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Macro-Aspartate Aminotransferase and Its Laboratory Detection: A Case Report

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Abstract. ***Background.*** Increased enzyme activity in human blood serum is usually associated with the existence of disease. On the other hand, enzyme activity can also be elevated in the presence of benign conditions, such as macro-enzymes. Macro-enzymes could lead to highly unnecessary and invasive procedures which may cause complications to the patient and an extra cost for the hospital. Therefore, it is important to diagnose this condition in order to avoid unnecessary clinical tests.

Case Presentation. We present a case of a 71-year-old asymptomatic female with persistent elevation of AST who was referred to our hospital for additional testing for underlying liver disease. By using polyethylene glycol (PEG) precipitation assay, we were able to identify macro-AST. This helped to avoid the high-risk liver biopsy procedure.

Conclusion. In the case of an isolated elevation of AST activity with no clinical indications of liver disease, diagnostic work-up for macro-AST should always be considered by physicians.

Keywords: macro-AST, PEG precipitation, macro-enzymes, liver

Lokalizuoto tenosinovinio gigantinių ląstelių naviko pėdoje ar kulkšnyje chirurginio gydymo baigtys: atvejų aprašymas

Santrauka. ***Kontekstas.*** Gigantinių ląstelių sausgyslių apvalkalo navikas (trumpinys GCTTS), kuris taip pat vadinamas tenosinoviniu gigantinių ląstelių naviku (trumpinys TGCT), yra lokalus agresyvus navikas, visų pirma pasireiškiantis sausgyslės apvalkale arba bursoje. Maždaug 3–5 % šių navikų atsiranda pėdoje arba kulkšnyje. Lokalūs šios srities pažeidimai dažnai pasireiškia susidarančia kieta mase arba gumbeliais, kurių

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lėtas, tačiau nuolatinis progresavimas gali trukti mėnesius ar net metus. Pacientų galimi nusiskundimai – skausmas, atsiradęs nešant svorį, ir sąnarių judesių ribotumas; tai priklauso nuo naviko vietos. GCTTS užtikrintai pašalinti būtina operacija. Tokiu būdu sunaikinama neoplazma ir atkuriamas apatinės galūnės funkcionalumas.

Metodai. Mūsų institucijoje nuo 2017 iki 2022 metų buvo nustatyta 13 GCTTS atvejų pėdoje ar kulkšnyje, kai prireikė chirurginės rezekcijos. Kiekvienu atveju registravome priešoperacinius ir pooperacinius simptomus. Taip pat fiksavome priešoperacinį ir pooperacinį funkcinį statusą pagal tiek MSTS, tiek ir AOFAS skalių vertinimus. Pateikėme ataskaitą apie vėlesnes komplikacijas ir vietinį išplitimą.

Rezultatai. Prieš chirurginį gydymą kiekvienas pacientas patyrė bent jau nestiprų skausmą. Vidutiniai priešoperaciniai MSTS ir AOFAS skalių vertinimai buvo atitinkamai 22,8 ir 70,7. Vidutinis naviko dydis – 17,7 mm. Kiekvienam pacientui buvo atlikta rezekcija su didelėmis pakraščio zonomis. Dviem atvejais (15,4 %) navikai vėl susiformavo. Nė vienam pacientui vėlesnio stebėjimo laikotarpiu nepasireiškė jokių sudėtingesnių komplikacijų. Atlikus operaciją, vidutiniai pooperaciniai MSTS ir AOFAS skalių balai išaugo atitinkamai iki 28,3 ir 92,2.

Išvada. Rezekcija su didelėmis pakraščio zonomis pėdos ar kulkšnies GCTTS atveju efektyviai padeda atkurti pacientų apatinių galūnių funkcionalumą ir yra susijusi su priimtinai nežymiu naviko išplitimo procentu.

Raktažodžiai: tenosinovinis gigantinių ląstelių navikas, gigantinių ląstelių sausgyslių apvalkalo navikas, pėda, kulkšnis, rezekcija, funkcionalumas, AOFAS ir MSTS skalės

Introduction

Increased enzyme activity in human blood serum is usually associated with the existence of disease. On the other hand, enzyme activity can also be elevated in the presence of benign conditions such as macro-enzymes. Macro-enzymes could lead to highly unnecessary and invasive procedures, which may cause complications to patient and extra cost for the hospital. Therefore, it is important to diagnose this condition in order to avoid unnecessary clinical tests. In the absence of organ-specific disease, macro-AST should be a part of the differential diagnosis.

