Screening for Macroamylase in a Community Hospital
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We evaluated the polyethylene glycol precipitation test (Gastroenterology 83: 378–382, 1982), looking for macroamylase in the serum of 66 patients whose values for serum amylase were above normal. Three patients (4.5%) were identified by this method as having macroamylase, and this was confirmed by gel-filtration chromatography and electrophoresis. We find this to be the best choice of a screening procedure for macroamylasemia because of its speed, simplicity, and apparent reliability. Diagnosis of macroamylasemia is important in preventing needless treatment and investigation for pancreatitis.

Additional Keyphrases: pancreatitis · screening · urinary amylase · misleading diagnostic sign

Macroamylasemia is a condition in which, for unknown reasons, normal circulating amylase binds to another serum protein to form a complex of higher molecular mass (1). Because this complex is relatively slowly cleared from the serum, an above-normal value for serum amylase is usually obtained. Clinically, its recognition is important to distinguish this condition from hyperamylasemia that is the result of pancreatic disease.

Previous methods for detecting macroamylasemia include ultracentrifugation, electrophoresis, and gel filtration chromatography—none of which is well-suited to routine laboratory use (1, 2). A recent polyethylene glycol precipitation (PEG) method (1) reportedly is rapid, simple, and appropriate for clinical testing. To examine this claim we undertook a study of patients in our hospital. We also wished to gain some idea of the prevalence of macroamylasemia in our population of patients with hyperamylasemia.

Materials and Methods

Serum amylase was determined by standard laboratory methods in an aca II discrete analyzer (Du Pont Co., Wilmington, DE 19898) or a centrifugal analyzer (Instruments Laboratory, Inc., Lexington, MA 02173). We saved serum specimens with supranormal values for amylase and tested them in a batch for macroamylase, using the PEG precipitation method. Serum was stored in the refrigerator if it was assayed within a week of collection, otherwise it was stored at −20 °C. Amylase activity after precipitation was determined in the centrifugal analyzer. All specimens that were positive for macroamylase by the PEG method were further tested by the gel filtration method (3) in our laboratory and sent to Mayo Medical Laboratories for confirmation by gel filtration (4) and agarose electrophoresis (5).

In the PEG precipitation method (1) a 120 g/L solution of polyethylene glycol 6000 is used to precipitate macroamylase selectively from serum, 0.2 mL of PEG (Eastman Kodak Co., Rochester, NY 14650) solution being added to one 0.2-mL aliquot of the serum and 0.2 mL of water to another. Both tubes are incubated at 37 °C for 10 min, then centrifuged (5000 × g, 10 min). Amylase activity in the supernates is determined by the usual methods. If 73% or more of the amylase activity is precipitated by PEG as compared with water, macroamylase is considered to be present (1). For amylase of normal molecular mass, less than 52% of amylase activity will be precipitated (1).

Results and Case Reports

Serum from three of 66 patients (4.5%) with hyperamylasemia was found positive for macroamylase by PEG precipitation and confirmed by gel filtration chromatography and agarose electrophoresis. The reported prevalence ranges from 0 to 10% (2). In all of these 66 patients, serum amylase assays were ordered by the attending physician to exclude the diagnosis of pancreatitis.

Case 1: A 33-year-old white woman was admitted with epigastric pain. At the time of admission, her serum amylase value was 164 U/L (normal 23–85), and her urinary amylase also was above normal, 97 U excreted per 2 h (normal 4–37 U/2 h). Four days later, urinary amylase had declined to 7 U/2 h, but the serum amylase activity remained above normal during hospitalization. On hospital day four, the ratio for amylase clearance/creatinine clearance was <1 (normal <4). Serum lipase activity was 23 U/L (normal <160 U/L). The patient's serum urea nitrogen concentration was 80 mg/L; the creatinine concentration was 9 mg/L. Of the total serum amylase, 82% was precipitated with PEG (normal <52%), a finding consistent with macroamylasemia. Hyperamylasemia had been detected in this patient during seven previous hospitalizations, and she had had intensive study, including endoscopic retrograde cholangiopancreatography, without confirmation of pancreatic disease. Serum amylase at followup two months later was 193 U/L (85% precipitated with PEG) and urinary amylase 3 U/2 h.

Case 2: A 61-year-old white woman was admitted because of postprandial gastrointestinal burning discomfort. Serum amylase was 228 U/L (96% precipitated with PEG) and urinary amylase was abnormal, 47 U/2 h. Her value for serum urea nitrogen was 90 mg/L, for creatinine 13 mg/L, and for lipase 58 U/L. The patient was treated with antibiotics and has remained well during the subsequent 11 months, at which time she was asymptomatic and had a serum amylase value of 347 U/L (93% precipitated with PEG).

