JOURNAL OF CLINICAL MEDICINE OF KAZAKHSTAN (E-ISSN 2313-1519)
Original Article DOI: https://doi.org/10.23950/jcmk/14398

Aminotransferases activity on additional therapy in rheumatoid arthritis patients with liver disease

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Received: 2023-10-30.
Accepted: 2024-03-01.

J Clin Med Kaz 2024; 21(2): 89-94

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Abstract

Aim. Investigate the effect of additional therapy of atorvastatin, essential phospholipids and their combination on activity of aminotransferases in RA patients with NAFLD.

Materials and Methods. We investigated 126 RA patients and 30 in control. 77 RA patients with NAFLD were divided into three groups. I: 25 RA patients received 10 mg of atorvastatin per day. II: 26 RA patients received essential phospholipids 1800 mg per day. III: 26 RA patients received essential phospholipids 1800 mg per day and atorvastatin 10 mg per day for 6 months.

The results. In the I group, a transient increase in ALT and AST activity was observed to 35.11±3.501 U/l and 30.51±2.19 U/l, respectively, and a spontaneous decrease in elevated transaminases was recorded after 6 months of atorvastatin use. In the II group, a decrease in ALT by 25.6% was observed compared to the indicators before treatment, and they remained unchanged even after 6 months. After 3 months of complex use of atorvastatin and essential phospholipids, ALT activity decreased by 33.8% and AST decreased by 8.2%, which was not observed in RA patients with NAFLD of groups I and II.

Conclusions. Use essential phospholipids 600 mg three times a day and atorvastatin 10 mg per day for 6 months in addition to antirheumatic therapy in RA patients with NAFLD allows to avoid a transient increase in aminotransferases, reduce the severity of hepatotoxic reactions, and avoid stopping or canceling antirheumatic therapy.

Keywords: rheumatoid arthritis, nonalcoholic fatty liver disease, aminotransferases, atorvastatin, essential phospholipids.

Introduction

In recent years, the development of nonalcoholic fatty liver disease (NAFLD) in patients with rheumatoid arthritis (RA). RA patients deserve more and more attention since fatty infiltration of the liver of varying intensity is observed in patients [1-2]. Most often, NAFLD develops in RA patients with high clinical and immunological activity of the disease [3]. Vassiliadis E. et al., showed that in the liver of RA patients, structural changes are observed, on which the functioning of this organ directly depends, namely: granular and fatty dystrophy, deposition of amyloid masses, less often - annular cirrhosis and necrosis of hepatocytes. Similar changes were found in the liver tissue of RA patients by other researchers [1,4]. Moreover, Radovanovic-Dinic B. et al., demonstrated a correlation between structural and functional liver disorders and the activity of the rheumatoid process [5]. According to a meta-analysis, 1 in 3 patients with RA had NAFLD, which is comparable to the overall prevalence in the general population [6].

The analysis of scientific information showed that the drugs used in RA – nonsteroidal anti-inflammatory drugs (NSAIDs) and the gold standard of treatment – methotrexate can contribute to liver damage with a high probability. Methotrexate can cause an increase in the activity of liver enzymes, the development of fibrosis and cirrhosis of the liver with long-term treatment [7-10]. However, data on the frequency of development and severity of liver fibrosis and cirrhosis when using antirheumatic drugs are ambiguous.

The clinical data, as well as a high percentage of changes in functional tests of the liver in RA patients, in the absence of a history of liver pathology, may indicate the benefit of the development of steatosis in patients. Currently, there is no specific biochemical marker that can confirm the diagnosis of NAFLD or help differentiate steatosis, non-alcoholic steatohepatitis (NASH) and liver cirrhosis [11-13]. Despite the fact that more than 50% of patients with NAFLD have no complaints, however, they have an increase in the size of the liver and a slight increase in the level of transaminases (2-4 times the upper limit of normal). The cause of liver dysfunction is autoimmune processes on the one hand, and the influence of drugs on the other. Hepatotoxic reactions that occur during the use of RA basic therapy depend on the duration of the disease, the timing of administration and the dose of drugs [14].

Thus, the problem of early diagnosis of NAFLD in RA while ensuring a minimum of negative effects on the body is extremely relevant both from the point of view of scientific and practical medicine.

