

Hemolytic Disease of the Newborn among Newborn  
Babies with ABO Incompatibility, at the University  
Teaching Hospital, Lusaka, Zambia.

**A dissertation submitted in partial fulfillment of the requirement for the award of  
the degree of Masters of Medicine in Paediatrics and Child Health**

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## **Declaration**

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other university.

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**Approval**

The University of Zambia approves this dissertation of Dr Monica Kapasa as fulfilling the requirement for the award of the degree of Master of Medicine of Paediatrics and Child Health.

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## **ABSTRACT**

**Introduction:** Hemolytic disease of the newborn is a clinical condition in which fetal red blood cells are destroyed by maternal allo-antibodies directed red blood cell antigens acquired from the father.

**Justification:** In many developing countries like Zambia, the true incidence of Hemolytic Disease of the New Born is not known. It may be postulated that hyperbilirubineamia due to ABO incompatibility may be an important problem in African infants and if not diagnosed and not treated, may be a major cause of developmental disabilities in children.

**Aim:** This study was undertaken to determine the prevalence of hemolytic disease of the newborn among newborns with ABO incompatibility.

**Method:** This was a prospective study at the University Teaching Hospital, from October 2008 and February 2009.

**Results:** 349 babies were successfully recruited in the study and the results showed that 49% of the mother/infant pair that were recruited were ABO incompatible and ABO hemolytic disease of the newborn was prevalent in 3.5% of all the pregnant mothers that were recruited and met the criteria for the study.

**Conclusion:** This supports the hypothesis that there is a relationship between ABO incompatibility and hemolytic disease of the newborn.

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## **Dedications**

To my husband, Esau and my children, Chiwoko, Kunda and Senkwe, for their understanding , love and support when I was away from them for long hours during my studies.

## TABLE OF CONTENTS

|   |          |
|---|----------|
| Declaration.....                        | i        |
| Copyright.....                          | ii       |
| Approval.....                           | iii      |
| Abstract.....                           | iv       |
| Acknowledgements.....                   | v        |
| Dedications.....                        | vi       |
| <b>Chapter 1</b>                        |          |
| 1.0 Introduction.....                   | 1        |
| 1.1 Statement of the problem.....       | 4        |
| 1.2 Study Justification.....            | 6        |
| <b>Chapter 2 Literature review.....</b> | <b>7</b> |
| <b>Chapter 3</b>                        |          |
| 3.0. Main Objectives.....               | 12       |
| 3.1 Specific objectives.....            | 12       |
| 3.2 Outcome Measures.....               | 12       |
| <b>Chapter 4</b>                        |          |
| 4.1 Study design.....                   | 13       |
| 4.2 Study site.....                     | 13       |
| 4.3 Study population.....               | 13       |
| 4.4 Selection of study subjects.....    | 13       |
| 4.5 Sample Size.....                    | 14       |
| 4.6 Procedures at enrollment.....       | 14       |
| 4.7 Data management.....                | 15       |
| 4.8 Data analysis.....                  | 15       |
| 4.9 Ethical considerations.....         | 16       |



|  |    |
|--|----|
| <b>Chapter 5 Results</b> .....                         | 17 |
| <b>Chapter 6 Discussion</b> .....                      | 21 |
| <b>Chapter 7 Conclusion</b> .....                      | 24 |
| <b>Chapter 8 Recommendations and Limitations</b> ..... | 25 |
| References .....                                       | 26 |
| Appendices.....  | 29 |

## CHAPTER 1

### **1.0 Introduction**

Hemolytic disease of the newborn is a clinical condition in which fetal red blood cells are destroyed by maternal allo-antibodies directed against red blood cell antigens acquired from the father. Immunoglobulin G (Ig G) antibodies that have been produced by the mother pass through the placenta and attack the red blood cells of the baby in circulation. The red blood cells are broken down and the fetus can develop reticulocytosis, jaundice and anemia (<sup>1</sup>).

Several systems of classifying blood types exist and these include the ABO system, Rhesus system and Kell system among others. The ABO system of classification is the most commonly used system and A, B and O are the three major blood types. The types of blood groups are based on small substances (antigens) that are attached to the surface of the blood cells.

The Rhesus system is subdivided into D, E, c, e and C. Rhesus D hemolytic disease of the newborn is one of the commonest causes of hemolytic disease of the newborn. The incidence of Rhesus disease in a population depends on the proportions that are Rhesus negative. Rhesus disease sensitization is about 10 times more likely to occur if the fetus is ABO compatible with the mother than if the mother and fetus are ABO incompatible(<sup>2</sup>).

ABO hemolytic disease of the newborn occurs almost exclusively in newborns of blood group A or B having mothers of group O, even though hemolytic disease of the newborn has been reported in a baby whose mother was group A with a high titres of anti B (<sup>3</sup>). Jaundice in hemolytic disease of the newborn is more frequent and severe in ABO incompatible black than white newborns and, furthermore, jaundice due to any other cause, is more likely to be more severe in ABO incompatible babies than compatible ones(<sup>4</sup>). The etiology of hemolytic disease of the newborn due to ABO incompatibility is complex because anti-A and anti-B antibodies are composed mainly of Immunoglobulin

M. Since only Immunoglobulin G antibodies cross the placenta, those pregnant women with high levels of Immunoglobulin G anti-A or B with an ABO incompatible fetus will be the ones to give birth to a newborn with ABO hemolytic disease of the newborn<sup>(5)</sup>.

