STUDIES IN NEONATAL JAUNDICE IN RESPECT OF ABO INCOMPATIBILITY IN PATIENTS OF AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, ZARIA

BY

ELLA, EKAH ELIJAH
(M. TECH/SSSE/2001/2002/775)

SEPTEMBER, 2004

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BEING A THESIS SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY, SCHOOL OF SCIENCE AND SCIENCE EDUCATION IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF TECHNOLOGY (M. TECH.) DEGREE IN MEDICAL MICROBIOLOGY OF THE FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA

SEPTEMBER, 2004

CERTIFICATION

This is to certify that this project work titled "Studies On Neonatal Jaundice in Respect of ABO Incompatibility in Ahmadu Bello University Teaching Hospital Zaria" was carried out under my supervision and has been examined, read and found to have met the regulations governing the award of Master of Technology (M.Tech) degree of the Federal University of Technology, Minna, Nigeria, and is approved for its contribution to knowledge and literacy presentation.

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EXTERNAL EXAMINER — **DATE**

DEDICATION

This research work is dedicated to my loving wife Mrs. Elizabeth E. Ella, and to the memory of mothers who spend precious days in pain and agony to see their jaundiced babies recover. It is my sincere prayers that this work would increase knowledge and adds succour to their pains by providing help to clinicians in the diagnosis and management of neonatal jaundice.

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ABSTRACT

The role of ABO compatibility was studied in relation to its contribution to neonatal jaundice and the severity of the jaundice caused using a total of 50 subjects admitted into the Special Care Baby Unit of A.B.U.TH within the months of March and June 2003. It was found that of the fifty cases analyzed, ABO incompatibility was responsible for 10% while Rhesus incompatibility constituted 12% with a Coomb's reactivity of 100%. Of these incompatible cases, the presence of hemolysin was established in all (100%) in the presence of complement while 81.81% were positive in the absence of complement. The mean PCV, reticulocyte count and white cell count values vary significantly when compared with the normal values (P = 0.05) with a mean bilirubin value of 20.77± 9.48 for the incompatible group indicating a case of hemolytic jaundice with a mean PCV value of 42.75 ±4.26.

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INTRODUCTION

The incidence of neonatal jaundice has witnessed appreciable increase as seen in the number of cases being admitted into hospitals (Campbell *et al.*, 1975). This has caused a lot of pains, expenses and loss of man-hours to parents who go all out to obtain cures for their jaundiced patients. Many of these neonates are usually hospitalized for periods ranging from one week to months depending on the severity of the condition. In some cases, the treatment involves the use of exchange blood transfusion (EBT) (Osborn, *et al.*, 1984a), while others would require long time of exposure to constant light otherwise known as phototherapy (Osborn *et al.*, 1984a, Kaplan *et al.*, 1992). This however is also flanked with the problem of epileptic light supply as always witnessed leading to longer stay in hospital beds.

Neonatal Jaundice as witnessed today is a common but poorly understood problem and it is often of uncertain clinical significance with approximately 50% of term newborn becoming visibly jaundiced in the first week of life (Maisels, 1982). There is a general concern about the increasing incidence of neonatal jaundice in many hospitals leading to suggestions that it might be due to some changes in clinical practices (Sims and Neligan, 1975). A visit to the pediatric wards would reveal the number of neonates that become visibly jaundiced often referred to as the yellow baby syndrome (California websites.com. 1998). Jaundice is the yellowing of the skin or whites of the eyes indicating excess bilirubin (a bile pigment) in the blood (the Oxford Concise Medical Dictionary,

1989). Bilirubin is a bile pigment, a product of the break down of the blood component hemoglobin and excreted in the bile. It is yellow or orange while it's oxidized form is green called biliverdin (Oxford Con. Med. Dictionary, 1989., Hansen, 2002). Bilirubin is formed mainly in the cells of the liver, spleen and bone marrow and also from the breakdown of heam- (iron) containing proteins such as myoglobin, catalase, cytochromes and peroxidases (Cheesbrough, 1987, Oxford Con. Med. Dic.1989.)

Jaundice is also referred to as icterus (Casbore, 2001). This is a condition characterized by a raised bilirubin level in blood (hyperbilirubinaemia) (Wood *et al.*, 1979). According to Wood and others (1979) hyperbilirubinaemia is a level of bilirubin greater than 205µmol/l (12mg/100ml) of blood. Minor levels of jaundice are only detectable by chemical analysis of the blood while severe cases are evident in the yellowing of the skin sclerae and mucosae otherwise referred to as overt or clinical jaundice (Churchill Liv. Poc. Med. Dic., 1987).

The heam (iron porphyrin) of the hemoglobin is first separated from the globin (globulin) and then converted to biliverdin, which is then reduced to bilirubin. Bilirubin accumulates in the blood and results in the yellowing of the skin, eyes, palms and other parts of the body (California websites. com., 1998, Hansen, 2002). Bilirubin can occur as conjugated (coupled to glucuronic acid) or unconjugated. Unconjugated bilirubin is insoluble in water and as such cannot be excreted in the urine. It is bound to albumin and transported by the blood to the liver. Conjugated bilirubin on the other hand, is bilirubin conjugated to glucuronic acid, a process catalyzed by the enzyme glucurony!-transferase in the liver cells.

It is water soluble and non-toxic (Hansen, 2002). The enzyme glucuronyl-transferase becomes fully active about three weeks of life (birth) (Hansen, 2002). Thus, in physiologic jaundice of the newborn, a rise in plasma bilirubin may occur within the first week of life. This is especially so in infants born premature (Cheesbrough, 1987). Conjugated bilirubin passes into the bile canaliculi through the bile duct and into the intestines where it is then excreted. Before excretion, it is converted by intestinal bacteria to urobilirubin (Cheesbrough, 1987). A few quantities is reabsorbed into the body circulation and excreted in the urine (Hansen, 2002).

The normal level of total bilirubin (conjugated and unconjugated) in an adult is 3-17 µmol/litre (0.2-0.9 mg/%) (Cheesbrough, 1987).

Jaundice is classified as follows according to Churchill Livingstone Pocket Medical Dictionary (1987): -

1. **Obstructive Jaundice**: This occurs when bile made in the liver fails to reach the intestine due to obstruction of the bile ducts.
2. **Hepatocellular Jaundice**: This is a disease of the liver cells (e.g. hepatitis). It occurs when the liver is unable to metabolize bilirubin. It accumulates in the blood producing dark urine.
3. **Hemolytic Jaundice**: In this case, there is excessive destruction of red cell in the blood. It is referred to as Icterus or physiologic jaundice and may be caused by incompatibility between mother and child.

4. **Cholestatic Jaundice: -Bile stasis** – This is the arrest of the flow of bile also referred to as intrahepatic cholestasis. The symptoms usually comprise of Jaundice, Itching, pale stools and dark urine.
5. **Acholuric Jaundice** :-(Sperocytosis). This is a case of jaundice without bile in the urine
6. **Infective Jaundice**: This is most commonly due to a viral infection, e.g. infective hepatitis.
7. **Leptospiral Jaundice**: It is also called Weil's disease caused by *Leptosoira interrogans.*
8. **Malignant Jaundice**: It is a case of acute diffuse necrosis of the liver.

OBJECTIVES

Neonatal Jaundice is caused by a number of factors depending on the state of health and well being of the mother and child. The choice of this research is informed by the desire to have a critical evaluation of ABO incompatibility as a contributing factor to neonatal jaundice.

1. Determination of blood parameters of neonates that are visibly jaundiced
2. Determination of the Bilirubin levels of neonates as an indication of hyperbilirubinemia,
3. To determine the contribution of ABO incompatibility to neonatal jaundice and

4. Assay for the presence of hemolysin and antibodies of maternal origin in the circulation of neonates.

It is hoped that the work shall help clinicians manage the case of neonatal jaundice, having had a proper and detailed knowledge of the etiology as it affects mother and child as well as stimulate interest and further research on neonatal jaundice, with particular attention on finding various options that would be used to manage affected newborn.

