Bilirubin Binding Capacity in the Preterm Neonate

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KEYWORDS

• Total serum bilirubin • Unbound bilirubin • Bilirubin:albumin molar ratio
• Bilirubin–albumin binding affinity • Bilirubin-induced neurotoxicity

KEY POINTS

- Bilirubin–albumin binding limits the use of total serum/plasma bilirubin (TB) as an indicator of bilirubin-induced neurotoxicity in premature infants.
- Bilirubin binding capacity and affinity are low and variable in premature infants.
- Specific exogenous drugs, intravenous lipids, and clinical factors such as metabolic acidosis, hypothermia, hypoxia, and sepsis may adversely affect bilirubin–albumin binding.
- The bilirubin:albumin molar ratio, in conjunction with TB, may be useful as a marker of bilirubin-induced neurotoxicity in more mature preterm and late preterm infants.
- Unbound bilirubin is a better vascular gauge of hyperbilirubinemia "severity" for bilirubin-induced neurotoxicity than the conventionally measured TB in premature infants.

INTRODUCTION

The primary goal of the evaluation and management of unconjugated hyperbilirubinemia in premature infants is to prevent acute and chronic bilirubin-induced neurotoxicity, a spectrum of neurodevelopmental disorders including, but not limited to, central apnea, sensorineural deafness, auditory neuropathy spectrum disorder, language disorders, autism, upward gaze palsy, and athetoid cerebral palsy.[1–10] It is generally believed that premature infants are at increased risk for bilirubin-induced neurotoxicity compared with term infants.[4,11] However, to date the evidence-based management of hyperbilirubinemia to prevent bilirubin-induced neurotoxicity remains elusive in premature infants less than 35 weeks gestational age (GA).[12] Consensus-based management guidelines for hyperbilirubinemia using total serum/plasma bilirubin (TB) levels, GA, and clinical risk factors for premature infants less than 35 weeks GA were recently published.[11] The TB level, the traditional parameter to

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evaluate and manage hyperbilirubinemia in premature infants, has not been a useful predictor of acute and chronic bilirubin-induced neurotoxicity.[4,13–19] This is not surprising; several biochemical and physiologic factors are involved in the pathogenesis of bilirubin-induced neurotoxicity.[20] More specifically, bilirubin–albumin binding limits the use of TB as an indicator of the amount of bilirubin in vascular and extravascular compartments, the magnitude of ongoing bilirubin production/excretion mismatch, and the overall risk of bilirubin toxicity in premature infants. There is growing evidence that non–albumin-bound (unbound) or free bilirubin (UB), resulting from altered bilirubin–albumin binding, is a more sensitive and specific biochemical measure of acute and chronic bilirubin-induced neurotoxicity than TB. In this article, bilirubin–albumin binding, a modifying factor for bilirubin-induced neurotoxicity, in premature infants is discussed. The endogenous and exogenous factors that may influence the bilirubin–albumin binding in premature infants is also examined. Furthermore, existing evidence for the role of bilirubin:albumin molar ratio (BAMR) and UB as predictors of bilirubin-induced neurotoxicity in premature infants is provided.

Bilirubin–Albumin Biochemical Structure and Binding

The predominant native form of unconjugated bilirubin in its usual form (*4Z,15Z*-IXα isomer) has its 2 rigid dipyrrole units internally hydrogen bonded to each other such that no polar (or ionizable) groups are exposed making it quite insoluble in water at a neutral pH.[21,22] Because of its low solubility in water, free unconjugated bilirubin can form detrimental dimers and higher aggregates.[22] Solubility increases with increasing pH owing to the ionization of the acidic groups on the molecule.[21,22] The hydrophobic native form of unconjugated bilirubin can readily cross the phospholipid layers of biomembranes, including the blood–brain barrier and neuronal cell membranes.[23] This native bilirubin or free unconjugated bilirubin (*4Z,15Z*-IXα isomer) is neurotoxic.[24,25]

Fortunately, native bilirubin, primarily a product of hemoglobin (Hgb) degradation, exists in the blood mostly bound to albumin, preventing it from crossing intact blood–brain barrier. Albumin serves as a vehicle for the transport of bilirubin to the liver, where the bilirubin dissociates from albumin and enters the hepatic cell for conjugation. More important, albumin is a highly abundant serum protein (0.6 mmol/L), which comprises 50% to 60% of the total plasma protein in humans. Moreover, albumin is a 66-kDa monomer containing 3 homologous helical domains (I–III), each divided into A and B subdomains.[26] It is therefore not surprising that, in addition to bilirubin, albumin binds a wide variety of endogenous ligands, including nonesterified fatty acids and hemin, all of them acidic and lipophilic compounds, with high affinity at multiple sites.[27–29] Among exogenous substances, many commonly used drugs with acidic or electronegative features (eg, ibuprofen) also bind to human serum albumin, usually at 1 of 2 primary sites located in subdomains IIA and IIIA.[27]

There are at least 2 types of binding sites for bilirubin and its photoisomers on albumin.[30,31] The strongest and key binding site for native bilirubin has a very high binding constant (affinity), approximately 1.4×10^7 L/mol at 37°C, and can be considered a specific site.[32,33] Other secondary sites have at least a 10-fold lower binding affinity.[34] Recent studies indicate that there are site-to-site interactions or allosteric interaction within albumin.[35–37] It is known that fatty acid and drug binding to albumin induces conformational changes in albumin, which may then influence bilirubin–albumin binding.[29,35–37]

Bilirubin–Albumin Binding Capacity and Affinity

Bilirubin–albumin binding is a function of the concentrations of bilirubin and albumin, and the binding affinity for bilirubin (strength of bilirubin binding to albumin).