Materials and methods

The study of laboratory biochemical indicators of blood, which can indicate liver damage, was conducted in a group of RA patients and a control group, compared by age and gender.

Were investigated 126 RA patients who had the following inclusion criteria: had written patient consent to participate in the study; female and male patients were aged 20 to 55 years old; the diagnosis of RA was established according to the criteria of ARA, 1987.

The control group consisted of 30 patients without RA who had the following inclusion criteria: had written patient consent to participate in the study; female and male persons were aged 20 to 55 years old; absence of any autoimmune pathology, inflammatory conditions and diseases; absence of any chronic diseases in the active phase.

The main data on the clinical and demographic characteristics of RA patients and the control group are shown in Table 1.

During the laboratory biochemical examination of blood, the following laboratory syndromes were studied: cytolytic,

Table 1 Clinical and demographic characteristics of RA patients and control group individuals

Indicator	Distribution feature	RA patients (n=126)		Control group (n=30)	
		n	%	n	%
Gender	Women	102	80.95	25	83.33
	Men	24	19.05	5	16.67
Age	Young	53	42.06	13	43.33
	Average	73	57.94	17	56.67
Grade of RA activity	I, DAS28≤3.2	7	5.56	-	-
	II, 3.2<DAS28≤5.1	79	62.7	-	-
		40	31.75	-	-

mesenchymal-inflammatory and cholestatic. To study the cytolytic syndrome, the blood serum level was studied - alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase (GGT), the de Ritis index was determined; mesenchymal-inflammatory – total protein, thymol test, cholestatic – total, direct and indirect bilirubin, alkaline phosphatase (ALP).

According to the patient selection criteria, RA patients and persons of the control group who had an increase in the level of ALT and AST three times or more from the upper limit of normal were not included in the study. All RA patients included in the study and individuals of the control group had negative results in the examination of markers of hepatitis B (HBsAg, AB-HBcor), hepatitis C (HCV, AB-HCV) and markers of autoimmune hepatitis (ANA - antinuclear (antinuclear) antibodies).

Laboratory biochemical research was carried out on an OLYMPUS AU-400 automatic biochemical analyzer (Japan) using "Beckman Counter" kits (USA) according to the manufacturer's method. For the study, venous blood was collected in the morning, on an empty stomach, from the elbow vein, using disposable sterile material.

Based on the results of a comprehensive clinical, laboratory, and instrumental examination of 126 patients with RA a diagnosis of NAFLD was established in 77 patients with RA.

All patients with RA and NAFLD had a proatherogenic serum lipid profile, which was expressed by a reduced level of HDL-C, a high level of TG, LDL-C, and LDL-C. Presumably, the accumulation of fat in the liver can be an independent factor of dyslipidemia and indicates the possible presence of a direct pathogenetic chain: liver steatosis - dyslipidemia - atherosclerosis. Changes in the lipid profile in blood serum may indicate metabolic disorders, changes in the quantitative and qualitative composition of lipids in the liver, and atherogenic dyslipidemia in patients with RA and NAFLD, in turn, is the most important risk factor for the development and progression of cardiovascular pathology.

For all patients with RA, dyslipidemia and NAFLD were prescribed additional therapy using atorvastatin, essential phospholipids, and their combination.

For dyslipidemia treatment in RA patients with NAFLD, we chose atorvastatin, as a representative of the highly effective class of drugs that inhibit HMG-CoA (3-hydroxy-3-methylglutarylcoenzyme A) reductase and is one of the most studied statins. In the TARA study, 2005 and meta-analysis, 2023, many pleiotropic effects of the use of atorvastatin in RA patients were shown, such as a decrease in RA activity by Disease Activity Score in 28 Joints (DAS28), a decrease in tenderness and swelling of the joints, as well as a decrease in the level of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and interleukin-6 (IL-6).

77 RA patients with NAFLD were divided into three studied treatment groups. All three groups were comparable in terms of age, gender, duration of RA disease, grade of RA activity and grade of fatty infiltration of the liver according to ultrasonography.

The first group consisted of 25 RA patients who received antirheumatic therapy for RA and atorvastatin in a daily dose of 10 mg in the evening, regardless of food intake, for 6 months.

The second group consisted of 26 RA patients who received antirheumatic RA therapy and essential phospholipids at a dose of 600 mg (two capsules) three times a day for 6 months.

