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Hot Topic Commentary

Prolonged Direct Hyperbilirubinemia Following Acute Hepatitis: When Not to Worry?

Danzhu Zhao[1*] and George Y. Wu[2]

[1]*Department of Medicine, University of Connecticut Health Center, Farmington, CT, USA;* [2]*Department of Medicine, Division of Gastroenterology-Hepatology, University of Connecticut Health Center, Farmington, CT, USA*

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Introduction

Serum direct bilirubin levels, consisting mostly of conjugated bilirubin, are often elevated in acute hepatitis due to cholestasis. This usually results in jaundice, which resolves as the hepatitis improves. However, serum direct bilirubin elevations can sometimes persist beyond 4 weeks.[1,2] Because bilirubin excretion is an important hepatic function, prolonged direct hyperbilirubinemia may raise concerns about the development of liver failure.[2] The incidence of acute liver failure has been reported to be approximately 3,000 cases per year in the U.S., with about 80% of these cases attributed to acute hepatitis.[3] Nevertheless, prolonged direct hyperbilirubinemia is not synonymous with cholestasis, nor is it necessarily a marker of liver failure. For instance, Dubin-Johnson syndrome[4] and Rotor syndrome are rare genetic causes of prolonged direct hyperbilirubinemia without cholestasis or liver disease.[1] A much more common condition leading to prolonged direct hyperbilirubinemia without cholestasis or liver failure is delta hyperbilirubinemia. Because delta hyperbilirubinemia is relatively common yet often underappreciated, the aim of this article is to update the pathogenesis, clinical significance, and management of delta hyperbilirubinemia.

Delta bilirubin

Delta bilirubin is defined as bilirubin that is covalently bound to serum albumin, making the molecules non-dissociable.[2] In contrast, unconjugated bilirubin binding to albumin is dissociable, allowing for the transfer of bilirubin to hepatocytes for elimination.[2]

Mechanism of formation

Studies on *in vitro* incubation of albumin and conjugated bilirubin have concluded that delta bilirubin formation is spontaneous and non-enzymatic.[5,6] Several mechanisms have been proposed for the formation of delta bilirubin,[5,7–10] including transesterification, glycation (similar to hemoglobin A1c formation), and acyl transfer or acyl migration. The most recent data support glycation of the carbonyl group of glucuronic acid to the epsilon amino group of lysine in albumin.[11]

Structural analysis of delta bilirubin from the serum of icteric patients with liver disease has identified a specific covalent binding site of bilirubin to serum albumin.[7] Adachi *et al*. digested delta bilirubin and isolated and purified specific peptide fractions containing a bilirubin derivative (azodipyrrole). Subsequent amino acid sequencing revealed that the peptide comprised residues 187–191 of serum albumin, with lysine 190 being the specific residue linked to bilirubin.[7] Okhawa *et al*. stabilized the bilirubin linkage to albumin, allowing enzymatic digestion and generating peptide fragments with the bilirubin linkage intact. These fragments, analyzed by liquid chromatography time-of-flight mass spectrometry, were compared to digested control human serum albumin.[8] Molecular weight analysis of the fragments confirmed that the predominant covalent binding site was lysine residue 190.[7] Additionally, it was confirmed that the linkage occurred by a glucuronic acid through glycation (Fig. 1).[7]

The chemical linkage of bilirubin to albumin to form delta bilirubin requires conjugated bilirubin, either in the form of monoglucuronide (BMG) or diglucuronide (BDG). As unconjugated bilirubin lacks glucuronic acid, it does not participate in delta bilirubin formation.[9] These results are supported by findings of van Breeman *et al*., in which employed high-performance liquid chromatography was used to show that mixing either BDG or BMG, but not unconjugated bilirubin, with human serum albumin *in vitro* resulted in the spontaneous non-enzymatic formation of delta bilirubin. BDG was found to be more reactive with albumin than BMG.[5] In a clinical study of patients with post-operative jaundice, Weiss *et al*. observed that serum levels of delta bilirubin decreased more slowly than BMG and BDG levels.[10] By day 18 post-operation, BMG and BDG fractions were no longer present in the serum, and bilirubinuria was absent. However, direct bilirubin was still present, with delta bilirubin accounting for 90% of the total bilirubin.[10] Evidence has shown that delta bilirubin concentrations can increase for up to 50 days before reaching a steady state.[12] Serum delta bilirubin levels correlate with levels of serum conjugated bilirubin and the duration of cholestasis.[9]

*Correspondence to: Danzhu Zhao, Department of Medicine, University of Connecticut Health Center, 263 Farmington Ave, Farmington, CT 06032, USA. ORCID: https://orcid.org/0009-0009-0732-2209, Tel: +1-360-790-1216, Fax: +1-860-679-6582, E-mail: dazhao@uchc.edu.

