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ANALYSIS OF SERUM BILIRUBIN/ SERUM ALBUMIN RATIO IN NEONATAL HYPERBILIRUBINEMIA AMONG PREMATURE NEONATES

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Background

Neonatal hyperbilirubinemia or neonatal jaundice is a common and potentially serious condition, particularly in premature infants. It is characterized by elevated levels of bilirubin in the blood, which can cause jaundice—a yellowish discoloration of the skin, sclera, and mucous membranes. Neonatal hyperbilirubinemia is the most commonly encountered medical problem in the first two weeks of life and a common cause of readmission to the hospital after birth. [1] Approximately 60% of full-term and 80% of preterm newborns develop jaundice in their first week of life.[2] Over the years, the understanding of neonatal jaundice has evolved, encompassing both severe and milder forms.[3] While jaundice is often a benign and self-limiting condition, known as "physiological jaundice," it can also present as "pathological jaundice" when bilirubin levels rise to dangerous levels. In severe cases, this can lead to complications such as bilirubin encephalopathy or kernicterus, which can result in long-term neurological damage or even death.

Premature neonates are at an even greater risk of developing severe hyperbilirubinemia due to their immature liver function, reduced albumin levels, and increased susceptibility to bilirubin neurotoxicity. The immaturity of the hepatic enzymes responsible for conjugating bilirubin contributes to the buildup of unconjugated bilirubin in the bloodstream. [4-5] This poses a significant risk to the central nervous system, particularly when free (unbound) bilirubin crosses the blood-brain barrier, leading to bilirubin-induced neurological dysfunction (BIND).[6]

The bilirubin/albumin (B/A) ratio has emerged as a valuable marker for assessing the risk of bilirubin neurotoxicity in neonates with hyperbilirubinemia.[7-9] Since albumin binds to unconjugated bilirubin in the blood, the B/A ratio serves as an indirect measure of free bilirubin, which is the neurotoxic form. An elevated B/A ratio indicate a higher likelihood of unbound bilirubin being present, thus increasing the risk of neurological damage.[10] Despite the potential significance of the B/A ratio, there is limited data on its use in clinical practice, particularly in premature neonates, who are at greater risk due to lower albumin levels and higher bilirubin production.

This study aims to analyze the serum bilirubin/serum albumin ratio in premature neonates with hyperbilirubinemia and to assess its potential as a clinical tool for predicting the severity of hyperbilirubinemia and the associated risk of neurological complications. By focusing on premature

infants, this study seeks to provide valuable insights into the management of neonatal hyperbilirubinemia and the role of the B/A ratio in guiding treatment decisions.[11]

Material and Methods

This case-control study was conducted at the Department of Biochemistry and the Department of Pediatrics, LN Medical College (LNMC) and J.K. Hospital, Bhopal. The study aimed to evaluate serum bilirubin and albumin levels, as well as the bilirubin/albumin (B/A) ratio, in healthy preterm neonates with hyperbilirubinemia.

Inclusion Criteria:

- Healthy preterm neonates diagnosed with hyperbilirubinemia.
- Neonates aged between 0 to 5 days of life.
- Gestational age between 32 to 36 weeks.

Exclusion Criteria:

- Neonates with signs of sepsis or other significant morbidities such as respiratory distress syndrome (RDS) or hypoxic-ischemic encephalopathy (HIE).
- Neonates beyond 5 days of life.
- Preterm neonates with gestational age less than 32 weeks.
- Term neonates (gestational age ≥37 weeks).

Sample Collection and Laboratory Measurements: Whole blood samples were collected from all neonates and immediately centrifuged to separate serum for analysis. The following parameters were measured:

- **Total Bilirubin (TB):** Serum total bilirubin levels were measured using the Dimethyl Sulfoxide (DMSO) method.
- **Serum Albumin:** Serum albumin levels were determined using the bromocresol green (BCG) dye-binding method with a spectrophotometer.

For each neonate, the first available test results of TB and albumin within the study period were used to avoid selection bias, especially in cases where multiple tests were performed on the same day.

Bilirubin/Albumin Ratio Calculation: The bilirubin/albumin (B/A) ratio was calculated as: B/A ratio=Total Bilirubin (mg/dL)/ Serum Albumin (g/dL)

Clinical Characteristics: Data on gestational age (GA), birth weight (BW), sex, and serum TB and albumin levels up to the 5th day of life were recorded for all neonates.

Statistical Analysis: The statistical analysis was performed using the Student's *t*-test to compare serum bilirubin, albumin levels, and B/A ratios between the case and control groups. A *p*-value of <0.05 was considered statistically significant. Data are presented as mean ± standard deviation (SD).

Results

In this study, we compared serum bilirubin levels, serum albumin levels, and the serum bilirubin/serum albumin ratio between neonates categorized as cases (n=50) and controls (n=25) on the 1st, 3rd, and 5th days of life. The purpose of this comparison was to evaluate the difference in these biochemical markers between the groups and assess the significance of these differences.