The third group consisted of 26 RA patients who, in addition to RA antirheumatic therapy, received essential phospholipids at a dose of 600 mg (two capsules) three times a day and atorvastatin at a daily dose of 10 mg for 6 months.

Control of the effectiveness of the use of essential phospholipids, atorvastatin and their combination were carried out 3 and 6 months after the start of treatment.

The analysis and processing of statistical data of clinical studies was carried out on a personal computer using the STATISTICA 10.0 StatSoft for Windows and MS Excel XP application program package. Using the sampling method, the following were determined: average value, error of the average value and standard deviation. The sample parameters given in the tables of the article have the following designations: M - the average value, m - the error of the average value, SD - the standard deviation, and n - the volume of the analyzed group. With the help of parametric methods, in the case of a normal distribution of signs for unrelated and related groups, the Student's t-test and Fisher's exact test were used, in the case when one of the indicators in the group is less than 5. The level of significance was considered critical at $p<0.05$. The following designations are given in the tables of the article: p – the level of significance reached.

Results

The results of the conducted studies showed that the grade of activity has a small effect on the changes in the enzymatic activities of ALT and AST in blood serum. Thus, in patients with the 1st grade of activity, the enzymatic activity of ALT and AST is 1.3 and 1.2 times higher than that of the control group, respectively. However, as the disease progresses, there is an increase in the activity of transaminases in the blood serum, with the maximum values in patients with the III grade of RA activity (Figure 1).

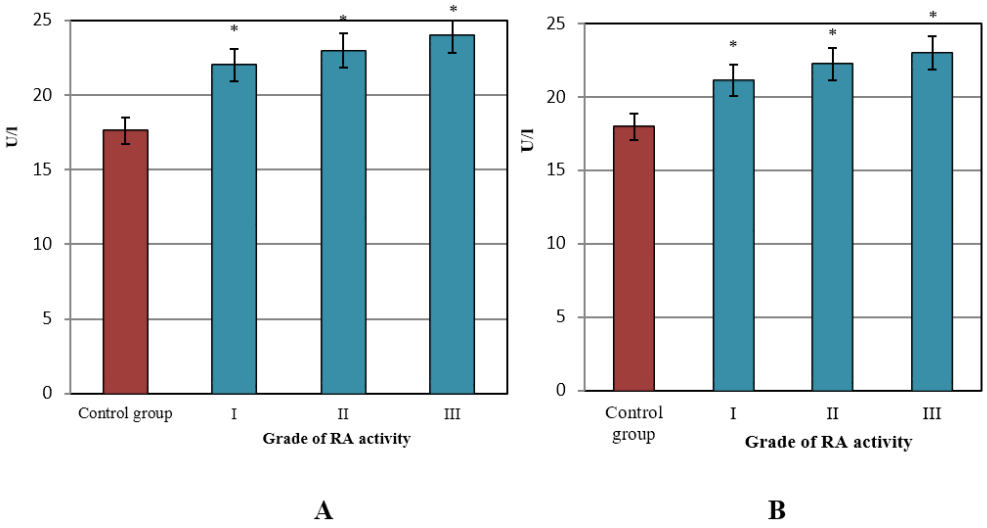

Figure 1 – Alanine aminotransferase (A) and aspartate aminotransferase (B) activity in blood serum of rheumatoid arthritis patients and the control group

* – statistically significant difference compared to the indicators of the control group, $p<0.05$.

Table 2

Dependence of indicators of cytolytic, cholestatic and mesenchymal-inflammatory syndromes in blood serum on the grade of rheumatoid arthritis activity, M±SD

Indicator	Grade of disease activity			Control group (n=30)
	I (n=7)	II (n=79)	III (n=40)	
Indicators of cytolytic syndrome				
LDH, U/l	173.57±6.07	177.80±1.54	178.35±2.24	174.20±1.8
Indicators of cholestatic syndrome				
Total bilirubin, mmol/l	7.03±0.28	6.95±0.09	6.87±0.13	7.64±0.26
direct bilirubin, mmol/l	1.21±0.04*	1.09±0.02*	1.09±0.02*	0.96±0.01
indirect bilirubin, mmol/l	5.81±0.27	5.86±0.09	5.78±0.12	6.68±0.26
ALP, U/l	67.57±5.15*	74.73±1.43*	74.28±2.21*	51.10±0.84
GGT, U/l	26.00±0.85	26.53±0.21	26.58±034	27.83±0.70
Indicators of mesenchymal inflammatory syndrome				
Total protein, g/l	72.00±0.85	69.89±0.24	69.68±0.31	71.23±0.66
Albumin, g/l	41.43±0.65*	41.99±0.15*	42.05±0.20*	33.07±0.42
Thymol test, units	2.80±0.16*	3.11±0.05*	3.35±0.09*	2.43±0.04

* – statistically significant difference compared to the indicators of the control group, $p<0.05$.