Case 3: A 27-year-old black man was admitted with alcohol abuse and epigastric pain. The value for serum amylase was 226 U/L (97% precipitated with PEG), for urinary amylase 9 U/2 h. His serum urea nitrogen concentration was 80 mg/L, creatinine 11 mg/L, and lipase 67 U/L. The diagnosis of pancreatitis was excluded and a peptic ulcer was detected. The patient did not return for followup examination.

Discussion

Macroamylasemia is not a marker for any specific disease.
It is simply an incidental finding in many unrelated conditions or in apparently normal individuals. As evidenced by our patients and documented in the literature (2, 6, 7) it is most commonly found in patients who are experiencing abdominal pain and who are often diagnosed as having pancreatitis. Macroamylase assays were not requested in any of our three patients; pancreatitis was a principle diagnosis being considered or treated in all of these patients.

Three types of macroamylasemia have been recognized. Type I, the classical form, is characterized by high serum and low urinary amylase activity in the presence of a normal rate of excretion of creatinine. In type II, urinary amylase is above normal and the proportion of macroamylase to normal amylase in the serum is less than in type I. Thus, the traditional criterion that above-normal urinary amylase activity excludes macroamylasemia is invalid. Type III macroamylasemia is associated with normal amylase activity in both the serum and the urine (4); it has no known clinical significance.

We conclude that assay for macroamylase by the PEG method of Levitt and Ellis (1) is indeed rapid, reproducible, and suitable for routine use in the laboratory. The results from this method compared favorably with those by gel-filtration chromatography or electrophoresis. PEG precipitation thus is our method of choice for routine screening for macroamylase in this community-hospital laboratory.

References
1. Levitt MD, Ellis C. A rapid and simple assay to determine if macroamylase is the cause of hyperamylasemia. Gastroenterology 83, 378–382 (1982).

CLIN. CHEM. 30/5, 742–744 (1984)

Improved Assay of Unconjugated Estriol in Maternal Serum or Plasma by Adsorption and Liquid Chromatography with Fluorimetric Detection
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In this improved assay only a 500-μL sample is needed and a single assay can be done within 30 min, 10 samples within 90 minutes. The sample of serum or plasma is diluted 20-fold with water, and the estriol is adsorbed from it onto graphitized carbon black (Carbopack B, Supelco). After two washings the estriol is desorbed with chloroform/methanol (60/40 by vol), which then is evaporated. The residue is redissolved in 50 μL of water/acetonicile, and 20 μL is injected into the chromatograph. Analytical recovery for estriol-supplemented serum or plasma averaged 98.6%. Day-to-day CVs ranged from 3.9% at 2 μg/L to 2.1% at 20 μg/L. The limit of sensitivity is 0.3 μg/L, which makes this procedure suitable for determination of estriol even in the first half of pregnancy. Our method is inexpensive, and shows that liquid chromatography can be used to determine estriol in pregnancy serum or plasma. It also is more sensitive and precise and requires less sample than other such methods.

Additional Keyphrases: monitoring pregnancy • steroids

Since Gurpide et al. (1) first elaborated an immunoassay for determining estrogens in biological liquids, many radioimmunoassay (RIA) methods have been proposed for monitoring estriol in maternal serum, and this technique is currently the most widely used. Sensitivity, specificity, simplicity, and rapidity are features claimed for many RIAs. Specificity, however, is not so excellent as claimed. Sample purification by extraction with solvent or adsorbent before RIA improves accuracy, but at the expense of rapidity and simplicity (2, 3). Moreover, for clinical laboratories with a low demand for serum estriol assays the short shelf-life of the reagent makes these techniques uneconomical. Only a few years ago the only valid alternative technique for measuring estriol concentrations in maternal serum was gas chromatography with use of a capillary column (4). This time-consuming technique involves many steps in sample preparation and requires highly specialized technicians.

"High-performance" liquid chromatography (HPLC) is increasingly becoming more reliable and practicable for routine laboratory assays. During the course of our experiments, two papers appeared reporting determination of unconjugated estriol in pregnancy serum by HPLC (5, 6).

We describe here a simple procedure involving HPLC with fluorimetric detection, which has a sensitivity comparable with that of RIA methods and requires only 500 μL of serum or plasma. The sample is purified by adsorption onto graphitized carbon black (Carbopack B) (7–9).

Materials and Method

Instrumentation
We used a Series 3B liquid chromatograph equipped with a Model 650 S LC fluorescence detector having a 20-μL flow cell and a Rheodyne Model 7125 injector with a 20-μL loop