FULL TERM TRAINING REPORT SUBMITTED TO

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

MASTER OF SCIENCE

IN

CLINCAL BIOCHEMISTRY

SUBMITTED BY

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Certificate

This is to certify that the work done by **Mr. Shahi Dul Islam** during the internship program from 1st January 2016 to 30th April 2016, on the topic entitled ***"Neonatal hyperbilirubinemia"*** in the Department of Clinical Biochemistry, SKIMS Medical College Bemina, Srinagar, under the supervision of **Dr. Ruqaya Aziz** (Associate Professor and HOD Biochemistry, SKIMS MCH, Srinagar), submitted to Lovely Professional University, Punjab India, as one of the requirements for a degree of Master of Clinical Biochemistry.

Ruqaya aziz
Dr. Ruqaya Aziz
(Associate Professor & Head)

No.: SKIMS-MC/Bio/2016/**29**
Dated: 04/05/2016

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CERTIFICATE

This is to certify that present full term training report entitled "**Neonatal Hyperbilirubinemia**" is the outcome of the original piece of work carried out by **Mr. SHAHI DUL ISLAM (11407876)** himself under my guidance and the contents of this project did not form a basis of the award of any previous degree to him and to the best of my knowledge to anybody also.

This project is fit for submission to the partial fulfillment of the conditions for the award of **M.Sc in Clinical Biochemistry**. Further certified that the candidate in habit and character is a fit and proper person for the award

Dr. Ekta Chitkara
Internal supervisor
Associate professor
Lovely Professional University
Phagwara Punjab, India

DATE 10/5/2016
Place:-LPU, Punjab

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I once again thank all those not mentioned here but have contributed to the successful completion of the study.

Date:10-5-2016 Shahi Dul Islam

Place: LPU, Punjab

DECLARATION

This is to certify that this written submission in the form of full time training report entitled **" Neonatal hyperbilirubinemia"** represents original ideas in my own words and where others ideas and words have been included. I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated any idea/fact/source in my submission. I understand that any violation of the above will be cause for disciplinary action by the school and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required.

This project encompasses the information generated by me based on experimental work carried out in the **SKIMS Medical college hospital Bemina Srinagar.** I assure and hold full responsibility for its genueness.

Date: 10-5-2016 Shahi Dul Islam

Place: LPU, Punjab

List of Abbreviations

TSB	Total serum bilirubin
UDPGT	Uridine diphosphoglucuronosyl transferase
PT	Phototherapy
CP	Cerebral palsy
SBA	Severe birth asphyxia
HIE	Hypoxic ischemic encephalopathy
G-6-PD	Glucose-6- phosphate dehydrogenase
UGT	Uridine Glucoronosyl transferase
NICU	Neonatal intensive care unit
FMC	Federal medical centre
NNJ	Neonatal jaundice
CRP	C-Reactive protein
DPD	Dichlorophenyl diazonium Tetrafluorobrate
MG/DL	Mille gram per Deci Litre

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ABSTRACT

Jaundice is mostly the life's first hurdle which a newborn faces immediately after birth and becomes the reason for his/her admission in neonatal ward .Jaundice is characterized by Hyperbilirubinemia which leads to yellowish color of sclera, mucosa, and skin. Physiological jaundice is always there due to the physiological immaturity of neonate to conjugate increased bilirubin production due to excessive RBC lyses and the bilirubin which is conjugated by liver entered into intestine along with bile. Here sterile gut if neonates have enzyme β-glucoronidase which de conjugate the conjugated bilirubin which again enter blood .Total serum bilirubin (TSB) level between 6 to 8 mg/dl by the first three days is in the physiological range. However, if TSB exceeds 5mg/dl on the first day, 10mg/dl on the second day and 15 to 20mg/dl on the third day of life, it is then the pathological condition and needs immediate treatment. Prolonged pathological jaundice may lead to kernicterus and neonate may suffer lifelong disability. This study was conducted on the neonates of SKIMS medical college hospital Bemina Srinagar to evaluate the bilirubin levels in neonates.100 neonates were included in the study conducted from January 2016 to April 2016. The neonates were clinical assessed and brief history taken with the aid of quesestionnaire. The parameters studied were total serum bilirubin, Direct Bilirubin.

Key Words:-Neonatal hyperbilirubinemia, Kernicterus.

Chapter 1

Introduction

CHAPTER 1

1:-INTRODUCTION

Neonatal jaundice refers to the yellow discoloration of the skin caused by the accumulation of bilirubin in skin and mucous membranes. This is associated with hyperbilirubinia. Neonatal jaundice is one of the most common conditions confronting neonatologists .Epidemiologist studies say that about 60% of term and 80% of preterm babies develop jaundice in first week of life[1].the goal of the management of the unconjucated hyperbilirubinemia is to avoid toxicity[2]. Exchange transfusion and phototherapy are two leading treatments for severe jaundice .although the need for exchange transfusion has markedly decreased after the availability of effective phototherapy .A small proportions of infants with severe hyperbilirubinemia need exchange transfusion. Which leads to increased risk of infections and death in most infants, unconjugated hyperbilirubinemia reflects a normal phenomenon. However in some infants serum bilirubin levels may raise excessively, which can be cause for concern because uncnjugated bilirubin is neurotoxic and can cause death in newborns and lifelong neurologic problem in infants who survive (kernicterus).the term bilirubin encephalopathy is used to refer acute neurological dysfunction associated with hyperbilirubinemia. While kernictures is strictly speaking a pathological term and it is often used to refer to the long term neurodevlopmental effects of bilirubin encephalopathy in most cases the etiology of this disorder is multifactorial. The term " kernicterus" was introduced in the early 1900's to refer to the yellow staining of the basal ganglia observed in infants who died in severe jaundice(3).Because of high incidence of RH hemolytic disease that is breakdown of RBC's and kernicterus ,pediatricians were aggressive in treating jaundice(4).How ever several factors have changed the management of jaundice. The

