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## Concept of BuBc in neonatal jaundice : needs a change

Kalpana Singh, Auxilia Borrison, Abbas Ali Mahdi

**Key words:** conjugated bilirubin, neonatal jaundice, photobilirubin, unconjugated bilirubin

**Corresponding author:** Kalpana Singh, Assistant Professor, Department of Biochemistry, King George's Medical University, Lucknow U.P 226003, India; Mobile: +91-9532155750; E-mail : kalpanasinghdr@gmail.com

Neonatal jaundice is of two types, physiological jaundice which is more common and harmless and occurs on 3rd day after birth and disappears within 14 days as compared to the pathological jaundice which occurs within 24 hrs of life and needs attention. Physiological jaundice occurs due to increased erythrocyte destruction or due to low activity of uridine diphosphoglucuronyl transferase enzyme (UDPG). While causes of pathological jaundice are hemolysis, breast feeding, infections, hypothyroidism and extrahepatic biliary atresia and needs investigation and medical intervention. Physiological jaundice is characterized by elevated unconjugated bilirubin which decreases as the activity of UDPG enzyme increases. Causes of pathological jaundice have to be ruled out by estimation of conjugated and unconjugated bilirubin along with other relevant investigations. An elevated level of conjugated bilirubin in the blood is characteristic of extrahepatic obstruction. Congenital disorders of bilirubin metabolism also result in hyperbilirubinemia for eg. Crigler-Najjar Syndrome and Gilbert's Syndrome due to deficiency or defect of glucuronyl transferase enzyme. The latter is characterized by elevated levels of unconjugated bilirubin, while in Rotor and Ruben-Johnson Syndrome conjugated bilirubin increases. High level of unconjugated bilirubin can cross the blood brain barrier resulting in permanent brain damage known as kernicterus [1]. Early recognition of hyperbilirubinemia and its causes can help in reducing serum bilirubin levels. Till date the approach of clinicians for managing neonatal jaundice solely depends on total serum bilirubin and

direct bilirubin while indirect bilirubin is calculated by subtracting direct from total bilirubin. The term conjugated and unconjugated bilirubin is often confused with direct and indirect bilirubin, however, they are not the same. Clinicians have to understand the difference between the terminologies unconjugated bilirubin (Bu), conjugated bilirubin (Bc), direct (DBil) and indirect bilirubin as the management of neonatal jaundice strictly depends on the laboratory findings.

Total bilirubin (TBil) is estimated by traditional diazo method (van den Bergh and muller reaction) which estimates all the fractions of bilirubin i.e conjugated (mono- and di-glucuronide), delta bilirubin (fraction of bilirubin covalently bound to albumin), unconjugated and photo bilirubin. The traditional method used for total bilirubin estimate all the fractions of bilirubin. Direct bilirubin contains conjugated and delta bilirubin while indirect bilirubin which is calculated include unconjugated and photo bilirubin. Thus limitation of diazo method for estimation of direct bilirubin is that delta bilirubin, being water soluble, will falsely elevate DBil values. HPLC is the only technique by which different fractions of bilirubin can be estimated, but the method is laborious and time consuming [2].

Orthoclinical diagnostics Vitros microslide came with a method by which Bu and Bc can be estimated directly. The advantage of this microslide technology is that conjugated bilirubin (Bc) does not contain delta bilirubin and the fractions i.e BuBc are actually estimated. TBil estimation is also available based on traditional diazo method on the same instrument.

Delta bilirubin can also be calculated by formula TBil- (Bu+Bc). It is negligible in neonates, however, if present it is associated with an elevated Bc results. Neonatal bilirubin (NBil) is calculated by addition of Bu and Bc. Neonates predominantly have higher levels of unconjugated bilirubin as compared to adults and its level decreases within 14 days, as the conjugation begins and they can be monitored by estimation of BuBc. Elevated levels of conjugated bilirubin due to biliary atresia can also be estimated by BuBc slide [3]. Limitation of this method is that in vivo exposure of light may alter the structural and chemical properties of bilirubin, due to formation of photobilirubin which will result in noticeable increase of Bc. Thus newborns in neonatal care unit, under phototherapy may exhibit an average increase of 0.3 mg/dl in measured Bc, due to photobilirubin.

Bu and Bc, which gives the exact concentration of unconjugated and conjugated bilirubin in the blood is currently available on Vitros microslide technology and may be considered as a technique equivalent to HPLC for estimating different fractions of bilirubin [4]. However, total bilirubin and BuBc (Neonatal bilirubin) results cannot be correlated for neonatal samples, as the method for their estimation is entirely different. Before processing the sample for neonatal bilirubin, laboratory person must have complete information about the method they are using for bilirubin estimation. On the other hand clinicians must also be aware about the method used for their requisition. In the end it is once again emphasized that direct and indirect bilirubin should not be confused for conjugated and unconjugated bilirubin, as their meaning is not the same.

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