ORIGINAL ARTICLE

# Assessment of Response to Phototherapy in Neonatal Jaundice in a Tertiary Care Hospital in West Bengal, India

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## ABSTRACT

Most of the neonates suffer from unconjugated hyperbilirubinemia due to rapid destruction of fetal haemoglobin. This condition is successfully treated by phototherapy. A descriptive, cross-sectional study was conducted in the Department of Biochemistry, College of Medicine and Sagore Dutta Hospital, West Bengal, India, in collaboration with Department of Pediatrics, to compare unconjugated bilirubin versus total bilirubin in neonates over the course of treatment and to determine which one is best prognostic marker in phototherapy. Blood samples were collected from 148 neonates with jaundice undergoing phototherapy for unconjugated hyperbilirubinemia on commencement of phototherapy i.e., 3rd and 5th, 7th days of commencement of phototherapy and their unconjugated bilirubin, conjugated bilirubin and total bilirubin were measured using micro slide technology on Vitros 250 Dry Chemistry analyzer. All the neonates were thoroughly clinically evaluated to monitor the prognosis of phototherapy. Over the days, mean values of unconjugated bilirubin of the 1st, 2nd and 3rd samples of neonates showed a significant decrease ($p<0.001$), when baby undergoes successful phototherapy; however, the total bilirubin showed no significant change ($p>0.05$). In case of delta bilirubin, we did not find any statistically significant findings in any within group or between group comparisons ($p>0.05$).

**Keywords:** Total Bilirubin, unconjugated Bilirubin, delta Bilirubin, phototherapy, neonatal jaundice

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## INTRODUCTION

Bilirubin is a breakdown product of heme. In phagocytes of the reticuloendothelial system (in liver & spleen), the enzyme heme oxygenase converts heme to biliverdin and carbon monoxide. Biliverdin is then reduced to bilirubin by biliverdin reductase. Bilirubin formed in the reticuloendothelial cells is insoluble in water. The lipophilic bilirubin is therefore transported in plasma bound to albumin. Albumin binds bilirubin in loose combination. So when present in excess, bilirubin can easily dissociate from albumin. The binding sites for bilirubin on albumin can be occupied by aspirin, penicillin, etc. Such drugs can, therefore, displace bilirubin from albumin. Hence, care should be taken while administering such drugs to newborn babies to avoid kernicterus[1].

The liver plays the central role in the further disposal of bilirubin. Inside the liver cell, the bilirubin is conjugated with glucuronic acid, to make it water soluble. About 80% molecules are in the diglucuronide form, while 20% are monoglucuronides. The water soluble conjugated bilirubin is excreted into the bile by an active process and this occurs against a concentration gradient. This is the rate limiting step in the

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catabolism of heme. Bilirubin is excreted subsequently through bile & urine[1].

During in vivo life, the developing fetus requires a high hemoglobin concentration to extract oxygen effectively from maternal blood and deliver it to fetal tissues. Immediately after birth, with abundant supply of oxygen from its own lungs, this requirement drops dramatically. The neonate's reduced red cell lifespan compared with adults' means that all newborns have a considerable excess load of hemoglobin to convert into bilirubin and excrete in the immediate postnatal period. Following delivery, a significant proportion of neonates may have additional hemoglobin to be disposed of as a result of bruising or other losses. Heme oxygenase, the enzyme responsible for breakdown of heme is further induced by inflammatory mediators, leading to a further increase in heme breakdown in premature infants with co-morbidities, such as respiratory distress syndrome & sepsis. With such an exceptional load on an immature hepatic conjugation system, it is therefore not surprising that approximately half of all infants become jaundiced in the first week of life.

Physiological Jaundice is also called as neonatal hyperbilirubinemia. In all newborn infants after the 2nd day of life, mild jaundice appears. This transient hyperbilirubinemia is due to an accelerated rate of destruction of RBCs and also because of the immature hepatic system of conjugation of bilirubin. In such cases, bilirubin does not increase above 5 mg/dl. It disappears by the second week of life.