CHAPTER TWO

LITERATURE REVIEW

2.1. HISTORICAL PERSPECTIVE

The fundamental observations in the late 1940s begat Pauling revolutionary idea of sickle cell anemia as a molecular disease. It was on the heels of this understanding that a series of studies led to the definition of the sickle hemoglobinopathies, an abnormality of the hemoglobin. Amid this excitement, which was 50 years ago, Heldrich reported a proven case of symptomatic sickle cell anemia in a new born, notable for the very young age at presentation. He described a newborn African American boy with a moderate hemolytic anemia of 4 weeks duration for which several transfusions of whole blood was given (Quim, 2001). This was however, found not to be a case of sickle cell anemia as neonatal hemoglobin, the predominant hemoglobin of any newborn prevents sickling and the attendant anemia. It was also found that the postnatal switch to adult hemoglobin production including sickle hemoglobin begins to occur gradually over a few months. This allows the expression of the disease with time and not at birth (Quim, 2001).

However Halbrecht's report on icterus precos in that same year addressed an important question in the period between initial recognitions of antibody mediated neonatal hemolytic diseases and the later prevention of Rhesus (Rh) disease. In his report, some babies without evidence of Rh diseases showed

early significant jaundice whereas others with evidence of maternal antibodies against fetal cells were only mildly affected (Halbrebt, 1951, Casbore, 2001).

This latter discovery led to the conclusion that what was reported by Heldrich was a case of icterus, neonatal jaundice as is known today.

2.2 JAUNDICE:

Jaundice in neonate is a case of hyperbilirubinaemia- a case associated with the accumulation of bilirubin in the circulation of the neonate due to inability to metabolize it (Dennery *et al.,* 2001Hansen, 2002).

2.2.1 Hyperbilirubinemia Is a case or the incidence of a high concentration of un-conjugated bilirubin in the circulation due to impaired hepatic uptake or diminished conjugation and excretion by the liver (Bernstein and Landing, 1962). This often leads to Kernicterus in neonates, a case in which there is accumulation of un-conjugated bilirubin reaching levels at which deposition of bile occurs in the mid and hindbrain with irreversible consequences (Culley, *et al.,* 1970). Apart from neuro-toxicity renal medullary and cortical necrosis have been found at autopsy-apalysis (Culley, *et al.,* 1970). Hyperbilirubinemia is indicated when the un-conjugated bilirubin level is in the excess of 205 µmol/L (12mg/100ml) of blood (Wood *et al.,* 1979).

Mild jaundice of the newborn is accepted as normal, requiring no detailed medial regimen but severe jaundice is regarded as a serious problem. As the number of factors influencing perinatal bilirubin metabolism increases

so does the number of mechanisms, which could theoretically lead to severe jaundice (Campbell, *et al.,* 1975). A common presentation of jaundice in neonates is referred to as *Icterus gravis neonatum* (Churchill Liv. Poc. Med. Dic., 1987).

2.2.2 *Icterus gravis neonatum*: Jaundice in neonate is often present at birth or appears within hours. It is more intense and persistent than the jaundice of simple hepatic immaturity (Bernstein and Landing, 1962). In most cases, the fall in erythrocytes and hemoglobin which normally takes place after birth is exaggerated though insufficient to be diagnosed as a case of anemia. The symptom includes enlarged liver (hepatomegaly) with the spleen becoming palpable, the grey matter of the hemisphere, midbrain, cerebellum and medulla may be affected and become bile-stained (kernicterus) and the ganglion cells degenerate (Brimblecombe and Barltrop, 1978). When these are observed, death may occur within days (Brimblecombe and Barltrop, 1978).

2.3. BILIRUBIN AND ITS METABOLISM.

Bilirubin is the end product of heme (iron porphyrin) catabolism and is formed through oxidation-reduction reactions (Hansen, 2002). Approximately 75% at bilirubin, according to Hansen, (2002) is derived from hemoglobin. The heam of the hemoglobin is first separated from the globulin (globin) and then converted to bilirubin by an oxidation reaction catalyzed by heme oxygenase with the release of iron and carbon monoxide. The iron is conserved for reuse. The

water-soluble biliverdin is then reduced to bilirubin, which because of the intra-molecular hydrogen bonds is insoluble in water in its most common isomeric form (bilirubin IXZZ) (Hansen, 2002). Due to its hydrophobic nature, it remains un-conjugated (Cheesbrough, 1987) and is transported in the erythrocytes. The presence of endogenous and exogenous binding competitions such as drugs decreases the binding affinity of albumin for bilirubin (Hansen, 2002) leaving a minute fraction of un-conjugated bilirubin is the serum. This free fraction is able to cross lipid-containing membranes, including the blood brain barrier, leading to neuro-toxicity (Culley, *et al.*, 1970, Watchko and Oski, 1992, Hansen, 2002).

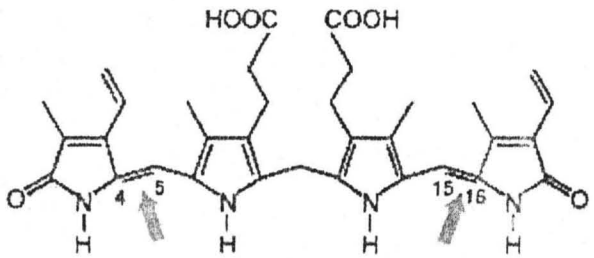

Fig 1. The Bilirubin molecule

When the bilirubin-albumin complex reaches the hepatocyte (liver cells), bilirubin is transported into the cell where it is partially bound to ligandin (Hansen, 2002). The uptake by hepatocyte is facilitated according to Hansen, (2002) by increasing ligandin concentration. Ligandin concentrations are low at birth but increases rapidly over the first few weeks of life. It may be increased by the administration of Phenobarbital (Valaes, *et al.*, 1980). In the hepatocyte endoplasmic reticulum, bilirubin is conjugated to glucuronic acid in a reaction catalyzed by uridine diphosphoglucuronyltransferase, (UDPGT) (Cheesbrough,

1987, Hansen, 2002). This conjugation is critical as it transforms the water insoluble (un-conjugated) bilirubin molecule to a water-soluble (conjugated) bilirubin molecule. Water solubility allows bilirubin to be excreted in the bile. The activity of UDPGT is low at birth (Hansen, 2002) but increases to adult values by age 4-8 weeks. Certain drugs like Phenobarbital, dexamethasome; clofibrate can be administered to increase UDPGT activity (Valaes *et al.,* 1980, Hansen, 2002).

Conjugated bilirubin is excreted into bile and transferred to the intestines where it eventually is reduced to a colourless tetrapyrroles by microbes in the colon (Hansen, 2002). In the intestine de-conjugation could occur in the proximal small intestines through the actions of β-glucuronidases located in the borders: (Hansen, 2002). This un-conjugated bilirubin could be reabsorbed into the circulation and increase the plasma level. This cycle uptake-conjugation – excretion de-conjugation- re-absorption is termed the enterohepatic circulation, and is extensive in the neonate partly because nutrient intake is limited in the first days of life. This prolongs the intestine transit time (Bernstein and Landing, 1962, Dennery, *et al.,* 2001 Hansen, 2002)

2.4. PATHO-PHYSIOLOGY OF JAUNDICE:

Jaundice in neonates is facilitated by two predisposing phenomena:

- The rate of bilirubin is higher in infants than adults and this is because their red blood cells have a shorter half-life and a higher turn over. Infants have a very high level of red cells with a P.C.V

value of 50-60 within the first week of life (Gupta, 1978, Dennery, *et al.,* 2001, Hansen, 2002).

- The hepatic excretory capacity is low due to low concentration of the binding protein ligandin in the hepatocytes and low activity of glucuronyl transferase, the enzyme responsible for binding bilirubin to glucuronic acid for efficient elimination (Sims and Neligan, 1975, Dennery, *et al.,* 2001, Hansen, 2002,).

All these predisposing factors in the neonate, tend to contribute to jaundice and thus mild jaundice are usually not considered to constitute a serious problem as it would normally disappear within few days after birth, even without special treatment (californiawebsites.com.,2003). To a lesser extent, it has been shown that variations in populations appear to be related to the tendency for the development of jaundice (Jo @ anesthetist. com. 2001). These include a mutation in UDPGT (Asians) and high incidences of glucose -6- phosphate dehydrogenase deficiency (Greeks). The predisposing factors earlier considered, coupled with the following conditions play significant role in the onset of jaundice in newborn.

