FACTORS ASSOCIATED WITH NEONATAL HYPERBILIRUBINEMIA IN THE FIRST 2 WEEKS OF LIFE IN OLA DURING CHILDREN’S HOSPITAL IN FREETOWN, SIERRA LEONE

A Dissertation in Partial fulfillment for the Degree of Master of Medicine in Pediatrics and Child Health at the University of Nairobi

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2014
DECLARATION

I certify that this dissertation is my original work, and has not been presented for any other award in any other University.

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This dissertation is dedicated to my parents whose love and encouragement in life made me what I am.
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May the Almighty God richly bless you all.
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<tr>
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<th>Full Form</th>
</tr>
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<tbody>
<tr>
<td>AAP</td>
<td>American Academy of Pediatrics</td>
</tr>
<tr>
<td>BBB</td>
<td>Blood Brain Barrier</td>
</tr>
<tr>
<td>BIND</td>
<td>Bilirubin Induced Neurologic Dysfunction</td>
</tr>
<tr>
<td>DAT</td>
<td>Direct Anti-globulin Test</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylene diamine tetracetic acid</td>
</tr>
<tr>
<td>ET</td>
<td>Exchange Transfusion</td>
</tr>
<tr>
<td>GA</td>
<td>Gestational Age</td>
</tr>
<tr>
<td>G6PD</td>
<td>Glucose-6-Phosphate Dehydrogenase</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>KNH</td>
<td>Kenyatta National Hospital</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide Adenine Dinucleotide Phosphate</td>
</tr>
<tr>
<td>NICU</td>
<td>Neonatal Intensive Care Unit</td>
</tr>
<tr>
<td>NNJ</td>
<td>Neonatal Jaundice</td>
</tr>
<tr>
<td>ODCH</td>
<td>Ola During Children’s Hospital</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cell</td>
</tr>
<tr>
<td>Rh</td>
<td>Rhesus</td>
</tr>
<tr>
<td>TSB</td>
<td>Total Serum Bilirubin</td>
</tr>
<tr>
<td>UDPGA</td>
<td>Uridine diphosphoglucuronic acid</td>
</tr>
<tr>
<td>UON</td>
<td>University of Nairobi</td>
</tr>
<tr>
<td>USL</td>
<td>University of Sierra Leone</td>
</tr>
<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
</tr>
<tr>
<td>VLBW</td>
<td>Very Low Birth Weight</td>
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</table>
# DEFINITIONS

**Jaundice:** Also called icterus, refers to yellow color of the skin and sclera caused by deposition of bilirubin secondary to increase bilirubin level in the blood\(^5\).

**Neonatal Jaundice** Presence of visible jaundice in neonates if bilirubin level is greater than 85 µmol/l.

**Neonatal Hyperbilirubinemia:** Bilirubin level greater than 85 µmol/l.

**Visible Jaundice:** Jaundice detected by a visual inspection.

**Significant Neonatal Jaundice:** An elevation of the serum bilirubin to a level requiring treatment: either by phototherapy or exchange transfusion.

**Preterm:** Less than 37 weeks gestational age.

**Term:** 37 weeks or more gestational age.
ABSTRACT

**Background:** Jaundice which result from increased levels of bilirubin is a common and important condition that require medical attention in the newborn period. In most infants, unconjugated hyperbilirubinemia reflects a normal transitional phenomenon but when serum bilirubin levels excessively rise, death may occur and infants who survive may get lifelong neurologic sequelae. Knowledge of the contributing factors for neonatal jaundice in a given setting would be valuable to help identify strategies for risk reduction.

**Objectives:** To describe the factors associated with neonatal hyperbilirubinemia in the first 2-weeks of life in Ola During Children’s Hospital in Freetown, Sierra Leone.

**Methods:** This hospital based descriptive cross-sectional study was carried out on neonates less than 2 weeks of age in Ola During Children’s Hospital (ODCH). Demographic, clinical and laboratory data: bilirubin levels, glucose-6 phosphate dehydrogenase (G-6PD) activity, blood group of both mother and baby, complete blood counts, VDRL, peripheral blood film and reticulocyte count, were collected to describe the factors associated with jaundice. A sample size of 95 neonates was studied.

**Results:** Out of 95 neonates 68.4% were male and 31.6% female. The median age of neonates was 5.00 days (IQR 3.00 – 8.00). The associated factors in the 95 jaundiced babies were: prematurity 26 (27.4%), probable sepsis 24(26.7%), ABO incompatibility 11(11.6%), cephalohematoma 9(9.5%), G6PD deficiency 7(7.6%) and Rhesus incompatibility 6(6.3%). Jaundice occurred in 24(25.2%) in whom no possible associated factor was found. Twenty one mothers (22.1%) knew their blood group and 15 (16.1%) was done during pregnancy, of 7 tested for syphilis, none was positive and 41 (43.2%) indicated they took herbal medicine during pregnancy.

**Conclusions:** The prevalence of prematurity, sepsis, ABO incompatibility, Rhesus incompatibility, Cephalohaematoma and G6PD deficiency was high in neonates with jaundice. Only 5(5.2%) of the mothers had HIV, Urinalysis, VDRL and blood group tested during pregnancy.
**Recommendations:** screening of babies with jaundice should include blood group, direct coombs test, reticulocyte count, complete blood count and bilirubin levels. All women should be tested for ABO and RhD as early as possible during each pregnancy, preferably at their first trimester visit. RhD negative women should be administered prophylactic RhD immunoglobulin both during pregnancy and after delivery. ANC record that covers prenatal screening for blood group, HIV, VDRL, or bacteriuria, should be held by women to ensure that all essential information is readily available to the caregiver.
1. INTRODUCTION AND LITERATURE REVIEW:

1.1. INTRODUCTION
Jaundice is the yellowish discoloration of the skin, sclera and mucous membranes resulting from deposition of bilirubin. Neonatal hyperbilirubinemia is defined as serum bilirubin greater than 85µmol/l (5mg/dl). Neonatal jaundice is a very common condition worldwide occurring in up to 60% of term and 80% of preterm newborns in the first week of life. Jaundice is one of the most common conditions requiring medical attention in newborn babies. The most common cause of readmission within neonatal period is jaundice as Ransome-Kuti reported that the condition was the commonest cause of neonatal admission to the Children’s Emergency room in Lagos University Teaching hospital. Studies done in Warri, Delta State, Nigeria, and in Federal Medical Centre Abakaliki, South East Nigeria revealed that neonatal jaundice accounted for 33% of the babies admitted and 35% of all Neonatal Intensive Care Unit (NICU) admissions respectively.

Neonatal Jaundice (NNJ) is also a common pediatric problem associated with high morbidity and mortality. Neonatal hyperbilirubinaemia is a recognized cause of brain damage, with unconjugated bilirubin causing kernicterus, which results in a dyskinetic movement disorder, gaze abnormalities and sensori-neuronal hearing loss as long-term sequelae. A retrospective study in a district hospital in rural Kenya found neonatal jaundice subjects had significantly more neurological, motor and developmental difficulties with 43% of the neonatal jaundice subjects unable to sit and/or stand independently and 18% died after discharge. Neonatal jaundice accounted for 24% of deaths of over 1000 admissions during the first 7 days of life, in a similar study carried out in a hospital in rural Kenya.

1.2. LITERATURE REVIEW

1.2.1. Pathophysiology of Bilirubin Metabolism
Bilirubin is produced in the reticuloendothelial system as the end product of heme catabolism and is formed through oxidation-reduction reactions. Approximately 75% of bilirubin is derived from hemoglobin, but degradation of myoglobin, catalase and cytochrome also contributes. In the first oxidation step, biliverdin is formed from heme through the action of hemeoxygenase, releasing iron and carbon monoxide (CO) that is the rate-limiting step. The iron is conserved for
reuse, but CO is excreted through the lungs and can be measured in the patient’s breath to quantify bilirubin production. Next, water-soluble biliverdin is reduced to bilirubin. Due to its hydrophobic nature, unconjugated bilirubin is transported in the plasma tightly bound to albumin. A minute fraction of unconjugated bilirubin in serum is not bound to albumin.