studies in the 1990's suggests that kernicterus from jaundice was rare and that too many infants were treated unnecessarily [5-6].Also newborn infants were being discharged from the hospital sooner after birth, limiting the ability of physicians to detect jaundice during the period when the serum bilirubin concentration is likely to rise [7-8]. Low concentration of bilirubin may have some antioxidant benefit, suggesting that it should not be completely eliminated [9]. Because of these factors, physicians became less likely to treat jaundice in neonates, which in turn led to increase in reports of almost forgotten and deadly kernicterus [10-11] .Neonatal hyperbilirubinemia results from a pre-disposition of to the production of bilirubin in newborn infants and their limited ability to excrete it. Infants, especially preterm infants have higher rates of bilirubin production than adults, because they have red cells with higher turnover and shorter life span (12) .In newborn infants, uncnjugated bilirubin is not readily excreted, and the ability to conjugate bilirubin is limited. Together, these limitations lead to physiologic jaundice that is high serum bilirubin concentrations in the first days of life in full term infants, followed by decline during the next several weeks to the values commonly found in adults. The average full term newborn infant has a peak serum bilirubin concentration of 5 to 6 mg per deciliter. serum bilirubin concentration higher than 17mg per deciliter in full term infants are no longer considered physiologic, and a cause of pathologic jaundice can usually be identified in such infants[13].now apart from this many a time it is physiological in newborn because liver is not enough mature to conjugate all the bilirubin the neonates have about 1% of uridine diphosphoglucuronosyl transferees (UDPGT) activity as that of an adult [14].apart from this there is an increased load of bilirubin in neonates as they have higher volume of circulation erythrocytes, a shorter mean erythrocyte life span and a larger early labeled bilirubin peak[15].this hyperbilirubinemia is due to uncnjugated bilirubin which is toxic to central nervous system . There are significant differences

in TSB levels in different populations and is difficult to define as normal or abnormal or obtain diagnostic and therapeutic cut of levels [16].The bilirubin level as physiological can be misleading and potentially dangerous. It is difficult to predict the course of hyperbilirubinemia on day one of neonate .There have been reports of cord blood bilirubin as a predictor of hyperbilirubinemia that would require phototherapy (PT). Early prediction will help in early discharge and prevent in unnecessarily hospitalization of babies and mothers. Albumin is synthesized by liver and it helps in transport in unconjugated bilirubin. There is a paucity of reports on serum albumin or cord blood albumin levels as a predictor of hyperbilirubinemia.

Neonates with hyperbilirubinemia are also associated with cerebral palsy. Cerebral palsy (CP) is a disorder of posture and movement due to defect or disease of the brain appearing the early years of life. The disease is not progressive although the manifestations may alter[17].severe birth asphyxia (SBA) with hypoxic ischemic encephalopathy (HIE) are major causes of cerebral palsy (CP).According to WHO ,Between four and nine million newborn develop birth asphyxia each year .

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1.1:-Pathophysiology

Jaundice is a symptom of an underlying condition that impairs the excretion of bilirubin from body .As the 120 days life span of a red blood cell comes to end or cell become damaged, the cell membrane becomes weak and susceptible to rupture .As this old or damaged cell circulates through the reticuloendothelial system , the fragile membrane eventually ruptures and the contents of the cell are expelled into the bloodstream .One of the cellular components released when this happens is hemoglobin ,which is ingested by phagocytic cells called macrophages . This phagocytosis split the hemoglobin into its constituent heme and globin portions. The globin portion is a protein that breaks down into amino acids and plays no role in the pathogenesis of jaundice. The heme on the other hand undergoes an oxidation reaction catalyzed by the enzyme oxygenase, to give biliverdin, iron and carbon monoxide .The biliverdin is a green colored pigment which then undergoes a reduction reaction to yield a yellow pigment called bilirubin. This reaction is catalyzed by cytosolic enzyme biliverdin reductase. Nearly 4mg of bilirubin for each kg of blood is produced in the body every day, mainly as a result of heme from expired red blood cells being broken down. The remainder is produced from other sources of heme such as failed red blood cell synthesis and from the breakdown of proteins that contain heme such as cytochromes and myoglobin. This insoluble bilirubin is referred to as “free”, “indirect” or “unconjugated” bilirubin and it moves towards the liver through the blood stream, while bound to albumin . In the liver bilirubin is conjugated with glucoronic acid reaction catalyzed by (UDP-glucoronosyl transferase) to give “conjugated” bilirubin, which is the water soluble form of bilirubin that can be excreted. This conjugated bilirubin leaves the liver and enters the bilary tree and cystic ducts as part of bile. Bacteria in the intestine then convert the bilirubin into

urobilinogen. This urobilinogen is then either converted into stercobilinogen and excreted nail beds and whites of eyes that is caused by a buildup of bilirubin in the body tissues.

