (154 - 982)
FIB-4 index	1.57 ± 0.08	1.54 (1.46 – 1.76)
Liver fibroscan **(Kpa)**	4.89 ± 1.3	4.7 (2.5 – 7.6)
Fibrosis **Significant fibrosis (≥ 7.1 kPa)** **No significant fibrosis (< 7.1 kPa)**	 12 93	 11.4% 88.6%

Table **[2]** demonstrates a statistically insignificant variance has been observed between the cases with and without significant fibrosis concerning the age, BMI or the sex distribution. There was a statistically significant reduction of ESR and albumin in the cases with significant fibrosis. There was a statistically significant rise of the median methotrexate cumulative dose and period of methotrexate use in the cases with significant fibrosis. There was a statistically significant rise of Fibrosis-4 in the cases with significant fibrosis.

Table [2]: Comparison of the demographic data, laboratory data, variables of methotrexate, and FIB-4 depending on the absence or existence of significant fibrosis

Variables	No signficnt fibrosis **[N=93]**	Significant fibrosis **[N= 12]**	Test of Sign.
Age **(years)**	49.73 ± 9.68	54.08 ± 5.02	t = -1.527 P = 0.130
BMI **(Kg/m2)**	26.33 ± 1.68	26.92 ± 1.88	t = -1.119 P = 0.266
Sex			
Male	14 (15.1%)	2 (16.7%)	FET P = 1.000
Females	79 (84.9%)	10 (83.3%)	
Laboratory data			
Hemoglobin **(gm/dl)**	10.32 ± 0.86	10.38 ± 0.73	t = - 0.238 P = 0.812
TLC **(x 10 3 /fl)**	5.52 ± 1.06	4.79 ± 1.12	t = 2.231 P = 0.028*
Platelets **(x 10 3 /fl)**	230.76 ± 34.93	230.83 ± 36.30	t = - 0.006 P = 0.995
CRP **(mg/dl)**	13 (4 – 40)	15 (9 – 20)	z = - 0.788 P = 0.431
ESR **(mm/hr)**	54.24 ± 6.38	42.92 ± 2.70	**t = 2.302** **P = 0.023***
AST **(UI/l)**	38.27 ± 7.07	36.42 ± 6.02	t = 0.867 P = 0.388
ALT **(UI/l)**	27.48 ± 6.32	27.25 ± 6.11	t = 0.121 P = 0.904
Albumin **(gm/dl)**	4 ± 0.13	3.62 ± 0.43	**t = 6.637** **P = 0.001***
Creatinine **(mg/dl)**	0.98 ± 0.10	0.98 ± 0.18	t = - 0.000 P = 1.000
FBG **(mg/dl)**	96.35 ± 5.23	93.25 ± 4.52	t = 1.962 P = 0.052
Methotrexate			
Median methotrexate cumulative dose **(gm)**	8.1 (2.3 – 22.7)	14 (4.6 – 18)	**z = - 2.600** **P = 0.009***
Median duration of methotrexate **(Weeks)**	499 (154 – 893)	804 (467 – 982)	**z = - 3.696** **P < 0.001***
FIB-4 index	1.56 ± 0.08	1.64 ± 0.10	**t = -3.293** **P = 0.002***

Data are presented as mean ± standard deviation, median (Range), or frequency (%), t= Independent samples t-test, z= Mann-Whitney u-test, FET: Fischer's exact test, *: Statistically significant (p-value below 0.05)

Table **[3]** demonstrates a statistically significant positive association has been observed between methotrexate dose with liver fibroscan (rs= 0.228) and FIB-4 index (rs= 0.454). Table **[4]** shows that the liver stiffness score and the FIB-4 index demonstrated a significant elevation with an elevation in the period of methotrexate.

Table [3]: Correlation between methotrexate dose with fibrosis indices

Variables		Methotrexate dose
Liver stiffness (Kpa)	rs	**0.228**
	p	**0.019***
FIB-4 index	rs	**0.454**
	p	**< 0.001***

rs: Spearman's correlation, *significant p-value below 0.05.

Table [4]: Comparative analysis of the liver stiffness (Kpa) and FIB-4 index according to duration of methotrexate use (weeks)

Variables	Dration (< 352 weeks) **[N= 26]**	Dration (352-523 weeks) **[N= 27]**	Dration (> 523 weeks) **[N= 52]**	Test of Sign.
Liver stiffness (Kpa)	4.25 ± 1.04	4.97 ± 1.48	**5.36 ± 1.12**	**F = 4.795 P = 0.001***
FIB-4 index	1.38 ± 0.06	1.56 ± 0.09	**1.79 ± 0.11**	**t = 4.122 P = 0.003***

F= One-way ANOVA test**,** *Statistically significant (p-value below 0.05).