When it reaches the liver, bilirubin is transported into liver cells, where it binds to ligandin. Bilirubin is bound to glucuronic acid (conjugated) in the hepatocyte reticulum in a reaction catalyzed by uridine diphosphoglucuronyl transferase (UDPGT) making it water-soluble. Water-solubility allows conjugated bilirubin to be excreted into bile. Once excreted into bile and transferred to the intestines, bilirubin is eventually reduced to colorless tetrapyroles by microbes in the colon. However some deconjugation occurs in the proximal small intestine through the action of B-glucuronidases located in the brush border. This unconjugated bilirubin can be reabsorbed into the circulation, increasing the total plasma bilirubin pool. This cycle of uptake, conjugation, excretion, deconjugation and reabsorption is termed ‘enterohepatic circulation’.11

1.2.2. Risk factors for Hyperbilirubinemia in Newborns

Physiological Jaundice
Under normal circumstances, the level of indirect reacting bilirubin in umbilical cord serum is 17-51µmol/l and rises at a rate of less than 85µmol/l/24hrs. Thus jaundice becomes visible on the 2nd -3rd day usually peaking by the 3rd day at 85-102µmol/l and decreasing to below 34µmol/l between 5th and 7th day of life.12 This type of jaundice is referred to as physiological and may result from defective transport of bilirubin into hepatocyte, low activity of bilirubin conjugating enzyme, excessive load of bilirubin to liver than it can conjugate.13

Pathological Jaundice
Appearance of jaundice within 24hours, increase in serum bilirubin beyond 85µmol/l/24hrs, serum bilirubin more than 255µmol/l, direct bilirubin greater than 34µmol/l at any time, presence of clinical jaundice beyond 2weeks and conjugated bilirubin (dark urine staining the clothes) would be categorized under pathological jaundice.14 Infants without identified risk factors rarely
have TSB level above 205µmol/l. As the number of risk factors increases, the potential to develop markedly elevated bilirubin levels also increases.

Maternal factors include blood type ABO or Rh incompatibility, breastfeeding, drugs such as diazepam and oxytocin, maternal illness such as gestational diabetes. Neonatal factors include birth trauma such as cephalohaematoma or cutaneous bruising; drugs such as chloramphenicol, sulfisoxazole acetyl with erythromycin ethylsuccinate; excessive weight loss after birth; infections; infrequent feedings; male gender; polycythemia; G-6PD deficiency; hereditary spherocytosis; cholestatic syndromes; Crigler- Najjar syndrome; prematurity and previous sibling with hyperbilirubinemia.¹¹

The incidence and risk factors for NNJ vary according to ethnic and geographical differences. In developed countries risk factors include hemolytic diseases (Rhesus isoimmunization and ABO hemolytic disease), prematurity and sepsis. In Nigeria, neonatal sepsis, prematurity, G-6PD deficiency, native herbs and contact with naphthalene balls contaminated clothes have been identified as risk factors for NNJ.¹⁵ Severe NNJ can therefore be said to have modifiable risk factors particularly in developing countries. Palmer and Drew, in a review of jaundiced newborn infants in Australia over a ten-year period found that prematurity was the most common etiological factor (20%) followed by ABO erythroblastosis (7%), sepsis (3%), Rhesus erythroblastosis (3%), bruising (2%) and G-6PD deficiency (0.5%).¹⁶ Trotman et al in their study found that the etiology of jaundice in the infant was attributed to ABO incompatibility in 35%, infection in 18%, prematurity in 11%, G-6PD deficiency in 5%, rhesus incompatibility in 3.5% and no cause was identified in 9% infants.¹⁷

**Rh isoimmunization:**

Erythroblastosis due to Rh incompatibility is still an important cause of hyperbilirubinemia. There are no inborn antibodies in the Rhesus blood group system. The Rh antibody is produced by an Rh negative mother in response to the presence of Rh antigen on the fetal red blood cell (RBC) membrane. The initial maternal response to this antigenic stimulus produces IgM antibodies, which do not cross the placenta. Later IgG antibodies are formed that cross into the fetus and attaches to antigenic sites on the RBC membrane.¹⁸
ABO incompatibility
ABO incompatibility can occur if the mother and the baby have different blood types. It most commonly occurs when the mother has type O blood and the baby has type A, B or AB blood. The cause of ABO incompatibility is reaction of maternal anti-A or anti-B antibodies to the A or B antigen on the red blood cells of the fetus or newborn. It is seen usually only in type A or B neonates born to type O mothers because these mothers make anti-A or anti-B antibodies of the IgG class which crosses the placenta, while mothers of type A or B usually make anti-A or anti-B antibodies of IgM class which do not cross the placenta. Jaundice of ABO incompatibility usually appears within the first 24-72hrs after birth.12, 13 Trotman et al in their study found out that the prevalence of NNJ due to ABO incompatibility was 35% and due to Rh isoimmunization was 3.5%.17

G-6PD deficiency
G-6PD deficiency is the commonest inherited red cell enzymopathy worldwide, affecting about 400 million people globally and affecting as many as 4,500,000 newborns worldwide each year.13,19 The G-6PD gene is located on the X chromosome and hemizygous males have the full enzyme deficiency. G-6PD deficiency affects all races, the highest rates (up to 34%) are found in tropical Sub-Saharan Africa, where it has also been associated with a protective effect against plasmodium falciparum malaria. The interaction between G-6PD deficiency and NNJ is often exacerbated in populations such as in West Africa, where polymorphism for the UGT1A1 gene commonly associated with Gilbert’s syndrome is prevalent either in its heterozygous or homozygous form.20

G-6PD is an enzyme found in normal RBC that catalyzes the first reaction in the pentose phosphate pathway. The G-6PD enzyme catalyzes the oxidation of glucose-6-phosphate to 6-phosphogluconate while concomitantly reducing the oxidized form of NADP+ to NADPH. NADPH, a required cofactor in many biosynthetic reactions, maintains glutathione in its reduced form. Reduced glutathione acts as a scavenger for dangerous oxidative metabolite in the cell. Since RBCs do not contain mitochondria, the pentose phosphate pathway is their only source of NADPH; therefore defense against oxidative damage is dependent on G-6PD.20 In a study done in Port Harcourt Nigeria, 400 neonates with jaundice were recruited out of whom 210 (52.5%)
had G-6PD deficiency. Among the G-6PD deficient neonates, 69% were males while 31% were females.  

**Infectious causes**

Sepsis is one of the important treatable problems associated with bilirubin production. The hyperbilirubinemia in septic neonates is thought to be a consequence of rapid hemolysis. Neonatal erythrocytes are susceptible to cell injury in response to oxidative stress. In addition hemeoxygenase is induced by oxidants and its induction could lead to increased catabolism of heme to bilirubin. Intrauterine infections may cause giant cell hepatitis and jaundice anytime during neonatal period. Jaundice is a recognized feature of congenital and neonatal malaria. Onyearuugha et al from Nigeria, in their study found out that the leading aetiological factors of NNJ were septicaemia (32.5%) and prematurity (17.5%).

**Breastfeeding Jaundice**

Breastfeeding jaundice usually occurs early in life (2-3 days) after birth due to insufficient milk intake. Decreased frequency of breastfeeding is associated with exaggeration of physiological jaundice. The jaundice associated with breastfeeding in the first few days after birth appears to be related to an increase in the enterohepatic circulation of bilirubin. This occurs in the first few days because until the milk has ‘come in’ breastfed infant nutrient intake is limited, with fewer calories thereby prolonging the intestinal transit time and passage of fewer stools in the first few days of life suggesting that increased amount of bilirubin is absorbed into the enterohepatic circulation. Another factor in decreased breast milk is reduction in Y protein synthesis which leads to decreased uptake of bilirubin in hepatocytes.