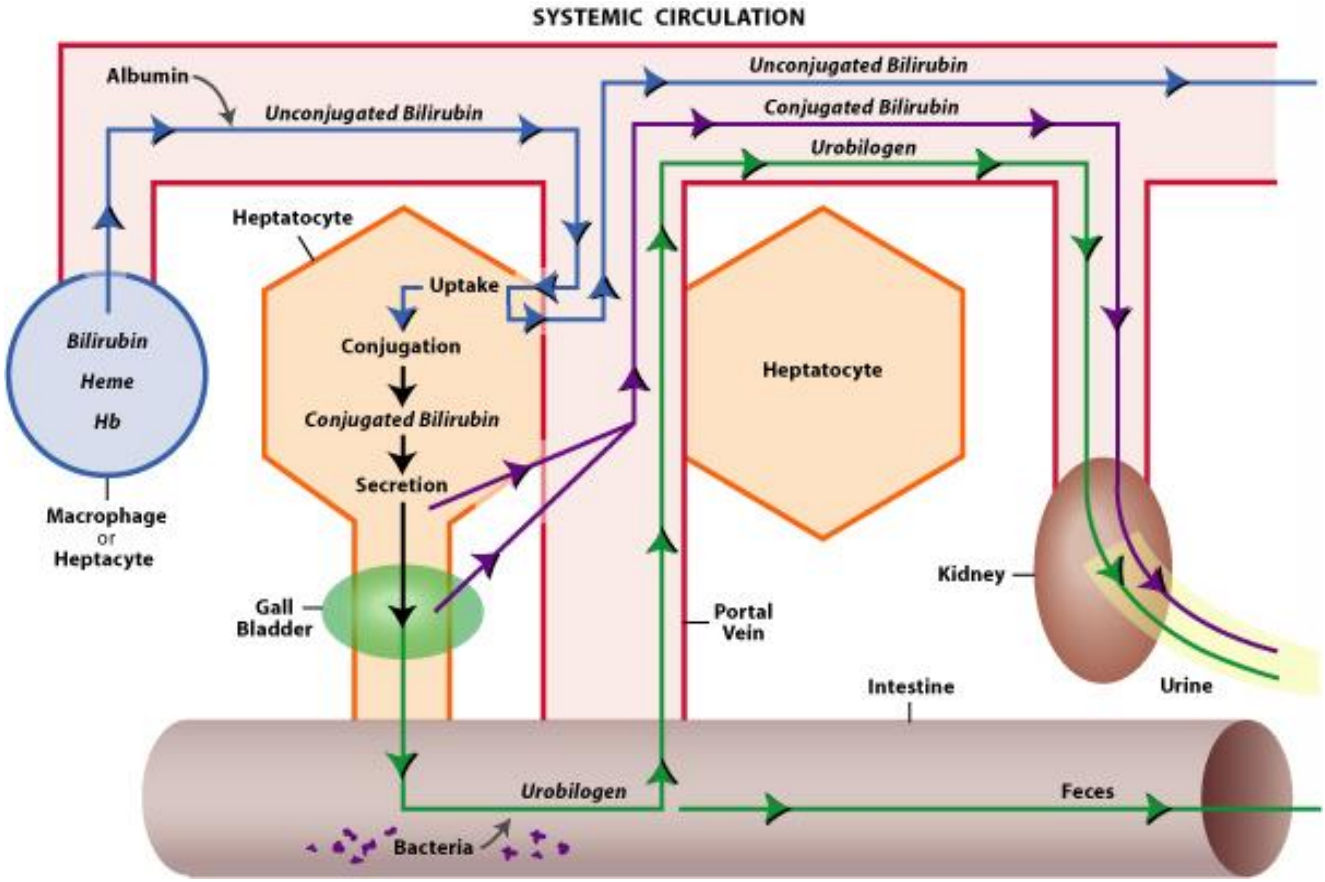

Fig 1:- Path physiology of Neonatal Jaundice.

The predominant source of bilirubin is the breakdown of hemoglobin in senescent or or hemolysed red cells.Haeme is degraded by heme oxigenase, resulting in the release of iron and the formation of carbon monoxide and biliverdin. Biliverdin is further reduced to bilirubin by biliverdin reductase .Bilirubin then enters the liver and is modified to an extractable conjugated form that enters the intestinal lumen but can be deconjugated by bacteria so that the bilirubin is reabsorbed into the circulation.

Increased production of bilirubin, deficiency of hepatic uptake, impaired conjugation of bilirubin, and increased enter hepatic circulation of bilirubin account for most cases of pathologic jaundice in newborn infants [18].increased production of bilirubin occurs in infants of various racial groups, as well as in infants with blood group incompatibilities, erythrocyte-enzyme deficiencies or structural defects of erythrocytes[19-20].

Another reason for pathologic hyperbilirubinemia is deficient hepatic uptake of bilirubin as occurs in patients with Gilbert's syndrome [21]. Deficiency of uridine diphosphate glucoronosyltansferase, the enzyme required for the conjugation of bilirubin, is another important cause of neonatal jaundice .Although all newborn infants are relatively deficient in this enzyme, those with Crigler-Najjar syndrome type 1, in whom the deficiency is severe, have bilirubin encephalopathy in the first days or months of life [22].encephalopathy is rare in infants with Crigler-Najjar syndrome type 2. In which serum bilirubin values rarely exceed 20mg per deciliter. In glucose-6-phosphate dehydrogenase deficiency, there is increased risk of hemolysis and impaired conjugation of bilirubin. Infants with Gilbert's syndrome also have mildly decreased uridine diphosphate glucoronosyltansferase activity. This decrease has been attributed to an expansion of thymine-adenine (TA) repeats in promoter region of the UGITA gene, the principal gene encoding this enzyme [23]. Racial variation in numbers of TA repeats and a correlation with uridine diphosphate glucoronosyltansferase activity suggest that these polymorphisms contribute to variations in bilirubin metabolism. A Common DNA-sequence variant (Gly71Arg), resulting in an amino acid change in the UDGT protein is associated with neonatal hyperbilirubinemia [24]. In addition the combination of glucose-6-phosphate dehydrogenase deficiency and Gilbert's syndrome increases the likely hood of severe hyperbilirubinemia. Increased enter hepatic circulation of bilirubin in the fasting state can also

exaggerate hyperbilirubinemia [25-26]. Newborn infants who are not feeding well are who are exclusively breast-fed have low levels of the intestinal bacteria that are capable of converting bilirubin to nonresorbable derivatives and the entrohepatic circulation of bilirubin may be increased in infants

