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Idiosyncratic LDH Elevation in a Young and Healthy Female Patient, a Case of Macro-LDH Detection Using PEG Precipitation

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Abstract Lactate dehydrogenase (LDH) is an unspecific marker predominantly for hemolysis, thrombotic, infectious, and malignant diseases. On rare occasions, it can also be idiosyncratically elevated. We present a case of a 30-year-old otherwise healthy female patient who is referred to outpatient internal medicine with a persistent LDH elevation for years. Extensive laboratory and radiological diagnostics are performed without any indications of disease. Macro-LDH is suspected, and serum immunocomplex precipitation with polyethylene glycol is performed. Laboratory results make the presence of macro-LDH likely. Therefore, it is suggested that macroenzymes be considered early in the diagnostic journeys of patients with elevated serum enzyme activities unsuitable to the corresponding clinical context to spare them unnecessary procedures and provide them with a diagnosis of this probable benign condition.

Keywords: *lactate dehydrogenase, macroenzymes, macro-LDH, PEG precipitation, idiosyncratic LDH elevation*

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1. Introduction

Lactate Dehydrogenase (LDH) serves as an unspecific marker of cell death in vitro [1,2] and human disease in vivo [3]. Serum LDH is commonly elevated in oncological patients [4,5], in hemolysis and associated disease [6,7], during infections and thrombosis [8] as well as in rheumatic conditions like rheumatoid arthritis [9]. In rare cases, this enzyme forms a complex with another protein, primarily immunoglobin, resulting in macro-LDH and elevated enzyme activity in laboratory assays due to increased serum half-life [10].

We present the case of a young female patient who underwent extensive diagnostic evaluation for asymptomatic elevation of serum LDH.

2. Case

A 30-year-old woman is referred to internal medicine by her general practitioner for evaluation of persistent elevation of LDH. Recent laboratory work shows an LDH concentration of 382 U/l (reference: 70-240 U/l). Complete blood count (CBC), liver function parameters, TSH (thyroid-stimulating hormone), and creatinine are unremarkable. The patient complains of no current or recent health problems. In further detail, she denies weight loss, fever (not continuous nor cyclical), night sweats, or staying abroad before detection of LDH elevation. She does not take any regular medications or drugs, has never had any medical condition, and is a mother of two healthy children. According to her, LDH enzyme activity has been elevated "for years", but she has never undergone extensive diagnostics, which she now wishes to be performed. Physical examination, electrocardiogram, and abdominal sonography show no abnormal findings, except for asymptomatic cholecystolithiasis. Blood work is repeated by atraumatic venipuncture, without a tourniquet, using a large bore needle. The hemolysis parameters haptoglobin and bilirubin are within the normal range, the CBC including reticulocyte count is unremarkable. Direct and indirect Coombs tests are negative. Coagulation studies, infection serology, autoantibody studies, muscle parameters, serum electrophoresis, immunophenotyping, immunoglobulin concentrations, electrolyte concentrations (sodium, potassium, calcium, chloride), liver and renal parameters (including urine status) do not show abnormalities. LDH is elevated (511 U/l, reference range: 125 - 220 U/l) and folic acid is mildly reduced (6,8 nmol/l, reference range: 7,9 – 46,4) without evidence of macrocytic anemia, the blood sedimentation rate is 80 mm after one hour and 100 mm after two hours. Pneumological evaluation for sarcoidosis and latent tuberculosis, as well as a computed tomography scan of

the thorax and abdomen, remain without significant findings. Table 1 shows an overview of the full patient evaluation. Folic acid 5 mg once a day for one month is prescribed for possible subclinical ineffective hematopoiesis. Elevation of serum enzyme activity is suspected in the context of macro-LDH.