**Breast milk Jaundice**

Breast milk jaundice is of late onset and has an incidence in term newborns of 2 to 4%. A strong familial predisposition is suggested by the recurrence of breast milk jaundice in siblings. It is characterized by indirect hyperbilirubinemia in a breastfed newborn that develops after the first 4-7 days of life, persists longer than physiologic jaundice (can persist for 3-12 weeks) and has no other identifiable cause. If breastfeeding continues, bilirubin returns to normal by 4-12 weeks of
age. If breastfeeding is stopped, the bilirubin level will fall rapidly in 48 hours. These neonates show good weight gain, have normal liver function test results and show no evidence of hemolysis. The etiology of breast milk jaundice is not clearly understood, but the following factors have been suggested to play a role: an unusual metabolite of progesterone inhibits UDPGA glucuronyltransferase; increased concentration of nonesterified free fatty acids; increased enterohepatic circulation of bilirubin due to increased content of beta glucuronidase activity in breast milk; inflammatory cytokines in human milk; and high epidermal growth factor levels in breast milk may be responsible for jaundice in these neonates. The reduced gastrointestinal motility and increased bilirubin absorption and uptake are thought to be the mechanisms. 12,13,21

1.2.3. Bilirubin Encephalopathy
Unconjugated bilirubin is potentially toxic to neural tissue (brain and spinal cord). In the neonate unconjugated bilirubin can penetrate the membrane that lies between the brain and the blood (the blood brain barrier).22 Regions most commonly affected include the basal ganglia; hippocampus; geniculate bodies and cranial nerve nuclei, such as the oculomotor, vestibular and cochlear. The cerebellum can also be affected. Kernicterus refers to an anatomic diagnosis made at autopsy based on a characteristic pattern of staining found in babies who had marked hyperbilirubinemia before they die. Bilirubin induced neurologic dysfunction (BIND) refers to the clinical signs associated with bilirubin toxicity (i.e. hypotonia followed by hypertonia and/or opisthotonus) and is typically divided into acute and chronic phases.22 Acute features include lethargy, irritability, abnormal muscle tone and posture, temporary cessation of breathing (apnea) and convulsions. Features of chronic bilirubin encephalopathy include athetoid cerebral palsy, hearing loss and visual and dental problems. There are certain factors that influence the passage of bilirubin into the brain and hence increase the risk of acute bilirubin encephalopathy. These include preterm birth, sepsis, hypoxia, seizures, acidosis and hypoalbuminemia. The rate of rise of the level of bilirubin is equally important hence the increased risk of kernicterus in babies with hemolytic disease such as G-6PD deficiency, ABO or Rhesus hemolytic disease. Kernicterus can also occur in the absence of severe hyperbilirubinemia; in this situation factors influencing
permeability of the BBB (e.g. acidosis, infection) and the amount of unbound (versus albumin-bound bilirubin may play a role).  

2. STUDY JUSTIFICATION

Neonatal morbidity and mortality remain very high in developing countries of Sub-Saharan Africa and one of the important contributors to this is neonatal jaundice. In Africa, NJ is commonly associated with sepsis which is a major contributor to neonatal mortality. Neonatal jaundice was the primary diagnosis in 17% of over 1000 admissions during the first 7 days of life, to a rural Kenyan hospital, accounting for 24% of deaths. Until recently Sierra Leone had the highest under-five mortality rate (262 per 1,000 live births in 2008) in the world. Neonatal mortality account for 20% of all under-five mortality, thus by 2008, Sierra Leone’s neonatal mortality rate was 56 per 1,000 live births. Mortality among children under five appears to be decreasing but there has been little progress in reducing neonatal deaths. As child health programs succeed in reducing deaths after the first month and year of life, an increasing proportion of under five deaths will be neonatal and action must now be taken to reduce newborn deaths. Global estimates suggest that over two thirds of newborns could be saved through existing maternal and child health programs. Newborn deaths can be reduced by strengthening newborn care within existing child and maternal programs. There are no data available on neonatal jaundice in Sierra Leone. Therefore determining the risk factors for neonatal jaundice (NNJ) is of importance, to reduce the risk for future babies to develop jaundice, for early intervention and for prophylaxis e.g. anti-D and treatment. It is hoped that the result would be a necessary tool in formulating measures of prevention, early detection and management of severe NNJ thereby reducing newborn deaths by strengthening newborn care and maternal health programs.

3. RESEARCH QUESTION

What are the associated factors for Neonatal hyperbilirubinemia in Ola During Children’s Hospital in Freetown?
4. STUDY OBJECTIVES

4.1. Primary Objective

To describe the associated factors of jaundice in the first 2 weeks of life in Ola During Children’s Hospital in Freetown. The main associated factors determined included: maternal and infant blood group (ABO and Rhesus), neonatal infections and G-6PD deficiency.

5. RESEARCH METHODOLOGY

5.1. Study design

The research design of the study was a hospital based descriptive cross-sectional study.

5.2. Study setting

Sierra Leone is located on the West African coast, bounded on the west by the Atlantic Ocean, on the north and east by Guinea and on the southwest by Liberia. The population of Sierra Leone was estimated to be 5.4 million for the year 2008.
The study was carried out at the Ola During Children’s Hospital (ODCH) in Freetown, the capital city of Sierra Leone between August and December, 2013. ODCH is Sierra Leone’s only Specialist Children’s hospital, located in the poor and densely populated eastern part of Freetown. Neonates from all over Freetown with jaundice are mostly referred to ODCH for management. ODCH serves as teaching hospital for the College of Medicine and Allied Health Sciences, University of Sierra Leone. More than 15,000 patients are treated at the hospital each year.

5.3. **Study Population**
The study subjects comprised of neonates (aged 0-14 days) with clinical jaundice admitted in Neonatal ward in Ola During Children’s Hospital.

5.4. **Inclusion Criteria**
1. All neonates with visible jaundice up to 14-days old admitted in the newborn unit of Ola During Children’s Hospital.

5.5. **Exclusion Criteria**
1. Those neonates whose guardians or parents declined to give consent.

5.6. **Sampling method**
The study applied consecutive sampling where any neonate meeting the inclusion criteria qualified for the study.

5.7. **Sample Size Determination**
The sample size was calculated using Fisher’s Formula for sample size determination:

\[
n = \frac{z^2 \times p(1-p)}{d^2} = \frac{1.96^2 \times 0.55 \times 0.45}{0.1^2} = 95
\]

- \(n\) = Sample Size
- \(z\) = Normal Standard Deviation taken with a 95% Confidence Interval; set at 1.96.
- \(p\) = Prevalence of G-6PD deficiency; Estimated at 55% as per Chime et al’s Study in Nigeria.
- \(d\) = Study Precision taken as 10%. (This was the most conservative estimate, using ABO incompatibility (37%) and septicaemia (32.5%, Onyearugha et al) yielded sample sizes of 90 and 85 respectively.)
6. DATA COLLECTION

6.1. Study Procedures
All babies admitted to the newborn unit were screened consecutively for jaundice and those with jaundice aged 14 days and below whose parents gave consent were subjected to further examination. Newborn babies (aged 0-14 days) were assessed visually for jaundice at the time of admission to the nursery and subsequently. The interviewer then informed the parents of the identified clinically jaundiced newborns in the hospital about the aim of the study and requested the parents’ consent. Consenting parents had a questionnaire administered to them to provide demographic data, gestational age determination using date of their last menstrual period, and other questions related to the study. A systemic general examination with particular attention to the factors known to be associated with hyperbilirubinemia was carried out. Four ml (4ml) of blood was collected aseptically from a superficial vein of each neonate, which was used for all parameters to be tested. A standard laboratory investigation was carried out: serum bilirubin level (total and direct), complete blood count, reticulocyte count, blood group of mother and infant, direct Coombs test on the baby’s blood, G-6PD activity, VDRL on the baby’s blood, and immature: total (IT) ratio. It was not possible to do bacteriological tests because blood culture bottles were not available. All test results were recorded and used for the purpose of this study. The results were also communicated to the guardian or parent and the doctor serving the ward.

6.3. Laboratory Methods

6.3.1. Determination of Complete Blood Count:
Blood collected into a tube containing ethylene diamine tetra acetic acid (EDTA) was analyzed for full blood count parameters by standard Coulter gram. Peripheral smear for RBC morphology, differential leukocyte count and reticulocyte count was also done.27

6.3.2 Determination of serum bilirubin
Blood collected into plain bottles was analyzed for bilirubin determination using the Jendrassik-Grof (1938) colorimetric method. For blood specimen which had to be delayed overnight or for
more than 2hrs without analysis, the blood was allowed to clot. The serum was separated from the cells into plain bottles, sealed in an envelope and refrigerated at 4°C to minimize breakdown of bilirubin by light. Direct bilirubin determination was based on the fact that direct bilirubin reacts with diazotized sulphanilic acid to form a blue colored complex in an alkaline medium. Total bilirubin was determined by the addition of caffeine which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid. The total minus the conjugated bilirubin, gave the value of unconjugated bilirubin.  

6.3.3. Blood group determination

The ABO and Rhesus blood grouping was done using the tile method. A drop of blood from each neonate was placed on a clean white tile in three places. A drop of each of the antisera, anti A, anti B and anti D were added and mixed with each blood sample with the aid of glass rods. Blood groups were determined on the basis of agglutination.