Table 2 shows the results of a laboratory study of indicators of cytolytic, cholestatic and mesenchymal-inflammatory syndromes in blood serum in the control group and in RA patients and their dependence on the grade of RA activity.

The next task of our study was to investigate the effect of additional therapy using atorvastatin, essential phospholipids and their combination on the activity of aminotransferases in 77 RA patients with NAFLD.

No differences between the groups were found when evaluating the initial enzymatic activities of ALT and AST in blood serum, which proved their comparability (Table 3).

The analysis of the results of RA patients with NAFLD of the I group showed that after three months of atorvastatin use, the enzymatic activities of ALT and AST in blood serum increased to 35.11±3.50 U/l and 30.51±2.19 U/l, respectively (Table. 3). However, after 6 months of atorvastatin use, there was a spontaneous reduction of elevated transaminases in patients (Table 3).

As for the introduction of essential phospholipids in patients of the II group, already 3 months after their use, a decrease in alanine aminotransferase activity by 25.6% was observed compared to the indicators observed before treatment, ($p<0.05$). The indicators remained at the same level 6 months after the start of the use of essential phospholipids (Table 3). The values of the de Ritis index at the level of 1.03±0.06 - after 3 months and at the level of 1.02±0.10 - after 6 months of the use of essential phospholipids testify to their

Table 3

Enzymatic activity of aminotransferases in blood serum of rheumatoid arthritis patients under the conditions of use of atorvastatin and essential phospholipids, M±m

Term	Treatment indicator		
	ALT, U/l	AST, U/l	de Ritis index
I group (n=25)			
Before treatment	29.72±2.58	24.63±1.93	0.82±0.01
After 3 months	35.11±3.50*	30.51±2.19*	0.87±0.05
After 6 months	26.94±3.93#	26.34±4.09 #	0.98±0.05*,#
II group (n=26)			
Before treatment	28.69±1.83	23.64±3.12	0.82±0.07
After 3 months	21.34±1.12*	22.01±4.03	1.03±0.06*
After 6 months	21.27±2.29*	21.79±3.92	1.02±0.10*
III group (n=25)			
Before treatment	28.71±2.53	23.24±2.10	0.81±0.09
After 3 months	19.01±1.92*	21.34±1.83	1.12±0.07*
After 6 months	17.09±1.25*,#	19.88±1.97*	1.16±0.13*,#

* – statistically significant difference compared to indicators characteristic of treatment, p<0.05; # – statistically significant difference compared to the indicators observed after 3 months of treatment, p<0.05.

protective effect in the treatment of RA patients with NAFLD (Table 3).

As can be seen from Table 3, the most pronounced positive result in the form of the absence of the formation of cytolysis syndrome and faster and more significant normalization of ALT and AST indicators, when comparing the data of the I, II and III comparison groups, was achieved with the simultaneous use of atorvastatin and essential phospholipids. After 3 months of complex use of atorvastatin and essential phospholipids, ALT activity decreased by 33.8% and AST decreased by 8.2%, which was not observed in RA patients with NAFLD of groups I and II.

Discussion

ALT and ACT are considered the most sensitive and specific indicators of hepatocellular damage. Since ALT and AST belong to intracellular enzymes, an increase in their activity in blood serum indicates hepatocyte damage and inflammatory processes in the liver. With deeper hepatocellular damage, an increase in the activity of the mitochondrial isoforms of the studied transaminases is observed in the blood serum, which is due to the damage to the mitochondria [15]. Probably, the metabolic processes that occur in the body of RA patients lead to destructive changes in the liver, as a result of which ALT and AST enter the bloodstream [16]. However, AST in the body is localized not only in the liver but also in the heart, so AST hyperfermentemia may be associated with damage to the liver or heart muscle as a result of cell cytolysis. To identify the origin of hyperenzymatemia of transaminases, the AST/ALT ratio (de Ritis index) is determined, the increase of which indicates a violation of the cardiovascular system, including damage to the myocardium. The de Ritis index value below 1.0 indicates liver damage [17].