The diagnosis of Hemolytic disease of the newborn due to ABO incompatibility cannot be made serologically using one single test; however several tests together make the diagnosis more probable. In contrast to Rhesus hemolytic disease, the immunological findings in hemolytic disease of the newborn due to ABO incompatibility do not correlate well with the severity of the clinical course<sup>(6)</sup>. Sometimes it is impossible to differentiate between hemolytic disease of the newborn due to ABO incompatibility and non-antibody mediated hyperbilirubinemia.

Diagnostic challenges and disease burden of ABO-Rh incompatibility are reviewed in Chapter 2.

Hemolytic disease of the newborn due to Rhesus incompatibility is preventable and preventable measures are in place in many countries. In contrast there are currently no preventable measures for Hemolytic disease of the newborn due to ABO incompatibility. ABO incompatibility hence is now the single most common cause of neonatal jaundice, with an incidence of 54.4 per 1000 births in the United States of America<sup>(2)</sup>. Rhesus incompatibility is prevented by giving a Rhesus negative mother, Rhesus immunoglobulin (anti D), which combines with fetal antigens and prevents the mother from forming antibodies to fetal Rhesus group antigens.

Hemolytic disease of the newborn can be managed by using any of the following modalities; phototherapy, exchange transfusion or intravenous immunoglobulins. Early application of any of these methods in the treatment of hemolytic disease of the newborn prevents bilirubin encephalopathy and kernicterus with subsequent death or development of severe neurological sequelae. Nearly 50% of babies with hemolytic disease of the newborn due to ABO incompatibility do not require treatment. Of the remaining 50%, half of these become extremely jaundiced and without treatment, 90% of them will die and 10% become severely affected by kernicterus. The other half are severely affected in utero and become hydropic<sup>(7)</sup>.

Currently the mortality rate due to ABO hemolytic disease of the newborn is 16% in the United States of America (<sup>7</sup>). Due to lack of studies in Zambia, the mortality rate due to ABO hemolytic disease of the newborn is not known.

Whereas, some studies have been done in other countries, such as Zimbabwe in Africa, to determine the incidence of hemolytic disease of the newborn, information to this effect is not available in Zambia. The aim of this study, therefore, is to find out the prevalence of hemolytic disease of the newborn among newborns with ABO incompatibility in Lusaka, Zambia.

### **1.1 Statement of the problem:**

The clinical presentation that often alerts pediatricians to hemolytic disease of the newborn is: a well term neonate, presenting within the first 48 hours of life, with early onset jaundice. The association between ABO incompatibility and jaundice was first described by Halbrecht in 1944, and called uterus praecox. Since then a lot has been written about this disease which has shed some light on the seriousness of this disease in Blacks particularly in Africa (<sup>8</sup>). Screening of mothers for different blood types antenatally will help in detecting which neonates are most at risk of developing hemolytic disease of the newborn. These babies can then be monitored closely and treatment started, if need be, to prevent future complications such as death or severe neurological sequale. However in Zambia, maternal blood group and antenatal antibody screening are not routinely done in most cases thereby delaying the institution of treatment early in those babies with hemolytic disease of the newborn particularly those who are ABO incompatible. Currently grouping and antibody screening is only done when a baby presents with jaundice as part of the investigations to determine the cause of the jaundice.

The prevalence of ABO incompatibility and the incidence of hemolytic disease of the newborn due to ABO incompatibility is not known in Zambia. This is due to the fact that not much research has been done here in Zambia in particular and generally in Africa.

In his study on Perinatal and Neonatal Mortality and Morbidity in Lusaka, Chintu et al noted a high number of neonates with ABO incompatibility admitted to the neonatal unit who required exchange transfusion for hyperbilirubemia, ratio of 1:400(<sup>9</sup>).

Hemolysis leads to elevated bilirubin levels in the body. After delivery bilirubin is no longer cleared (via the placenta) from the neonate's blood leading to accumulation of higher levels, and symptoms of jaundice increase within 24 hours after birth. Like any other severe neonatal jaundice, there is a possibility of kernicterus. The most common sequelae of kernicterus is cerebral palsy. Other complications of elevated bilirubin include hepatosplenomegaly, and hemolytic anemia. When severe this anemia may require multiple transfusions putting the baby at risk of acquiring blood borne infections like HIV and hepatitis, and iron overload.

Some children with cerebral palsy are being followed up in the Pediatric Neurology Clinic of UTH. Most of these children have severe developmental disabilities like not being able to walk and talk, deafness and blindness, convulsive disorders, mental insufficiency with learning disabilities, and some have chronic malnutrition due to difficulties in feeding .Some of these children are first seen in the outpatient department and often present with a delay in the acquisition of milestones, and a history of jaundice usually in the first 48 hours of life is given by the mother.

Since there is no mechanism to detect ABO incompatibility at birth, there is need to conduct a study to find out the incidence of hemolytic disease of the newborn due to ABO incompatibility at UTH.

## **1.2 Study justification:**

There is lack of information regarding the true incidence of ABO hemolytic disease and the significance of various laboratory investigations commonly employed in its evaluation (<sup>10</sup>). In many developing African countries, like Zambia, the true incidence of ABO hemolytic disease is not known.

There is no single test that can be used to confirm the diagnosis of ABO-hemolytic disease of the newborn. Several tests have to be used and if positive make the diagnosis likely. This study is therefore important to establish the frequency of ABO incompatibility and the true incidence of ABO-HDN in black African infants particularly at the University Teaching Hospital, in Zambia so that treatment can be instituted early to prevent developmental disabilities like cerebral palsy. The information can also be passed on to the policy makers so that routine anti body screening for blood grouping can be done in mothers antenatally so that 'high risk' patients are closely monitored.