Table no. 1: Comparison of serum bilirubin level

Parameter	Cases(n=50) Mean± SD	Controls(n=25) Mean± SD	'P' value
On 1st day	5.483±2.31	1.150±0.17	(<0.001)
On 3rd day	8.39±2.3	1.080±0.18	(<0.001)
On 5th day	10.01±3.04	0.968±0.20	(<0.001)

Serum bilirubin levels in cases were significantly elevated compared to controls across all three time points (1st, 3rd, and 5th days).
The data are as follows:

- **1st Day:** The mean serum bilirubin level in cases was 5.483 ± 2.31 mg/dL, while in controls it was 1.150 ± 0.17 mg/dL. This difference was statistically highly significant with a *p* value of <0.001. This indicates that cases had markedly higher bilirubin levels at birth compared to controls.
- **3rd Day:** On the 3rd day, the mean bilirubin level in cases further increased to 8.39 ± 2.3 mg/dL, compared to 1.080 ± 0.18 mg/dL in controls. The *p* value remained highly significant (<0.001). This suggests a continued rise in bilirubin levels in the cases during the early days of life.
- **5th Day:** By the 5th day, the mean serum bilirubin level in cases peaked at 10.01 ± 3.04 mg/dL, while in controls it was relatively stable at 0.968 ± 0.20 mg/dL ($p < 0.001$). This sharp rise in bilirubin in the cases is indicative of hyperbilirubinemia in this group, whereas the bilirubin levels in controls remained within the normal range.

Thus, a progressive and highly significant rise in bilirubin levels was observed in cases compared to controls over the first five days of life, with *p* values <0.001 at all three time points.

Table no. 2: Comparison of serum albumin level

Parameter	Cases(n=50) Mean± SD	Controls(n=25) Mean± SD	'P' value
On 1st day	2.886±0.63	3.348±0.73	(<0.01)
On 3rd day	2.74±0.59	3.427±0.69	(<0.001)
On 5th day	2.584±0.60	3.5 ±0.61	(<0.001)

Serum albumin levels, on the other hand, were significantly lower in cases compared to controls across all three time points:

- **1st Day:** On the 1st day, the mean serum albumin level in cases was 2.886 ± 0.63 g/dL, while in controls it was 3.348 ± 0.73 g/dL. This difference was moderately significant with a *p* value of <0.01. This suggests that albumin levels in cases were already lower at birth compared to controls.
- **3rd Day:** By the 3rd day, the mean albumin level in cases slightly decreased to 2.74 ± 0.59 g/dL, compared to 3.427 ± 0.69 g/dL in controls. The difference in albumin levels became highly significant ($p < 0.001$), indicating that the gap between the two groups widened over time.
- **5th Day:** On the 5th day, the mean serum albumin level in cases dropped further to 2.584 ± 0.60 g/dL, while controls had a mean level of 3.5 ± 0.61 g/dL ($p < 0.001$). This suggests a continuing decline in serum albumin levels in cases, while controls maintained stable albumin levels.

Thus, serum albumin levels in cases showed a gradual decline over the first five days, with highly significant differences between cases and controls on the 3rd and 5th days ($p < 0.001$)

Table no. 3: Comparison of serum bilirubin /serum albumin ratio

Parameter	Cases(n=50) (Mean± SD)	Controls(n=25) (Mean± SD)	'P' value
On 1st day	1.915±1.04	0.356±0.10	(<0.001)
On 3rd day	3.103±0.98	0.322±0.08	(<0.001)
On 5th day	4.001±1.37	0.284±0.09	(<0.001)

The ratio of serum bilirubin to serum albumin was markedly higher in cases compared to controls across all three time points:

- **1st Day:** The mean bilirubin/albumin ratio in cases was 1.915 ± 1.04, compared to 0.356 ± 0.10 in controls. This difference was highly significant ($p < 0.001$), indicating that the balance between bilirubin and albumin was already disrupted in cases at birth.
- **3rd Day:** By the 3rd day, the mean bilirubin/albumin ratio in cases rose sharply to 3.103 ± 0.98, while in controls it remained stable at 0.322 ± 0.08 ($p < 0.001$). This indicates that the bilirubin load was increasing rapidly relative to albumin in cases, leading to a marked imbalance.

- **5th Day:** On the 5th day, the ratio reached its peak in cases, with a mean of 4.001 ± 1.37, while controls had a mean ratio of 0.284 ± 0.09 ($p < 0.001$). This dramatic increase in the bilirubin/albumin ratio in cases indicates a critical imbalance, which could reflect a higher risk of bilirubin-induced neurotoxicity in this group.

The highly significant difference ($p < 0.001$) in the bilirubin/albumin ratio between cases and controls at all three time points suggests that cases were at a significantly higher risk for complications associated with hyperbilirubinemia due to the lower buffering capacity of albumin to bind and neutralize bilirubin.