Table 1. full patient evaluation overview

lab work / organ system	parameters	results and interpretation
cell turnover	LDH	511 U/l, reference range: 125 - 220 U/l, markedly elevated
cellular blood components	Complete blood count (including differential blood count), reticulocyte count, Vitamin B12, folic acid, immunophenotyping, direct and indirect Coombs test, haptoglobin	Unremarkable but folic acid is mildly reduced (6,8 nmol/l, reference range: 7,9 – 46,4)
liver function parameters	aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatases (ALP), gamma-glutamyl transferase (GGT), bilirubin, serum electrophoresis	Unremarkable
thyroid gland	TSH (thyroid-stimulating hormone)	Unremarkable
kidney function parameters	potassium, creatinine, urine status	Unremarkable
coagulation studies	partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, D-dimer	Unremarkable
infection serology	cytomegalovirus (CMV), Epstein–Barr virus (EBV), human immunodeficiency virus (HIV), serology for hepatitis A, B and C, Treponema Pallidum hemagglutination assay, Plasmodia serology, ferritin, C-reactive protein	Unremarkable
autoantibody studies	antinuclear antibody (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), rheumatoid factor, anti-cyclic citrullinated peptides	Unremarkable
muscle studies	creatine kinase (CK), myoglobin, troponin T	Unremarkable
serum electrolytes	sodium, chloride, potassium, calcium	Unremarkable
blood sedimentation rate	80 mm after one hour, 100 mm after two hours	Unremarkable
other tests and evaluations	Physical examination, electrocardiogram, sonography of the abdomen, computed tomography scans of the thorax and abdomen	Unremarkable, except for asymptomatic cholecystolithiasis which should not cause LDH elevation

3. Method

PEG precipitation is performed using a modified method based on the procedure proposed by Davidson et al. [11]. 25 w/v % polyethylene glycol 6000 (Merck Millipore, Article # 817007) is prepared in 0,9 % sterile sodium chloride solution (Fresenius Kabi, free-flex infusion bag). 2 ml of patient serum is carefully mixed with 2 ml of 25 % PEG and incubated for 5 minutes at room temperature (verum sample). For the control specimen, 2 ml of patient serum is mixed with 2 ml of a 0,9 % sterile NaCl solution and incubated for 5 minutes at room temperature, resulting in a dilution of 1:1. The control and verum samples are centrifuged in Vacuette tubes with serum separator gel (Greiner Bio-One, Article # 455071) at a relative centrifugal force (RCF) of 1 521 for 8 minutes (Hettich Rotofix 32 A). The supernatant is transferred to a new Vacuette tube and sent for laboratory analysis. A third tube containing naïve blood serves as a reference and for the determination of folic acid concentration. The PEG precipitation process is displayed in the following flowchart.

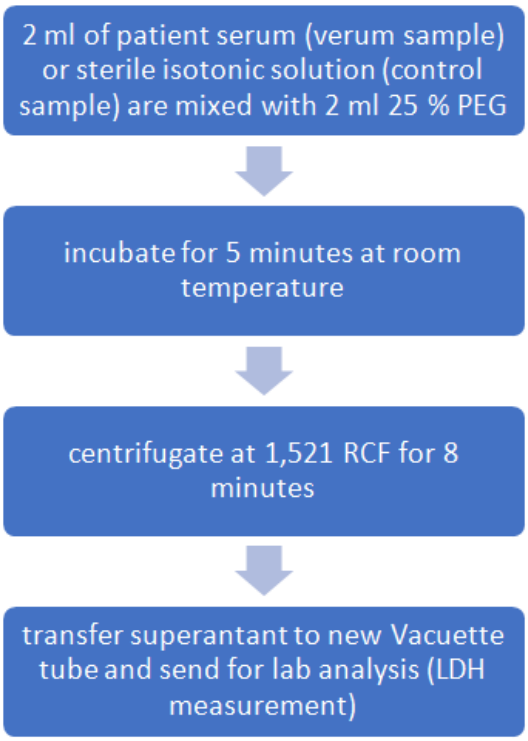

Figure 1. PEG precipitation process

PEG is used due to its ability to precipitate immunoglobins and their corresponding complexes, largely sparing uncomplexed serum enzymes [12].

Bürki et al. and Dedeene et al. [13,14] recently tried to establish reference values in healthy patients for PEG precipitation activities (PPA), a measurement for the serum enzyme activity after PEG precipitation. However, it should be noted that reference values vary significantly in the literature and appear to depend on analysis assays, lab machine used (Roche versus Abbott), precipitation protocol, and initial serum enzyme activity.

4. Results

Laboratory results are shown in the following table. Cholesterol and immunoglobin G (IgG) concentrations were assessed as control markers for successful PEG precipitation.

Serum gamma globulins in the naïve sample were in the normal range, as increased serum globulins may generate false-positive results [15].