Hyperbilirubinemia may be associated with developmental disability and a history of neonatal jaundice is common in African infants with cerebral palsy. Animashaun(<sup>11</sup>) found that in nearly 50% of children with cerebral palsy in Lagos ,Nigeria had a history of neonatal jaundice and kernicterus.Holmes(<sup>12</sup>), in her paper, also found a high etiological incidence in the African children with cerebral palsy. For these reasons, it may be postulated that hyperbilirubinemia due to ABO incompatibility may be an important problem in African infants and if undiagnosed and untreated, may be a major cause of developmental disability in these children . With this in view, Paeditricians looking after black newborns should pay special attention to babies with ABO incompatibility in order to prevent long-term sequelae.

## CHAPTER 2

### Literature Review:

Several studies have been done to establish the incidence of hemolytic disease of the newborn and it has been noted that the incidence of hemolytic disease of the newborn due to ABO incompatibility is significantly greater in black Africans than in the Caucasians (<sup>13</sup>). There is an increased incidence and an increased severity of ABO-hemolytic disease of the newborn in certain populations i.e. Arabs, African-Americans, south-east Asians and Latin Americans (<sup>13, 14</sup>). Bucher et al observed an incidence in blacks two to three times that of Caucasians in a single hospital study of full-term infants (<sup>14</sup>).

Hemolytic disease of the newborn due to ABO incompatibility is diagnosed by the appearance of jaundice in the first 24-48 hours of life, together with evidence of hemolysis with spherocytes (<sup>13</sup>). Serologic evidence and clinical manifestations are variable and previous studies have shown poor correlation between serological tests on cord blood and clinical course in affected infants with hemolytic disease of the newborn. Although hemolytic disease of the newborn as a result of ABO incompatibility is clinically milder than Rhesus incompatibility, severe hemolysis occasionally occurs that in some cases requires exchange transfusion (<sup>15</sup>). It has been noted that hemolytic disease of the newborn due to ABO incompatibility frequently occurs during the first pregnancy, and about 50% of infants are affected unlike Rhesus hemolytic disease of the newborn in which the first born- babies are usually spared or free of the disease and subsequent babies are the ones that are affected. ABO incompatibility is present in about 12% of pregnancies, with evidence of fetal sensitization in 3% live births. Less than 1% of births which are ABO incompatible are associated with significant haemolysis (<sup>16</sup>). In general 15-20% of all maternal/fetus pairs are ABO incompatible, but hemolytic disease of the newborn due to ABO incompatibility is confined to the approximately 1% of such group O mothers who have high titres of Immunoglobulin G anti A/B antibodies (<sup>17</sup>). Hemolysis due to anti A is more common (1 in 150) than that due to anti-B.



### Comparison between Rhesus and ABO incompatibility

|                    | Rhesus disease   | ABO disease   |
|--------------------|--|---|
| Incidence          | First born usually spared<br><br>More in Caucasians than blacks                | First born affected in 50% of cases<br><br>More in blacks than Caucasians                   |
| Severity           | Severe in both racial groups<br><br>Subsequent siblings more severely affected | More severe in blacks than Caucasians<br><br>Subsequent siblings may or may not be affected |
| Direct Coombs test | Always positive  | May be positive or negative   |
| Haematology        | No spherocytes<br><br>Aneamia very severe                                      | Microspherocytosis<br><br>Aneamia generally mild to moderate                                |
| Prevention         | Preventable  | Not yet preventable   |

Previous studies done have shown poor correlation between serological tests on cord blood and clinical course in affected infants with hemolytic disease of the newborn. In a retrospective analysis of 254 cases of ABO hemolytic disease of the newborn, the relation of laboratory parameters to incidence and severity of jaundice was studied; 65% of infants who had positive direct agglutination test experienced jaundice, compared with about 35% of control infants or infants who had ABO hemolytic disease with negative direct agglutination test results (<sup>2</sup>). Erythrocyte characteristics such as reticulocytosis, microspherocytes and positive indirect Coombs test in the control infants were similar to those in ABO hemolytic disease with hyperbilirubinemia and black infants predominated over white with a ratio of 2.5:1(<sup>18</sup>). In a study done in Puerto Rico, indirect Coombs test in cord sera, representing the passive transfer from mother to fetus of antibody directed towards antigen on the fetal erythrocytes, were positive in 58.8% of infants at the University of Puerto Rico hospital as compared to 40.4% of the infants at the North

Carolina Baptist hospital (<sup>19</sup>). In another study, 1391 cord blood specimens were tested for Rhesus, direct agglutination test, indirect Coombs test, total and direct bilirubin tests. The study showed that 53.3% were A, B or AB; 19.3% of both A and B infants and 7% of AB had immune antibodies in their sera. Direct agglutination test was neither diagnostic nor predictive of severity. A study done in Canada collected data prospectively through the Canadian Pediatric Surveillance Program from 2002-2004. Infants were included if they had a peak serum bilirubin level of more than 425umol/l or underwent exchange transfusion. Infants with rhesus iso-immunisation or who were born at less than 36 weeks gestation were excluded. Results showed that of 365 cases reported, 258 were confirmed to be severe neonatal hyperbilirubemia for an estimated incidence of 1 in 2480 live births. Causes of severe hyperbilirubinemia were identified in 93 as follows; ABO incompatibility-48, Glucose 6-phosphate dehydrogenase deficiency-20 and other incompatibilities-12 (<sup>20</sup>).

There have been no studies done in Zambia to establish the prevalence of Rhesus incompatibility, despite records from the blood bank at the University teaching hospital showing a prevalence of 7% from the period 2006-2010 of the blood donors.

