The Method Matters: Multiple Macroenzymes Detected in the Presence of Hypergammaglobulinemia

Sara P. Wyness¹, Sonia L. La’ulu¹, Michael Yee², Lorraine Tosiello³, Joely A. Straseski¹,⁴

¹ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT, ²SUNY, StonyBrook School of Medicine, StonyBrook, NY, ³Department of Medicine Jersey City Medical Center, Jersey City, NJ ⁴Department of Pathology, University of Utah, Salt Lake City, UT


ABSTRACT

Background: We report the finding of an individual with hypergammaglobulinemia, elevated creatinine kinase (CK), elevated liver enzymes, and amylase concentrations at the high end of the normal range. Patient denied any symptoms associated with these values (myalgia, fevers, rashes, chest pain, or muscle weakness). The elevated CK was determined to be due to immunoglobulin bound macro-CK type 1, thus macroenzymes were considered as a possible source of the elevated liver enzymes. The presence of multiple macroenzymes, and the possible role of hypergammaglobulinemia, has not been previously reported in the literature. Methods: Polyethylene glycol (PEG) precipitation and ultrafiltration (UF) were used to evaluate the presence of seven macroenzymes (alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), CK, lactate dehydrogenase (LD) and lipase (LIP)). Monomeric recoveries were determined by dividing the activity of the supernatant or ultrafiltrate by the neat activity and converting to a percent. Results were compared to previously reported reference intervals established in healthy populations. Results: PEG monomeric recoveries suggested the presence of 6 of the 7 macroenzymes tested (all but ALP). UF revealed the presence of three macroenzymes (CK, AST and AMYL). These results were observed on two separate occasions, 10 months apart. Previous data had indicated that UF was the more precise method of macroenzyme detection of CK, AST, AMYL, LD, and LIP (Wyness et al., Clin Chim Acta, 2010 and 2011). Conclusions: The macroenzymes identified by UF supported the clinical presentation of elevated CK and AST. MacroAMYL was also detected by UF, despite high-normal AMYL values. However, normal serum AMYL in the presence of macroAMYL is well documented. A previous report has shown that when globulins are present in excess, PEG may co-precipitate monomeric enzymes along with serum globulins, causing false-positive reporting of macroenzymes (Ram et al., Ann Clin Biochem, 2008). This mechanism may explain the discrepancy between PEG and UF results in the presence of hypergammaglobulinemia, making UF a better method of detection in these circumstances. The presence of multiple macroenzymes in a single patient is novel, however, the preferential use of UF needs to be confirmed in other hypergammaglobulinemic patients.

INTRODUCTION

- Macroenzyme: complex formed by self-polymerization or association with other plasma components such as immunoglobulins G and A [1].
- Several methods of macroenzyme detection exist:
  - Polyethylene glycol precipitation (PEG)
  - Ultrafiltration (UF)
  - Electrophoresis
  - Gel-filtration chromatography

- Hypergammaglobulinemia: increased concentrations of gamma globulins (predominately immunoglobulins) in the blood.
- A previous report has shown that in the presence of hypergammaglobulinemia [2] PEG may co-precipitate monomeric enzymes along with serum gamma globulins;
- This may result in false-positive macroenzyme reporting.

Hypothesis: Could hypergammaglobulinemia play a role in the development of multiple macroenzymes in a single patient?

MATERIALS AND METHODS

- PEG precipitation was performed using a PEG 8000 solution (250 g/L in PBS) in a 1:2 dilution with patient samples. Specimens were vortexed for a minimum of 30 seconds, incubated at room temperature for 10 minutes, then centrifuged at 10,000 x g for 5 minutes.
- UF was performed using Amicon Ultra-0.5 centrifugal filter units with Ultracel-100 membranes, 100 kDa molecular mass cutoff, from Millipore (Billerica, MA). Specimen (500 µL) was added to the sample reservoir, which was inserted into a filtrate vial. Specimens were centrifuged at room temperature for 18 hr at 150 x g.

DISCUSSION AND CONCLUSIONS

- PEG precipitation identified the presence of 6 of the 7 tested macroenzymes. As this seemed an unlikely result, an alternative method of macroenzyme detection was attempted (UF).
- UF identified the presence of 3 of the 7 macroenzymes tested. These correlated with the relevant clinical findings in this hypergammaglobulinemic patient:
  - Elevated CK and AST
  - AMYL at the high end of normal

- PEG precipitation of monomeric enzyme in the presence of excess serum globulins (hypergammaglobulinemia) has been reported previously [2].

- When hypergammaglobulinemia is present, the method of macroenzyme detection should be considered. Due to PEG co-precipitation concerns and previously reported precision data [3,4] UF may be the better method of detection in these cases.

- Additional studies of hypergammaglobulinemia and the presence of multiple macroenzymes are needed in order to confirm the relationship between these two states.

ACKNOWLEDGEMENTS

The ARUP Institute for Clinical and Experimental Pathology provided financial support.

REFERENCES