TO STUDY THE CORRELATION BETWEEN UMBILICAL CORD BLOOD SERUM TOTAL BILIRUBIN LEVELS AND THE DEVELOPMENT OF SIGNIFICANT NEONATAL HYPERBILIRUBINEMIA IN TERM HEALTHY NEONATES

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INTRODUCTION

Hyperbilirubinemia is a common and in most cases benign problem in neonates. Jaundice is observed in first week of life in approximately 60 % of term infants and 80% of preterm infants. Although bilirubin may have a physiological role of an antioxidant, elevated levels of unconjugated bilirubin is potentially neurotoxic.**[**1**]** For these reasons careful monitoring of all jaundiced new-borns is required to ensure proper management.

In healthy term infants TSB peaks on 3rd or 4th day of life, attaining clinically detectable levels in approximately 2/3 of population **[**2**]**. Hence when the newborn stays at the hospital for a 72-hour post-delivery period; it is possible to observe for the non-physiological early onset jaundice, thus allowing medical intervention, if necessary. However, in the current era of early discharge of healthy term newborns after delivery, because of medical and social reasons and economic constrains3, the newborn may hence develop high levels of jaundice after discharge which may go unnoticed or untreated resulting in significant morbidity and mortality. It has been shown that newborns whose post delivery hospital stay is <72 hours are at a significantly greater risk for readmission than those whose stay is >72 hours **[**4**]**. Severe jaundice and even kernicterus can occur in some full term healthy newborns discharged early with no apparent early findings of haemolysis **[**4**]**.

Hyperbilirubinemia is the most commonly reported cause for readmission during the early neonatal period5. Such readmission, besides involving extra. expenses for the family, exposes a probably healthy newborn to the hospital environment **[**6**]**.

The American Academy of Paediatrics (AAP) recommends that newborns discharged within 48 hours should have a follow up visit after 2-3 days to detect significant jaundice and other problems.**[**7**]** Bilirubin toxicity remains a significant problem despite recent advances in the care of jaundiced neonates **[**8**]**. Kernicterus, though infrequent, is the cause of 10% of mortalities and at least 70% of long-term morbidities among newborns.**[**9**]**

In recent years many efforts have been made to identify the infants likely to develop significant hyperbilirubinemia requiring intervention. A few recent studies investigated the value of umbilical cord blood TSB levels as a tool in predicting the subsequent development of significant hyperbilirubinemia in healthy term newborns, because early prediction and detection of threatening bilirubin levels permit initiation of phototherapy and prevent higher risks, cost of other therapies and kernicterus.

In a country like India where majority of deliveries takes place in resource poor settings, evaluation of newborn for hyperbilirubinemia by estimating venous blood TSB levels has got some practical difficulties like availability of staff that has expertise in collection of venous sampling in a neonate. Umbilical cord blood can be easily obtained at the time of delivery by the cut end of the umbilical stump thereby overcoming the difficulties of venous sampling in neonate and also alleviating the need for painful pricking of the neonate for the sampling. There have been no such studies done till date in India to assess the correlation between umbilical cord blood TSB level and risk of development of neonatal hyperbilirubinemia.

The objective of the this study was to assess whether the TSB levels found in the umbilical cord blood at birth could be used as a tool to predict the risk of the development of significant hyperbilirubinemia in full-term healthy newborns.

AIMS AND OBJECTIVES

1) To study the correlation between umbilical cord blood TSB level at the time of birth and subsequent development of neonatal hyperbilirubinemia in healthy term neonates.

2) To assess whether the TSB levels found in the umbilical cord blood sample at birth in a full term healthy neonate could be used as a tool to predict the risk of development of subsequent significant unconjugated hyperbilirubinemia.

3) To assess if, measuring cord blood TSB at birth can reduce the need for doing 'pre-discharge' serum bilirubin sampling subsequently before discharge of the baby from the hospital.

4) To determine a 'cut-off value' of umbilical cord TSB which determines the risk of subsequent development of significant neonatal hyperbilirubinemia, this may thereby help in taking decision for early and safe hospital discharge of healthy newborns.

METHODOLOGY

A. STUDY DESIGN:

Prospective Observational Study.

B. STUDY POPULATION:

This study included full term healthy newborn neonates delivered in Manipal hospital, in Bangalore. Newborn infants fulfilling the following selection criteria were enrolled in the study after informed parental consent.

C. SELECTION CRITERIA:

a) Inclusion Criteria:

- All modes of delivery
- Term gestation i.e. gestation age more than or equal to 37 weeks
- Birth weight more than or equal to 2500gms
- APGAR score more than or equal to 7 at 1 and 5 min of life.
- Irrespective of the maternal –foetal blood group incompatibility(ABO or Rh)

b) Exclusion Criteria:

- Preterm neonates i.e. gestation less than or equal to 36+6 weeks
- Low birth weight neonates i.e. weight less than 2500
- Sick neonates i.e. neonates diagnosed to have Neonatal sepsis,

Respiratory distress syndrome, perinatal asphyxia, Meconium aspiration syndrome or Neonatal seizures etc.

D. PERIOD OF STUDY: JUNE 2010 TO DECEMBER 2011

E. PLACE OF STUDY: Neonatal Division, Manipal Hospital, Old Airport Road. Rustam Bagh, Bangalore.

F. METHOD OF EVALUATION:

Full term healthy neonates delivered in Manipal hospital neonatal unit were included in the study as per previously mentioned selection criteria. An informed consent was taken from the father or relatives at the time of delivery. Umbilical cord blood sample was collected at the time of delivery from the placental end of the umbilical stump. Blood was directly collected in the plain serum containers and immediately transported to the biochemistry laboratory and all samples so obtained were estimated for total serum bilirubin level by Diazotized sulfanilic acid method using Siemens Dimention® clinical chemistry system. Subsequently neonates were assessed clinically every day 3 times as per neonatal unit protocol. Icterus was assessed clinically and graded according to Kramer's rule of dermal icterus zone method10. Decision of sending a blood sample for laboratory assessment of serum bilirubin level along with the routine screening investigations was taken by the investigator based on the clinical severity as per Kramer's rule of dermal icterus.

'Significant hyperbilirubinemia requiring phototherapy' was defined as total serum bilirubin falling in the phototherapy zone as per AAP criteria.**[**9**]** Need for phototherapy was decided based on American Academy of Paediatrics guidelines for phototherapy as in practice parameter for treatment of unconjugated hyperbilirubinemia in healthy new born infants based on gestation weight bloodgroup incompatibility and other risk factors**[**9**]**.(fig 1)

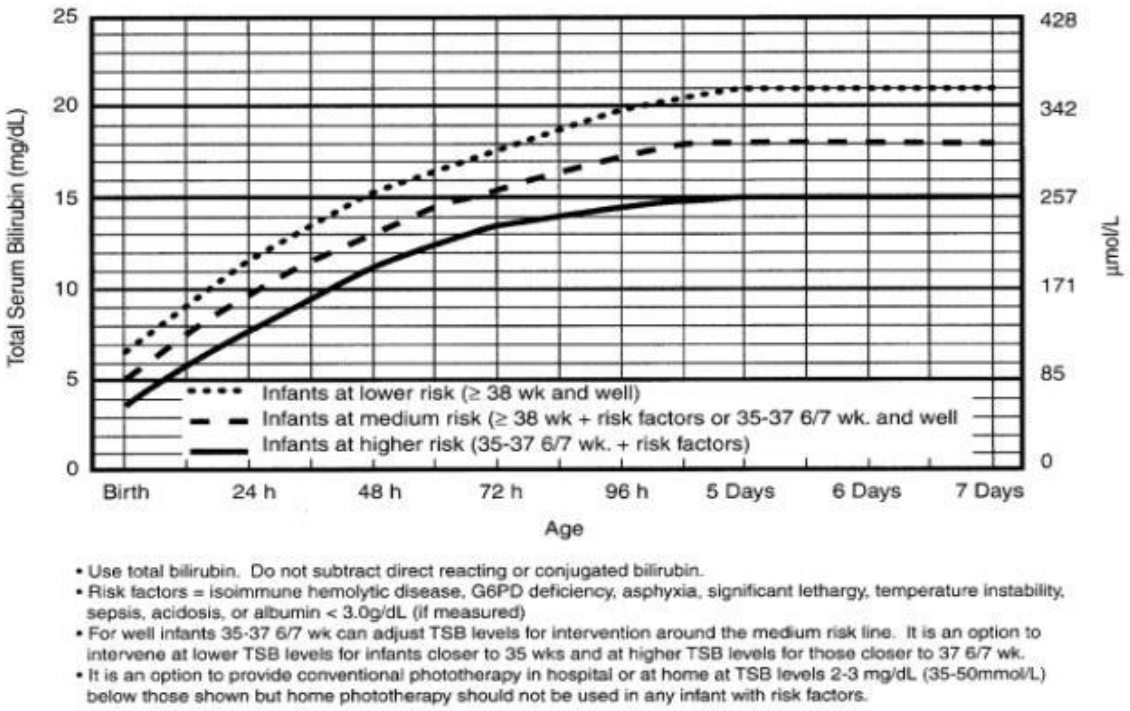

Fig.no.1: Guidelines for phototherapy for hospitalized infants, 35 weeks or more. (AAP-2004 guidelines for clinical practice.) **[**4**]**

The unit protocol was such that those newborn infants who did not require phototherapy were advised to return for followup as per their risk zone in the 'Hour specific bilirubin normogram' 11 published by AAP9. And those who required phototherapy were given double surface phototherapy and monitored by lab estimation of TSB as per protocol.

The unit protocol also determines follow-up visit of the neonates as per AAP guidelines. When serum bilirubin is estimated before 48 hours of life, decision on discharge and follow up was made by predicting the risk of subsequent hyperbilirubinemia by plotting TSB values on the 'Hour specific bilirubin normogram' 11 published by AAP.

For all newborn neonates enrolled under this study, data was collected on the Total serum bilirubin level in umbilical cord blood at the time of birth, Gestational age, Birth weight, Blood group of baby and mother, Clinical evaluation for the jaundice and TSB levels in subsequent blood sampling at 48 to 72 hours, and requirement of photo therapy (see appendix).

If the baby got readmitted in view of persistent or increasing jaundice, that data was also included in the study. Peak serum TSB levels estimated was noted. After compilation of data further analysis to correlate between umbilical cord blood TSB level and subsequent development of significant neonatal hyperbilirubinemia was done.

H. STATISTICAL ANALYSIS.

Data was analysed using Statistical Package for Social Sciences – 16 (SPSS-16) software for windows. Results were expressed as mean ± SD and percentage. To compare mean value between groups Students t-test and Chi-square test were applied appropriately. Sensitivity, specificity and positive predictive value for predicting hyperbilirubinemia was determined ROC analysis. Microsoft word and excel were used to generate graphs, tables etc.