Table **[5]** demonstrates that with univariate regression analysis, increased median methotrexate cumulative dose, increased duration of methotrexate use, decrease ESR, decrease serum albumin and increase FIB-4 were shown as risk factors for significant liver fibrosis. However, with univariate regression analysis, decrease serum albumin and increase FIB-4 were the only parameters that revealed independent risk prediction for significant liver fibrosis.

Table [5]: Univariate and multivariate regression analysis for prediction of significant fibrosis (≥ 7.1 kPa)

Predictors	Univariate regression				Multivariate regression			
	P value	Odds ratio	95% C.I. for odds ratio		P value	Odds ratio	95% C.I. for odds ratio	
			Lower	Upper			Lower	Upper
Age	0.133	1.051	0.985	1.123				
BMI	0.266	1.220	0.859	1.733				
Male gender	0.884	1.129	0.223	5.709				
Median MTX cumulative dose	**0.008***	1.166	1.040	1.307	0.071	1.365	.973	1.913
Duration of methotrexate	**0.002***	1.008	1.003	1.012	0.135	1.123	0.844	1.976
Hemoglobin	0.810	1.091	0.535	2.224				
TLC	0.845	1.068	0.553	2.061				
Platelets	0.995	1.000	0.983	1.018				
CRP	0.502	1.033	0.940	1.136				
ESR	**0.029***	0.949	0.905	0.995	0.239	0.936	0.839	1.045
AST	0.386	0.964	0.887	1.048				
ALT	0.903	0.994	0.903	1.094				
Albumin level	**0.003***	0.011	0.002	0.092	**0.030***	**0.011**	**0.003**	**.158**
Serum creatinine	0.914	1.334	0.007	6.012				
FBG	0.060	0.899	0.804	1.004				
FIB-4	**< 0.001***	0.773	0.673	0.888	**0.008***	**0.663**	**0.489**	**0.898**

OR: Odds ratio, CI: Confidence interval, *Statistically significant (p-value below 0.05).

DISCUSSION

Rheumatoid arthritis is a chronic, inflammatory, systemic autoimmune illness that is related to systemic complications and developmental disability. Rheumatoid arthritis is marked by hyperplasia and synovial inflammation, releasing of autoantibodies involving RF and anti-citrullinated protein antibody (ACPA), bone and cartilage defects, and systemic characteristics involving pulmonary, psychological and cardiovascular illnesses [19]**.** Methotrexate was suggested as the first-line management in the American College of Rheumatology (ACR) and European League Against Rheumatism (EULAR) protocols for rheumatoid arthritis. Hepatic fibrosis is a known side-effect of prolonged methotrexate use and it is related to great cumulative methotrexate dosage and metabolic syndrome [20].

The purpose of the recent research was to evaluate fibrosis of the liver by FIB-4 index and fibroscan in prolonged methotrexate treated cohort of Egyptian RA cases. The current research involved 105 cases with rheumatoid arthritis who were diagnosed depending on ACR/EULAR (2010) categorization characteristics for rheumatoid arthritis. Concerning the demographic information, the current research demonstrated that the mean age of the cases group was 50.23 ± 9.35 years with a range between 30 and 75 years. The mean body mass index was 26.4 ± 1.7 kg/m2 with a range between 33 and 29 kg/m^2. There were 16 males (15.2%) and 89 females (84.8%). Also, **Avouac *et al.*** [21] conducted their

study on 170 cases with established rheumatoid arthritis: 141 (eighty-three percent) were females with a mean age of methotrexate 59±12 years and mean illness period of 15±11 years.

Regarding the median methotrexate cumulative dose, the present study revealed that the median methotrexate cumulative dose was 8.95 ± 5.02 gm with a range between 2.3 and 22.7 gm. The duration of methotrexate intake was 555.94 ± 230.53 weeks with a range between 154 and 982 weeks. Likewise, **Bafna *et al.*** [22] conducted their cross-sectional research on adult cases (not less than eighteen years of age) of rheumatoid arthritis that had been on methotrexate for ≥ three years. They revealed that the median period and cumulative dose of methotrexate were 336 weeks (interquartile range,144-912 weeks) and 6300 milligrams (interquartile range, 2400-22,000 milligrams), correspondingly.

