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RESEARCH ARTICLE

# A STUDY ON CORD BLOOD BILIRUBIN LEVELS IN A TERTIARY CARE CENTRE OF HARYANA IN INDIA

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**ABSTRACT**

To determine the level of cord blood bilirubin in all healthy term newborns and asses its usefulness in predicting neonatal jaundice. Neonatal jaundice (NNJ) is an interesting, complicated and controversial clinical problem. It is a cause of concern for the parents as well as pediatricians. The concept of prediction of jaundice offers an attractive option to pick up babies at risk of neonatal hyperbilirubinemia. Physical examination is not a reliable measure of serum bilirubin. Under these circumstances it would be desirable to be able to predict the risk of jaundice, in order to implement early treatment and thereby minimize the risk of bilirubin dependent brain damage.Neonatal Hyperbilirubinemia has been defined as the bilirubin levels > 12.9 mg/dl in term babies and 15 mg/dl in preterm babies.Neonatal jaundice is visible manifestation in skin and sclera of elevated serum concentrations of bilirubin and this usually occurs in neonates if serum bilirubin level is >5 mg/dl. Most adults are jaundiced when total serum bilirubin (TSB) levels exceed 2.0 mg/dL. Kernicterus and near miss kernicterus are neonatal conditions that are associated with irreversible or reversible brain injury respectively.[5] Concerns regarding jaundice have increased after reports of bilirubin encephalopathy occurring in healthy term infants without hemolysis.The study was conducted in 100 term newborns in neonatal unit, Department of Pediatrics, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, from September 2012- July 2013. Serum bilirubin estimation was done in the biochemistry department of MMIMSR , Mullana. Serum bilirubin estimation was done by Diazo method in which a detergent is used to accelerate the reaction is used. Incidence of NNJ in our study is 14 %. Mean total bilirubin on third post natal day was 9.14 mg/dl. Using CBB level of ≥ 1.9 mg/dl as a cut-off, NNJ can be predicted with sensitivity of 92.8%, specificity of 83.7 %, positive predictive value of 48.1 % and negative predictive value of 98.6 %. The Negative Predictive Value (98.6%) in the present study suggests that in healthy term babies (without RH and ABO incompatibility with Cord Blood Bilirubin ≤ 1.9 mg/dl ) cord serum bilirubin can help to identify those newborns who are unlikely to require further evaluation and intervention. These newborns can be discharged with assurance to parents. Babies with CBB level ≥ 1.9 mg/dl should be followed more frequently.

**Keywords:** Newborn, Neonate, Neonatal Jaundice, Cord blood bilirubin.

## INTRODUCTION:

Health is defined as a state of complete physical, mental and social well being and not merely an absence of disease. Health is a fundamental human right[1].Inspite of completing more than 60 years of independence and with so many prevailing health programmes, Infant Mortality rate is high compared to developed countries. Infant mortality rate is one of the most universally accepted indicator of health status not only of infants but also the whole population[2].

Developing countries like India must be fully aware of this limitation on the development of neonatal care, particularly neonatal intensive care. The ultimate aim should be to benefit maximum number of newborn babies with improved survival and reduced mortality.

Clinical jaundice is seen in 60-70% of term and about 80% of preterm newborns[3]. 6.1% of well term newborn have a serum bilirubin over 12.9mg%. Serum bilirubin over 15 mg% is found in 3 % of normal term newborns.[4]The potential risk of developing bilirubin encephalopathy or even kernicterus is high in babies with elevated serum bilirubin level. The sequalae could be serious as patients may develop cerebral palsy, sensorineural deafness and mental retardation[5]. Concerns regarding jaundice have increased after reports of bilirubin encephalopathy occurring in healthy term infants without hemolysis[6].



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Factors like a why worry attitude about jaundice among doctors, financial constraints, family and medical consideration, shortage of hospital beds and personnel frequently influence the decision about early hospital discharge of the mother and infant after birth[7]. Total serum bilirubin (TSB) in infants discharged within 48 hours of life, generally shows an increasing trend and some of these infants later develop significant hyperbilirubinemia requiring treatment[8].