6.3.4. Determination of G-6PD activity

Blood samples collected in ethylene diamine tetra acetic acid (EDTA) tubes were delivered immediately to the laboratory. The specimen was stored at 4°C if the screening could not be done immediately. The definitive diagnosis was based on the estimation of enzyme activity by quantitative spectrophotometric analysis of the rate of NADPH production from NADP. The red cell G-6PD activity, expressed as units per gram of hemoglobin (u/gHb), was determined by an enzymatic calorimetric assay for the quantitative determination of G-6PD deficiency using a commercial kit. The assay was performed according to the instructions included in the kit.
6.2. Criteria for diagnosis:

6.2.1. Neonatal Sepsis causing jaundice

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life.

Blood culture bottles were not available therefore sepsis was not proven. Probable sepsis was diagnosed when clinical signs and symptoms and hematological findings were consistent with bacterial infection without a positive culture. No sepsis was considered when clinical signs/symptoms subsided within 24 hours and no hematological findings attributable to sepsis.

Clinical findings: simultaneous presence of any two of seven signs: history of difficulty feeding; history of convulsions; movement only when stimulated; respiratory rate of 60 breaths per minute or more; severe chest in-drawing and temperature of 37.5ºC or more or below 35.5ºC.\(^{31}\)

Hematological findings: total leucocyte count <5,000 or >20,000/cmm, Immature/Total neutrophil >0.2, neutropenia/neutrophilia: as per Manroe chart for term and Mouzinho’s chart for Very Low Birth Weight (VLBW) infants, and platelet counts <50,000/mm.\(^{32-34}\)

Criteria for diagnosis: simultaneous presence of any 2 of 7 signs and the criteria of Manroe with 2 of 3 indices (total PMN count, immature PMN count, and I/T ratio) abnormal was used as the criteria for probable sepsis.

6.2.2. Blood group incompatibility causing neonatal hyperbilirubinemia

For the purpose of this study, ABO hemolytic disease as a cause of neonatal hyperbilirubinemia was diagnosed when mother was blood group O and infant blood group A, B or AB.

Rh incompatibility was diagnosed when mother was Rh negative and the baby Rh positive with the presence of hemolysis. Hemolysis was considered to be present if: the baby developed a reticulocytosis of greater than 6% and/or direct Coombs test on the baby’s blood is positive.\(^{13}\)

6.2.3 G-6PD deficiency:

Any neonate with an activity below 6.4u/gHb, was considered G-6PD deficient.\(^{35}\)
7. DATA MANAGEMENT AND STATISTICAL ANALYSIS

Data was collected using structured proforma and entered into a password protected Microsoft Access Database. The hard copy data forms were stored in a lockable cabinet in the Principal Investigator’s office during data collection and in the statistician’s office during entry. Upon completion of Data entry, hard copy forms were compared with the entered data to identify errors and corrections made appropriately.

Descriptive statistics were carried out where discrete variables were summarized with frequencies and percentages while continuous variables were summarized using measures of central tendency such as mean, median, mode and standard deviation.

To describe risk factors for neonatal jaundice, proportion of children who had sepsis, ABO incompatibility, Rhesus incompatibility, G6PD deficiency and Prematurity were estimated and the result compared with socio-demographic statistics and other health factors such as bilirubin levels

Data was presented in the form of graphs, pie charts, tables and narrative.

ETHICAL CONSIDERATIONS

The study was undertaken after approval by the Department of Paediatrics, University of Nairobi, the Kenyatta National Hospital-University of Nairobi Ethical Review Committee and the Ola During Children’s Hospital Medical Advisory Committee.

Informed written consent to participate in the study was obtained from the parent or guardian of the neonate after explanation of the study and voluntary nature of participation. Caregivers were informed that refusal to participate in the study will not affect treatment of their child. Confidentiality was maintained. Personal details such as name of the patient were not recorded.

Any information pertinent to the management of the child discovered during the interview was communicated to the attending doctor. No neonate suffered delayed treatment as a result of the study. The parent/guardian was informed on the laboratory findings. The primary doctor of the neonates under study had access to data regarding laboratory findings. Standard protocols were used to institute therapy for neonatal jaundice.36,37
RESULTS

Between August 2013 and December 2013, ninety five babies who were jaundiced and their mothers were enrolled into the study, at Ola During Children’s Hospital.

**Figure 1**: Flow chart for selection of study subjects
Neonatal characteristics

Neonatal demographics are shown in Table 1. The median age of neonates on admission was 5 days (IQR 3.00 – 8.00). The majority 65 (68.4%) of infants were male while 30 (31.6%) were females. The median gestational age was 39 weeks (IQR 37.00 – 42.00). There were 26 (27.4%) preterm and 69 (72.6%) term neonates. The median birth weight of the study population was 2.9 kilograms (IQR 2.30 – 3.40). Thirty four (36.2%) had low birth weight.

Table 1: Neonatal Socio-demographic characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Frequency (%)</th>
<th>Median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonatal Age in Days (IQR)</td>
<td>5.00(3.00 – 8.00)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>65(68.4)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>30(31.6)</td>
<td></td>
</tr>
<tr>
<td>Gestational Age in Weeks (IQR)</td>
<td>39(37.00 – 42.00)</td>
<td></td>
</tr>
<tr>
<td>Birth weight in Kgs (IQR)</td>
<td>2.9(2.30 – 3.40)</td>
<td></td>
</tr>
<tr>
<td>Age at presentation of jaundice in Days (IQR)</td>
<td>3.00(2.00 – 3.00)</td>
<td></td>
</tr>
<tr>
<td>Previous jaundice Sibling</td>
<td>6(6.3)</td>
<td></td>
</tr>
</tbody>
</table>

Majority 30 (31.9%) of babies presented on 2nd day of life, followed by 29 (30.9%) on 3rd day of life, then 12 (12.8%) on 1st day and 9 (9.6%) on 4th day of life. The median age of jaundice presentation was at 3 days of life (IQR 2.00 – 3.00). Of all enrolled babies, 6 (6.3%) had a history of jaundice in previous siblings. Forty – one (47.1%) infants were exclusively breastfed, 5 (5.7%) were fed formula and the rest received mixed feeding 19 (20.00%) or fluid 24 (25.2%). The median Haemoglobin level was 14.2 g/dL (IQR 11.75 – 16.00). The median total bilirubin was 87.50 µmol/l (IQR 52.50 – 157.50). None of the babies had a positive VDRL result.
Maternal characteristics

The median maternal age of mothers was 23 years (IQR 19.00 -32.00). The majority of the mothers 51 (54.8%) were primiparous. Fourteen (15.1%) of the women had more than 3 prior pregnancies. Maternal demographics are shown in Table 2.

Table 2: Maternal Socio-demographic Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Frequency (%)</th>
<th>Median(IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Age (Years)</td>
<td></td>
<td>23.00(19.00 – 32.00)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Para 0</td>
<td>51(54.8)</td>
<td></td>
</tr>
<tr>
<td>Para 1</td>
<td>9(9.7)</td>
<td></td>
</tr>
<tr>
<td>Para 2</td>
<td>19(20.4)</td>
<td></td>
</tr>
<tr>
<td>Para 3+</td>
<td>14(15.1)</td>
<td></td>
</tr>
<tr>
<td>Knows blood group</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>21(22.1)</td>
<td></td>
</tr>
<tr>
<td>Blood group done during pregnancy</td>
<td>15 (16.1)</td>
<td></td>
</tr>
<tr>
<td>HIV test during pregnancy</td>
<td>85(89.5)</td>
<td></td>
</tr>
<tr>
<td>HIV positive</td>
<td>2(2.5)</td>
<td></td>
</tr>
<tr>
<td>Urinalysis test during pregnancy</td>
<td>51(53.7)</td>
<td></td>
</tr>
<tr>
<td>Positive Bacteriuria</td>
<td>16(31.4)</td>
<td></td>
</tr>
<tr>
<td>VDRL test during pregnancy</td>
<td>7(7.4)</td>
<td></td>
</tr>
<tr>
<td>Herbal drug use during pregnancy</td>
<td>41(43.2)</td>
<td></td>
</tr>
<tr>
<td>Prolong Rupture of membrane (≥ 18 hrs)</td>
<td>27(29.0)</td>
<td></td>
</tr>
<tr>
<td>Oxytocin use</td>
<td>39(48.1)</td>
<td></td>
</tr>
<tr>
<td>Place of Delivery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td>16(16.8)</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>79(83.2)</td>
<td></td>
</tr>
<tr>
<td>Delivery Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal</td>
<td>82(86.3)</td>
<td></td>
</tr>
<tr>
<td>Caesarian Section</td>
<td>11(11.6)</td>
<td></td>
</tr>
<tr>
<td>Forceps/Vacuum</td>
<td>2(2.1)</td>
<td></td>
</tr>
</tbody>
</table>
Of 27 (29.0%) women who had prolonged rupture of membrane, 8 (29.6%) of them had rupture of membrane for more than 48 hours. Thirty nine (48.1%) women indicated receiving oxytocin during labor but 6 (7.4%) did not know whether they had been given or not. Sixteen (16.8%) of the women had delivered at home. Eighty two (86.3%) of the women had spontaneous vertex delivery, 11 (11.6%) had caesarian section and 2 (2.1%) had vacuum/forceps mode of delivery.

