Original Article

The Effect of Delayed Transportation of Blood Samples on Serum Bilirubin Values in Neonates

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Abstract

Background: **Delays in transporting blood samples may cause inaccurate results. Samples may be exposed to light or heat during delays, resulting in the degradation of analytes, for example, bilirubin. This study was done to determine the effect of delays in the transportation of blood samples on serum bilirubin test results.**

Methods: **Samples taken from neonates admitted to a tertiary hospital with jaundice were included in the study. The samples were collected through venipuncture in 3 labelled containers. The first container was sent immediately to the laboratory, while the second and third containers were sent after being kept in the ward for 1 and 3 hours, respectively. Bilirubin values were measured colourimetrically at a wavelength of 578 nm using a Roche Hitachi 912 Chemistry Analyser upon arrival in the laboratory.**

Results: **A total of 36 serum samples were studied. The mean of the indirect bilirubin measurements for 0-, 1-, and 3-hour samples were 174 (SD 68.65), 186.97 (SD 60.47), and 184.56 (SD 66.93), respectively. There was a significant difference in the mean indirect bilirubin measurement of 1-hour samples (*P* = 0.047, 95% CI -24.66 to -1.18) and 3-hour samples (*P* = 0.045, 95% CI -19.77 to -0.23) compared with 0-hour samples. There were no significant differences observed in either the mean total bilirubin or the mean direct bilirubin measurements of different time intervals.**

Conclusion: **This study confirms that delays in the transportation of blood samples influence the bilirubin test results.**

Keywords: *bilirubin, neonatal jaundice, transportation, time factors, specimen handling, medical sciences*

Introduction

Delay in the transportation of blood samples to the laboratory may cause alteration in test results (1,2). Of particular concern is the testing of serum bilirubin level. Unconjugated bilirubin is known to be sensitive to light, and although it is hypothesized that serum bilirubin levels decrease with delays in sample transportation to the laboratory (3), the effects of such delays on actual serum bilirubin level have not been studied.

Accurate testing of serum bilirubin level is important, especially in the treatment of neonatal jaundice since bilirubin level often dictates decision to initiate or discontinue treatment (3). Inaccurate testing of bilirubin level may cause under-treatment, putting the neonates at a risk of developing bilirubin encephalopathy; or over-treatment, leading to unnecessary separation of the baby and the mother (4).

If delays in the transportation of the blood sample systematically cause a decrease in serum bilirubin level, it may be worthwhile to undertake a large trial to generate a formula that would allow for the correction of bilirubin level if there was an inadvertent delay in transportation of sample to the laboratory. This study was performed with the aim of determining the effects of a delay in the transportation of blood samples on serum bilirubin values in neonates.

Materials and Methods

The study was conducted at the Neonatal Intensive Care Unit (NICU) and Special Care Nursery (SCN) at Hospital Universiti Sains

Malaysia (HUSM) from May 2007 until July 2007. Neonates requiring serum bilirubin values were included in this study. Written informed consents were obtained from the parents or guardians.

Blood sample of each neonate was collected through venipuncture into 3 separate containers, labelled A, B, and C. Container A was sent to the laboratory immediately after blood collection. The containers labelled B and C were sent after 1- and 3-hour delays, respectively. A dedicated person ensured timely delivery of the samples to the laboratory, and care was taken in the laboratory to perform the test upon arrival of the samples. Containers B and C were kept on a table in the ward while waiting to be sent to the laboratory. Each neonate was subjected to only 1 blood taking in this study.

Total bilirubin was determined by the colourimetric method using Roche Hitachi 912 Chemistry Analyser (Japan) bought by HUSM in 1999. Bilirubin level was determined based on the method described by Jendrassik and Groft (5), which involved the reaction of bilirubin with diazotised sulphanilic acid (for direct bilirubin measurement) in the presence of caffeine benzoate(for total bilirubin measurement). Caffeine benzoate was used to split the bilirubin–protein complex, thereby enabling bilirubin reaction with diazotised sulphanilic acid. The reaction was measured at a wavelength of 578 nm using the Roche Hitachi 912 Chemistry Analyzer. Indirect bilirubin level was calculated from the difference between total and direct bilirubin measurements (6).

