

## Measurement and Clinical Usefulness of Delta Bilirubin in Liver Disease

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Delta bilirubin is albumin-bound conjugated bilirubin, which can be calculated using the formula [Total bilirubin -(conjugated + unconjugated bilirubin)]. Newer technologies, such as dry chemistry, provide separately conjugated and unconjugated bilirubin that is more specific and informative than direct and indirect bilirubin by the conventional method, in which only approximate values of conjugated bilirubin can be measured. In cholestasis, conjugated bilirubin combines non-enzymatically with albumin to form delta bilirubin in the circulation and remains for a long time. This review describes the utility of delta bilirubin in cholestasis, chronic liver disease, childhood jaundice, transplant rejection, and other jaundices. The clinical correlation of delta bilirubin in all conditions was summarized and their future perspectives were discussed.

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## Introduction

Bilirubin is an orange-yellow pigment that is derived from senescent red blood cells. After formation in reticuloendothelial cells, bilirubin is taken to and biotransformed mainly in the liver and excreted in bile and urine.<sup>1,2</sup> Increased plasma bilirubin levels are a common clinical finding. An increase in bilirubin may be due to changes in any phase of its metabolism : (a) excessive production of bilirubin (ie pathological hemolysis); (b) impaired uptake in the liver with an increase in indirect bilirubin; (c) defective conjugation caused by a defect in UDP-glucuronosyl transferase; and (d) impaired biliary clearance with elevated direct bilirubin secondary to protein clearance defects or inability of bile to enter the small intestine of the bile duct.<sup>3</sup> It has been found in several sera to contain the third known species of bilirubin as delta-bilirubin [total bilirubin- (unconjugated bili + conjugated bili)], which is formed by spontaneous transesterification of conjugated bilirubin esters to exposed carboxyl groups on various serum proteins (mainly albumin) in subjects with cholestasis. Delta-bilirubin is a non-toxic and non-enzymatically covalently bound complex with albumin, it has a long half-life regardless of hepatorenal function, it is not excreted in

urine or bile.<sup>4</sup> Because this occurs only when conjugated bilirubin is present in excess, significant circulating delta bilirubin is seen only in patients with cholestasis.<sup>5</sup>

*Determination of delta bilirubin in clinical laboratories*

The method used by most laboratories today to measure bilirubin is based on the diazo method, first described in 1938 by Jendrassik and Gróf. Estimation of conjugated and unconjugated bilirubin fraction is based on the relative insolubility of unconjugated bilirubin in aqueous solutions. Total bilirubin reacts with the accelerator, resulting in "total bilirubin". Unconjugated bilirubin is estimated "indirectly" as the difference between total and direct bilirubin.<sup>6</sup> The division of bilirubin into direct and indirect fractions by solubility is always approximate.<sup>7,17</sup> A century ago, we were not aware that direct bilirubin is conjugated and indirect bilirubin is not. This difference is an empirical fact and is useful because it relates to disease. Today, we know more about bilirubin and its different forms. Bilirubin is separated into four peaks using a high-performance liquid chromatography technique (HPLC) (Table 1).<sup>3</sup>

HPLC is not feasible for routine bilirubin estimation. However, the tests available in dry chemistry (Ortho Clinical Diagnostics VITROS 5600 and 250 Clinical Chemistry Systems) measure three different bilirubin

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**Table 1:** Bilirubin is separated into four peaks using HPLC

No.	Bilirubin peak in HPLC	Alpha peak	Beta peak	Gamma peak	delta peak
Total bilirubin contains all the fractions.					
1	Type of bilirubin	Unconjugated bilirubin	bilirubin monoglucuronate	Bilirubin diglucuronate	bilirubin conjugated to albumin
2	BY Vitros slide method	Unconjugated (Bu)	Conjugated Bilirubin (Bc)		Delta Bilirubin[Total-(BU+Bc)]
3	By other traditional methods	Indirect bilirubin (Total-Direct)	Not defined clearly		

