CORD BLOOD BILIRUBIN AS A PREDICTIVE MARKER OF NEONATAL HYPERBILIRUBINEMIA IN ABO AND Rh INCOMPATIBLE BABIES-A PROSPECTIVE STUDY

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BRANCH – VII

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DEPARTMENT OF PEDIATRICS

CHENGALPATTU MEDICAL COLLEGE

CHENGALPATTU-603 001

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DECLARATION

I, **Dr. S. KAYALVIZHI** have proposed study titled **"CORD BLOOD BILIRUBIN AS A PREDICTIVE MARKER OF NEONATAL HYPERBILIRUBINEMIA IN ABO AND Rh INCOMPATIBLE BABIES-A PROSPECTIVE STUDY"** in the department of Pediatrics at Chengalpattu Medical College and Hospital. I hereby ensure that I will abide by the rules of the institutional ethical committee.

A PROSPECTIVE STUDY

A bonafide work done by me in the Department of Pediatrics, Chengalpattu Medical College, Chengalpattu under the guidance of **Prof. Dr .J. SATHYA, M.D, DCH,** Head of the department, Department of Pediatrics, Chengalpattu Medical College, Chengalpattu. This dissertation is submitted to, **THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY**, Chennai towards the partial fulfillment of the requirements for the award of M.D degree in Pediatrics.

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Place: Chengalpattu

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This is to certify that the dissertation titled **"CORD BLOOD BILIRUBIN AS A PREDICTIVE MARKER OF NEONATAL HYPERBILIRUBINEMIA IN ABO AND Rh INCOMPATIBLE BABIES-A PROSPECTIVE STUDY"** is the bonafide work of **Dr. S.KAYALVIZHI** in partial fulfillment of the requirements for **M.D.BRANCH-VII (PEDIATRICS)** examinations of **THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY** to be held in 2020, done under the guidance of **Dr. J. SATHYA, M.D, DCH, Professor & H.O.D, Department of Pediatrics, Chengalpattu Medical College, Chengalpattu,** during the academic year 2017-2020.

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Title of Work	:	Cord blood bilirubin as a predictive marker of neonatal hyperbilirubinemia in ABO and Rh incompatible babies – A prospective study
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Co-Investigators	:	Dr. J.Sathya, MD., DCH., Professor & HOD Department of Paediatrics Chengalpattu Medical College Chengalpattu
Department	:	Paediatrics

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 27.03.2018 at the Medical Education Unit, Chengalpattu Government Medical College, Chengalpattu at 11.00 AM.

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NH NEONATAL HYPERBILIRUBINEMIA

DAT	DIRECT ANTIGLOBULIN TEST
G6PD	GLUCOSE 6 PHOSPHATE DEHYDROGENASE
RBC	RED BLOOD CORPUSCLES
OATP	ORGANIC ANION TRANSPORTER PROTEIN
DNA	DOUBLE STRANDED NUCLEIC ACID
NMDA	N-METHYL-D-ASPARTATE
BAER	BRAINSTEM AUDITORY EVOKED RESPONSE
ABR	AUDITORY BRAIN STEM RESPONSE
MRI	MAGNETIC RESONANCE IMAGING
TCB	TRANSCUTANEOUS BILIRUBINO METER
ETCOc	END-TIDAL CARBON MONOXIDE
CBC	COMPLETE BLOOD COUNT
TSB	TOTAL SERUM BILIRUBIN
CO	CARBON MONOXIDE
CBB	CORD BLOOD BILIRUBIN
PT	PRETERM
FT	FULLTERM
UCB	UMBILICAL CORD BILIRUBIN
UCSB	UMBILICAL CORD SERUM BILIRUBIN
STB	SERUM TOTAL BILIRUBIN

CHAPTER 1

INTRODUCTION

1.1 GENERAL

Hyperbilirubinemia is the most common clinical problem in the neonatal period requiring evaluation and treatment (1). It has been a significant public health problem in parts of South East Asia, the tropical Africa, and the Pacific islands and in some countries bordering the Mediterranean Sea during the past 20 decades. Although it is mostly due to physiological process, few newborns develop potentially high bilirubin value posing serious damage to the brain.

Normal adults have serum bilirubin of <1mg/dl in contrast almost all newborns have serum total bilirubin of >1mg/dl (1). This high level may be due to shorter life span of RBCs, excess bilirubin production, inability to handle this excess load by neonatal immature liver enzymes, increased beta glucuronidase enzyme activity, poor intestinal colonization of bacteria and increased enterohepatic circulation.

Almost 85% of all term newborns and most preterm's develop clinical jaundice. 6.1% of all well term newborns have a peak total serum bilirubin of >12.9mg/dl and 3% of normal term infants have peak total serum bilirubin of >15mg/dl (1).

Hyperbilirubinemia is also the most common cause of readmission to hospital of babies who have been discharged earlier .Routine hospital stay for

mothers and newborns have been reduced over the past few years due to various reasons (2, 3). This allowed the family members to return to their work earlier and also reduced the economic burden of the country.

Although there is no clear cut definition for early discharge, American academy of Pediatrics states that early and very early discharge as 48 and 24 hours respectively following uneventful normal vaginal delivery (4).

The practice of early hospital discharge has led to an increased rate of readmission to hospitals due to conditions that may not be evident in the first 2-3 days of life (5) with a large number of these readmissions has been attributed by jaundice (6-11). In a study done by Sola A et al in 1995, showed that there is reemergence of bilirubin induced encephalopathy and kernicterus related to early discharge of neonates (12).

Kernicterus is chronic and permanent sequelae of bilirubin toxicity to brain that occur during the first year of life. Most infants who develop kernicterus would have had a very high serum bilirubin >20mg/dl in their neonatal period. Initiation of phototherapy at a correct time would have definitely reduced the risk of kernicterus (13, 14).

Major risk factors for the development of neonatal hyperbilirubinemia include cephalhematoma, significant bruising, previous sibling with jaundice, immune or other haemolytic disease, gestational age of 35-36 weeks, predischarge total bilirubin in high risk zone(> 95^{th} percentile for age in hours according to Bhutani normogram) or high intermediate risk zone. Though our

knowledge on neonatal hyperbilirubinemia has been improved over the past few years, still we are not able to precisely predict the newborns at risk of developing jaundice.

Roberson et al in his study on “clinical and laboratory findings in heterospecific pregnancy, with a note on the incidence of ABO hemolytic disease” as early as 1960 reported that cord blood bilirubin of >3mg/dl has been associated with the development of jaundice in ABO incompatible babies (15).

Similar study done by Risenberg et al in 1977 in his study of “correlation of cord bilirubin levels with hyperbilirubinemia in ABO incompatibility” reported that cord blood bilirubin >4mg/dl predicts the risk of developing neonatal hyperbilirubinemia (16). In 2005, Amar Taksande et al in his study showed that cord blood bilirubin of >2mg/dl had sensitivity and specificity of 89.5% and 85% respectively.

Since then many authors studied the usefulness of cord blood bilirubin in predicting neonatal hyperbilirubinemia. However only limited studies were done to find the cut off value of cord blood bilirubin in ABO and Rh incompatible babies in predicting hyperbilirubinemia so as to ensure early phototherapy and protect those babies from developing bilirubin encephalopathy.

Early treatment of jaundice with phototherpy is an effective and simple method as compared to exchange transfusion in the treatment of severe neonatal hyperbilirubinemia and also it prevents the risk of later development of

kernicterus. Thus early prediction of newborns at risk of NH is a good option.

The American academy of pediatrics recommends that the babies should have a follow up visit after 2-3 days for the detection of jaundice and other problems if they are discharged before 48 hours(4). However in developing countries like India, this is not practical. Hence an effective and easy measure should be available to predict the newborns at high risk of developing jaundice so that these babies can be monitored frequently and can be discharged later whereas the low risk babies can be discharged earlier.

ABO and Rh incompatibility occurs in 25-30% of pregnancies. But only 1-2% of these babies develop significant hyperbilirubinemia requiring therapy (17). High bilirubin value and bilirubin induced toxicity can occur in these ABO and Rh incompatible babies even without significant haemolysis and DAT positive as shown by Chen JY in his study of "prediction of the development of neonatal hyperbilirubinemia in ABO incompatible babies" (17).

Hence the present study is to find the predictive value of cord blood bilirubin in neonatal hyperbilirubinemia in ABO and Rh incompatible babies.

1.2 BILIRUBIN

Jaundice is a term derived from the French word "jaune" which means yellow. It is a yellowish discoloration of skin and mucous membrane caused by the deposition of bilirubin produced by degradation of heme.

Only a moderate degree of jaundice develops in healthy term neonate as long as the balance between the production and the excretion of bilirubin is

maintained. If this balance is broken either due to increased production or reduced excretion, there will increased circulating bilirubin. It gets deposited in brain producing acute or chronic bilirubin encephalopathy and permanent neurological damage.

Various steps in bilirubin metabolism include:

- Bilirubin production
- Transport
- Hepatic uptake
- Conjugation
- Hepatic excretion and
- Enterohepatic absorption.

Bilirubin was discovered by Rudolf Virchow in 1847 (18). Bilirubin is derived from the breakdown of heme containing proteins in the reticuloendothelial systems. A normal newborn produces 6-10mg/kg/day as compared to adult production of 3-4mg/kg/day (1).

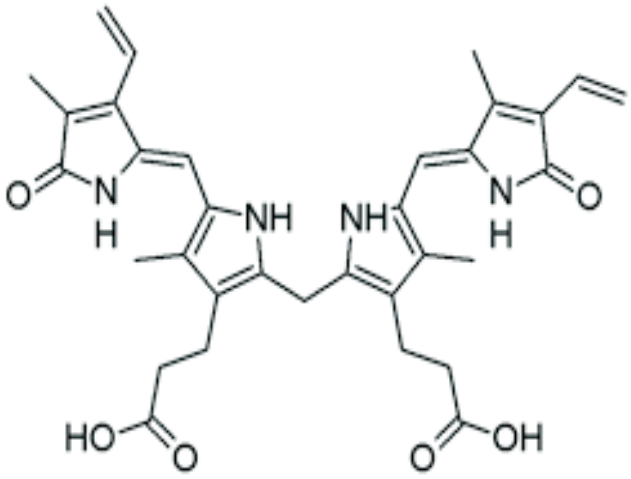


FIGURE 1.1: STRUCTURE OF BILIRUBIN

Bilirubin is a open chain tetrapyrrole formed by the cleavage of porphyrin in heme. The structure of bilirubin is similar to the pigment phytochrome and phycobilin which are used by plants and algae to capture light respectively as shown in Figure 1.1. Both of these pigments contain four pyloric ring chains. The double bonds in these pigments get isomerizes on exposure to light. This process of isomerisation also takes place in double bonds of bilirubin when exposed to light.

This property has been utilized in phototherapy where the more soluble E, Z isomers are formed from the insoluble Z,Z isomers upon light exposure as the intermolecular hydrogen bond is removed (19). Then the more soluble bilirubin gets excreted through bile.

1.2.1 Bilirubin production

Bilirubin is produced by the breakdown of heme containing proteins of reticuloendothelial system. The major heme protein responsible for the production of 80-90% of bilirubin is hemoglobin. 34 mg of bilirubin is produced from 1gm of hemoglobin. Another 10-20% of bilirubin is produced from non-heme proteins like catalase, cytochromes, peroxidase and tryptophan pyrolase (20, 21).

Bilirubin is produced by a two step sequential catalytic reaction that takes place in the cells of reticuloendothelial systems like spleen and in phagocytes and kupffer cells of liver.

Heme is taken up into these cells and acted upon by an enzyme heme oxygenase. It releases the chelated iron and produces an equimolar amount of carbon monoxide which will be excreted through the lungs. This reaction leads to the formation of the green product, biliverdin which is then acted upon by NADPH dependent enzyme, biliverdin reductase resulting in the end product of bilirubin as shown in Figure 1.2. Since heme breakdown yields equimolar amount of CO and biliverdin, CO measurement can give the indirect assessment of bilirubin production.

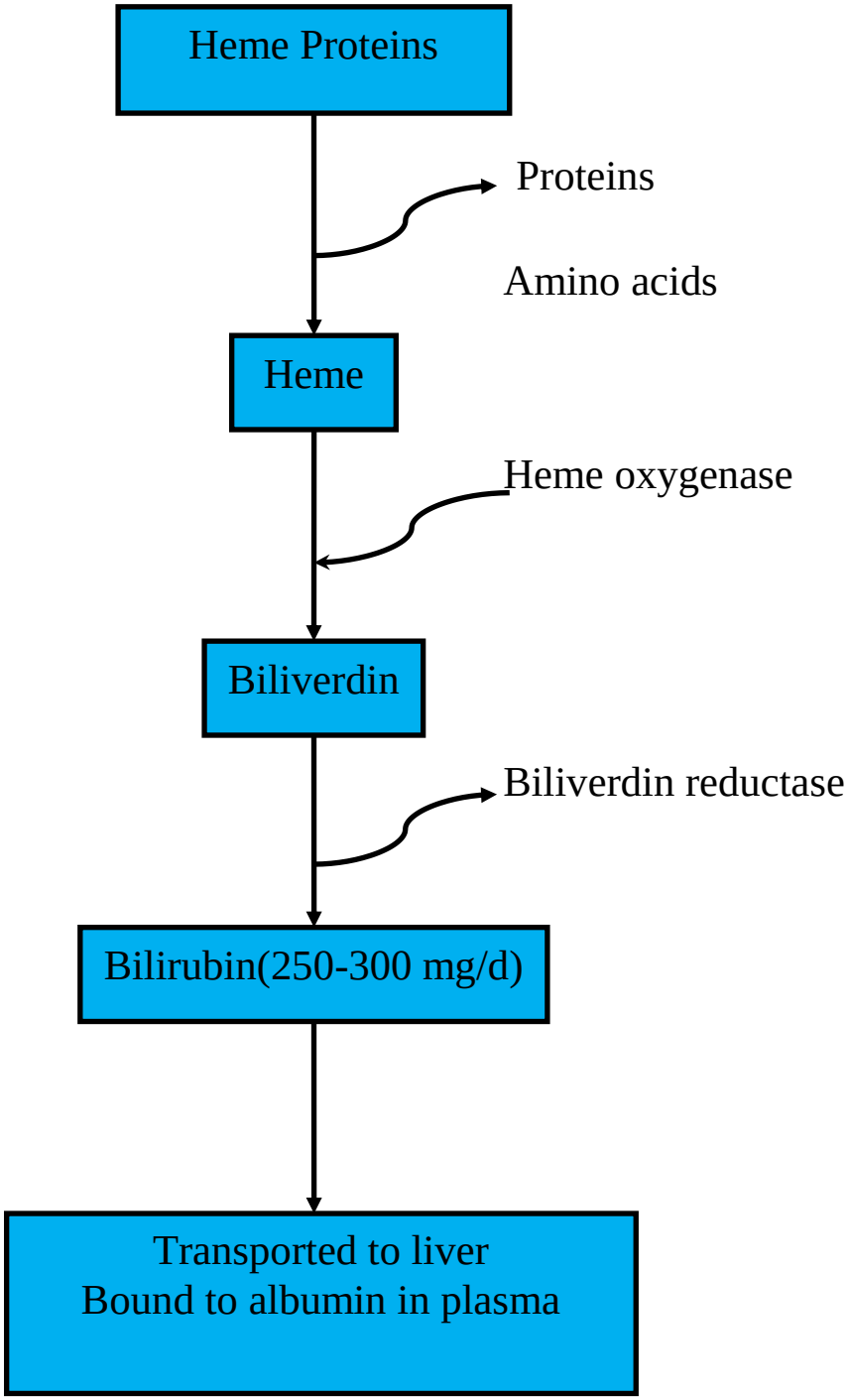


FIGURE 1.2: PRODUCTION OF BILIRUBIN

1.2.2 Albumin binding

Albumin acts as a transporter once bilirubin is released into the plasma.

Albumin has a very high affinity for bilirubin and it bounds almost all bilirubin. No free non-albumin bound bilirubin is found in plasma under ideal condition. In case of hypoalbuminemia, bilirubin also bounds with high density lipoproteins to a lesser degree.

Reduced serum concentration and reduced molar binding capacity of albumin in newborns accounts for the larger amount of unbound bilirubin in plasma as compared to adults. Unbound bilirubin crosses the blood brain barrier and is toxic to neurons.

Albumin bound bilirubin remains within the vascular space and there is no leakage and precipitation in tissues. There is also limited glomerular filtration. When this albumin-bilirubin complex reaches the liver, it is been taken up by the hepatocytes and the bilirubin gets dissociated from the albumin.

1.2.3 Hepatic uptake

Hepatocytes take up the bilirubin by two mechanisms-

- Passive diffusion and
- Receptor mediated endocytosis.

Passive diffusion is not energy consuming and it allows the flow bi-directional. Active transport of bilirubin is mediated by carrier proteins. Most of

the bilirubin entering the hepatocytes are transported into the periportal system with the lesser amount remaining within the hepatocytes is transported back into the sinusoidal space.

1.2.4 Hepatic conjugation

Conjugation is necessary to make the bilirubin soluble and its secretion into the canalicular membrane. Bilirubin is conjugated by an enzyme UDPGT (uridine diphosphoglucuronic glucuronosyltransferase) to form monoglucuronide and diglucuronide bilirubin as shown in Figure 1.3. There are many forms of UDPGT. UDPGT1A1 is the most important physiological isomer. This process of conjugation is one of the most vital detoxification mechanisms in our body.

Term newborns have only 1% of UDP enzyme level when compared to adults. This reduced enzyme level is one of the reason for neonatal hyperbilirubinemia. UDP enzymes rise slowly to reach the adult value by 3 months of age.