2.5 FACTORS AFFECTING THE INCIDENCE OF NEONATAL JAUNDICE:

2.5.1. Infection: - Bacterial infection is a recognized cause of hyperbilirubinemia in the newborn and some reports suggest that unexplained indirect hyperbilirubinemia may be the only manifestations of sepsis in seemingly

"healthy" newborns (, Rooney, *et al.,* 1971, Chavalitdhamrong, 1975, Linder, *et al.,* 1988). Neonatal bacterial infection occurs significantly among low birth weights, after prolonged rupture of membrane and in the course of other neonate illnesses leading to septicemia (Maisels and kring, 1992). Some investigations have reported that the appearance of jaundice in the first few days after birth may be the first sign of bacterial sepsis in healthy looking neonates (Rooney *et al.,* 1971). In one of such documented cases, 22 newborns with bacterial infection and hyperbilirubinemia were identified with serum bilirubin levels which were exceptionally high 484 to 856 umol/L (28.3 to 50mg /100ml) and several with significant elevations of direct reacting bilirubin. (Rooney *et al.,* 1971). Linder *et al.,* (1988) in their research, reported cases of term neonates younger than 7days old having serum bilirubin levels that exceeded 17μmol/L (10mg/100ml) on the first day of birth. This level in their report rose to 25μmol/L (15mg/100m) after 48 hours (Linder *et al.,* 1988) with some showing positive blood culture of bacterial infection. Generalized neonatal bacterial infections (septicemia) are frequent and their prognosis is still poor (Bennet, *et al.,* 1981, Guillois, *et al.,* 1989,).

Common organisms isolated (associated with neonatal septicemia) include;

2.5.1.1. Group B-Streptococcal Infections in the newborn have been described in numerous reports (Barton *et al.,* 1973, Franciosi, *et al.*, 1973, Lloyd and Reid, 1976). In their report, they considered

the organism, the pattern of illness it engenders, and frequency of isolations as a major cause of neonatal sepsis. It has further been suggested that the disease has an early onset and mimics the respiratory distress syndrome even to the extent of hyaline membrane formation (Ablow, *et al.,* 1976). The age of onset of sepsis as well as the time at which death occurs is early in streptococcal bacteremia as compared to other Gram positive and Gram-negative bacteremia (Xanthou, 1972, Kuchler, *et al.,* 1976).

2.5.1.2. Meningitis: Cases of meningitis in neonates have also received wide attention and reports (Drew and Arroyave, 1981). Organisms commonly isolated include *Escherichia coli group D-streptococcus Staphylococcus aureus, klebsciella, Enterobacter cloacae, Acinetobacter, Serratia, and Aeromonas aeruginosa* (Guillois, *et al.,* 1989) Other pathogens often encountered with an early onset, within 48 hours of birth include *Listeria monocytogenes Clostridium welchii, Haemophilus influenza, Ps. alkaligenes* and *Streptococcus viridans* (Jeffery, *et al.,* 1977).

2.5.2. Prematurity:

In infants born pre-maturely (preterm) a rise in plasma bilirubin usually occurs within the first week of life, a phenomenon referred to as physiological jaundice of the newborn (Cheesbrough, 1987). The rate of the fall of Hemoglobin and the nadir of its fall are greater in pre-term

infants, the anaemia of prematurity. This is occasioned primarily due to the very rapid rate of growth of preterm infants whose expanding blood volume quickly outgrows their shrinking erythrocyte mass (Merck manual, 2003). In infants the liver and intestinal systems are immature and cannot excrete bilirubin as fast as the body produces it. About one-half of all newborn develops jaundice and premature infants are much more likely to develop it (Edgren, 2003). Preterm infants are infants delivered before the gestation period of 36 weeks (Jo @ anaesthetist.com., 2001). Other factors closely related to prematurity and known to contribute to the unset of jaundice include low birth weight (below 2500g) resuscitation at birth, poor feeding and use of drugs.

2.5.3. Glucose –6- Phosphate Dehdrogenase Deficiency (G-6- P.D D). G-6-P-D deficiency has been found to be associated with the incidence of jaundice (hyperbilirubinemia) especially among the Greeks (Sheba, *et al.,* 1962). This however is associated with only males and is notable in those with ethnic origins in Greece, Turkey, Nigeria, Sardinia and Sephardic Jews from Kurdistan, Iraq, Iran and Syria (Sheba *et al.,* 1962, Jo @ anesthetist. com., 2001). Surprisingly, however, despite the high frequency of the enzyme deficiency in selected groups, very few controlled studies have addressed the issue of G-6- PD deficiency associated neonatal hyperbilirubinemia (Milbauer *et al.,* 1973, Ashkenazi *et al.*, 1983). The incidence of this phenomenon is traceable to the Jewish population

(Sheba, *et al.,* 1962), which, during their long stay in dispersion, remained a distinct group maintaining their religious and cultural bonds and keeping marriages to non-Jews to a minimum. This relative isolation in addition to a high rate of consanguineous marriage in some groups may well have influenced the genetic makeup and resulted in the propagation of deleterious genes differing significantly from the indigenous Jewish population (Sheba *et al.,* 1962, Kaplan and Abramox, 1992). The mass return of Jewish exiles following the establishment of the State of Israel in 1948 provided a unique opportunity for studying the genetic tracts. It was rare among Ashkenazi Jews (Eastern European origin), of low incidence among North African Jews and of high incidence in Asian Jews especially Jews from Kurdistan in whom the incidence was found to be as much as 58.2% (Sheba, *et al.,* 1962, Kaplan and Abramox, 1992). It has been established that G-6-P.D. deficient neonates are prone to developing jaundice in the neonatal period than normal neonates (Piomelli, 1987). This jaundice is also associated with the ingestion of or contacts with certain drugs, chemicals and breast milk of a mother who recently ate fava bean (Kaplan and Abramox, 1992). The etiology of this jaundice is obscure. Exposure to hemolytic agents such as naphthalene (Valaes *et al.,* 1963), quinine (Glass *et al.,* 1973), herbal remedies (Brown and Boon, 1968), triple dye (Ramot *et al.,* 1964) and menthol (Owa, 1989) may trigger the jaundice. However jaundice do occur even in the absence of known trigger materials (Kaplan and Abramox, 1992), and is due to an alternative

factor, which may be defective glucuronidation of bilirubin in the liver due to defective G-6-PD activity in the hepatocyte. This is supported by the works of Oluboye *et al.,* (1979) who reported decreased G-6-PD activity in liver biopsy tissues of G-6-P.D. deficient adults and that of Berry and Melmed (1977) whose work associated severe jaundice in G-6-PD deficient patients with infectious hepatitis. Glucose–6-phosphate dehydrogenase deficiency is a frequent occurring X-lined enzymatic defect (WHO, 1989, Beutler, 1991). The distribution of this type of the enzymatic defect has also been described in a variant population in Nigeria (Bienzle, *et al.,* 1976). It has also been described among U.S. term neonates (Lopez and Cooperman, 1971). The incidence is also high in infants with mutations in the gene coding for UDPGT (Gilbert syndrome) and in infants with homozygous in heterozygous G-6-PD deficiency (Hansen, 2002).

2.5.4. Oxytocin And Drug Induced Jaundice In Neonates: The increase in the frequency and severity of neonatal jaundice have been linked with the increased use of oxytocic agents for the active management of labour (Campbell *et al.*, 1975). The increasing use of oxytocic drugs to induce labor and its incrimination in the onset of neonatal jaundice was also described by Ghosh and Hudson (1972), Sims and Neligen, (1975). They attributed the rise in the incidence of jaundice in neonates to be largely due to the frequent use of these drugs in inducing labour.