Regarding the analysis of fibrosis of the liver the present study showed that the Fibrosis-4 index was 1.57 ± 0.08 with range between 1.46 and 1.76. The mean liver fibroscan was 4.89 ± 1.3 Kpa with a range between 2.5 and 7.6 Kpa. According to the liver fibroscan score, there were 12 cases (11.4%) with significant fibrosis (≥ 7.1 kPa).

Also, **Bafna *et al.*** [22] revealed that the mean stiffness of the liver was 5.22 ± 2.03 kPa. 12 cases (sixteen percent) had Fibroscan score not less than 7.1 kPa, of which three cases had severe stiffness of the liver (9.5 to 12.5 kPa) and 1 case had stiffness of the liver in the cirrhosis range (above 12.5 kPa). In addition, **Darabian *et al.*** [23] conducted their study on cases with inflammatory arthritis who were managed with methotrexate and provided screening with fibroscan. They recorded in their study that, 12.7% had phase three or four (F3+) fibrosis of the liver. Among individuals in group 1 (Methotrexate not higher than 499 milligrams), 13.3% had F3+ fibrosis, while in group four (Methotrexate above 6000 milligrams) the fibrosis rate was 9.1%.

In terms of the comparison of the demographic data depending on the absence or existence of significant fibrosis, the current research displayed a statistically insignificant difference has been observed between the cases with and without significant fibrosis regarding the age, BMI or the sex distribution.

Similarly, **Lertnawapan *et al.*** [24] conducted their study on RA cases prescribed methotrexate who have been assessed for fibrosis of the liver with fibroscan. They were in agreement with the current research regarding the absence of significant correlation among both age and sex and fibrosis degree, while they were against the current study regarding BMI being significantly associated with fibrosis as they revealed that body weight (P = 0.03), BMI (P-value below 0.001), were greater in the fibrosis of the liver group with statistical significance.

The comparison of the laboratory data depending to the absence or existence of significant fibrosis, the current research demonstrated that there was a statistically significant decrease of ESR (p-value equal to 0.023) and albumin (p-value equal to 0.001) in the cases with significant fibrosis, whereas insignificant association between the remaining laboratory parameters (HB, TLC, CRP, PLTs ALT, AST and FBG).

While **Lertnawapan *et al.*** [24] in their biochemical results, revealed that there were no variances in platelet, fasting blood glucose, hemoglobin, albumin, total bilirubin, HbA1c, direct bilirubin, alkaline phosphatase, creatinine or lipid profiles between non-fibrosis and fibrosis groups. The fibrosis group had greater laboratory values shown in aspartate transaminase (P-value equal to 0.03), alanine transaminase (P-value equal to 0.003), INR (P-value below 0.001), GGT (P-value below 0.001) and ultrasound abnormality (P-value below 0.001).

Concerning the comparison of the Fibrosis-4 index according to the absence or existence of significant fibrosis, the current research demonstrated that there was a statistically significant rise of Fibrosis-4 (p-value equal to 0.001) in the cases with significant fibrosis.

In accordance, **Sun *et al.*** [25] and **Ucar *et al.*** [26] displayed that the noninvasive scoring system FIB-4 could be used as a valid predictor for hepatic fibrosis. On the contrary, **Lertnawapan *et al.*** [24] revealed that there were no statistical differences in FIB-4 among the non-fibrosis and fibrosis group.

Regarding the comparison of the diseases related criteria depending to the absence or existence of significant fibrosis the current research demonstrated that there was a statistically significant rise of the median methotrexate cumulative dose (p-value equal to 0.009) and period of methotrexate use (p-value below 0.001) in the cases with significant fibrosis. Regarding the correlation between methotrexate dose with fibrosis indices, the current research revealed a statistically significant positive association that has been observed between methotrexate dose with liver fibroscan (rs= 0.228, p-value equal to 0.019) and FIB-4 index (rs= 0.454, p-value below 0.001). The cumulative dose of methotrexate has been observed as a risk factor for significant fibrosis of the liver in numerous researches [27-29]. In agreement, **Bafna *et al.*** [22] revealed that fibroscan scores significantly related to cumulative dose of methotrexate (r= 0.30, p-value equal to 0.008). They concluded that prolonged methotrexate treatment in rheumatoid arthritis has been related to elevated stiffness of the liver on fibroscan.