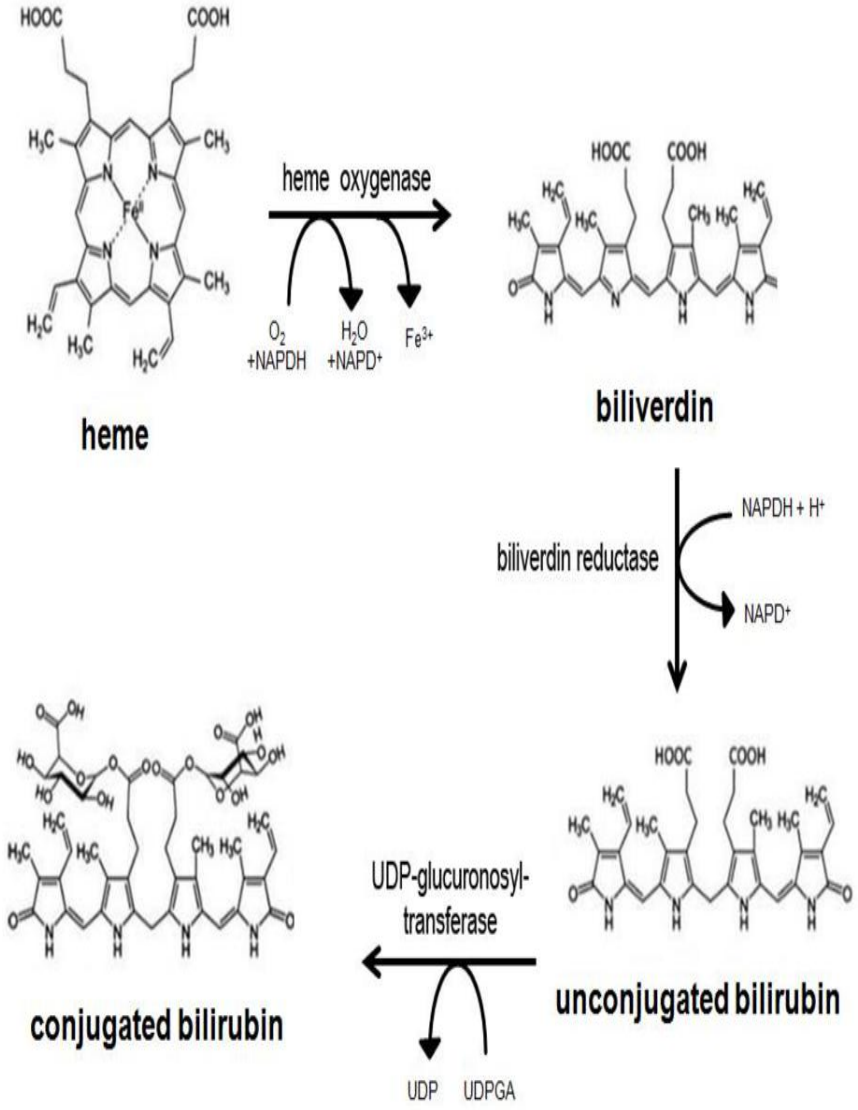


FIGURE 1.3: HEME TO CONJUGATED BILIRUBIN

1.2.5 Excretion of conjugated bilirubin

The conjugated bilirubin is actively transported across the bile canalicular system of hepatocytes through four known canalicular proteins. The most important one being the multidrug resistant associated protein 2 (MRP2). A fraction of conjugated bilirubin is transported into the sinusoids and portal circulation by multidrug resistant protein3 (MRP3). This fraction is then taken

back into the hepatocytes through the sinusoidal proteins, organic anion transport protein 1B1 and 1B3 (22, 23).

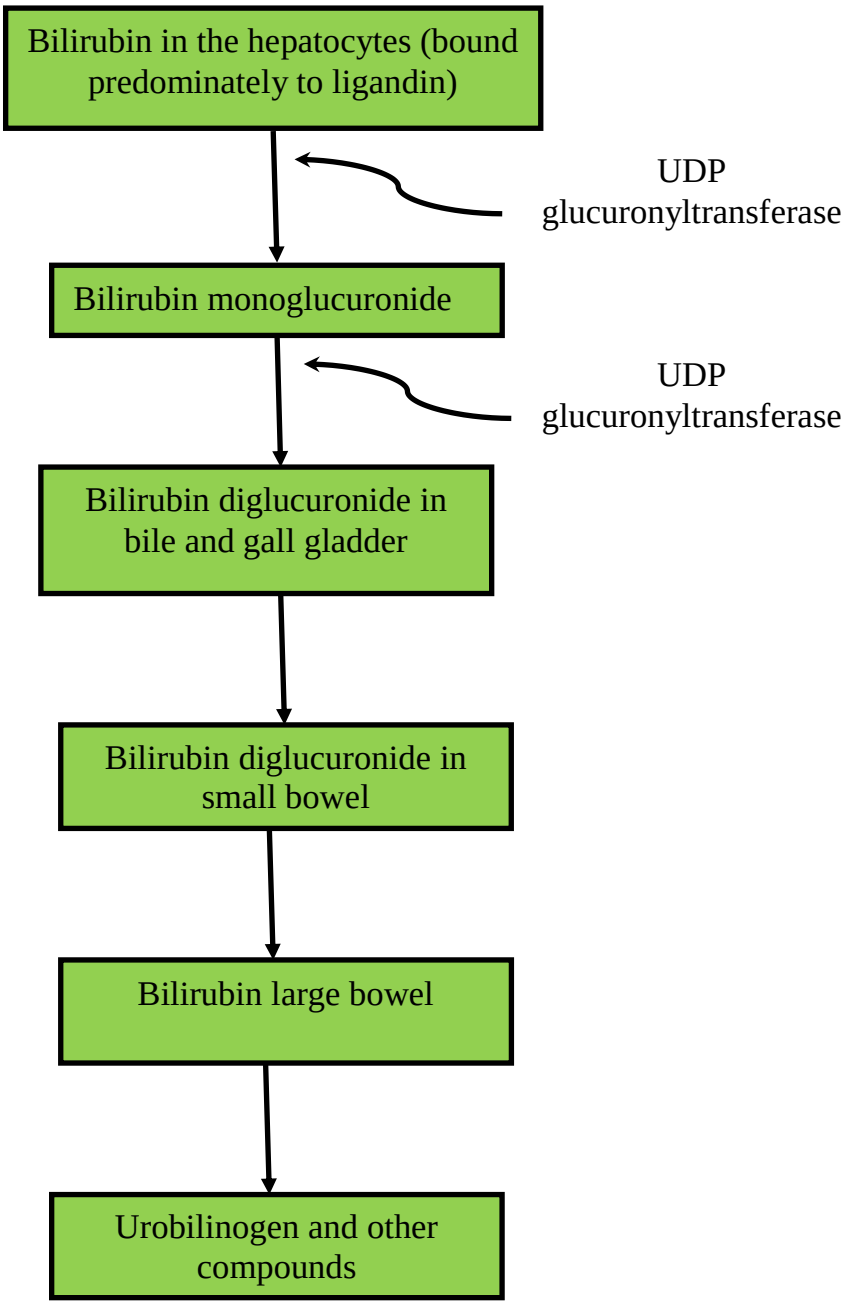


FIGURE 1.4: EXCRETION OF BILIRUBIN

Hence some conjugated and unconjugated bilirubin taken back into the hepatocytes is released into plasma where it binds with albumin and is circulated

in the blood stream. Only the conjugated bilirubin enters the gall bladder from the canalicular system and is excreated into the small intestine as shown in Figure 1.4.

Hepatic uptake of bilirubin and its conjugation is more restrictive than hepatic excretion in neonates when compared to adults.

1.2.6 Enterohepatic circulation

There is no additional metabolism or absorption of conjugated bilirubin in proximal small intestine. When it reaches the distal ileum and colon, it is deconjugated by bacterial flora to urobilinogen which are then excreted through urine and stool.

In adults, high intestinal bacteria convert the conjugated bilirubin to urobilinogen which is not a substrate for beta glucuronidase activity. In newborns , there is reduced bacterial flora and increased beta glucuronidase activity. This enzyme causes deconjugation of bilirubin and absorption through intestinal mucosa to enter the enterohepatic circulation in newborns. This to some extent accounts for neonatal hyperbilirubinemia.

1.2.7 Fetal handling of bilirubin

During fetal life, most of the unconjugated bilirubin formed is transported across the placenta to maternal blood. But the placenta is

impermeable to conjugated bilirubin. Formation of conjugated bilirubin is limited in fetus due to reduced hepatic blood flow, reduced uptake through ligantin, reduced level of enzyme activity. This lesser amount of conjugated bilirubin being formed is excreted into the fetal gut, metabolized by beta glucuronidase and reabsorbed.

Increased level of bilirubin in amniotic fluid is found in hemolytic disease and in intestinal obstruction below the bile ducts. Normally bilirubin begins to appear in amniotic fluid by 12 weeks and disappear by 37 weeks of gestation.

1.3 PHYSIOLOGICAL HYPERBILIRUBINEMIA

The term “physiologic bilirubinemia” refers to the “normal” elevation of unconjugated bilirubin that almost occurs in every term newborn and this should be distinguished from pathological elevation (24).

In most newborns total serum bilirubin rises to >2mg/dl at first day of life. By 3-5 days of life, this rises to reach peak of 6-8mg/dl and then falls. A rise of 12mg/dl in first week of life is in physiological range. Until 1 month of life, total bilirubin <2mg/dl can never been seen.

This non-pathological jaundice is due to:

A. Increased bilirubin production due to

1. Reduced RBC life span – 90 days when compared to 120 days in adults
2. Increased RBC volume per kilogram
3. Increased turnover of non-heme proteins

4. Increased ineffective erythropoises

B. Reduced hepatic uptake due to

1. Reduced hepatic blood flow
2. Decreased hepatic ligantin
3. Reduced binding of ligantin by other anions

C. Reduced hepatic clearance

Due to reduced level of UDP enzyme activity. UDP activity at 7 days of life in term infant is approximately 1% when compared to that of adults. It takes 3 months to reach the adult value.

D. Reduced hepatic excretion

1. Increased enterohepatic circulation
2. Raised beta glucuronidase activity
3. Reduced intestinal bacteria
4. Reduced gut motility with poor evacuation of bilirubin laden meconium
5. Formation of bilirubin monoglucuronide than diglucuronide

1.4 PATHOLOGICAL HYPERBILIRUBINEMIA

Defined as total bilirubin >95th percentile on hour specific Bhutani normogram and it includes the following conditions

1. Jaundice onset <24 hours of life
2. An elevated total bilirubin requiring phototherapy
3. A rate of rise of total bilirubin >0.2mg/dl/hr

4. Persistant jaundice >14 days of life
5. Associated with illness such as lethargy, poor feeding, vomiting, excessive weight loss, temperature instability, apnea or respiratory distress.

1.4.1 Causes of pathological hyperbilirubinemia

A. Increased production:

Hemolytic diseases like:

1. Abnormal RBC morphology like hereditary spherocytosis, hereditary elliptocytosis
2. RBC enzyme abnormalities like pyruvate kinase deficiency, G6PD deficiency
3. ABO or Rh or minor group incompatibility

B. Increased RBC breakdown as in

1. Cephalhematoma
2. Excessive bruising
3. Polycythemia
4. Sepsis

C. Reduced bilirubin clearance

1. Gilbert syndrome- most common inherited disorder of bilirubin conjugation due to mutation in promoter region of UGT1A1 resulting in reduced UGT production.
2. Crigler najjar syndrome- type 1 (absent UGT activity)
3. Criggler najjar syndrome –type 2(reduced UGT activity)
4. Organic anion transporter OATP-2 polymorphism
5. Mutation in the gene encoding UGT1A1 reducing bilirubin conjugation and hepatic clearance
6. Reduced clearance also occurs in babies of inherited metabolic disorders like galactosemia, hypothyroidism, and diabetic mother.

D. Increased Enterohepatic circulation

1. Delayed and reduced enteral feeding
2. Breast milk jaundice-
    a. Occurs due to a factor called beta glucuronidase in breast milk promoting deconjugation and intestinal absorption.
    b. 2.4% of all infants develop breast milk jaundice.
    c. It starts at 3-5 days of life, peaks at 14 days and reaches the normal level over 1 to 3 months of age if breastfeeding is continued (1).
    d. Baby will have adequate weight gain and no evidence of hemolysis.

e. Total bilirubin will fall in 48 hours if breastfeeding is discontinued and begins to rise if feeding restarted but will not reach the previous high value

3. Breastfeeding jaundice:
    a. It occurs due to failure of lactation
    b. Babies will have excess weight loss, dehydration with hypernatremia
    c. Reduced intake of milk leads to reduced bilirubin clearance and increased enterohepatic circulation resulting in hyperbilirubinemia.

Other causes of neonatal hyperbilirubinemia are:

1. Certain ethnic origins like East Asian, Greek, and American Indian
2. Infant of diabetic mother
3. Maternal intake of drugs like sulfonamides and antimalarials
4. Delayed cord clamping- polycythemia and then hyperbilirubinemia
5. Intestinal obstruction leading to increased enterohepatic circulation
6. Hypoxic ischaemia
7. Respiratory distress leading to delayed initiation of enteral feeding

1.5 ABO INCOMPATIBILITY

ABO incompatibility has become the most common cause of isoimmune hemolytic disease due to routine isoimmunisation with Rh Ig (25). It occurs in 15-20% of pregnancies (26).Only 0.5-1% of babies will develop

coombs positive hemolytic jaundice. Because of the higher frequency of blood group type A, ABO incompatibility occurs most commonly in babies with A blood group.

Anti A and anti B antibodies present in mother of O blood group are of IgG type and they will cross the placenta, attaches to A or B blood group fetus RBC membrane and these IgG coated RBCs are lysed by Fc receptor bearing cells in reticuloendothelial system.

DAT positive was seen in about one third of babies with blood group A or B born to mother with O blood group (27). In a study in turkey, there was 14.8% incidence of ABO incompatibility with 21.1% developed significant hyperbilirubinemia and 4.4% developed severe hemolytic disease (28). Various studies have been done showing the incidence of hyperbilirubinemia in DAT positive babies. Kaplan et al in his study showed 19.6% of DAT positive babies required phototherapy (29). Mebere A et al in his study found that only 20% of babies with hyperbilirubinemia were DAT positive (27).

Early and rapidly progressive jaundice also occurs in babies with ABO set up and DAT negative. This is been partly explained by the mechanism of interaction with polymorphisms for the (TA) 7 sequence in the promoter of the

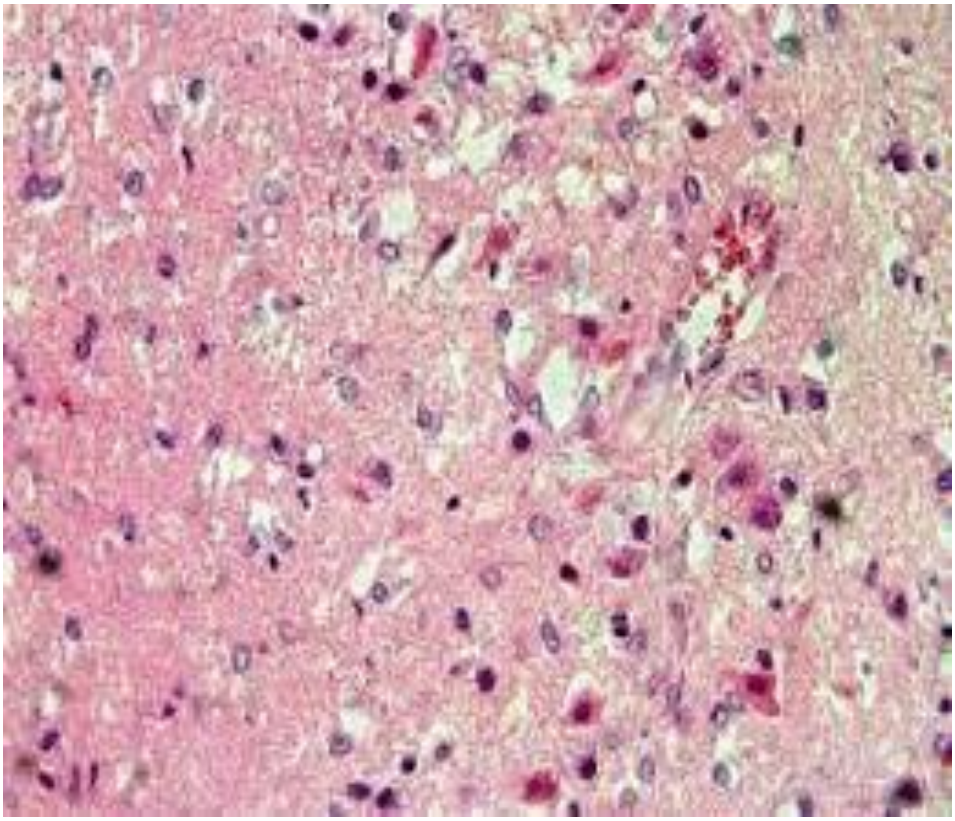
gene coding UGT1A1 (28).

1.6 BILIRUBIN TOXICITY

Hervieux was the first to describe the term brain jaundice (kernicterus) in 1847(30).The region's most commonly affected follows a topographic order of basal ganglion especially subthalamic nucleus and globus pallidus, hippocampus, various brainstem nuclei, including inferior colliculus, oculomotor, vestibular, cochlear, and cerebellum (dentate nucleus, vermis) (31,32). The involvement of these regions explains the clinical sequelae of bilirubin encephalopathy.

1.6.1 Histopathological finding in kernicterus

The Histopathological findings of brain in kernicterus are shown in Figure 1.5.



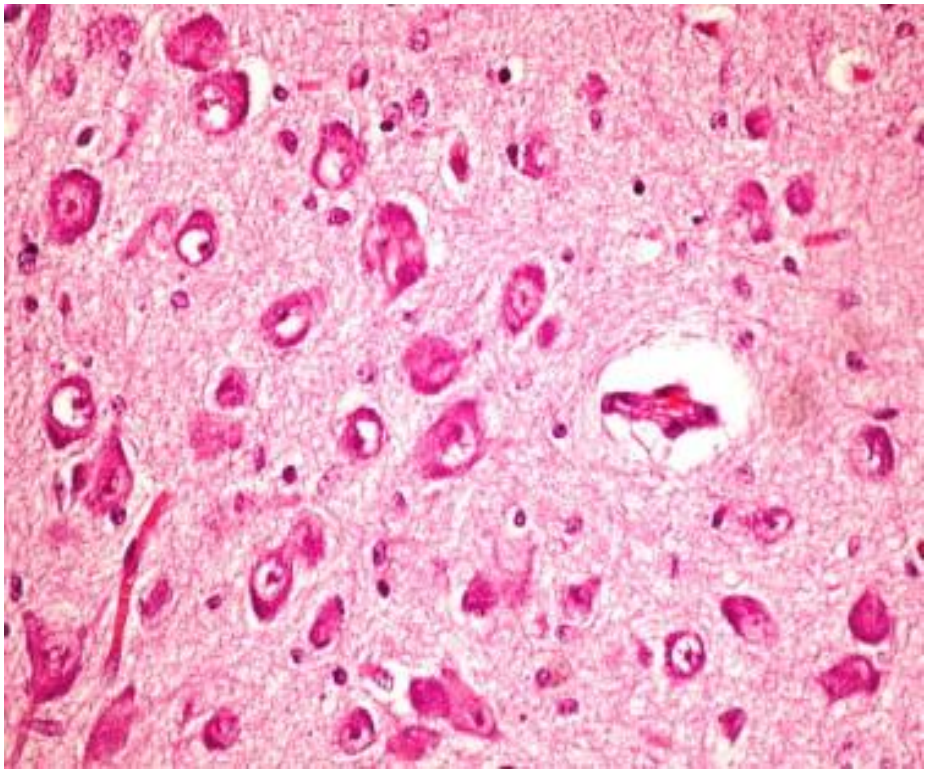


FIGURE 1.5 (a): HISTOPATHOGICAL FINDINGS IN KERNICTERUS

(a) Early

It occurs in 2-5 days. It is characterized by yellow pigment in neuronal cytoplasm, pyknotic nucleus, moth-eaten appearance of neuronal membranes, basophilic cytoplasm, loss of nissl substance.

(b) Subacute

It occurs in 6-10 days, characterized by hypertrophic bare astrocyte nuclei, spongy neutrophil, neuronal dissolution and granular mineralization of membranes

(c) Late

It occurs >10 days, characterized by neuronal loss, demyelination of optic tracts and fornix with dysmyelination and degeneration of globus pallidus and subthalamic nucleus

The combination of bright yellow orange staining of brain nuclei with evidence of neuronal damage and degeneration within the nuclei with the unique topographic pattern of nuclear involvement is necessary for the diagnosis of kernicterus (33).