2.5.4.1. **Oxytocic drugs:** They are agents' employed in the hastening of parturition as well as agents promoting uterine contractions. (Churchill Med. Poc. Dic., 1987). A common oxytocic drug employed in the management of labour is oxytocin (Sims and Neligen, 1975). It is a hormone obtained from the posterior pituitary gland found to contract muscles in milk ducts and hence causing milk ejection (Churchill Med. Poc. Dic., 1987). A preparation obtained from pituitary extract called syntocinon (Churchill Med. Poc. Dic., 1987) is found to cause uterine contraction. However, other researchers could not confirm these claims and found no correlation between the use of oxytocin and the incidence of neonatal jaundice (Osborn *et al.,* 1984b). In their findings (Osborn *et al.*, 1984b) administration of medications such as Demerol, magnesium sulfate, pitocin, and vistaril, during labour and delivery did not appear to influence an infant's risk for becoming jaundiced. Other workers (Wood *et al.,* 1979) found that if used in relatively low dosage, oxytocin has no effect and showed its effect in jaundice only in cases where post-maturity was excluded. The view supports that of Davies *et al* (1973) and Beazley and Alderman (1975) that the effects of oxytocic drugs are likely to be dose related. That oxytocic drugs have direct influence on neonatal jaundice therefore, remains a mirage and with further research, the true picture could emerge.

2.5.5. Breast Milk Jaundice: Maternal milk jaundice is interesting and complex. Human milk has received a worldwide acceptance, in responds to the campaign of UNICEF for exclusive breast-feeding, and as the most valuable and nearly perfect food for the newborn (Freier and Eldelman, 1980). Milk is also considered to be the most satisfactory single food substance elaborated by nature and found to contain 3.8% fats, 3.2% protein, 4.8% carbohydrates, 0.7% minerals and 87% water in addition to lactose, phosphates, protease, enzymes lysozymes and vitamins (Salle, 1973). The campaign for exclusive breast-feeding, the UNICEF method requires that newborn be exclusively breast-fed for six months from birth, and has receive wide acceptance in Nigeria with many hospitals designated Baby-friendly hospitals and centers. Despite the many advantages of Breast-feeding, there is ample documentation of the strong association between breast-feeding and an increase in the risk of neonatal hyperbilirubinemia (Jo @ anesthetist. com., 2001, Gourley, 2002). The association of Breast milk to jaundice was described by Arias *et al* (1963), Gartner and Arias, (1966) as a syndrome of prolonged neonatal un-conjugated hyperbilirubinemia related to the ingestion of certain abnormal human milks. The work of Maisels and Gifford (1986) also confirmed that breast-feeding is strongly associated with increased serum bilirubin levels within the neonatal age of 3-6 days as well as in later days. When compared with infants fed on infant formula, Kivlaham and James (1984) showed that the incidence of physiologic jaundice was significantly higher

in breast-fed newborn. Breast-fed infants thus have higher serum bilirubin levels than formula-fed infants and there is also evidence that breast-feeding is a significant risk factor for extreme hyperbilirubinemia and kernicterus (Gourley, 2002).

2.5.5.1. Human milk, Infant formula and neonatal Jaundice. In a review of 8000 infants (Schneider, 1986) it was found that moderate jaundice (Serum bilirubin greater than 12mg/100ml) was 3 times more likely among breastfed than formula-fed neonates while severe jaundice serum bilirubin >15mg (100ml) was 6 times more likely in the breastfed group. Another studies established that 6.8% of breastfed infants' developed unexplained serum bilirubin levels greater than 15mg/100ml (DeAngelis. *et al.,* 1980). The association between human milk feeding and jaundice is also seen in premature infants fed with banked human milk (Lucas and Baker, 1986), and a combination of human milk and infant formula (Sirotal *et al.,* 1988). This phenomenon was observed in many communities and various races that practice breast-feeding (Fisher, *et al.,* 1978). In the United States, increase in neonatal jaundice was recorded, occurring at a rate of 22% to 23% between 1959 and 1966 (Maisels and Gifford, 1986). This figure however rose to between 54% and 55% in 1980 and was seen to correlate with the period in which dramatic increase in breast-feeding was recorded (Martinez and Krieger, 1984)

2.5.5.2. **Physiopathology of Breast Milk Jaundice:** Inhibition of hepatic excretion of bilirubin was found to be associated with human milk consumption and early studies suggested that the steroid pregnane-3 α 20 β- diol inhibited glucurony/fransferase activity (Arias *et al.*, 1964). This enzyme is responsible for the conjugation of bilirubin with glucuronic acid prior to biliary excretion (Gourley, 1998). The inhibition of UDPGT by pregnane-3 α 20 β- diol impairs intestinal absorption which is a key step in the enterohepatic circulation of bilirubin since UDPGT is the conjugating enzyme responsible for this action to form glucuronides (Gourley, 2002). Another enzyme Beta-glucuronidase that is responsible for the de-conjugation of glucuronides has been identified in human milk (Gourley, 2002). Beta glucuronidase has been found in lysozyme, microsomes (Fishman *et al.*, 1964) and also isolated from a variety of mammalian tissues and fluids (Dutton, 1966). Studies on 34 breast fed infants (Gourley and Arend, 1986) revealed that human milk contained considerable levels of Beta-glucuronidase (418 $\pm$ 334 modified sigma units/ml). These levels were found to be 7 to 8 times higher than maternal serum Beta glucuronidase level while the levels of Beta-glucuronidase in standard infant formula was negligent (6.0-6.8 msu/ml) (Gourley and Arend, 1986). Another factor associated with breast milk and the onset of neonatal jaundice was the suggestion of Poland *et al,* (1980) that breast milk contain free fatty acids, which do inhibit hepatic bilirubin glucurony/transferase. Poland

et al, (1980) found that milk samples obtained from women whose infants exhibited the clinical syndrome of breast-milk jaundice, contained high level of non-esterified fatty acids (NEFA), which inhibited UDP-glucurony/transferase activity *in-vitro*. Poland *et al* (1980) attributed the high levels of NEFA to the hydrolysis of the milk triglycerides by abnormally high lipase activity. Others found that concentration of NEFA in milk and their UDPGT inhibitory activity increased with storage time even at 4^0C (Luzaeu *et al.*, 1974). At least 2 lipases have been described in human milk and in milk from other primates (Hernell, 1975). These are bile salt-stimulated and serum stimulated lipases. The action of the bile salt- stimulated lipase results in the hydrolysis of triglycerides to glycerol and tree fatty acids (Hernell and Olivectora, 1974 I &II). The milk with high lipase activity is often referred to as abnormal milk. Normal women milk contains little lipase activity (Poland *et al.*, 1980).

The mechanism of breast milk jaundice is therefore related to inhibition of UDPGT by substances in breast milk. These fatty acids increase neonatal levels, which are known to inhibit UDPGT (Jo @ anesthetist. com., 2001). Fatty acids themselves accumulate stored milk, which explains why such milk tends to raise bilirubin levels when taken. Breast milk may also increase bilirubin absorption from the intestines due to the beta-glucuronidase activity (Decarvallo *et al.*, 1985). However several other reasons have been proffered as possible reasons why breast milk jaundice is on the increase: One of such reason is the

fact that Breast fed infants loose more weight (and presumably receive less calories) in the first few days of life than bottle fed infants and an association between weight loss and hyperbilirubinemia have been documented (Wood *et al.*, 1979, Butler and Macmillan, 1983). Calorie deprivation is known to increase the plasma bilirubin concentration in normal adults as well as those with Gilbert syndrome (Barret, 1971). However, Kivlahan and James (1984) failed to confirm this in their research. Osborn *et al*, (1984b) related the onset on jaundice in Breast fed babies to calorie loss, confirming the works of Felsher *et al.*, (1970), Barret, (1971) and Bloomer *et al* (1971).

2.5.6. Rhesus Iso Immunization

Rhesus iso-immunization has been observed as one of the causes of jaundice of the hemolytic type (Kivlahan and James, 1984, Merck manual, 2003). The Rhesus (Rh) antigen was discovered by Landsteiner and Wiener (1940) when they found that by injecting blood from the monkey *Macacus rhesus* into rabbits and guinea pigs, antibodies were obtained which agglutinated the monkey's cells and about 85% of red cells from human donors. Those donors whose cells were agglutinated were termed Rh-positive while the remaining 15% were termed Rh-negative. Historically, the Rh antigen has served as an informative marker for studying the evolution of human population (Cavalli-Storza, 1991, Hyland *et al.,* 1994) and has become a major blood groups system in transfusion and clinical medicine. (Cartron and Agre, 1993).

