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# CORD BLOOD BILIRUBIN AND ALBUMIN - PREDICTOR OF NEONATAL JAUNDICE

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## ABSTRACT

Neonatal jaundice is very common finding in newborn babies.Albumin is synthesized in liver. Unconjugated bilirubin binds to albumin which helps in its transport. Bilirubin toxicity is reduced on the tissues This in turn reduces the bilirubin toxicity on the tissues. Transport and binding capacity are reduced and hence determination of at-risk neonates early on will help to avoid the complications associated with neonatal jaundice. The present study reviewed association between cord albumin and development of neonatal hyperbilirubinemia.

## INTRODUCTION

One of the most frequent aberrant findings in the first week of life in newborns is hyperbilirubinemia. Clinical jaundice often develops in one-third of newborn babies, and in most infants, it is a physiological phenomena that occurs naturally.[1]

Almost 60% of term infants and 80% of preterm neonates are affected in the first week of life.[2]Due to immature liver in newborns, poor gut microbiota, increase in beta glucuronidase activity and enterohepatic circulationleads to inability in handling the excessive bilirubin production in newborns,thus increases incidence of hyperbilirubinemia in neonates.

Neonatal jaundice has been ranked 7$^{th}$leading cause of mortality globally and accounts for almost 1309.3 deaths/100 000.Countries with low-middle socio-demographic index (SDI) values like Sub-Saharan Africa and South Asia.

ABO incompatibility are known to occur in 15% of pregnancies, however only 1% of newborns experience substantial hyperbilirubinemia that necessitates medical attention. Even in healthy term neonates with ABO incompatibility who do not exhibit severe hemolysis or a positive DAT (Direct antiglobulin test), extremely high bilirubin levels and kernicterus can develop.[3]

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Follow up visit is mandatory by American Academy of Pediatrics after 48 to 72 hours of discharge for all neonates who were earlier discharged before 48 hours of life todiagnose jaundice and other significant problems.[4]

Kernicterus is a highly fatal and irreversible occurrence that causes significant long-term morbidity and death, but it is currently prevented because phototherapy has a clear risk-reducing potential for bilirubin levels exceeding 20mg/dl.[5,6]

Numerous diseases and other circumstances put neonates at risk for developing hyperbilirubinemia, which necessitates medical care. RH incompatibility, cephalhematoma, substantial bruises, injuries, a prior sibling's history of neonatal jaundice, and pre-discharge TSB or TcB in a high risk level is a few examples. Even though our knowledge of neonatal hyperbilirubinemia has greatly advanced recently, it is still impossible to predict with absolute certainty which infants are most likely to experience substantial hyperbilirubinemia.

Since albumin and bilirubin bind at equimolar proportions, low serum albumin concentrations result in higher serum levels of free bilirubin. Since many years ago, similar associations between umbilical cord blood bilirubin and NH have also been discussed.

In 1960 Robinson et al. reported that cord blood bilirubin levels> 3mg/dl were highly suggestive of ABO incompatibility. [7]In 1977, Risemberget al. reported in their study that infants with ABO incompatibility and cord bilirubin levels >4 mg/dl are at higher risk of hyperbilirubinemia and that frequent re-evaluation is mandatory.[8] In 1989 Aage Knudsen found that 85% of babies became jaundiced, if cord bilirubin was more than 2.3mg/dl and 57 % of such babies required phototherapy.[9]

The researchers analyzed at how albumin and bilirubin in umbilical cord blood could be used to predict major NH. However, no study has determined a single cut-off value for the bilirubin and albumin levels in umbilical cord blood, particularly in situations of ABO incompatibility, allowing us to predict at birth which infants will experience substantial hyperbilirubinemia requiring therapeutic intervention.

Thus, in the present study we reviewthe predictive ability of the umbilical cord blood bilirubin and albumin in development of significant neonatal hyperbilirubinemia.

**Fetal bilirubin metabolism**

Due to lower foetal hepatic perfusion, decreased hepatic ligandin, and decreased Uridine diphosphoglucuronyl transferase (UDPG-T) activity, conjugated bilirubin synthesis is restricted in the foetus whereas the majority of unconjugated bilirubin is removed through the maternal circulation via the placenta. UDPG-T activity is detectable between 18 and 20 weeks, and levels in full-term and preterm neonates are typically less than 0.1% of adult values. Only 6 to 14 weeks after birth can this enzyme's adult value be seen.[10]

Normally in amniotic fluid the bilirubin is detected as early as 12 weeks of gestation, but disappears by 36- 37 weeks.[4]

During the neonatal period,bilirubinmetabolism shows a transition from the fetal to adult stage.The principal route ofeliminationin fetal stage is the placenta (lipid soluble

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unconjugated) whereas during adult stage the hepatic cellsis water-soluble conjugated form is excreted into the biliary system and gastrointestinal tract from hepatic cells.