Although the indicators of transaminase activities were within the permissible reference values (men less than 41 U/l; women less than 31 U/l), however, changes in the de Ritis index indicate deviations in the work of the hepatobiliary system. Analyzing another indicator of cytolytic syndrome – LDH, its value did not differ from normal indicators (Table 2).

Studies of biochemical markers of cholestatic syndrome showed that the level of enzymatic activity of ALP in blood serum increases compared to the indicators of a control group of individuals, and as RA activity progresses, the enzymatic activity of ALP in blood serum increases (Table 2). Hyperfermentemia of ALP may be associated with obstruction of the intrahepatic or extrahepatic bile ducts or inflammatory processes in the liver. It should be noted that the increase in alkaline phosphatase activity occurs against the background of unchanged indicators of total and indirect bilirubin, that is, before the level of bilirubin increases. Such changes in indicators are observed in liver diseases.

As for direct bilirubin, its value significantly exceeds the indicator of the control group of individuals at all stages of RA development (Table 2). However, in patients with the 1st grade of RA activity, its indicator is higher than in patients with the 2nd and 3rd grades of activity. The established fact indicates that as RA progresses in the liver, the conjugation of indirect bilirubin and its transition to direct bilirubin is disrupted. It is believed that hyperbilirubinemia due to the direct fraction has a hepatic origin and may be associated with impaired excretion of direct bilirubin due to cytolysis of hepatocytes. In addition, an increase in the concentration of bilirubin in the blood can indicate cholestasis or volumetric damage to the liver parenchyma. The level of GGT in the blood serum of RA patients did not statistically differ from the indicators found in the control group (Table 2).

The analysis of indicators of mesenchymal-inflammatory syndrome showed that against the background of a stable level of total protein in the blood serum of patients with the 1st grade of RA activity, the level of albumin increased by 1.3 times and the indicator of thymol test by 1.2 times compared to the indicators of the control group (table 2). As the grade of activity progresses, the values of these indicators increase and reach a maximum in patients with the III grade of RA activity. Thus, the highest level of thymol test was observed at the III grade of disease activity, 3.35±0.09 units, which is 1.2 times higher than at the first grade of disease activity, 2.80±0.16 units, and 1.4 times higher than in the control group – 2.43±0.04 units (p<0.05) (Table 2).

An increase in the level of albumin in the blood can be a consequence of slight dehydration of the body, which will increase the negative course of RA. So, for the normal functioning of the articular cartilage, a sufficient amount of water is needed, which is the basis of the intercellular substance and 75% of the weight of the cartilage tissue. There are no blood vessels in the cartilage, and all the nutrients in the chondrocytes come from the liquid environment of the extracellular matrix, which consists mainly of water. Dehydrated cartilage and joints are gradually destroyed, and in the later stages of arthritis, the process becomes irreversible. At the same time, elevated values of the thymol test indicate an increase in the concentration of α-, β- and γ-globulins and lipoproteins in the blood, which is most often observed in liver diseases.

Therefore, as the grade of RA activity increases, there is a violation of the functioning of the organs of the liver, which is expressed by a violation of the synthetic and, possibly, detoxification function of the liver and the excretory function of the gallbladder. However, since the values of the studied indicators are within the permissible norm, it is difficult to say about violations on the part of hepatobiliary system based only on laboratory biochemical indicators. Therefore, only a comparison of the complaints of RA patients with the data of clinical and

instrumental studies allows us to conclude that in patients with the development of RA, the work of the hepatobiliary system is disturbed, which is expressed by the development of NAFLD.

Therefore, under the conditions of use of atorvastatin, the increase in the level of ALT and AST is transient, since their activities returned to the initial level and even lower without the need to cancel statins. This is confirmed by many studies, the results of which showed the effectiveness and safety of the use of statins in the treatment of NAFLD [18-20].

The correcting ability of phospholipids on the functional state of the disease is obviously related to their amphiphilic properties, which can regulate the penetration of the cell skin. Thus, the use of phospholipids leads to the following hepatotropic effects: reduction of lipid peroxidation processes, restoration of enzyme systems, normalization of protein metabolism, improvement of the metabolism of the disease [21-24]. This contributes to the restoration of plasma membrane cells and the regeneration of damaged cells [25-27].