A retrospective cross-sectional study was done at Perirenyatwa hospital in Zimbabwe to establish the incidence of hemolytic Disease of the Newborn. 22493 infants at Perirenyatwa hospital during 1995-1997 and 2002-2003 periods were studied and the main outcome measures were ABO and Rh blood group results. 191(0.85%) infants had hemolytic disease of the newborn and 163(85.34%) of these were due to anti AB, 25(13.09%) were due to anti A and 3(1.57%) were due to anti kell. The incidence of hemolytic disease of the newborn during the 1995-1997 and 2002-2003 periods were 0.93% and 0.64% respectively (<sup>21</sup>). There was no mention on the prevalence of Rhesus incompatibility.

In a study done in South Africa to establish the role of fetomaternal ABO incompatibility as a cause of overall neonatal jaundice, its relationship to the degree of jaundice and its etiologic contribution to severe jaundice necessitating exchange transfusion in black infants, showed that ABO incompatibility was a major cause of jaundice in almost 58% of infants. ABO incompatibility accounted for 41.7% of those severely jaundiced with

serum bilirubin above 301 $\mu$ mol/l and 44.4% of newborn infants had exchange transfusion<sup>(22)</sup>.

A study done in the Czech Republic observed that the rate of occurrence of incompatibility states between blood group O mothers and their blood group A or B offspring amounted to 14%. In the same study in Czech, it was also observed that every tenth newborn that was incompatible manifested hemolytic disease of the newborn requiring treatment <sup>(23)</sup>. In a study done in Venezuela, the frequency of ABO incompatibility in the population of Caracas attending the maternity hospital “concepcion palacios” was 16% and the incidence of ABO hemolytic disease of the newborn was near 5% <sup>(24)</sup>.

In Puerto Rico, a prospective study was done at the University of Puerto Rico hospital and the North Carolina Baptist Hospital which showed that the incidence of hemolytic disease of the newborn due to ABO incompatibility was 28.3% or 1 in 3.5 and 18.4% or 1 in 5.4 respectively <sup>(19)</sup>.

In a study of ABO and Rh (D) phenotype frequencies of different racial/ethnic groups in the United States, a 10 year demographic data base on 3.1 million allogeneic and autologous donors giving blood showed that the highest percent of group O was found in Hispanic (56.5%), North-American Indians (54.6%) and black non Hispanic (50.2) donors .Hispanic and black non- Hispanic donors had a lower percentage of Rh negativity of 7.3 and 7.1 respectively compared to white non-Hispanic donors (17.3)<sup>(25)</sup>.

Even though clinical manifestations of hemolytic disease of the newborn due to ABO incompatibility are said to be milder, some studies have shown fatal results. A study that was done in Turkey reported twin newborns both afflicted with significant hemolytic disease of the newborn due to ABO incompatibility but showing different degrees of clinical severity. Fatal hydrops developed in one of the twins. Two cases of hemolytic disease of the newborn due to ABO incompatibility were reported and in both cases blood erythroblastosis was 300 per 100 leukocytes was found at birth <sup>(26)</sup>. In a study done to determine the incidence of ABO hemolytic disease of the newborn among Hong Kong Chinese infants, showed that 1 in 5 infants had ABO hemolytic disease of the newborn with a serum bilirubin level of 300 $\mu$ mol/L or more. The study compared “expected”

frequency of various mother-infant ABO combinations (based on the ABO distribution of the local population) with the “observed” frequency of a cohort of infants with severe neonatal jaundice <sup>(27)</sup>. In America a case was described of a B/Rh positive term newborn from an O/Rh negative African-American mother demonstrating aggressive hemolysis and a robust response of the bone marrow .This case was successfully managed with phototherapy and a simple RBC transfusion without the need for exchange transfusion <sup>(5)</sup>.

A study was done in Iran to determine the etiology and complications of exchange transfusion performed for neonatal hyperbilirubinaemia. This study showed that the most common causes of exchange transfusion overall was ABO incompatibility (22.1%) and glucose-6-phosphate dehydrogenase deficiency (19.1%) <sup>(28)</sup>.

**Research question:**

Is there a relationship between newborns with ABO incompatibility and hemolytic disease of the newborn in Zambia?

**Hypothesis:**

There is a relationship between ABO incompatibility and hemolytic disease of the newborn in Zambia.

**Null Hypothesis:**

There is no relationship between ABO incompatibility and hemolytic disease of the newborn in Zambia.

Cut off level is P value of less than or equal to 0.05.

## **CHAPTER 3**

### **STUDY OBJECTIVES**

#### **3.0 Main objective:**

To find out the prevalence of hemolytic disease of the newborn, in ABO blood group incompatibility in newborn infants at UTH.

#### **3.1 Specific objectives:**

1. To determine the frequency of ABO incompatibility.
2. To determine the prevalence of ABO- hemolytic disease among newborns.
3. To measure umbilical cord bilirubin levels.
4. To measure umbilical cord hemoglobin levels.
5. To co-relate the above with ABO hemolytic disease.

#### **3.2 Outcome Measures:**

1. Blood grouping (ABO)
2. Level of cord bilirubin
3. Level of cord hemoglobin level
4. Jaundice
5. Coombs test
6. Spherocytosis
7. Reticulocytosis
8. Weight of baby
9. Age and parity of mother

## **CHAPTER 4**

### **STUDY METHODS**

**4.1 Study design:** The study was a prospective cross-sectional and observational study and was done between October 2008 and February 2009.