**Neonatal Bilirubin metabolism**

Neonatal jaundice is defined as yellowish discoloration of the skin and sclera of the eyes in a newborn baby due to high bilirubin levels.Most common physical finding during first week of life is jaundice.

**Sources of Bilirubin**

Breakdown of different heme containing proteins produces bilirubin inside the reticuloendothelial system.

1. Hemoglobin of RBC isthe major heme containing protein almost contributes 75% of total bilirubin production.
2. Early labeled bilirubin is the rest 25% mostly released due to ineffective erythropoiesis in the bone marrow and otherheme containing proteins like myoglobin, cytochromes, catalase, peroxidase and from free heme.

**Bilirubin synthesis**

Formation of biliverdin is the first step in synthesis (alinear tetrapyrrole) from heme. Heme releases 1 mol of ferrous and 1 mol of $CO_2$. Itis a rate limiting step and gets up regulated during hemolysis.[12]

Second step of bilirubin involves conversion of biliverdin to bilirubin by Biliverdin reductase found in cytosol of the cells.[12]

**Bilirubin transport in the plasma**

Transport of bilirubin in plasma occurs in bound state with serum albumin; this complex usually does not enter the CNS and is thought to be nontoxic.

**Bilirubin uptake**

Dissociated bilirubin from albumin and its non-polarfat-soluble crossesplasma membrane of the hepatocyte and binds mainly to cytoplasmic ligandin (Yprotein) to get transported in the smooth endoplasmic reticulum.Ligandin concentration is increased by Phenobarbital.

**Bilirubin conjugation**

Unconjugated bilirubin (UCB) is usually fat soluble but converted to water soluble conjugated(direct) bilirubin (CB) in the SER by the enzyme UDPG-T. This enzyme is inducible by phenobarbital and catalyzesformation of bilirubin monoglucuronide. Both bilirubin monoglucuronide and bilirubin diglucuronide are able to be excrete in the bile canaliculi against a concentration gradient.

**Bilirubin excretion**

Conjugated bilirubin finally enters the GI tract and is eliminated from the body as stool, which containing large amount of bilirubin. Excretion is known to be the rate limiting step of the bilirubin clearance from the plasma.

**Enterohepatic circulation of bilirubin**

Conjugated bilirubin is not usually reabsorbed in the bowel until it isconverted to unconjugated bilirubin by the intestinal enzyme β-glucuronidase. Intestinal bacteria can prevent the enterohepatic circulation by converting the conjugated bilirubin to urobilinoids, which are not substrates of β-glucuronidase.

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**Albumin Metabolism and its role in Neonatal Hyperbilirubinemia**

Albumin is usually 69 kDaconstituting a major fraction of the protein in the human plasma contributing almost 60% of the total plasma protein. About 40% of albumin is present in the plasma. Albumin is initially synthesized as a **preproprotein.** Its **signal peptide** is removed as it moves through the cisternae of the rough endoplasmic reticulum (RER), and a **hexapeptide** at the amino terminal is cleaved subsequently along the secretorypathway.[13]

Endogenous albumin can be synthesized in the animal and human fetusfrom early gestational period. All albumin producedby the fetusisusually fetalin origin because albumin cannot cross the hemochorial placenta as in other animalslike rat, guinea pig.[14]

Synthesis of albumin usually starts by 7th-8th week in the human fetus and increasesinversely to that of (AFP) α-fetoprotein, which is the dominant fetal protein. In neonates Albumin concentrations are low almost (~2.5 g/dL), reach adult levels (~3.5 g/dL) after several months.

Albumin constitutes 70 – 75% and has variable functions includingmaintenance of plasma oncotic pressure, loadingof bilirubin, cysteine, free fatty acids, calcium, drugs and its antioxidant property.[15]

Albumin can be utilized as an index of the hepatocyte ability to carry out synthetic function. $T_{1/2}$ of albumin is usually 19–21 days and may not reflect acute changes in liver synthetic ability[15]

Little data is available regarding reference ranges for serum albumin concentrations in preterm and term infants. Serum albumin lower limit in term babies is 2.8 gm/dL.and in term mean serum albumin level is 3.1 gm/dL.[15]

Hence at term the normal range of Serum albumin is 3.1±3 g./dL

Postnatal albumingenerally follows the gestational trend and increases with gestational age. Considering the functions of albumin, which includeacting as an antioxidant and transporting bilirubin and free fatty acids[15]