During past 100 years, few major themes have dominated the thinking about pathogenesis and treatment of neonatal jaundice beginning with an understanding of the chemical basis of jaundice and identification of the substance causing jaundice as bilirubin. Bilirubin when conventionally illustrated has ridge like conformation[9].

Bilirubin is derived from the heme containing proteins in the reticuloendothelial system. The normal newborn produces 6 to 10 mg of bilirubin per kilogram per day that is nearly 2 to 3 times as compared to adult. The destruction of circulating red blood cells accounts for 75% of daily bilirubin production in the neonate[10]. 1 gm of hemoglobin produces 34mg of bilirubin. The remaining 25% of bilirubin called early labeled bilirubin[11]. It is derived from ineffective erythropoiesis in bone marrow from other heme containing proteins in tissues (e.g., myoglobin, cytochromes, catalases and peroxidases and from free heme).[12] The heme ring from heme containing proteins is oxidized to bilirubin by heme oxygenase. This enzyme is called the *rate limiting enzyme*. Heme oxygenase is a microsomal enzyme found in reticuloendothelial system and also in tissue macrophages and in gut mucosa, liver, spleen. In the fetus, hepatic microsomal heme oxygenase activity has been measured to be 8 times higher than in adult liver. Heme oxygenase must be present by the time bilirubin appears in human fetus at 14 weeks gestation. The subsequent step, reduction at the C-11 carbon of biliverdin IX$\alpha$ to bilirubin IX$\alpha$ takes place in cytosol and it is catalyzed by biliverdin reductase[13]. Both of these processes are dependent on nicotinamide adenine dinucleotide phosphate oxidase (NADPH). This reaction releases carbon monoxide (CO) (excreted from lung) and iron (reutilized). Catabolism of 1 mmol of hemoglobin produces 1 mmol each of CO and bilirubin. The metabolism of heme results in the formation of free iron. CO and biliverdin, which in turn is reduced to bilirubin[14]. Unconjugated bilirubin is transported in plasma bound to albumin with a binding affinity of $10^7$ to $10^8 M^{-1}$ at the primary binding site. This bound form does not cross the blood brain barrier and is nontoxic[15]. In addition to albumin, bilirubin can also bind to other proteins (e.g., alpha-fetoprotein and ligandin) as well as to lipoproteins. Lysine is involved in bilirubin binding to both albumin and ligandin and possibly to other proteins. Recent findings suggest that binding to lysine may play a role in mediation or modulation of bilirubin toxicity[16].

Bilirubin dissociates from albumin at the sinusoidal surface of hepatocyte, being taken up by facilitated diffusion. Inside the hepatocyte bilirubin binds to cytosolic *glutathione-s-transferases* initially termed ligandins. Binding to glutathione-s-transferases keeps unconjugated bilirubin soluble in cytosol of hepatocyte and increases the net uptake of bilirubin by reducing its efflux from the cell[17]. Conjugation (glucuronidation) of bilirubin is essential for the efficient biliary excretion of bilirubin. Bilirubin glucuronidation is catalyzed by specific isoform of *uridinediphosphoglucuronate glucuronyl transferase*, termed UGT1A1. It catalyzes the binding first one molecule of glucuronic acid to bilirubin, forming bilirubin monoglucuronide. Conjugated bilirubin is not absorbed from the intestine, but the small amount of unconjugated bilirubin that appears in the bile is partially reabsorbed. The gut bacteria reduce the conjugated bilirubin to sterecobilinogen, which is excreted with feces. The newborn is born with sterile gut and intestinal microbial flora, and there is an enzyme, called *β-glucuronidase*, which converts bile glucuronide into unconjugated bilirubin that is reabsorbed into the circulation. This is called *enterohepatic circulation* and is particularly important in babies who are not fed by mouth from birth, as with introduction of feeds bacteria destroys this enzyme[18]. Meconium also contains a significant amount of bilirubin estimated to be as much as 5-10 times daily production. Half of this is unconjugated and thus capable of being reabsorbed. Intestinal bacteria degrades bilirubin into urobilinogen, most of which is absorbed from intestine and undergoes enterohepatic circulation[19]. A minor fraction is then excreted in the urine and stool. Unconjugated bilirubin in the mammalian fetus can be disposed of either by crossing the placenta into maternal circulation or by passing through fetal liver and being excreted into fetal bile. Placental membranes are essentially impermeable to polar compounds such as biliverdin and bilirubin conjugate but non-polar compounds such as unconjugated bilirubin can diffuse across[20]. The transplacental gradient of bilirubin from fetus to mother was between 5:1 to ~10:1. In early life , bilirubin binds to fetoprotein and liver plays minor role in the excretion. Bilirubin has been found in amniotic fluid from 12$^{th}$ week of gestation but gradually disappears as the volume of amniotic fluid increases by 36-37 weeks. As amniotic fluid is primarily a fetal product, it is thought that bilirubin in this fluid most likely comes from the fetus