Therefore, against the background of the liver protective action of essential phospholipids and the use of atorvastatin, antirheumatic therapy drugs do not show a pronounced hepatotoxic effect, as evidenced by a decrease in the activity of transaminases in blood serum.

Conclusion

1. The pathology of hepatobiliary system in RA patients occurs in 61.10% of patients and does not depend on age, duration of the disease, and activity of the inflammatory process.

2. Determining only laboratory biochemical indicators of hepatobiliary system work is not enough to assess the state of the liver and control the progression of liver damage. For a reliable determination of liver pathology, it is necessary to carry out a comprehensive assessment of the patient's complaints, clinical, laboratory and instrumental data.

3. Using the combination of atorvastatin and essential phospholipids helps to avoid the formation of cytolysis syndrome and decreases the activity of ALT by 33.8% and AST by 8.2%, compared to the use of atorvastatin alone.

4. In RA patients with NAFLD recommended to use of essential phospholipids in a dose of 600 mg three times a day and atorvastatin in a daily dose of 10 mg for 6 months in addition to antirheumatic therapy. This will allow for avoiding a transient increase in aminotransferases, reducing the severity of hepatotoxic reactions, to avoid stopping or cancelling the antirheumatic drug therapy.

Author Contributions: Conceptualization, I.K. and L.K.; methodology, I.K. and L.K.; validation, I.K. and L.K.; formal analysis, I.K. and L.K.; investigation, I.K. and L.K.; data curation, I.K. and L.K.; writing – original draft preparation, I.K. and L.K.; writing – review and editing, I.K. and L.K.; visualization, K.H.S.; supervision, I.K. and L.K. All authors have read and agreed to the published version of the manuscript.

Disclosures: There is no conflict of interest for all authors.

Acknowledgments: None.

Funding: None.

References

1. Lee YH, Bae SC, Song GG. Meta-analysis of associations between the peroxisome proliferator-activated receptor-γ Pro12Ala polymorphism and susceptibility to nonalcoholic fatty liver disease, rheumatoid arthritis, and psoriatic arthritis. Genet Test Mol Biomarkers. 2014;18(5):341-348. doi:10.1089/gtmb.2013.0503
2. Radovanović-Dinić B, Tešić-Rajković S, Zivkovic V, Grgov S. Clinical connection between rheumatoid arthritis and liver damage. Rheumatol Int. 2018;38(5):715-724. doi:10.1007/s00296-018-4021-5
3. Wendt MMN, de Oliveira MC, Franco-Salla GB, et al. Fatty acids uptake and oxidation are increased in the liver of rats with adjuvant-induced arthritis. Biochim Biophys Acta Mol Basis Dis. 2019;1865(3):696-707. doi:10.1016/j.bbadis.2018.12.019
4. Vassiliadis E, Oliveira CP, Alvares-da-Silva MR, et al. Circulating levels of citrullinated and MMP-degraded vimentin (VICM) in liver fibrosis related pathology. Am J Transl Res. 2012;4(4):403-414.
5. Radovanović-Dinić B, Tešić-Rajković S, Zivkovic V, Grgov S. Clinical connection between rheumatoid arthritis and liver damage. Rheumatol Int. 2018;38(5):715-724. doi:10.1007/s00296-018-4021-5
6. Zamani M, Alizadeh-Tabari S, Chitkara P, Singh S, Loomba R. Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Rheumatoid Arthritis: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2023 Oct;21(11):2789-2796. doi: 10.1016/j.cgh.2023.02.021. Epub 2023 Mar 5. PMID: 36871771.
7. Lucas CJ, Dimmitt SB, Martin JH. Optimising low-dose methotrexate for rheumatoid arthritis-A review. Br J Clin Pharmacol. 2019;85(10):2228-2234. doi:10.1111/bcp.14057
8. Conway R, Carey JJ. Risk of liver disease in methotrexate treated patients. World J Hepatol. 2017;9(26):1092-1100. doi:10.4254/wjh.v9.i26.1092
9. Shetty A, Cho W, Alazawi W, Syn WK. Methotrexate Hepatotoxicity and the Impact of Nonalcoholic Fatty Liver Disease. Am J Med Sci. 2017;354(2):172-181. doi:10.1016/j.amjms.2017.03.014
10. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World J Gastroenterol. 2014;20(2):475-485. doi:10.3748/wjg.v20.i2.475
11. Lewis JR, Mohanty SR. Nonalcoholic fatty liver disease: a review and update. Dig Dis Sci. 2010;55(3):560-578. doi:10.1007/s10620-009-1081-0
12. Hashimoto E, Tokushige K, Ludwig J. Diagnosis and classification of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis: Current concepts and remaining challenges. Hepatol Res. 2015;45(1):20-28. doi:10.1111/hepr.12333
13. Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. J Hepatol. 2013;58(5):1007-1019. doi:10.1016/j.jhep.2012.11.021
14. Karlsson Sundbaum J, Eriksson N, Hallberg P, Lehto N, Wadelius M, Baecklund E. Methotrexate treatment in rheumatoid arthritis