\* Alpha, unconjugated bilirubin

\* Beta, mono-conjugated bilirubin, mostly with glucuronic acid

\* Gamma, bis conjugated bilirubin

\*Delta, bilirubin covalently bound to albumin

fractions corresponding to the molecular type recognized by HPLC. The VITROS test (reflection spectrophotometry) method for bilirubin consists of two separate tests. The VITROS TBIL slide measures total bilirubin using the diazo method. The VITROS BuBc slide uses a mordant (a product used in the textile and photographic industries to bind dyes to substrates such as fabric or films) to simultaneously measure unconjugated bilirubin (Bu) and conjugated bilirubin (Bc). In the presence of a stain, the visible spectrum of conjugated and unconjugated bilirubin is different. For the VITROS test for bilirubin, unconjugated bilirubin (Bu) corresponds to the HPLC alpha peak; conjugated bilirubin (Bc) corresponds to the number of beta and gamma peaks; total bilirubin (TBIL) includes the total HPLC fraction of TBIL and the sum of Bu + Bc corresponding to the d peak of HPLC.<sup>16,19,20</sup> In obstructive jaundice, conjugated bilirubin is not excreted with bile and then passes into the blood. Once in circulation, it slowly binds to albumin to form delta bilirubin. This non-enzymatic process is similar to the formation of glycated hemoglobin from glucose and hemoglobin. Half life of Delta bilirubin is same as albumin, that is 2 to 3 weeks. Patients with jaundice should have a decrease in conjugated bilirubin immediately following treatment. However, due to its long half-life, delta bilirubin remains elevated for weeks after the reaction has resolved. Since they contain delta bilirubin, total and direct bilirubin results are also increased, falsely indicating that the patient needs more treatment. On the other hand, with the new preparation of BuBc, conjugated bilirubin (Bc) (measured directly) drops rapidly, indicating that the patient is well.<sup>9,20</sup> In this article, we have reviewed various studies on delta bilirubin.

### **Delta Bilirubin as a Marker of Cholestasis**

Nita Garg and Abhinav Manish conducted a 6-month prospective study involving 110 randomly selected

patients, 60 patients, and 50 controls aged 15 to 75 years. Inclusion criteria included patients with a cholecystectomy history, ultrasound-proven duct obstruction, and medical jaundice. Patients with alcoholic cirrhosis, viral hepatitis and other organ defects were excluded from the study. They found that serum delta-bilirubin and conjugated bilirubin, ALP, GGT and transaminase levels increased in case of cholestasis. Statistics were performed on the data and the value was Delta bilirubin ( $0.332 \pm 0.169 \pm 0.023$ ) and cases ( $1.21 \pm 0.91 \pm 0.332 \pm 0.169 \pm 0.023$ ) and cases ( $1.21 \pm 0.91 \pm 0.127$ ), p-value <0.0001, which is significant.<sup>5</sup>

Arpita Bhakta *et al.* studied obstructive, hemolytic, hepatocellular, and normal patients (50 each). While delta bilirubin constitutes 20.29% of total bilirubin in normal patients, this rate is 43% in obstructive jaundice 50%, hepatic jaundice 35.05%, hemolytic jaundice 28.03%, delta percentage was higher in all three cases. Bilirubin is higher than normal. The percentage of delta bilirubin is highest in obstructive jaundice.<sup>14</sup>

### **Increased delta bilirubin in the serum with special regard to the chronicity of the disease**

Dr. Susruta Sen *et al.* studied 50 patients with hemolytic jaundice. In addition, 50 control subjects who did not have hepatobiliary disease but underwent routine physical examination were also included in this study. Subjects with childhood hyperbilirubinemia, immunosuppressive drugs, alcoholic or non-alcoholic, cancer, Wilson's disease, and Gilbert's syndrome were excluded from this study. Obstructive, hepatic, or hemolytic jaundice is diagnosed by the patient's history, radiographs, and other medical records. Compared to the control group, serum delta bilirubin increased significantly in chronic, hepatic, and hemolytic jaundice groups. Patients with chronic jaundice have higher delta bilirubin levels than those with chronic jaundice or hemolytic jaundice. The percentage of delta bilirubin (%) was higher in patients

with jaundice and severe jaundice compared to controls, but there was no significant difference between the controls and the hemolytic group. The percentage of delta bilirubin was higher in patients with acute jaundice and acute jaundice compared with the hemolytic jaundice group. Patients with acute jaundice, hepatic jaundice and hemolytic jaundice had higher serum bilirubin levels compared to controls. In addition, the serum bilirubin level increased in patients with jaundice when compared to the hepatitis group or the hemolysis group, but the difference was not significant.<sup>15,18</sup>