The required sample size was calculated using the sample size for the paired t test equation in the Power and Sample Size Calculation PS Software (Version 2, 2003). Sample size calculation was done by using the mean and standard deviation of total bilirubin from a study by Wilson et al. (7). A sample size of 36 neonates was needed to achieve a power of 80% with a significance level of 0.05.

Data entry and analysis were performed using SPSS version 11.0 (SPSS Inc, Chicago, IL). The mean and standard deviation of serum bilirubin values for each delay interval were calculated. Differences in total bilirubin, direct bilirubin, and indirect bilirubin levels between the 0- and 1-hour, as well as 0- and 3-hour delay intervals were determined by a paired t test. The study was approved by the Ethical Committee and Research Universiti Sains Malaysia, Kubang Kerian, where the research was done.

Results

A total of 36 serum samples were studied. All samples reached the laboratory within 5 minutes of the planned timing and contained sufficient blood for testing. The mean and standard deviation for total bilirubin, direct bilirubin, and indirect bilirubin levels at different hours are presented in Tables 1, 2, and 3, respectively.

There was a significant difference in the indirect bilirubin measurements of 1-hour samples (P = 0.047) and 3-hour samples (P = 0.045) in comparison with 0-hour samples (Table 3). There were no statistically significant differences observed in the total bilirubin (Table 1) and direct bilirubin (Table 2) measurements taken at different delay intervals. The tests of assumption for the paired t test were not violated.

It was observed that 17% of the results after the 1-hour delay and 14% of the results after the 3-hour delay in transportation were very similar to the results of the samples sent immediately after sampling (Figures 1 and 2). On the other hand, 38% of the results after the 1-hour delay and 32% of the results after the 3-hour delay showed decrease in values compared with non-delayed samples, and 45% of the results after the 1-hour delay and 54% of the results after the 3-hour delay showed increase in values. The highest increases in the results after the 1-hour and 3-hour delays were 142 mmol/L and 164 mmol/L, respectively, while the greatest decreases were -28 mmol/L and -35 mmol/L, respectively.

Discussion

This is the first study undertaken to determine whether a delay in the transportation of neonatal blood samples for the measurement of bilirubin level would systematically affect the results. It was found that for some patients, delays in the transportation of bilirubin samples to the laboratory had no major effect on the treatment plan, especially on deciding the need for phototherapy. However, for a substantial minority of patients, the results were markedly different after a delay in the transportation of the samples, resulting in significant differences in the mean indirect bilirubin values between the immediate samples and the delayed samples.

It is impossible to predict which patient will still have reliable bilirubin measurement after a delay in sample transportation. Increase in bilirubin level after a delay could be due to

Table 1: Total bilirubin levels in different delay intervals (*n = 36*)

	Mean (SD)	Mean difference (95% CI)#	*t* value (P value)#
0-hour	179.03 (69.73)	-	-
1-hour	189.72 (60.03)	-10.69 (-23.22 to 1.83)	-1.733 (0.092)
3-hour	186.75 (67.98)	-7.72 (-18.32 to 2.87)	-1.480 (0.148)

Comparison with 0-hour

Table 2: Direct bilirubin levels in different delay intervals (*n = 36*)

	Mean (SD)	Mean difference (95% CI)	*t* value (P value)#
0-hour	4.75 (4.82)	-	-
1-hour	3.61 (4.66)	1.14 (-2.64 to -0.37)	1.536 (0.133)
3-hour	3.86 (4.21)	0.89 (-2.77 to -0.99)	0.966 (0.343)

