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Prevalence of aminotransferase macroenzymes in rheumatoid arthritis patients and impact on their management

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CASE REPORT

Introduction

Rheumatoid arthritis (RA) treatment can be hepatotoxic, but liver enzymes can be falsely elevated due to macroenzyme presence. Macroenzymes are often found in autoimmune diseases, but prevalence and effect on treatment is unclear. This study aimed to determine aminotransferase macroenzyme prevalence and effect in RA patients.

Materials and methods

This study included consecutive RA patients without liver disease sent for laboratory tests. Samples with elevated AST or ALT were processed for macroenzymes. Presence was determined using polyethylene glycol precipitation (PEG).

Results

Out of 126 patients, 21 had elevated aminotransferase levels. Due to liver disease, 6 patients were excluded, another 3 were unavailable for informed consent, leaving 12 patients for inclusion. Out of 12 patients, 1 had increased AST levels, 2 increased ALT levels, and 9 both. Macro-ALT was detected in 5/11 patients, 1 also had macro-AST. Out of 5 patients with macroenzymes, treatment change was seen in 3/5 patients, imaging in 2/5, both in 2/5.

Conclusion

Elevated liver enzymes in RA patients is not always indicative of hepatotoxicity, as shown by the fact that about half of patients in our study had macroenzymes detected. Before assuming drug hepatotoxicity and changing treatment or ordering imaging, rheumatologists could consider macroenzyme presence.

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INTRODUCTION

Macroenzymes are serum enzymes bound by serum components, namely immunoglobulins, where research has found various enzymes (such as aspartate [AST] and alanine transferases [ALT], lactate dehydrogenase [LDH], creatinine kinase [CK], amylase [AMY], and lipase [LIP]) form such complexes causing falsely elevated values, and often are found in autoimmune diseases (1,2).

Rheumatoid arthritis (RA) is one such autoimmune disease where macroenzymes can be found (1,2). RA is a chronic inflammatory disease, exact cause is unknown, which manifests as symmetrical joint destruction, especially synovial joints (3). Therapeutic drug of choice, methotrexate, as well as other commonly used drugs, have well-documented hepatotoxicity (4,5). Elevated liver enzymes may lead to change in treatment or additional diagnostics, which may be unnecessary, or even detrimental, considering macroenzymes may be to blame, as seen in one example of a case of macro-AST which lead to an invasive liver biopsy (6).

This prospective study aimed to evaluate and determine the prevalence of aminotransferase (AT) macroenzyme complexes in RA patients. A secondary goal was to evaluate the rate of unnecessary changes in treatment or additional imaging.

MATERIALS AND METHODS

This study included consecutive RA patients with increased AST and/or ALT levels without known liver disease, based on laboratory requisitions with a diagnosis of RA as sent by rheumatologists during followups from Rheumatology Outpatient Clinics at Clinical Hospital Center Rijeka between 16th May and 14th of Dec 2018. AST and ALT were measured on a Beckman Coulter AU5800 (Beckman Coulter, California, USA) biochemistry analyser using the IFCC Reference Method modified without pyridoxal phosphate, at 37°C. The Croatian Chamber of Medical Biochemistry recommended, age and gender specific, ALT and AST reference ranges were used (Table 1). Macroenzyme presence was determined using polyethylene glycol precipitation (PEG) according to Levitt and Ellis (7) with a 1:2 25% PEG solution. PEG solution was prepared by adding 2.5 g of PEG 6000 (for synthesis) solution (Mercks KGaA, Darmstadt, Germany) to 6 mL of deionised water, after the PEG 6000 had dissolved, deionised water was added to the 10 mL mark in the measuring tube. Solution was kept at 4°C. For testing macroenzyme presence, 200 µL of patient serum was pipetted in a tube, then another 200 µL of PEG solution was added and vortexed for 10 secs. The mixture was incubated in a water bath at 37°C for 10 mins before being centrifuged at 3000 rpm for 20 mins at room temperature. For a blank, 200 µL of deionised

water was added instead of PEG solution. The sample and blank probes were simultaneously treated the same way. PEG-precipitable activity (PPA) was calculated by measuring aminotransferase levels in the probe supernatants, and using the formula: %PPA = 100 x ((activity blank x activity PEG)/activity blank). Electrophoresis was not used — besides being unavailable, a strong correlation exists between the methods (8). Cut-off values for PPA according to Davidson and Watson (9) were used, which are cut-off of 76% PPA for macro-ALT and cut-off of 54% PPA for macro-AST.

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Clinical Hospital Centre Rijeka. All participants have provided informed consent for inclusion in the study.

Table 1 AST and ALT reference values as recommended by the Croatian Chamber of Medical Biochemistry (2004)

Test	Recommended method and/or procedure	Recommended method and/or procedure	Unit	References		
				Sex	Age (years)	Interval
Alanine-aminotransferase (ALT)	UV photometry, IFCC method, 37°C, Tris buffer, L-alanine, α-ketoglutarate, pyridoxal phosphate, nicotinamide adenine dinucleotide (NADH), lactate dehydrogenase, pH 7,15	UV photometry, 37°C, L-alanine without pyridoxal phosphate, Tris buffer	U/L	Male Female Male Female Male Female Male Female Male Female	0-2 0-2 3-7 3-7 8-12 8-12 13-19 13-19 ≥ 20 ≥ 20	11-46 11-46 9-20 9-20 11-37 11-37 10-33 10-29 12-48 10-36
Aspartate aminotransferase (AST)	UV photometry, IFCC method, 37°C, Tris buffer, L-aspartate, α-ketoglutarate, pyridoxal phosphate, nicotinamide adenine dinucleotide (NADH), malate dehydrogenase, lactate dehydrogenase pH 7,65	UV photometry, 37°C, L-aspartate without pyridoxal phosphate, Tris buffer	U/L	Male Female Male Female Male Female Male Female Male Female	0-2 0-2 3-7 3-7 8-12 8-12 13-19 13-19 ≥ 20 ≥ 20	26-75 26-75 24-49 24-49 14-39 14-39 11-38 14-32 11-38 8-30