REVIEW OF LITERATURE

Jaundice is defined as yellowish discolouration of skin and sclera due to elevated serum bilirubin levels. Level of bilirubin at which skin becomes icteric is around 2mg/dl in adults. In neonates clinical icterus is not evident until 5 mg/dl 1. Chemical hyperbilirubinemia, defined as a TSB level of 2.0 mg/dL or more, is virtually universal in newborns during the first week of life.

Significant Jaundice is observed during the 1st wk of life in approximately 60% of term infants and 80% of preterm infants. The yellow colour usually results from the accumulation of unconjugated, nonpolar, lipid-soluble bilirubin pigment in the skin.**[**1**]**

Although bilirubin may have aphysiologic role as an antioxidant, elevations of indirect, unconjugated bilirubin is potentially neurotoxic.**[**1**]**

Neonatal jaundice is classified into benign physiologic jaundice and hyperbilirubinemia, which is either pathologic in origin or severe enough to be considered deserving of further evaluation and intervention. This latter entity has been called “non-physiologic”, although frequently no disease is identified as being causative or consequent**[**12**]**.

Criteria for physiological jaundice **[**1**]**:

- The level of indirect bilirubin in umbilical cord serum is 1-3 mg/dL
- Level rises at a rate of <5 mg/dL/24 hr;
- Jaundice becomes visible on the 2nd or 3rd day,
- Peaking between the 2nd and 4th days at 5-6 mg/dL and decreasing to <2 mg/dL between the 5th and 7th days of life.

Physiologic mechanisms of neonatal hyperbilirubinemia:

Distinctive aspects of normal newborn physiology that contribute to neonatal hyperbilirubinemia include :

- Increased bilirubin synthesis;
- Less effective binding and transportation;
- Less efficient hepatic conjugation and excretion;
- Enhanced absorption of bilirubin via the enterohepatic circulation.

Criteria for pathological jaundice:

- Clinical jaundice in the first 24 hours of life
- TSB concentration increasing by more than 0.2 mg/dL (3.4 mol/L) per hour or 5 mg/dL (85 mol/L) per day
- TSB concentration exceeding the 95th percentile forage in hours
- Direct serum bilirubin concentration exceeding1.5-2 mg/dL (26-34 mol/L)
- Clinical jaundice persisting for more than 2 weeks in a full-term infant
- Mechanisms of pathological jaundice1:
- Increases the load of bilirubin to be metabolized by the liver (hemolytic anemias, polycythemia, bruising or internal hemorrhage, shortened red blood cell life as a result of immaturity or transfusion of cells, increased entero-hepatic circulation, infection);
- Damages or reduces the activity of the transferase enzyme or other related enzymes (genetic deficiency, hypoxia, infection, thyroid deficiency);
- Competes for or blocks the transferase enzyme (drugs and other substances requiring glucuronic acid conjugation); or
- Leads to an absence or decreased amounts of the enzyme or to reduction of bilirubin uptake by liver cells (genetic defect, and prematurity).

Physiology of bilirubin synthesis [13**]**

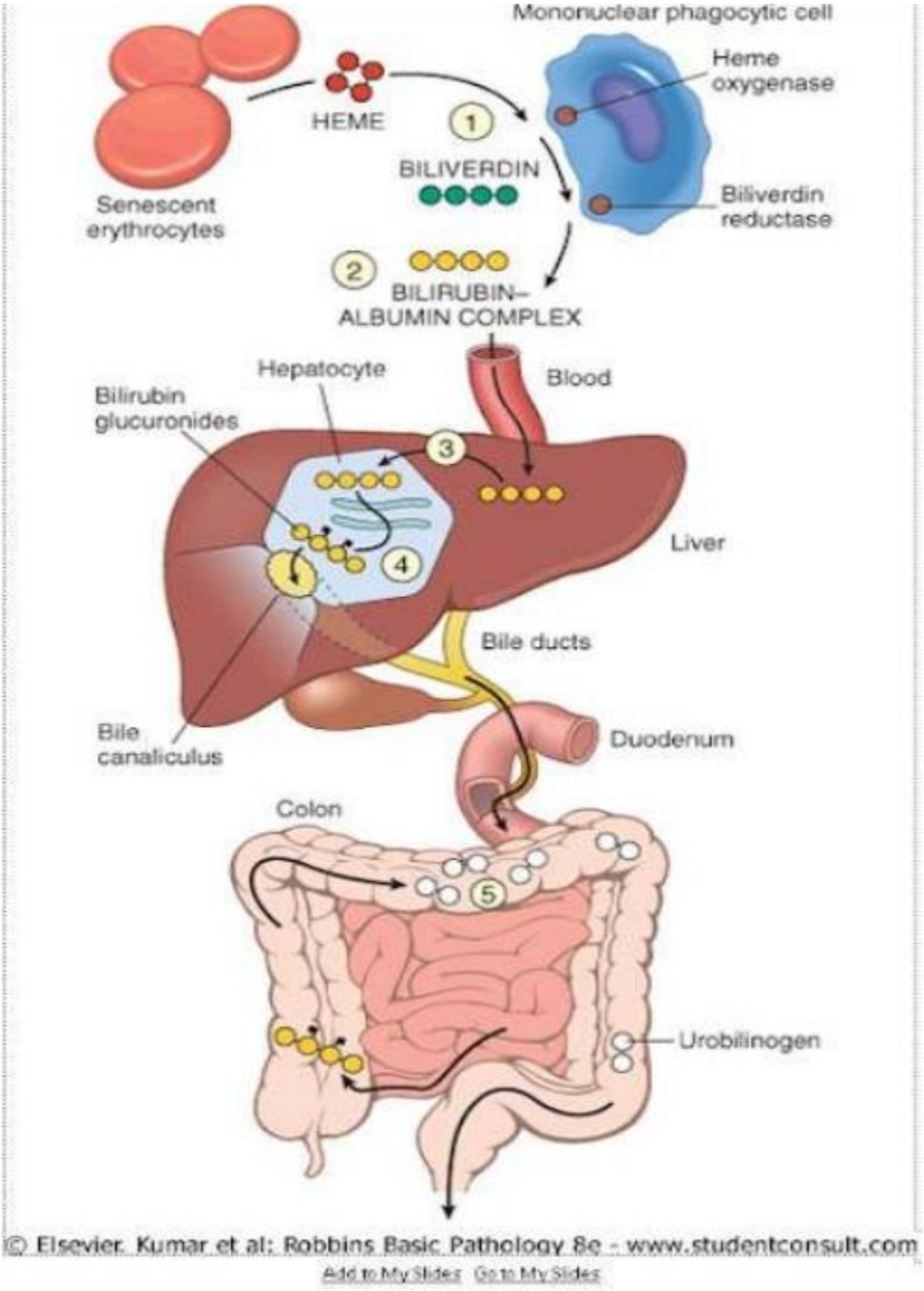

Fig.no.2: Pathway of bilirubin metabolism.

1. The heme ring of heme containing proteins is oxidezed in reticuloendothelial system tobiliverdin by microsomal enzyme heme Oxygenase, this reaction releases carbon monoxide. Biliverdin converted in bilirubin by enzyme beliverdin reductase.

2. Nonpolar bilirubin binds to serum albumin and transported to liver.

3. In lever bilirubin is uptaken by hepatocytes with the help of ligandin for transport o SER.

4. Bilirubin is conjugated in the hepatocytes with the help of the enzyme UDP glucuronide transferase. This conjugated bilirubin is excreted in the duodenum.

5. In colon this conjugated bilirubin is converted by intestinal bacteria intourobilinoids. Some part of conjuagated bilirubin is converted back to unconjugated bilirubin by intestinal enzyme beta glucuronidase which is reabsorbed into circulation – Entero Hepatic circulation.

Bilirubin synthesis:

Bilirubin is a breakdown product of hemoglobin. The bilirubin load is greater in neonates because in this population, not only does hemoglobin break down at two to three times the adult rate (Maisels et al, 1971),**[**14**]** but also there is an increased rate of red blood cell (RBC) degradation in the marrow even before release. In addition, bilirubin synthesis in healthy neonates results from a greater erythrocyte mass at birth and a shorter half-life of neonatal RBCs. Normal term newborns have a hemoglobin level of approximately 17 to 19 g/dL, and a hematocrit of approximately 50% to 60%. Polycythemia, defined as a hematocrit greater than 65%, occurs in 1.4% to 1.8% of infants born at sea level and in 4% of those born at higher altitudes. The life span of erythrocytes is less than 70 days for pre- mature infants. It is estimated to be approximately 70 to 90 days in healthy term infants, compared with 120 days in adults.

Binding & Transport:

Circulating bilirubin is bound to plasma albumin. It is believed that the neurotoxicity associated with hyperbilirubinemia is primarily the result of unbound or “free” bilirubin, so the amount of albumin available for binding is important.

The full-term newborn infant has a significantly lower plasma albumin level than that for the adult and, correspondingly, fewer bilirubin binding sites. The albumin level is inversely correlated with gestational age, so this lack of binding sites is more pronounced in preterm infants. Plasma albumin level increases rapidly over the first few days after birth, resulting in a mean increase over the first 7 days of almost 30%. Adult levels are reached by about 5 months of age (Notarianni, 1990).**[**15**]**

Albumin binding of compounds in neonates is similar to that in adults in that acidic drugs and bilirubin are bound to albumin, but the binding affinity maybe altered. In particular, affinity maybe altered in the first several days after birth. This may be due to the presence of endogenous displacing agents in newborns, as well as structural differences in the albumin that resolve with maturity, adult characteristics being attained by 10 to 12 months of age (Miyoshi et al, 1966).**[**16**]**

Conjugation & Excretion:

During intrauterine life, fetal removal of bilirubin is accomplished by way of the placenta and maternal-fetalcirculation, and the bilirubin in cord blood is virtually all unconjugated. At birth, blood supply to the right lobe of the liver changes from the high oxygen content of the umbilical vein to the low oxygen content of the portal vein. Blood flow through the hepatic arteries develops only in the first week of extrauterine life. In addition, the ductus venosus may remain partially patent for several days, allowing blood to bypass the liver completely. All of these factors can contribute to a delay in the plasma clearance of bilirubin.