and elevated liver enzymes: A long-term follow-up of predictors, surveillance, and outcome in clinical practice. Int J Rheum Dis. 2019;22(7):1226-1232. doi:10.1111/1756-185X.13576
15. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther. 2011;34(3):274-285. doi:10.1111/j.1365-2036.2011.04724.x
16. Zhao S, Mysler E, Moots RJ. Etanercept for the treatment of rheumatoid arthritis. Immunotherapy. 2018;10(6):433-445. doi:10.2217/imt-2017-0155
17. Li Y, Zhang W. Sheng Wu Gong Cheng Xue Bao. 2017;33(1):36-43. doi:10.13345/j.cjb.160241
18. Eslami L, Merat S, Malekzadeh R, Nasseri-Moghaddam S, Aramin H. Statins for non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. Cochrane Database Syst Rev. 2013;(12):CD008623. Published 2013 Dec 27. doi:10.1002/14651858.CD008623.pub2
19. Chatrath H, Vuppalanchi R, Chalasani N. Dyslipidemia in patients with nonalcoholic fatty liver disease. Semin Liver Dis. 2012;32(1):22-29. doi:10.1055/s-0032-1306423
20. Lewis JH. Clinical perspective: statins and the liver--harmful or helpful?. Dig Dis Sci. 2012;57(7):1754-1763. doi:10.1007/s10620-012-2207-3
21. Dajani AI, Abu Hammour AM, Zakaria MA, Al Jaberi MR, Nounou MA, Semrin AI. Essential phospholipids as a supportive adjunct in the management of patients with NAFLD. Arab J Gastroenterol. 2015;16(3-4):99-104. doi:10.1016/j.ajg.2015.09.001
22. Gundermann KJ, Gundermann S, Drozdzik M, Mohan Prasad VG. Essential phospholipids in fatty liver: a scientific update. Clin Exp Gastroenterol. 2016;9:105-117. Published 2016 May 5. doi:10.2147/CEG.S96362
23. Dajani AI, Popovic B. Essential phospholipids for nonalcoholic fatty liver disease associated with metabolic syndrome: A systematic review and network meta-analysis. World J Clin Cases. 2020;8(21):5235-5249. doi:10.12998/wjcc.v8.i21.5235
24. Lüchtenborg C, Niederhaus B, Brügger B, Popovic B, Fricker G. Lipid Profiles of Five Essential Phospholipid Preparations for the Treatment of Nonalcoholic Fatty Liver Disease: A Comparative Study. Lipids. 2020;55(3):271-278. doi:10.1002/lipd.12236
25. Jamwal R, Barlock BJ. Nonalcoholic Fatty Liver Disease (NAFLD) and Hepatic Cytochrome P450 (CYP) Enzymes. Pharmaceuticals (Basel). 2020;13(9):222. Published 2020 Aug 29. doi:10.3390/ph13090222
26. Udut VV, Vengerovsky AI, Dygai AM. Effects of phospholipid hepatoprotectors on apoptosis during experimental liver pathology induced by isoniazid and paracetamol. Bull Exp Biol Med. 2013;154(5):614-617. doi:10.1007/s10517-013-2012-9
27. Vasilevskaia AS, Butov MA, Uzbekova DG, Mnikhovich MV, Nikiforov AA. Eksp Klin Gastroenterol. 2013;(12):79-82.