Effects of Lipofundin® on the Measurement of Total Bilirubin by Spectrophotometry

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Abstract

Background: Jaundice occurs frequently in neonates and can cause severe neurological complications; hence, hyperbilirubinemia is usually monitored by direct spectrophotometry. However, lipemia, resulting from inborn disorders or parenteral feeding of preterm neonates with lipid emulsion, may interfere with certain laboratory assessments. Here, we evaluated whether artificial lipemia also interferes with bilirubin measurement by direct spectrophotometry.

Methods: Total bilirubin levels were assessed by the spectrophotometry when serial concentrations of Lipofundin®, medium-chain triglycerides, or a stabilizer solution, were added to cord blood samples from five full-term and five pre-term newborn infants.

Results: In blood specimens from ten neonates, spectrophotometry-determined bilirubin levels proportionally and significantly increased in the presence of Lipofundin® at least 1% v/v or 10% medium-chain triglycerides at least 10% v/v in all pre-term and full-term infants. The stabilizer solution caused no interference.

Conclusion: Lipofundin® in the cord blood interferes with spectrophotometric measurement of total bilirubin; this effect is mainly related to triglyceride levels and has implications for management of neonates with jaundice.

Keywords: Bilirubin; Lipofundin®; Medium-chain triglycerides; Spectrophotometer

Introduction

Neonatal jaundice is common in newborns, affecting nearly 70% of full-term and 80% of pre-term neonates during the first week of life [1]. Risk factors for neonatal jaundice include prematurity, breast feeding, ABO blood type and unrecognized maternal-fetal Rh incompatibility, infection, cephalohematoma, asphyxia, glucose-6-phosphate dehydrogenase deficiency, family history, and bruising at birth [2]. Simple and non-invasive phototherapy is the first approach to reducing high levels of bilirubin in neonates; if this is not successful, an exchange transfusion is required [3]. Given that neonatal jaundice, which is characterized by increased blood bilirubin levels, can reflect a physiological condition or a severe disease leading to brain damage [4-7], all newborns, especially pre-term infants, should be assessed for jaundice.

Pre-term and small-for-dates infants are particularly reliant on fats for normal body growth and development, as they are usually intolerant to carbohydrates and nitrogen; in the absence of sufficient fats, they could face an energy deficit at a time of very high energy demand. Typically, high-caloric fat solutions, which provide a significant portion of their daily caloric needs, are delivered intravenously; such parenteral feeding is indispensable during the first days or weeks of life due to the incomplete development of the gastrointestinal tract of these pre-term neonates. However, when premature infants and neonates are subjected to parenteral nutrition involving lipid emulsions, transient lipemia may occur, which may interfere with certain laboratory chemistry assays.

Bilirubin levels can be measured by direct spectrophotometry, diazotization method, high-performance liquid chromatography (HPLC), and enzymatic methods [8-10]. For neonates, methods that can provide fast and reliable results from very small volumes of whole blood are ideal. Direct spectrophotometry is such a method, and is both easy and fast to apply in a nursery. Simply, blood specimens, obtained from neonates by heel prick, are collected in capillary tubes, and after centrifugation, the serum is assayed using a spectrophotometer [11]. Bilirubin has a yellow color with a typical spectrographical peak at 460 nm. The decrease in intensity of light striking the detector in the spectrophotometer, after passing through the serum sample, is attributable to absorption by the sample, and the absorbance is proportional to the concentration of bilirubin in the serum [12].

However, lipemia has been reported to interfere with such light-based methodology [12,13]. To investigate the effect of lipemia on laboratory analysis of various serum substances, soy-based lipid emulsions have been used to simulate lipemia in interference studies [13,14]. For instance, Bornhorst et al. [15] added IntraLipid®; a commonly used synthetic lipid emulsion, to serum to simulate lipemic samples; their results indicated that laboratory evaluation of

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ceruloplasmin, prealbumin, and transferrin by light-based immuno-
turbidimetric assays rendered incorrectly low levels of these substances. However, lipemia alters light scattering in samples, caused by cloudiness or turbidity of the sample; therefore, in absorbance assays, levels of serum substances should increase, rather than decrease [12].

To determine whether the measurement of bilirubin levels was affected by excessive lipids, the following experiments were designed to show the effect of Lipofundin® and medium-chain triglycerides on the measurement of total bilirubin in the newborn infants by direct spectrophotometry.

**Material and Methods**

Ten cord blood specimens were randomly collected from 5 pre-
term and 5 full-term neonates between May and June 2011. The collection and use of cord blood specimens were approved by the Ethics Committee of the Tri-service general hospital, Taiwan (100-05-017). Oral consent was obtained from the parents of the neonates.