**4.2 Study site:** The study was done at the maternity and neonatal wards of the University Teaching Hospital, Lusaka, Zambia.

**4.3 Study population:** Full-term babies with a birth weight of 2.5kg and above of blood group O mothers were recruited in the study. Mothers with blood group O were recruited consecutively once over the stated period. All the mothers that were recruited came from within Lusaka.

#### **4.4 Selection of study subjects:**

##### **4.4.1 Inclusion criteria**

Mothers with blood group O.

Mothers with gestational age of 37 completed weeks.

Full term black newborns with birth weight of 2.5kg and above.

Mothers with written consent.

##### **4.4.2 Exclusion criteria**

Preterm infants- infants born before 37 completed weeks of gestation.

Infants with a birth weight less than 2.5kg.

Mothers, with a history of draining of liquor for more than 24 hours before delivery.

Mothers with signs of infection during labour e.g. fever, tender abdomen.

Mothers with chorioamnionitis.

Infants with birth trauma.

Infants born with severe congenital malformations.

Infants with birth asphyxia.

Infants with other known causes of jaundice and hemolysis.

**4.5 Sample size:** Sample size was calculated using the formula

$n = Z^2PQ/d^2$  for cross sectional studies, where

P- estimate of prevalence in this case 50%, Z-1.96, d-desired width of confidence interval, Q- 100-P

$$n = 1.96^2 \times 50 \times 50 / 5^2$$

n =384, assuming 95 percent confidence interval.

Sample size calculated was 384 but only 349 samples were eligible for enrollment in the study.

#### **4.6 Procedures at enrollment**

The purpose of the study and the procedures were explained to the Mothers first. Written consent was obtained by signature or thumb print .Mothers who consented to be in the study were interviewed by using a structured questionnaire in the ante-natal clinic and then revisited again when they went into labor to collect the blood. Some mothers were also interviewed in the labor ward before going into active labor and monitored until delivery when the blood was collected.

Bed side blood grouping was done for those mothers who did not have their blood group done in the antenatal clinic.

Information that was obtained included age, parity, history of jaundice in previous babies and outcome. For the consenting mother, about 3mls of umbilical cord blood was collected, 1ml of blood was put in a plain bottle taken to the blood bank for assessing blood group and direct Coombs test, 1ml of blood was be put in a lithium containing bottle and sent to the biochemistry laboratory for measuring bilirubin levels. The last 1ml of blood was put in an EDTA bottle and taken to the hematology laboratory for measuring hemoglobin levels, reticulocyte count and for peripheral smear. Peripheral smear was to check for spherocytes and reticulocytes. The results from the laboratories were entered on a baby's laboratory form and included the baby's weight, Apgar score, and sex.

Babies who developed jaundice within the first 24 hours of birth were admitted to the neonatal intensive care unit in D-block and management was instituted according to the neonatal intensive care unit protocol on neonatal jaundice. Treatment options were also documented on the questionnaire.

For the purposes of this study the criteria for ABO hemolytic disease of the newborn were:

1. Born at this hospital
2. Birth weight of 2.5kg or greater
3. Jaundice observed within 48 hours of birth
4. Infants of blood group A,B or AB born to mother of blood group O
5. No Rhesus incompatibility
6. Positive Coombs test
7. Spherocytosis +/- microcytosis

#### **4.7 Data management:**

##### **Data tools;**

A structured questionnaire was used for collection of relevant information on the mother. A laboratory form was used for details and results of the blood samples that were collected from the baby at birth.

**4.8 Data Analysis:** Frequencies were calculated for the blood groups and percentages calculated to answer the first objective. Outcomes were categorized into independent and dependant variables and based on these the type of measurement was established. Unordered categories for example, sex and blood type were calculated using chi-square, proportions and Mantel-Haentzel tests. Ordered categories with intervals that are not quantifiable for example classification jaundice (mild, moderate and severe) were calculated using proportions, rank correlation and chi-square tests. Ranked spectrum with quantifiable intervals for example, weight, bilirubin and hemoglobin levels were calculated using t-test, analysis of variance and rank correlation. A computer and SPSS was used for data entry and analysis



#### **4.9 Ethical Considerations**

Ethical clearance was sought and obtained from the University of Zambia, Research and Ethics Committee before initiation of the study. Informed consent was also obtained from the mothers before they and their babies were recruited in the study. A written consent form was made available to the mother to sign it or thumbprint it if she was not able to write.

## CHAPTER 5

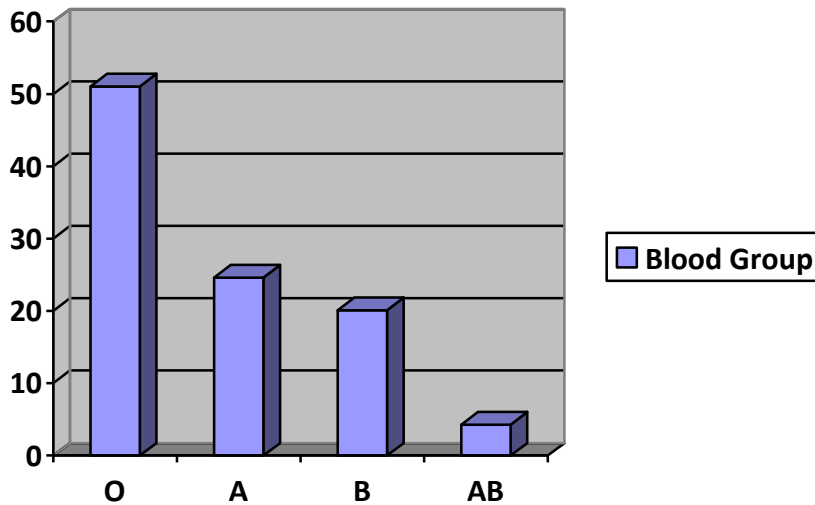
### RESULTS

The study recruited 349 mothers with blood group O and their babies .Of these, 318 were Rh+ (91%) while 31 were Rh – (9%). The results for blood grouping for the babies are shown in table 1. The mother/infant pair that was ABO incompatible from the study was 171 accounting for 49%.