Of all enrolled mothers 21 (22.1%) knew their blood group and 15 (16.1%) had blood group testing during pregnancy. All 95 mothers had their blood group tested during the study. Among 85 (89.5%) who were tested for HIV during pregnancy, 2 (2.5%) were HIV positive. Fifty one (53.7%) of the women did urinalysis test for bacteriuria during pregnancy, 16 (31.4%) had a positive bacteriuria result. Of 7 tested for syphilis, none was positive and 41 (43.2%) indicated they took herbal medicine during pregnancy. Only 5 (5.2%) of the women received complete assessment including HIV, Urinalysis for bacteriuria, VDRL and blood group tests during pregnancy as shown in table 3.

Table 3: Proportion of mothers tested for HIV, VDRL, urinalysis and blood group during pregnancy

<table>
<thead>
<tr>
<th>Tests conducted during pregnancy</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>85 (89.5)</td>
</tr>
<tr>
<td>Blood group</td>
<td>15 (16.1)</td>
</tr>
<tr>
<td>Urinalysis</td>
<td>51 (53.7)</td>
</tr>
<tr>
<td>VDRL</td>
<td>7 (7.4)</td>
</tr>
</tbody>
</table>

Blood Group distribution

The distribution of blood group among the mothers and the babies is shown in Figure 2. Blood group O+ was the most common in both the mothers and the babies at 54 (56.8%) and 40 (42.1%) respectively. The majority of mothers had blood group O 42 (44.5%), followed by A 26 (27.4%), then B 24 (25.3%) and AB 3 (3.2%). There were 6 (6.3%) Rhesus negative mothers. Four (66.7%) of the rhesus negative mothers were multiparous, with three or more previous pregnancies and two (33.3%) were primiparous. There were 54 (56.8%) infants with blood group
O, followed by B 22 (23.2%), then A 15 (15.8%) and AB 4 (4.2%). None of the babies had a positive direct Coombs result.

![Blood group distribution of mothers and their babies](image)

Figure 2: Blood group distribution of mothers and their babies

Factors associated with neonatal jaundice (Table 4)
The leading associated factors of neonatal jaundice in the 95 babies were prematurity found in 26 (27.4%), septicemia 24 (26.7%) and ABO incompatibility 11 (11.6%). Out of the remaining 34, cephalohaematoma was diagnosed in 9 (9.5%) neonates, G6PD deficiency in 7 (7.6%) neonates, Rhesus incompatibility in 6 (6.3%) neonates and polycythaemia in one neonate. Jaundice occurred in 24(25.3%) in whom no possible risk factor was found.
Table 4: Associated factors in neonates with jaundice

<table>
<thead>
<tr>
<th>Associated factors</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 95</td>
<td></td>
</tr>
<tr>
<td>Prematurity only</td>
<td>26 (27.4)</td>
</tr>
<tr>
<td>Probable sepsis</td>
<td>24 (26.7)</td>
</tr>
<tr>
<td>ABO incompatibility</td>
<td>11 (11.6)</td>
</tr>
<tr>
<td>Cephalohaematoma</td>
<td>9 ( 9.5)</td>
</tr>
<tr>
<td>G6PD deficiency</td>
<td>7 ( 7.6)</td>
</tr>
<tr>
<td>Rhesus incompatibility</td>
<td>6 ( 6.3)</td>
</tr>
<tr>
<td>Probable Sepsis + ABO incompatibility</td>
<td>5(5.3)</td>
</tr>
<tr>
<td>ABO incompatibility + G6PD deficiency</td>
<td>1(1.1)</td>
</tr>
<tr>
<td>G6PD deficiency + Sepsis</td>
<td>4(4.2)</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>1 ( 1.2)</td>
</tr>
<tr>
<td>Unknown</td>
<td>24 (25.3)</td>
</tr>
</tbody>
</table>

Babies with G6PD deficiency, prematurity, ABO or Rhesus incompatibility and Sepsis had a higher median serum bilirubin than those without.

Preterms

Of the 95 neonates with jaundice, 26 (27.4%) were preterms with majority of the neonates 17 (65.3%) delivered at 34-36 weeks gestation followed by 5 (19.2%) at 31-33 weeks gestation as shown in table 5 below.

Table 5: Distribution of gestational age among preterms

<table>
<thead>
<tr>
<th>Gestation (weeks)</th>
<th>frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28-30</td>
<td>4 (4.2)</td>
</tr>
<tr>
<td>31-33</td>
<td>5 (5.3)</td>
</tr>
<tr>
<td>34-36</td>
<td>17 (17.9)</td>
</tr>
<tr>
<td>&gt;36</td>
<td>69 (72.6)</td>
</tr>
</tbody>
</table>
Comparing Associated factors in Preterm and Term neonates

Twenty three (88.5%) of the 26 preterms had associated factors for neonatal jaundice. The associated factors as presented in Table 6: twelve (46.2%) Preterms had probable sepsis, 4 (15.4%) had Rhesus incompatibility, 3 (11.5%) had ABO incompatibility, 3 (11.5%) with G6PD deficiency and 1(3.8%) had polycythemia. Three (11.5%) had no known associated factor. Rhesus incompatibility was significantly higher in preterm than in term babies with neonatal jaundice ($X^2= 4.98$, p-value= 0.02).

Table 6: Comparison of associated factors in Preterm and Term neonates

<table>
<thead>
<tr>
<th>Associated Factor</th>
<th>No. Preterm (%)</th>
<th>No. Term (%)</th>
<th>$X^2$-test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 26</td>
<td>n = 69</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Probable sepsis</td>
<td>12(46.2)</td>
<td>23(33.3)</td>
<td>1.01</td>
<td>0.315</td>
</tr>
<tr>
<td>ABO incompatibility</td>
<td>3(11.5)</td>
<td>8(11.6)</td>
<td>&lt;0.0001</td>
<td>0.994</td>
</tr>
<tr>
<td>Cephalohaematoma</td>
<td>0(0.0)</td>
<td>9(13.0)</td>
<td>3.74</td>
<td>0.053</td>
</tr>
<tr>
<td>G6PD deficiency</td>
<td>3(11.5)</td>
<td>4(5.8)</td>
<td>1.10</td>
<td>0.293</td>
</tr>
<tr>
<td>Rhesus incompatibility</td>
<td>4(15.4)</td>
<td>2(2.9)</td>
<td>4.98</td>
<td>0.026</td>
</tr>
<tr>
<td>Polycythemia</td>
<td>1(3.8)</td>
<td>0(0.0)</td>
<td>2.76</td>
<td>0.097</td>
</tr>
</tbody>
</table>

Bilirubin levels in relation to day of presentation

7(33.4%) neonates had significant hyperbilirubinemia (bilirubin > 240µmol/L) within 48hours, whereas it was found in 6 (11.5%) neonates above 48hours (p-value = 0.474). Neonates with G6PD deficiency 4(9.8%) and ABO incompatibility 7(16.7%) presented earlier in the first 48hrs, it was not significant (p-value = 0.503 and 0.178). However probable sepsis 20 (38.5%), prematurity 13 (25%) and rhesus incompatibility 3(7.7%) presented above 48 hours, (p-value = 0.802, 0.697 and 0.563).
DISCUSSION

This is the first study on neonatal jaundice in Sierra Leone. There was a general male preponderance in this study 65 (68.4%), which is in accordance with other studies. In a study carried out at the University Hospital of the West Indies, there were 103 (61%) males and 67 (39%) females. Also Onyearugha et al in Nigeria found, out of the 83 inborn babies, 46(55.4%) were males and 37(44.6%) were females. In this study, majority of neonates 75.6% developed jaundice in early neonatal life (within 1-3 days of birth) with majority 30 (31.9%) presenting on 2nd day of life, followed by 29 (30.9%) on 3rd day and 12 (12.8%) on 1st day of life. These results are comparable with the studies in which it is also mentioned that neonatal jaundice usually appears 2-4 days after birth. This observation could be readily explained by the fact that most causes of neonatal jaundice including prematurity and septicaemia, even as observed in this study often manifest in early neonatal life.