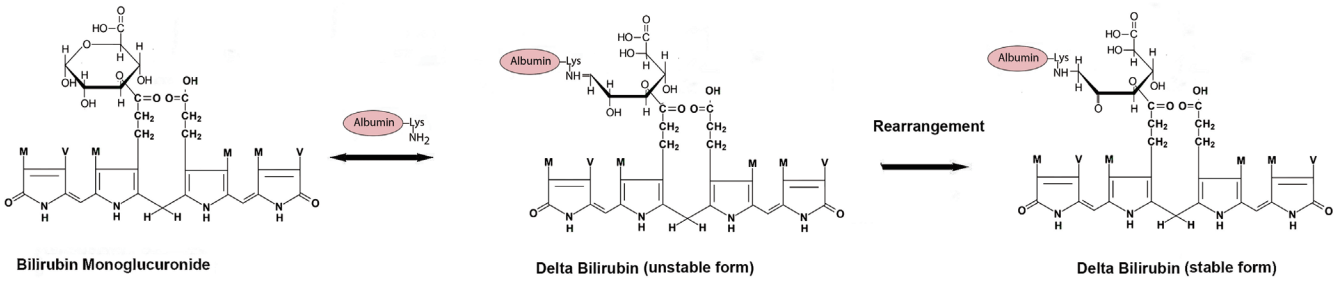

Fig. 1. Diagram of a proposed pathway for the formation of delta bilirubin. Bilirubin glucuronides, either BMG (bilirubin monoglucuronide) or BDG (bilirubin diglucuronide), can bind to albumin in a reversible Schiff's base link, producing an unstable form of delta bilirubin. Over time, a molecular rearrangement can occur, resulting in the formation of stable delta bilirubin. Serum levels of delta bilirubin correlate with past serum levels of conjugated bilirubin and the duration of hyperbilirubinemia.

Detection

The albumin linkage in delta bilirubin prevents bilirubin folding, thereby exposing the diazotization reaction site in a manner similar to the simple glucuronic acid linkage in conjugated bilirubin. Therefore, in clinical laboratories, delta bilirubin is measured as direct bilirubin and cannot be distinguished from conjugated bilirubin.[2]

Elimination

Due to disrupted internal hydrogen bonds, conjugated bilirubin is water-soluble, making elimination by hepatic and renal pathways possible.[13] The vast majority of serum-conjugated bilirubin is rapidly excreted by the liver, resulting in a normal half-life of about 2–4 hours.[2] In contrast, delta bilirubin, because of its covalent linkage to albumin, assumes the half-life of albumin, which is 17–21 days.[9,14] This accounts for the prolonged duration of jaundice observed with delta bilirubin.

Because the renal glomerular pore diameter is about 4 nm, if serum levels of conjugated bilirubin exceed the renal threshold of approximately 0.16 mg/dL, conjugated bilirubin will be filtered into the urine, although there is considerable threshold variability.[15] However, because delta bilirubin is similar in size to albumin (about 14 nm), it cannot be excreted in the urine (with an ultrafiltrate fraction <1%). Therefore, delta bilirubin can be present at very high levels in serum but be undetectable in urine.[13]

Because it is covalently bound, bilirubin is primarily released from delta bilirubin only when albumin is catabolized.[2] Albumin is normally removed from circulation by scavenger receptors of hepatic sinusoidal endothelial cells.[15] Therefore, the liver is likely the primary site for delta bilirubin catabolism.[2] The resolution of delta hyperbilirubinemia depends on liver function and does not require specific treatment.