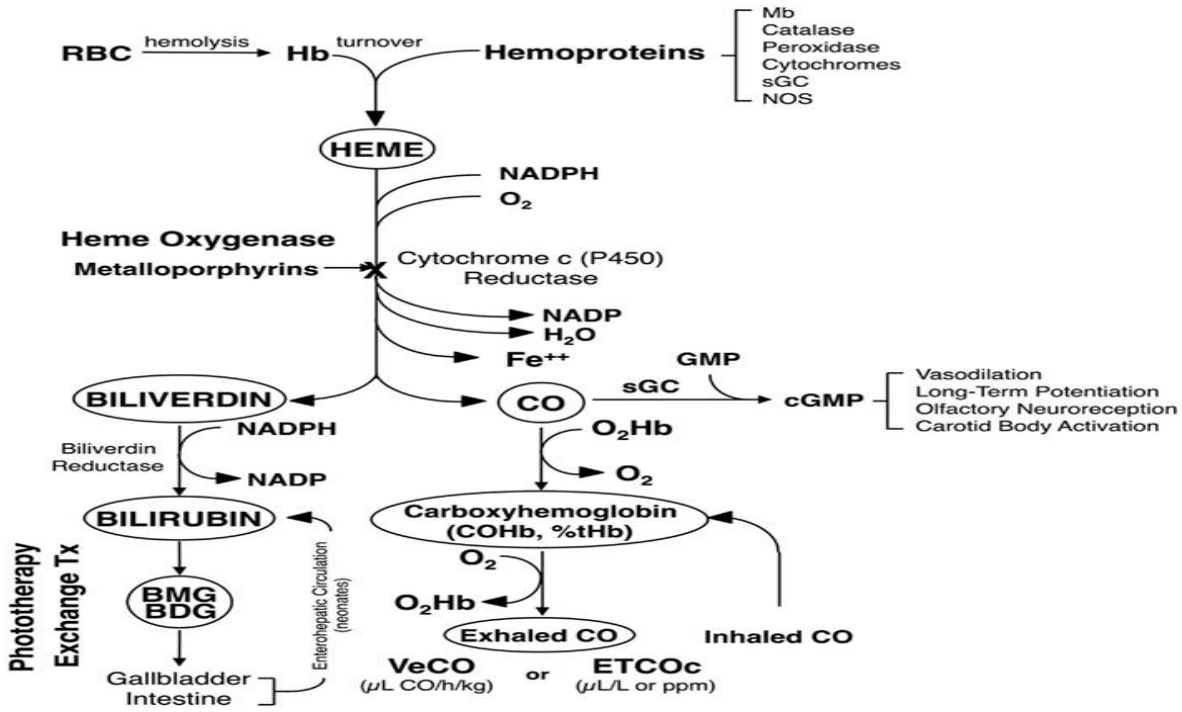

Fig 2:- Metabolic pathway of the degradation of heme and the formation of bilirubin [27].

Physiological jaundice:-

Jaundice attributed to immaturity usually appears between 24-72 hours of age, peaks by 4-5 days in term and 7th day in preterm neonates and disappears in 10-12 days of life .it is predominantly unconjugated and levels usually do not exceed 15mg/dl. This pattern of physiological jaundice

has been described in predominantly in artificially fed babies. Bilirubin levels upto 17-18mg/dl may be accepted as normal in term healthy newborns.

Pathological jaundice:- Bilirubin levels that deviate from the normal range and requiring intervention would be defined as pathological jaundice. Appearance of jaundice within 24 hours, increase in serum bilirubin beyond 5mg/dl/day, peak levels above the expected normal range, presence of clinical jaundice beyond 2 weeks and conjugated bilirubin would be categorized under pathological jaundice.

Breast feeding and jaundice:- Exclusively breast fed infants have a different pattern of physiological jaundice as compared to artificially fed babies [28-29]. Jaundice in breast fed babies usually appears between 24-72 hours of age, peaks by 5 to 15 days of life and disappears by the third week of life. They have also been reported to have higher bilirubin levels. Studies have shown that 13% breast fed babies had peak bilirubin levels of 12mg/dl or higher as compeered to 4% artificially fed babies [30]. One third of all breast fed babies are detected to have mild clinical jaundice in third week of life which may persist into the 2nd and 3rd month of life in a few babies.

Authors have stated that this increased frequency is not related to characteristics of breast milk but rather to the pattern of breast-feeding. Decreased frequency of breast feeding is associated with exaggeration of physiological jaundice. Encouraging mother to breast feed her baby 10-12 times /day would be helpful in the management of jaundice in a term healthy baby.

Classification of jaundice:-

1) - Haemolytic and prehepatic jaundice:-

In this type there is increased break down of hemoglobin. So that liver cells are unable to conjugate all the increased bilirubin formed.

Causes:- abnormalities within the red blood cells by various haemoglobinopathies, hereditary spherocytosis, G-6-PD deficiency in red cells and fauvism. Auto immune hemolytic anemia, in malaria.