Biochemical investigations have shown that the Rhesus antigen is composed of a set of antigens, the main ones being D, Cc, Ee, and G (Race *et al.*, 1944, Mourant, 1945). The D antigen and those of the Cc and Ee series are carried by at least three distinct but homologous integral membrane protein of apparent Mr=30,000.-32,000 (Blanchard *et al.,* 1988). Many attempts at determining the chemical structure of the D antigenic determinants have been made using the techniques of inhibition of agglutination (Watkins, 1966). It has been postulated that the Rh antigen structure is a large complex containing Rh and Rh-related proteins as well as other related glyco-proteins (Cartron and Agre, 1993). It is well established that the Rh gene is located on chromosome IP34-IP36 (Marsh *et al.*, 1974, and Cherif-zahar *et al.,* 1991), and is composed of the homologous RhD and RhCE genes in RhD positive individuals but of the RhCE gene only in the RhD negative individuals (Colin, *et al.*, 1991). The number of the D sites on the human erythrocyte has been variably estimated to be about 20,000 to 33,000 per red blood cell (Watkins, 1966). Rhesus (Rh) incompatibility may occur when an Rh-negative woman carries an Rh-positive fetus. Maternal isommunization occurs after some incompatible fetal red blood cell (RBC) crosses the placenta and induces an immunologic response with specific maternal anti-Rh antibodies some of which subsequently cross the placenta into the fetus and lead to hemolysis of fetal red blood cells (Merck manual, 2003).

2.5.6.1.. Rhesus Antibodies

The antibodies to the Rh antigen were first recognized by Levine and Stetson (1939). The most common anti-Rh-antibodies occurs as

incomplete agglutinin requiring colloids as suspending media for serologic tests (Diamond and Denton, 1945). The employment of antiglobulin techniques discovered by Coombs also enhanced the rapid discovery of anti-Rh sera with many different specificities and serologic reactivity (Coombs *et al.*, 1945). The major characteristics of the Rh antibodies according to Bryant (1976) are as follows.

I. Majority of Rh-antibodies are IgG (75%) reacting at 37^0C. Early stages of the immune response are prominently IgM.

II. IgM, the early agglutinins are saline agglutinins and do not cross the placenta

II. IgG produced in response to stimulation crosses the placenta but do not agglutinate saline suspended Rh-positive cells.

IV. Rh antibodies are capable of causing severe hemolytic transfusion reactions.

V. Naturally occurring anti-D is extremely rare.

Another related phenomenon associated with hemolytic jaundice and the basis for this research is the association of ABO isoimmunization as a possible cause of neonatal jaundice.

2.5.7. ABO Isoimmunization And Neonatal Jaundice

Despite the advances made in the diagnosis and treatment of Rh hemolytic diseases of the newborn, the role of the A and B factors, as a cause of a similar disease has not received sufficient treatment (Rosenfield, 1955).

Zuelzer and Cohen (1957) pointed out the importance of ABO incompatibility and Robinson, *et al.*, (1960) associated hyperbilirubinemia to ABO incompatibility.

The factors that hindered the general appreciation of A-B hemolytic disease according to Rosenfield (1955) could generally be classified as follows.

I. Whereas Rh disease is readily recognizable with the aid of the direct anti-globulin lest, A-B disease may not be recognized easily.

II. An unusual antibody is not present in the serum of the mother since anti-A and anti- B are normally present

III. The usual form of the disease has little or no anemia and a variable degree of jaundice and

IV. Less than 10 percent of all severe cases of hemolytic disease of the newborn are due to the A-B factor (Rosenfield, 1955, Risemberg, *et al.*, 1977).

2.5.7.1 The ABO System

Studies on agglutination of erythrocytes and bacterial antigens began in the 1900s and this has provided very useful information on many fields of study (Grubb, 1951). One such research areas is the field of blood group antigens discovered by Karl Landsteiner in 1901 (Bloodbook. com., 2001). In his work, Landsteiner obtained blood from six of his colleagues and reacted the serum and cells obtained from each with the cells of the others. His work produced an interesting agglutination pattern from which he proposed what is known today as the ABO blood group

antigens (Zmijewski and Fletcher, 1972). A summary of his work is presented in the Table 1 below.

TABLE 1. THE RESULT OF LANDSTEINER EXPERIMENT.

Sera	Cells						Antibody.
	Drs ST	Ple	Sturl	Erd	Zar.	Landst	
Dr St	-	+	+	+	+	-	α and β
Dr Ple	-	-	+	+	-	-	β
Dr Sturl	-	+	-	-	+	-	α
Dr Erd	-	+	-	-	+	-	α
Dr Zar	-	-	+	+	-	-	β
Dr.Lanst	-	+	+	+	+	-	α and β
Antigen	0	A	B	B	A	0	

His discovery was closely followed in 1902 by the work of his colleagues Decastrello and Sturli (Bloodbook. com., 2001) who reported the findings on the AB group in that same year. Since these discoveries, a lot of works have been done in the attempts to discover blood group antigens, and its attending reactions. Other workers discovered related blood groups to the ABO and these include A_1,A_2 A- A_1B and A_4 (Zmijewski and Fletcher, 1972, Bryant, 1976).

2.5.7.2: The Chemistry of the ABO Blood Antigens.

Antigens of the ABO (H) systems are found on the erythrocyte surface from fetal life but may not express themselves of full strength until about three

years after birth (Bryant, 1976). It forms an integral part of the cell membrane and the estimate of the number of "A" sites on a single erythrocyte vary widely from as low as 120,000 to as high as 1,170,000 while the number of "B" sites have been estimated at 310,000 to 830,000 per cell (Barret, 1978). The ABO system is determined by the terminal glycosyl residue attached to a common carbohydrate chain of the red cell surface with N-acetylgalactosaminyl-transferase an enzyme found in group A people and is responsible for the production of the A-antigen while galactosyl-transferase present in B-patients catalyses the production of the B-antigen. Both enzymes are however absent in group O individuals (Hearn *et al.,* 1968, Race *et al.*, 1968, Schenkel-Brunner and Tuppy, 1969). Red cell membranes contain a protein precursor substance, which is converted to H-substance by the product of the dominant H-gene (Bryant, 1976, Baker, *et al.*, 2001). A and B antigens are synthesized by the addition of specific sugar molecules to the H-substance. In the absence of H genes, this conversion does not occur and the result is an absence of the ABH antigens and is known as the Bombay phenotype (Baker, *et al.,* 2001). According to Bryant, (1976) the expression of the A and B antigens is coded for by the A and B genes, which is dependent on the H gene. He gave the sequence of events leading to the expression of the ABH on the red cells as follows.

I. A precursor muco-polysaccharide substance is first converted by the gene H to H substance.

II. The H substance is partly converted by the A or B genes into A or B antigens. Some H substance remains unconverted.

III. The O-gene being amorphic, effects no conversion of the H-substance.

The pathway is presented in Fig 2 below.

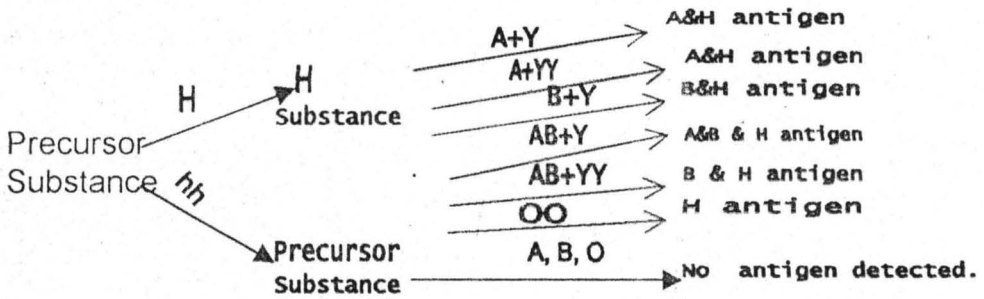

Fig 2. Genetic pathway leading to the production of A, B and H antigens on the red blood cells (Watkins and Morgan, 1959).