RESULTS

Out of 126 RA patients, 21 had elevated AT levels. A total of 6 patients were excluded due to liver disease or conditions which could cause elevated levels, as noted in medical documentation (4 with non-alcoholic fatty liver disease [NAFLD], 1 pancreatic cancer,1 unspecified liver lesion), and another 3 patients were excluded as they were unavailable to obtain informed consent for inclusion in the study, leaving a total of 12 patients for inclusion. Demographically, median patient age was 63 (40 to 78), 2 patients were male, and 10 female. Out of 11 patients, 1 had increased AST levels, 2 increased ALT levels, and 9 both. Macro-ALT was detected in 5/11 patients, 1 of which also had macro-AST. Due to technical issues with the analyser, 1 patient was excluded since the sample could not be treated in the same way as the others.

A change in treatment was observed in 5/12 patients, additional imaging was ordered in 6/12 patients, both in 4/12 patients, and no change or imaging in 5/12 patients. Of patients with macroenzymes, change in treatment was seen in 3/5 patients, imaging was ordered in 3/5 patients, both in 2/5 patients, no action in 1/5 patients (Table 2). Liver biopsy or other invasive procedures were not noted.

Examining the macroenzyme patients further, one patient with both complexes present was

Table 2 Breakdown of AST and ALT values, AST and ALT PPA, change in treatment or further imaging by patient

Patient	AST (U/L)	ALT (U/L)	PPA AST (%)	PPA ALT (%)	Change in treatment	Further imaging
1	61	135	53,85	76,67	Yes	No
2	60	84	53,33	80,95	Yes	Yes
3	52	54	71,43	85,71	Yes	Yes
4	48	31	50,00	62,50	Yes	Yes
5	42	54	50,00	80,77	No	No
6	40	51	50,00	66,67	No	No
7	40	41	11,11	37,50	No	No
8	39	67	47,37	68,75	No	No
9	38	56	50,00	84,62	No	Yes
10	25	41	0,00	50,00	No	No
11	23	43	42,86	69,23	Yes	Yes

diagnosed with methotrexate induced hepatotoxicity, and treatment was discontinued. Treatment was subsequently reinstated after imaging results were found to be normal. Another patient was also diagnosed with methotrexate induced hepatotoxicity, and treatment has been postponed for the time being. Methotrexate was temporarily discontinued for a third patient for 3 weeks, and continued after follow up laboratory test results were shown to be static, with no change in AT levels; no imaging was done. The final two patients with complexes present were without change in treatment, but one had an ultrasound done yearly to monitor for liver changes, and the other had no imaging done within the last year.

DISCUSSION

Although it is known that macroenzymes are present in autoimmune diseases such as RA (1,2), an exact prevalence is unclear. Unfortunately, our study did not yield enough of a sample size to determine the prevalence of macroenzymes in RA, this is a clear limitation, but it did confirm that macroenzymes are present. It should be noted there is a possibility that some requisitions were incorrectly labelled with diagnosis of RA, however, all patients tested in our study had a diagnosis of RA either confirmed or suspected at the time.

With only 21/126 RA patients with elevated liver enzymes in our study, 6 of which with known liver disease, it could be commented that although hepatotoxicity of drugs in RA is known (4,5), it is well-managed. Still, with examples in the literature such as ordering invasive procedures, that is, liver biopsy (6), and keeping in mind that treatment which may be adequate or beneficial is often changed if hepatotoxicity is suspected, the importance of recognising and detecting possible macroenzymes remains relevant. About half of the patients with elevated AT had either some form of change in treatment, either pausing or discontinuation; imaging ordered, that is, abdominal ultrasound; or both. It is possible that the patients with macroenzymes did not require any change in treatment or imaging done. If macroenzymes are to blame for the elevated liver enzymes, then it is clear how such laboratory results can influence management. However, it should be noted that macroenzymes can be present without elevating laboratory results outside of reference ranges (1), and any acute or dramatic change warrants further investigation.

Considering that PEG is available in a number of diagnostic laboratories, and the test is simple, inexpensive, and non-invasive, testing for AT macroenzyme presence in patients with RA, or other autoimmune diseases, may be warranted as a differential diagnosis for elevated AT levels, especially when considering drug hepatotoxicity as changing treatment which is adequate or beneficial, or ordering further tests, especially invasive tests such as liver biopsy, should be avoided.

To clarify, the PPA cut-off values for macro-AST and ALT used were taken, as mentioned, from a study by Davidson and Watson (9). The study tested several enzymes, taking 40 patients with elevated levels, and measured PPA after excluding macroenzyme using electrophoresis (9). By calculating mean enzyme and PEG-precipitable activity, reference ranges for each enzyme were proposed, for AST 18 to 53 U/L and for ALT 38 to 76 U/L (9), for our study, the upper limit of reference ranges were used.

In comparison, according to Davidson and Watson the cut-off value for PPA for macroamylase is 60 % (9), and an accepted value for macroprolactin is 50 % (10), both of which are routinely used in our laboratory, however, these differences are not explained.

In conclusion, elevated liver enzymes in RA patients may not always be indicative of hepatotoxicity, as shown by the fact that about half of patients in our study had macroenzymes detected. Before assuming drug hepatotoxicity and changing treatment or ordering other diagnostics, rheumatologists and laboratory personnel could consider aminotransferase macroenzyme presence.

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