Oxygen tension during intrauterine life in blood is low, thereby generating only low amounts of radicals, thatcan destroy albumin. The lowered oxygen tension will becompensated by the increased oxygen affinity to the fetal hemoglobin. Atbirth,HbFis broken down, hence releasing huge amount of bilirubin that needs to be transported by albumin. Also, during the start of the 3$^{rd}$trimester, fatty acid concentrations are usually lowered and will be of no burden to albumin. The increased albumin synthesis will therefore be seen just before term birth, as a preparation against an increased radical exposure and for a higher transport load consisting of hemoglobin breakdown products and fatty acids, the latter found in high amounts in postnatal breast milk.

Bilirubin and albuminusually bind in equimolar ratio. Free bilirubin is anticipated when the molar bilirubin- to- albumin (B: A) ratio is > 0.8. Around 8.5mg of bilirubin will bind tightly to 1 g of albumin.

It is the free bilirubin which can cross the blood brain barrier. There are no precise data to correlate a specific bilirubin value or albumin value withneurotoxicity.

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**Complications of neonatal jaundice**

The most significant side effect of newborn jaundice is bilirubin encephalopathy. The effect of bilirubin on the central nervous system is known as bilirubin encephalopathy, whereas kernicterus describes the neuropathological alterations brought on by pigment deposition in particular regions of the CNS, such as the basal ganglia, pons, and cerebellum.[15]

It is a complex process that necessitates the presence of susceptible nerve cells in the brain, a critical level of free bilirubin, and its ability to pass the blood-brain barrier. The degree and length of hyperbilirubinemia, albumin's binding capacity, structural maturity, cell membrane makeup, metabolic status, and physiological environment all likely have a significant impact on how neurodysfunction develops.

**Entry of bilirubin into the brain**

The mechanism by which indirect bilirubin enters the brain and causes damage is not clear.A number of hypotheses studies have been proposed about entry of bilirubin into the brain.[17]

One hypothesis isdue to the lipophilic nature of free bilirubin, in equilibrium with bound bilirubin, has access to tissues. Thus, any increase or decrease in the concentration of free bilirubinor binding capacity of albumin can result in increased level of free bilirubin within the brain tissue, saturating membranes and leading to precipitation of bilirubin acid in the nerve cell membrane.[15]

Second hypothesis is based on the rate of tissue uptake of bilirubin depends on both the concentration of albumin-bound bilirubin and the pH, Low pH is known to increase precipitation and tissueuptake.[15]

Third hypothesis suggests that a damaged blood-brain barrier is the cause of entry of bound bilirubin into the brain.[15]

Recent studies have suggested that unconjugated bilirubin is a substrate for Pglycoprotein (P-gp) and therefore the blood-brain barrier P-gp may play a role in limiting the passage of bilirubin into the CNS. It is an ATP – dependent integral plasma membrane transport protein that translocates a wide range of substrates across biologic membranes.[16]

**Factors that increase susceptibility to Neurotoxicity associated withHyperbilirubinemia**areAsphyxia**,**Septicemia**,**Hyperthermia**,**AcidosisHypoalbuminemia**,** Caloriedeprivation**,** Low birth weight**,**Young gestational ageandExcessive hemolysis.

**Bilirubin toxicity at cellular level**[16]

There are four possible ways:

Interruption of normal neurotransmission

Dysfunction of mitochondria

Cellular and intracellular membrane impairment

Interference with enzyme activity

Clinical features may includelethargy, poor feeding, high pitched cry, hypotonia,Irritability,opisthonous, seizures, apnea, oculogyric crisis,hypertonia, retrocollis.

All infants who survive this phase develop chronic bilirubin encephalopathy(clinical diagnosis of kernicterus)

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Pronounced opisthonous, piercing cry, apnea, seizures, coma, and death are examples of advanced features.

**Chronic bilirubin encephalopathy (kernicterus)**

Kernicterus is defined asbrain dysfunction induced by bilirubin.[17]This term was given by Schmorl in 1904. It happens in hyperbilirubinemia and is brought on by an accumulation of bilirubin in the central nervous system's grey matter, which may result in irreparable neurological damage. Athetosis, athetoid cerebral palsy, partial or whole loss of high frequency sensorineural hearing, paralysis of the upward gaze, dental abnormalities, and intellectual deficiencies are its defining features.[4]

**Evaluation and diagnosis of neonatal jaundice**

NH affects almost 60% of term and 80% of preterm neonates during first week of life.