In some breast-fed infants, prolongation of the jaundice has been attributed to high level of an estrogen derivative in maternal blood, which is excreted through the milk. This would inhibit the glucuronyl transferase system. Sulpha and such other drugs may release bilirubin from albumin, and may cause jaundice in newborn[2].

When blood level of bilirubin is more than 20 mg/dl, the capacity of albumin to bind bilirubin is exceeded. In young children before the age of 1 year, the blood-brain barrier is not fully matured, and therefore free bilirubin enters in the brain (Kernicterus).It is deposited in brain, leading to mental retardation, fits, toxic encephalitis and spasticity[3,4]. Phototherapy with blue light (440 nm wave length) isomerizes the insoluble bilirubin to more soluble isomers. These can be excreted through urine without conjugation[5-7].

Universal screening of unconjugated bilirubinemia is recommended by experts worldwide. Several different methods for its estimation are available now days but satisfactory and appropriate results might not be obtained due to interference of delta bilirubin. Delta bilirubin is the conjugated bilirubin covalently bound to Albumin and it stays for a prolonged period in the circulation (approx.. 21 days).[8] In majority of the laboratories; unconjugated bilirubin is measured indirectly after subtracting direct bilirubin from total bilirubin. Dry chemistry based methods excludes delta fraction from total bilirubin and exclusively measures Unconjugated fraction which aids the treatment.

## METHODS

The descriptive, cross-sectional study was done on 148 newborns suffering from neonatal jaundice admitted into the College of Medicine & Sagore Dutta Hospital, Kamarhati, West Bengal, India, between March 2022 and February 2025.

We included clinically icteric neonates admitted in post natal ward and SNCU suffering from exclusively neonatal hyperbilirubinemia undergoing phototherapy during the study period. However, neonates suffering from sepsis (based on clinical and laboratory parameters), NCS/IEM/TORCHES related neonatal jaundice and babies born to HepB/HIV/COVID positive mothers were excluded from the study.

Blood samples were collected from admitted neonates undergoing phototherapy on their day 3, day 5 and day 7 of life. 2 ml of Blood was drawn in clotted vial following that it was centrifuged and serum obtained were used for the estimation of unconjugated, direct and total bilirubin using micro slide technology on Vitros 250 Dry Chemistry analyzer (Ortho Clinical Diagnostics, USA).

Data obtained was scrutinized, compiled and analyzed using SPSS software version 23.0 for Windows. One way ANOVA and paired t-test were applied. A p-value $<0.05$ was considered statistically significant. Results obtained were arranged in tabular forms.

## RESULTS

We were able to collect the data of all 148 newborn samples on day 3 after initiation of phototherapy after being diagnosed of neonatal hyperbilirubinemia. We could be able to follow up them on day 5 (after 48 hours of initiation of phototherapy) also; but among 148 newborn only 98 babies could be followed on day 7. From the descriptive statistics (Skewness and kurtosis) it is clear that unconjugated bilirubin (day3, day 5, day 7), total bilirubin (day3, day 5, day 7), delta bilirubin (day3, day 5, day 7) are following

normal distributions of data. Their skewness are well between +/- 1 and Kurtosis +/- 2. But the conjugated bilirubin (day3, day 5, day 7) is not following the normal distribution as skewness and kurtosis are beyond the acceptable range for parametric distribution. It is quite explainable as liver conjugation process is very poorly developed in neonates at their earlier days and also if differ from one baby to another and also it doesn't have any clinical significance in management and prognosis of neonatal hyperbilirubinemia. So we are exempting conjugated bilirubin from our further analysis and only concentrating on unconjugated bilirubin (day3, day 5, day 7), total bilirubin (day3, day 5, day 7), delta bilirubin (day3, day 5, day 7) (Table 1). From ANOVA (post hoc analysis) test, while comparing unconjugated bilirubin within and between groups we found significant statistical difference between the mean values ($p<0.001$). But in case of total bilirubin, we only found statistically significant difference between the mean values of day 3 (at 0 hours of initiation of phototherapy) and day 7 (96 hours of phototherapy) ($p<0.05$). Other between groups comparison didn't show any statistically significant findings. In case of delta bilirubin, we did not find any statistically significant findings in any within group or between group comparisons (Table 2). By paired t-test we found statistically significant difference and good correlation between mean values of three groups (mentioned in the table) of unconjugated bilirubin ($p<0.001$). None of the groups of total bilirubin showed statistically significant difference of mean values or good correlation (Table 3).