Macro-AST

Since 1964 macro-enzymes are known as complexes formed by the binding of an enzyme and macromolecules [1]. The first scientist who reported macro aspartate aminotransferase (macro-AST) was Konttinen in 1978 [2]. Macro-AST is known for forming complexes of high molecular mass which are unable to undergo renal clearance because of their size, thus resulting in persistent elevated serum AST activity [3]. Moreover, the activity of macro-AST can remain stable over time or fluctuate naturally. Usually, the patients are female, younger than 60 years of age, who are also at high-risk group for autoimmune disease and the AST activity in serum is higher than 500 U/l [4, 5].

Aspartate aminotransferase (AST) is a pyridoxal phosphate dependent transaminase enzyme which catalyzes the reversible transfer of an alpha amino group between aspartate and glutamate [6]. This enzyme is found in the liver, myocardial cells, renal, cerebral, skeletal muscle and red blood cells [1]. There are two isoenzymes – one located in the mitochondria (m-AST) and the other in the cytosol (c-AST) and are encoded by *GOT1* and *GOT2* on chromosomes 10q24 and 16q12, respectively [7]. Both isoenzymes can form macro-enzymes.

Macro-enzymes can be categorized in two types: type I and type II. The explicit pathogenesis mechanism remains unclear but most likely the mechanism of type I is based on autoimmunity [8]. The immune reaction or the dysregulation of immune tolerance seems to be linked with the formation of macro-enzymes [4]. One of the possible theories is that Fab portion of immunoglobulins IgG, IgM or IgA binds serum enzymes as antigens in the setting of a molecular mimicry phenomenon [9]. Type II macro-enzymes are formed when the enzyme either self-polymerises or

associates with external chemicals such as drugs [8]. Macro-AST is a type I macro-enzyme and is produced by formation of a 250-kDa complex. In 2017, Kulecka *et al.* performed *GOT1* sequence analyses for patients with familial macro-AST and found out a missense mutation (p. Gln208Glu and rs374966349) in glutamate oxaloacetate transaminase 1 gene which is associated with macro-AST and has unknown inheritance and penetrance [10]. The study also demonstrated that at this position is a negatively charged amino acid glutamate which may be crucial for a firm anchorage of serum immunoglobulins on the GOT1 surface. However, this mutation was observed only in 20 of 29 families, thus it suggests that macro-AST could have polygenic inheritance requiring further genetic research.

Macro-AST is rare among the general population, reported prevalence is approximately 0.014% in gastroenterological patients and more than 33% in children with an isolated increase in AST activity [11]. Usually, the patients with macro-AST are completely asymptomatic and thus it is a benign condition which requires no specific treatment [11]. However, there are cases of macro-AST's association with malignancies (lung, breast, gastrointestinal tract cancers and multiple myeloma) or with rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, celiac disease, chronic HCV, Kawasaki disease in children [7, 9, 12, 13].

Laboratory diagnostic methods of macro-AST

There are numerous methods for macro-AST identification in human blood serum samples such as ultracentrifugation, gel filtration chromatography (GFC), immunofixation electrophoresis (IE) or polyethylene glycol (PEG) precipitation assays (Table 1) [14–17].

One of the most frequently used methods in routine clinical laboratory for macro-AST detection is PEG precipitation assay due to its short time, low cost and simple procedure. Macro-AST detection is based on PEG ability to precipitate Ig-AST complexes by absorbing available solvent and increasing their concentration in solution. In order to confirm presence of macro-AST, AST activity is measured after the precipitation and AST recovery (%) and PEG precipitation activity (%) are calculated [15, 18]. Even though this method is fast, simple and cost-effective, it is only suitable for screening as it has low specificity due to false precipitation of monomeric AST, lacks reference ranges and the result has to be confirmed by more reliable method [15, 16, 19, 20].

Ultracentrifugation method is rarely used for macro-enzyme identification in routine clinical laboratories due to its complexity. The basis of macro-enzyme separation by ultracentrifugation is usage of filters which allows to separate high molecular weight molecules, such as Ig complexes, from low molecular weight molecules – monomeric enzymes. This method also requires assessing AST activity prior and after separation in order to calculate AST recovery (%) and activity post-ultracentrifugation (%). Even though this method is more specific than PEG precipitation assay, it lacks applicability due to long analysis time and expensive machinery needed [16, 18].