Kramer,dermal staging may be used to gauge the severity of jaundice.[8]

The infant is inspected in broad daylight. Digital pressure should be used to blanch the skin, and the color of the subcutaneous tissue and underlying skin should be documented. Table 1 is a basic overview for the degree of dermal staining with the degree of bilirubin.

| Area of body | Level of bilirubin |
|---|---|
| Face | 4-6 mg/ dl |
| Chest, upper abdomen | 8-10 mg/dl |
| Lower abdomen, thighs | 12-14 mg/dl |
| Arms, lower legs | 15-18 mg/dl |
| Palms, soles | 15-20 mg/dl |

**Table 1: Kramer's staging for clinical diagnosis of jaundice[18]**

Dermal staining progresses in cephalo-caudal direction. The Cephalo-caudal progression is based on relative thickness of skin at various regions, skin is thinnest on the face and extremely thick on the palms and soles.The skin is relatively thinner in premature babiesand therefore jaundice shows more readily even at low serum bilirubin level.

High riskfactorsmay include Prematurity,perinatal asphyxia, blood group in compatibility,Perinatalasphyxia,Infant of diabetic mother, Intrapartum use of oxytocin, Problem in breastfeeding, h/o Jaundice in previous siblings.Cephalhematoma or significant bruising.

**HIGH RISK FACTORS for JAUNDICE[19]**

Prematurity.
Perinatal asphyxia.
Blood group in compatibility.
Low birth weight
Infant of diabetic mother.
Intrapartum use of oxytocin.
Problem in breastfeeding.
H/o Jaundice in previous siblings.

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Cephalhematoma or significant bruising.

**APPROACH TO NEWBORN with jaundice.[20]**

- High risk newborns to be identified.
- follow up for Jaundice should be ensured.
- Early and exclusive breast feeds should be ensured adequacy of breast feeding.
- clinical condition to be assessed
- birth weight &gestation to be ascertained
- Jaundice with post-natal age should be evaluated
- systematic evaluation – history and physical examination should be performed.
- Jaundice is physiological orpathological should be decided
- Observation is required in case of physiological and baby well.
- Signs of bilirubin encephalopathy (lethargy, poor, feeding, shrill cry, asymmetric Moro reflex, hypertonia, opisthotonus or convulsions) should be looked for if jaundice is severe
- Lab tests should be performed.
- Appropriate measures should be incorporated toreduce elevated bilirubin, counsel parents

**Criterion for physiological jaundice[57]**

Indirect bilirubin,
Direct bilirubin never > 2mg/dl or <15% of total bilirubin,
Appearance - after 36 hours of age,
Rate of rise of bilirubin – < 5mg /dl/day,
Severity of jaundice – Usually does not >15mg/dl

**Laboratory Evaluation of JaundicedNewborn[9]**

These tests are individualized to a newborn to know the cause for NH. Even after detailed investigations, the cause of NH remains uncertain in about one-third of cases. Investigation list as follows**:**

- **Maternal:** Blood grouping and Indirect Coombs Test (ICT) to test for Isoimmune hemolytic disease, Serology to rule out syphilis.

  **Infant:**
- Total serum bilirubin (TSB) or Transcutaneous bilirubin.
- Blood grouping, Rh typing and Direct coomb test to test for hemolytic disease.
- Hemoglobin and Hematocrit. Anemia suggests hemolytic disease and large entrapped hemorrhage.
- Polycythemia cause jaundice.
- Reticulocyte count is elevated in hemolytic anemia
- Red cell morphology – By peripheral blood smear
- Red cell fragmentation seen in disseminated intravascular coagulation(DIC)
- Spherocytes suggests ABO incompatibility or Hereditary Spherocytosis.
- Platelet count is decreased in infections.

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- White blood cellcounts< 50,000 cells/cumm or BNR> 0.2 suggest infection.
- Urine analysis for reducing substance to diagnose Galactosemia.
- Screening of G6 PD deficiency.
- Serum protein and albumin to estimate albumin binding capacity and reserve albumin binding site.
- pH
- Proteinbinding (2,4 hydroxy benzene azobenzoic acid (HABA),Salicylates)
- These tests help to measure the quantity of binding of bilirubin in the serum of jaundice infants.

## CONCLUSION

Neonatal hyperbilirubinemia occurs in 5-10% term neonates. Studies have shown that serum albumin <2.8 g/dl is considered as a risk factor for neonatal hyperbilirubinemia. Cord albumin levels > 3.4 g/dl is considered safe. Low albumin indicates less binding of bilirubin and hence decreased transport leading to increased deposition in tissues. Therefore measuring cord blood albumin and bilirubin levels may be used as an early predictor of neonatal hyperbilirubinemia.

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