The allelic genes A, B, and O located on chromosome 9 and H and h on chromosome 19 respectively control the ABO system (Baker, *et al.*, 2001). Bryant (1976) indicated that the antigens consist of four molecules of three different sugars, N-acetylgalactosamine, D-galactose N-acetylglucosamine. The addition of a sugar known as L-fucose to the terminal D- galactose producing the H-substance. The presence of A gene results in the attachment of the sugar N-acetylgalactosamine to the H-substance to produce the A specificity while the B-gene results in the attachment of D-galactose to the terminal end unit (Barret, 1978). The absence of the A and B gene leads to the inability of group O individuals

to produce the antigens coded by these genes. Thus the H-substance remains without the terminal sugar antigens (Bryant, 1976, Barret, 1978).

2.5.7.3. Theories Of Inheritance Of The ABO H "System"

Many postulates have been presented regarding the hereditary probabilities of the ABO system. Bernstein proposed the presently adopted and most accepted scheme (Zmijewski and Fletcher, 1972). He invoked the existence of a single tri-allelic system with genes A, B and O operative at a single genetic locus (Zmijewski and Fletcher, 1972). The resulting phenotype and genotype based on his theory are as in Table 2

TABLE 2. ABO PHENOTYPE AND THEIR CORRESPONDING GENOTYPE

Phenotype	Genotype
A.	A/A, A/O
B.	B/B, B/O
O.	O/O
AB	AB

From his theory, the A and B genes are co-dominant and both are dominant to the O gene, which is recessive. He observed that there are 21 possible mating combinations that would give rise to a varying number of genotypes and phenotypes in the offspring (Zmijewski and Fletcher, 1972). The method of gene exchange and recombination is said to follow the simple

Mendelian theory of inheritance (Bryant, 1976). The mating and expected offspring are presented in the Table 3 below: -

TABLE 3. GENOTYPE EXPECTED IN OFFSPRING RESULTING FROM MATING BY BERNSTEIN'S THEORY.

Matings	Expected offspring.
A/A x A/A	A/A
A/A x A/O	A/A, A/O
A/A x B/B	A/B
A/A x B/O	A/B, A/O
A/A x A/B	A/A, A/B
A/A x O/O	A/O
A/O x A/O	A/A,A/O,O/O
A/O x B/B	A/B, B/O
A/O x B/O	A/B,O/O,A/O,B/O
A/O x A/B	A/A,A/B,A/O,B/O
A/O x O/O	A/O,O/O
B/B x B/B	B/B
B/B x B/O	B/B, B/O
B/B x A/B	A/B, B/B
B/B x O/O	B/O
B/O x B/O	B/B, B/O, O/O
B/O x A/B	A/B, B/B, A/O, B/O
B/O x O/O	B/O, O/O
A/B x A/B	A/A, A/B, B/B
A/B x O/O	A/O, B/O
O/O x O/O	O/O

2.5.7.4. Antibodies to the ABO "H" System

Alongside his discovery, of the ABO system in 1901, Karl Lansteiner also reported the presence of antibodies directed against the A and B antigens in the sera of his colleagues (Zmijewski and Fletcher, 1972, Bryant, 1976). He reported that individuals belonging to Blood group A has antibodies against B antigen in

their sera while those with group B blood has antibodies against the A antigen in their sera. Group O individuals have both anti-A and anti-B antibodies in their sera while those with AB cells possess neither of the antibodies. These antibodies are referred to as iso-antibodies (Zmijewski and Fletcher, 1972, Blood book. com., 2001, Hull, 2003,).

2.5.7.4.1. Iso-antibodies:- anti- A and anti-B was found to occur with precise regularity in the sera of all individuals as reported by Landsteiner (Bryant, 1976). Their production in infants does not begin units about 10days of age, with 50% of infants expressing them of 40 days of age and almost all by the age of 4 months (Baker, *et al.,* 2001). Studies reported by Baker, *et al,* (2001) revealed the existence of natural antibodies, which is also referred to as allo-antibodies. They gave their properties as follows:

i. Reacting maximally at 4^0C but the thermal range of activity includes 37^0C,
ii. They are cold agglutinin i.e. agglutinate cells suspended in saline,
iii. The agglutinated cells adhere strongly and are difficult to break up (Baker, *et al.,* 2001).

The presence of an immune type of anti-A and anti-B was reported and these are the main causes of ABO hemolytic disease often associated with jaundice in neonate (Hull, 2003).

2.5.7.5 Physiopathology of ABO Related Neonatal Jaundice: Human red blood cells bear on their surface membrane the ABO antigens and thus antigenic (Barret, 1978). Red cells that present foreign antigenic

configuration to the host are also considered harmful and specific antibodies (immune antibodies) are produced against them (Bryant, 1978). This, according to Bryant (1978) can occur in any of the following ways.

2.5.7.5.1. **By Transfusion**: If red cells of a different antigenic group is given to an individual that host would respond by producing antibodies against such cells leading to lyses of the offending red cells (Bryant, 1978, Hull, 2003).

2.5.7.5.2. **Through Pregnancy**. The placental transfer of red cells between infant and mother do initiate an immune response if the infant red cells bear antigens that are not present in the mother (Bryant, 1978, Hull, 2003).

It is this second phenomenon that results in the hemolytic reaction of the newborn often associated with mild jaundice which may progress with time if it is not detected and treated early (Hull, 2003 and Merck, manual 2003). The major response in the immune reaction is the production of antibodies.

Antibodies are serum proteins that are produced in response to antigenic stimulation and have the ability to react specifically with the antigen that stimulated their production (Barret, 1978). This means that the existence of naturally occurring antibodies in humans maybe as a result of some sort of antigenic stimulation. Actually, these anti-A and anti-B were stimulated and appear to be related to stimulations by the inhalation, or ingestion of bacteria, seeds and common foodstuffs which

have similar chemical structures to the ABO antigens (Zmijewski and Fletcher, 1972, Bryant, 1976, Baker, *et al,* 2001). Generally, antibodies are immunoglobulin and have been classified, based on their structures and molecular weight into IgM, IgA, IgE, IgD, and IgE (Rowe and Faley, 1965, Barret, 1978).

As indicated earlier (Baker, *et al,* 2001) the naturally occurring antibodies are cold agglutinins and most of them belong to the IgM class. They are not known to exhibit hemolytic properties *in vitro* and do not fix complement. However on immunization with blood group antigens, the titer of iso-antibodies showed increase and they acquire hemolytic properties (Zmijewski and Fletcher, 1972). This is referred to as immune anti A and B. The properties of naturally occurring and immune antibodies for anti-A are presented in Table 4 below.

Table 4. Properties of Naturally occurring and Immune Anti-A.

	Natural Anti-A	Immune Anti A
Lytic properties	No	Yes
Specificity	$A_{common} + A_1$	A_1
Reactivity in agglutination	Complete	Incomplete
Immunoglobulin type	IgM or IgG	IgG (IgA?)
Reaction with soluble A substance	Easy to neutralize	Difficult to neutralize
Optimum reactive temperature	20^0C	37^0C
Reaction with Pig A cells	No	Yes

In the event of an incompatible pregnancy between mother and child it has been established that fetal cells cross the placenta from the 28th weeks of gestation though the number of cells are usually small to cause appreciable antibody production (Mollison, 1972). At term, a relatively large transfusion of fetal cells into the maternal circulation occurs initiating appreciable immune response, and since the mother already possess antibodies of the naturally occurring type, a secondary response is initiated resulting in large production of IgG, which has the ability to cross the placenta (Bryant, 1976, Weir, 1983). The route and degree to which the various classes of immunoglobulin are transferred to the offspring vary in different species in mammals (Brambell, 1970, Hemmings, 1974). The rate of transfer of immunoglobulin varies according to their size and structure. Another factor is dependent upon the capacity of the cells of the placenta membranes to allow endocytosed proteins to be transferred across it (the placenta) without being degraded (Wild 1973). Other studies have showed that the transfer of plasma proteins across the placenta is most likely by diffusion in the case of albumin whereas an active transport is involved in the process of transfer of immunoglobulin molecules (Gitlin, 1974). In man, the transmission of immunoglobulin across the placenta occurs exclusively by the way of the chorioallantoic membrane applying the principle of selection (Brambell, 1970). Maternal 1gG molecules are detectable in the fetal blood after 10 weeks gestation and by the end of the normal gestation period of 36 weeks, the mean level of IgG in the newborn sera reaches a level slightly higher than that seen in the corresponding maternal blood (Beard and Nathanielsz 1976).