**Table 1:** Descriptive statistics of day 3 samples (at 0 hours initiation of phototherapy), day 5 (48 hours after phototherapy) day 7 (96 hours after phototherapy)

| Name of the parameter | Total no of samples | Mean±SD (mg/dl) | Range at 95% confidence interval (mg/dl) | Skewness | Curtosis |
|---|---|---|---|---|---|
| Unconjugated bilirubin on day 3 (at 0 hours initiation of phototherapy) | 148 | 13.52±2.42 | 6.70–21.00 | 0.170 | 0.766 |
| Unconjugated bilirubin on day 5 (48 hours after initiation of phototherapy) | 148 | 12.08±2.35 | 7.30–21 | 0.874 | 1.48 |
| Unconjugated bilirubin on day 7 (96 hours after initiation of phototherapy) | 98 | 11.56±2.46 | 5.90–18.30 | 0.049 | 1.12 |
| Conjugated bilirubin on day 3 (at 0 hours of initiation of phototherapy) | 148 | 0.174±0.21 | 0.0–1.60 | 3.89 | 18.34 |
| Conjugated bilirubin on day 5 (48 hours of initiation of phototherapy) | 148 | 0.172±0.22 | 0.10–1.90 | 5.039 | 31.705 |
| Conjugated bilirubin on day 7 (96 hours of initiation of phototherapy) | 98 | 0.20±0.24 | 0.01–1.0 | 2.674 | 6.482 |
| total bilirubin on day 3 (at 0 hours of initiation of phototherapy) | 148 | 14.72±2.70 | 6.80–21.90 | - 0.007 | 0.322 |
| total bilirubin on day 5 (48 hours after initiation of phototherapy) | 148 | 13.28±2.48 | 8.30–21.7 | 0.584 | 0.691 |
| total bilirubin on day 7 (96 hours after initiation of phototherapy) | 98 | 12.88±2.73 | 6.40–19.5 | -0.178 | 0.877 |

| Name of the parameter | Total no of samples | Mean±SD (mg/dl) | Range at 95% confidence interval (mg/dl) | Skewness | Curtosis |
|---|---|---|---|---|---|
| Delta bilirubin on day 0 (at 0 hours of initiation of phototherapy) | 148 | 1.03±0.62 | 0.0–2.8 | 0.483 | 0.039 |
| Delta bilirubin on day 5 (48 hours after initiation of phototherapy) | 148 | 0.999±0.55 | 0.0–2.6 | 0.329 | -0.088 |
| Delta bilirubin on day 7 (96 hours after initiation of phototherapy) | 98 | 1.05±0.72 | 0.0–3.90 | 0.78 | 0.59 |

**Table 2:** One way ANOVA within groups of unconjugated bilirubin (day3, day 5, day 7)

| Variable | | F value | Significance |
|---|---|---|---|
| Unconjugated Bilirubin | Between groups | 17.832 | 0.000 ** |
| | Within groups (Post Hoc analysis) | | 0.0 |
| | Between Day 3 (0 hour) & day 5 (48 hours) | | 0.001 ** |
| | Between Day 5 (48 hours) & day 7 (96 hours) | | 0.000 ** |
| | Between Day 3 (0 hour) & day 7 (96 hours) | | 0.000** |
| Total bilirubin | Between groups | 4.511 | 0.07 |
| | within groups (Post Hoc analysis) | | |
| | Between Day 3 (0 hour) & day 5 (48 hours) | | 0.10 |
| | Between Day 5 (48 hours) & day 7 (96 hours) | | 0.08 |
| | Between Day 3 (0 hour) & day 7 (96 hours) | | 0.046 |
| Delta bilirubin | Between groups | 0.546 | 0.540 |
| | within groups (Post Hoc analysis) | | |
| | Between Day 3 (0 hour ) & day 5 (48 hours) | | 0.776 |
| | Between Day 5 (48 hours) & day 7 (96 hours) | | 0.871 |
| | Between Day 3 (0 hour) & day 7 (96 hours) | | 0.567 |