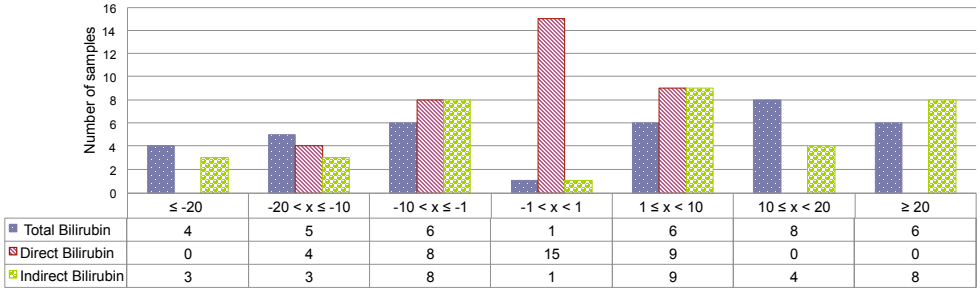
Comparison with 0-hour

Table 3: Indirect bilirubin levels in different delay intervals (*n = 36*)

	Mean (SD)	Mean difference (95% CI)#	*t* value (P value)#
0-hour	174.56 (68.65)	-	-
1-hour	186.97 (60.47)	-12.42 (-24.66 to -1.18)	-2.059 (0.047)*
3-hour	184.56 (66.93)	-10.00 (-19.77 to -0.23)	-2.078 (0.045)*

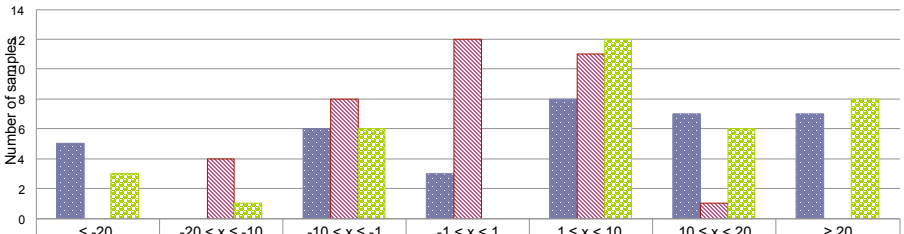
Comparison with 0-hour

* Significant difference observed ($P < 0.05$)



	≤ -20	-20 < x ≤ -10	-10 < x ≤ -1	-1 < x < 1	1 ≤ x < 10	10 ≤ x < 20	≥ 20
Total Bilirubin	4	5	6	1	6	8	6
Direct Bilirubin	0	4	8	15	9	0	0
Indirect Bilirubin	3	3	8	1	9	4	8

Figure 1: Differences in total, direct, and indirect bilirubin levels (mmol/L) between 0- and 1-hour (n=36)



	≤ -20	-20 < x ≤ -10	-10 < x ≤ -1	-1 < x < 1	1 ≤ x < 10	10 ≤ x < 20	≥ 20
Total Bilirubin	5	0	6	3	8	7	7
Direct Bilirubin	0	4	8	12	11	1	0
Indirect Bilirubin	3	1	6	0	12	6	8

Figure 2: Differences in total, direct, and indirect bilirubin levels (mmol/L) between 0- and 3-hour (n=36)

haemolysis before the sample was processed (1). Decrease in serum bilirubin level could be explained by the breakdown of bilirubin due to light exposure (3). Developing a formula to adjust for delays in transportation does not seem to be feasible considering the erratic differences in bilirubin level. Inevitable delays in analysing the sample can also occur in the laboratory despite timely arrival of the samples. As such, strict measures were taken in this study to analyse the samples immediately upon arrival in the laboratory.

Conclusion

There was no consistent trend towards the increase or decrease in serum bilirubin level in delayed transportation of the sample to the laboratory. However, in a substantial number of cases, the decision to start or discontinue phototherapy could be altered due to erroneous test results that may be caused by such delays.

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Authors' contributions

Conception and design: PS, KV, NR, JO, HVR
Provision of study materials or patients: PS, MRM
Analysis and interpretation of data: PS, KV, JO, HVR
Statistical expertise: KV
Drafting of the article: PS, NR
Critical revision of the article: PS, NR, JO, HVR
Final approval of the article: HVR
Administrative, technical, or logistic support: PS, MRM

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