Gel filtration chromatography is the most common chromatographic technique currently used for macro-enzyme identification. GFC is based on macro-enzyme and monomeric enzyme separation by their size difference. Monomeric enzymes elute earlier than their Ig complexes with bigger molecular mass and thus it allows accurate and specific isolation of these molecular species. Due to its specificity GFC is considered as reference method in laboratory diagnosis of macro-AST. Even though this method is highly specific, there are several drawbacks, such as long analysis time and high cost. Therefore, only reference laboratories use it for macro-AST identification [16, 21, 22].

Table 1. Macro-AST identification methods, principles, common utility characteristics and properties.

Method	Analysis Time	Basis of macro-AST removal	Cost	Difficulty	Properties
Polyethylene glycol precipitation	Short	Concentrating and precipitating proteins in the sample	Low	Low	• Low specificity • The need of confirmatory assay • No reference ranges
Ultracentrifugation	Long	Separation by size (filtration)	High	High	High specificity
Gel filtration chromatography	Long	Separation by size (filtration)	High	High	• High specificity • Reference method
Immunofixation electrophoresis	Long	Separation by size and charge, identification by specific antibodies and dyes	High	High	• High specificity • Reference method

Immunofixation electrophoresis is another analysis method applied to macro-enzyme detection and identification. It separates monomeric enzymes from their Ig-complexes by size and electric charge and allows their identification and visualization by enzyme specific monoclonal antibodies and dyes. Even though IE is highly specific for macro-AST identification, it is labour intensive and lengthy, thus it is seldom used in routine clinical and is restricted to reference laboratories as a reference method [16, 23].

Case report

A 71-year-old woman was referred to the clinic for persistently isolated elevated serum AST which was first detected 6 months ago during work-up for gastro-oesophageal reflux disease and hepatosteatosis. She complained of reflux and dysphagia and was being successfully treated for grade A erosive esophagitis with proton pump inhibitors (PPIs) and bismuth oxide. She did not have any abdominal pain, myalgias or jaundice and her clinical exam was otherwise unremarkable. She had no history of alcohol abuse. Her past medical history included hypertension, dyslipidaemia, paroxysmal atrial fibrillation and spinal osteoarthritis. She was taking the following medications: Esomeprazole, Pancreatic enzymes, Bismuth oxide, Itopride, Silymarin, Valsartan/Hydrochlorothiazide, Valsartan/Amlodipine, Rivaroxaban, Zopiclone, Vinpocetine, nonsteroidal anti-inflammatory drugs (NSAIDs), Nitrofurantoin as needed for urinary tract infection (UTI), "Aterolip" (herbal medication for dyslipidaemia).

Her laboratory work-up showed elevated AST at 578 U/l (reference interval ≤40 U/L), while alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (AP), creatine kinase (CK), lactate dehydrogenase (LDH) and bilirubin test results were normal. Coagulation work-up and albumin were not indicative of any abnormalities. Earliest medical records revealed that 10 years ago the activity of AST was normal, but 6 months ago markedly increased at 118 U/l, and continued to increase up to 653 U/l over the following 2 months (Figure 1). The rest of patient's laboratory work-up was unremarkable: complete blood count, erythrocyte sedimentation rate, C-reactive protein, ferritin, myoglobin, ceruloplasmin, creatinine, sodium, potassium and ionized calcium were normal. Work-up for autoimmune hepatitis included antinuclear antibodies and antimitochondrial antibodies which were both negative and hepatitis C serology, as well as, hepatitis B surface antigen were negative. Tissue transglutaminase antibodies for celiac disease were negative. Faecal test for occult blood was negative. Urinalysis and urine sediment microscopy were normal. Radiographic studies included abdominal ultrasound which showed signs of hepatic and pancreatic

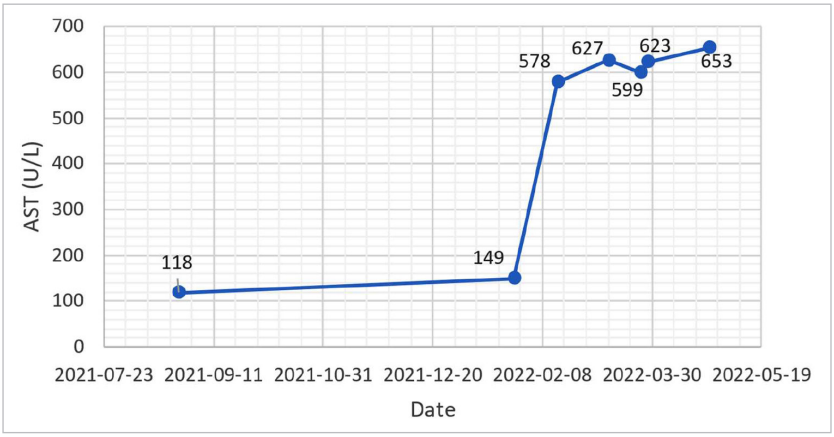

Figure 1. The increase of AST activity in patient`s serum in ten months interval.