 

itself [21]. The fetus swallows significant amounts of amniotic fluid and it has been speculated that this ingested bilirubin may be absorbed across the intestinal mucosa. One of the reason, the body converts biliverdin, a polar, hydrophilic, excretable, non-toxic molecule to bilirubin a non polar, hydrophobic, non excretable and toxic molecule is that biliverdin , unlike bilirubin doesn't cross the placenta easily[22]. This conversion protects the fetus from biliverdin overload. For same reason the fetus has a defective hepatic bilirubin conjugated mechanism but active unconjugated mechanism[23].

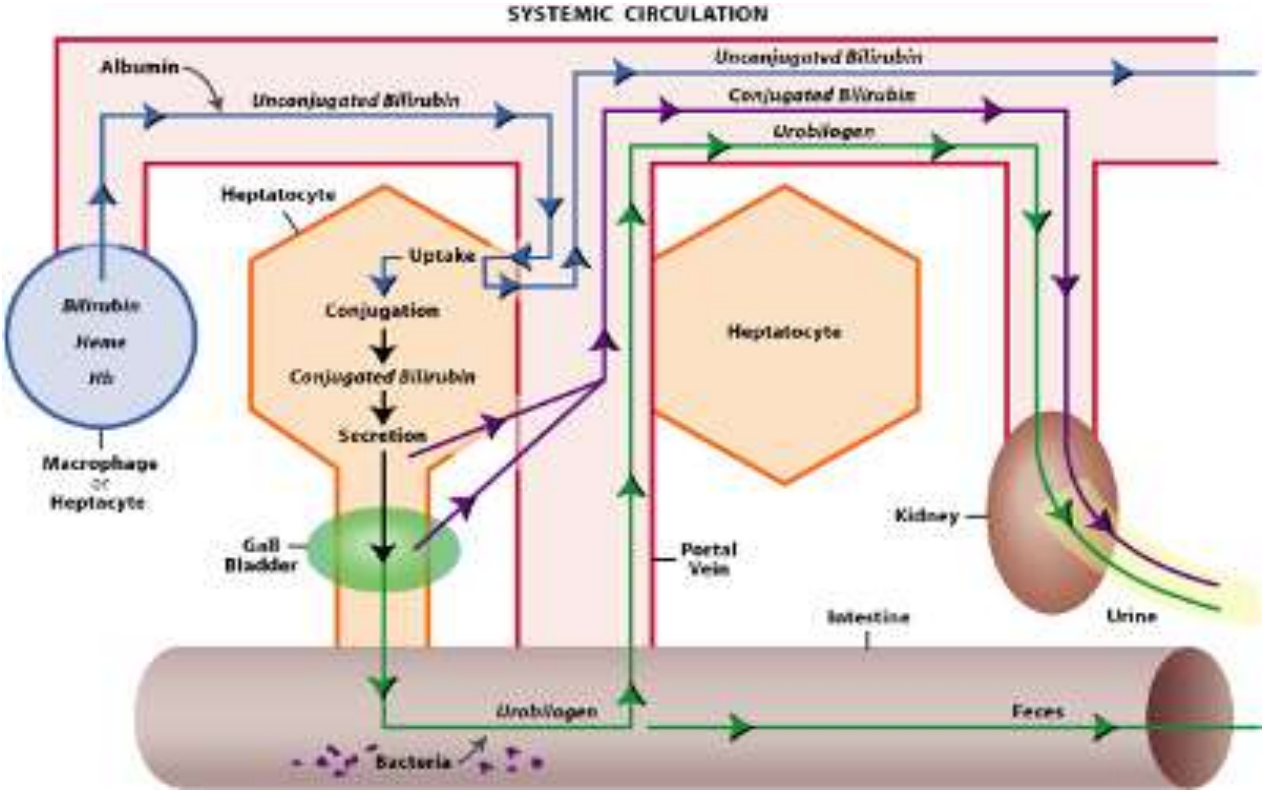


**Figure 1: Schematic illustration of bilirubin metabolism in fetal and neonatal life**

## MATERIALS AND METHODS:

This study was conducted in 100 term newborns in Neonatal unit , Department of Pediatrics, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, from September 2012-July 2013. Serum bilirubin estimation was done at birth from cord blood and then at 72 hours of life from peripheral venous blood sample.

A prospective cohort study model was adopted, in which the group of newborns according to the inclusion criteria were followed up clinically and by laboratory investigation during the period of their hospital stay. At the time of this study, neonates were observed for three days post-delivery period, prior to discharge.

Inclusion Criteria was all full term neonates delivered by normal vaginal delivery (NVD) or lower segment casearen section (LSCS) ,with birth weight >2.5 kg and no birth asphyxia.

The study parameter included mother's interview to record clinical history in a specially designed proforma. Cord blood samples for analysis of conjugated, unconjugated and total bilirubin levels were collected from all newborns that complied with the protocol inclusion criteria.