The conjugating capacity of normal infants varies greatly, with delayed conjugation and excretion, in some cases, related to the immaturity of the liver cell itself. The activity of the uridine diphosphate- glucuronosyltransferase (UGT) system in the newborn liver must be induced. Elevated conjugated bilirubin evels at birth, in cases of severe fetal hemolysis, suggest that elevated TSB levels may be necessary to induce the conjugating enzymes. Production of UGT has been shownto be enhanced when certain drugs are administered. Phenobarbital is known to have this effect, and its clinical application is discussed later. Other pharmacologic substances , however , can inhibit UGT activity. Steroids structurally related to estrogen and progesterone have been shown to have this effect in vitro and in vivo, as have phenothiazines and the ester propionate preparation of erythromycin (Hsia et al, 1960).**[**17**]**

Enhanced enterohepatic circulation:

Intestinal absorption of bilirubin successfully excreted into the intestine is enhanced by several features of newborn physiology, thereby adding to the tendency of newborns to become jaundiced. Conjugated bilirubin, as either the mono or diglucuronide, is unstable and can be spontaneously or enzymatically hydrolyzed back to unconjugated bilirubin, which can be easily reabsorbed through the mucosa. In addition, absorption is enhanced by the sterility of the intestinal contents. Older children and adults have intestinal flora, which can metabolize conjugated bilirubin to the water-soluble and readily excretable breakdown products urobilin and stercobilin. Newborns have no such advantage; instead, the neonatal intestinal mucosa has a greater concentration of glucuronidase than is found in the adult. This enzyme can deconjugate bilirubin to form more unconjugated bilirubin, which can be absorbed via the enterohepatic circulation, adding further to the circulating unconjugated bilirubin load. Two other factors accelerating the deconjugation of bilirubin glucuronides in the newborn intestine are the mildly alkaline pH of the proximal intestine, which facilitates nonenzymatic hydrolysis, and the predominance of monoglucuronides as the main excretion form of bilirubin in the first few days of life.

Causes of Unconjugated Hyperbilirubinemia:[12**]**

A. Excessive production of bilirubin (hemolytic disease of newborn)

1. Blood group heterospcificity (incompatibility)

a. Rh isoimmunization

b. ABO incompatibility

c. Minor blood group incompatibility

2. Red blood cell enzyme abnormalities

a. Glucose-6-phosphate dehydrogenase deficiency

b. Pyruvate kinase deficiency

3. Sepsis

4. Red blood cell membrane defects

a. Hereditary spherocytosis

b. Elliptocytosis

c. Poikilocytosis

5.Extravascular blood

6. Polycythemia

B. Impaired conjugation or excretion

1. Hormonal deficiency

a. Hypothyroidism

b. Hypopituitarism

2. Disorders of bilirubin metabolism

a. Crigler-Najjar syndrome type I

b. Crigler-Najjar syndrome type II (Arias disease)

c. Gilbert disease

d. Lucey-Driscoll syndrome

3. Enhanced enterohepatic circulation

a. Intestinal obstruction, pyloric stenosis

b. Ileus, meconium plugging, cystic fibrosis

CAUSES OF JAUNDICE ON THE BASIS OF AGE OF ONSET:[18]

A. Within 24 hrs of birth:

1. Hemolytic disease of the newborn due to feto-maternal blood group incompatibility in the rhesus,ABO and minor blood group systems.

2. Intra-uterine infections

3. Deficiency of red cell enzymes-G6PD, pyruvate kinase ,hexokinase

4. Hereditory spherocytosis

5. Criggler-najjar syndrome

6. Homozygous alfa thallasemia

7.Lucey Driscoll syndrome

8. Drugs-vit.k, salicylates, sulfasoxazole, etc., to the mother.

B. Within 24-72 hrs of age:

Physiologic jaundice appears during this period but can be aggravated and prolonged by immaturity, birthasphyxia, acidosis, hypothermia, hypoglycemia, cephalhematoma or any concealedhemorrhage, polycythemia, hypothyroidism, breast feeding, sepsis and mild hemolytic states due to feto-maternalblood group in compatibility,spherocytosis, and deficiency of red cell enzymes.

C. After 72 hrs of age (and within first 2 weeks):

1. Sepsis

2. Neonatal hepatitis

3. Extra hepatic biliary atresia

4. Inborn errors of metabolism.

5. Hypertrophic pyloric stenosis and intestinal obstruction.

(IN APPROXIMATE ORDER OF IMPORTANCE)[19**]**

RISK FACTORS FOR DEVELOPMENT OF SEVERE

HYPERBILIRUBINEMIA IN INFANTS ≥35 WEEKS OF GESTATION

MAJOR RISK FACTORS
• Predischarge TSB or TcB level in the high-risk zone (see Fig. 96-8) • Jaundice observed in the first 24 hr • Blood group incompatibility with positive direct antiglobulin test, other known hemolytic disease (glucose-6-phosphate dehydrogenase deficiency), elevated end-title CO concentration • Gestational age 35-36 wk • Previous sibling received phototherapy • Cephalohematoma or significant bruising • Exclusive breast-feeding, particularly if nursing is not going well and weight loss is excessive • East Asian race*
MINOR RISK FACTORS

• Predischarge TSB or TcB level in the high intermediate-risk zone
• Gestational age 37-38 wk • Jaundice observed before discharge • Previous sibling with jaundice • Macrosomic infant of a diabetic mother • Maternal age ≥25 yr • Male gender
DECREASED RISK (these factors are associated with decreased risk of significant jaundice, listed in order of decreasing importance)
• TSB or TcB level in the low-risk zone (see Fig. 96-8) • Gestational age ≥41 wk • Exclusive bottle-feeding • Black race • Discharge from hospital after 72 hr

From AAP Subcommittee on Hyperbilirubinemia: Management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation, Paediatrics114:297–316, 2004.

Clinical features of neonatal jaundice:[1**]**

Jaundice usually becomes apparent in a cephalo-caudal progression, starting on the face and progressing to the abdomen and then the feet, as serum levels increase. Dermal pressure may reveal the anatomic progression of jaundice (face, ≈5 mg/dL; mid-abdomen, ≈15 mg/dL; soles, ≈20 mg/dL), but clinical examination cannot be depended on to estimate serum levels.

Infants with severe hyperbilirubinemia may present with lethargy and poor feeding and, without treatment, can progress to acute bilirubin encephalopathy (kernicterus)

BILIRUBIN TOXICITY, ENCEPHALOPATHY, AND KERNICTERUS [12**]**

Neonatal jaundice can be an entirely benign physiologic process, or it can also be the first sign of serious illness with associated toxicity manifested in the nervous system. The terms bilirubin encephalopathy and kernicterus represent clinical and pathologic abnormalities resulting from bilirubin toxicity in the central nervous system. Often the term kernicterus is used to include an entire spectrum of clinical and pathologic manifestations attributed to bilirubin. However, kernicterus, by strict definition, includes only the neuropathologic changes that are characterized by pigment deposition in specific regions of the brain, especially the basal ganglia, pons, and cerebellum. Of all infants in whom kernicterus develops, 50% die, and the survivors may have choreoathetoid cerebral palsy, high- frequency auditory nerve deafness, and mental retardation. The term bilirubin encephalopathy is correctly applied to the clinical manifestations of the effects of bilirubin on the central nervous system; a broad spectrum of neurologic signs is attributed to bilirubin, ranging from subtle behavioral changes such as lethargy and irritability to seizures, hearing deficits, mental retardation, and death. Because it is not clear under what conditions neurotoxicity develops or at what TSB concentration this damage occurs, little agreement exists about what constitutes a “safe” level of bilirubin. The effect of even moderate increases in TSB levels on early development remains a source of controversy, especially because some clinical manifestations are reversible on reduction of the STB concentration.

Before exchange transfusions were used to control TSB levels in isoimmune hemolytic disease, kernicterus was a common postmortem finding in infants dying with severe jaundice. Kernicterus has been documented in ill, low birth weight (mainly premature) infants whose TSB levels remained much lower than the levels formerly associated with kernicterus. Whether this “low-bilirubin kernicterus” is associated with prematurity alone or is necessarily associated with stresses such as hypoxia, acidosis, respiratory distress, and neonatal septicemia is uncertain.

Clinical manifestations of bilirubin toxicity:

Bilirubin toxicity usually does not become overt until high TSB levels have been established for several hours. Acute bilirubin encephalopathy typically progresses through three stages.

Stage 1: occurs during the first few days, with the infant having decreased activity, poor sucking, hypotonia, and a slightly high-pitched cry. If the TSB level is rapidly decreased (e.g., by way of exchange transfusion), the abnormalities often can be reversed.

Stage 2: develops after a week, with the infant demonstrating the features of stage 1 and also rigid extension of all four extremities, tight-fisted posturing of arms, crossed extension of the legs, and a high- pitched, irritable cry. Sometimes these changes are accom- panied with seizure activity, backward arching of the neck (retrocollis) and trunk (opisthotonos), and fever.

Stage 3: typically begins after the first week, with the infant demonstrating hypertonia with marked retrocollis and opisthotonos, stupor or coma, and a shrill cry. After several months in patients who survive, chronic bilirubin encephalopathy develops and has three major features: movement disorder (chorea, ballismus, tremor), gaze abnormalities (especially limitation of upward gaze), and auditory abnormalities.

The most common findings later in childhood are choreoathetosis, ocular paralysis, and eighth nerve deafness; severe mental retardation and spastic cerebral palsy occur in a minority. In general, the motor findings are the most obvious abnormalities in long-term survivors. In addition to the neurologic abnormalities, some children with bilirubin encephalopathy have dental enamel hypoplasia.

Factors that Increase Susceptibility to Neurotoxicity Associated with Hyperbilirubinemia:**[**18**]**

- Asphyxia
- Hyperthermia
- Septicemia
- Hypoalbuminemia

- Acidosis
- Caloric deprivation
- Prolonged hyperbilirubinemia
- Young gestational age
- Low birth weight
- Excessive hemolysis

MANAGEMENT OF NEONATAL HYPERBILIRUBINEMIA [19**]**

The aim of therapy is to ensure that serum bilirubin is kept at a safe level and brain damage is prevented. Exchange blood transfusion remains the single most effective and reliable method to lower the bilirubin when it approaches the critical levels. However, other supportive and therapeutic measures are useful to prevent excessive raise of serum bilirubin and reduce the need for exchange transfusion.

METHODS OF ASSESSMENT OF JAUNDICE:

A. CLINICAL ASSESMENT:**[**10**]**

The severity of jaundice should be assessed in natural daylight by observing the cephalo-caudal progression, and by using Krammer's staging **[**10**]**

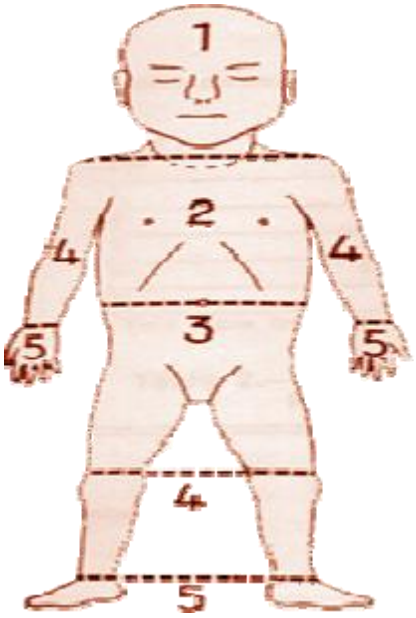

Fig.no.3: Krammer's method of dermal icterus:

ZONE	1	2	3	4	5
Range (mg/dl)	4-6	8-10	12-14	15-18	15-20

B. NON-INVASIVE METHODS:

(a) Transcutaneous bilirubinometry

(b) End-tidal CO estimation

C. INVASIVE METHOD:

Measuring Total serum bilirubin levels (TSB).