On the contrary, **Erre *et al.*** [30] conducted their study on 140 consecutive rheumatoid arthritis cases below methotrexate management (methotrexate-treated rheumatoid arthritis; mean management period: 6.2 years; mean methotrexate cumulative dose: 4.67 grams), 33 rheumatoid arthritis cases naive to methotrexate (methotrexate-naive rheumatoid arthritis) and hundred age and sex-matched healthy blood donors (HD). They demonstrated that values of the hepatic stiffness, though within the usual range, were

significantly greater in rheumatoid arthritis cases against controls, irrespective of methotrexate management. rheumatoid arthritis cases taking methotrexate didn't have a greater occurrence of significant fibrosis of the liver when compared to methotrexate naive rheumatoid arthritis cases and the general population (4.95 ± 0.7 kPa in methotrexate below one gram, 4.90 ± 1.1 kPa in methotrexate one to three grams and 4.80 ± 0.9 in methotrexate above three grams, p-value equal to 0.610). Significant fibrosis of the liver has been diagnosed in four cases in the methotrexate-treated rheumatoid arthritis (highest kPa value equal to 7.6; no hepatic function test anomalies or clinical signs of liver failure) and in none in all the methotrexate-naive rheumatoid arthritis and healthy blood donors groups (p-value equal to 0.145). Also, **Azzam *et al.*** [31] conducted a systematic review and meta-analysis and observed insignificant correlation among hepatotoxicity and methotrexate cumulative dose, all regarding vote counting and with concern to the meta-analysis of correlation coefficients from researches, which utilized fibroscan. However, this study was not specific on RA cases as it included large number of researches of different disease and different doses of methotrexate, which may explain such discrepancy.

Regarding univariate and multivariate regression analysis for expectation of significant Fibrosis (≥ 7.1 kPa), the current study revealed in their univariate regression analysis that increased median methotrexate cumulative dose, increased duration of methotrexate use, decrease ESR, decrease serum albumin, and increase FIB-4 were shown as risk factors for significant fibrosis of the liver. However, with univariate regression analysis, decrease serum albumin and increase FIB-4 were only those who revealed independent risk prediction for significant fibrosis of the liver.

In the same line, **Lertnawapan *et al.*** [24] in their univariate analysis showed that body mass index (odds ratio [OR] 1.27; ninety-five percent CI 1.11-1.45; P-value equal to 0.001), IFG (odds ratio 3.4; ninety-five percent CI 1.24-9.32; P-value equal to 0.02), fatty liver (odds ratio 3.65; ninety-five percent CI 1.11-11.97; P-value equal to 0.03), HLP (odds ratio 4.39; ninety-five percent CI 1.80-10.72; P-value equal to 0.001), rheumatoid arthritis period (odds ratio 1.12; ninety-five percent CI 1.02-1.23; P-value equal to 0.02), the cumulative methotrexate dosage (odds ratio 1.01; ninety-five percent CI 1.01-1.03; P-value below 0.001), prescribed statin (odds ratio 3.39; ninety-five percent CI 1.37-8.37; P-value below 0.001), tender joint count (TJC; odds ratio 1.31; ninety-five percent CI 1.01-1.68; P-value equal to 0.04), alanine transaminase (odds ratio 1.04; ninety-five percent CI 1.01-1.07; P-value equal to 0.01), GGT (odds ratio 1.13; ninety-five percent CI 1.08-1.18; P-value below 0.001), INR (odds ratio 48853.68; ninety-five percent CI 169.31-1.41; P-value below 0.001) and ultrasound abnormality (odds ratio 6.96; ninety-five percent CI 2.67-18.18; P-value below.001) have been related to the existence of fibrosis of the liver observed through transient elastography. Nevertheless, in the multivariate analysis only body mass index (odds ratio 1.22; ninety-five percent CI 1.05-1.41; P-value equal to 0.01); fatty liver (odds ratio 2.32; ninety-five percent CI 1.58-9.19; P-value equal to 0.02); alanine transaminase (odds ratio 1.04; ninety-five percent CI 1.02-1.09; P-value equal to 0.04) and cumulative methotrexate dosage (odds ratio 1.03; ninety-five percent CI 1.01-1.04; P -value equal to 0.001) were independently related to fibrosis of the liver.

Lertnawapan *et al.* [32] performed a cross-sectional research to explore the correlation between a cumulative methotrexate dose, metabolic syndrome, and fibrosis of the liver in cases diagnosed with rheumatoid arthritis. They displayed that following multivariate analysis, only body mass index (odds ratio = 14.73; ninety-five percent CI 2.90–74.79; p-value equal to 0.001), insulin resistance (odds ratio = 312.07; ninety-five percent CI 6.19–15732.13; p-value equal to 0.04), and cumulative methotrexate dosage (odds ratio 1.03; ninety-five percent CI 1.01–1.10; p-value equal to 0.002) have been related to liver fibrosis.