**Table 1. Frequency of blood groups Among the babies (N=349).**

| Blood group | Frequency | Percent | Valid Percent | Cumulative Percent |
|-------------|-----------|---------|---------------|--------------------|
| O           | 178       | 51.0    | 51.0          | 51.0               |
| A           | 86        | 24.6    | 24.6          | 75.6               |
| B           | 70        | 20.1    | 20.1          | 95.7               |
| AB          | 15        | 4.3     | 4.3           | 100.0              |
| Total       | 349       | 100.0   | 100.0         |                    |

Diagram 1: Frequency of Blood Group among Babies



The hemoglobin concentrations collected from the cord blood of the babies recruited in the study ranged from 8.9g/dl to 18.3g/dl. Low levels of hemoglobin were noted in those

babies with blood group A or B as compared to those babies who had blood group O (Table 2).

The bilirubin concentrations collected from the cord blood of the babies recruited ranged from 25umol/l to 360umol/l .It was noted that babies with blood group O had low cord bilirubin as compared to babies with blood group A or B( Table 2).

The cord hemoglobin and cord bilirubin concentrations of groupA babies were not significantly different from those of group B babies.

**Table 2: Cord hemoglobin and bilirubin concentrations**

| Blood group | Number of subjects | Bilirubin+/-<br>1SD(umol/l) | Hemoglobin +/-<br>1SD(g/dl) |
|-------------|--------------------|-----------------------------|-----------------------------|
| O           | 178                | 68+/-0.56                   | 18.3+/-1.71                 |
| A           | 86                 | 174+/-0.81                  | 12.99+/-1.71                |
| B           | 70                 | 168+/-0.75                  | 12.78+/-2.02                |
| AB          | 15                 | 165+/-0.78                  | 12.8+/-1.9                  |

Analysis of variance showed a significant difference (P<0.001) of babies from blood group A and B when compared to Group O babies.

Spherocytes were noted in some babies in blood group A and B but not in the babies with blood group O.

**Table 3. Results of the Coombs test Among babies (N=349)**

| Coombs test | Number | Percentage |
|-------------|--------|------------|
| Positive    | 97     | 27.8%      |
| Negative    | 252    | 72.2%      |
| Total       | 349    | 100%       |

All babies with blood group O had a negative Coombs test where as 50 babies with blood group A, 38 babies with blood group B and 9 babies from AB had positive Coombs test (Table 3 and 4).

**Table 4: Results of Coombs test among Blood groups (N=349)**

| Blood group | Coombs positive | Coombs negative |
|-------------|-----------------|-----------------|
| O           | 0               | 178             |
| A           | 50              | 36              |
| B           | 38              | 32              |
| AB          | 9               | 6               |

Out of all the babies recruited in the study, 86 babies developed jaundice of varying degrees. 80 of them had mild jaundice which was noted after 48 hrs of birth. Jaundice developed within the first 24hrs in 1 baby with a positive Coombs test and another 5 babies with a positive Coombs test developed jaundice between 24-48 hours. Out of the 80 babies who developed jaundice, 10 were blood group O where as the others were either blood group A or B (Table 5 and 6)

**Table 5. Comparing jaundice and Coombs test**

| Blood group | Jaundice present | Coombs positive | Coombs negative |
|-------------|------------------|-----------------|-----------------|
| O           | 10               | 0               | 10              |
| A           | 48               | 5               | 43              |
| B           | 20               | 1               | 19              |
| AB          | 2                | 0               | 2               |

**Table 6. Timing of jaundice**

| Blood group | Within 24hrs | 24-48hrs | After 48hrs |
|-------------|--------------|----------|-------------|
| O           | 0            | 0        | 10          |
| A and B     | 1            | 5        | 70          |

The study showed that the majority of the mothers were aged between 15-34 years accounting for 64.5% (225) of the enrolled mothers, 26.6% (93) were above 35 years of age and 8.9% (31) were below 15 years of age. The age ranges of the mothers enrolled whereas shown in table 7.

**Table7. Maternal age (N=349)**

| Age(years) | Frequency | Percent | Valid Percent | Cumulative Percent |
|------------|-----------|---------|---------------|--------------------|
| ≤15        | 31        | 8.9     | 8.9           | 8.9                |
| 16-35      | 225       | 64.5    | 64.5          | 73.4               |
| ≥35        | 93        | 26.6    | 26.6          | 100.0              |
| Total      | 349       | 100.0   | 100.0         |                    |

Most of the mothers enrolled in the study had more than 1 child but less than 5 accounting for 45.3% (158), whereas primigravidas accounted for 44.4% (155). Mothers with 5 or more children accounted for 10.3% (36).The results for the parity of mothers enrolled in the study whereas shown in table 8.