Modes of Intervention to Reduce Serum Bilirubin Concentration **[**12**]**

1. Hydration

2. Phototherapy

3. Exchange transfusion

4. Drugs to increase conjugation

5. Inhibition of reabsorption (binding in the gut)

6. Inhibition of bilirubin production

1. HYDRATION:

There is no evidence that excess fluid administration affects the TSB concentration. Some infants however, especially those being breast-fed, may be mildly dehydrated and may need supplemental fluid intake to correct their dehydration. Reduction in TSB concentration, which appears to be a response to rehydration, especially with increased oral nutrition, may be related to the correction of dehydration, to more effective intestinal motility (with the increased oral intake leading to elimination of conjugated bilirubin via stool), or to enhanced removal of photoproducts in both urine and stool. Because milk-based formula is less likely to enhance reabsorption of bilirubin by the enterohepatic circulation, it often is recommended for use for oral correction of mild dehydration in hyperbilirubinemic children.

2. PHOTOTHERAPY:

Phototherapy for neonatal hyperbilirubinemia was first proposed in 1958 by Cremer and colleagues in England (Cremer et al, 1958).20 Subsequently, this therapy has been used for the reduction of elevated TSB levels and for the “prophylactic” prevention of hyperbilirubinemia in premature infants. With the development of Rh-immune globulins to prevent maternal isoimmunization and the introduction of phototherapy, the need for exchange transfusions in healthy term infants was reduced significantly. Phototherapy has remained the mainstay of treatment for hyperbilirubinemia.

The efficacy of phototherapy is influenced by the following factors:

(1) The spectrum of light delivered, the blue- green region of the visible spectrum being the most effective;

(2) The energy output or irradiance of the phototherapy light, measured in W/cm2/nm; and

(3) The surface area of the infant exposed to phototherapy (Landry et al,1985).**[**21**]**

Bilirubin is known to undergo photodegradation in vivo and in vitro, resulting in photoproducts that are more polarized and therefore more water soluble and potentially excretable than native bilirubin. Bilirubin is a yellow pigment with a peak absorption at a wavelength of 450 nm. The initial and most rapid reactions occurring as a result of phototherapy produce configurational isomers, characterized by changes in the shape of the bilirubin molecule but not its structure (McDonagh and Lightner, 1988).**[**22**]** These isomers are the most abundant serum photoisomers and may account for up to 20% of TSB in infants undergoing phototherapy. The most prominent of these configurational isomers is called 4Z,15E bilirubin (native bilirubin being 4Z,15Z bilirubin). The second most rapid photochemical reaction leads to the formation of structural isomers, the most prominent known as lumirubin. Formation of this compound appears to be irreversible. Although certain phototherapy reactions reach their peak at relatively low levels of irradiance (6 to 9 W/cm2/nm), the production of lumirubin is directly proportional to the energy output on the skin (Garg et al,1995).**[**23**]** High-intensity phototherapy results in greater amounts of lumirubin among the photoisomers.

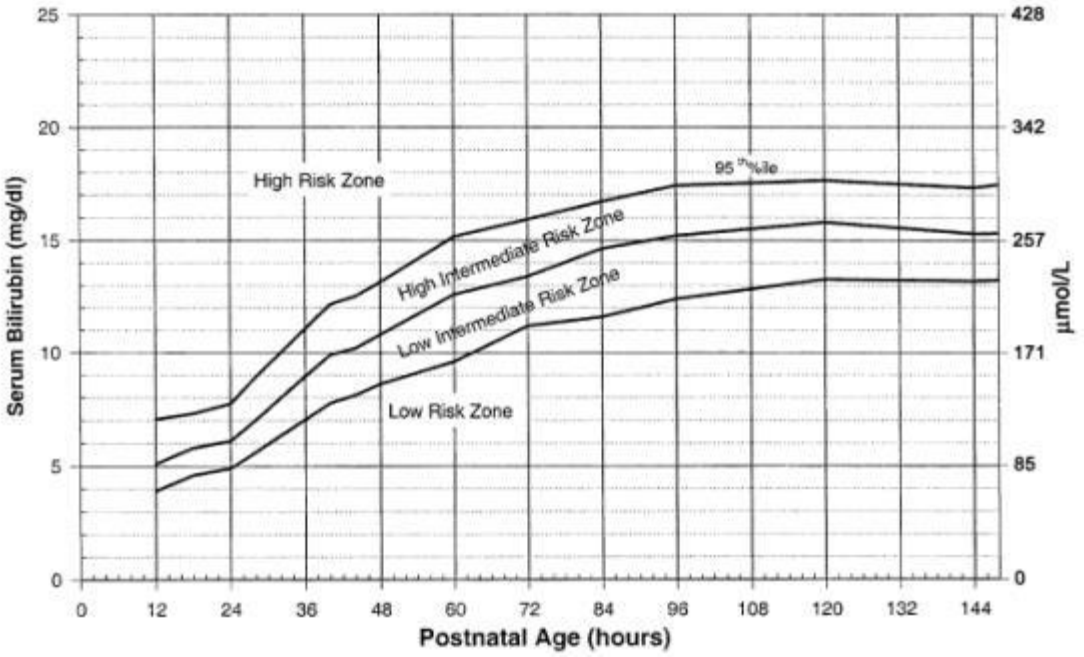

Fig .No.4: Nomogram displaying risk designation of term and near-term well infants based on hour-specific serum total bilirubin values. The high-risk zone is designated by the 95th percentile track. The intermediate risk zone is subdivided into upper and lower risk zones by the 75th per- centile track. The low risk zone is below the 40th percentile. (From Bhutani VK, Johnson L, Sivieri EM: Predictive ability of a predischarge hour–specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy and near- term newborns. Paediatrics103:6-14, 1999).**[**11**]**

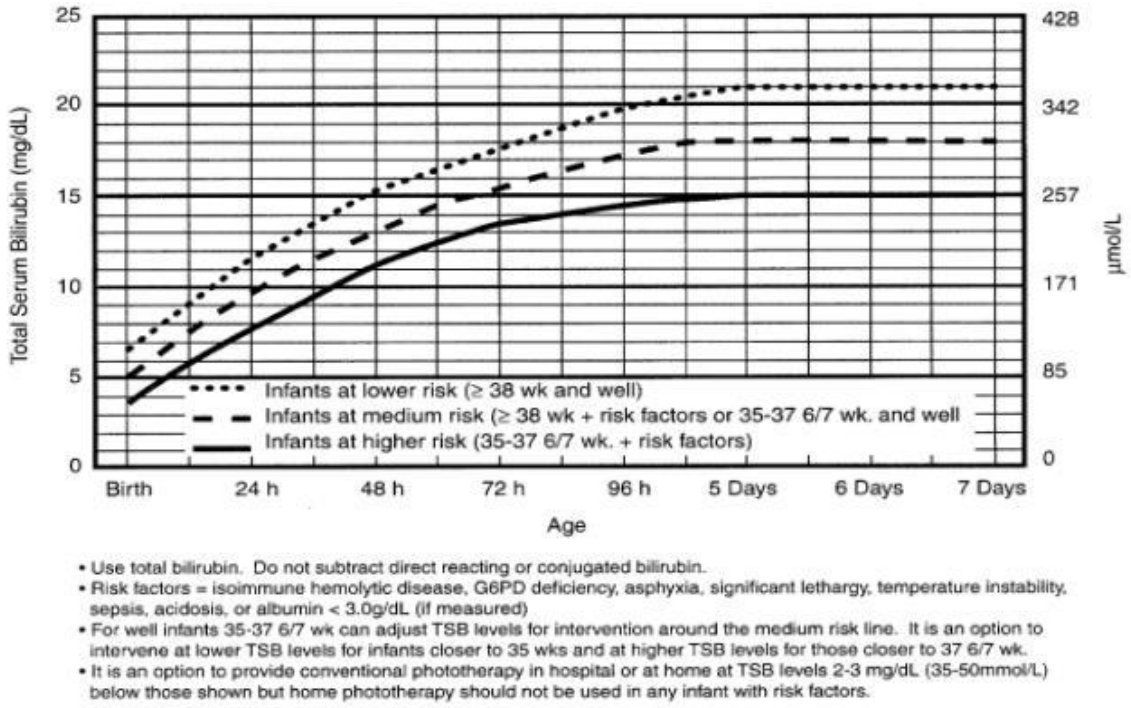

Fig.no.1: Guidelines for phototherapy for hospitalized infants,35 weeks or more. (AAP-2004 guidelines for clinical practice.) **[**4**]**

Commonly used phototherapy units contain a number of “daylight,” “cool white” or “special blue” fluorescent tubes. Others use tungsten-halogen lamps. These lamps may be part of a radiant warming device, included as a bank of lights shining on the baby, or included in fiberoptic vests or blankets, which are used to increase the surface area exposed or to aid in continuing phototherapy while a baby is held or fed. The special blue fluorescent tubes make the baby appear blue, occasionally causing discomfort to nursery personnel. Recently, high-intensity gallium nitride light-emitting diodes (LEDs) have been proposed as a potential light source for delivering phototherapy (Vreman et al, 1998). LEDs deliver high-intensity narrow-band light with minimal heat generation, are lightweight and portable, and can be used in a variety of applications. In a preliminary clinical trial, blue LED phototherapy was as efficacious as conventional fluorescent photo therapy (Seidman et al, 2000).**[**24**]** The potential benefits and versatility of this type of phototherapy are promising.

3. EXCHANGE TRANSFUSION:

Although the first successful exchange transfusion performed on an infant with familial icterus gravis was reported in 1925 (Hart, 1925),**[**25**]** this mode of intervention was not accepted until hemolytic disease of the newborn was conceptually understood in the 1940s. Exchange transfusion decreases the risk of bilirubin encephalopathy by reducing the total bilirubin load, increasing the binding sites of plasma albumin, and shifting bilirubin out of tissue into the plasma as well as providing erythrocytes less apt to haemolyse.

