Serum Lecithin Level in Patients with Obstructive Jaundice

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Abstract

In this study, a colorimetric method for the determination of serum lecithin is examined using LPL, phospholipase C and glycerol dehydrogenase. The results showed that serum lecithin concentration in the obstructive jaundice patients was increased compared to that in the control group [185.47±16.12 (mean ±SD, no.=57) vs. 112.38±9.27 (mean ±SD), no.=39, P<0.05] mg/100 ml. The increase of serum lecithin in the patients with obstructive jaundice is a good indicator of liver disease, especially obstructive jaundice. Our results showed that measurement of lecithin is considered to be one of the most specific and sensitive methods for the detection of obstructive jaundice.

Keywords: Obstructive jaundice, lecithin, colorimetric method

Introduction

Phospholipids are essential structural component of plasma lipoprotein. They play important roles in the transport of cholesterol and triglycerides through plasma.1 The determination of serum phospholipids is an important clinical test in diagnosis of liver disease, especially obstructive jaundice.2 Serum phospholipid is increased in patients with obstructive jaundice.3 The variations of lecithin cholesterol acyltransferase activity caused by the degree of Jaundice.4 The decrease of serum bilirubin and the sequential changes of bile contents after relief of obstruction are quite similar in gallstone and tumor induced obstructive jaundice.5 Of the methods that have been developed for measuring phospholipids, those based on chemical analysis of phosphorus content are the most widely used.6,7 We were prompted to look for an alternative more rapid method, and report here a colorimetric method for measuring serum lecithin, which eliminates the need for acid digestion and color development. The purpose of this study was to determine a very sensitive biochemical indicator of obstructive jaundice patients.

Materials and Methods

Materials: Lipoprotein lipase, glycerol dehydrogenase, phospholipase C, NAD, Phenazine methosulfate (PMS), iodonitro tetrazolium chloride (INT), Glycerol Triton x-100, between 20 and lecithin were purchased from Sigma Chemical Company, St. Louis, Mo. USA. All of the chemicals used were guaranteed-grade reagents and were used without further purification. All solutions were prepared with distilled deionized water.

Color reagent

Iodonitro tetrazolium chloride (40mg), 10mg phenazine methosulfate, and 0.5mg between 20 were dissolved in 50ml distilled deionized water.

Standard solution

Lecithin was dissolved in a aqueous solution containing 5g Triton x-100 per liter.

Study population

Selected patients with obstructive jaundice admitted at Shaheed Yahyanejad Hospital in Babol were prospectively enrolled. For each patient, either the history of the clinical situation suggested the possibility of obstructive jaundice was done. Subjects were divided into the following two groups:

Group 1: The control group consisted of 106 healthy volunteers, 47 males and 59 females, aged 24-51 (mean 39.7±5.9) years, with no history of obstructive jaundice were used as a control group. These subjects were deemed free of clinical complication or medications on the basis of interviews. The laboratory data of this group were used as a reference.

Group 2: The study group consisted of 57 patients with obstructive jaundice, 31 males and 26 females, age between 18 to 72 years (mean 46.5±8.3) years. Fifty seven patients with obstructive jaundice underwent percutaneous transhepatic catheter drainage or endoscopic nasobiliary drainage were classified into two groups, depending on the cause of obstruction: Common bile duct stones (no.=22) and biliary tract tumors (no.=35). All patients in the gallstone group presented with acute cholangitis while only four patients in the
tumor group had a positive bacteria culture in bile. Fasting bile and serum were collected on the day of catheter placement and the 2nd, 4th, 6th, 8th and 10th day thereafter. The sequential changes of serum concentration of lecithin were checked and compared the two groups.