**Table 8. Maternal Parity (N=349)**

| Parity  | Frequency | Percent | Valid Percent | Cumulative Percent |
|---------|-----------|---------|---------------|--------------------|
| Valid 1 | 155       | 44.4    | 44.4          | 44.4               |
| 2-5     | 158       | 45.3    | 45.3          | 89.7               |
| >5      | 36        | 10.3    | 10.3          | 100.0              |
| Total   | 349       | 100.0   | 100.0         |                    |

ABO hemolytic disease affects 50% of first-born infants without necessarily affecting subsequent infants (<sup>1</sup>) as compared to Rhesus incompatibility where the first born is usually spared.

## CHAPTER 6

### DISCUSSION

The frequency of newborns with blood group A, B, and AB born from blood group O mothers from this study was found to be 51%, 24.6% and 4.3% respectively.

The results obtained from this study for the frequency of blood groups ABO were comparable to those that have been done in other African countries. A study on the frequency of blood groups ABO and Rhesus D in the Guinean population showed the phenotypical frequencies of the antigens of blood groups A, B, AB and O to be respectively equal to 22.5%, 23.86%, 4.72% and 48.8%. From the study in Guinea it was concluded that these frequencies were not significantly varying between the regions and the ethnic groups and they were close to those observed in sub-Saharan Africa<sup>(29)</sup>.

Another study was done in Nigeria on the gene frequencies of ABO and Rh (D) blood group alleles in a healthy infant population in Ibadan, in order to, provide gene frequency values, 4748 healthy infants were typed for ABO and Rh (D) blood groups over a period of five years. Overall 2575 (54.2%) were blood group O, 1023 (21.6%) were blood group A, 1017 (21.4%) were blood group B and 133 (2.8%) were blood group AB<sup>(4)</sup>.

From this study, the frequency of ABO incompatibility was found to be 49%. Most studies done on ABO incompatibility have been done on Caucasian infants and has shown variable frequencies. A study in Czech republic showed the frequency to be 14%<sup>(14)</sup> where as in Venezuela, it was shown to be 16%<sup>(23)</sup>. In a study in Puerto Rico at two different hospitals, the frequency was 28.3% at Puerto Rico hospital and 18.4% at North Carolina hospital<sup>(24)</sup>. In his study, Bucher concluded that the incidence of ABO incompatibility and hemolytic disease of the new born was 2-3 times greater in Africa-Americans as compared to Caucasians<sup>(13)</sup>.

From this study, the mean cord hemoglobin was found to be 13.7g/dl with a range of 8.9g/dl to 18.3g/dl. It was noted that babies who had low hemoglobin levels were from blood group A and B and this is due to that fact that there is hemolysis taking place in these babies with incompatible blood with their mothers. Conversely, these same babies were noted to have higher levels of cord bilirubin as compared to babies with blood group O for the same reason. The mean cord bilirubin was found to 192umol/l with a range of 25umol/l to 360umol/l. The cord blood hemoglobin and cord bilirubin concentrations of group A babies were not significantly different from those of group B babies. All values in the group A and B babies were significantly higher than the group O babies ( $p < 0.01$ )

From this study, jaundice was present in 50.3% of the babies with ABO incompatibility and Coombs test was positive in 56.7% of ABO incompatible babies. It was noted that all the babies with blood group O had a negative Coombs test despite some babies developing jaundice after 48 hrs. This jaundice was thought to have been physiological. However many of the babies who had a positive Coombs test had jaundice. One baby had severe jaundice which developed within 24 hours and 5 other babies had moderate jaundice which developed within 48 hours. These babies were admitted to the neonatal intensive care unit and were treated with phototherapy. Babies with mild jaundice were discharged home and mothers told to bring back the baby if they noticed that the jaundice had worsened.

Some studies have shown the cut off level of serum bilirubin to be above 425umol/l to perform exchange transfusion (<sup>18</sup>) whereas others studies have used serum bilirubin of above 300umol/l as the cut off level for exchange transfusion (<sup>20</sup>). In this study the highest level of cord bilirubin was noted to be 360umol/l, however exchange transfusion was not being done in the neonatal unit at the time of the study due to non availability of exchange transfusion sets.

It has already been alluded to that there is no single test to diagnose ABO hemolytic disease of the newborn but several tests put together make the diagnosis likely. In this study, the criteria for hemolytic disease of the new born due to ABO incompatibility

included, jaundice in the first 48 hrs, blood group A or B, positive Coombs test and presence of spherocytes in peripheral blood smear. Six babies from this study met the criteria and were said to have hemolytic disease of the newborn as a result of ABO incompatibility. These babies were also noted to have low cord hemoglobin level of 10g/dl and below, high cord bilirubin level of 300umol/l and above, microcytes and/or spherocytes on peripheral smear, and appearance of jaundice within 48 hours. In his study, Desjardin et al showed that ABO incompatibility represents a spectrum of hemolytic disease extending from those in which there is little laboratory evidence of erythrocyte sensitization, but evidence of hemolysis, to severe hemolytic disease in which erythrocyte sensitization is easily demonstrable<sup>(30)</sup>.

From this study it was concluded that ABO hemolytic disease of the new born was prevalent in 3.5 % of all the pregnant mothers that were recruited. It was also found that 49% of all pregnancies in this study were ABO incompatible. In Caucasians , evidence of ABO hemolytic disease of the newborn occurs in up to 3%<sup>(31)</sup>, of all pregnancies despite the fact that 20-25% of all pregnancies are ABO incompatible<sup>(32)</sup>. Several other studies have shown the incidence of ABO hemolytic disease of the newborn to be 2 to 3 times more common in blacks as in Caucasians<sup>(11, 4, 33)</sup>.

This study shows that ABO hemolytic disease is a spectrum of disease occurring in most group A or B of group O mothers. In its mildest form, sensitization is minimal and the clinical pictures such as hyperbilirubinaemia, hemolysis and anaemia are absent or minimal. It is a spectrum from mild laboratory abnormality to severe clinical disease.