Exchange transfusion should be considered in cases of hemolysis in which intensive phototherapy has failed to decrease the TSB levels by 1 to 2 mg/dL (17 to 34 mol/L) in 4 to 6 hours or when the rate of rise of TSB indicates that the level will reach 25 mg/dL (428 mol/L) within 48 hours. It also should be performed in infants with high TSB concentrations and early signs of kernicterus and in cases of hemolysis with anemia and hydrops. Exchange transfusion removes much of the circulating bilirubin and "sensitized" red cells (erythrocytes with maternal antibodies attached), replacing them with red cells compatible with the mother's antibody rich serum and providing fresh albumin with binding sites for bilirubin. The process is tedious in that 5- to 10-mL aliquots are removed and replaced sequentially until about twice the volume (blood volume is 85 to 90 mL/kg) of the neonate's circulating blood volume has been exchanged. The anticoagulant of choice in the blood being infused is citrate-phosphate-dextrose. Because the citrate chelates calcium ions, there may be a need for cal- cium gluconate infusion during the course of the exchange. High concentration of glucose in the infusate may stimu- late insulin production, increasing the risk of severe hypoglycemia (Rubaltelli and Griffith, 1992).**[**26**]**

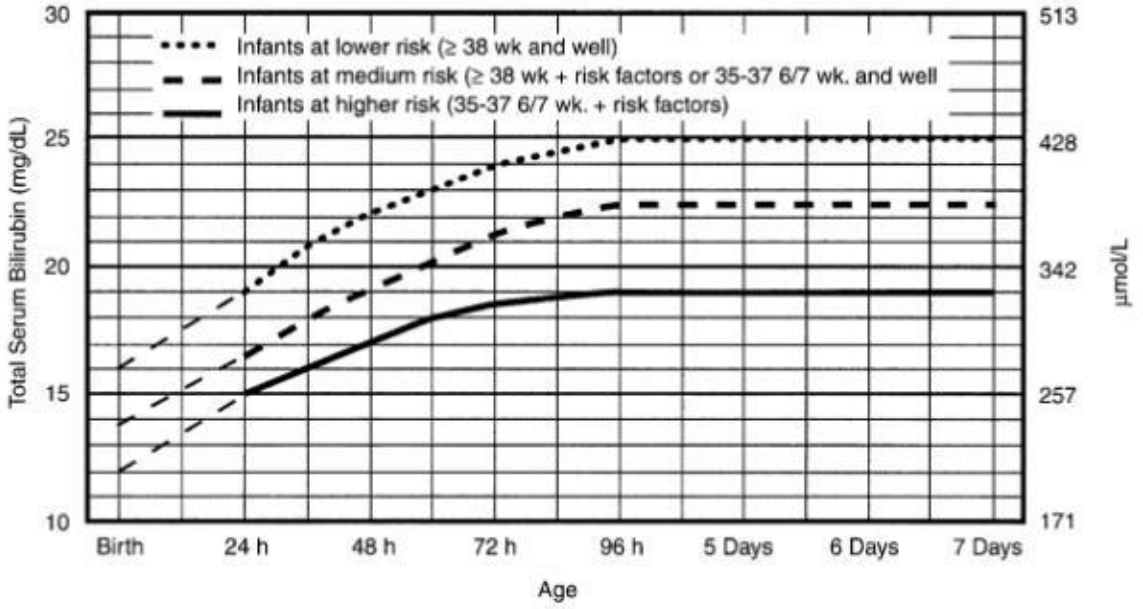

Fig. no. 5: Guidelines for exchange transfusion for neonates > 35 weeks of gestation (AAP-2004) **[**19**]**

Blood stored for more than 4 days has excessive potassium levels, and such blood requires erythrocyte washing and resuspension in compatible plasma. All of these issues, as well as the temperature of the infusate and the environment, lead to possible stress and instability in the infant, especially in one who is ill and of low birth weight. The amount of bilirubin removed by each transfusion is a function of the initial TSB level and the amount of blood exchanged. After an exchange transfusion, low levels of TSB concentration may increase rapidly for several hours as bilirubin in tissues "migrates" back into the circulation. Typically, TSB levels fall to one half of the pre-exchange value. In some cases (particularly in infants with hemolytic disease), the procedure may need to be repeated to lower the TSB concentration sufficiently.

Exchange transfusions are not free of risk. Some reports estimate a risk of morbidity resulting from the procedure at 5%, with apnea, bradycardia, cyanosis, vasospasm, and hypothermia being the most common problems. Other risks include coagulation disturbances, electrolyte imbalances, thrombocytopenia, necrotizing enterocolitis, portal vein thrombosis, blood- borne infections, cardiac arrhythmias, and sudden death. Monitoring of electrolytes, platelet count, coagulation parameters, and arterial blood gases is recommended during the procedure. The procedure should there- fore be instituted only when intensive phototherapy does not control the rapid rate of rise of TSB levels and the risk of bilirubin encephalopathy outweighs the risk from the procedure itself. Mortality rate estimates run as high as 0.5%.

4. INDUCING ENZYMES TO INCREASE CONJUGATION:

Several pharmacologic agents have been identified as liver enzyme inducers, and those inducing UGT have been shown to increase the conjugation and excretion of bilirubin. Phenobarbital and nicotinamide were the first agents used for prevention and treatment of hyperbilirubinemia in newborns, and phenobarbital continues to be used in Gilbert disease and Crigler-Najjar syndrome type II (Arias disease). The effect of phenobarbital (at a dosage of 2.5 mg/kg/ day) on TSB levels begins within a few days of its administration. This characteristic has made it less than optimal for use in the usual neonatal hyperbilirubinemia, because the TSB is already high or rapidly increasing in the first few days. Phototherapy has been shown to be more effec- tive in this situation, and adding phenobarbital to phototherapy has not been shown to have any advantage. However, postnatal phenobarbital administration may provide a useful approach to later elevation of TSB levels.

Several chemicals used in traditional Chinese medicine have an enzyme- inducing effect similar to that of pheno- barbital (Yin et al, 1991a). In studies involving rats and rabbits, yin zhi huang in a dose of 30 to 60 mg/kg/day has been shown to accelerate the plasma clearance and conjugation of bilirubin to an even greater degree than occurs with phenobarbital at 60 mg/kg/day. Both drugs increase glucuronyltransferase activity, but because they have dissimilar effects on other liver enzymes, it appears that their mechanisms of action may be different.

5. BLOCKING BILIRUBIN REABSORPTION:

Neonatal hyperbilirubinemia is exacerbated by, if not caused by, enhanced re absorption in the enterohepatic circulation. The absence of intestinal flora in neonates prevents the degradation of bilirubin in meconium and stool to products such as urobilinogen, which could be excreted. Bilirubin glucuronide arriving in the intestines is readily deconjugated to bilirubin and reabsorbed. Some of the products of phototherapy, especially the configurational isomers of bilirubin, also return to the native bilirubin form and may be reabsorbed. To counteract this process, various strategies have been used to bind the bilirubin in the intestinal lumen to substances that resist absorption.

Seen as an effective low-risk, low-cost therapy, products such as activated charcoal and dried agar (an extract of seaweed) have been used with inconsistent results.

More recent studies using doses of 500 mg/kg of agar every 6 hours suggest that agar therapy can be used to augment the efficacy of phototherapy, and perhaps it could be used as therapy by itself (Caglayan et al, 1993).27 Besides resulting in a more rapid decline in STB levels, the use of agar increased stool frequency, suggesting enhanced clearance of intraluminal bilirubin regardless of binding. Cholestyramine was similarly considered but was found to cause hyperchloremic acidosis (Nicolopoulos et al, 1978).28

6. INHIBITING BILIRUBIN PRODUCTION:

Whereas other modes of intervention are directed at disposing of excess bilirubin already produced, attempts to decrease the production of bilirubin have met with moderate success. Studies in vitro and in both animals and humans have shown that certain metalloporphyrins can reduce the production of bilirubin, substantially reducing TSB levels. These studies suggest that, in cases of hyperbilirubinemia caused by excess bilirubin production (i.e., catabolism of heme), these metalloporphyrins could be used to prevent the accumulation of dangerous TSB levels, thus obviating more expensive, time-consuming, or hazardous interventions such as phototherapy and exchange transfusion. The mechanism of action of these compounds is competitive inhibition of the activity of HO, the rate-limiting enzyme in heme catabolism. Tin or zinc protoporphyrin, tin or zinc mesoporphyrin, and other synthetic analogues of the natural metalloporphyrin (ferroporphyrin-heme) are potent competitors of HO, the critical enzyme in the catabolism of heme. Their action is based on the fact that the catalytic site of HO recognizes metalloporphyrins with central metal ions other than iron.HO actually favors some of these metalloporphyrins over heme as a substrate, sometimes by a large factor (Cornelius and Rodgers, 1984; Valaes et al, 1994; Vreman et al, 1990, 1991). The potency of metal lo porphyrin s and their side effects are influenced by the central metal cation and the nature of the side chains. Tin porphyrins are more potent than zinc or cobalt porphyrins.

Since early detection of neonatal jaundice and interventions to control the increasing bilirubin levels can prevent significant proportion of infants from developing kernicterus, many parameters have been used to predict the hyperbilirubinemia . Recently following tools have

been used in monitoring newborn infants for jaundice and early development of pathologically high level of jaundice.

A. NON-INVASIVE METHODS:

(a) Transcutaneous bilirubinometry

(b) End-tidal CO estimation

B. INVASIVE METHOD:

Measuring First day total serum bilirubin and Predischarge Total serum bilirubin levels(TSB).

Umbilical cord TSB is also a useful tool to predict risk of development of significant neonatal hyperbilirubinemia.

Many studies have been done to assess the predictability of risk of development of significant hyperbilirubinemia in a term healthy newborn based on TSB levels in umbilical cord blood.

J. Maxwell Johnstone,**[**29**]** et al in 1953 showed that in ABO heterospecific pregnancies there is a slight but significant increase in the mean cord-blood serum bilirubin value above that for homospecific pregnancies, and that this difference is unaffected by the Rh status of the pregnancy, provided that cases of maternal Rh iso-immunization are excluded.

Risemberg, **[**30**]** et al in 1977 showed that the risk to infants with ABO incompatibility and cord TSB levels greater than 4 mg/100 ml (68 mol/l) is so consistent that frequent re-evaluation is mandatory in this group, and there may be an indication for therapeutic intervention even earlier in the course of the disease than is currently the practice.

In 1986, Rosenfeld**[**31**]** et al, analyzed a group of 108 full-term newborns according to their risk of developing severe hyperbilirubinemia and concluded that neonates with an umbilical cord blood TSB level of lower than 2 mg/100 ml had a 4% chance of developing significant jaundice, in comparison with a 25% chance presented by the ones with levels higher than 2 mg/100 ml. In addition, the latter group also presented a higher chance of needing to undergo phototherapy.

Knudsen[32] et al, in 1989, carried out a study to demonstrate that jaundiced newborns presented higher umbilical cord blood TSB levels than newborns without clinical jaundice. In addition, the number of jaundiced newborns undergoing phototherapy was significantly higher when these levels were higher than 2.3 mg/100 ml, in comparison with the number of jaundiced newborns with no need for treatment and whose cord blood TSB levels were lower than or equal to 2.3 mg/100 ml. This proved the possibility of defining a newborn risk group for developing neonatal hyperbilirubinemia at birth.