These newborns were followed up for the 3-day period of their hospital stay. They were physically examined daily and also whenever necessary bilirubin levels were done. Values of bilirubin at third day were compared with those of cord blood bilirubin. In all cases the recommendation for phototherapy was followed according to the schedule proposed by the AAP.[22] Establishment of the mother's and newborn's blood groups were also done.

2 ml each of plain cord blood samples were collected from the umbilical cord and subjected to following investigations:

- ABO Blood grouping and Rh typing.
- Total and differential serum bilirubin assessment.



 

- 2 ml plain venous blood was collected from the baby after 72 hours and total and differential serum bilirubin were assessed.
- Blood collected was transported to laboratory within 2 hours of collection.

Serum bilirubin estimation was done in the biochemistry department of MMIMSR , Mullana. Serum bilirubin estimation was done by Diazo method in which a detergent is used to accelerate the reaction is used. The diazo reagent used is 2, 5 – dichlorophenyl diazonium tetrafluoroborate. This method for bilirubin estimation is based on the principle that bilirubin reacts with diazotized sulphanilic acid in acidic medium to form pink coloured azobilirubin with absorbance directly proportional to the serum bilirubin concentration. Direct bilirubin, being water soluble, directly reacts in acidic medium. However, indirect or unconjugated bilirubin is solubilized using a surfactant and then it reacts similar to direct bilirubin.

The main outcome of this study was analyzed in terms of hyperbilirubinemia requiring phototherapy serum bilirubin level of ≥15 mg/dL was taken as NNJ.[22]

All babies were classified into four groups depending on the UCS bilirubin levels <0.9 (group-I), 1.0-1.9 (group-II), 2.0-2.9 (group-III), >3 (group-IV). Serum bilirubin estimation was done after 72 hours of postnatal life. Babies were categorized according to the need for phototherapy.

Statistical tests of significance (chi-square) were applied and the predictive values (sensitivity, specificity, PPV, NPV) were calculated using the conventional formulae.