1.6.2 Pathophysiology of bilirubin toxicity

When the concentration of bilirubin exceeds its solubility, bilirubin will aggregate and precipitate from solutions (34). The mechanisms by which bilirubin exerts its toxicity includes, (35, 36, 37, 38)

1. Inhibits cellular respiration and protein phosphorylation
2. Inhibits mitochondrial enzymes
3. Inhibits DNA and protein synthesis

4. Alters cellular glucose metabolism
5. Initiates a mitochondrial pathway of apoptosis and inhibits the function of NMDA receptor ion channels.

1.6.3 Mechanism of bilirubin entry into brain

Bilirubin does not require membrane transporters for its entry into brain. Only unbound bilirubin has the capacity to enter the brain cells. When the serum level of unbound bilirubin increases the probability of toxic level of bilirubin entering the brain also increases. However, if the blood brain barrier is disrupted, both bilirubin and albumin can enter.

1.6.4 Clinical features of bilirubin toxicity

1.6.4.1 Acute bilirubin encephalopathy

Term refers to acute clinical manifestation of bilirubin toxicity that occurs in first week of life. It progress through 3 distinct phases (39, 40, and 41). According to Volpe, definite neurological signs occur only in 50-60% newborns with bilirubin encephalopathy.

(a) Early phase

- Lethargy
- Poor sucking
- Hypotonia, paucity of movements
- Slightly high pitched cry

(b) Intermediate phase

- Moderate stupor, irritability
- Hypertonia some with retrocollis, opisthotonus
- Minimal feeding
- High pitched cry

(c) Late phase

- Pronounced opisthotonus, retrocollis
- Deep stupor to coma
- Fever
- Apnea
- Seizures
- Sometimes death

Subsequently after 1 week, hypertonia is replaced by hypotonia. Babies who developed hypertonia in second phase almost always develop chronic bilirubin encephalopathy sometimes exchange transfusion can reverse CNS manifestations.

1.6.4.2 Kernicterus

The term kernicterus has been reserved for chronic and permanent neurological damage occurring as sequelae of bilirubin toxicity.

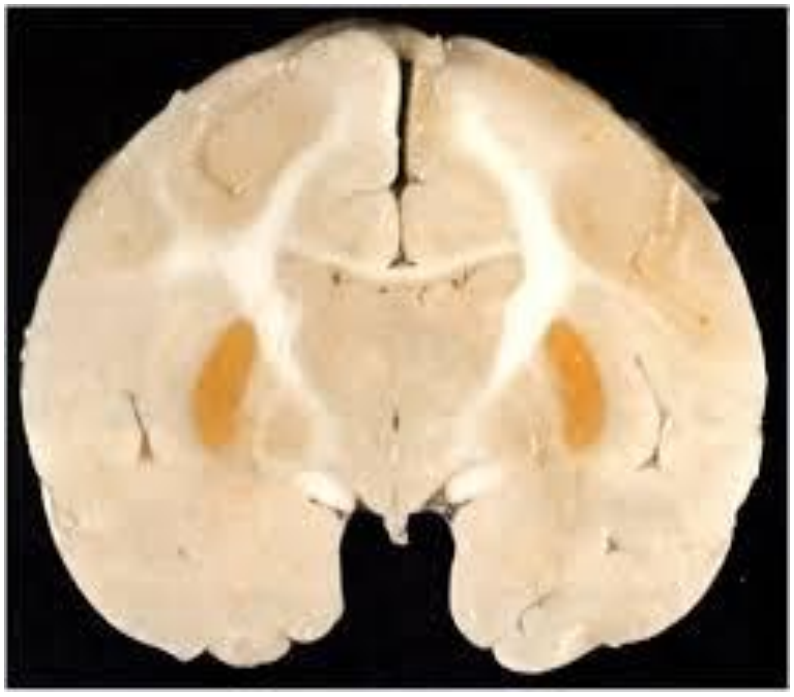


FIGURE 1.6: MACROSCOPIC APPEARANCE

Chronic bilirubin encephalopathy follows a typical pattern of temporal evolution (42) which includes high pitched cry, poor feeding, hypotonia with brisk deep tendon reflexes, persistent tonic neck reflex, and motor delay. Other typical features are not apparent until 1 year of age and these children are hypotonic for the first 6-7 years and then become hypertonic. The most commonly affected areas are the basal ganglion (Figure 1.6), the hippocampus and the brainstem nuclei.

1.6.4.2.1 Clinical features

Consists of tetrad of extrapyramidal disturbances, auditory abnormalities, gaze palsy and dental hyperplasia (43).

(a) Extrapyramidal symptoms

As early as 18 months, athetosis may develop but it can be delayed until 8-9 years of age. As per Perlstein, the absence of athetosis or other forms of extrapyramidal signs makes the diagnosis of chronic bilirubin encephalopathy doubtful (43). On severe affection, children may show facial grimacing, drooling, dysarthria, difficulty swallowing and chewing.

(b) Auditory abnormalities

Injury to the cochler nuclei is the principle cause of hearing loss. Some studies also shows possible involvement of peripheral auditory system as well (44).Auditory neuropathy in the presence of abnormal BAER and normal inner ear function has been described by Shapiro in his study (45).

(c) Gaze abnormalities

Supranuclear and nuclear palsies has been described by deposition of bilirubin in rostral midbrain and oculomotor nuclei respectively. Children presents with upward gaze palsies.

(d) Dental dysplasia

Dental dysplasia has been found in 75% of children with kernicterus with smaller number having greenish discoloration of teeth.

1.6.4.2.2 Diagnostic modality

(a) Magnetic resonance imaging

MRI confirms the diagnosis of acute bilirubin encephalopathy and kernicterus. Bilateral symmetrical high intensity signal in globus pallidus is the characteristic image. Sometimes hippocampus and thalamus also shows high intensity lesions (Figure 1.7).

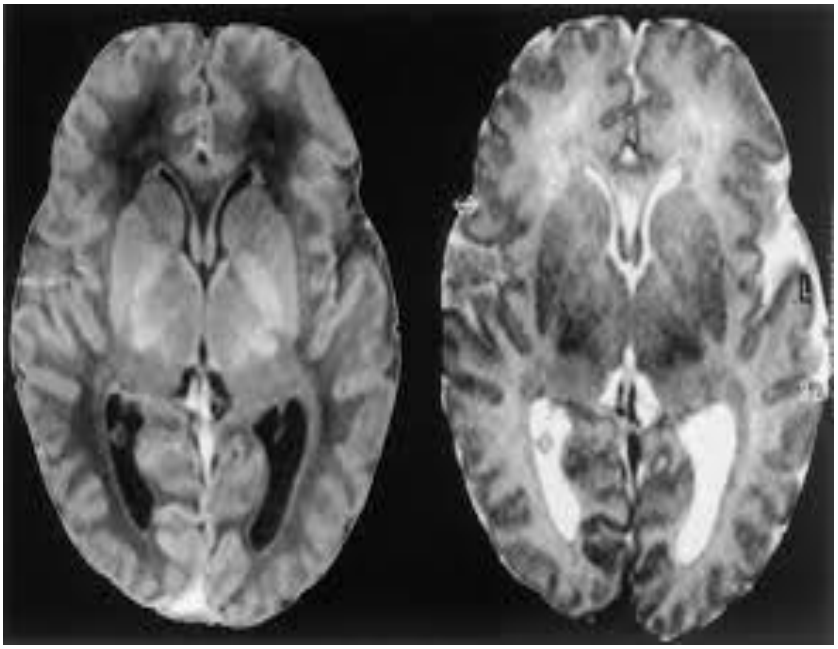


FIGURE 1.7: MRI SHOWING HIGH INTENSITY IN GLOBUS PALLIDUS

(b) Auditory neuropathy spectrum disorder

The most sensitive part of the central nervous system to bilirubin induced toxicity is the auditory pathway. Even a moderately elevated bilirubin can cause damage to auditory pathway and manifests clinically as auditory neuropathy spectrum disorder.

The gold standard for the diagnosis of bilirubin induced neurological damage is ABR. It shows increased latencies of ABR waves at 3 and 4 with reduced amplitudes.

1.7 ACUTE AND LONG TERM SEQUELAE OF HYPERBILIRUBINEMIA – A "NEVER EVENT"

"Never events" are entirely preventable serious incidents with a potential to cause patient harm or death. Kernicterus has been enlisted as one of the Never Events in UK by National Institute of Health and Clinical Excellence (NICE) and in US by National Quality Forums

National institutes of various countries (AAP, NICE – UK, etc) have proposed guidelines for screening of significant neonatal hyperbilirubinemia and treatment thresholds.

American Academy of Pediatrics recommends that newborns discharged within 48 hours of life should have a follow-up visit after 2-3 days. The AAP also has developed a resource kit and bilitool a web based program as a practical instrument for plotting hour specific TSB/TcB measurements.

NICE 2010 recommend risk assessment in every newborn and review within 48 hours of birth of babies with known risk factors for significant hyperbilirubinemia. NICE has provided a billi-wheel to display the treatment thresholds and assist in precise measurement of baby's age in hours.

A recent meta-analysis showed that infants at risk of severe hyperbilirubinemia in low and middle-income countries are associated with the following maternal and neonatal factors that can be effectively managed with available interventions to curtail the disease burden. At risk newborns include,

- Primiparity
- delivery outside the public hospital
- ABO incompatibility
- Rhesus hemolytic disease
- G6PD deficiency
- UGT1A1 polymorphisms
- Low gestational age
- Under weight / weight loss
- Sepsis
- High TcB/TSB

1.8 EPIDEMIOLOGY OF NEONATAL JAUNDICE

Factors associated with increased risk of neonatal jaundice includes

- RACE-East Asians, native Americans, Greek, Mexicans have greater risk for jaundice

- Maternal and familial factors like primipara, maternal age >25 years, maternal diabetes, hypertension, oral contraceptive usage at the time of conception, first trimester bleeding, use of oxytocin at the time of delivery, decreased zinc level, previous sibling with jaundice.
- Drugs used in mother like epidural analgesia, diazepam, promethazine
- Types of deliveries like forceps, vacuum extraction, breech delivery, delayed cord clamping, elevated cord bilirubin
- Other factors like cephalhematoma, significant bruising, male gender, delayed meconium passage, increased weight loss after birth, reduced breastfeeding, jaundice observed before discharge, shorter hospital stay after birth, predischarge serum bilirubin in high risk zone.

1.9 IDENTIFICATION OF JAUNDICED NEWBORN

All infants should be routinely monitored for the presence of jaundice by blanching the skin with digital pressure and it should be done in well lit room in daylight near the window.

1.10 CEPHALOCAUDAL PROGRESSION OF JAUNDICE

Dermal icterus is first seen in the face and then progresses to trunk and extremities in a caudal manner (Figure 1.8 and Table 1.1). It is a useful clinical tool but less reliable after TSB exceeds 12mg/dl.

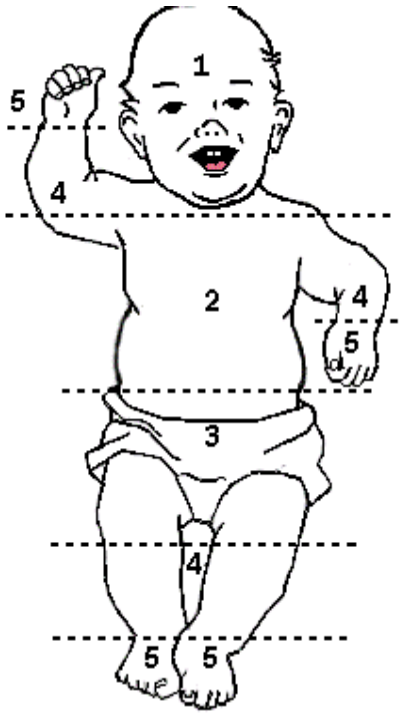


FIGURE 1.8: KRAMERS CEPHALOCAUDAL PROGRESSION OF JAUNDICE

S.NO	Area of body	Serum Bilirubin levels (mg/dl)
1	Face	4.8
2	Chest, Upper abdomen	8-10
3	Lower abdomen, thighs	12-14
4	Arms, Lower legs	15-18
5	Palms, soles	15-20

TABLE 1.1: CEPHALOCAUDAL PROGRESSION OF JAUNDICE

1.11 EVALUATION OF JAUNDICED INFANT >35 WEEKS OF GESTATIONAL AGE

The indication and evaluation of jaundiced infant for >35 weeks of gestational age are tabulated in Table 1.2.

Indications	Evaluation
Jaundice in first 24 hours	Transcutaneous bilirubin/or TSB
Jaundice appearing excessive for age	TcB and /TSB
TSB in exchange range or not responding to treatment	Retic count, albumin, G6PD, ETCOc
Infant on phototherapy/ Serum bilirubin rising rapidly	CBC, smear, coombs test, blood group, direct bilirubin
Jaundice persisting beyond 3 weeks of life	TSB and direct bilirubin. Thyroid and galactosemia screening If direct high- evaluate for cholestasis
Elevated direct bilirubin	Do cultures. Evaluate for sepsis

TABLE 1.2: EVALUATION OF JAUNDICE IN NEWBORN

1.11.1 Non-invasive measurement of serum bilirubin

1. Ingram icterometer

It is a simple and inexpensive screening tool that can be used by nurses and even by parents at home (46, 47). It is a piece of transparent plastic on which 5 transverse strips of graded yellow hue is present as shown in Figure 1.9. If

pressed against the nose, the yellow color of the blanched skin is matched with appropriate yellow strip and grading is done.

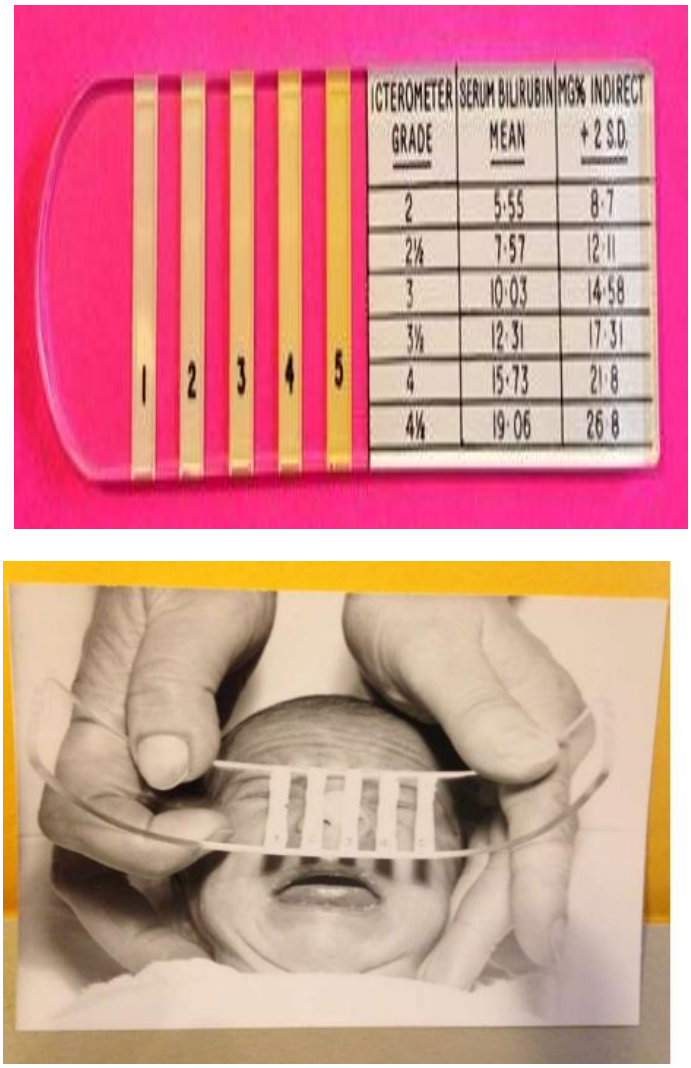


FIGURE 1.9: INGRAM ICTEROMETER

2. Transcutaneous device

1. Minolta air shield jaundice meter:
    a. It was the first electronic device marketed for transcutaneous bilirubin measurement (Figure 1.10). The principle includes formation of two beams one of which enters the shallow area and

the other enters the deeper area of subcutaneous tissues. The differences between the optical densities are detected by blue and green photocells.

FIGURE 1.10: MINOLTA AIR SHIELD JAUNDICE METER

2. Bilichek device:

   a. It uses the multiple spectrum of visible light reflected by skin by employing multiple wavelengths (Figure 1.11).

FIGURE 1.11: BILICHEK DEVICE

3. Another device called JM103 (Figure 1.12) was also used for transcutaneous bilirubin measurement. It has some acceptable level of diagnostic accuracy as a screening tool.

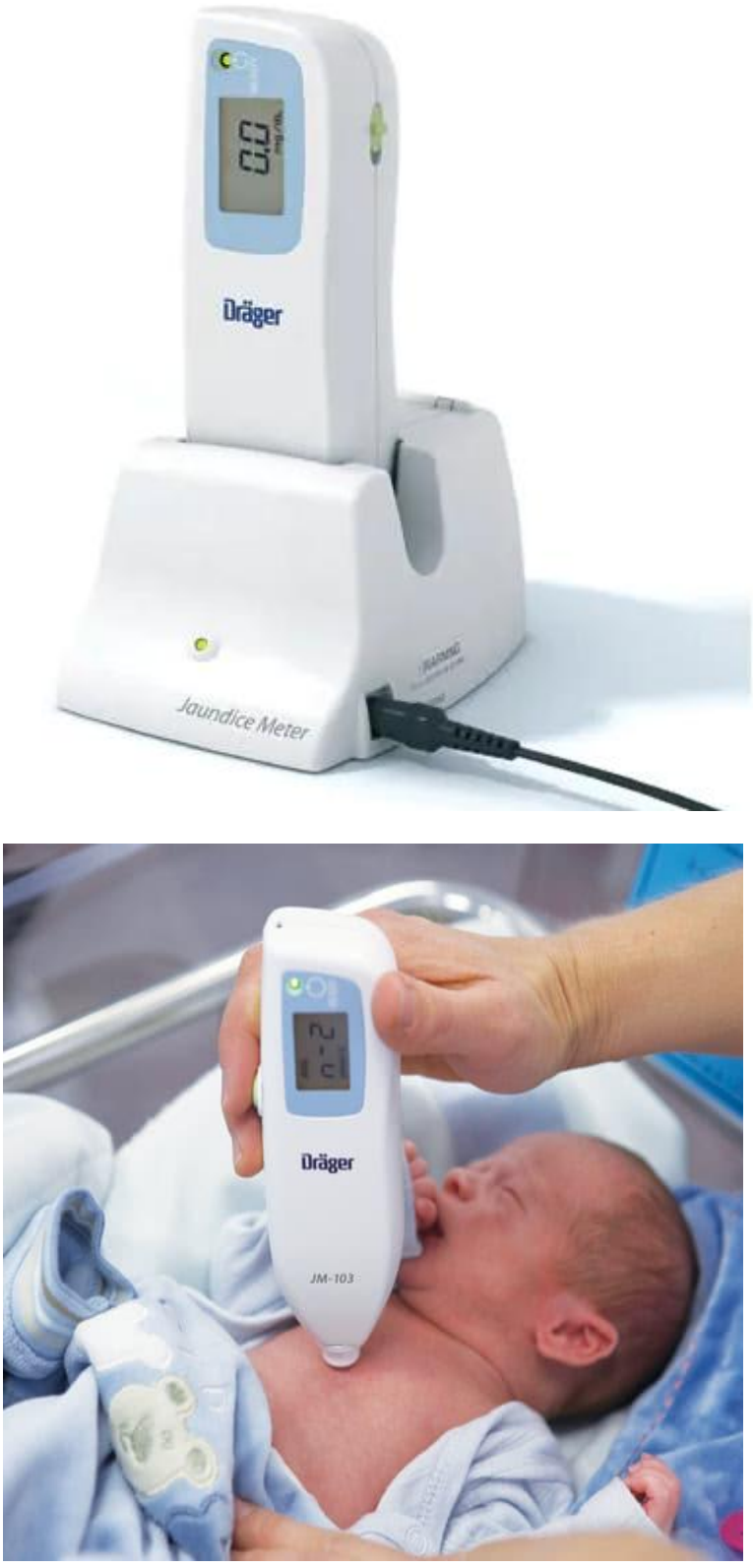


FIGURE 1.12: JM103

Disadvantage of TcB measurement (48, 49)

1. It is less precise with decreasing gestational age
2. It is not reliable for infants under phototherapy as it bleaches the skin.
3. It is used only as a screening tool and the decision cannot be taken on single isolated TcB measurement.