Bernaldo[33] et al in 1997 ; showed that unconjugated bilirubin levels in cord blood were indicative of the jaundice severity developed by full-term newborns without complications, up to their third day of life. Levels that were equal to or greater than 2 mg/100 ml indicated a 53% probability of the need for further treatment by phototherapy.

Suchonska B, Wielgos.M[34], et al in 2004 showed that the concentration of TSB in the umbilical blood can be useful indicator of risk of icterus in newborns. The special care is need for newborns whose concentration of TSB in umbilical blood is over 1 mg%.

In 2005, Rostami and Mehrabi Y[35]et al, have studied around 640 neonates for cord TSB as a predictor for pathological jaundice. They demonstrated that 92.4% of infants with umbilical cord TSB level would not develop significant hyperbilirubinemia. They concluded that cord TSB level below 3 mg/dl identifies those infants who are not at risk of developing pathological jaundice.

Recently in 2009, Zakia Nahar, Md. Shahidullah, et al. [36] have done a similar study of 84 newborns. They concluded that in umbilical cord TSB samples of 84 neonates 77% had level of <2.5mg/dl and only 4.1% developed pathological jaundice. At a cord blood TSB cut off of 2.5mg/dl has got a positive predictive value of 91% and negative predictive value of 96%, and sensitivity and specificity of 77% and 98% respectively. Hence they concluded that at a cutoff value of 2.5mg/dl in umbilical cord TSB level can predict significant hyperbilirubinemia with high negative and positive predictive value and high levels of sensitivity and specificity.

RESULTS

In this study a total of 300 neonates were studied during the study period from June 2010 to December 2011. The mean gestation age of these neonates was 38.37 ± 1.18 weeks and Mean birth weight was 3.072 ± 0.370 kg.

GA in weeks	Number(n)	Percentage(%)
37 - 37+6	78	26.0
38 - 38+6	82	27.3
39 - 39+6	75	25.0
40 - 40+6	65	21.7
Total	300	100.0

Of these 300 neonates studied 149 (49.7%) were male and 151(50.3%) were female.

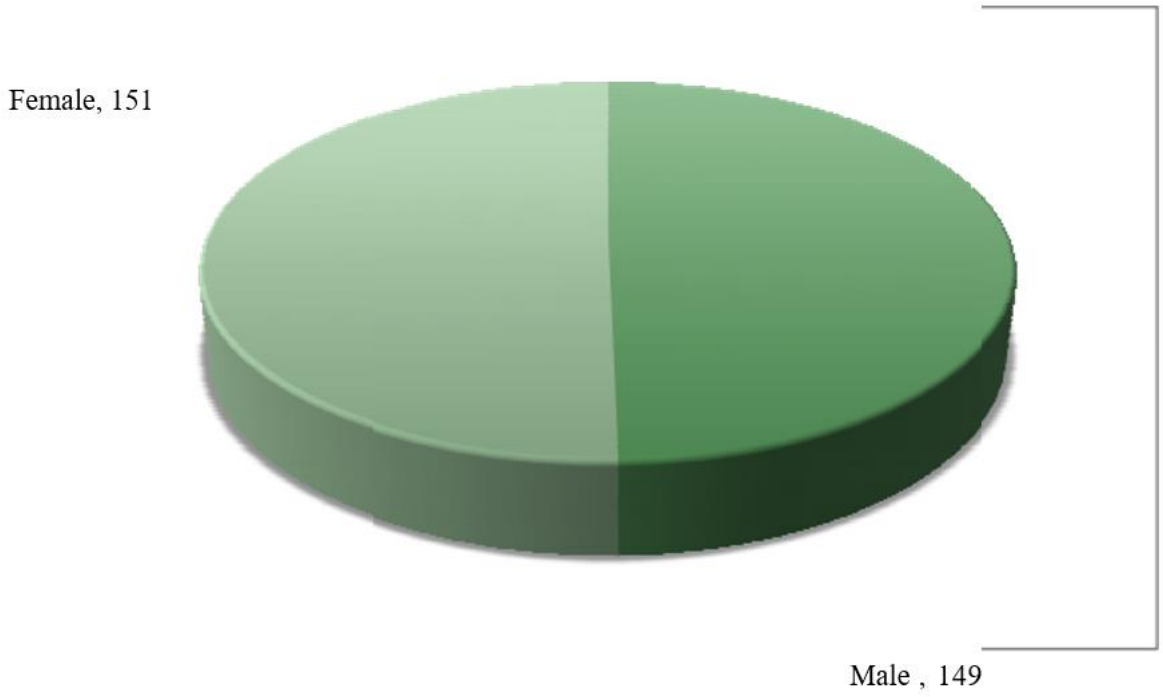

Fig 6 : Gender distribution of study neonates

Mother's blood group	Number	Percentage
A-v	7	5.7
A+v	61	20.3
AB-	3	1.0
AB+	21	7.0
B-v	18	6.0
B+v	84	0
O-v	20	6.7
O+v	76	25.3
Total	300	100.0

Table 2: Distribution of mother's blood group of the study neonates.

Predominant blood group among study neonate'smothers was B +ve in 84 cases (28%), followed by O +ve in 76 cases (25.3%) and A +ve in 61 cases (20.3%).

Baby'sblood groups	Number	Percentage
A-v	7	2.3
A+v	61	20.3
AB-	3	1.0

AB+	17	5.7
B-v	11	3.7
B+v	83	27.7
O +	1	.3
O-v	10	3.3
O+v	107	35.3
Total	300	100.0

Predominant blood group among neonates was O +ve in 107 cases (35.3%), followed by B +ve in 83 cases (27.7%) and A +ve in 61 cases (20.3%).

Of 300 neonates in the study 37 (12.3%) newborns had ABO incompatibility with mother and 47 (15%) had Rh incompatibility with mother.

The cord blood TSB and peak post natal TSB in study neonates is as shown in the annexure E.

The ‘mean Cord blood TSB’ level was 1.75 ± 0.46 mg/dl and ‘mean peak post-natal TSB’ level was 10.88 ± 3.26 mg/dl.

Of these 300 neonates, 88 (29.3%) neonates developed significant hyperbilirubinemia requiring phototherapy in immediate neonatal period and 25 neonates (8.8%) required readmission for significant hyperbilirubinemia necessitating phototherapy.

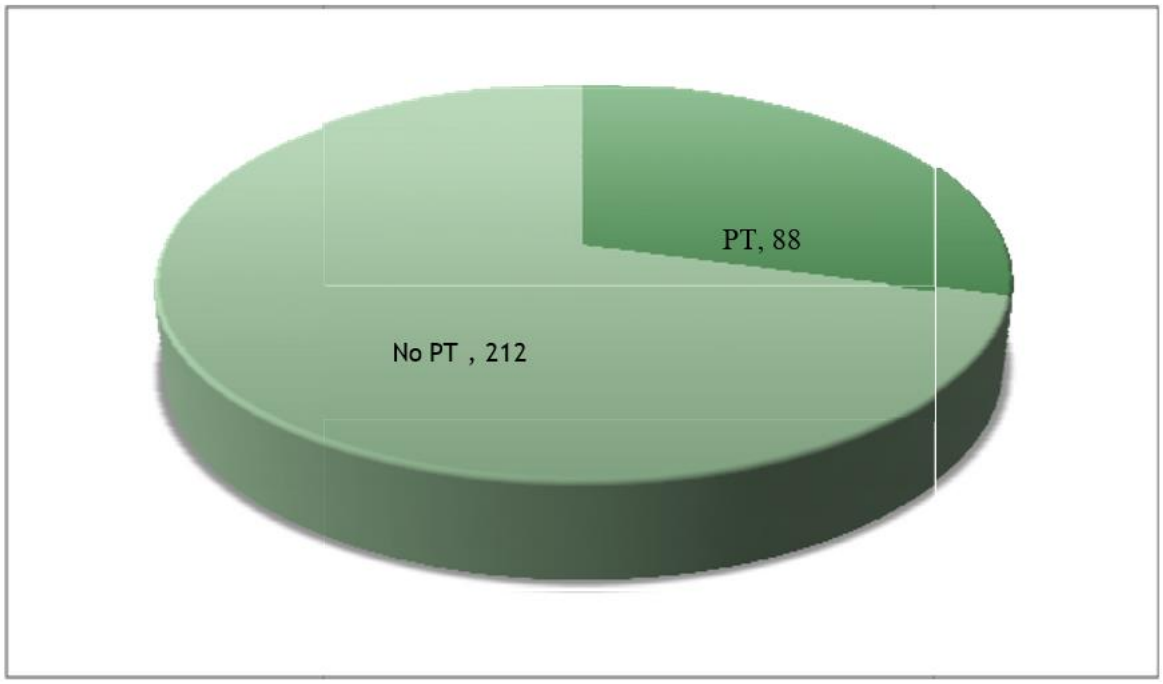

Fig 7: Distribution of study neonates who required PT and those who did not required PT

Mean TSB level among study neonates who required PT was 14.3±2.8 and those who do not required PT was 9.4±2.1, this difference was statistically significant ($p<0.01$)(Table 4) (Fig 8)

PT	Mean Bili	Number	Std. Deviation
0(no)	9.4245	212	2.13030
1(yes)	14.3943	88	2.82022
Total	10.8823	300	3.26400

Mean TSB Bilirubin

Table 4: Mean peak TSB levels in study neonates

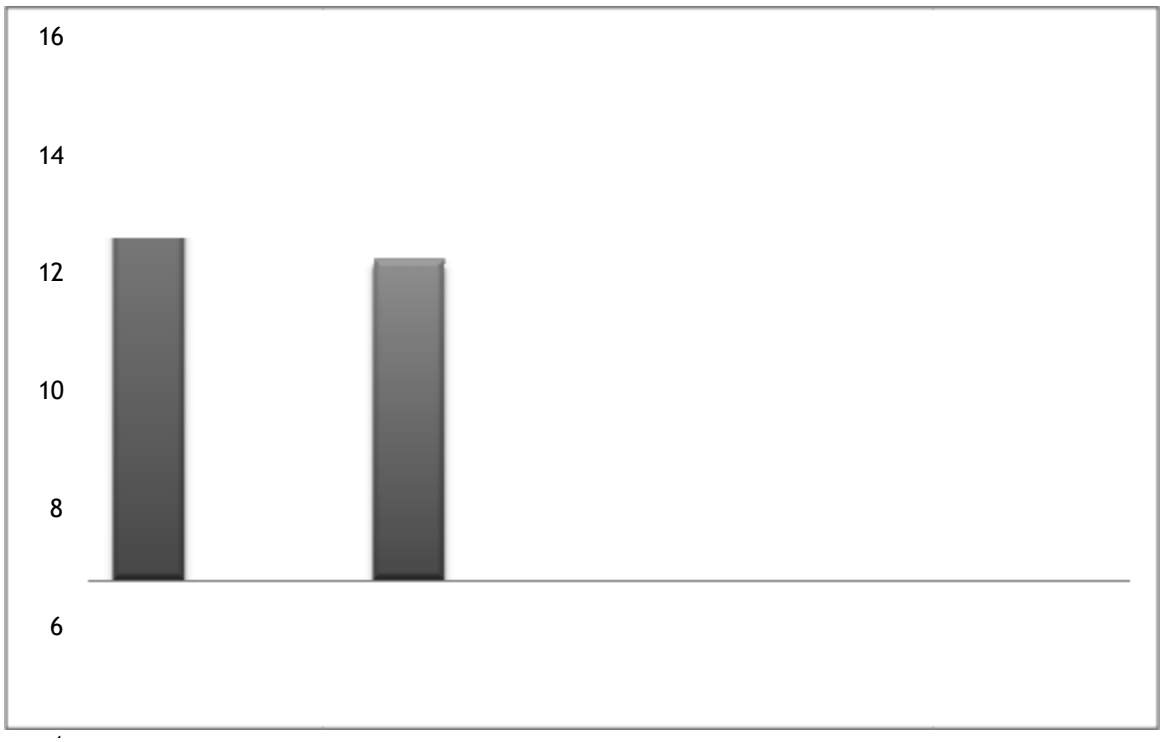

Fig 8: Mean peak TSB levels in study neonates in those who required PT and those who did not require PT.