This came in disagreement with **Avouac *et al.*** [21] who demonstrated in their several parameters logistic regression analyses that there was an independent correlation between elevated Fibrosis-4 (above1.45) and male sex, reduced illness activity, and management with tocilizumab and leflunomide. No association has been detected among FIB-4 and methotrexate management period.

On the contrary to the present study **Darabian *et al.*** [23] revealed that in several parameters linear regression analysis, statistically significant factors for stiffness of the liver were body mass index, male gender, waist circumference, and age. Insignificant association among the cumulative methotrexate dosage and stiffness of the liver, even at great methotrexate doses, has been detected.

An explanation might be that cases on prolonged methotrexate management are being closely followed up with serial blood examinations through their rheumatologist, proper action (e.g., decrease in dosage or stoppage of methotrexate, regarding hepatic biopsy, and/or referral to hepatology) was obtained with anomalous hepatic enzymes, and those with anomalous outcomes were selected out [23].

CONCLUSION

In the context of methotrexate-prescribed RA patients, prolonged usage of methotrexate seemed to be related to a higher possibility of fibrosis of the liver as revealed through FIB-4 index and fibroscan. In addition, such association is believed to have a positive correlation with both methotrexate dose and duration.

Conflict of interest: All authors declare no conflicts of interest pertinent to the content of this review.

Funding: This review was conducted without any sources of funding.