Toxicity

Because conjugated bilirubin is covalently bound to albumin, delta bilirubin is relatively non-toxic. Delta bilirubin cannot readily cross cell membranes or the blood-brain barrier and, therefore, does not have the toxic effects that unconjugated bilirubin has on the central nervous system.[13]

Other potential clinical uses of delta bilirubin measurement

Delta bilirubin measurement has been reported to have additional clinical uses. The concentration of serum delta bilirubin has been shown to predict the effectiveness of biliary drainage, with higher percentages of delta bilirubin correlating with greater effectiveness in biliary drainage.[16]

Delta bilirubin has also been demonstrated to be a potentially sensitive marker for acute rejection in liver transplant recipients.[17] Lower levels or declines in delta bilirubin levels were observed in patients experiencing acute rejection, while non-rejection patients exhibited elevated levels.[17]

Clinical implications of delta bilirubin and prolonged direct hyperbilirubinemia: When to worry and when not to worry

When direct hyperbilirubinemia is profound and prolonged in acute cholestatic hepatitis, concern should be raised if symptoms persist and liver function tests decline while liver injury markers (aminotransferases, alkaline phosphatase, and gamma-glutamyl transferase) remain elevated.[18] Monitoring these parameters is typically sufficient for assessing the status of liver failure. In such cases, although delta hyperbilirubinemia may be present and reflect severe liver damage and deteriorating liver function, its measurement is unnecessary, and does not add useful information for management.[18]

In patients with acute cholestatic hepatitis who show resolution of symptoms and normalization of aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase levels, liver failure is unlikely, even if direct hyperbilirubinemia and jaundice persist.[18] In these cases, negative or trace bilirubinuria in the presence of normal renal function suggests that delta hyperbilirubinemia, rather than conjugated hyperbilirubinemia, is the cause of jaundice. In such instances, patients can be reassured that the jaundice, even if profound, is not indicative of liver failure and will resolve completely, albeit slowly, over the course of weeks to months.[10]

Summary of important clinical points

1. Delta bilirubin reacts the same as conjugated bilirubin in standard laboratory diazotization assays so the sum of conjugated plus delta bilirubin is reported as direct bilirubin.[13] It cannot measure delta separate from conjugated bilirubin.
2. Levels of delta, plus conjugated, plus unconjugated bilirubin are reported as total bilirubin.[9]
3. The presence of delta hyperbilirubinemia should be considered in patients with prolonged, severe jaundice and direct hyperbilirubinemia who show recovery of liver function and symptom improvement without bilirubinuria.[10,13] With recovery, hepatic excretion of conjugated bilirubin normalizes, leaving delta bilirubin as the pre-

dominant form of bilirubin in circulation.[10]

4. Delta hyperbilirubinemia is only a marker of past prolonged conjugated hyperbilirubinemia, usually due to prior cholestasis.[9] The severity of liver disease should be determined by clinical signs and symptoms, serum markers of liver injury, cholestasis, and liver function.[18]
5. The lack of glomerular filtration of delta bilirubin results in an absence of bilirubinuria, even in the presence of very high serum levels of delta bilirubin.[10] If liver function deteriorates, conjugated bilirubin excretion becomes impaired, and if serum levels reach or exceed the renal threshold, bilirubinuria will appear. However, this bilirubinuria will be solely due to conjugated bilirubin[10] as delta bilirubin cannot be filtered due to its size.
6. While usually unnecessary, in cases where the diagnosis is unclear, the presence of delta bilirubin can be confirmed using an Ortho-Clinical Diagnostics VITROS 250 BuBc System, which measures total, conjugated, and unconjugated bilirubin.[9] In this assay, delta bilirubin is not directly measured but can be estimated by subtracting the sum of conjugated and unconjugated bilirubin (measured individually) from total bilirubin.[9]

Understanding the role of delta bilirubin in chronic cholestasis can provide reassurance and an explanation for patients who present with an atypically prolonged course of jaundice but demonstrate improvement in symptoms, resolution of cholestasis, and normalization of aminotransferases and liver function tests.