Among the 37 neonates with ABO incompatibility between mother and baby, 20 (54.1%) require PT and 17 (45.9 %) did not require PT (Table 5). Among 47 neonates with Rh incompatibility setting 17 (36.2 %) require PT and 30(63.8%) did not require PT (Table 6). Since the number of neonates in these groups was small we could not correlate the risk of development of pathological jaundice with cord blood TSB.

		PT		Total number
.		0(no)	1(yes)	
ABO setting	0(no) Count % within ABO	195	68	263
		74.1%	25.9%	100.0%
	1(yes) Count % within ABO	17	20	37
		45.9%	54.1%	100.0%
Total number	Count % within ABO	212	88	300
		70.7%	29.3%	100.0%

** *Number not sufficient to generate any statistical significant conclusion*

Table 5: Mother-Baby ABO incompatibility in neonates Vs requirement of PT in them **.

		PT		Total number
		0(no)	1(yes)	
Rh setting	0(no) Count % within Rh	182	71	253
		71.9%	28.1%	100.0%
	1(yes) Count % within Rh	30	17	47
		63.8%	36.2%	100.0%
Total number	Count % within Rh	212	88	300
		70.7%	29.3%	100.0%

Table 6: Mother-Baby Rh incompatibility in the neonates Vs requirement of PT in them. **

We found was that at a 'cord blood TSB' cut off value of < 2.5mg/dl these term healthy neonate were found to have a Sensitivity of 70.9%, specificity of 33.3%, and Negative Predictive Value of 6.8% in predicting the low risk of developing significant hyperbilirubinemia . Positive predictive value of this was 94.3%. (Table 7).

		PT		Total number
		0(no)	1(yes)	
Umbi cord TSB	CB < 2.5 mg/dl Count	200	82	282
	% within umb1	70.9%	29.1%	100.0%
	% within pt	94.3%	93.2%	94.0%
	CB > 2.5 mg/dl Count	12	6	18
	%within umb1	66.7%	33.3%	100.0 %
	% within pt	5.7%	6.8%	6.0%
Total number	Count	212	88	300
	% within umb1	70.7%	29.3%	100.0%
	% within pt	100.0%	100.0%	100.0%

Table 7: Correlation of Cord TSB Vs Need for PT with 2.5mg/dl as cut off: Sensitivity 70.9% specificity 33.3%: PPV94.3%; NPV6.8%

Receiver operating characteristic (ROC) curves are used in medicine to determine a cutoff value for a screening test. The ROC curve is a graph of sensitivity (y-axis) vs. 1 – specificity (x-axis). An important measure of the accuracy of the clinical test is the area under the ROC

curve. ROC curve areas are typically between 0.5 and 1.0. The clinician will have to decide which cut- off value will provide the sensitivity and specificity values that have the greatest clinical value in the diagnosis of any screening test.

Receiver operator characteristics curve analysis of our study data showed that predictive value of cord blood TSB for low risk of developing significant hyperbilirubinemia is even better at a cut off value of < 2.3 mg/dl with a sensitivity of 90.9 % (Table 8). Area under the curve was 0.526 (95% CI: 0.456, 0.597). Hence our study suggest that a cut off value of 2.3mg/dl in cord blood TSB is a good tool for predicting that the term healthy neonate will have a very low risk of developing significant hyperbilirubinemia in postnatal period.

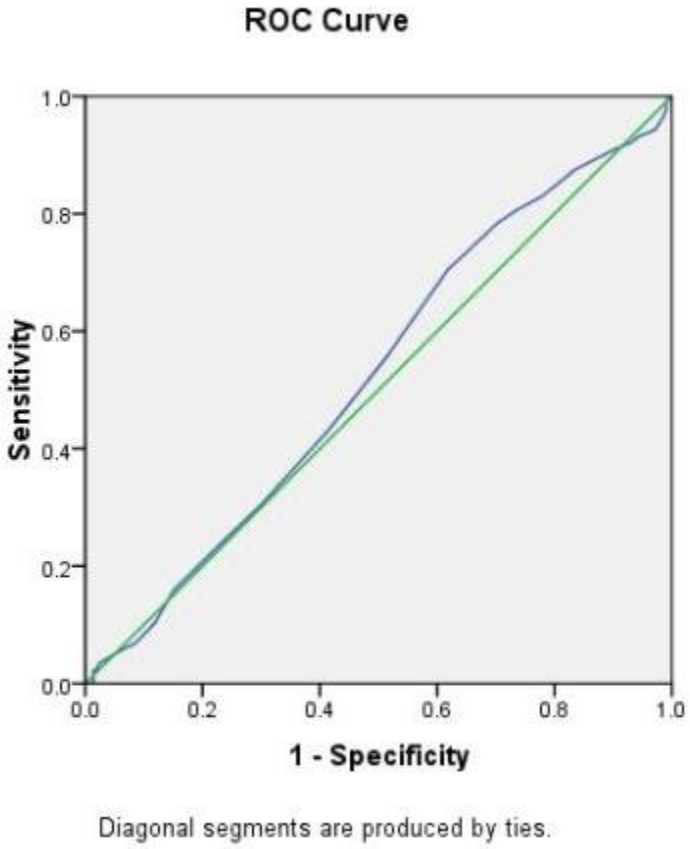

Fig 9: ROC curve analysis of study neonates. Area under the curve was 0.526 (95% CI: 0.456, 0.597).

Umbilical cord TSB cutoffs	Sensitivity	1 - Specificity
-.7000	.000	.000
.4000	.000	.005
.5500	.000	.014
.6500	.023	.014
.7500	.023	.019
.8500	.034	.024
.9500	.045	.042
1.0500	.057	.061
1.1500	.068	.085
1.2500	.102	.118
1.3500	.159	.151
1.4500	.239	.231
1.5500	.307	.302
1.6500	.432	.415
1.7500	.557	.514
1.8500	.705	.618
1.9500	.784	.703
2.0500	.807	.736

2.1500	.830	.778
2.2500	.875	.835
2.3500	.909	.901
2.4500	.920	.929
2.5500	.932	.943
2.6500	.943	.972
2.7500	.966	.986
2.8500	.977	.991
2.9500	.989	.991
3.1000	1.000	.995
4.2000	1.000	1.000

Table 8 : Co-ordinates generated from the ROC analysis of cord blood TSB

Test Result Variable(s):cord blood TSB

We analyzed our data further and classified all the study newborns into three groups based on the Mean peak postnatal TSB. We used following classifications,1) Mild jaundice (peak TSB <10mg/dl),2) Moderate jaundice (10-15mg/dl) and 3) Severe jaundice (>15mg/dl); Then we intrapolated the cord blood TSB of these neonates to each group and analyzed the same. We found that the difference in mean cord blood TSB among these 3 groups was not statistically significant (p>0.05) (Table 10). Using a cut off umbilical cord blood TSB of >2.5mg/dl, we found that 38.9% of neonates developed mild jaundice, 50% developed moderate jaundice and 11.1% developed severe jaundice (Table 11). These differences were not statistically significant (p>0.05). Hence cord blood TSB in a term healthy neonate cannot be used for predicting whether neonate develops mild, moderate or severe jaundice in postnatal period.

Severity of PN Jaundice	Number	Mean cord blood TSB	Std. Deviation
Mild(<10)	134	1.7470	.45334
Moderate(10-15)	131	1.7557	.49352
Severe(>15)	35	1.7714	.41200
	300	1.7537	.46548

Table 10: Mean cord blood TSB of study neonates Vs severity of PN jaundice

		Severity of Jaundice			Total
.		Mild	Moderate	Severe	
Umbilical cord TSB	CB < 2.5 mg/dl Count	27	122	33	282
	% within umb1	45.0%	43.3%	11.7%	100.0%
	CB > 2.5 mg/dl Count		9	2	18
	% within umb1	38.9%	50.0%	11.1%	100.0%
Total number	Count	134	131	35	300
	% within umb1	44.7%	43.7%	11.7%	100.0%

Table 11: Cord blood TSB cut off of 2.5mg/dl Vs severity of hyperbilirubinemia

DISCUSSION

In a country like India where birth-rate is high and availability of resources is limited, hospitals are discharging the healthy term neonates earlier than 48 to 72 hours with poor follow-up assessment for jaundice. This has led to increase in incidence of the kernicterus in healthy term newborns. Neonatal hyperbilirubinemia is one of the most common reasons for the readmission of the newborn infants. This early discharge policy in hospitals from developing countries and limited followup facilities hence necessitates for a tool to predict the risk of development of significant neonatal hyperbilirubinemia in a term healthy neonate requiring phototherapy.

In this study we have assessed the potential ability of neonate's umbilical cord blood TSB level as a tool for predicting the risk of development of unconjugated hyperbilirubinemia in early neonatal period.

We had studied 300 healthy term neonates during this study period, out of which 88 subsequently required phototherapy and 212 neonates did not required phototherapy (Fig 7). Of total 300 study neonates 18 neonates had cord blood TSB of >2.5mg/dl of these 6 babies (33.3%) required phototherapy. And of the 88 babies who required PT only 6 babies (6.8%) had a cord blood TSB >2.5mg/dl.

As in this study, cord blood TSB could not predict the high risk of development of jaundice we further looked whether we can contrarily predict the low risk of pathological jaundice using cord blood TSB.