#### **Predictive marker of delta bilirubin for acute rejection in liver transplant recipients**

Deepa Gupta and colleagues studied 80 patients (56 men and 24 women) who had undergone a major liver transplant in Medanta-Medicity, Gurgaon. The mean age of the patients was 43±19 years. Patients with chronic liver disease, alcoholic liver disease, cholestatic liver disease, cryptogenic cirrhosis, chronic respiratory failure, extrahepatic biliary atresia, NAFLD, Alagille syndrome, Budd-chiari syndrome, hemochromatosis, type I tyrosinemia, Wilson's disease. Student's t-test was used to reveal significant differences ( $p < 0.05$ ) between rejected and non-survival transplant patients. Of the 54 rejections, 50 had decreased delta bilirubin (DB) scores or DB scores (<40% TBIL). When calculating the mixed bilirubin score (>50% TBIL) in the separate group, only 23 were found. Of the 26 excluded, 21 had their CB score decreased while their DB score increased more. The results showed that delta bilirubin had the highest sensitivity with 93% and conjugated bilirubin was 43%, while the sensitivity of SGOT, SGPT, GGT and alkaline phosphatase was 61, 81, 80 and 80%, respectively.<sup>11</sup>

#### **Delta bilirubin in the serum of pediatric patients**

EM Brett and colleagues tested serum from 539 infants and children. Bilirubin (B) delta ratios appear to be associated with age and chronic diseases. In infants younger than 28

days of age, delta bilirubin is usually less than 2% of total bilirubin; delta bilirubin is 35% in infants and children with hyperbilirubinemia. In neonates, increased delta bilirubin (more than 50% of total bilirubin) is associated with stomach and kidney disease, intestinal obstruction, biliary atresia, and liver disease. The percentage of delta bilirubin is low (less than 10%) in infants and children with hemolytic anemia, sepsis, shock, and other jaundice. The outcome is poor in a few cases because of the low delta bilirubin value and high conjugated bilirubin.<sup>12</sup>

Wen Ye *et al.* conducted a prospective study on infants with biliary atresia and children with cholestasis and found that the level of B $\delta$  (delta bilirubin) increased with an increase in conjugated bilirubin (Bc) and total bilirubin (TB) Level up. In addition, the duration of cholestasis partially determines the level of B $\delta$ . A 1 mg/dL increase in Bc is associated with approximately 0.36 mg/dL increase in delta bilirubin ( $p < 0.0001$ ). at the same time, cholestasis of 100 days was associated with an increase in delta bilirubin of approximately 1.0 mg/dL ( $p < 0.0001$ ). Serum albumin levels were not associated with delta bilirubin ( $p = 0.89$ ).<sup>13</sup>

#### **Hyperbilirubinemia after relief of obstruction**

After endoscopic or surgical resection of mechanical biliary obstruction, pruritus and serum bilirubin resolve rapidly, but jaundice does not resolve. He also noticed that the urine had cleared, but the jaundice was still there. Studies have shown that this discrepancy is due to the presence of "bilirubin" or "a bilirubin conjugate covalently bound to albumin" (delta bilirubin) in the blood. This delta bilirubin is diazo-positive and has a high molecular weight (due to albumin binding), so it cannot be ultrafiltered in the kidney. Therefore, they are not seen in the urine. While these bile proteins are catabolized in plasma, the albumin portion of these bile proteins undergoes proteolysis. Therefore, they have a plasma half-life of 17 days, similar to that of albumin. This explains the jaundice one week after the blockage is cleared.<sup>21,22</sup>

**Table 2:** Summary of studies showing the importance of Delta bilirubin

Author	Type of study	Number of cases	Participant Group	Results
Nita Garg <i>et al.</i>	Prospective case control	110 patients 15-75 years	60 cases of obstructive jaundice and 50 controls	Delta Bilirubin in controls (0.332±0.169±0.023) and in cases (1.21±0.91±0.127) with p value <0.0001
Arpita Bhakta <i>et al.</i>	Observational case control study	200 adults middle aged	Obstructive jaundice, hepatocellular jaundice, hemolytic jaundice and normal each 50	In normal patients, 20.29% of total bilirubin is delta bilirubin, 43.50% is obstructive jaundice, 35.05% is hepatic jaundice and 28.03% is hemolytic jaundice.