2) - Hepatocelluler or hepatic jaundice:-

In which there is disease of the parenchymal cells of liver. This may be divided into three groups

Conditions in which there is defective conjugation: There may be a reduction of number of functioning of liver cells e.g., in chronic hepatitis in this all functions are impaired or there may b a specific defect in conjugation process for example Gilbert's syndrome , Crigler Najjar syndrome etc.

Cholestatic jaundice: A yellowing of the skin caused by thickening of bile, Obstruction of hepatic ducts, or changes in liver cell function.

3) - Obstructive or post hepatic jaundice:-

In which there is obstruction to flow of bile in the extra hepatic ducts, example due to gall stones .carcinoma of head of pancreas, enlarged lymph glands pressing on bile duct etc [31].

CHAPTER 2

AIM

AND

OBJECTIVE

Chapter 2

AIM

Effect of Hyperbilirubinemia in newly born babies.

Objectives

To assess the effect of increased levels of Bilirubin in newly born Babies.

CHAPTER 3

Review

Of

Literature

Chapter 3

Kavehmanesh et al(2008)[32]:- This cross sectional study was conducted among women who gave birth to their children at Najmieh hospital , Tehran , a questionnaire was filled for each mother infant pair at the time of delivery and completed at the time of nursery discharge .mothers were asked to bring their babies to hospital in case of jaundice. They were also followed by phone on the 3rd, 7th, 10th and 14th of life to ask about jaundice or readmission. They were asked to bring their infant if they were jaundiced. The medical records of infants who were readmitted were reviewed and in case of readmission abstracted.

Serum total bilirubin was tested in any infant who has visible icterus before or after discharge. Data recorded from mothers included their age, race, blood group and Drug consumption during pregnancy, oxytocin consumption during labor, and history of rupture of amniotic membrane. Data recorded from the babies include their sex, birth weight, maturity, gravity, and length of nursery stay. Subjects were selected from the population of mother- infant pairs in which the infants were singletons and weighing $\geq$ 2500 gr. A total no of 3112 women gave birth during the period of this study. New born weighing less than 2500 gram (236 cases), twin babies (37cases) and those hospitalized for jaundice appearing prior to nursery discharge or any other reasons (137 cases) were excluded, remaining 2702 infants, gestational age of less than 37 weeks were considered as premature .Bilirubin levels of more or equal 95% on bilirubin namogram on the first 48 hours and more or equal 15mg/dl thereafter was considered as indication for hospitalization. Those infants who had been readmitted within the neonatal period because of jaundice were compared with all other infants who were not readmitted within the period of time. The data of mother infant were analyzed in SPSS (version 11.5), using chi-square or fisher exact

tests and logistic regression for categorical and t-test for continuous data. There were 340(12.6%) rehospitalizations for hyperbilirubinemia and remaining 2362 infants were non hospitalized cases. Maternal mean (SD) age was 27071(5.4) years and didn't differ between the two groups of icteric and non icteric babies. Mean standard deviation neonatal birth weight was 3301.1 (395) grams totally [3284.6 (393.0) in icterus and 3303.5(385.9) in non icterus which do not differ between the two groups. neonatal gestational age was 39. The incidence of hyperbilirubinemia among this study group was 12.6%, the incidence of significant hyperbilirubinemia being 3.1%. Other studies report incidences of significant hyperbilirubinemia 1.7% to 12%[33-34], although an incidence of readmission was as low as 4.2 per 1000 newborns has been documented in some studies[35].These differences may be attributed to ethnic and geographic variations . In this study the maternal age did not differ between the two groups of icteric and non icteric babies. The association of Asian race with jaundice has been well established before [36-37]. There were no significant difference between maternal blood groups of icterus and non icterus, but RH negative mothers had significantly more icteric babies than RH positive mothers. Although there are some studies that show increased risk of jaundice in maternal O blood group, but there is no difference between maternal ABO or RH group in some other studies [38]. In this study birth weight does not differ significantly in these two groups. Although male sex has been found to be more prone to jaundice in some other studies [39].in this study only prematurity of newborn had a significant effect on neonatal readmission for jaundice. Similar results are shown in other studies [40].

Ezham A et al(2011)[41] :- This study shows in prevalence of uridineglucoronosyltransferase 1A1(UGT1A1) mutations in Malay neonates with severe jaundice .discovered that out of 250 neonates that were enrolled in the study , one hundred and twenty five neonates of Malay ethnic

parentage were admitted with severe unconjugated hyperbilirubinemia . They found that there was an equal distribution of gender between the severely jaundiced neonates and the control cases.

Onyearugha et al (2011)[42]:-this retrospective study conducted in the neonatal intensive care unit(NICU) of federal medical centre(FMC), Abakaliki, Ebony state, south east Nigeria from 1st January 2008 to 31st December 2009.during the study 160 neonates were managed for neonatal jaundice (NNJ) while the overall number of neonatal admissions in the neonatal intensive care unit was 457, Hence neonatal jaundice accounted for 35.0% of all NICU admissions , six of the newborns were excluded due to inadequate data , so 154 comprising 83 inborn and 71 out born babies were used for further analysis. Out of the 83 inborn babies, 46(55.4%) were males and 37(44.6%) were females .giving a male female ratio of 1:2:1. Also 40 of the out born babies (56.3%) were males and (43.7%) were females . significant bilirubinemia (SB>169 micro mole/L) occurred significantly more in out born babies 66(63%) than in inborn babies 40(48.2%).The leading etiologic factors of NNJ in 40 inborn babies with significant bilirubinemia were septicemia 17(42.5%) and prematurity 13(32.5%).also the predominant etiologic factors of neonatal jaundice in the out born babies were septicemia 33(50%) and prematurity 14(21.2%). Significantly more out born than inborn babies have septicemia, The mean maximum of serum bilirubin (SB) of the out born subjects was 334.2 micromole/L(range 156.6-526.2 micromole/L) and that of the inborn subjects 276.7 micromole/L(range 96.4-364.4 micromole/L).the mean gestational age of the inborn subjects was 37.2 weeks(range 28-43 weeks) while that of out born subjects was 38.7weeks (range 32-43 weeks). Preterm delivery occurred more in the inborn babies 25(30.1%) than in the out born subjects 14(19.7%).The occurrence of neonatal jaundice of 35% of neonatal intensive care unit (NICU) admissions