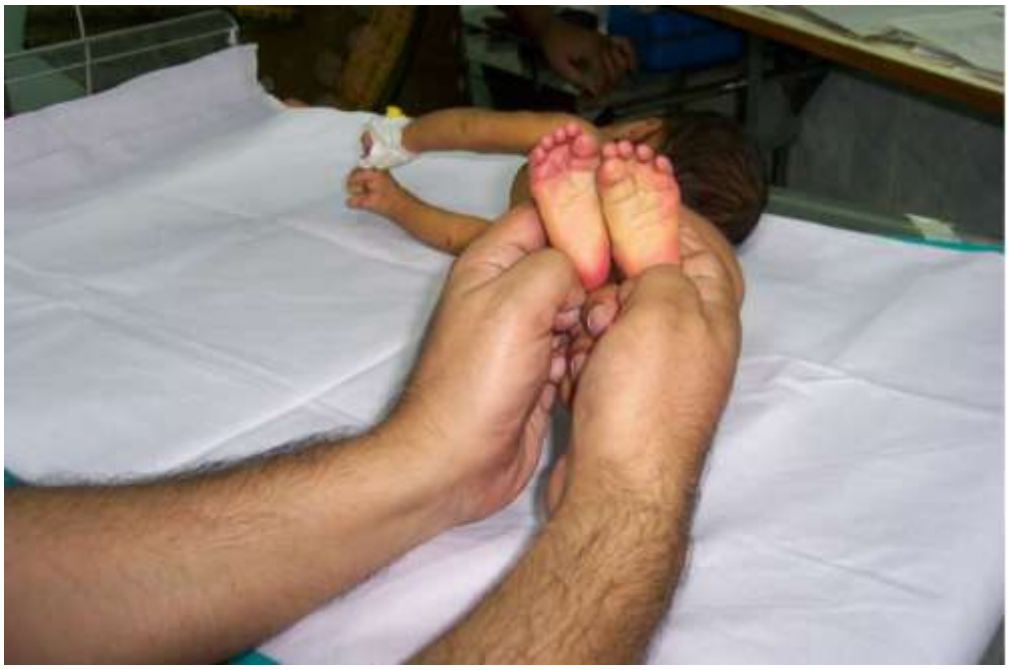

**Figure 2: Clinical estimation of neonatal jaundice**

**Results and Discussion:**

The incidence of hyperbilirubinemia in the present study was 14 %, 58% of which are males and 42% are females.

Incidence was more in babies born through LSCS (77.77%) than in NVD (23.23%)

**Table 1: Basal parameters**

| **Study ( No.of Cases )** | ***UCS Bilirubin*** (mg/dl) | **Incidence of Jaundice(%)** |
|---|---|---|
| Present study | 0-0.9 | 0 |
| | 1 – 1.9 | 1.9 |
| ( 100 ) | 2 – 2.9 | 40.9 |
| | >3 | 80 |



 