Susruta Sen <i>et al.</i>	observational case control study	200 middle aged adults	Obstructive jaundice, hepatocellular jaundice, hemolytic jaundice and normal each 50	Serum delta bilirubin was elevated in patients with obstructive jaundice ( $0.13 \pm 0.18$ vs. $1.66 \pm 1.68$ ; $p = 0.0001$ ), jaundice ( $0.13 \pm 0.18$ vs $1.0 \pm 0.99$ ; $p = 0.0001$ ) and patients with hemolytic jaundice ( $0.13 \pm 0.18$ vs $1.11 \pm 1.39$ ; $p = 0.0001$ ) compared to controls.
Deepa Gupta <i>et al.</i>	Case control study	Eighty patients (56 men and 24 women) undergoing major liver transplants at Medanta-Themedicity, Gurgaon were studied. The mean age of the patients was $43 \pm 19$ years.	Chronic liver disease (n=31), alcoholic liver disease (n=11), cholestatic liver disease (n=11), cryptogenic cirrhosis (n=7), liver malignancy (n=5), liver dysfunction (n=4) ), extrahepatic biliary atresia (n=2), NAFLD (n=4), Alagille syndrome (n=1), Budd-chiari syndrome (n=1), hemochromatosis (n=1), Type I tyrosinemia (n= 1) ) , Wilson's disease (n=1)	Delta bilirubin (DB) score decreased in 50 of 54 rejections or DB score remained low (<40% of TBIL)
E M Brett <i>et al.</i>	Observational case control	539 infants and children	539 specimens from 347 different patients with hyperbilirubinemia: 242 from an acute-care referral facility and 105 from the well-baby nursery of a community hospital.	In neonates, elevated B $\delta$ (more than 50% of total bilirubin) is associated with systemic diseases and liver diseases, cirrhosis, biliary atresia, and liver disease.
Wen Ye, etal	prospective study (PROBE: Clinicaltrials.gov NCT00061828) of biliary atresia.	Subjects were Infants with Cholestasis from the Pediatric Liver Disease Research and Education Network (ChiLDREN) and its predecessors, the Biliary Atresia Study and BA Infants from the Child Study (PROBE: ClinicalTrials.gov NCT00061828). Consortium (BARC) from Jun 1, 2004 to Mar 1, 2012	129 children out of 66 male and 63 females	every 100 days of cholestasis is associated with an approximately 1.0 mg/dL increase in delta bili. ( $p < 0.0001$ ) given the same level of Bc.
Deepa Gupta <i>et al.</i>	Case control study	Eighty patients (56 men and 24 women) undergoing major liver transplants were studied. The mean age of the patients was $43 \pm 19$ years	Chronic liver disease (n=31), alcoholic liver disease (n=11), cholestatic liver disease (n=11), cryptogenic cirrhosis (n=7), liver malignancy (n=5), liver dysfunction (n=4) ), extrahepatic biliary atresia (n=2), NAFLD (n=4), Alagille syndrome (n=1), Budd-chiari syndrome (n=1), hemochromatosis (n=1), Type I tyrosinemia (n= 1) ) , Wilson's disease (n=1)	Delta bilirubin(DB) score decreased in 50 of 54 rejections or Db score remained low(<40%of TBIL)w

## Conclusion

Delta bilirubin levels increase with increasing conjugated bilirubin and total bilirubin levels and also depend on the duration of cholestasis. The availability of percent delta bilirubin can help in interpreting the degree of cholestasis in cases of hepatobiliary obstruction and also helps in the prognosis of these patients. It can be calculated using newer dry chemistry where the conjugated unconjugated fractions are measured and give a calculated delta bilirubin fraction. Serum delta-bilirubin indicates the efficiency of biliary drainage. Analysis of serum delta-bilirubin over 7 days can differentiate well-drained patients from poorly-drained patients. Delta bilirubin also predicts disease chronicity with an inverse relationship to serum albumin. It may also be a useful marker for liver graft rejection and acceptance. Delta bilirubin can be useful in diagnosing and treating many liver diseases. Further large-scale studies are required, so we recommend more randomized control trials and large population studies addressing this question.

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