Out of the 212 neonates who did not require PT, Umbilical cord blood TSB level in 200 neonates was <2.5mg/dl i.e. 94.3% of the neonates who do not require PT had umbilical cord TSB <2.5mg/dl (Table 6). Out of total 300 study neonates 282 had umbilical cord blood TSB level < 2.5 mg/dl of these 70.9% did not develop pathological jaundice. Hence by this study we infer that umbilical cord TSB cut off of < 2.5mg/dl can predict that a healthy term newborn

is at a lower risk of developing significant hyperbilirubinemia we found this to have a sensitivity of 70.9% and specificity of 33.3% and Positive predictive value of 94.3% (Table 6).

ROC curve analysis of our data shows that a cut off of < 2.3 mg/dl had an even better predictability as a screening tool for risk of developing subsequent pathological jaundice with a sensitivity of 90.9 %, Area under the curve was 0.526 (95% CI: 0.456, 0.597). (Table 8 and Fig 9).

Similar reports like us were obtained in a study done in 2005 by Rostami and Mehrabi Y, et al,[35]. They studied around 640 neonates for cord blood TSB as a predictor for pathological jaundice. They demonstrated that 92.4% of infants with cord blood TSB level below 3 mg/dl would not potentially develop significant hyperbilirubinemia[35].

In 1986, Rosenfeld[31] et al, analysed a group of 108 full-term newborns according to their risk of developing severe hyperbilirubinemia and concluded that neonates with an umbilical cord blood TSB level of lower than 2 mg/100 ml had a 4% chance of developing significant jaundice, in comparison with a 25% chance presented by the ones with levels higher than 2 mg/100 ml.

In 1989 Knudsen[32], et al carried out a study to demonstrate that clinically jaundiced newborns presented with higher umbilical cord blood TSB levels than newborns without clinical jaundice. In addition, the number of jaundiced newborns undergoing phototherapy was significantly higher when these cord blood TSB levels were higher than 2.3 mg/100 ml, in comparison with jaundiced newborns with no need for treatment had cord blood TSB lower than or equal to 2.3 mg/100 ml. This proves the possibility of defining a newborn risk group for developing neonatal hyperbilirubinemia at birth[32]. This study also showed that if cord TSB was below 1.2mg/dl, 2.9% of infants developed jaundiced as opposed to 85% if cord TSB was above 2.5mg/dl.

Bernaldo[33] et al in 1997; studied 380 newborns for cord blood TSB and concluded that a cut off value for cord blood TSB as 2.0 mg/dl , 53% of neonates who had cord blood TSB more than 2.0mg/dl develop pathological jaundice. This study also showed that TSB levels in cord blood were indicative of the jaundice severity developed by full-term newborns without complications, up to their third day of life.

Recently in 2009, Nahar and Shahidullah, et al.[36] have done a similar study of 84 newborns. They concluded that in the umbilical cord blood samples of 84 neonates, applying the critical value of 2.5 mg/dl, demonstrated that 73 (87%) infants had their cord blood TSB concentrations <2.5 mg/dl. 3 of them (4.1%) subsequently developed significant hyperbilirubinemia. This critical value of 2.5 mg/dl had a negative and positive predictive value of 96% and 91% respectively with sensitivity 77% and specificity 98.59%.

In our study we have taken a sample size of 300 neonates which was smaller when compared to few of the other studies done previously. This was because our study was time bound and also there were some constraints in obtaining the informed consent, hence we could take only 300 study subjects. In our study neonates, difference in the mean peak TSB levels at 48-72 hours between those neonates who required phototherapy(14.39mg/dl) versus those who do not require phototherapy(9.42mg/dl) is statistically significant ($p<0.01$) (Table 4). However the difference in mean cord blood TSB between those neonates who required PT (1.76 mg/dl) and those who do not required PT (1.73mg/dl) are not statistically significant (Table 9) .i.e., in our study umbilical cord TSB could not predict the high risk of development of pathological jaundice.

Similar results were obtained by study done by Jacobson et al[37], in 1982, compared cord TSB levels of 87 neonates who received standard phototherapy for neonatal jaundice with the cord TSB levels of 95 neonates without neonatal jaundice. There were no significant differences in the cord TSB levels between the two groups leading to the conclusion that cord TSB levels could not predict the risk of development of significant hyperbilirubinemia in their study.

Rostami and Mehrabi Y. et al,[35] in 2005, had a similar results like us . In their study the difference in mean cord blood TSB between those neonates who required PT (37.4mmol/L) and those who do not required PT (34mmol/L) are not statistically significant. Hence they concluded that cord TSB levels cannot identify newborn infants who are at increased risk for developing significant hyperbilirubinemia.

Hence keeping this aspect in mind we analyzed our data further and classified all study newborns into three groups based on the Mean peak TSB postnataly. We used following classifications, Mild jaundice (peak TSB <10mg/dl), moderate jaundice (10-15mg/dl) and severe jaundice (>15mg/dl); then we intrapolated the cord blood TSB of these neonates to each

group and analyzed the same. We found that difference in mean cord blood TSB among these groups was not statistically significant ($p>0.05$). At a cut off umbilical cord blood TSB of >2.5mg/dl, 38.9% developed mild jaundice, 50% developed moderate jaundice and 11.1% developed severe jaundice (Table 10). Hence cord blood TSB cannot be used for predicting whether neonate develops mild, moderate or severe jaundice.

Results obtained in our study indicate that a healthy term neonate with a cord blood TSB level of < 2.3mg/dl has a very low risk of development of significant hyperbilirubinemia in early neonatal period with a sensitivity of 90.9 %. (Table 8). This implies that in a setup where monitoring a neonate in postnatal period for pathological jaundice by doing repeated venous blood sampling in not feasible, decision on early discharge can be taken based on cord blood TSB levels and if cord blood TSB found to be <2.3mg/dl neonates can be discharged early without the need for pre-discharge TSB estimation as the risk of hyperbilirubinemia is low. Obtaining cord blood sampling during the delivery is technically an easy procedure which does not require specially trained staff to collect it and it also alleviates the need for painful pricking of the neonate.

Our study has got certain limitations. This study considered only single parameter of umbilical cord TSB levels for assessing the risk of significant hyperbilirubinemia in a term healthy neonate. But in actuality there are multiple factors which determine the risk of postnatal hyperbilirubinemia such as feeding difficulty, dehydration and weight loss, which come into play mostly during the late neonatal period. Hence even if early discharge of neonate is planned based on the cord blood TSB being <2.3mg/dl it is necessary to advice the parents regarding the infant feeding.

In this study we also found that a cord blood TSB of <2.3mg/dl has a sensitivity of 90.9% in predicting the low risk of significant hyperbilirubinemia but remaining 9.1% of the study neonates who had cord blood TSB levels <2.3mg/dl but still developed significant hyperbilirubinemia cannot be overlooked. That means if a neonate is discharged home based on the cord blood TSB level of <2.3mg/dl then there is still a 9.1 % chance of that neonate developing significant hyperbilirubinemia later.

Similar data was seen in some of the other studies as well. Rostami and Mehrabi Y, et al,[35] . In 2005, have demonstrated that 7.6% of the neonates who had a cord blood TSB levels <3mg/dl had developed hyperbilirubinemia requiring phototherapy.

In 1986, Rosenfeld[31] et al concluded that neonates with an umbilical cord blood TSB level of lower than 2 mg/100 ml had a 4% chance of developing significant jaundice.

Recently in 2009, Nahar and Shahidullah, et al.[36] have concluded that in the umbilical cord blood samples of 84 neonates, applying the critical value of 2.5 mg/dl, demonstrated that 73 (87%) infants had their cord blood TSB concentrations <2.5 mg/dl. 3 of them (4.1%) subsequently developed significant hyperbilirubinemia.

In our study total serum bilirubin estimation in umbilical cord blood was done using the standard method and equipment (Diazotized sulfanilic acid method using equipment Siemens Dimention® clinical chemistry system). These instruments are periodically assessed for quality control by using a QC material of known total bilirubin concentration as per hospital's quality control policy. Such standard equipments for TSB estimation may not be available in peripheral setup due to financial constraints. However other commonly used equipments are non chemical photometric devices like Twin Beam (Ginevri), ABL 735 (Radiometer), and Roche OMNI S (Roche Diagnostics). These equipments estimate TSB by multiple wavelength photometry. Other equipments are clinical chemical analyzers namely Hitachi 912 (Roche Diagnostics), Dade RxL (Dade Behring), and Vitros 250 (Ortho Clinical Diagnostics). These chemical analyzers estimate TSB on the principle of Diazo reaction (Hitachi), Jendrassic groff method (Dade RxL) or spectrophotometric method (Vistro 250). In a study published by AAP in 2006 Grohmann and Roser, [38] et al, have compared these frequently used method of TSB estimation in neonates and concluded that these non chemical photometric analyzers and clinical chemical analyzers correlate well with each other in TSB estimation in neonates. This observation suggests that various methods and equipments of total bilirubin estimation frequently used in other urban hospitals and also in peripheral healthcare centres give a reliable estimation of total serum bilirubin. Hence we can adopt this umbilical cord TSB as a screening tool in all kind of healthcare centres whether it's a major referral hospital or a peripheral hospital.

Thus from this present study we can conclude that umbilical cord blood TSB in a term healthy neonate can be estimated at the time of delivery and if it is found to be <2.3mg/dl, a decision on 'early and safe discharge' of the neonate can be taken as our study shows that these neonates have a very low risk of development of significant postnatal hyperbilirubinemia. Also as the method of obtaining a blood sample from umbilical cord is technically easy as compared to obtaining neonate's venous blood sampling as also different techniques used by any kind of

healthcare setup for estimation of total serum bilirubin are also fairly reliable, this screening tool can be adapted widely across the country. This will help in taking decisions on ‘early and safe discharge’ of neonates in resource limited healthcare setups also, without the burden of worry regarding subsequent development of severe hyperbilirubinemia or kernicterus. This screening tool of cord blood TSB however needs to be further validated through a large multi centre trial with larger sample size before implementing universally.

CONCLUSION

From this study it can be concluded that

1. Umbilical cord blood TSB level measured at the time of birth can be used in predicting low risk of developing significant hyperbilirubinemia in a term healthy neonate during early neonatal period.

2. A cord blood TSB level less than 2.3 mg/dl can be used as a cut of value for identifying a neonate at low risk of developing significant postnatal hyperbilirubinemia.

3. However cord blood TSB may not be useful in predicting the severity of postnatal hyperbilirubinemia in term healthy neonates.

4. Thus newborn infants having cord blood TSB levels <2.3 mg/dl can be safely discharged home early as they have a low risk of developing pathological jaundice however, parents should be advised to come for followup if there is visible increase in the jaundice.

5. This can reduce the need for blood sampling for estimating pre- discharge Total Serum Bilirubin in resource limited settings.

6. This pilot study however needs to be further validated through a multicentre control trail with a larger sample size.

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