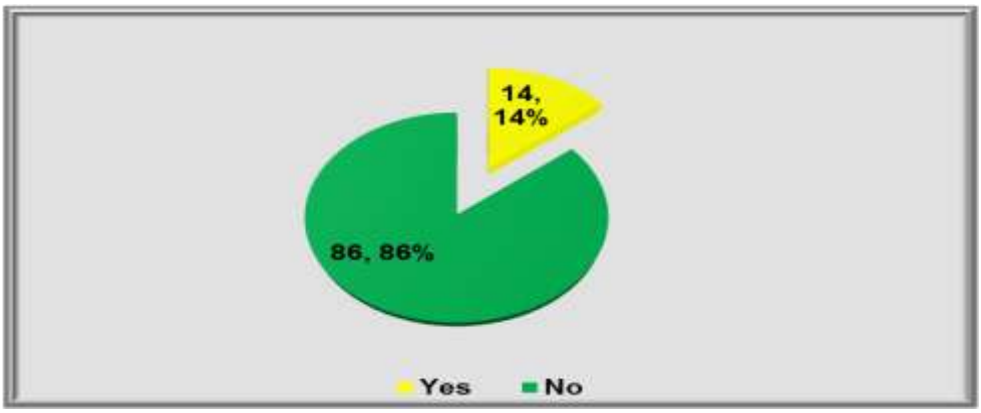


**Figure 3: Distribution of study population**

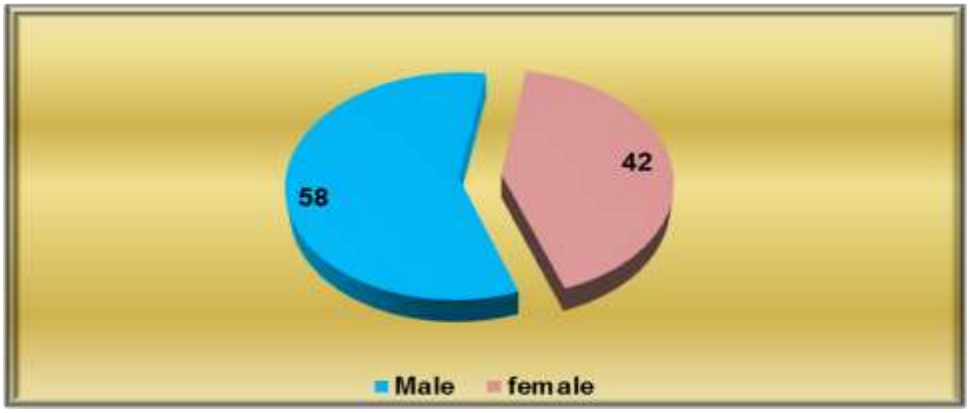


**Figure 4: Sex wise distribution of study group**

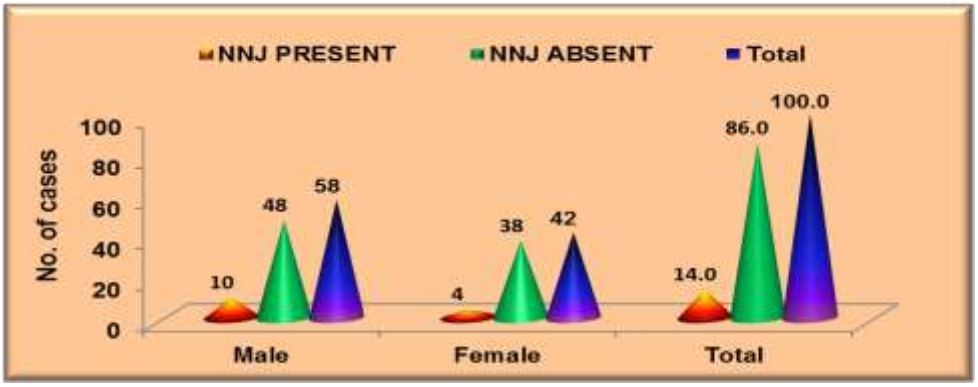


**Figure 5: Association between sex of newborn and NNJ**

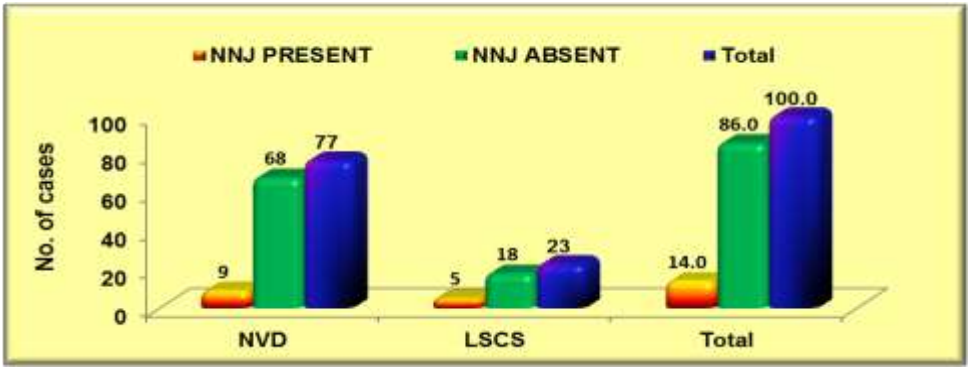


**Figure 6: NNJ and Mode of Delivery**

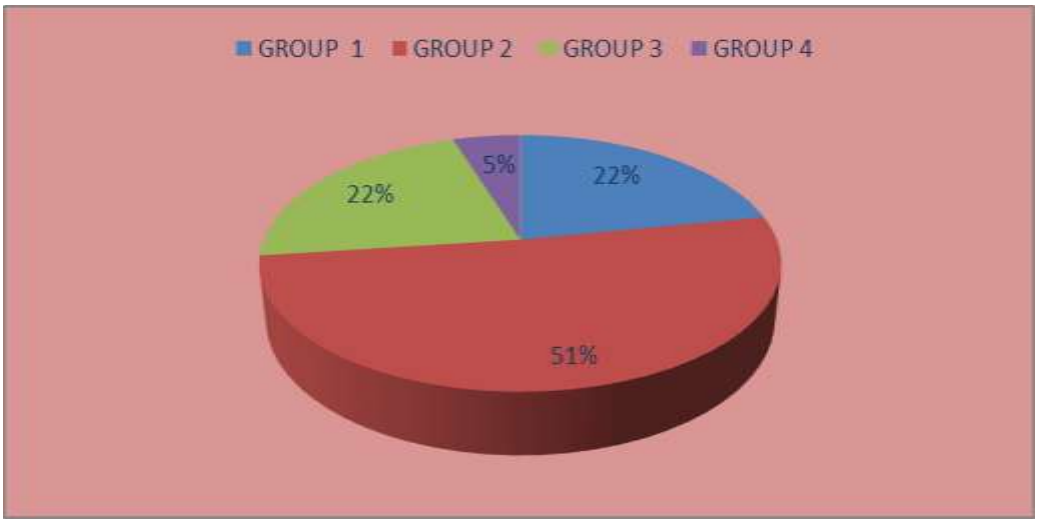


**Figure 7: Incidence of NNJ in each group**

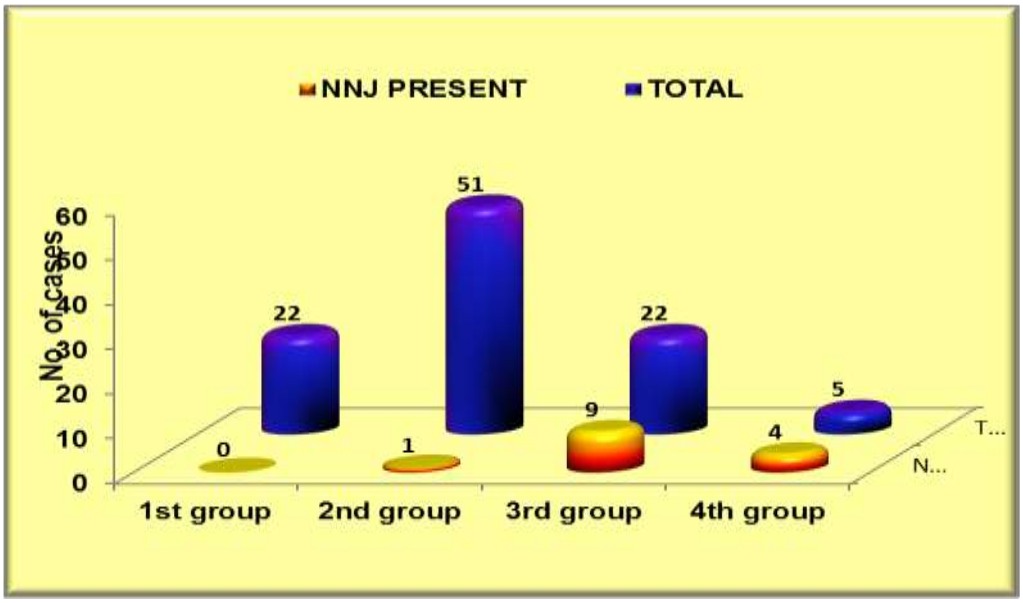


**Figure 8: Incidence of NNJ in each group**

**Discussion and Conclusion:**

The study group consisted of 100 full-terms Appropriate for gestational age neonates delivered in MMIMSR, Mullana between September 2012- September 2013. Incidence of hyperbilirubinemia was 14% in study population. Mean umbilical cord serum bilirubin was 1.63±0.73. There was no significant association between neonatal hyperbilirubinemia and route of delivery (p>0.005). No association was found between neonatal hyperbilirubinemia and sex of the baby and maternal age (p>0.05, not significant). The correlation between between the mother's blood group and the increased risk of hyperbilirubinemia in neonates was insignificant. Significant association was seen between neonatal hyperbilirubinemia and increasing umbilical cord serum bilirubin (p= 0.001).Using umbilical cord blood bilirubin level of ≥1.9 mg/dL hyperbilirubinemia could be predicted with sensitivity of 92.8% , specificity of 83.7% , positive predictive value of 48.1% and negative predictive value of 98.6%.

Our data suggest that umbilical cord blood can be utilized for estimation of serum bilirubin to predict development of neonatal hyperbilirubinemia and decide need for appropriate intervention in healthy term neonates.

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