1.11.2 Invasive methods

- ✓ Filter paper with bilirubinometer
- ✓ Capillary bilirubin estimation by spectrophotometry
- ✓ Measurement of total serum bilirum by
    - Van den bergh reaction or Diazo reaction- this test utilizes ehrlich diazo reagent which reacts with direct bilirubin in serum to give pink to reddish purple colored azobilirubin. It can be read at 1 minute.
    - Bilirubinometer-
        - The Jerdraussik Grof method
        - The Malloy Evelyn method
        - Direct spectrophotometry
    - High pressure liquid chromatography- it is the gold standard and rapid and quantifies all fractions of bilirubin.
    - Enzymatic method-
        - Peroxidase method
        - Peroxidase diazo method
    - Simple colorimetric method

1.11.3 End tidal carbon monoxide measurements

Equimolor quantities of carbon monoxide and biliverdin are formed when heme is broken down by heme oxygenize. So this measurement of CO in end tidal breath can be used as a bedside index test of bilirubin production (50).

1.11.4 Bilirubin to albumin molar ratio

The molor ratio of bilirubin to albumin correlates well with unconjugated bilirubin levels. It can be used as a surrogate marker for unbound bilirubin. Bilirubin to albumin ratio can be used as an adjunct to TSB measurement in deciding for exchange transfusion.

1.12 TREATMENT OF HYPERBILIRUBINEMIA

1. Phototherapy

All total bilirubin levels should be interpreted in terms of infant's age in hours and treatment should be started for the following babies.

i. A neonate with TSB of >95th percentile

ii. The rate of rise crosses percentiles or

iii. The rate of rise exceeds 0.2mg/dl/hr

There are various factors which determine the dose of phototherapy is

1. Irradiance of light source
2. Spectrum of light emitted
3. Design of phototherapy unit

4. Surface area of infant exposed to light
5. Distance of infants from light source

Mechanism of action of phototherapy

Phototherapy detoxifies bilirubin by converting it to photoproducts that are less lipophilic and are excreted by by-passing liver.

(a) Configurational isomerisation

Phototherapy converts the stable Z-Z isomer to Z-E isomer. The formation of Z-E isomer is spontaneously reversible in the dark. It is rapidly converted to unconjugated bilirubin in bile and is excreted. When the neonate is exposed to phototherapy, there is rapid isomerization in skin but there is slow clearance of Z-E isomer. Hence it plays only minor role in lowering bilirubin concentration (51).

(b) Structural isomerisation

An irreversible process of cyclization of bilirubin occurs in the presence of light to lumirubin and is excreted in bile and also some amount in urine (51). There is more rapid clearance of lumirubin from the serum than the Z-E isomer which is responsible for the reduction of serum bilirubin on phototherapy.

(c) Photo-oxidation

There is photo-oxidation of bilirubin to water soluble substance that can be excreted in the urine. But it is a slow process and it contributes only minor to

the reduction of serum bilirubin level.

Various light sources used are:

1. Fluorescent tubes
2. Light emiting diodes
3. Halogen lamps
4. Fiberoptic systems.

2. Hydration and feeding

Because lumirubin is excreted in the urine, maintaining adequate hydration improves the efficacy of phototherapy.

3 Exchange transfusion

Exchange transfusion is used when serum bilirubin raises inspite of phototherapy to protect infants from bilirubin induced neurotoxicity. Fresh whole blood of O Rh negative irradiated packed RBCs that are suspended in AB plasma is used. Interpretation for the need of exchange transfusion is done using hour specific normogram.

Common complications of exchange transfusion are:

- Thrombocytopenia
- Coagulation abnormalities
- Hypoglycemia
- Hyperkalemia
- Hypocalcemia
- Acid base abnormalities

4. Pharmacological therapy

1. Phenobarbitol

   It is a potent microsomal enzyme inducer that increases bilirubin conjugation and excretion. It is effective in lowering the serum bilirubin level when given in adequate dosage to mother or neonate or both (52, 53).

2. Tin mesoporphyrin

   It inhibits the enzyme heme oxygenase that is involved in the production of bilirubin and thus reduces its level.

3. Intravenous immunoglobulin

   It decreases bilirubin production by inhibiting hemolysis.

CHAPTER 2

REVIEW OF LITERATURE

As early as 1940s in the United States, a practice of early postpartum discharge was initiated (54). Now other countries are also moving on to the trend of shortening the postpartum length of hospital stay due to various reasons like cost containment, hospital bed availability and a movement towards "demedication of childbirth" (55).

In addition to shorter hospital stays, early postpartum discharge for healthy mothers and newborns was introduced to promote a more family-centered approach to birth allowing greater involvement of fathers, less sibling rivalry, improved rest and sleep for the mother, less exposure of the mother-newborn to nosocomial infections, enhancement of maternal confidence in caring for the baby and finally, less conflicting advice on breastfeeding (56).

However, concerns about early discharge arose regarding potential adverse outcomes such as delay in detecting and preventing maternal morbidities and neonatal pathologies (57), earlier weaning, lack of professional support, higher prevalence of postpartum depression and increased hospital readmissions for both mothers and infants due to many problems most commonly hyperbilirubinemia.

Robert L et al studied the association between early discharge from hospital after birth and readmissions to hospital for jaundice in healthy term neonates. They found that the neonate discharged earlier in the first 2 days after

birth are more likely to be readmitted for jaundice when compared with infants stayed more than 3 days (58).

The risks and benefits of early discharge of the mother and the newborn were studied by Keily M et al. They found that early discharge had a benefit of increased exclusive breastfeeding rates and positive effect on mental status of the mother but there was a higher risk of readmission to the hospital due to jaundice (59).

"Length of hospital stay, jaundice, and hospital readmission" study done by M.Jeffrey Maisels and Elizabeth Kringin concluded that the babies discharged earlier than 3 days are at risk of readmission to hospital mainly due to hyperbilirubinemia (60).

Although a milder form of jaundice occurs in all healthy term newborns, in some babies it progresses to severe form by 4 to 6 days of life. If this is missed, the baby may develop acute bilirubin encephalopathy and chronic neurological dysfunctions.

Hence, an easier and effective method of predicting newborns at risk of developing jaundice is an option to solve this problem. Various studies have been done to predict hyperbilirubinemia in newborns using cord blood bilirubin in healthy tern newborns.

Amar taksande et al in their study, which included healthy term newborns, concluded that a critical value of cord blood bilirubin more than 2 mg/dl had the high sensitivity (89.5%) and specificity (85%) for predicting

neonatal hyperbilirubinemia (61).

Rudy Saltrya et al in their study “Correlation between cord blood bilirubin level and incidence of hyperbilirubinemia in term newborns” concluded that cord blood bilirubin level of more than or equal to 2.54 mg/dl can predict the development of neonatal hyperbilirubinemia requiring phototherapy (62).

Sun et al in his study of predicting the subsequent jaundice using cord serum bilirubin found that the babies with UCB level >2 can have neonatal hyperbilirubinemia with sensitivity of 77% and specificity of 98.6% (63).

Zakia Nahar et al in their prospective study of “predictive value of umbilical cord blood bilirubin in neonatal hyperbilirubinemia” found that a cord blood bilirubin level of more than or equal to 2.5mg/dl in full term newborns to predict the development of significant hyperbilirubinemia with negative predictive value of 96% suggesting that it is less likely for newborn to develop jaundice when UCB is < 2.5mg/dl (64).

Knufer et al in his study on 2005 found a total cord serum bilirubin level of more 2.34 mg/dl, there is higher incidence of neonatal hyperbilirubinemia in the first week of life (65).

Bernaldo and Segre investigated the predictability of umbilical cord blood unconjugated bilirubin of 2.0 mg/dl, they showed that 53% of babies needed phototherapy and raising the cutoff value to 2.5 mg/dl, they predicted that 72% of babies needed phototherapy (66).

Chen JY et al in 1994 conducted a study on “Prediction of neonatal

hyperbilirubinemia in ABO incompatibility" in eighty-eight healthy full-term newborn infants born to O blood group mothers. Titers of IgG anti-A and anti-B antibodies were measured in mothers. Direct Coombs' test and serum bilirubin level was measured in cord blood .He concluded that ABO incompatible newborn infants with cord bilirubin levels $\geq$ 4 mg/dl represent a "high risk" category and should be placed in hospital for frequent re-evaluation and appropriate therapy (17).

Allam bhat et al studied the correlation of cord blood bilirubin with hyperbilirubinemia in healthy term newborn babies in Haryana, Delhi. CBB was done for all babies and they were followed up for the first 5 days for the development of jaundice. Babies who developed jaundice were started on phototherapy and others were observed. In his study, the development of significant hyperbilirubinemia was 11.2%. Cord blood value of >3.5 mg/dl had a high sensitivity (97.06%), specificity (99.22%), positive predictive value (94.29%) and negative predictive value (99.61%) in predicting future pathological jaundice (67).

Zeiten et al study population consists of 50 males, 44 females with the mean GA of 38.70 ± 1.38 weeks in full term compared to 35.62 ± 0.64 in late preterm. He showed that 40.4% of PT needed treatment when compared to 29.8% of FT. The mean total cord bilirubin was higher among males, preterm, caesarean and ABO and Rh incompatibility newborns. He found that when UCB in late PT newborns was $\geqslant$1.75 mg/dl and $\geqslant$1.85 mg/dl in FT newborns,

there was a probability that those newborns may need phototherapy and when the levels of total cord bilirubin were ⩾2.05 mg/dl in PT newborns and ⩾2.15 mg/dl in FT it means that those babies are in actual need of phototherapy. Thus he showed that cut-off points for total cord bilirubin level in PT and FT groups were 2.05 and 2.15 mg/dl respectively (68).

Knudson et al investigated the predictive ability of umbilical cord bilirubin for postnatal hyperbilirubinemia. For the prediction of need for phototherapy using a UCS bilirubin cut-off level of 30 μmol/l had a sensitivity of 90% and a negative predictive value of 99.1%, indicating that all patients with UCS bilirubin values below 30 μmol/l were at a very low risk of developing dangerous hyperbilirubinemia (69).

Nutan Kamath et al conducted his study in Mangalore. His study had a population of 500 term, appropriate for gestational age with a APGAR score >7. Significant hyperbilirubinemia was defined as STB >15mg/dl. 14% was found to be prevalence of significant hyperbilirubinemia in his study. Serum bilirubin level was done in case of clinical jaundice presenting before 5 days. 5^{th} day jaundice was related with that of UCB. He found that mean UCSB was 1.56 ± 0.70 mg/dl. There was a significant association between birth order, route of delivery and hyperbilirubinemia requiring phototherapy (P<.005). Hyperbilirubinemia could be predicted with sensitivity of 90% and specificity of 82.55% , positive predictive value of 45.65% and negative predictive value of 98.07% using UCSB of >3.19mg/dl (70).

Bindhu et al conducted her study in tertiary care center in Kerala during the year 2014-2015. 254 out of 450 newborns (56%) neonates developed clinically significant hyperbilirubinemia. Majority of babies had cord blood bilirubin levels ranging from 1.5-2.4mg/dl. Levels ≥3 mg/dl was found only in 1.3%. Majority of newborn (60%) in her study had an intermediate risk of developing hyperbilirubinemia, and only 1.3% belonged to the high risk category on risk stratifications. According to her study, cord bilirubin cut off value of 1.9 mg/dl predicted subsequent hyperbilirubinemia with sensitivity of 91.8% and specificity of 52.4% (71).

Jayashree Vasudevan et al conducted her study in a total of 1114 term and near term babies born between January 2008 and December 2009. Umbilical cord bilirubin levels were taken in all the children. Serum bilirubin was obtained from neonates on day three who were clinically jaundiced. 12.6% developed hyperbilirubinemia out of 1114 study subjects. AOC was 0.6, which is closer to the null value of 0.5 (95% CI 0.55 to 0.66, p-value 0.001) when cord bilirubin level of 1.5 mg/dl was used as screening test. The negative predictive value was 96% with cord bilirubin level above 1.5 mg/dl and consistently maintained above 90% with increasing levels of cord bilirubin up to 3mg/dl (72).

There are only few studies predicting the cut off value of cord blood bilirubin for neonatal hyperbilirubinemia in ABO and Rh incompatible babies. Hence the present study was done to find the usefulness of cord blood bilirubin in predicting neonatal jaundice in babies of A or B blood group born to O

positive mother and in babies of positive blood group born to negative mother.

CHAPTER 3

AIMS AND OBJECTIVE

The primary aim of the study is

- to predict the usefulness of cord blood bilirubin in identifying subsequent hyperbilirubinemia in ABO and Rh incompatible babies requiring therapeutic intervention so that early postpartum discharge of both mother and those newborns can be planned

Secondary aim:

- To reduce the rate of babies requiring exchange transfusion

CHAPTER 4

METHODOLOGY

The study of "cord blood bilirubin as a predictive marker of neonatal hyperbilirubinemia in ABO and Rh incompatible babies" is conducted in Chengalpattu medical college and hospital in healthy term newborn babies over a period of one year.

- **Study design:** Prospective study
- .
- **Period of study:** September 2018 to May 2019
- **Study Population:** Babies born with A or B to O+ mothers or mother negative and baby positive blood group in Chengalpattu Medical College Hospital including both caesarean and labor natural
- **Sampling Method:** Convenient Sampling
- **Sampling size :** Sample size 93 is calculated using formula:

$$N = \frac{2 \times S.D^2 \times \left(Z_\alpha + Z_\beta\right)^2}{d^2}$$

where,

- d is the mean difference
- S.D is the standard deviation
- N is the number of samples
- α is the significance level

- β is the power, probability of detecting a significant result (typically 80%, 90%)
- Z points on normal distribution to give required power and significance

The inclusion and the exclusion criteria for the study are as follows,

INCLUSION CRITERIA

- Newborn with A or B born to O+ mothers or mother negative and baby positive blood group
- Newborn with Gestational Age (GA) >37 weeks
- Newborn with birth weight 2.5-4kg
- Newborn with APGAR score >7

EXCLUSION CRITERIA

- Absence of significant illness or of major congenital malformation
- Neonatal problems like sepsis, hypothyroidism, respiratory distress syndrome.
- Trauma conditions like Cephalhematoma

Considering the above inclusion and exclusion criteria, 100 newborns delivered by both cesarean and labor natural in Chengalpattu Medical College were selected for the study.

Informed and written consent was obtained for all cases. To obtain the required data, questionnaire method, maternal case file and examination of the newborn were used.

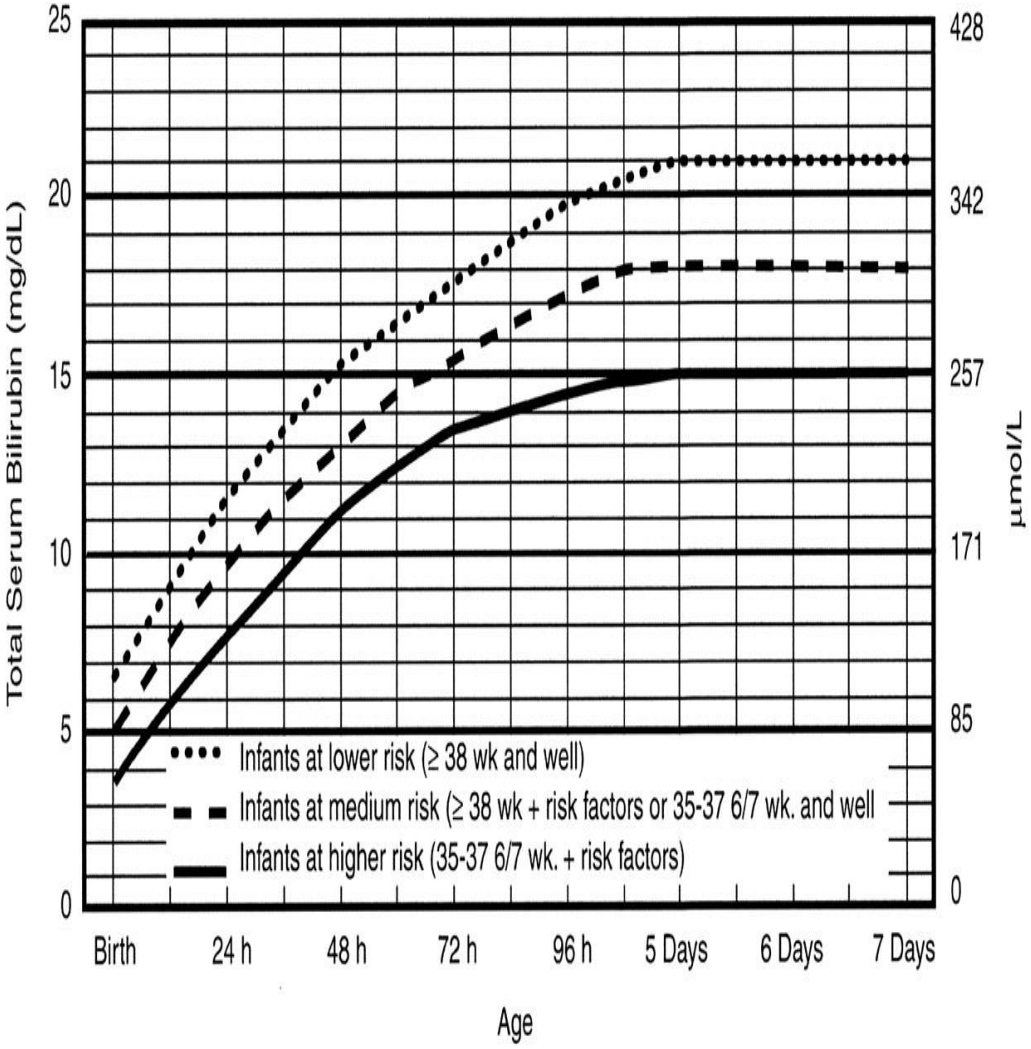
Cord blood was collected from term babies born to O positive or any negative blood group mother and sent for total serum bilirubin and blood grouping evaluation. Healthy babies of A or B born to O positive mother and babies of positive blood group born to negative group mother were enrolled in the study.

Various neonatal factors like weight at birth, gestational age, sex, APGAR at 5mins, delayed adaptation, perinatal depression were collected. Maternal factors like age, number of births, blood group, and mode of delivery, previous sibling with jaundice, maternal diabetes mellitus, and gestational hypertension were collected from maternal file.