Methods

Reaction mixture: In 50ml of 0.15 M potassium phosphate buffer (pH=7.25) were dissolved 10000 units of LPL, 500 units of glycerol dehydrogenase, 50 mg of color reagent and 50 mg of triol x-100 to prepare reagent solution. 100 ml of a serum sample or a standard glycerol solution was incubated with 0.5 ml of phospholipase C (50U/ml) dissolved in 10mM Tris-HCl buffer (pH=7.25) at 37°C for 45min. Next, One ml of the above reagent solution was added to the reaction mixture, followed by incubation at 37°C for 10 min. Adding 5ml of 0.15mHCl stopped the reaction and the absorbance was read at 490nm in a spectrophotometer. A 1mM solution of NADH was freshly prepared in distilled deionized water. Aliquots of graded concentrations of NADH (0.0 - 200nmol) were reacted with the color reagent (0.5ml) and after color formed, 2.0ml of potassium phosphate buffer (pH=7.25) was added to each tube and the absorbance read at 490nm. The absorption maximum was found to be at 490 nm. It was also found that the reaction was completed within 30min at 37°C. The color development was stable for at least 30 min.

Statistical analysis

Sensitivity and specificity were calculated for lecithin relative to the clinical diagnosis. Receiver operating characteristic (ROC) curve was developed for lecithin. Student t-test was used for between group comparison of results. P<0.05 were considered to indicate statistical significance.

Results

The calibration curve showed linearity up to 300-mg serum lecithin/100ml, the line passing through the origin for lecithin (Fig 1).

Serum lecithin concentration in the obstructive jaundice patients group [185.47±16.12mg/ 100ml (mean±SD, n=57)] was increased compared to that in the control group [112.38±9.27], mg/100ml, n=39], p<0.05. Fifty-seven samples of human serum were analyzed by the proposed method, and the results compared with those obtained by the chemical method. As shown in Fig.3, the correlation between the two methods is excellent and its coefficient value is 0.97.

Furthermore, comparison of the proposed method with the enzymatic method \(^1\) was performed. The coefficient is 0.96 and the value is satisfactory as shown in Fig.4.

![Graph](image-url)
**Discussion**

Serum lecithin is more effective test for obstructive jaundice than alkaline phosphatase, since serum alkaline phosphatase is raised in the third trimester of pregnancy. Also, alkaline phosphatase is found in many cancers metastatic to bone, Pagets disease of bone, parathyroid disease with bone involvement, rickets and osteomalacia. Lecithin is the most sensitive and specific marker for obstructive jaundice. Lecithin as a test for the confirmation of an obstructive jaundice, would replace alkaline phosphatase as the "gold standard". We report here a simpler and more rapid method, which allows measurements of serum lecithin in the range 0.05-300mg/100ml. The assay is based on the formation of NADH due to enzymatic reaction of LPL, phospholipase C and glycerol dehydrogenase which is coupled to the reduction of the tetrazolium. Because of the low concentration of the lecithin in serum and due to assaying the concentration by measuring NADH formed at 340 nm is often not possible. A chemical method for lecithin determination used as a clinical test is an indirect assay which measures inorganic phosphorus derived from lecithin. Therefore, the chemical method requires the isolation of lecithin from serum and hydrolysis of it before determination. The application of enzymes for clinical tests have the advantage of determining one component in the presence of various components such as in serum or urine, on the basis of substrate specificity and catalytic action under mild condition. The correlation between our results with those obtained by the chemical and enzymatic methods is acceptable. The results confirm that the proposed method has characteristics of simplicity, precision and rapidity. The present method requires no procedures of extraction and isolation of lecithin from serum, since the addition of various compounds demonstrated that the proposed method was affected little or not at all. The proposed method can be satisfactorily employed as a routine clinical laboratory test and could be used in the concurrent determination of triglyceride and phospholipids in serum and adapted to an automatic analyzer system. Our results showed that serum lecithin is increased in patients with obstructive jaundice and were in good agreement with those reported previously. These findings suggest that lecithin has several advantages over traditional serological markers of obstructive jaundice. The unique properties of this marker and, in particular, its high diagnostic specificity (91%) and sensitivity (96%), the high concentration gradient between obstructive jaundice and normal blood. Finally, the determination of lecithin in combination with early markers of obstructive jaundice, such as alkaline phosphatase allows a reliable diagnosis of obstructive jaundice. The choice of lecithin as an innovative biochemical marker for the diagnosis of obstructive jaundice had led to the development to a new rapid assay that is very sensitive and highly specific.

**Reference**


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