Sepsis and prematurity were the leading associated factors identified in this study occurring in 27.4% and 26.7% of the study population respectively. Studies within and outside Africa; have also observed neonatal jaundice in association with these two clinical entities. A study conducted by Onyearugha et al on Prevalence and associated factors of neonatal jaundice as seen in Federal Medical Centre Abakaliki, Southeast Nigeria, found the leading aetiological factors of neonatal jaundice to be septicaemia (32.5%) and prematurity (17.5%).

Sepsis is a cause of jaundice in the newborn period. Babies who have sepsis are likely to develop high levels of bilirubin from increased haemolysis and defective conjugation of bilirubin. In this study 51 (53.7%) of the mothers were tested for bacteriuria during pregnancy with 16 (31.4%) of them having positive bacteriuria. Also 27 (29.0%) of the women had prolonged rupture of membrane for more than 18 hours. From this study 16 (16.8%) pregnant women delivered at home, even though health care has been made free for all pregnant women and lactating mothers. Often, the traditional birth attendants and employees in the churches are inadequately trained, and practice in unhygienic environment ultimately resulting in septicaemia in the newborn. Early
booking, effective and regular attendance to antenatal care, optimum maternal nutrition and delivery in appropriate health care facility can help to reduce the incidence of prematurity and septicaemia thereby reducing the incidence of severe neonatal jaundice in the community.\(^{45}\) In Africa, neonatal jaundice is commonly associated with sepsis.\(^{23,24,25}\) A retrospective study by Israel et al in Nigeria, found sepsis and prematurity were major diagnosis identified in 45% and 20% of the study population respectively.\(^{15}\)

Prematurity was found in 26 (27.4%) neonates. Preterm newborns are prone to developing jaundice due to immaturity of their bilirubin conjugating system, higher rate of haemolysis, increased enterohepatic circulation and decreased caloric intake.\(^{46}\) Preterm babies are also prone to other clinical conditions like sepsis that may affect outcome in them.\(^{42,47}\) Twelve (46.2%) of the preterms had sepsis, 4(15.4%) had rhesus incompatibility, 3(11.5%) of the babies with prematurity also had G6PD deficiency and 3 (11.5%) had ABO incompatibility and the only neonate with polycythemia was also preterm. The prevalence of rhesus in preterms neonates with neonatal jaundice was significantly higher than in term neonates but the actual numbers were very small.

Majority of neonates in our study were having blood group O rhesus positive, followed by B rhesus positive with frequencies of 56.8% and 21.1% respectively, then A 15 (14.7%) and AB 4 (4.2%). In order to provide gene frequency values for the ABO and Rh (D) alleles in a healthy infant population in south west Nigeria by Omotade et al, 4,748 healthy infants were typed for ABO and Rh (D) blood groups over a five year period (1988-1992). Overall, 54.2% were blood group O, 21.6% were blood group A, 21.4% were blood group B and 2.8% were blood group AB.\(^{48}\) The distribution of ABO blood group varies worldwide. Blood group O was the most common blood group found in international studies except in Nepal where blood group A was the most common (34%) and O was 1.5% less common (32.5%) than A.\(^{48-50}\) In our study the majority of mothers had blood group O 42 (44.5%), followed by A 26 (27.4%), then B 24 (25.3%) and AB 3 (3.2%). There were 6 (6.3%) rhesus negative mothers. A study on blood group distribution among blood donors and antenatal care attendant by Mwangi at the Aga Khan hospital in Nairobi; blood group O was found to be the most frequent 49% followed by blood
group B 24%, then A 22% and AB 5% with 3% rhesus D negative in ANC attendants. There was no study found on blood group distribution in Sierra Leone.

ABO incompatibility is the most common form of haemolytic disease in the newborn period. Group A and group B newborns of group O mothers are defined as having ABO incompatibility. There were 11 babies with either blood group A or B and whose mother’s blood group was O. In our study out of 95 neonates, the prevalence of ABO incompatibility in jaundiced neonates was 11.6%. In this study out of 95 neonates, the frequency of Rh incompatibility in jaundiced neonates was 6.3%. Direct Coombs test on babies’ blood cells was negative in all babies. Our results are in agreement with other studies. They all agree that ABO incompatibility was more prevalent than Rh incompatibility in their studies.

Oseni et al in South-West Nigeria, found 50 (38%) mothers were ABO incompatible with their babies and Direct Coombs test (DCT) done on all the red cells of the babies were negative. In another study carried out by Jeremiah et al in Nigeria on distribution of ABO and Rh blood groups among 500 pregnant women found out that group O was 48.0%, A 41.2%, B 7.6%, and AB was 3.2%. No anti-D was identified despite 8.6% of the study population being Rhesus D (Rh D) negative.

In a study by Israel et al, sepsis and prematurity were major diagnosis identified in 45% and 20% respectively, ABO incompatibility was found in 7.6% of babies.

At University Hospital of the West Indies, ABO incompatibility (35%), infection (19%) and prematurity (11%) were more common causes of hyperbilirubinaemia than Rhesus isoimmunization (4%). Sgro et al from Canada, in their study found ABO blood group incompatibility (n= 48) to be the most common cause, followed by G6PD deficiency (n = 20), other antibody incompatibility (n = 12) and hereditary spherocytosis (n = 7).

Of the 95 mothers in the study, only 21 (22.1%) knew their blood group and 11 (11.6%) with ABO incompatibility, 74 (77.8%) of the neonates were therefore at risk of blood group incompatibility.

Glucose-6-phosphate dehydrogenase enzyme deficiency is the most common red cell enzymopathy that causes neonatal haemolysis and jaundice. It is an X- linked recessive disorder that affects males; however, deficient females may also present with haemolysis and
jaundice. Babies who are G-6-PD deficient are three times more prone to developing neonatal jaundice than G-6-PD deficient infants.\textsuperscript{43} The most devastating clinical consequence of G6PD deficiency is neonatal hyperbilirubinemia, which can be severe and result in kernicterus or even death.\textsuperscript{59} Pathogenesis of hyperbilirubinemia in G6PD-deficiency neonates include: hemolysis, decreased conjugating capacity in infants with total serum bilirubin > 15 mg/dl, and association with variant UGT-promoter for Gilbert syndrome.\textsuperscript{60,61} G6PD was diagnosed in 7.6\% neonates out of 95 neonates included in this study. G-6-PD was deficient in 4 of 65 males (6.2\%) and 3 of 30 females (10.0\%). One neonate with this enzyme deficiency also had ABO incompatibility. Three babies of the enzyme deficient group were preterm. A study by George et al to determine the prevalence G6PD deficiency among neonates at University of Port Harcourt Teaching Hospital (UPTH) Nigeria; a total of 210 neonates were G6PD deficient giving a prevalence of 52.5\%.\textsuperscript{62} In another study in Iran where a prospective descriptive study was carried out on 244 neonates with jaundice, G6PD deficient rate was 5.7\% (14/244).\textsuperscript{63} The interaction between G-6PD deficiency and neonatal jaundice is often exacerbated in populations such as in West Africa, where polymorphism for the UGT1A1 gene commonly associated with Gilbert’s syndrome is prevalent either in its heterozygous or homozygous form.\textsuperscript{20,64}

While Ahmed et al. (1995) from Nigeria reported septicaemia and G6PD deficiency as leading causes of neonatal jaundice, Mohammad from Asia documented ABO incompatibility and prematurity as the leading causes.\textsuperscript{25,65} Mohammad et al from Pakistan got a frequency of ABO incompatibility and Rh-incompatibility in jaundiced neonates to be 22.5\% and 12.5\% respectively; prematurity in 15\% neonates and G6PD in 9.5\% neonates.\textsuperscript{65} Koosha et al from Singapore found the prevalence of sepsis, ABO incompatibility, Rhesus incompatibility, cephalohaematoma, and G6PD deficiency to be 15.7\% (59 neonates), 3.7\% (14 neonates), 2.1\% (8 neonates), 0.5\% (2 neonates), and 2.1\% (8 neonates), respectively.\textsuperscript{59}

Majority of the babies, 47.1\% in this study were exclusively breastfed. Severe neonatal jaundice occurring in exclusively breast-fed infants even as in this study, has been documented previously by Olusanya et al.\textsuperscript{66} This could be explained, at least in part, by reduced intake of calories, dehydration and jaundice associated with breastfeeding. Rooming-in and breastfeeding on
demand which provide adequate calories and hydration help to reduce the incidence of severe neonatal jaundice in exclusively breast-fed newborns.\textsuperscript{5} Onyearugha et al in his study on neonatal jaundice in Nigeria, found an overwhelming majority of the inborn babies, 90.4\% were exclusively breastfed.\textsuperscript{5}

In our study only 5.2\% of women received the full screening test for HIV, syphilis, bacteriuria and blood group. Majority 85 (89.5\%) had HIV test done during pregnancy with 51 (53.7\%) tested for bacteriuria, 15 (16.1\%) had their blood group done during pregnancy and 7 (7.4\%) screened for syphilis. Since blood was drawn for HIV screening, this blood could also be used for blood grouping as there were only 2 (2.5\%) mothers found to be HIV positive whilst 17 (17.9\%) neonates had ABO or Rhesus incompatibility.