Babies were examined daily and looked for the evidence of jaundice, sepsis and development of any illness for the first 5 days. Serum blood was drawn at 72 hours of life for all babies. Blood for evaluation of total bilirubin was also drawn at less than 72 hours of life from babies who showed clinical evidence of jaundice. Peripheral venous blood was used to measure serum bilirubin.

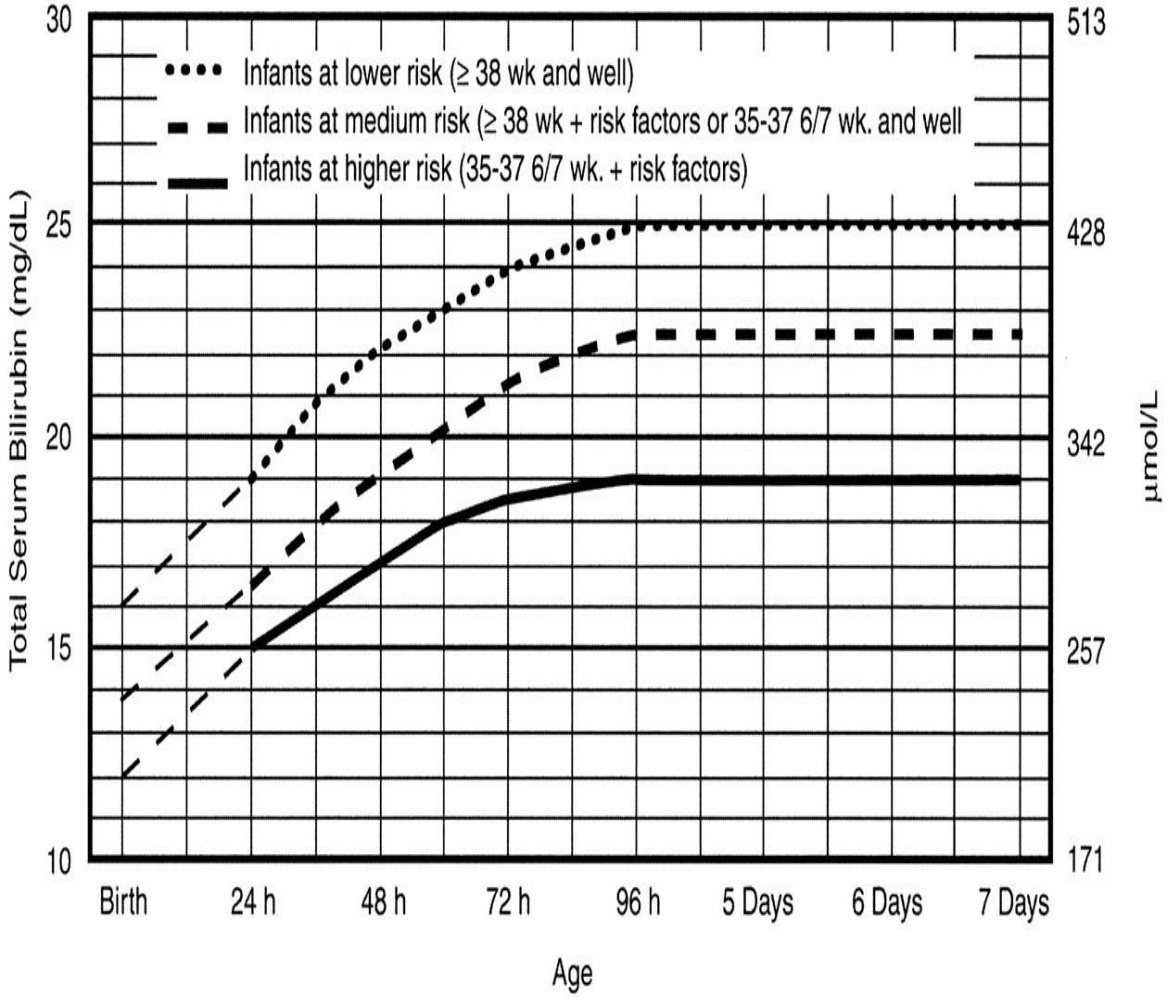
Serum bilirubin estimation was done within 12 hours of collection by Diazitized sulfanilic test. The blood sample collected was stored away from light and was refrigerated between 2-8^{0}C till the estimation was done.

The main outcome of the study was inferred in terms of hyperbilirubinemia. Babies developing significant hyperbilirubinemia are treated with phototherapy and exchange transfusion as per the American academy of paediatrics practice parameter, 2004 as shown in Figure 4.1 and 4.2 respectively.



- Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin.
- Risk factors = isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis, or albumin < 3.0g/dL (if measured)
- For well infants 35-37 6/7 wk can adjust TSB levels for intervention around the medium risk line. It is an option to intervene at lower TSB levels for infants closer to 35 wks and at higher TSB levels for those closer to 37 6/7 wk.
- It is an option to provide conventional phototherapy in hospital or at home at TSB levels 2-3 mg/dL (35-50mmol/L) below those shown but home phototherapy should not be used in any infant with risk factors.

FIGURE 4.1: HOUR SPECIFIC NORMOGRAN FOR PHOTOTHERAPY



- The dashed lines for the first 24 hours indicate uncertainty due to a wide range of clinical circumstances and a range of responses to phototherapy.
- Immediate exchange transfusion is recommended if infant shows signs of acute bilirubin encephalopathy (hypertonia, arching, retrocollis, opisthotonos, fever, high pitched cry) or if TSB is ≥5 mg/dL (85 µmol/L) above these lines.
- Risk factors - isoimmune hemolytic disease, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis, acidosis.
- Measure serum albumin and calculate B/A ratio (See legend)
- Use total bilirubin. Do not subtract direct reacting or conjugated bilirubin
- If infant is well and 35-37 6/7 wk (median risk) can individualize TSB levels for exchange based on actual gestational age.

FIGURE 4.2: HOUR SPECIFIC NORMOGRAM FOR EXCHANGE TRANSFUSION

IAP-NNF also recommends considering phototherapy with neonatal serum bilirubin levels of ≥15 mg/dl after 48 hours of life. So, in the present study

babies with serum bilirubin level of ≥15 mg/dl are considered hyperbilirubinemia and needs phototherapy after 48 hours of postnatal life. Maternal, neonatal and natal variables were compared between neonates with 2 days follow up.

CHAPTER 5

STATISTICAL ANALYSIS

5.1 INTRODUCTION

Data analysis was carried out using SPSS version 26 software. Sensitivity, specificity, mean cord bilirubin, positive predictive value and negative predictive value of different cut-points of cord blood serum bilirubin were derived. For determining the significance of each test, P <0.05 was used.

Chi-square test was used to find the significance in categorical data. Fishers exact was used if the expected cell frequency is less than 5 in 2*2 Table. The receiver operating characteristic analysis was used to find the cut off value with sensitivity and specificity of the study.

5.2 DATA SOURCE

Out of 107 newborns enrolled, 4 could not be followed up, 2 were admitted in ICU and 1 refused to give consent. Hence a total of 100 newborns were followed up for the 1st five days with clinical assessment and lab investigations.

Out of the 100 enrolled newborns, 53% (n=53) were male, 47% (n=47) were females, 42% (n=42) of the delivery was by labor natural and 58% (n=58) of the delivery was by cesarean. 57 % (n=57) newborns developed jaundice which were in physiological range and hence went home without any treatment. Remaining 43% (n=43) neonates developed significant hyperbilirubinemia

which required treatment. Schematic representation of the study is shown in Figure 5.1.

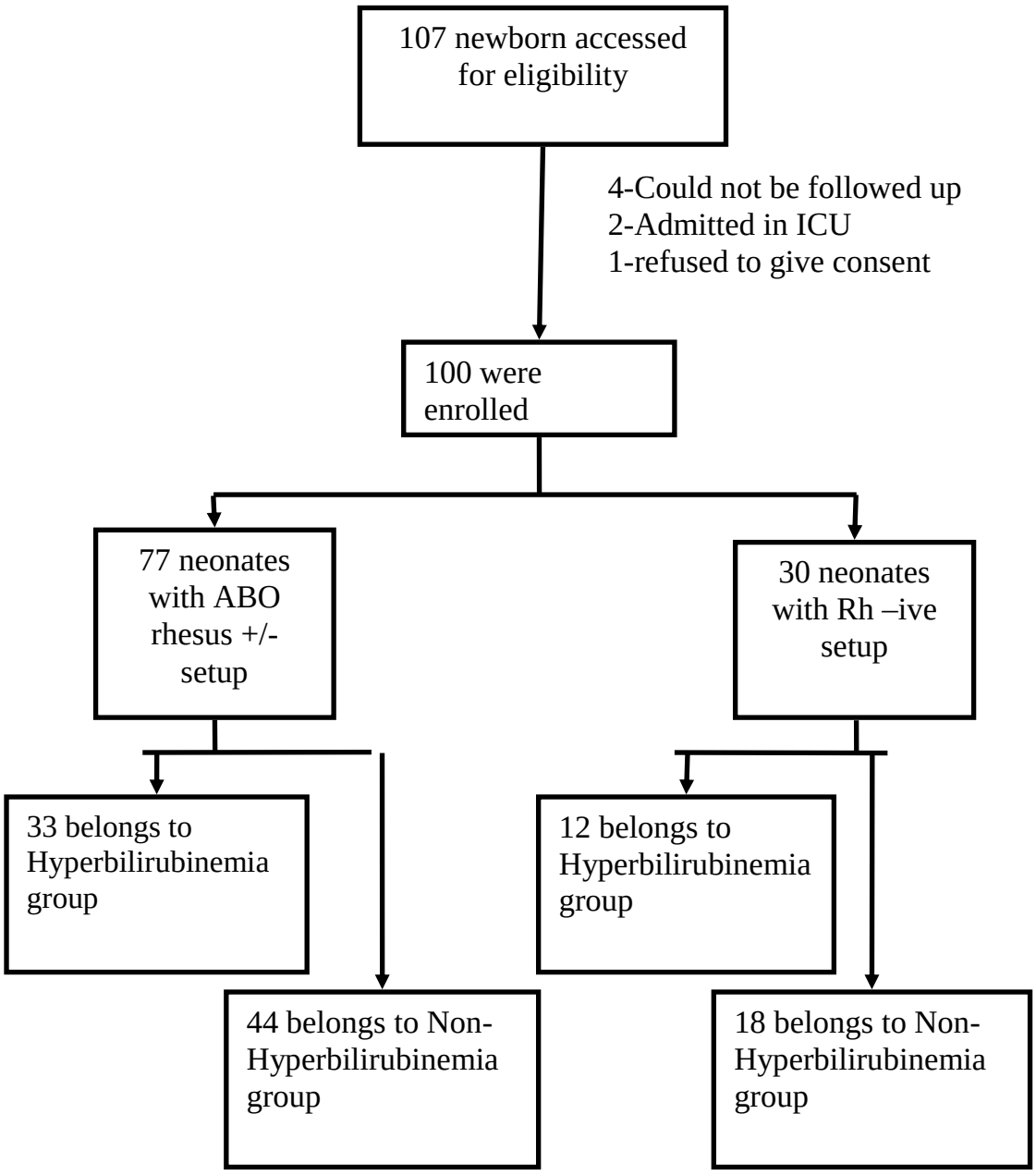


FIGURE 5.1: SCHEMATIC REPRESENTATION OF STUDY GROUP

The association between the serum bilirubin level and the different neonatal and maternal factors like: Sex, mode of delivery, weight at birth, order

of birth, perinatal depression, delayed adaptation, maternal age, maternal diabetes mellitus, systemic hypertension, maternal anemia, hypothyroidism, previous sibling jaundice were analyzed and discussed in the following section.

5.3 GENDER DISTRIBUTION OF CASES

Out of the 100 enrolled newborns, 53 %(n=53) were male, 47 %(n=47) were females (Figure 5.2). There was no significant difference in the number of sex of babies enrolled in the study.

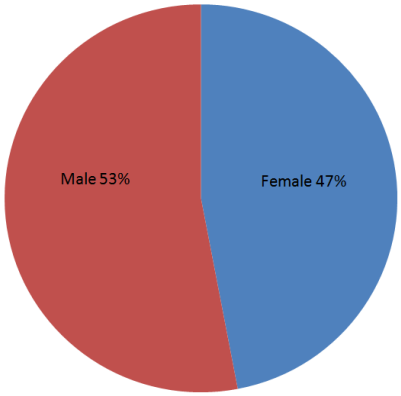


FIGURE 5.2: GENDER DISTRIBUTION

5.4 ASSOCIATION BETWEEN NEONATAL JAUNDICE AND SEX

Table 5.1 shows the sex wise distribution of cases and the development of hyperbilirubinemia. Out of 53 %(n=53) male newborn cases, 46.5% (n=20) developed significant hyperbilirubinemia and out of 47 % (n=47) female babies, 53.5% (n=23) developed neonatal jaundice.

Sex	Frequency (%)	Hyperbilirubinemia(%)(n)

		Yes	No
Male	53	46.5(20)	57.9(33)
Female	47	53.5(23)	42.1(24)

TABLE 5.1: SEX WISE DISTRIBUTION OF CASES

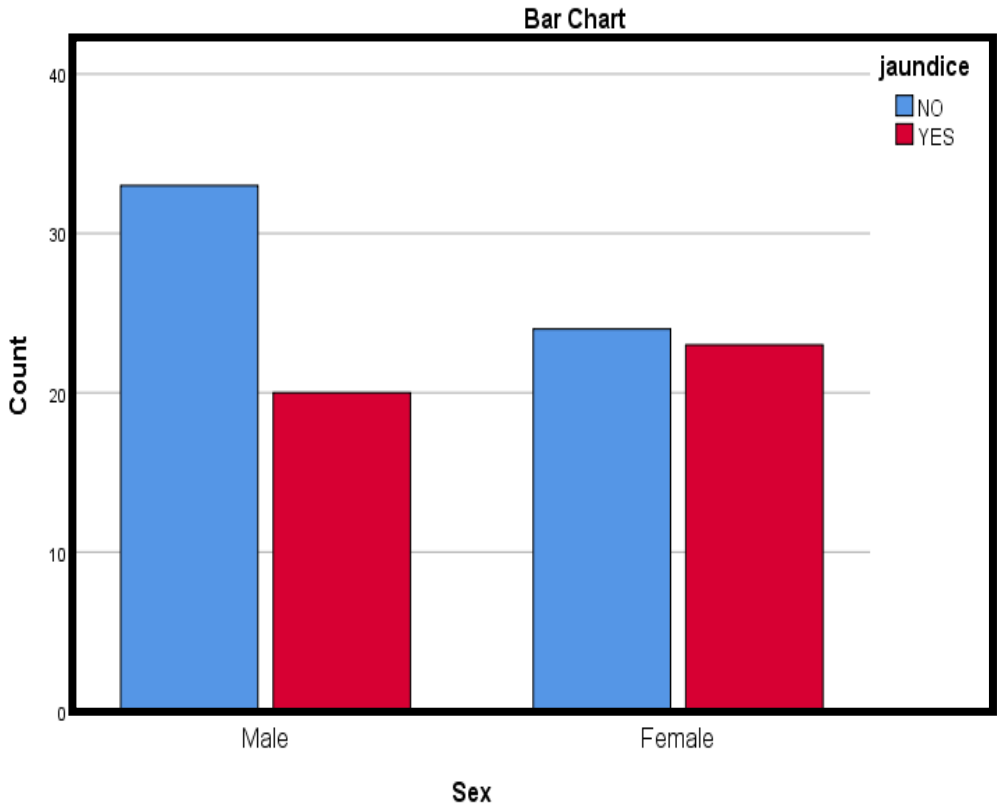


FIGURE 5.3: JAUNDICE AND SEX OF THE NEWBORN

The significance between serum bilirubin level and the sex of the newborn was also analyzed and found to be p=0.25, which indicates that they are insignificant. Hence the present study infers that the serum bilirubin level is independent of the sex of the newborn. The number of male and female babies developing significant hyperbilirubinemia is shown in Figure 5.3.

5.5 ASSOCIATION BETWEEN NEONATAL HYPERBILIRUBINEMIA AND MODE OF DELIVERY

Out of 42 % (n=42) delivered by labor natural, 44.2% (n=19) developed jaundice and out of 58% (n=58) of cesarean delivery, 55.8% (n=24) developed significant hyperbilirubinemia.

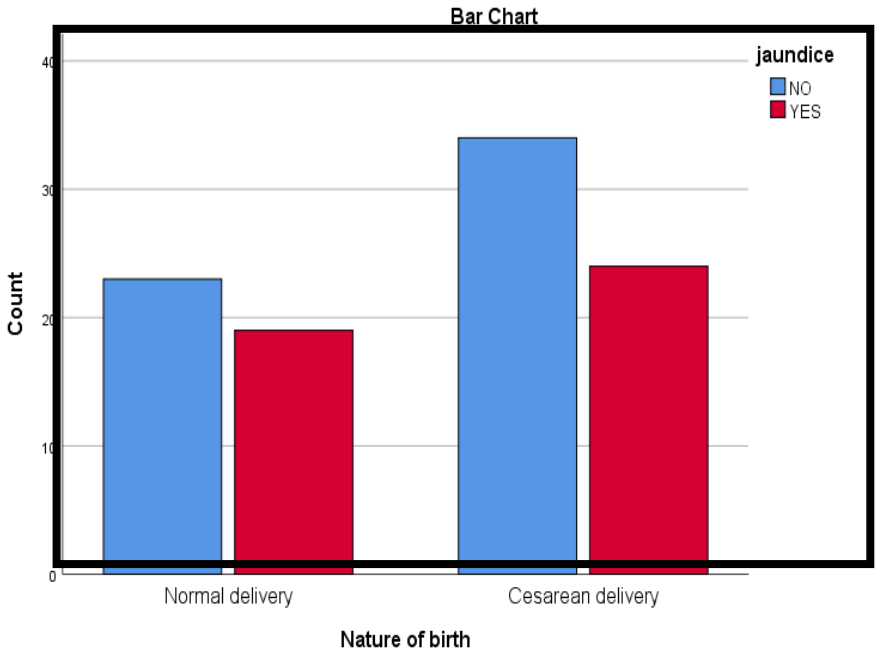


FIGURE 5.4: JAUNDICE AND NATURE OF BIRTH

Figure 5.4 represents the number of babies delivered by labor natural and cesarean section developing jaundice. There is no significant difference (p = 0.7) in the serum bilirubin level in both the modes of delivery. Hence the present study infers that the serum bilirubin level is independent of the mode of delivery.

5.6 ASSOCIATION BETWEEN PERINATAL DEPRESSION AND NEONATAL JAUNDICE

Perinatal depression is a clinical term refers to the condition of infant including depressed mental status, hypotonia and disturbance in spontaneous

respiration and circulation in first hour of life. In the present study, 6% (n=6) had perinatal depression and 2.3% (n=1) out of perinatally depressed babies developed jaundice.