## **CHAPTER 7**

### **CONCLUSION**

This study showed that the frequency of blood groups A, B and AB blood groups from blood group O mothers was 51%, 24.6% and 4.3% respectively and that 49% of all pregnant mothers recruited were ABO incompatible.

The mean cord hemoglobin level was found to be 13.7g/dl with a range of 8.9g/dl to 18.8g/dl and the mean cord bilirubin level was found to be 246umol/l with a range of 25umol/l to 360umol/l. Most babies from blood group A and B had low cord hemoglobin and high cord bilirubin levels.

It was noted that hemolytic disease of the newborn due to ABO incompatibility was present in 3.5% of the pregnancies that were recruited in the study according to the criteria for the study.

The study therefore supports the hypothesis that there is a relationship between ABO incompatibility and hemolytic disease of the newborn in Zambia.

## **CHAPTER 8**

### **RECOMMENDATIONS**

1. Blood grouping for pregnant mothers should be done in the antenatal clinics so that at risk infants can be identified early and appropriate management instituted.
2. Blood grouping for babies should also be routinely done so that in infants presenting with jaundice ABO hemolytic disease of the newborn is excluded.
3. Follow up studies are needed on children with high cord bilirubin levels but with no apparent jaundice to see if they can present with cerebral palsy.

### **LIMITATIONS**

Early discharge of infants from the postnatal wards hindered the diagnosis of ABO hemolytic disease of the newborn as jaundice may take up to 24 hours to develop and jaundice may have been missed due to some babies being dark skinned. Babies are discharged early due to a large number of deliveries and limited bed spaces.

It was not possible to exclude full term babies with G6PD deficiency as a cause of jaundice in black infants due to non-availability of reagents.

Since the study was done at the University Teaching Hospital, only high risk patients sent to the hospital were enrolled and this may not be a true reflection of the general population.

Most studies ,in most countries, on the frequency of ABO incompatibility and the incidence of hemolytic disease of the new born due to ABO incompatibility `were done over 5 years ago and therefore most of the references used are old.

Reticulocytosis was not done.

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## **APPENDICES**

- A. Questionnaire
- B. Participation information sheet
- C. Consent form
- D. Baby's laboratory form
- E. Letter of clearance from ethics committee

## QUESTIONNAIRE FOR THE MOTHER

1. Identity No .....
2. Age: .....
3. Parity: .....
4. Gravidity.....
5. Blood group: 1. group O   
2. group A   
3. group B   
4. group AB
6. History of neonatal deaths: 1.Yes  2. No  
If yes, what was the cause: .....
7. History of jaundice in previous babies: 1. Yes  2. No   
If yes, what was the cause.....
8. How was it treated: 1. Phototherapy   
2. Exchange transfusion   
3. Immunoglobulin

## LABORATORY FORM FOR THE BABY

Identity No .....

Age:.....

Birth weight:.....

Apgar score:.....

Blood group: 1. group O

2. group A

3. group B

4. group AB

Cord hemoglobin.....

Cord serum bilirubin.....

Coombs test 1. Positive  2. Negative

Reticulocyte count.....

Peripheral smear.....

Jaundice Present  Absent

If present, what is the severity: 1.mild

2.moderate

3.severe

Time of appearance of jaundice: 1. 0-24 hrs

2. 24-48 hrs

3. Over 48 hrs

Treatment of jaundice: 1. Phototherapy

2. Exchange transfusion

3. Immunoglobulin



- Outcome: 1. Discharged
2. Died

## **PARTICIPATION INFORMATION SHEET**

My name is Monica Kapasa. I am a doctor working here at UTH and training to be a pediatrician (doctor who looks after children). I am conducting a study as part of my training.

The study is looking at hemolytic disease of the newborn. This is a disease that occurs in babies when the mother has blood group O and the baby has blood group A or B. These babies will have their red blood cells destroyed and this will lead to a substance called bilirubin to be high in the body and will lead to the baby developing jaundice which is the yellowing of eyes and skin. This bilirubin if not treated will enter the brain of the baby and lead to a condition called cerebral palsy where the brain does not develop .Because of this the baby will delay in doing things like sitting, walking, talking and will have problems with feeding and will develop malnutrition. The red blood cells that are destroyed will reduce the amount of blood in the body and the baby may have problems with the heart and may have problems with breathing.

In order to check if your baby is at risk of developing Hemolytic disease of the newborn, blood will be collected from the umbilical cord of the baby after its born and will be sent to the laboratory for investigations.

If the baby is found to have Hemolytic disease of the newborn, he/she will be admitted to the neonatal intensive care unit where appropriate treatment will be given.

If you agree to take part in this study, you will be required to sign a consent form or thumbprint if you are not able to write. You are free to withdraw from the study if you so wish for any reason and the treatment of the child will not be affected in any way.

Please note that there will be no payments or gifts offered if you agree to take part in the study.

If you have any questions, feel free to call me anytime on 0966768139 or email at lkapasa@yahoo.co.uk

**CONSENT FORM**

I..... have been invited to take part in the study on hemolytic disease of the newborn. I have read the information, or it has been read to me and I voluntarily consent to have my child participate in the study. I understand that I have the right to withdraw from the study at anytime without affecting the care of the child and that I will be given no special services or any payments or gifts.

.....   ...../...../.....  
Signature/ thumb print of participant                         Date

.....   ...../...../.....  
Name/signature of witness   Date

.....   ...../...../.....  
Name/signature of researcher   Date