WHO recommends that screening tests for haemoglobin, syphilis, HIV, proteinuria, blood/Rh group, and bacteriuria be carried out during the first (8-12 weeks) antenatal visit and test for bacteriuria repeated during the second (24-26 weeks), third (32 weeks) and fourth (36-38 weeks) visits.\textsuperscript{67} These recommendations supported by scientific evidence, are low cost, and can be implemented in first level facilities in all countries in Africa including Sierra Leone.

There was no record held by these women to show that these tests were done and the result was as they reported. A number of studies have shown benefits of home-based ANC records held by women. Women who hold their own records are more likely to keep follow up appointments, ask questions about their health, and feel in control of their pregnancy. WHO recommends that in designing their own ANC records, countries should ensure that all essential information is readily available to the caregiver.\textsuperscript{67} This was not the case in this study.

Significant number of mothers with 43.2\% of babies with hyperbilirubinaemia took herbal drugs. Maternal use of herbal medications being associated with severe neonatal jaundice has been reported previously from Lagos, southern Nigeria.\textsuperscript{66} Israel et al on Risk factors for neonatal jaundice in babies presenting at the University of Benin Teaching Hospital, Benin City found native herbs were used in 33 (7.0\%) cases.\textsuperscript{15} Jaundice occurred in 24 (25.3\%) in whom no possible associated factor was found. In a similar study carried out in Benin by Israel et al, there was no identifiable risk factor in 171 (36.3\%) of subjects.\textsuperscript{15}
STUDY LIMITATIONS

There were some limitations to this study. Not all the associated factors such as hypothyroidism, galactosemia, pyruvate kinase deficiency, etc. could be assessed in this study due to financial constraints. Also blood culture bottles were not available and as a result sepsis could not be confirmed. The prevalence of neonatal sepsis may have been underestimated. ANC records were not held by the mothers, therefore making recall difficult and the information given not verified.
CONCLUSIONS

There is a high prevalence of sepsis, prematurity and blood group (ABO and rhesus) incompatibility as associated factors for neonatal jaundice in the first 2 weeks of life in Ola During Hospital.

A large number of mothers did not know their blood group and their blood group was not done during pregnancy with no record to show for it. Also none of the mother’s with Rhesus incompatibility knew their blood group or received anti-D.

Majority of the mothers did not receive the full antenatal screening for HIV, syphilis, blood group and bacteriuria as per WHO recommendation.
RECOMMENDATIONS

1. Women should be offered testing for blood group and rhesus D status in early pregnancy. Also routine antenatal anti-D prophylaxis should be offered to all pregnant women who are rhesus D-negative. Identifying blood group, rhesus D status and red cell antibodies in pregnant women is important to prevent haemolytic disease of the newborn (HDN).

2. A standard laboratory investigation should be carried out on jaundice neonates, including: serum bilirubin level (total and direct), complete blood count, reticulocyte count, blood group of mother and infant, direct Coombs test on the baby’s blood, VDRL, septic and G6PD screening whenever indicated.

3. ANC record that covers prenatal screening for blood group, HIV, VDRL, bacteriuria or hemoglobin; and childbirth, should be held by women to ensure that all essential information is readily available to the caregiver.
REFERENCES


12. Chawla G. Prediction of significant neonatal hyperbilirubinemia in healthy term newborns using cord bilirubin and 24th hour serum bilirubin. Shri BM Patil Medical College Hospital and Research centre, Bijapur, 2006; 135.


42. MacDonald MG, Seshia MMK, Mullet MD editors. *Avery’s neonatology. 6th ed.* Lippincott Williams and Wilkins; 2005.


APPENDIX I: CONSENT EXPLANATION FORM

Study Title: Associated factors for neonatal hyperbilirubinemia in the first 2 weeks of life in Ola During Children’s Hospital in Freetown, Sierra Leone.

Investigator: Dr. Isha Kadijah Kamara

Supervisors: Prof. R. Musoke, Dr. D. Wamalwa

Investigator’s Statement: I am Dr. Isha Kamara from the department of Paediatrics and Child Health of the University of Nairobi. I am carrying out a study entitled, “Risk factors for neonatal hyperbilirubinemia in the first 2 weeks of life in Ola During Children’s hospital in Freetown, Sierra Leone” as part of my postgraduate training in the said department. I am requesting you and your child to kindly participate in this study. Participation in this study is voluntary and you can choose to opt out of the study without any penalty. The purpose of this consent form is to give you information you will need to help you decide whether to participate in the study. Please read this form carefully. You are free to ask any questions about the study. The investigator will be available to answer any questions that arise during the study and afterwards.

Introduction: Neonatal jaundice is a common condition which occurs in approximately 60% of full term babies (37 weeks and over) and 80% of premature babies. Jaundice is the name given to the yellow appearance of the skin and the white of baby’s eyes due to the accumulation of bilirubin in the body. Newborn infants are constantly making new red blood cells, and breaking down the old ones. One of the waste products of old blood cells is a yellow substance called bilirubin. Bilirubin is processed by the liver into an easily disposable form and then eliminated from the body in the bowel movements. Some babies make bilirubin faster than they can get rid of it, causing the bilirubin to build up in the body and make the skin appear yellow. The amount of jaundice that most newborns have is not harmful. However, at very high levels, bilirubin can damage the brain or the hearing.

This study aims to determine the risk factors for neonatal jaundice. Your participation in this study will help us identify the risk factors for neonatal jaundice. The result of this study will help health workers in this facility and beyond to improve care given to all neonates with jaundice and to prevent jaundice and its complications from happening in other neonates. All the
information obtained will be held in strict confidentiality. Any information that may identify you or your child will not be published or discussed with any unauthorized persons. We will however discuss overall findings regarding all children who participated in the study without revealing you or your child’s identity. **Your participation in this study is purely voluntary and there is no monetary gain.** It will not cost you financially to participate in this study. You are free to withdraw from the study if you so wish without any penalty.

**Procedure:** The study will involve specific questions and examination of your child. If the child is jaundice and you agree for your baby to part of this study, I will ask you some questions about you and your child. Blood samples will be taken from you and your baby, for tests that are routinely done on newborns with jaundice. 4ml of blood will be collected from a superficial vein to carry out investigations to determine the risk factors for jaundice, including: serum bilirubin level (total and direct), complete blood count, reticulocyte count, blood group of mother and infant, direct Coombs test on the baby’s blood, G-6PD activity, VDRL, septic screen and bacteriological examination of the cord swab whenever indicated.

For blood specimen which has to be delayed overnight or for more than 2 hours without analysis, the blood will be allowed to clot. The serum will be separated from contact with cells into plain bottles, sealed in an envelope and refrigerated at 4°C to minimize breakdown of bilirubin by light. The specimen will be stored at 4°C if the screening for G-6PD deficiency could not be done immediately.

**Risks:** There is a risk of pain and being bruised during drawing of blood samples.

**Benefits:** The result will be interpreted to you and the doctor taking care of your baby to assist in further management of your child.

**Confidentiality:** If you agree to be part of the study, the information will be held in strict confidence and only used for the purpose of this study. No specific information regarding you, your child or your family will be released to any person without your written permission.

**Problems or Questions:**
If you ever have any questions about the study or about the use of the results you can contact the principal investigator, **Dr Isha Kamara** by calling **076-837589**.

If you have any questions on your rights as a research participant you can contact the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee by calling **+254-020 2726300 Ext 44355**.

I ……………………………………………confirm that the study has fully been explained to me and I have had a chance to ask questions about the research, to which satisfactory answers have been given. I understand I can withdraw from the study at any time without any penalty. I give consent to participate in this study.

Signature/Thumb print………………………………..Date……………………

I confirm I have explained to the parent/caregiver all relevant information about the study as indicated above.