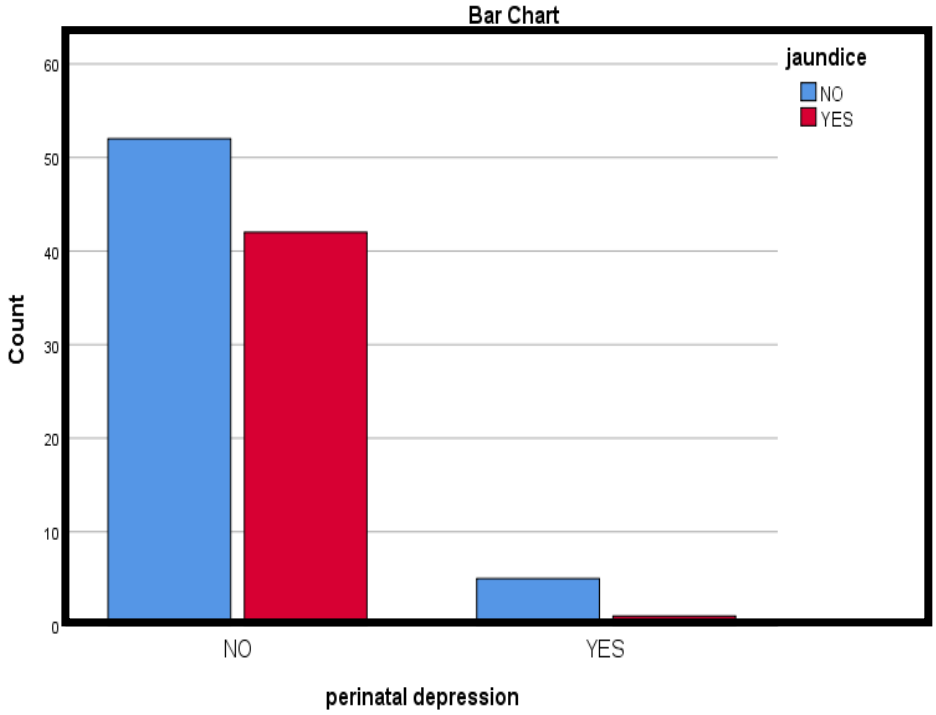


FIGURE 5.5: JAUNDICE AND PERINATAL DEPRESSION

Figure 5.5 represents number of perinatally depressed and non-depressed babies developing hyperbilirubinemia. There is no significant difference (p = 0.179) in the serum bilirubin level in depressed and non depressed babies. Hence the present study infers that the serum bilirubin level is independent of the perinatal depression.

5.7 ASSOCIATION BETWEEN NEONATAL JAUNDICE AND SGA BABIES

SGA refers to the neonate with birth weight with <10th percentile or <2SD below the mean for infants gestational age. Figure 5.6 presents the number of SGA and AGA babies developing jaundice. There is no significant difference (p

= 0.921) in the serum bilirubin level between SGA and AGA babies. Hence the present study infers that the serum bilirubin level is independent of the weight of the babies.

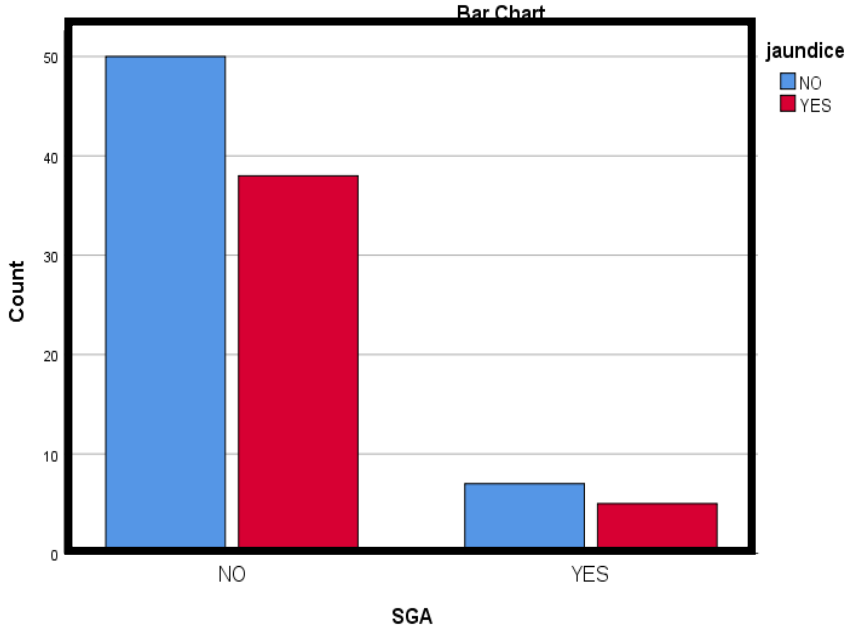


FIGURE 5.6: SGA AND JAUNDICE

5.8 ASSOCIATION BETWEEN DELAYED ADAPTATION AND JAUNDICE

In the present study, 13 %(n=13) had delayed adaptation out of which 16.3 %(n=7) developed hyperbilirubinemia requiring phototherapy as shown in Figure 5.7. There is insignificant association between serum bilirubin and delayed adaptation (p=0.416).

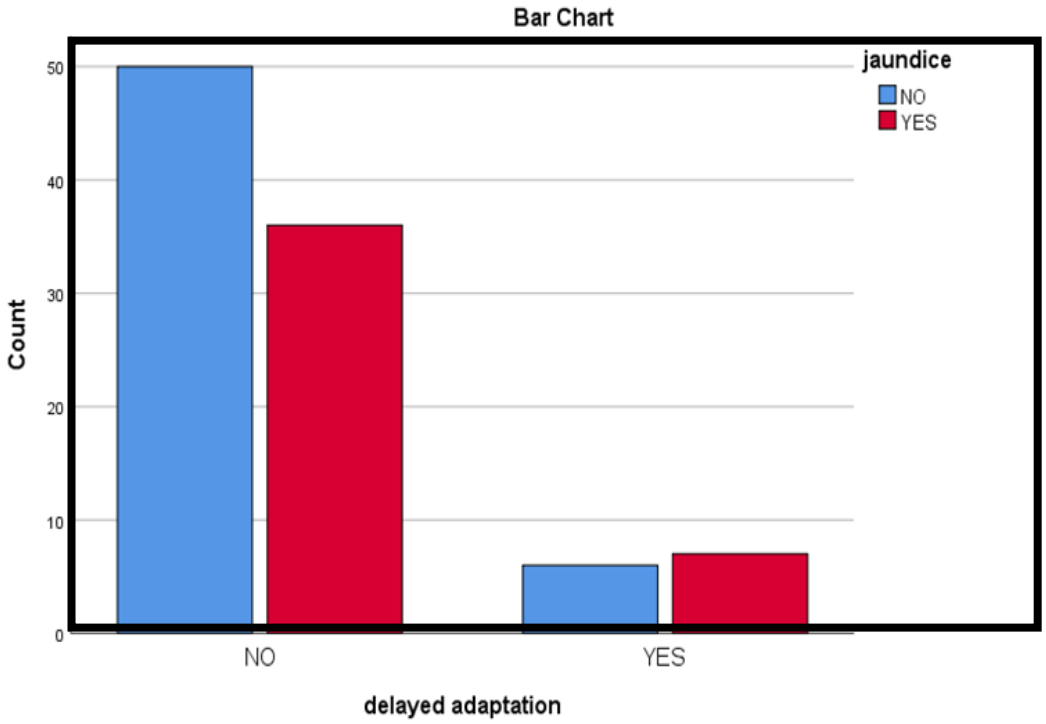


FIGURE 5.7: JAUNDICE AND DELAYED ADAPTATION

5.9 ASSOCIATION BETWEEN MATERNAL DM AND JAUNDICE

Out of total 14% (n=14) babies of diabetic mother, 11.6% (n=5) of babies developed significant hyperbilirubinemia with a p value of 0.553, which infers there is no significant association between babies born to diabetic and non-diabetic mother (Table 5.2).

Maternal DM	**Neonatal (%) (n) Hyperbilirubinemia**		**Total (n)**
	YES	**NO**	
Without DM	88.7(38)	84.2(48)	86
With DM	11.6(5)	15.8(9)	14
Total(n)	43	57	100

TABLE 5.2: MATERNAL DM AND JAUNDICE

5.10 ASSOCIATION BETWEEN MATERNAL HYPERTENSION AND JAUNDICE

Out of total 22% (n=22) hypertensive mother, 25.6% (n=11) of babies developed significant hyperbilirubinemia with a p value of 0.453, which infers there is no significant association between babies born to hypertensive and non-hypertensive mother (Table 5.3).

Maternal Hypertension	**Neonatal (%) Hyperbilirubinemia(n)**		**Total(n)**
	YES	**NO**	
Without Hypertension	74.4(32)	80.7(46)	78
With Hypertension	25.6(11)	19.3(11)	22
Total(n)	43	57	100

TABLE 5.3: MATERNAL HYPERTENSION AND JAUNDICE

5.11 ASSOCIATION BETWEEN MATERNAL ANAEMIA AND JAUNDICE

Out of 19 babies born to anemic mother, 11(21.1%) babies developed significant jaundice as shown in Figure 5.8. The association between maternal anemia and hyperbilirubinemia is insignificant (p=0.214).

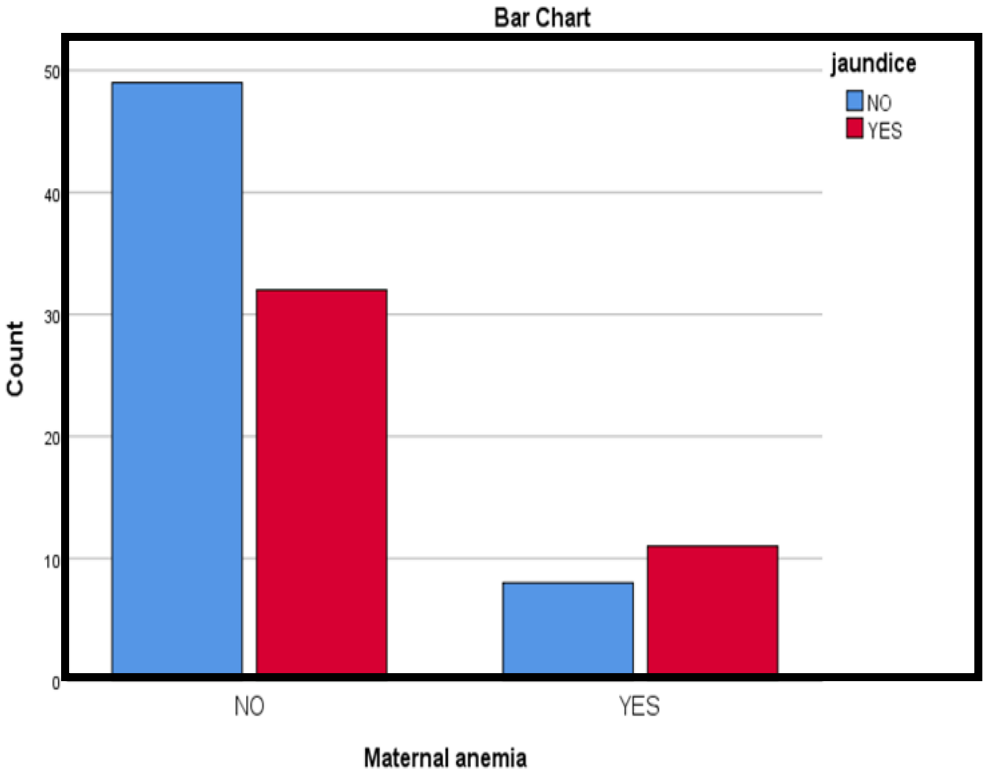


FIGURE 5.8: JAUNDICE AND MATERNAL ANAEMIA

5.12 ASSOCIATION BETWEEN Rh INCOMPATIBILITY AND JAUNDICE

Table 5.4 shows the relationship between Rh compatibility and jaundice. Out of 30 Rh -ve cases, 12 babies (27.9%) developed hyperbilirubinemia and out of 70 Rh +ve cases, 31 babies (72.1%) developed hyperbilirubinemia as shown in Table 5.4 and the same is represented in bar diagram (Figure 5.8).

Rh	Frequency (%)	Hyperbilirubinemia(%)(n)	
		Yes	No
Rh -ve	30	27.9(12)	31.6(18)
Rh +ve	70	72.1(31)	68.4(39)

TABLE 5.4: Rh INCOMPATIBILITY AND JAUNDICE

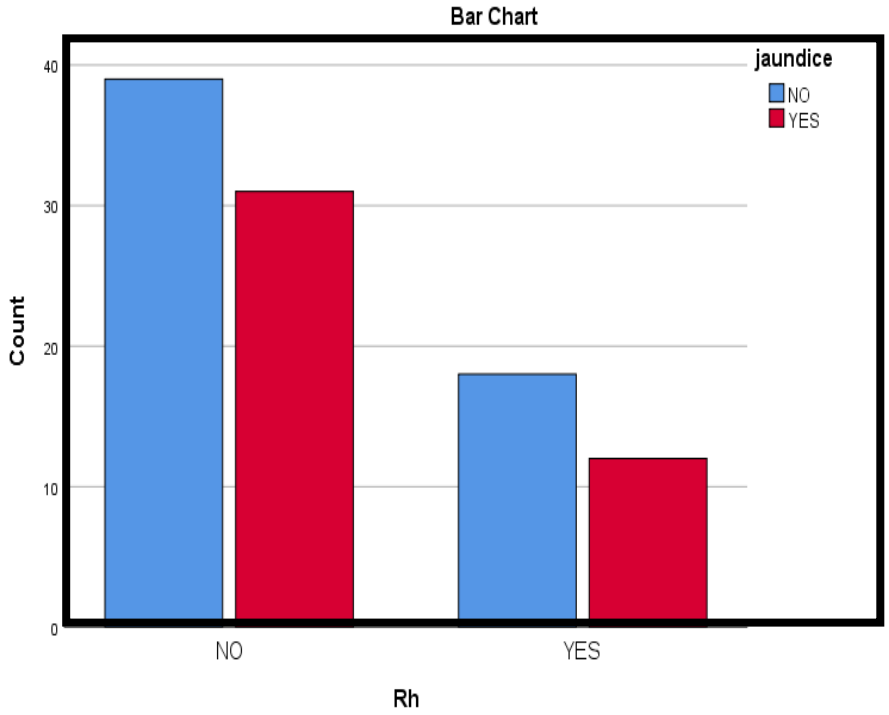


FIGURE 5.9: Rh AND JAUNDICE

Figure 5.9 presents the number of babies developed jaundice in Rh positive and negative groups. There is no significant difference (p = 0.692) in the serum bilirubin level of two groups.

5.13 ASSOCIATION BETWEEN ABO AND JAUNDICE

The relationship between ABO and jaundice is shown in Table 5.5. 33 babies (76.7%) out of 77 ABO set up babies developed significant hyperbilirubinemia with p value of 0.958.

ABO	Frequency (n)	Hyperbilirubinemia(%)(n)	
		Yes	No
YES	77	76.7(33)	77.2(44)
NO	23	23.3(10)	22.8(13)

TABLE 5.5: ABO AND JAUNDICE

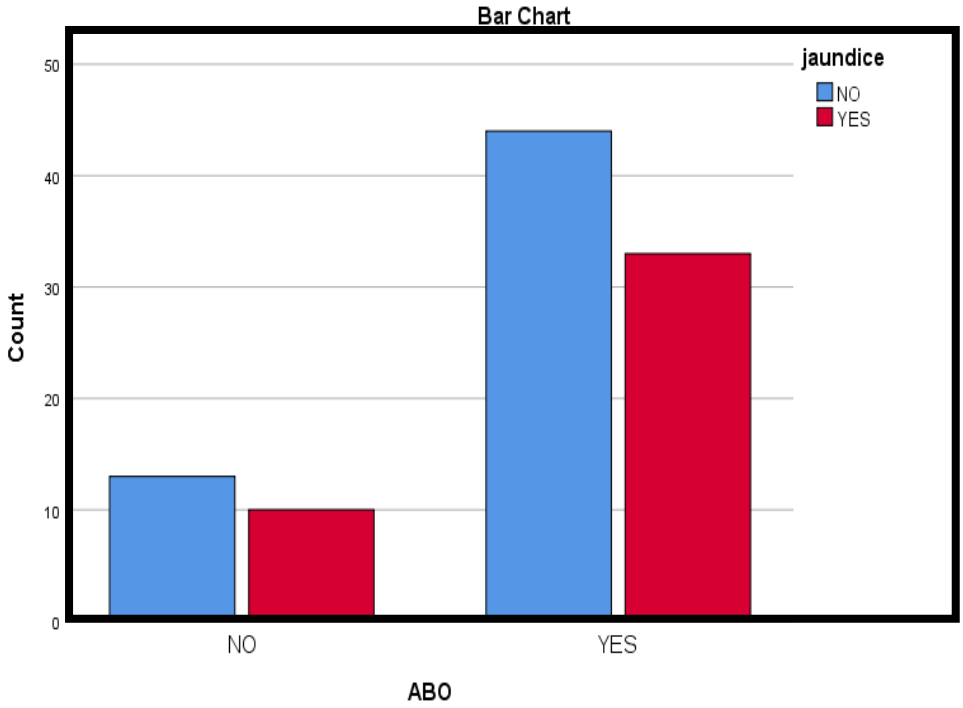


FIGURE 5.10: ABO AND JAUNDICE

Figure 5.10 presents the mean serum bilirubin level and ABO blood group. There is no significant difference (p = 0.958) in the serum bilirubin level of two groups. Hence the present study infers that the serum bilirubin level is independent of ABO blood group.

5.14 ONSET DAY OF JAUNDICE

In addition to all of the factors analyzed in the previous sections, the percentage of babies developing jaundice in first 5 days of life was recorded and the following is in Figure 5.11. Day 3 recorded the highest percentage (74%) of hyperbilirubinemia, while Day 4 had 9%.

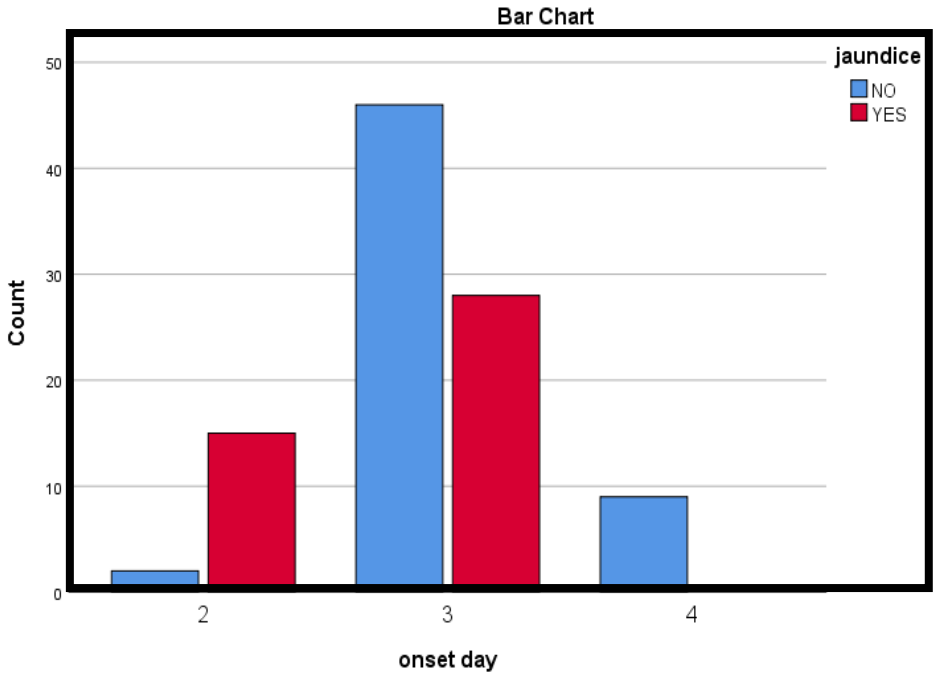


FIGURE 5.11: ONSET DAY

5.15 MEAN SERUM BILIRUBIN

Mean serum bilirubin on day 2 was 17.36 and day 3 was 14.34. The reduction of mean total serum bilirubin on day 3 may be attributed due to starting of therapy.

	onset day	N	Mean
serum bilirubin	2	17	17.364706
	3	74	14.343243

TABLE 5.6: MEAN SERUM BILIRUBIN

Apart from the comparative study, the baseline neonatal and maternal characteristics for all the factors in the study are shown in the Table 5.7 and 5.8 respectively.