observed in this study goes to confirm neonatal jaundice as among leading causes of neonatal morbidity as noted in previous reports in Nigeria and other parts of the world ranging between 10to 35% of neonatal admissions (Udo et al 2008). Bilirubinemia occurred significantly occurred more in out born babies than in the inborn subjects. This agrees with the observation in previous surveys (Owa and Ogunlesi 2009) [(43)] .The study has also demonstrated septicemia followed by prematurity as the leading etiological factors of neonatal jaundice. Southern Nigeria reported septicemia and glucose -6-phosphate (G6PD) as well as prematurity and G6PD deficiency as leading causes of neonatal jaundice respectively. (Ho 2002)[(44)] from Asia documented ABO incompatibility and glucose 6 phosphate as a leading causes . The 6 subjects developed severe jaundice on exposure to naphthalene ball contaminated cloths. Naphthalene is one of the drugs causing hemolysis and jaundice in glucose 6 phosphate deficient subjects. Mothers of the out born babies with significant bilirubinemia took herbal drugs. Maternal use of herbal medications being associated with severe neonatal jaundice has been reported previously from southern Nigeria. (Olusanya et al 2009)[(45)] .Prematurity is the second leading cause of neonatal jaundice in both the inborn and out born babies. Preterm newborns are prone to developing jaundice due to immaturity of their bilirubin conjugation system. Higher rate of hemolysis, increased entrohepatic circulation and decreased caloric intake. In this study majority of the majority of the inborn babies were exclusively breastfed and were significantly more than out born babies fed likewise. This also shows the neonatal jaundice occurs exclusively in breastfed babies. From this eight deaths were recorded 6 occurred in out born babies and this was because the out born babies presented in a more severely ill state with higher SB levels and relatively late.

Onyearugha CN et al(2011)[(46)]:- reported a high prevalence of neonatal jaundice in Abakaliki , south east Nigeria and recommended that effective and sustained health education of

the citizenry and particularly expectant women on the need for early booking ANC , regular antenatal supervision of pregnancy and delivery in appropriate health facility as well as an early signs of neonatal jaundice and prompt presentation of affected newborn for appropriate medical care be implemented forth with to curb this un acceptable situation.

Sahu et al (2011) [47]**:-** the study conducted in the departments of pediatrics and biochemistry at the Pondicherry institute of medical science, Pondicherry India. The study included forty newborns with the inclusions such as sequentially born term babies (gestational age > 37 weeks) from any mode of delivery both genders etc. The exclusion criteria were preterm babies (gestational age < 37 weeks), neonatal sepsis and any complication arising during the hospital stay .with the informed consent of parents 2ml of cord blood was collected from newborn and serum separated . They were estimated for serum albumin by BCG method uses Dade Behring UK .From then on the newborns was followed by clinically for jaundice according to the evaluation of the Kramer dermal zones [48]. Where ever necessary further laboratory tests were done for bilirubin and managed accordingly. The results showed that the babies who had the albumin levels <208gm/dl were developing hyperbilirubinemia nearly 82% of the newborns were involved. The babies having albumin levels ranging from 2.8-3.3 gm/dl were also having increased bilirubin. But those who had albumin >3.3 gm/dl were negative for increased bilirubin levels [49-50-51] .Albumin is the major binding protein in the human neonates. Low production of albumin will lower its transport and binding capacity [52].Albumin binds to potentially toxic products like bilirubin and antibiotics bilirubin binds to albumin in equimoler ratio. It is the free bilirubin which can cross the blood brain barrier .The clinical manifestations of acute bilirubin encephalopathy can be insidious and progress rapidly to severe life threatening illness.

Kernicterus is the chronic sequelae of acute bilirubin encephalopathy. The incidence of kernicterus is unknown.

Najib ks et al(2013)[53]:- This prospective study had been performed on all infants less than 28 days referred to Namazi hospital from February 2009 to February 2010 due to severe indirect hyperbilirubinemia (bilirubin more than 95 percentile on the hour specific Bhutani namogram[54]. History including birth weight, level of education of their mother , mothers age , mothers knowledge about the effect of hyperbilirubinemia on neonate, the onset time of hyperbilirubinemia , the onset of breast feeding, history of formula feeding, history of intravenous oxytocin infusion during labor , technique of delivery , times of first medical visit of neonate, the family history of jaundice and their cause in other siblings were taken from all mothers , complete physical examination including weight on admission , signs of kernicterus (e.g. opistotonus and convulsion) were performed carefully. Lab data including complete blood count, direct serum bilirubin, G6PD level, blood group and RH type of mother and neonate and C- reactive protein (CRP) were taken in all those entire neonate to define the cause of hyperbilirubinemia. Bilirubin tested with diazo method, and G6PD level was checked with spot Florence method. Statistical analysis was done with using Chi- square test and T-test. From 1134 neonates with indirect hyperbilirubinemia re referred to Namazi hospital from February 2009 to February 2010.170neonates were included in this study according to inclusion and exclusion criteria. Ninety of them were male (52.2%) and 71(41.8%) were female.125 neonate were delivered with normal vaginal method and 45 neonates with cesarean section method, 66 neonate used oxytocin during labor. Birth weight of neonates was 3068 g (min: 1550g max: 4300g). In this study 19 neonate developed jaundice in first 24 hours after birth, 137 neonate developed jaundice after discharging from hospital. Time of first feeding of neonate was 3.99 hours after