Table 2. lab values of naive serum, after PEG precipitation and control serum

		Sample		
		Naïve	**PEG (verum)**	**0,9 % NaCl (control)**
Lab Value	**LDH (reference: 125 – 220 U/l)**	629 U/l	83 U/l	257 U/l
	Cholesterol (reference: 120 – 200 mg/dl)	202 mg/dl	25 mg/dl	101 mg/dl

IgG was not detectable in the verum sample and cholesterol was reduced by 75 % comparing control and verum; therefore, success of the PEG precipitation reaction is likely. The folic acid concentration was now normal with still elevated LDH, excluding the hypothesis of subclinical ineffective hematopoiesis due to folic acid deficiency.

The PEG precipitation activity (PPA) can be calculated using the following equation, indicating how much LDH is not complexed to another protein and therefore does not precipitate during PEG exposure [11,13]:

$$\%\ PPA = \frac{enzyme\ activity(naïve) - enzyme\ activity(verum) * 2}{enzyme\ activity(naïve)} * 100$$

The reference values for the lower limits of LDH PPA vary in the literature ranging from 45 % (99. percentile upper limit of normal) to 65 % [13,14]. Davidson et al. [11] propose a 12 – 70 % reference spectrum for PPA in patients without macro-LDH.

The PPA in the patient sample is 73,6 % and therefore above the upper normal limits for PPA, suggesting the presence of macro-LDH.

LDH-isoenzyme electrophoresis is performed and confirms the presence of macro-LDH using a second test method for verification.

5. Discussion

Macroenzymes develop when serum enzymes form complexes with serum proteins, especially immunoglobins. These complexes with higher molecular mass than uncomplexed enzymes lead to an elevated enzyme activity in laboratory assays due to increased serum half-life [10,16].

As a result macroenzymes can lead to misinterpretation of laboratory values, prompting rigorous patient evaluation for a possible benign condition [17].

Historically macroenzymes have been described as causative factors for elevated serum enzyme activities of amylase [18] and successively for AST, lipase, CK, and LDH [10,16]. LDH macroenzymes were first found in lupoid liver cirrhosis and leukemia in 1967 [19,20]. Cases of LDH elevation due to complex formation have also been described in patients with ulcerative colitis and myocarditis, in a post-burn patient, after interferon-alpha therapy for hepatitis C, after streptokinase therapy and after myocardial infarction. Hence they are usually associated with some kind of disease [19,20,21,22,23,24,25].

Nevertheless, there is also evidence, that macro-LDH formation is possible in totally healthy people: Perry et al. [8] presented a case of a healthy woman with a highly elevated serum LDH concentration of 4 457 U/l (normal range: 297 – 618 U/l), where LDH has formed a complex with immunoglobulin G to produce IgG-LDH. In 2011 Fillee et al. [26] described another case of a clinically healthy patient with an LDH concentration greater than two times the upper limit of normal, who had undergone splenectomy for assumed lymphomatous splenic lesions, only later to be diagnosed with LDH-macroenzyme condition presumably without clinical relevance.

Precipitation of macroenzyme complexes with polyethylene glycol 6000 and comparison of enzyme activity in the supernatant after centrifugation to an unprecipitated sample have been proposed as an easy-to-perform method in suspected cases of macroenzymes [11].

This method showed practical applicability and has already been used, for example, in a clinical case to prove the presence of AST-macroenzyme in a patient serum [27].

To our knowledge, this is the first case in which macro-LDH was suspected in a completely healthy young individual, and the PEG precipitation method was used first line in an outpatient setting for detection of an LDH enzyme complex.

6. Conclusion

In the case presented, extensive laboratory and radiological diagnostics had been performed before the possibility of macro-LDH was considered. After reviewing the literature, a procedure easy to perform in an outpatient setting and without laboratory equipment was found and performed, which demonstrated the existence of macro-LDH in the patient's serum.

In general, macroenzyme conditions are characterized by persisting increased serum enzyme activities. It is therefore suggested that the possibility of macroenzyme existence should be considered early in patients in whom elevated serum enzyme values do not match the clinical context. As the prevalence of macroenzymes remains without an exact figure, it is unclear whether withholding or delaying extensive diagnostic testing for other conditions (possibly including radiation exposure and not insignificant cost for patients or health systems) in otherwise healthy patients is reasonable. Further research is necessary to establish validated PPA reference values to enable a broad adaption of the PEG precipitation method in the low-threshold detection of most likely benign macroenzyme conditions.

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Conflicts of Interest

Patrik Wintersberger: Ongoing employment with MSD Animal Health, reimbursement of expenses by Recordati.

Jürgen Harauer: Nothing to declare.

Patient Consent

Informed consent from the patient for publication was obtained.

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