Features	**Types**	**Hyperbilirubinemia group (N %)**	**Non-hyperbilirubinemia group (N%)**	**p**

Gender	Male Female	20(46.5) 23(53.5)	33(57.9) 24(42.1)	0.259
Delivery Mode	Labor Natural Cesarean Section	19(44.2) 24(55.8)	23(40.4%) 34(59.6%)	0.7
Birth order	1 2 3 4	26(60.5) 12(27.9) 4(9.3) 1(2.3)	35(61.4) 19(33.3) 2(3.5) 1(1.8)	0.649
Delayed Adaptation	Present Absent	7(16.3) 36(83.7)	6(10.7) 50(89.3)	0.416
Perinatal Depression	Present Absent	1(2.3) 42(97.7)	5(8.8) 52(91.2)	0.179
SGA	Present Absent	5(11.6) 38(88.4)	7(12.3) 50(87.7)	0.921
Blood Group	ABO Rh	33(76.7) 12(27.9)	44(77.2) 31(72.1)	0.958 0.692

TABLE 5.7: NEONATAL CHARACTERISTICS

Features	**Types**	**Hyperbilirubinemia group (N%)**	**Non-hyperbilirubinemia group(N%)**	**p**
Maternal Age	18-24 25-30 >30	26(60.5) 16(37.3) 1(2.3)	29(50.8) 24(42.2) 4(7.1)	0.017
Maternal	Present	5(11.6)	9(15.8)	0.553

DM	Absent	38(88.4)	48(84.2)	
Maternal SHT	Present Absent	11(25.6) 32(74.4)	11(19.3) 44(80.7)	0.453
Maternal hypothyroid	Present Absent	5(11.6) 38(88.4)	12(21.1) 45(78.9)	0.214
Maternal anemia	Present Absent	11(25.6) 32(74.4)	8(14) 49(86)	0.145
previous sibling jaundice	Present Absent	8(18.6) 35(81.4)	5(8.8) 52(91.2)	0.148

TABLE 5.8: MATERNAL CHARACTERISTICS

5.16 RECEIVER OPERATING CHARACTERISTICS (ROC) ANALYSIS

The ROC curve was first developed by engineers during World War II for detecting enemy objects in battlefields and was then introduced to psychology for perceptual detection of stimuli. It is a plot of the true positive rate against the false positive rate for the different possible cut points of a diagnostic test.

An ROC curve demonstrates several things:

1. It shows the tradeoff between sensitivity and specificity (any increase in sensitivity will be accompanied by a decrease in specificity).
2. The closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate the test.
3. The closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test.
4. The slope of the tangent line at a cut point gives the likelihood ratio (LR) for that value of the test..
5. The area under the curve is a measure of text accuracy.

ROC analysis was done using the SPSS software and the curve is shown in Figure 5.12. Based on the receiver operating characteristic (ROC), cut off value for cord bilirubin was chosen as >2.3 with the larger area under the curve (0.972).

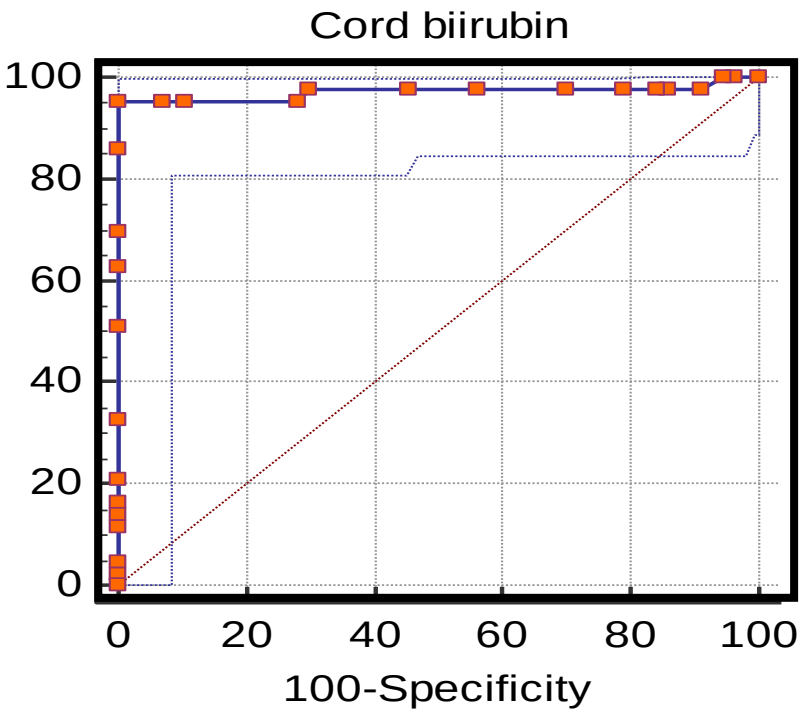


FIGURE 5.12: ROC CURVE

5.16.1 Area under the roc curve (AUC)

Area under the curve for umbilical cord serum total bilirubin in the study population is shown in Table 5.9.

Area under the ROC curve (AUC)	0.972
Standard Error	0.0226
95% Confidence interval	0.927 to 1.000
z statistic	20.908
Significance level P (Area=0.5)	<0.0001

TABLE 5.9: AREA UNDER THE CURVE

It clearly signifies that umbilical cord serum total bilirubin has a large area under the curve of 0.972 for prediction of significant hyperbilirubinemia at birth.

5.17 SENSITIVITY SPECIFICITY TABLE

Below Tables shows that this study revealed cord blood bilirubin of >2.3 has sensitivity and specificity of 95.35% and 100%.

Criterion	Sensitivity	95% CI	Specificity	95% CI	+LR	-LR
>1.3	97.67	87.7 - 99.9	14.04	6.3 - 25.8	1.14	0.17
>1.4	97.67	87.7 - 99.9	15.79	7.5 - 27.9	1.16	0.15
>1.5	97.67	87.7 - 99.9	21.05	11.4 - 33.9	1.24	0.11
>1.6	97.67	87.7 - 99.9	29.82	18.4 - 43.4	1.39	0.078
>1.7	97.67	87.7 - 99.9	43.86	30.7 - 57.6	1.74	0.053
>1.8	97.67	87.7 - 99.9	54.39	40.7 - 67.6	2.14	0.043
>1.9	97.67	87.7 - 99.9	70.18	56.6 - 81.6	3.27	0.033
>2	95.35	84.2 - 99.4	71.93	58.5 - 83.0	3.40	0.065
>2.1	95.35	84.2 - 99.4	89.47	78.5 - 96.0	9.06	0.052
>2.2	95.35	84.2 - 99.4	92.98	83.0 - 98.1	13.59	0.050
>2.3	95.35	84.2 - 99.4	100.00	93.7 - 100.0		0.047
>2.5	86.05	72.1 - 94.7	100.00	93.7 - 100.0		0.14
>2.6	69.77	53.9 - 82.8	100.00	93.7 - 100.0		0.30
>2.7	62.79	46.7 - 77.0	100.00	93.7 - 100.0		0.37
>2.8	51.16	35.5 - 66.7	100.00	93.7 - 100.0		0.49

Youden index J	0.9535
Associated criterion	>2.3
Sensitivity	95.35
Specificity	100.00

TABLE 5.10: SENSITIVITY SPECIFICITY TABLE

5.18 SCATTERED DIAGRAM

The correlation between cord bilirubin and serum bilirubin is shown in Figure 5.13. Figure shows that there was a significant association between the total bilirubin in cord blood and newborn serum bilirubin

level.

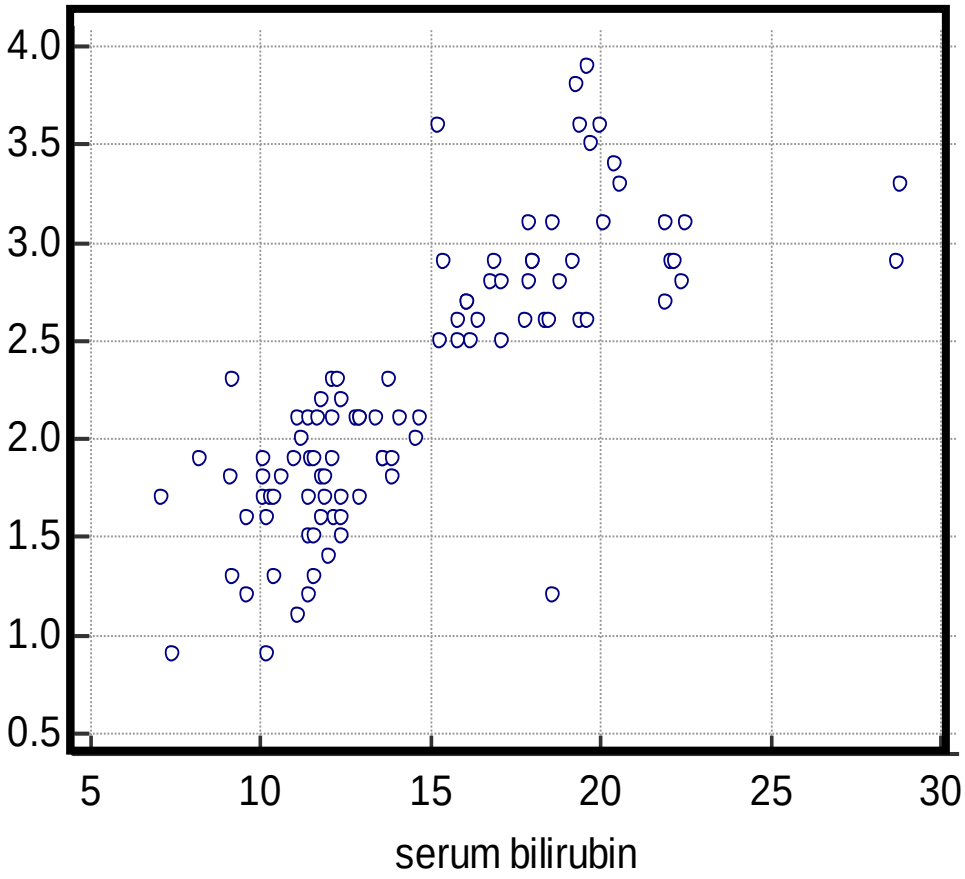


FIGURE 5.13: SCATTERED DIAGRAM

5.19 POSITIVE AND NEGATIVE PREDICTIVE VALUE

Table 5.11 shows high negative predictive value of 96.61% with diagnostic accuracy of 98% and cohen's kappa 0.959.

Parameter	Estimate	Lower - Upper 95% CIs	Method
Sensitivity	95.35%	(84.54, 98.72)	Wilson Score
Specificity	100%	(93.69, 100)	Wilson Score

Positive Predictive Value	100%	(91.43, 100)	Wilson Score
Negative Predictive Value	96.61%	(88.46, 99.07)	Wilson Score
Diagnostic Accuracy	98%	(93, 99.45)	Wilson Score
Cohen's kappa (Un weighted)	0.959	(0.7631 - 1.155)	

TABLE 5.11: POSITIVE AND NEGATIVE PREDICTIVE VALUE

5.20 REGRESSION

Logistic regression predicting the likelihood of developing hyperbilirubinemia requiring phototherapy is shown in Table 5.13.

Sample size	100
Coefficient of determination R^2	0.6242
Residual standard deviation	0.4223

TABLE 5.12: LEAST SQUARES REGRESSION

y = 0.4443 + 0.1232 x (y is the Cord_bilirubin and x is the serum_bilirubin)					
Parameter	Coefficient	Std. Error	95% CI	t	P
Intercept	0.4443	0.1480	0.1506 to 0.7380	3.0017	0.0034
Slope	0.1232	0.009657	0.1040 to 0.1424	12.7573	<0.0001

TABLE 5.13: REGRESSION EQUATION

Source	DF	Sum of Squares	Mean Square
Regression	1	29.0286	29.0286
Residual	98	17.4798	0.1784

F-ratio	162.7487
Significance level	P<0.0001

TABLE 5.14: REGRESSION TABLES

CHAPTER 6

DISCUSSION

Hyperbilirubinemia is one the most common problems in neonates. Almost 60% of term babies and 80% of preterm babies develop significant hyperbilirubinemia in first week of life (1). ABO and Rh incompatibility is one among the important cause of neonatal hyperbilirubinemia. Normally umbilical cord serum bilirubin is 1-3 mg/dl and rises at a rate of 5mg/dl/day making jaundice visible on 2^{nd} or 3^{rd} day.

The presumption of our study is that high serum bilirubin at birth would predict high peak in later part of life. Our aim was to quantify the relationship between cord blood bilirubin and peak serum bilirubin levels in first 5 days of life.

There are cases of bilirubin related encephalopathy and neurological damage occurring as a result of missing of cases due to early discharge from hospital (59). So it is very essential to have an easy and more reliable test to identify newborns at risk of developing significant hyperbilirubinemia so that these neonates can be monitored closely and frequently. It also facilitates the early discharge of other low risk babies.

There is also no major statistically significant difference in the serum bilirubin of babies with perinatal depression, delayed adaptation, small for gestational age babies and also with maternal factors like gestational diabetes, hypertension, anemia, hypothyroidism, previous sibling jaundice. There is no

statistically significant difference in the development of hyperbilirubinemia between the ABO and Rh incompatible babies.

Out of 53% male babies, 46.5% developed jaundice and out of 47% female babies, 53.5% developed significant hyperbilirubinemia. There is no statistically significant difference in the serum bilirubin level between the sexes of babies. This was similar to the findings noted by Awasthi and Rehman in 1998 and Alpay et al in 2000, a prospective cohort study conducted in Kings George medical college, Lucknow (73).

Umbilical cord blood has been chosen to estimate the initial serum bilirubin as it is non invasive, easy and the results can be obtained within few hours. Thus the well appearing ABO and Rh incompatible newborns that are discharged within few days postnatally can be predicated for the development of significant hyperbilirubinemia (74). If they are at low risk, then they can be discharged early without unnecessary prolongation of hospital stay.

Total of 100 newborns were followed up for 1st five days for the development of significant hyperbilirubinemia with clinical assessment and lab investigations. 42% (n=42) delivered by labor natural, Out of which 44.2 % (n=19) developed significant hyperbilirubinemia 58% (n=58) delivered by cesarean section. Out of which 55.8% (n=24) developed significant hyperbilirubinemia. There is no statistically significant difference in the serum bilirubin between two modes of delivery.

Umbilical cord bilirubin has been the area of interest since 1950 and has

been considered as an index of neonatal jaundice (77). Comparison of cut-off values, sensitivity, and specificity of various studies with the present study are shown in Table 6.1 and 6.2 respectively.

Studies	Year	Cut off value
Robinson et al	1960	>3
Risenberg et al	1977	>4
Chen JY et al	1994	>4
Rosenfeldj et al (75)	1986	>2
Knudsen et al	1989	>2.34
Rataj et al (76)	1994	>2.5
Knufffer et al	2005	>2.34
Sun et al	2007	>2
Satrya et al	2007	>2.54
D Bhat et al	2012	>2.5
Our study	2019	>2.3

TABLE 6.1: COMPARISON OF VARIOUS STUDIES SHOWING THE CUT OFF VALUE OF CORD BLOOD BILIRUBIN IN PREDICTING SIGNIFICANT HYPERBILIRUBINEMIA

Robinson et al in 1960 showed that the cut off value of umbilical cord blood bilirubin of >3 developed significant hyperbilirubinemia (15). Risenberg et al in 1977, Chen JY et al in 1994, in their study of predicting the cord blood

bilirubin in development of jaundice in ABO incompatible babies had cut off value of >4mg/dl, 4mg/dl respectively.(16,17) D Bhat et al in his study in 2012 found that the UCSB of ABO set up babies with cut off value >2.5mg/dl developed jaundice (74).

Studies	Cut off cord	Sensitivity (%)	Specificity (%)
Knudsen et al	>2.35	13	99
AmarTaksande et al	>2	89.5	85
Sun et al	>2	77	98.6
RudySatryaet al	>2.54	90.5	85
Nahar et al	2.5	77	98.6
Allam bhat et al	>3	97.06	99.22
present study	<1.5	97.67	21
	1.5-2.3	95.35	100
	2.3-2.9	32.56	100
	>3	11.63	100

TABLE 6.2: COMPARISON OF SENSITIVITY AND SPECIFICITY OF VARIOUS STUDIES WITH OUR STUDY

In the present study, the cut off value of > 2.3 has been considered predicting the development of significant hyperbilirubinemia in ABO and Rh incompatible babies.

According to Knudsen et al study, at cut off value of 2.35 the sensitivity

and specificity was 13% and 99% (69). In Nahar et al study, the sensitivity and specificity was 77% and 98.6% at cut off of 2.5 respectively (64). In Allam bhat et al study, it was 97.06 and 99.22 at cut off of >3 mg/dl (67). In our study the sensitivity and specificity at UCB of 2.3 was 95.35 and 100% with area under the curve of 0.972 which has a high predictive value. This wide variation in sensitivity and specificity may be due to different arbitrarily used cut off value of cord blood bilirubin.

CHAPTER 7

SUMMARY & CONCLUSIONS

7.1 SUMMARY

- 100 healthy term newborns belonging to ABO and or Rh set up delivered by both labor natural and cesarean section in Chengalpattu medical college and hospital during the period of one year from September 2018 to May 2019 were included in the study.

- Relevant history was obtained from the mother and maternal hospital records. A complete clinical examination of the neonate was done at birth.

- Blood grouping, Rh typing, serum total bilirubin was estimated from the umblical cord bilirubin at birth.

- Neonates were followed up daily for the 1st five days of life for the development of jaundice. Serum total bilirubin was done in all neonates at 72 hours of life. Blood for STB estimation was also collected from babies less than 72 hours of life if showed clinical evidence of jaundice.

- Development of significant hyperbilirubinemia requiring treatment in the form of either phototherapy or exchange transfusion is the main outcome of the study.

- The mean serum bilirubin on day 2 of life was found to be 17.36.

- Receiver operating characteristics was used to find the cut off values for umbilical cord bilirubin in predicting significant hyperbilirubinemia.

- In babies with ABO and Rh incompatibility, the UCSB level >2.3mg/dl was considered to predict the postnatal significant hyperbilirubinemia with sensitivity and specificity of 95.35% and 100% respectively suggesting that these high risk babies should be monitored very closely and frequently and warrants early discharge.