During the first week of life, the maternal IgG molecules are replaced gradually by similar immunoglobulin produced by the infants and at about one year, the immunoglobulin found in the infant would exclusively bear the infants markers (Beard and Nathanielsz, 1976). Cases of hemolytic diseases do result from pregnancies involving a group A, B, and AB infants in which the mother is O group. It is said to be an important diagnosis in cases of neonatal jaundice and anemia, with very severe cases requiring exchange blood transfusion (Brimblecombe and Barltrop, 1978). This often occurs where the infants inherit a blood group from the father that is not compatible with that of the mother, leading to the maternal response to the fetal cells (Bryant, 1976, Merck manual, 2003). Determination of hemolytic anemia is usually presumed in cases of an Rh negative woman immediately after birth once the baby's blood is known to be positive (Merck manual, 2003). This is not routinely carried out in cases involving the ABO incompatible births. Newborns incompatible with their mothers in the ABO system presents a potentially higher risk of severe hyperbilirubinemia especially in the presence of a positive direct coomb's test (Orzalesi *et al*, 1973).

2.6. ABO INCOMPATIBILITY AND COOMB'S REACTION

Coomb's test is a common diagnostic test employed in the detection of maternal antibodies in the fetal circulation. The presence of jaundice was significantly associated with coomb's test positivity in infants (Orzalesi, *et al*, 1973). In their report, Orzalesi *et al* (1973) stated that infants with ABO incompatibility the presence of a positive coomb's test carried a risk of neonatal

jaundice which they found to be four times higher than in infants with a negative coomb's test. However, Risemberg, *et al* (1977) warned that the coomb's test is not by itself a reliable method of predicting the severity of hyperbilirubinemia.

2.7. DIAGNOSTIC INVESTIGATIONS OF NEONATAL JAUNDICE.

The investigation carried out to establish the case of neonatal jaundice includes physical and laboratory studies according to Hansen (2002).

2.7.1. - **Physical Observation**. Neonatal jaundice first becomes visible in the face and fore head and progress gradually to the trunk and extremities. This phenomenon is referred to as cephalocaudal (or cephalopedal) progression (Kramer, 1969, Hansen, 2002 and Beeby, 2003). In most infants, the yellow coloration is the only finding in physical examination. Other symptoms include drowsiness, changes in muscle tone, seizures and altered crying characteristics (Hansen 2002). The characteristic color of a neonate at birth is pink.

2.7.2 Laboratory Findings-

2.7.2.1 **Transcutaneous Bilirubinometry**. This can be performed using a device to measure the skin color and estimate the total bilirubin level transcutaneously (Hansen 2002 and Beeby 2003). This is a presumptive diagnosis to allow for prompt attention while further investigation is being awaited.

2.7.2.2. **Serum Bilirubin (SBR).** This is regarded as the gold standard for deciding if a baby's jaundice requires intervention (Beeby, 2003).

One of the limitations is that it is an indirect method of measurement. Another limitation is that the SBR level that is save for a given baby is not known. Many authors have predicted that levels of 1.7mg/100ml (Davidson *et al* 1941) and 1.25mg/100ml (Johnstone, 1953) are safe and normal values in healthy infants. Other investigations include

2.7.2.3 **Blood Type And Rh Determination**

2.7.2.4 **Direct Coomb's Test**

2.7.2.5 **Hemoglobin and Hematocrit Values**

2.7.2.6 **Peripheral Blood Film For Erythrocyte Morphology**

2.7.2.7 **Reticulocyte Count**

2.7.2.8. **Liver Function Tests (Aspartate Aminotransferase –ASAT, and Alanine Aminotransferase –ALAT).** These are elevated in hepatocellular diseases.

2.7.2.9. **Full Blood Count** and film to reveal spherocytes or septic changes.

2.7.2.10. **Glucose -6-Phosphate Dehydrogenase Screen** (if male and of appropriate ethnic group).

2.7.2.11. **Sepsis Screen**.

2.7.2.12. **Galactosaemia Screen**. (Hansen, 2002 and Beeby, 2003).

2.7.2.13. **Screening Hypotonic Osmotic Fragility**

2.7.2.14. **Titration Of Maternal Antibodies**, and

2.7.2.15. **Hemolysin Titer** against infant red cells (Rosenfield, 1955)

2.8. THE HEMATOLOGICAL VALUES OF NORMAL NEONATES:

The total white cell count and differential counts are valuable parameters and guides in the diagnosis, treatments and prognosis of various childhood illnesses. These vary with age. During the first 3 days of age, the total white cell counts are usually high ranging from 9000 to 35000 cells per cubic milimetre (9.0 to 35.0 X 10^9 cells per liter) (Lanzkowsky, 1980). The lymphocyte count values and their ratio to the granulocytes (neutrophils, eosinophils and basophils) vary. The lymphocytes predominate in the first few years of life until 4 years and thereafter, the granulocytes dominate (Table 5). Other hematologic values are presented in Table 6 reflecting the normal values for reticulocyte and PCV.

Table 5. Normal Leukocyte counts.

	TOTAL LEUCOCYTE		NEUTROPHIL	LYMPHOCYTE
AGE	MEAN	RANGE	%	%
Birth	18.1	9.0-30.0	61	31
12hours	22.8	13.0-38.0	68	24
1 week	12.2	5.0-21.0	45	41
1 month	10.8	5.0-19.5	35	56
2 years	10.6	6.0-17.0	33	60
4 years	9.1	5.5-15.5	42	50
6 years	8.5	5.0-14.5	51	42

(Lanzkousky, 1980)

Table 6. Normal hematological values

	1 day	2day	6day	2weeks	Adult
Reticulocyte%	3 2 to 8	3 2 to 10	1 0.5 to5	0.4	0.5 to 2
FBC/cumm	17,000	17,000	13500	12000	7000
Eosinophils total	20 to 100	--	--	--	100-400
Lymphocytes%	20	20	37	55	25-33
Monocytes %	10	15	9	8	3-7
Hematocrit %	54 ± 10	--	51	50	37 -47

(Gupta, 1978)

2.9. MEDICAL INTERVENTION AND TREATMENT REGIMEN

After proper identification has been carried out using a series of investigations, management of the jaundice component can be commenced. These include the following.

2.9.1. Phototherapy. Phototherapy has been shown to be a safe and effective method for lowering the SBR level (Brown *et al*, 1985). Cremer *et al* (1958) was the first to report the use of light, both natural and artificial as a treatment for neonatal hyperbilirubinemia. Prompted by the observation of nursery staff that the yellow pigmentation seen on jaundiced neonates seem to fade after exposure to sunlight for a short while they placed several jaundiced infants in sunlight and discovered that the serum bilirubin level decreased after such exposure (Beeby, 2003). This began the use of phototherapy for the

management of neonatal jaundice, which is still in use today (Beeby, 2003). The relationship and effect of different lights employed in the management of jaundice as well as their intensity are presented in the reports of Lucey, (1972) Ostrow, (1972), Sisson, *et al* (1972) and Hansen (2002).

2.9.2. Intravenous Immunoglobulin (IVIG). The use of a combination of IVIG and phototherapy has been shown to significantly reduce the SBR and need for exchange blood transfusion especially in babies with isoimmune hemolytic jaundice as compared to phototherapy alone (Alcock and Liley, 2003)

2.9.3. Exchange Blood Transfusion. This is indicated for avoiding bilirubin neuro-toxicity when other therapeutic methods have failed. It may also be indicated in infants with erythroblastosis presenting severe anemia, hydrops, or both (Beeby, 2003). SBR levels at which exchange transfusion may be indicated is 350μmol/L (20mg/100ml)(Hansen, 2002 and Beeby, 2003)

2.8.4. Tin Mesoporphorin. This is a substance that acts by inhibiting hemoglobin oxidase and thus reduces bilirubin production. It is, however, being reviewed and yet to be employed widely (Suresh, *et al,* 2003)

2.8.5. Others. To a lesser extent, phenobarbitone has been found to be effective in decreasing the level of bilirubin (Arias, *et al*, 1969, Crigler and Gold, 1966). Agar and activated charcoal have also been used to reduce enterohepatic circulation and interruption of breast-feeding has been advocated in the control of breast milk jaundice (Beeby, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1. SCOPE AND AREA OF COVERAGE

3.1.1 Scope: - The project span a period of four months from the month of March to June 2003. Within this period, a total of 50 newborn admitted into the Hospital and diagnosed, as having jaundice were included in the study. The selected hospital was Ahmadu Bello University Teaching Hospital, (A.B.U.T.H.) Zaria.