Investigators Signature ………………………..Date………………
INFORMED CONSENT FORM IN KRIORIO

Wetin di städi de bɔt fɔ ɛp yu disayd if yu pikin fɔ tek pat

Taytul: Di tin dem we go mek bebi dem get bɔku bilirubin na dem blɔd insay di fɔs tu wiks we dem bɔn, na Ola During Children’s Hospital, Fritɔŋ, Salone.

Edman pan di städi: Dr. Isha Kadijah Kamara

Supavaysɔ dem: Prof. R. Musoke, Dr. D. Wamalwa

Di Edman ŋ not: Tenki fɔ we yu gri fɔ rid dis fɔm. Dis fɔm get impɔtɔnt infɔmshɔn dem we go ɛp yu disayd if yu wan fɔ mek yu bebi de pan dis stɔdi. Duya rid dis infɔmshɔn saful ɛŋ ask kwestiɔn dem bɔt ɛni tin we daut yu pan dis stɔdi.

ŋtrodɔkɔshɔn: Jɔndis pan yɔŋ bebi dem na kɔmɔŋ sik we kin kech 60% pan bebi dem we bɔn we di mami ŋ bele trɔŋ fayn fayn wan ɛŋ 80% we di mami ŋ bele ŋɔ trɔŋ fayn. Jɔndis na we di bebi ŋ kanda en di wayt say na dem yay tɔn yala bιkɔs bilirubin bɔku na dem bɔdi. Yɔŋ bebi dem de mek nyu blɔd ɔltɛm en de klia di ol wan. Wan pan di tin dem we de lef bien we di bɔdι klia blɔd, dem kɔl am bilirubin. Di liba de brok dis bilirubin ɛŋ mek am izhi fɔ kɔmɔt na di bɔdι wit pupu. Salir bebi dem de mek bilirubin kwik wan pas aw I de kɔmɔt na di bɔdι, dis kin mek di bilirubin bɔku na di bɔdι en mek di kanda yala. Di kayn jɔndis we plenti yɔŋ bebi dem kin get na kin kɔs prɔblem, bɔt bɔku bɔku bilirubin kin ambag di bren ɛŋ yes. Fɔ mek yu tek pat pan dis stɔdi lef to yusef, nɔ bɔdι nɔ go fos yu fɔ tek pat. Dis stɔdi wan fɔ no di tin dem we de kɔs dis. Wetin wi fɛn go ɛp fɔ tap di bren ŋ pwel wan.

Di we- ɛn-aw: If yu gri fɔ mek yu bebi de pan dis stɔdi, a go ask yu kwestiɔn dem bɔt yu bebi. Dɔn a go tek blɔd ʃrɔm yu bebi en yusef, fɔ du tɛst dem we dem kin du fɔ yɔŋ bebi dem we get jɔndis.

Wetin kin bi: Sɔmtem yu ɛŋ yu pikin go fil ʃɔml pen we dem de tek blɔd.

Benifit: Wi go brokɔdɔŋ wetin wi ʃɛn to yu ɛŋ di dɔkta, fɔ ɛp ʃɛn yu pikin bɛte wan.
**Yu sikrit tin dem:** If yu gri fo de pan di stadi, wetin wi no bot yu, wi go kip am to wis en go yuzh am fo di stadi namo. Wi nu go tel enibodi enitin bot yu, yu bebi c yu fambul dem, if yu nu rayt en gri fo mek wi do so.

**Problem a kwestion dem:**

If yu get eni kwestion bot di stadi c wetin wi fen bot pan di stadi, yu go klo di pasin we de bifo pan dis stadi, Dr Isha Kamara pan dis nomba 076837589.

If yu get eni kwestion bot yu rayt fo tek pat pan dis stadi, yu kin klo KNH/UoN Ethics and Research Committee pan dis nomba +254-020 2726300 Ext 44355.

Fo sho se yu andatand di tin dem pan dis stadi en yu gri fo de pan di stadi, du ya sayn c put yu big finga i mak de ya.

Mi …………………………………………………………………………………………………………………………………………………………………………………

grí se dem don brok-don ultin bot di stadi to mi en ah gri fo de pan dis stadi.

Edman pan di stadi…………………………………… Date …………………
APPENDIX II: QUESTIONNAIRE

STUDY TITLE: ASSOCIATED FACTORS FOR NEONATAL HYPERBILIRUBINEMIA IN THE FIRST 2 WEEKS OF LIFE IN OLA DURING CHILDREN’S HOSPITAL IN FREETOWN, SIERRA LEONE

A. Socio-demographic

Code .............................................
Date .............................................
Guardian’s contact.........................
Date of Birth.................................
Current Weight of the baby..............

B. Maternal

1. Mother’s age:

2. Date of last menstrual period:

3. Parity: a. 1   b. 2   c. 3   d. ≥ 4

4. Any history of jaundice in previous sibling? a. Yes   b. No

If yes, which treatment did he/she received?

   a. Phototherapy   b. Exchange blood transfusion

5. In the last 2 weeks of pregnancy, did you have vaginal discharge? a. Yes   b. No

   If yes, which color was it? a. white   b. yellow   c. brown

   Which medications did you receive?

6. Did you have any skin rash in the last trimester of pregnancy? a. Yes   b. No
If yes, which medications did you receive?

Specify:

7. Were you treated for malaria in the last 2wks of pregnancy?   a. Yes   b. No

If yes, which drugs were you given?

Specify:

8. Did you have urinalysis done during pregnancy?   a. Yes   b. No

If yes, what was the result?   a. Positive   b. Negative

If positive, which drug did you receive?


If yes, what was the result?   a. Positive   b. Negative

If positive, which drugs are you on?

Specify:

10. For how long did your membranes ruptured before delivery?
    a. < 18hrs   b. 18hrs - 24hrs   c. >24hrs


If yes, what was the result?   a. Positive   b. Negative

If positive, which drugs were you given?

Specify:

12. Do you know your blood group?   a. Yes   b. No

If yes, was it done during pregnancy?   a. Yes   b. No

14. Was oxytocin used during labor? a. Yes   b. No   c. Don’t know

15. Where did you deliver? a. At home   b. Hospital

If at home, what was used to cut the cord? a. surgical blade   b. ordinary blade   c. knife

16. Which type of delivery did you have?
   a. Normal vaginal delivery   b. Caesarean Section   c. Instrumentation: Vacuum/Forceps

C. Neonatal

1. Age of neonate:


3. What was the birth weight of the baby?

4. How old was the baby when you first notice jaundice?

5. What are you feeding the baby?
   a. Breast milk only   b. Breast milk + water   c. Breast milk + Formula   d. Formula only

6. How are you feeding the baby?
   a. Breastfeeding   b. Cup feeding   c. Nasogastric tube feeding

7. When did you initiate feeding?
   a. Within 1hr of birth   b. If more than 1hr, specify time

8. How many times has the baby been fed over the last 24hrs?

9. If cup or NG tube feeding, what volume are you feeding?

10. On examination, are there
    i. Cephalohaematoma or bruises? a. Yes   b. No
ii. Pallor?  a. Yes  b. No

iii. Petechiae?  a. Yes  b. No

iv. Skin rash?  a. Yes  b. No

v. Cataract?  a. Yes  b. No

vi. Para or Umbilical infection  a. Yes  b. No


viii. Splenomegaly?  a. Yes  b. No

ix. Hypotonia?  a. Yes  b. No

x. Opisthotonus?  a. Yes  b. No

xi. Bulging fontanel?  a. Yes  b. No

xii. High pitched cry?  a. Yes  b. No

D. Laboratory findings

1. Serum bilirubin

   a. Total-------------------µmol/l
   b. Direct-------------------µmol/l
   c. Indirect-------------------µmol/l

2. Blood grouping and Rh typing of mother and baby

   a. Baby’s blood group---------b. Mother’s blood group---------

3. Direct coombs test of baby’s blood

   a. Positive  b. Negative

4. Complete blood count

   a. Hemoglobin--------------------------g/dl
b. Total WBC count\(\times 10^9/l\)
c. Total neutrophil count\(\times 10^9/l\)
d. Total immature neutrophil count\(\times 10^9/l\)
e. I: T Ratio
f. Total platelet count\(\times 10^9/l\)

5. Peripheral blood film
   a. Reticulocyte count
   b. RBC morphology

6. G-6PD level

7. VDRL on the baby’s blood: a. Positive  b. Negative

   Pathogen isolated

9. Umbilical swab if indicated