birth .one hundred fifty four neonates has exclusive breast feeding, three neonates have exclusive formula feeding and 12 neonates had both breast feeding and formula feeding. 46 neonates (27.9%) had history of jaundice in siblings (12.4% need phototherapy 5.3% need exchange phototherapy. G6PD deficiency (30.7%) ABO and RH incompatibility (5.8%) and unknown (50%). Two neonates had signs of kernicterus .cause of severe hyperbilirubinemia was ABO and RH incompatibility (5.9%), G6PD deficiency (25.5%), sepsis (12%). Other causes such as immune hemolytic anemia (3.5%) and unknown (53.1%).in this study the prevalence of severe hyperbilirubinemia was 15% in all neonates icter. Mirfazeli et al reported that prevalence of hyperbilirubinemia was 12% in north Iran [(55)]. Gilbert syndrome is a common entity in iran probably due the higher number of consanguineous marriages in Fars province. In this study the cause of severe hyperbilirubinemia similar to other previous studies in the world was ABO and RH incompatibility (5.9%), G6PD deficiency (25.5%). Sepsis (12%), other causes (3.5%), causes of severe hyperbilirubinemia in this study were 53.1% unknown.

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CHAPTER 4

Material

And

Methods

Chapter 4

4.0 Material and Method

This cross sectional study was conducted among the women who gave birth to their children at SIKMS medical college hospital Bemina Srinagar, from 5 January 2016 to 20 April 2016. The study included a total of 100 subjects. All the subjects were the case of neonatal jaundice. They were diagnosed on the basis of the elevated level of bilirubin. Blood samples were collected from neonates for the estimation o serum bilirubin. History of all subjects was recorded. A questionnaire was filled for each mother infant pair at the time of delivery and completed at the time of nursery discharge. Mothers were asked to bring their babies to hospital in case of jaundice .they were also followed by phone in case of jaundice or readmission. They were asked to bring their infant if they were jaundiced. The medical records of infants who were readmitted were reviewed and, in case of readmission, abstracted.

4.1 METHOD ANALYSIS

All the serum samples were analyzed by automation using the Beckman coulter .The precision of instrument was checked on many occasions. All the analytical procedures were standardized the reagents were calibrated.

4.1.1 Estimation of total bilirubin by VD reaction

Summary:-

80-85% of bilirubin produced daily originates from hemoglobin released by the breakdown of senescent erythrocytes, the remaining 15 to 20 % results from the breakdown of heame containing proteins such as myoglobin, cytochromes, catalases and from bone marrow as a result of infective erythropoiesis. A number of diseases affect one or more of the steps. Involved in the production, uptake, storage, metabolism and excretion of bilirubin, Depending on the disorder unconjugated or the conjugated bilirubin or both are major contributors to the resulting hyperbilirubinemia.

Principle

A stabilized diazonium salt 3, 5-dichlorophenyldiazonium tetrafluoroborate (DPD), reacts with conjugated bilirubin directly and with unconjugated bilirubin in the presence of an acceleter to form azobilirubin. The absorbance at 540 nm is proportional to the Total bilirubin concentration. A separate sample blank is performed to reduce endogenous serum interference.

4.1.2 Estimation of bilirubin by JENDRASSIK / GROF METHOD

Summary

85% bilirubin originates on degradation of hemoglobin with the other 15% being derived from cytochromes, myoglobin and catalases, uncnjugated bilirubin which binds to plasma albumin, is produced in the course of degradation in the reticuloendothelial system, liver kupffer cells, spleen and bone marrow. Unconjugated (primary and indirect) bilirubin is soluble in lipids and toxic. With the aid of the enzyme glucoronyl transferase, bilirubin is conjugated primarily by glucoronic acid in the microsomes of hepatic parenchymal cells. In contrast to uncnjugated bilirubin, conjugated (secondary, direct) bilirubin is soluble in water and excreted through the kidneys. Bilirubin assays are suitable for evaluating the degree of severity of icteric clinical symptoms. Distinguishing between direct and indirect bilirubin is a valuable aid in the differential diagnosis < 20% total bilirubin is an indicator of jaundice of pre-hepatic origin. This value can increase to > 50% in hepatic and post hepatic jaundice.

Principle

In the presence of caffeine accelerator Total bilirubin couples with sulphanilic acid to form a red azobilirubin dye. The color of intensity of which is proportional to the bilirubin concentration. Determination of direct bilirubin is performed without caffeine additive.

Chapter 5

Result

And

Discussion

RESULTS

From 100 neonates with indirect hyperbilirubinemia referred to SKIMS medical college hospital Bemina Srinagar from January 2016 to April 2016, 100 neonates were included in this study according to inclusion and exclusion criteria. 30 of them (30%) were taken as control having mean total serum bilirubin level was 10.25, in preterm babies (n =15) mean total serum bilirubin level was 23.88 and in term babies (n=55) mean serum bilirubin level was 16.75 .this shows that serum bilirubin level is higher in case of preterm babies as compared to term babies.