Discussion

This study evaluated the serum bilirubin/serum albumin (B/A) ratio in premature neonates with hyperbilirubinemia, focusing on its potential as a predictor for the severity of the condition and the risk of bilirubin-induced neurotoxicity. Our findings demonstrated that the B/A ratio was significantly elevated in premature neonates with hyperbilirubinemia compared to controls, suggesting that it could serve as a valuable clinical marker for identifying infants at increased risk of severe complications.

Our results are consistent with previous studies that highlight the increased risk of severe hyperbilirubinemia in premature neonates due to immature liver function and lower albumin levels. Meena KJ and Singh S (2015) noted that albumin plays a key role in bilirubin transport and clearance, with lower albumin levels resulting in reduced bilirubin clearance, leading to significant hyperbilirubinemia.[12] Ahlfors et al. (1994) similarly observed a significant decrease in albumin levels with illness and gestational age, further heightening the risk of bilirubin toxicity in preterm infants.[13]

Studies by Sai Sunil Kishore M and P Tarakeswara Rao (2016) showed that newborns with serum bilirubin levels exceeding 4.75 mg/dL within 24 hours were at a higher risk of developing neonatal hyperbilirubinemia, which correlates with our findings regarding the predictive value of early elevated bilirubin levels. [14]

The B/A ratio has been established as a surrogate marker for free, unbound bilirubin, the neurotoxic form that poses a threat to the developing brain. Previous research by Maisels et al. showed that elevated B/A ratios are associated with an increased risk of bilirubin-induced neurological dysfunction (BIND) and kernicterus. [15] Dalia Mosallam et al. (2019) further highlighted the significant relationship between the total serum bilirubin (TSB) levels and the B/A ratio, reinforcing its predictive value. Similarly, Reem M. Soliman et al. (2021) reported mean TSB and B/A ratios that align with our findings in neonates admitted with hyperbilirubinemia. [16-17]

Govaert et al. (2003) described five premature infants (25–29 weeks gestational age) with clinical signs of kernicterus, showing a significantly elevated B/A ratio, which underscores the risk of neurotoxicity in this population [18]. The reduced binding capacity of albumin in preterm neonates with lower levels of albumin leads to increased levels of unbound bilirubin, allowing it to cross the blood-brain barrier and cause neurotoxicity. [19-21]

Clinical Significance of the B/A Ratio:

In our study, the B/A ratio showed a strong correlation with the severity of hyperbilirubinemia, particularly in neonates with higher total bilirubin levels. This supports the notion that the B/A ratio could serve as a valuable clinical tool for assessing the risk of bilirubin-induced neurotoxicity. [22-24] Unlike total bilirubin levels, which do not reflect the amount of free bilirubin, the B/A ratio provides a more comprehensive assessment of the bilirubin-binding capacity and thus the risk of neurotoxic effects. [25-26]

Implications for Premature Neonates:

Premature neonates are especially susceptible to bilirubin-induced neurotoxicity due to their underdeveloped blood-brain barrier, lower albumin levels, and decreased bilirubin conjugation capacity. Our findings suggest that those with elevated B/A ratios are at higher risk of developing

acute bilirubin encephalopathy or kernicterus. Monitoring both serum bilirubin and albumin levels in these neonates is essential to prevent severe outcomes.

Management Considerations:

The current management of neonatal hyperbilirubinemia relies primarily on phototherapy and exchange transfusion, with decision-making often based on total bilirubin levels. However, these may not adequately reflect the risk of neurotoxicity. Incorporating the B/A ratio into clinical practice could improve treatment decisions, allowing for earlier and more targeted interventions in high-risk infants. Neonates with disproportionately high B/A ratios might benefit from more aggressive management, even if their total bilirubin levels are not critically elevated by traditional standards.

While phototherapy and exchange transfusions are the mainstays of treatment, additional therapies such as intravenous immunoglobulin (IVIG) may be considered in certain cases. [27] Despite advances in the management of hyperbilirubinemia, it continues to be a significant cause of neonatal morbidity and mortality.

Study Limitations:

Although this study provides valuable insights, it has several limitations. The relatively small sample size restricts the generalizability of our findings, and a larger, multicenter study would help validate the role of the B/A ratio in managing neonatal hyperbilirubinemia. Additionally, we did not assess long-term neurodevelopmental outcomes, which would be crucial to determining whether the B/A ratio correlates with later cognitive and motor function. Future studies should focus on evaluating the long-term prognostic value of the B/A ratio and its association with neurodevelopmental outcomes.

Conclusion

The findings of this study highlight the potential of the serum bilirubin/serum albumin ratio as a clinically significant marker in the management of neonatal hyperbilirubinemia, particularly in premature neonates. Given the increased vulnerability of premature infants to bilirubin neurotoxicity, the B/A ratio could serve as a valuable tool for early identification of those at higher risk, allowing for timely and targeted interventions. Future research should focus on larger cohort studies and the exploration of long-term neurodevelopmental outcomes to further validate the use of the B/A ratio in routine clinical practice.

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