Before lipemia simulation test, all samples were first analyzed for total bilirubin using the diazotization method (Roche/Hitachi cobas c501 analyzer), which is not affected by lipemia [16], to provide reference for baseline data.

Lipofundin® (B. Braun Melsungen AG, D-34209 Melsungen, Germany), a commonly used synthetic lipid emulsion, is composed of medium-chain triglycerides, soybean oil, glycerol, egg yolk phospholipids, sodium oleat, and α-tocopherol. To assess the effects of these components of Lipofundin® on total bilirubin estimations, cord blood samples were diluted with 0, 1, 10, 20, 25, 50% volume of medium-chain triglycerides (Nu-Lipi®; Nutritec-Enjoy Nutrition Center Inc., Taipei, Taiwan; powder form, diluted 10% v/v in 0.9% normal saline), and a stabilizer solution, including 2.5% glycerol (1,2,3-Propanetriol, Glycerin, Sigma, Taipei, Taiwan), 0.02% α-tocopherol (1-α-Phosphatidylcholine, Sigma, Taipei, Taiwan), 1.2% egg lecithin (Sigma, Taipei, Taiwan), and a stabilizer solution, including 2.5% glycerol (1,2,3-Propanetriol, Glycerin, Sigma, Taipei, Taiwan), 0.02% α-tocopherol (Sigma, Taipei, Taiwan), and 0.03% sodium oleate (Sigma, Taipei, Taiwan). After centrifugation of the samples at 12000 rpm for 5 minutes, bilirubin levels were measured with a spectrophotometer (Leica UNISTAT® Bilirubinometer; Leica Microsystems, Buffalo, New York USA), at 454 nm and 540 nm (measurement at 2 wavelengths is used to diminish interference by hemoglobin). All the preparations and procedures were carried out at room temperature (22–25°C), and all test tubes used in this study were heparin-free.

Statistical analysis was performed using SPSS software (version 18; SPSS Inc., Chicago, IL, USA). Levels of total bilirubin for neonates before lipemia simulation test measured with the diazotization and the spectrophotometry method, were assessed using the paired t test. Levels of total bilirubin in the presence of variable volume of Lipofundin® 10% medium-chain triglycerides and the stabilizer solution were assessed using the repeated measures ANOVA.

**Results**

Ten newborn infants (GA range: 35 3/7 to 40 2/7 weeks, 5 females and 5 males, 5 full-term:case 1-5 and 5 pre-term: case 6–10) were enrolled in this study. Statistical analysis showed no significant difference in levels of total bilirubin measured with the diazotization method and the spectrophotometry before lipemia simulation test (Table 1) (p>0.05).

To assess the effects of Lipofundin® on the measurement of total bilirubin with light-based methodologies, the lipid emulsion was serially added to cord blood samples of full-term (case 1–5) and pre-term (case 6–10) neonates. Spectrophotometrically determined levels of total bilirubin in blood specimens from both pre-term and full-term infants were similarly increased in proportion to the increasing concentration of the Lipofundin®. Comparison the levels of total bilirubin between these groups showed no statistical significance (Figure 1) (p>0.05), and levels of the pre-term group were relatively higher than those of the full-term group.

When data of total bilirubin, measured by the spectrometry from the full-term group and pre-term group were pulling together, statistical analysis with repeated measures ANOVA showed significant when Lipofundin® was more than 1% v/v (Figure 2) (P<0.0001). Similarly, the presence of 10% medium-chain triglycerides also significantly interfered with the measurement of total bilirubin by spectrophotometry when 10% medium-chain triglycerides was more
than 10% v/v (Figure 3) (P<0.001).

To confirm that the interference effects of Lipofundin® were mainly related to that of medium-chain triglycerides, the stabilizer was serially added to cord blood samples. Results showed that the stabilizing solution caused no interference with the spectrophotometric measurement of total bilirubin (Figure 3).

Discussion

Direct spectrophotometry, using bilirubinometers, as well as diazotization methods are the two most common and popular assays for measuring serum bilirubin levels [17]. Total bilirubin levels, as measured using a diazotization method, vary with the concentration for measuring serum bilirubin levels [17]. Total bilirubin levels, as diazotization methods are the two most common and popular assays than 10% v/v (Figure 3) (P<0.001).

In conclusion, Lipofundin® and medium-chain triglycerides can interfere with light-based assays. To avoid infants receiving unnecessary phototherapy or even exchange transfusion, clinicians may need to exclude neonates with other potential conditions, such as hyperlipidemia or in parenteral nutrition involving lipid emulsions, which may interfere with certain laboratory chemistry assays, leading to false hyperbilirubinemia.

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References


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