steatosis but was otherwise unremarkable. Liver elastography was also done and was consistent with hepatosteatosis, the results were as follows: 4.0 kPa, CAP – 249 dB/m, IQR/med – 15%, IQR – 0,6. A trial of ursodeoxycholic acid proved to be ineffective. Discontinuation of PPI and herbal medication "Aterolip" also had no effect on AST activity.

Following extensive work-up with mostly negative results, macro-AST syndrome was hypothesised. AST activity in serum was evaluated spectrophotometrically using an enzymatic International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (NADH, pyridoxal-5'-phosphate) method by Architect i2000SR (USA, Illinois, Chicago). The sample was also assessed for the presence of macro-AST using PEG assay. 25% PEG solution was mixed with patient's serum in equal parts and vortex mixed for 1 minute. After mixing, centrifugation was performed at 554 x g for 10 minutes using Sigma 1-14 microcentrifuge (Germany, Osterode). Finally, supernatant was carefully removed from precipitate and used for post-PEG AST activity assay. Simultaneously, 100 µl of serum was mixed with 100 µl physiological saline solution and the AST activity was measured. Patient's AST activity was 653 U/l, post-PEG precipitation AST activity ($ASTactivity_{PEG}$) was 10 U/l and AST activity in saline diluted sample ($ASTactivity_{SAL}$) was 562 U/l. Effect on PEG precipitation can be defined by the recovery after precipitation [21]:

$$\text{Recovery (\%)} = (ASTactivity_{PEG}/ASTactivity_{SAL}) \times 100 = (10/562) \times 100 = 1{,}78\%$$

% PEG precipitation activity (%PPA) was also calculated:

$$\%PPA = 100 \times [(ASTactivity_{SAL} - ASTactivity_{PEG})/ASTactivity_{SAL}] = 100 \times [(562\text{-}10)/562] = 98{,}22\%$$

Post-PEG precipitation AST activity, PEG recovery and PPA results indicated presence of macro-AST.

Discussion

In the presented case, 71-year-old asymptomatic female with persistent elevation of AST was referred to the hospital for additional testing for underlying liver disease. The evaluation of elevated liver enzymes involved an extensive work-up mostly differentiating between infectious, environmental, autoimmune and hereditary causes of liver injury. In particular, most work-ups should screen for alcoholic and nonalcoholic fatty liver disease, viral hepatitis, autoimmune hepatitis, hemochromatosis, Wilson's disease, alpha-1 antitrypsin deficiency and medication related liver injury [24]. Nonhepatic causes of AST activity elevation are few and include acute conditions like cardiac muscle damage,

heat stroke, rhabdomyolysis, haemolysis and chronic conditions like macro-AST syndrome. Our patient had fatty liver disease but this could not explain significant isolated AST elevation without any increase of ALT. She was also tested for medication induced liver injury but both PPI and herbal OTC medication discontinuation had no effect on AST activity. This led to conclusion that AST elevation was most likely not related to liver injury.

Even though there are variety of methods for macro-enzyme identification not all routine clinical laboratories have them readily available at their disposal. Usually, routine clinical laboratories have PEG precipitation assay available for them as they often screen for Ig-hormone complexes, such as macro-prolactin [19]. It is known that PEG precipitation assay lacks specificity for macro-AST identification, but it is easily applied in routine clinical laboratories and preliminary results can facilitate clinical decision making until further results from reference laboratories could be obtained. Therefore, PEG precipitation assay, may be helpful in avoiding unnecessary additional interventions or laboratory testing and reduce hospital expenditures for patient care.

Conclusions

Using PEG precipitation assay we were able to identify that macro-AST was responsible for isolated elevation of AST. This helped to avoid high-risk liver biopsy procedure which was considered before macro-AST diagnosis. In case of isolated elevation of AST with no clinical indications of liver disease, diagnostic work-up for macro-AST should always be considered by physician. A timely detection of macro-AST allows to reduce the number of unnecessary procedures, testing and costs in a hospital setting.

Declaration of interests

The authors declare that they have no conflict of interest.

Informed consent

Written informed consent was obtained from the patient for the purposes of publication of this case report.

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