**Difference was statistically significant at p-value <0.05.

**Table 3:** Paired t-test for unconjugated and total bilirubin between three different groups depending upon duration of phototherapy

| Variable | Name of the comparing group | T value | Correlation | Significance |
|---|---|---|---|---|
| Unconjugated bilirubin | Between Day 3 (0 hour ) & day 5 (48 hours) | 7.908 | 0.567 | 0.006 ** |
| | Between Day 5 (48 hours ) & day 7 ( 96 hours) | 10.235 | 0.845 | 0.000** |
| | Between Day 3 (0 hour) & day 7 (96 hours) | 10.789 | 0.896 | 0.000 ** |
| Total bilirubin | Between Day 3 (0 hour ) & day 5 (48 hours) | 1.368 | 0.236 | 0.78 |
| | Between Day 5 (48 hours ) & day 7 ( 96 hours) | 2.089 | 0.312 | 0.1 |
| | Between Day 3 (0 hour) & day 7 (96 hours) | 2.712 | 0.382 | 0.06 |

**Difference was statistically significant at p-value <0.05.

## DISCUSSION

Neonatal jaundice remains one of the most common clinical conditions requiring medical intervention in the early neonatal period. Phototherapy is the standard and most widely used treatment for unconjugated hyperbilirubinemia. In the present study, the response to phototherapy was evaluated by measuring changes in total bilirubin and unconjugated bilirubin levels, providing insight into both the effectiveness of treatment and the biochemical behaviour of bilirubin fractions during therapy.

Our findings demonstrate a significant reduction, though statistically not significant in total bilirubin levels following phototherapy. The decline in unconjugated bilirubin was more pronounced than that of total bilirubin, reflecting the primary mechanism of phototherapy, which targets unconjugated bilirubin by converting it into water-soluble photoisomers that can be excreted without hepatic conjugation[9]. The observed reduction in unconjugated bilirubin is clinically important, as this fraction is responsible for bilirubin neurotoxicity. Elevated unconjugated bilirubin can cross the immature blood–brain barrier and potentially lead to bilirubin-induced neurologic dysfunction. Therefore, monitoring unconjugated bilirubin levels alongside total bilirubin may provide a more accurate assessment of neurotoxicity risk and therapeutic response than total bilirubin alone. Similar observations have been reported by Amin and Lamola, who emphasized the importance of bilirubin binding and unbound fractions in neonatal jaundice management[10].

In our study, the rate of bilirubin decline varied among neonates, which may be attributed to differences in gestational age, birth weight, baseline bilirubin levels, hematocrit, and albumin concentration. Preterm and low-birth-weight neonates are known to have reduced bilirubin-binding capacity and immature hepatic function, potentially influencing their response to phototherapy[11]. Another important observation was that the decline in unconjugated bilirubin closely paralleled clinical improvement in jaundice. This supports the utility of unconjugated bilirubin measurement as a valuable laboratory parameter for monitoring treatment efficacy. While total bilirubin measurement remains the standard practice due to its availability and simplicity, reliance solely on total bilirubin may underestimate the ongoing neurotoxic risk in certain clinical situations, such as hypoalbuminemia or hemolytic disease. The results of this study also highlight the importance of accurate bilirubin measurement methods. Analytical variability between assays can influence clinical decision-making, particularly at critical treatment thresholds. Therefore, standardized and reliable laboratory methods for both total and unconjugated bilirubin estimation are essential for optimal neonatal care.

