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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 indispensible 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), 1.2% egg lecithin (L-α-Phosphatidylcholine, 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

Case	Sex	G A (week)	Total bilirubin (mg/dL) by spectrophotometry	Total bilirubin (mg/dL) by diazotization method	P >0.05
1	F	39 3/7	0.5	0.6	
2	M	40 1/7	1.0	1.2	
3	F	40 2/7	1.1	1	
4	M	38 2/7	1.2	1.4	
5	M	39 5/7	0.5	0.3	
6	M	36 4/7	0.9	1.4	
7	F	35 4/7	1.1	1.2	
8	F	36 4/7	1.0	0.9	
9	F	35 3/7	1.4	1.2	
10	M	36 1/7	0.6	0.8	

Table 1: Sex, gestational ages, and levels of total bilirubin measured with spectrophotometry and diazotation method from cord blood samples.

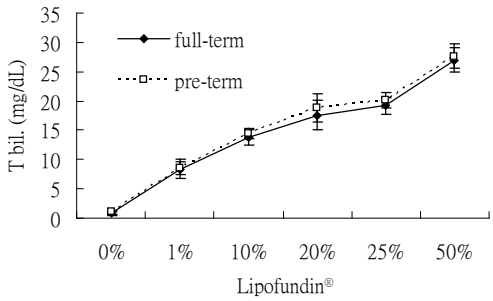

Figure 1: Spectrophotometrically determined levels of total bilirubin in blood specimens from both pre-term and full-term infants were similarly increased in proportion to the increasing concentration of the Lipofundin®.

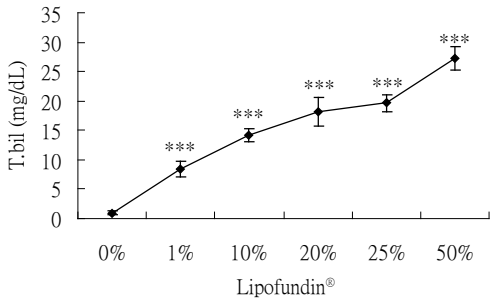

Figure 2: Influence of various concentration of Lipofundin® on total bilirubin levels in all cord blood samples, measured by the direct spectrometry. *** *p*<0.0005

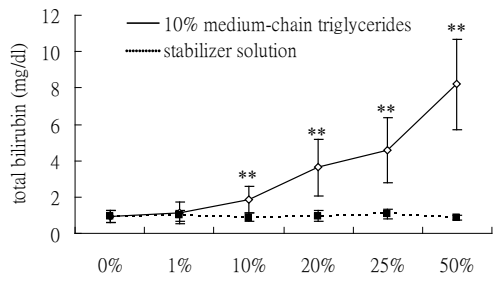

Figure 3: Influence of 10% medium-chain triglycerides and a stabilizer solution, including 2.5% glycerol + 1.2% egg lecithin + 0.02% α-tocopherol + 0.03% sodium oleate, on total bilirubin levels in all cord blood samples, measured with direct spectrometry. ** $p<0.005$

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 and type of the diazo reagent [18], the pH of the reaction mixture [19], and the duration of the reaction [20], but are not affected by the presence of lipemia [16]. Before the lipemia-simulation experiment, all blood specimens should be measured for the level of total bilirubin with a diazotization method to obtain a standard reference, and this reference should be similar to levels of total bilirubin, measured with the spectrometry method in the absence of lipid (Table 1).

Furthermore, as Doumas et al. [18], previously reported that blood samples collected with heparin almost invariably yielded turbid solutions, leading to inaccuracies in the measurement of absorbance, the tubes used in this study lacked a heparin coating. Additionally, because prematurity is a common cause of neonatal hyperbilirubinemia, it was necessary to rule out that the gestational age could confound the results of this study. In this study, we showed that Lipofundin® and medium-chain triglyceride caused interference with spectrophotometrically-determined bilirubin measurements in both pre-term and full-term samples (Table 1).

In this study, we clearly showed that the spectrophotometrically-determined levels of total bilirubin are falsely in proportion to the increase of Lipofundin® in the serum. Similarly, 10% medium-chain triglycerides also caused significant interference in this manner. Soybean oil, another component of Lipofundin® and a refined product containing neutral triglycerides, was expected to have the same effect as medium-chain triglycerides. Therefore, we did not evaluate soybean oil in this study.

Because a solution mimicking the stabilizer in Lipofundin® had no effect on bilirubin measurements (Figure 3), the main effects of Lipofundin® causing interference with spectrophotometrically-determined bilirubin measurements are due to the existence of medium-chain triglycerides. These lipids may form suspended particles that produce turbidity, leading to light scattering and thereby causing false increased measurements of absorption. Thus, our study shows that it is the concentration of triglycerides in the sample is the key *determining factor*.

Direct spectrophotometry is a much more appropriate method for measuring bilirubin levels in newborn infants. First, its execution is easy, and results can be obtained quickly using very small quantities of serum. Second, it is not affected by the variables associated with diazotization methods [18]. Third, no dilution or other chemistry is required to prepare the sample, so that the accuracy of the test is not affected by manipulation, and the unaltered sample is also available for further chemical analyses. However, despite these advantages, our results pointed out that the direct spectrophotometry is significantly influenced by lipemia, which is of special concern in premature infants and neonates that are submitted to parenteral nutrition using lipid emulsions to promote their rapid growth and address their high energy requirements, when they undergo a number of laboratory tests, multiple opportunities for artifactual abnormalities arise.

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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