Table 1:- Table shows the mean and standard deviation of the serum total bilirubin levels in controls, preterm and term babies

Parameters	Total serum bilirubin in Mg/dl		
	Control	Preterm	Term
Mean	10.25	23.88	16.76
Standard dev.	5.77	16.52	4.31
P-value	0.46	0.81	0.24

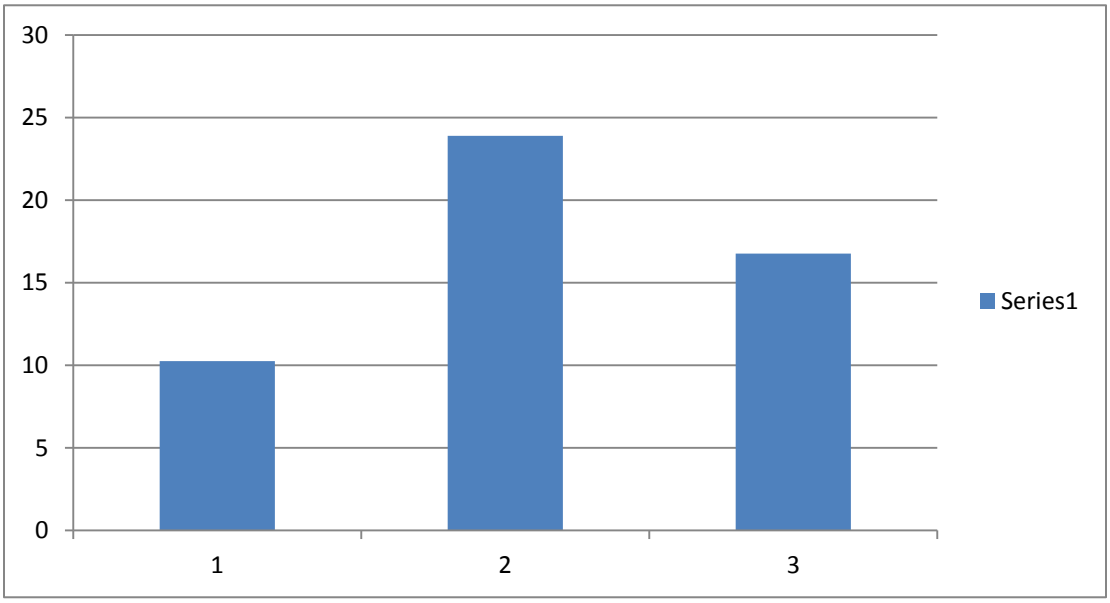

Fig. Graph showing mean total serum bilirubin level in 1- controls 2- preterm 3- term babies

DISCUSSION

In this study prevalence of severe hyperbilirubinemia was 15% in all neonates. The level of indirect bilirubin was high in neonates this is because due to the excessive lyses of RBC's. In this study we mostly see the hepatic jaundice because liver is unable to conjugate all the bilirubin. Increased production of bilirubin, deficiency of hepatic uptake, impaired conjugation of bilirubin and increased entrohepatic circulation of bilirubin account for most cases of pathologic jaundice in newborn infants. Increased production of bilirubin occurs in infants in various racial groups as well as in infants with blood group incompatibilities erythrocyte enzyme deficiencies [(56)].the another reason also for this hepatic jaundice is deficient hepatic uptake of bilirubin as occurs in patients with Gilbert's syndrome deficiency of uridine diphosphate glucoronosyl transferase. The enzyme required for the conjugation of the bilirubin [(57)]. Increased entrohepatic circulation of bilirubin in the fasting state can also exaggerate hyperbilirubinemia. New born infants who are not feeding well or who are exclusively breast fed have low levels of the intestinal bacteria that are capable of converting bilirubin to nonresorbable derivatives and the entrohepatic circulation of bilirubin may be increased in such infants. The prolonged jaundice may lead to kernicterus. Prematurity is also the second leading cause of neonatal jaundice in neonates. Preterm newborns are prone to developing jaundice due to immaturity of their bilirubin conjugation system, higher rate of hemolysis, increased entrohepatic circulation and decreased caloric intake. In this study we found that preterm babies more serum bilirubin levels as compared to term babies. Kernicterus refers to the neurologic consequence of the deposition of the uncnjugated bilirubin in brain tissue. Subsequent damage and scarring of basic ganglia and brain stem nuclei may occur.

if the serum bilirubin level exceeds the binding capacity of albumin, unbound lipid soluble bilirubin crosses the blood brain barrier if damage has occurred because of acidosis, hypoxia. If significantly elevated serum bilirubin levels are left untreated kernicterus may occur with associated disabling neurologic dysfunction and possibly death.

Conclusion

Results of this study show that there was a difference in the serum bilirubin levels in the term and preterm babies. This study shows that the serum total bilirubin level was high in the preterm babies as compared to term babies this is because due to underdeveloped liver. The liver is unable to conjugate all bilirubin. However in some infants serum bilirubin levels may raise excessively, which can be cause for concern because uncnjugated bilirubin is neurotoxic and can cause death in newborns and lifelong neurologic problem in infants who survive (kernicterus).the term bilirubin encephalopathy is used to refer acute neurological dysfunction associated with hyperbilirubinemia. While kernictures is strictly speaking a pathological term and it is often used to refer to the long term neurodevlopmental effects of bilirubin encephalopathy in most cases the etiology of this disorder is multifactorial

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Chapter 6

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