REFERENCES

1. **Schlenker C, Halterman T, Kowdley K (2011):** Rheumatologic disease and the liver. Clinics in Liver Disease, 15(1): 153-64.
2. **Smolen J, Landewé R, Breedveld F *et al.* (2014):** EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2013 update. Annals of the Rheumatic Diseases, 73(3): 492-509.
3. **Visser K, Katchamart W, Loza E *et al.* (2009):** Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. Annals of the Rheumatic Diseases, 68(7): 1086-93.
4. **Quintin E, Scoazec J, Marotte H *et al.* (2010):** Rare incidence of methotrexate-specific lesions in liver biopsy of patients with arthritis and elevated liver enzymes. Arthritis Research & Therapy, 12: 1-7.
5. **Dávila-Fajardo C, Swen J, Cabeza Barrera J *et al.* (2013):** Genetic risk factors for drug-induced liver injury in rheumatoid arthritis patients using low-dose methotrexate. Pharmacogenomics, 14(1): 63-73.
6. **Podgórska J, Werel P, Klapaczyński J *et al.* (2020):** Liver involvement in rheumatic diseases. Reumatologia/Rheumatology, 58(5): 289-96.
7. **Laharie D, Terrebonne E, Vergniol J *et al.* (2008):** Foie et méthotrexate. Gastroentérologie Clinique et Biologique, 32(2): 134-42.
8. **Kranidioti H, Manolakopoulos S, Kandili A *et al.* (2016):** Evaluation of liver fibrosis by transient elastography (FibroScan®) in rheumatic patients during methotrexate treatment. Mediterranean Journal of Rheumatology, 27(2): 40-7.
9. **Saag K, Teng G, Patkar N *et al.* (2008):** American College of Rheumatology 2008 recommendations for the use of nonbiologic and biologic disease-modifying antirheumatic drugs in rheumatoid arthritis. Arthritis Care & Research, 59(6): 762-84.
10. **Castera L (2011):** Invasive and non-invasive methods for the assessment of fibrosis and disease progression in chronic liver disease. Best Practice & Research Clinical Gastroenterology, 25(2): 291-303.
11. **Manolakopoulos S, Triantos C, Bethanis S *et al.* (2007):** Ultrasound-guided liver biopsy in real life: comparison of same-day prebiopsy versus real-time ultrasound approach. Journal of Gastroenterology and Hepatology, 22(9): 1490-3.
12. **Kumar A, Vasdev V, Manrai M *et al.* (2018):** Assessment of hepatic fibrosis in patients with rheumatoid arthritis on long-term methotrexate therapy using transient elastography. Indian Journal of Rheumatology, 13(4): 246-51.
13. **Sterling R, Lissen E, Clumeck N *et al.* (2006):** Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology, 43(6): 1317-25.
14. **Marcellin P, Ziol M, Bedossa P *et al.* (2009):** Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. Liver International, 29(2): 242-7.
15. **Aletaha D, Neogi T, Silman A *et al.* (2010):** 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis & Rheumatism, 62(9): 2569-81.
16. **Ishak K, Baptista A, Bianchi L *et al.* (1995):** Histological grading and staging of chronic hepatitis. Journal of Hepatology, 22(6): 696-9.
17. **Laurent S, Jennifer O, Cécile B *et al.* (2011):** Non-invasive assessment of liver fibrosis by vibration-controlled transient elastography (Fibroscan®). Liver Biopsy, 1:249-309.
18. **Bafna P, Sahoo R, Hazarika K *et al.* (2021):** Prevalence of liver fibrosis by Fibroscan in patients on long-term methotrexate therapy for rheumatoid arthritis. Clinical Rheumatology, 40(9): 3605-13.
19. **Jang S, Kwon E, Lee J (2022):** Rheumatoid arthritis: pathogenic roles of diverse immune cells. International Journal Of Molecular Sciences, 23(2): 905.
20. **Lertnawapan R, Chonprasertsuk S, Siramolpiwat S *et al.* (2023):** Correlation between cumulative methotrexate dose, metabolic syndrome and hepatic fibrosis detected by fibroscan in patients receiving methotrexate in rheumatoid arthritis in comparison with psoriatic arthritis. Medicina (Kaunas), 59(6):1029.
21. **Avouac J, Degrave R, Vergneault H *et al.* (2022):** Risk of liver fibrosis induced by methotrexate and other rheumatoid arthritis medications according to the Fibrosis-4 Index. Clin Exp Rheumatol, 40(1): 150-7.
22. **Bafna P, Sahoo R, Hazarika K *et al.* (2021):** Prevalence of liver fibrosis by Fibroscan in patients on long-term methotrexate therapy for rheumatoid arthritis. Clinical Rheumatology, 40: 3605-13.
23. **Darabian S, Wade J, Kur J *et al.* (2022):** Using FibroScan to assess for the development of liver fibrosis in patients with arthritis on methotrexate: a single-center experience. The Journal of Rheumatology, 49(6): 558-65.
24. **Lertnawapan R, Chonprasertsuk S, Siramolpiwat S (2019):** Association between cumulative methotrexate dose, non-invasive scoring system and hepatic fibrosis detected by Fibroscan in rheumatoid arthritis patients receiving methotrexate. International Journal of Rheumatic Diseases, 22(2): 214-21.
25. **Sun W, Cui H, Li N *et al.* (2016):** Comparison of FIB-4 index, NAFLD fibrosis score and BARD score for prediction of advanced fibrosis in adult patients with non-alcoholic fatty liver disease: a meta-analysis study. Hepatology Research, 46(9): 862-70.
26. **Ucar F, Sezer S, Ginis Z *et al.* (2013):** APRI, the FIB-4 score, and Forn's index have noninvasive diagnostic value for liver fibrosis in patients with chronic hepatitis B. European Journal of Gastroenterology & Hepatology, 25(9): 1076-81.
27. **Pongpit J, Porntharukchareon S, Kaewduang P *et al.* (2016):** Liver stiffness measurement in psoriasis: do metabolic or disease factors play the important role? BioMed Research International, 2016(1): 7963972.
28. **Maybury C, Jabbar-Lopez Z, Wong T *et al.* (2014):** Methotrexate and liver fibrosis in people with psoriasis: a systematic review of observational studies. British Journal of Dermatology, 171(1): 17-29.

29. **Berends M, Snoek J, De Jong E *et al.* (2006):** Liver injury in long-term methotrexate treatment in psoriasis is relatively infrequent. Alimentary Pharmacology & Therapeutics, 24(5): 805-11.
30. **Erre G, Cadoni ML, Meloni P *et al.* (2019):** Methotrexate therapy is not associated with increased liver stiffness and significant liver fibrosis in rheumatoid arthritis patients: a cross-sectional controlled study with real-time two-dimensional shear wave elastography. European Journal of Internal Medicine, 69: 57-63.
31. **Azzam A, Jiyad Z, O'Beirne J (2021):** Is methotrexate hepatotoxicity associated with cumulative dose? A systematic review and meta-analysis. Australasian Journal of Dermatology, 62(2): 130-40.
32. **Lertnawapan R, Chonprasertsuk S, Siramolpiwat S *et al.* (2023):** Correlation between cumulative methotrexate dose, metabolic syndrome and hepatic fibrosis detected by fibroscan in rheumatoid arthritis patients. Medicina, 59(6): 1029.