- High negative predictive value of 96.61% suggests that babies with UCSB of <2.3mg/dl are less likely to develop significant jaundice and these low risk babies can be discharged earlier without fear.

7.2 CONCLUSIONS

- One of the common problem in postnatal ward is hyperbilirubinemia

- Simple predictors can be used in early identification of at risk newborns for development of significant hyperbilirubinemia and to prevent bilirubin induced neurological damage

- In the present study , cut off value for cord blood bilirubin of >2.3 has sensitivity of 95.35 and specificity of 100% to predict hyperbilirubinemia.

- High negative predictive value of 96.61% in our study shows that in ABO and Rh incompatible babies with cord blood bilirubin of <2.3 are unlikely to develop significant hyperbilirubinemia and hence these babies can be discharged without fear

- We recommend that babies of ABO and Rh set up with cord blood bilirubin of > 2.3 should be monitored very frequently and closely to reduce the mortality and morbidity due to neonatal hyperbilirubinemia.

7.3 LIMITATIONS

- Only healthy term neonates were included in the study

- Evidence of hemolysis was not taken into consideration

ANNEXURE-1

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ANNEXURE-2

Name:

Date & Time of Birth:

Gender:

Gestational Age:

(As per USG/LMP)

Weight of baby:

APGAR 1MIN 5 MIN

HISTORY

Mothers Information

1. Maternal Blood group
2. Drug intake
3. Thyroid disorder
4. Maternal Illness
5. GHT,GDM
6. Previous sibling with jaundice or anemia
7. Family history
    a. Jaundice
    b. Anemia
    c. Splenectomy
    d. Early gall bladder disease
    e. Liver disease

BABY INFORMATION

1. Cephalhaematoma
2. Significant bruising
3. Congenital Malformation

4. Sepsis
5. Hypothyroidisim
6. Asphyxia
7. Respiratory distress syndrome

SIGNS OF ILLNESS (YES/N0)

1. Vomiting
2. Lethargy
3. Poor feeding
4. Excessive Weight Loss
5. Apnea
6. Tachypnea
7. Temperature instability

LAB VALUES

1. Cord Blood
    a. Total bilirubin
    b. Indirect
    c. Direct
2. Baby Blood Group
3. Serum Bilirubin
    a. Total Bilirubin
    b. Indirect
    c. Direct

FINAL DIAGNOSIS

OUTCOME

ANNEXURE-3

PATIENT CONSENT FORM

STUDY DETAIL:

"**CORD BLOOD BILIRUBIN AS A PREDICTIVE MARKER OF NEONATAL HYPERBILIRUBINEMIA IN ABO AND Rh INCOMPATIBLE BABIES-A PROSPECTIVE STUDY"**

STUDY CENTRE:

CHENGALPATTU MEDICAL COLLEGE & HOSPITAL, CHENGALPATTU

PATIENT NAME: PATIENT AGE:
IDENTIFICATION NUMBER:

- I confirm that I have understood the purpose of procedure for the above study.
- I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.
- I understand that my participation in the study is voluntary and that I am free to withdraw at anytime without giving any reasons, without my legal rights being affected.
- I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if withdraw from the study, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.
- I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperative with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms.
- I hereby give consent to participate in this study.
- I hereby give permission to undergo complete clinical examination and diagnostic test.

Signature/Thumb impression: Place:
Patient name and address: Date:

Signature of the investigator: Place:
Study Investigator's name: Date:

ANNEXURE-4

CHILD ASSENT FORM

STUDY DETAIL:

"**TOPS AND SNAP II-PE SCORES AS PREDICTORS OF NEONATAL MORTALITY AMONG TRANSPORTED NEONATES IN LEVEL 3 NICU ADMISSIONS"**

CHENGALPATTU MEDICALCOLLEGE& HOSPITAL,
CHENGALPATTU

PATIENT NAME: PATIENT AGE:
IDENTIFICATION NUMBER:

I confirm that I have understood the purpose of procedure for the above study. I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction. I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving any reasons, without my legal rights being affected.

I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if withdraw from the study, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperative with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms.

I hereby give consent to participate in this study. I hereby give permission to undergo complete clinical examination and diagnostic test.

Signature/Thumb impression: Place:
Patient name and address: Date:

Signature of the investigator: Place:
Study investigator's name: Date:

ANNEXURE-5

S.No	Name	GA	Nature of birth	5 min APGAR	Sex	Birth Weight	Birth Order	SGA	Perinatal depression	delayed adaptation	Mothers age	Maternal DM	Maternal SHT sibling jaundice	Maternal hypothyroid	Maternal anemia	previous sibling jaundice	Rh	ABO	Cord biirubin	serum bilirubin	onset day	jaundice	recovered day	Duration
2	B/o Sailaja	39	2	9	1	2.52	2	1	0	0	29	0	0	0	1	0	0	1	2.9	18	2	1	4	3
19	b/0 mahalakshmi	39	1	9	1	3.85	1	0	0	1	23	0	0	0	0	0	0	1	2.5	15.3	2	1	4	3
39	b/o Indhu	39	1	9	2	2.9	2	0	0	0	30	0	0	0	0	0	0	1	2.6	16.4	3	1	4	2
44	B/0 Rajeshwari	37	1	9	1	2.54	1	0	0	1	20	0	0	0	0	0	1	0	2.8	17.1	2	1	4	3
45	b/o nagalakshmi	37	1	9	1	2.58	1	0	0	0	25	0	0	0	0	0	0	1	2.5	15.8	3	1	4	2
63	b/o kavitha	40	2	9	1	2.75	4	0	0	0	29	0	0	0	0	0	0	1	2.7	16.1	2	1	4	3
76	B/O Kokila	38	2	9	1	2.6	1	0	0	0	18	0	1	0	1	0	0	1	2.7	16.1	3	1	4	2
1	B/o Devi	38	2	9	2	3.3	2	0	0	0	27	1	0	0	0	1	0	1	1.2	18.6	3	1	5	4
5	b/o parameswari	40	1	9	1	2.61	1	0	0	1	19	0	0	0	1	0	0	1	2.7	21.9	3	1	5	3
6	b/o Radhika	40	2	8	1	3.39	1	0	1	0	21	0	1	0	0	0	0	1	2.8	22.4	3	1	5	3
8	b/0 Malathi	39	2	9	2	2.58	1	1	0	0	20	0	0	0	0	0	0	1	3.6	20	2	1	5	4
9	b/0 kalaiselvi	40	1	9	2	2.5	1	0	0	0	23	0	0	0	1	0	1	0	2.6	18.5	3	1	5	3
12	b/o Nirmala	38	1	9	2	2.6	2	0	0	0	21	0	0	0	0	0	0	1	2.5	17.1	3	1	5	3
15	b/o Divya	39	2	9	2	3.25	2	0	0	1	29	0	0	0	0	0	0	1	2	14.6	3	1	5	3
17	b/o Lavanya	39	2	9	2	3.5	1	0	0	0	27	1	0	0	1	0	0	1	3.1	20.1	2	1	5	4
18	b/O Ellamal	39	2	9	2	3.58	3	0	0	0	31	0	0	0	0	1	0	1	2.6	19.4	3	1	5	3
21	B/O Rajeshwari	40	2	9	1	2.95	2	0	0	0	29	0	1	0	0	1	0	1	2.9	22.2	3	1	5	3
23	b/o dhanasundari	37	1	9	2	2.51	1	1	0	0	24	1	0	0	0	0	1	0	2.6	19.6	3	1	5	3
24	b/O pavithra	38	2	9	2	2.9	1	0	0	0	26	0	0	1	0	0	1	0	2.5	16.2	3	1	5	3
27	b/o Dhanalakshmi	39	2	9	1	3	2	0	0	0	24	0	1	0	1	0	0	1	3.6	19.4	2	1	5	4
30	b/0 kavitha	39	1	9	1	3.08	1	0	0	0	27	0	0	1	0	0	0	1	2.9	15.4	2	1	5	4
34	B/O Oviya	37	2	9	1	2.8	1	0	0	0	23	0	1	0	0	0	0	1	2.8	17.9	3	1	5	3

38	b/o sathya	37	2	9	2	2.7	2	0	0	0	24	1	1	1	0	1	0	1	2.9	19.2	3	1	5	3
41	B/o Nandhini	38	1	9	2	3.1	1	0	0	0	19	0	1	0	0	0	1	0	3.1	18.6	3	1	6	4
42	B/O Nageshwari	38	2	9	1	2.9	3	0	0	0	29	1	1	0	1	1	0	1	2.6	18.4	3	1	4	2
89	B/O Dilsatha begum	40	1	9	2	2.89	1	0	0	0	21	0	1	0	0	0	1	0	2.8	16.8	3	1	5	3
90	b/o bhuvaneshwari	37	2	9	2	2.75	1	0	0	1	23	0	0	0	1	0	0	1	3.1	17.9	2	1	5	4
91	b/o kavitha	40	1	9	1	2.75	1	0	0	0	23	0	0	0	1	0	0	1	2.9	16.9	3	1	5	3
92	b/o priya	39	2	9	1	3.5	1	0	0	0	20	0	1	0	0	0	0	1	2.6	17.8	3	1	5	3
98	b/0 revathi	37	2	9	2	2.7	1	0	0	1	25	0	0	0	0	0	1	1	2.9	18	3	1	5	3
99	B/O Girija	40	1	9	1	2.85	2	0	0	0	23	0	0	0	0	1	0	1	2.6	15.8	3	1	5	3
7	b/o Uma	39	1	9	2	2.65	1	0	0	0	22	0	0	0	0	0	1	0	2.9	28.7	3	1	6	4
10	b/o Sarawathi	40	1	9	2	2.59	2	0	0	0	25	0	0	0	0	1	0	1	3.1	21.9	3	1	6	4
20	b/o logu	37	1	9	2	2.5	1	1	0	0	24	0	0	0	0	0	0	1	3.3	20.6	2	1	6	5
25	b/o Sangeetha	38	2	9	2	2.8	1	0	0	0	20	0	0	1	0	0	0	1	3.8	19.3	2	1	6	5
32	B/O Seeth	38	1	9	2	2.75	1	0	0	0	18	0	0	0	1	0	1	1	3.5	19.7	2	1	6	5
33	B/O Megala	38	2	9	1	3	3	0	0	0	30	0	0	0	1	0	1	0	3.3	28.8	3	1	6	4
51	B/O Rupa	38	1	9	2	2.65	1	0	0	0	23	0	0	0	0	0	0	1	2.8	18.8	3	1	6	4
56	B/O Sivagami	39	2	9	2	2.51	2	1	0	0	30	0	0	0	0	0	0	1	3.4	20.4	3	1	6	4
64	b/o rashitha	37	2	9	1	3	1	0	0	0	20	0	0	1	0	0	0	1	2.9	22.1	3	1	6	4
78	b/o manimegalai	37	2	9	1	2.93	3	0	0	0	21	0	0	0	0	1	1	0	3.1	22.5	2	1	6	5
3	b/o Jayalakshmi	38	1	9	1	2.75	1	0	0	0	23	0	1	0	0	0	0	1	3.6	15.2	2	1	7	6
37	b/o premA	38	2	9	2	3.5	2	0	0	1	29	0	0	0	0	0	1	0	3.9	19.6	2	1	7	6
50	B/0 Mythili	39	2	9	2	2.9	1	0	0	0	19	0	0	0	0	0	0	1	1.9	11.6	3	0	0	0
52	b/o anupriya	39	2	9	2	2.6	1	1	0	0	27	0	0	0	0	0	1	0	2.1	13.4	3	0	0	0
54	B/O Thanitha	37	1	8	2	2.6	1	0	1	0	19	0	0	0	1	0	0	1	2.1	12.8	3	0	0	0
69	b/o shobana	38	2	8	2	2.7	1	0	1	0	23	0	0	0	0	0	0	1	1.9	13.6	3	0	0	0
71	b/o ammu	38	1	9	1	3.3	2	0	0	0	28	1	1	1	1	1	1	0	1.7	12.9	3	0	0	0
82	b/o geethA	40	1	9	1	3.7	1	0	0	0	20	0	0	0	0	0	0	1	1.7	11.9	3	0	0	0

83	b/O pavithra	39	2	9	1	2.9	1	0	0	0	21	0	0	0	0	0	0	1	2.3	13.8	3	0	0	0
87	b/o prabhavathi	40	2	9	1	3.9	1	0	0	0	24	0	1	0	1	0	0	1	2.1	12.9	3	0	0	0
88	b/O JAYA	37	2	8	2	3.03	2	0	1	0	25	0	0	1	0	0	0	1	1.9	13.9	3	0	0	0
4	b/o Radha	39	2	9	1	2.55	2	0	0	0	26	0	0	1	0	0	0	1	0.9	10.2	4	0		
11	b/o kalaivani	37	1	9	1	2.6	1	0	0	0	19	0	0	0	0	0	0	1	1.5	11.6	2	0		
13	b/O Ranjani	38	2	7	1	3.1	2	0	1	0	28	0	0	0	0	0	0	1	2.3	12.3	4	0		
14	b/o kalaivani	38	2	9	2	2.7	1	0	0	0	23	0	0	1	0	0	0	1	1.5	12.4	3	0		
16	b/o Jayalakshmi	37	2	9	2	3	3	0	0	0	26	0	0	0	0	0	1	0	1.7	12.4	3	0		
22	b/o krishnaveni	37	1	9	2	2.4	1	1	0	0	20	1	0	0	0	0	0	1	2.3	12.1	3	0		
26	b/o Devi	37	2	9	1	2.6	1	0	0	1	28	1	0	0	0	0	0	1	2.2	12.4	4	0		
28	b/o Rooba	39	1	9	1	3	2	0	0	0	32	0	0	0	0	0	0	1	2.1	11.7	3	0		
29	b/o Malathi	39	1	9	2	2.5	1	0	0	0	28	0	0	0	0	0	1	1	0.9	7.4	2	0		
31	B/O sarasu	38	1	9	2	2.8	3	0	0	0	33	0	0	0	0	1	1	0	2.2	11.8	3	0		
35	b/o bharathi	38	2	9	1	2.5	2	0	0	0	26	0	0	0	1	1	0	1	1.3	10.4	3	0		
36	b/o parameswari	38	2	9	2	3.32	1	0	0	0	20	1	0	0	0	0	0	1	2.1	11.4	3	0		
40	b/o Devi	38	1	9	1	2.98	4	0	0	0	32	0	0	0	0	0	0	1	1.3	9.2	3	0		
43	b/O Gnasundari	40	2	9	2	3.04	1	0	0	0	27	0	0	0	0	0	1	0	1.8	9.1	3	0		
46	b/o anujam	38	2	9	2	2.9	2	0	0	0	26	0	1	1	0	0	0	1	2.1	14.7	4	0		
47	b/0 Shenbagam	37	1	9	1	3.4	1	0	0	0	24	0	0	1	0	0	0	1	1.6	10.2	3	0		
48	b/o mariyammal	39	2	9	1	2.5	2	0	0	0	21	0	0	1	0	0	0	1	1.2	9.6	3	0		
49	b/O Rajeshwari	37	2	9	1	2.5	2	0	0	0	26	1	1	0	0	0	0	1	1.7	10.1	3	0		
53	b/o Sangeetha	38	2	9	1	3.36	2	0	0	0	27	0	0	0	0	0	0	1	1.1	11.1	3	0		
55	b/o banupriyA	37	1	9	1	2.64	1	0	0	0	25	1	0	0	0	0	1	0	1.2	11.4	3	0		
57	b/0 gowri	38	1	9	1	2.9	1	0	0	1	21	0	0	1	0	0	0	1	1.6	12.4	3	0		
58	b/o kalaivani	39	2	9	1	3.2	1	0	0	1	24	0	0	0	0	0	0	1	1.8	11.9	3	0		
59	b/o sheela	38	2	9	1	2.7	1	0	0	0	32	0	1	0	0	0	1	0	1.6	12.2	3	0		
60	b/o padmavathi	37	2	9	1	2.7	2	0	0	0	23	0	0	0	0	0	0	1	1.7	11.4	3	0		

61	b/o kamatchi	37	1	9	2	2.5	1	0	0	0	27	0	0	0	0	0	0	1	1.8	11.8	3	0		
62	b/o Nirmala	37	1	9	2	2.9	2	0	0	0	24	0	0	0	0	1	1	0	1.9	11.5	3	0		
65	b/o gomathi	38	1	9	2	2.8	2	0	0	0	24	1	0	0	0	0	0	1	1.8	10.6	3	0		
66	b/o rekha	40	2	8	2	3	1	0	1	1	23	0	0	0	0	0	1	0	2.1	12.9	3	0		
67	b/o sharmila	39	2	9	1	2.7	2	0	0	0	25	0	0	0	0	1	0	1	1.8	10.1	3	0		
68	b/o papitha	37	2	9	1	2.5	2	1	0	0	23	0	0	0	0	0	1	0	1.6	11.8	3	0		
70	b/o kavitha	39	1	9	1	3.35	1	0	0	0	27	0	0	1	0	0	0	1	1.5	11.4	3	0		
72	b/o manjula	39	2	9	1	2.7	2	0	0	0	24	1	1	0	0	0	0	1	1.4	12	3	0		
73	b/o arpudha	38	2	9	2	2.6	1	0	0	0	26	0	0	0	0	0	0	1	1.7	7.1	3	0		
74	b/o priya	38	2	9	1	2.8	2	0	0	1	30	0	0	0	1	0	0	1	1.9	10.1	3	0		
75	B/O Komala	39	1	9	1	3.2	1	0	0	0	28	0	1	0	0	0	0	1	1.3	11.6	4	0		
77	B/O Nagavalli	39	2	9	2	2.52	1	1	0	0	19	0	0	1	1	0	1	1	1.9	12.1	3	0		
79	b/o manjupriya	39	1	9	1	2.9	1	0	0	1	23	0	1	0	0	0	0	1	1.7	10.3	3	0		
80	b/o sunitha	39	2	9	1	3.32	1	0	0	0	21	0	0	0	0	0	1	0	1.9	13.6	4	0		
81	B/o Nandhini	39	1	9	1	3.2	1	0	0	0	27	0	1	1	0	0	1	0	1.6	9.6	4	0		
84	b/o poornima	40	2	9	2	3.2	2	0	0	0	27	0	1	0	1	0	1	1	1.9	8.2	3	0		
85	B/O venilla	38	1	9	2	2.8	1	0	0	0	21	0	1	1	1	0	0	1	1.9	11	3	0		
86	b/o renuga	38	2	9	2	2.5	1	1	0	0	26	0	0	0	0	0	1	1	1.8	13.9	4	0		
93	b/o kamatchi	37	2	9	1	2.6	2	0	0	0	21	1	0	0	0	0	0	1	2	11.2	3	0		
94	b/o sandhya	39	1	9	2	3.1	1	0	0	0	26	0	0	0	0	0	1	1	1.7	10.4	3	0		
95	b/o kowsalya	38	2	9	2	2.56	1	1	0	0	21	0	0	0	0	0	0	1	2.1	14.1	4	0		
96	b/o kavitha	39	2	9	1	3.65	1	0	0	0	24	0	0	0	0	0	0	1	2.3	9.2	3	0		
97	b/o sarasu	38	1	9	1	2.5	1	1	0	0	20	0	0	0	0	0	1	0	2.1	11.1	3	0		
100	b/o radha	33	1	9	1	2.8	1	0	0		21	0	0	0	0	0	0	1	2.1	12.1	3	0		