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Conflict of interest

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References

[1] Lu L, Chinese Society of Hepatology and Chinese Medical Association. Guidelines for the Management of Cholestatic Liver Diseases (2021). J Clin Transl Hepatol 2022;10(4):757–769. doi:10.14218/JCTH.2022.00147, PMID:36062287.
[2] Kalakonda A, Jenkins BA, John S. Physiology, Bilirubin. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. [cited 2024 May 17]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK470290/.
[3] Stravitz RT, Lee WM. Acute liver failure. Lancet 2019;394(10201):869–881. doi:10.1016/S0140-6736(19)31894-X, PMID:31498101.
[4] Liu T, Zhao J, Feng JY, Lu Y, Sheps JA, Wang RX, *et al*. Neonatal Dubin-Johnson Syndrome and its Differentiation from Biliary Atresia. J Clin Transl Hepatol 2023;11(1):163–173. doi:10.14218/JCTH.2021.00460, PMID:36406324.
[5] Van Breemen RB, Fenselau C, Mogilevsky W, Odell GB. Reaction of bilirubin glucuronides with serum albumin. J Chromatogr 1986;383(2):387–392. doi:10.1016/s0378-4347(00)83484-2, PMID:3558567.
[6] Gautam A, Seligson H, Gordon ER, Seligson D, Boyer JL. Irreversible binding of conjugated bilirubin to albumin in cholestatic rats. J Clin Invest 1984;73(3):873–837. doi:10.1172/JCI111283, PMID:6707206.
[7] Adachi Y, Murata N, Tsunasawa S, Kamisako T, Yamamoto T. Lysine 190 is a covalent bilirubin-binding amino acid in human delta bilirubin. Int Hepatol Commun 1996;5(4):282–288. doi:10.1016/0928-4346(96)00309-X.
[8] Ohkawa T, Norikura R, Yoshikawa T. Rapid LC-TOFMS method for identification of binding sites of covalent acylglucuronide-albumin complexes. J Pharm Biomed Anal 2003;31(6):1167–1176. doi:10.1016/s0731-7085(02)00733-1, PMID:12667932.
[9] Kesarwani A, Nichat P, Rajak N, Potey G. Measurement and Clinical Usefulness of Delta Bilirubin in Liver Disease. Cent India J Med Res 2023;2:10–14. doi:10.58999/cijmr.v2i02.116.
[10] Weiss JS, Gautam A, Lauff JJ, Sundberg MW, Jatlow P, Boyer JL, *et al*. The clinical importance of a protein-bound fraction of serum bilirubin in patients with hyperbilirubinemia. N Engl J Med 1983;309(3):147–150. doi:10.1056/NEJM198307213090305, PMID:6866015.
[11] Tiribelli C, Ostrow JD. New concepts in bilirubin chemistry, transport and metabolism: report of the International Bilirubin Workshop, April 6-8, 1989, Trieste, Italy. Hepatology 1990;11(2):303–313. doi:10.1002/hep.1840110222, PMID:1968418.
[12] Ye W, Rosenthal P, Magee JC, Whitington PF, Childhood Liver Disease Research and Education Network. Factors Determining δ-Bilirubin Levels in Infants With Biliary Atresia. J Pediatr Gastroenterol Nutr 2015;60(5):659–663. doi:10.1097/MPG.0000000000000690, PMID:25564820.
[13] Fevery J. Bilirubin in clinical practice: a review. Liver Int 2008;28(5):592–605. doi:10.1111/j.1478-3231.2008.01716.x, PMID:18433389.
[14] Tripathi N, Jialal I. Conjugated Hyperbilirubinemia. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. [cited 2024 Feb 13]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK562172/.
[15] Gburek J, Konopska B, Gołąb K. Renal Handling of Albumin-From Early Findings to Current Concepts. Int J Mol Sci 2021;22(11):5809. doi:10.3390/ijms22115809, PMID:34071680.
[16] Kozaki N, Shimizu S, Higashijima H, Kuroki S, Yamashita H, Yamaguchi K, *et al*. Significance of serum delta-bilirubin in patients with obstructive jaundice. J Surg Res 1998;79(1):61–65. doi:10.1006/jsre.1998.5357, PMID:9735241.
[17] Gupta D, Gupta A, Raizada A. Delta bilirubin: a sensitive and predictive marker for acute rejection in liver transplant recipients. Int J Res Med Sci 2018;6(3):945–949. doi:10.18203/2320-6012.ijrms20180620.
[18] Hernaez R, Solà E, Moreau R, Ginès P. Acute-on-chronic liver failure: an update. Gut 2017;66(3):541–553. doi:10.1136/gutjnl-2016-312670, PMID:28053053.