Delta bilirubin, a covalently bound bilirubin–albumin complex, exhibited a different kinetic pattern compared with unconjugated bilirubin. Unlike unconjugated bilirubin, delta bilirubin showed minimal or delayed reduction following phototherapy. This finding is expected, as delta bilirubin is tightly bound to albumin and has a longer half-life corresponding to albumin turnover rather than bilirubin metabolism. Phototherapy does not directly affect delta bilirubin, and its persistence contributes to sustained elevations in measured total bilirubin despite clinical improvement[8]. The persistence of delta bilirubin has important clinical implications. In some neonates, total bilirubin levels may remain elevated even after effective phototherapy due to the presence of delta bilirubin, potentially leading to misinterpretation of treatment response. Our findings highlight that reliance on total bilirubin alone may overestimate residual hyperbilirubinemia and prompt unnecessary continuation of phototherapy. In contrast, unconjugated bilirubin levels more accurately reflected the immediate therapeutic response and reduction in neurotoxicity risk.

Furthermore, delta bilirubin accounted for a larger proportion of total bilirubin in neonates with prolonged jaundice or higher baseline bilirubin levels. This observation aligns with previous studies demonstrating that delta bilirubin becomes more prominent when conjugated bilirubin levels are elevated or when hyperbilirubinemia persists. Although delta bilirubin itself is not considered neurotoxic due to its strong albumin binding, its contribution to total bilirubin underscores the importance of fractionated bilirubin analysis in neonatal jaundice[11]. The inclusion of delta bilirubin measurement also enhances laboratory interpretation, particularly when evaluating rebound bilirubin levels after discontinuation of phototherapy. Persistently elevated total bilirubin due to delta bilirubin may not warrant further intervention if unconjugated bilirubin levels remain within safe limits. Therefore, fractionated bilirubin assessment can support more precise clinical decision-making and reduce overtreatment[11].

It is quite logically explainable as delta bilirubin being albumin bound fraction of bilirubin, its clearance depends on the half-life of albumin and take time to clear out from the circulation[8]. Total bilirubin is a summation of unconjugated, conjugated, delta fraction of bilirubin. Though we can measure it directly but change in total bilirubin with earlier day of phototherapy often cannot be appreciated properly as conjugated and delta fraction do not change that fast as unconjugated one.

Though a significant difference of mean values of total bilirubin between day 3 and day 7 (found by Post hoc ANOVA) is possible because there is a statistically significant difference in unconjugated bilirubin values; we did not have that statistical significant difference or correlation while performing paired T test. Hence, as a prognostic marker of phototherapy if we become able to measure unconjugated fraction instead of total bilirubin we can appreciate the change at very early stage of phototherapy (as soon as with-in 48 hours) and also with time which we couldn't find in case of total bilirubin.

### Limitations

1. In the present study, we could only be able to follow up 98 babies on Day 7 of photo therapy, if we could follow up all the 148 babies statistical analysis would have been more robust.
2. We could not perform inter instrument analysis (e.g., dry chemistry vs wet chemistry platform) due to inadequacy of serum as from a new born we can't draw sufficient blood to perform the tests in different instrument. Inter instrument comparison could add new dimensions (comparisons of sensitivity, specificity) to this study.

## CONCLUSION

Unconjugated bilirubin was significantly reduced in the three groups of neonates after they underwent phototherapy. This could further aid in the treatment showing that phototherapy was successful in them and reduce the hospital stay. Total bilirubin, on the other hand showed no significant decrease among the three groups. Thus, Vitros dry chemistry platform allows us to measure the unconjugated fraction separately proving to be a game changer for treatment of neonatal bilirubinemia.

**Conflict of interest:** The authors would like to declare that there was no conflict of interest related to the study.

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**Ethical approval:** Ethical clearance was obtained from the Institutional Ethical Committee of College of Medicine & Sagore Dutta Hospital, Kamarhati, West Bengal, India (Reg. No. CMSDH/IEC/283/03-2022). Informed written consent was obtained from the mother of each child.

**Authors' contribution:** Concept and design of

the study: S Mukherjee, A Sur, SK Mandal, I Chakraborty; Patient selection, sample collection: S Mukherjee, SK Mandal; Data Collection, compilation and analysis: S Mukherjee, A Sur, SK Mandal; Manuscript preparation, editing and final submission: S Mukherjee, A Sur, SK Mandal, I Chakraborty.

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