3.1.2 Subject Study: - The period under study, only neonates (ages 0-28 days) admitted into the hospital and clinically diagnosed, as having jaundice was included. This excluded every other admission in respect of illnesses other than jaundice as well as children above 28 days old.

3.1.3. Area of Coverage: - ABUTH Zaria serves the Health needs of a wide population of people from all parts of the country. It is the biggest teaching hospital in the northern part of Nigeria and properly equipped for effective management of neonatal patients. It also serves for referral from other hospitals outside the State. The sample size was significant considering the scope of coverage and health needs served by the Hospital under study.

3.2 SAMPLES

A total of three (3) milliliter of venous blood was obtained from neonates admitted into the Special Care Baby Unit (S.C.B.U) of the Ahmadu Bello University Teaching Hospital (A.B.U.TH) Zaria. Similarly, another three 3 milliliter was collected from their mothers. Sample collection was limited to neonates within the ages of 0 to 7 days as this is the age bracket in which neonatal jaundice is mostly observed (Maisel, 1982). The samples were collection were within the months of March to June 2003 and within this period, a total of 50 neonates were sampled and included in the analysis. All samples were collected aseptically and separately into sterile heparinized tubes using a five mls syringe and needle and immediately transported to the laboratory shielded from light (Light breaks down bilirubin) in an ice park. All the samples were labeled and given a code number corresponding to the month of collection and ready for subsequent analysis.

3.3 SAMPLE TREATMENT

In the laboratory the samples were centrifuged at 2500rpm for 10 minutes to obtain serum using a bench centrifuge (MSE Minor bench centrifuge BA 6063). However before separation of the cells from the serum blood group determination, packed cell volume, white cell count, differential leukocyte count, and reticulocyte count were carried out. After separation of cells, the remaining tests that included Coomb's, bilirubin, and titration of sera, hemolysin and saline agglutination were performed.

3.4 BLOOD GROUP DETERMINATION

Blood grouping was carried out using the slide agglutination method (Baker, *et al.*, 2001). Four drops of the baby's blood and four drops of the mother's blood were placed at different spots on a floor tile i.e. one drop per spot. A drop of monoclonal Antibody A (Anti-A) was added to the first pair of blood (baby and mother). Antibody B (Anti B) was added to the second set of drops, Antibody AB (Anti AB) was added to the third while Antibody D (Anti D)-Rhesus factor- was added to the forth set of drops. The mixtures were rocked by an orbital rotation manually and observed for agglutination after five minutes. All monoclonal antibody reagent used were from Biotec laboratories (U.K.)

3.5 RETICULOCYTE COUNT

This was also performed for the neonate's blood. Three drops of the whole blood was placed in a Kahn tube and three drops of 10 percent New Methylene blue stain was added (New Methylene blue in citrate-saline). The mixture was incubated at 37°C for 30 minutes in an incubator (Gallenkamp model 150). After staining a thin film was prepared from it and was allowed to air dry .It was then viewed using the high power objective (x100) of a binocular microscope. Reticulocytes were counted and percentage occurrence calculated. This is the total number of reticulocytes (nucleated red cells) per 1000cells counted.

$$\% \text{ Reticulocyte} = \frac{X}{1000} \times 100$$

where: X = number of cells counted (reticulocytes)

3.6 BILIRUBIN DETERMINATION:

This was determined by the method of Malloy and Evelyn of 1937 as reported by Baker, *et al* (2001). The method is based on the formation of a purple compound Azobilirubin, when bilirubin reacts with the diazotized sulphanilic acid introduced by Vander Bergh.

3.6.1. Conjugated Bilirubin: Into 2.7mls of distilled water was added 0.1ml of serum in two different tubes. Exactly 0.7mls of a mixed diazo reagent was added to the test while the blank was mixed with 0.7mls of diazo A reagent only. A standard was prepared using 0.2mls of standard bilirubin as control. 2.6mls of distilled water was added to a third tube containing 0.2mls of the standard bilirubin in 3.5mls of methanol to which was also added 0.7mls of mixed diazo reagent. All three tubes were incubated at room temperature for 5 minutes. The absorbance was read at 540nm using a spectrophotometer (Cecil 1000 Series). The conjugated bilirubin was calculated using the formula;

$$\text{Con. Bilirubin} = \frac{T-B}{S-B} \times 171\mu mol/L$$

Key. T=Test
S=Standard
B=Blank

3.6.2. Total Bilirubin: This was determined using the method for conjugated bilirubin and then 3.5mls of methanol was added to the test and blank leaving the standard (the standard already containing methanol). The absorbance was read after 5 minutes at 540nm using the same spectrophotometer above and the total bilirubin was calculated using the formula.

Key: T=Tests

Total bilirubin = $\frac{T-B}{S-B}$ × 342 μmol/L S=Standard B=Blank

N B- **Diazo A:**-1gm of sulphanilic acid (Analar) in concentrated hydrochloric acid (BDH) and made up to 1L with distilled water.

Diazo B:-0. 5gms of sodium nitrite (BDH) 100mls of distilled water.

Diazo reagent: - 0.3mls of solution B to 10.0mls of solution prepared fresh always.

3.7 PACKED CELL VOLUME (P.C.V.)

This was done using whole blood for the neonate only. The heparinised blood was allowed to flow into a capillary tube by capillary action. One end of the capillary tube was then sealed using the flame of the Bunsen burner. It was placed in the haematocrit centrifuge (Hawksley microhaematocrit centrifuge Patent No 891481) and centrifuged at 5000rpm for 10 minutes. At the end of centrifugation the haematocrit was read using the PCV reader (Hawksley micro-haematocrit reader 850179). The haematocrit reading is equal to the P.C.V. value.

$$PCV = \frac{\text{Height of packed cell column.}}{\text{Height of whole blood column.}} \times 100$$

3.8 WHITE BLOOD CELL COUNT

The total white blood count was determined for the neonate blood using the haemocytometer (Neubauer improved chamber S.748) In the process 0.05ml

of heparinised blood was pipetted into the white blood cell pipette and it was filled with the white cell diluting fluid (3.0 percent Acetic acid containing 0.01gm of gentian violet in distilled water) to the 1ml mark to make a dilution of 1:20. It was allowed to stand at room temperature for 10 minutes after which a Pasteur's pipette was used to fill the haemocytometer chamber with a cover slip in place by capillary action. The preparation was placed on the binocular microscope (Olympus model BH2) stage and the cells counted using the medium power objective (x40), and the total white blood count determined per cubic mm of blood, using the formula:

$$W.B.C. = \frac{N \times 20 \times 10^6}{5 \times 0.1}$$

where – N = Number of cells counted
20 = dilution factor.
5 = total area counted
0.1 = depth of hemocytometer.

3.9 DIFFERENTIAL LEUCOCYTE COUNT

A thin blood film was prepared using the neonate blood on a clean grease free microscope slide and stained with Wright's stain. The stained film was air dried and observed using the high power objective (x100) of a binocular microscope (Olympus model BH2) and the various cells and their percentages determined i.e. number of each cell type per 100 white cells counted.

$$\% \text{ Cell count} = \frac{X}{N + L + E + M + B} \times 100$$

where – N = Neutrophils
L = Lymphocytes
E = Eosinophils
M = Monocytes
B = Basophils.

{N.B. For normal values refer to Tables - 5 & 6}

TABLE 8: BLOOD GROUP DISTRIBUTION OF VISIBLY JAUNDICED NEONATES AND THEIR MOTHER.

BABY	MOTHER	NO OF CASES	%
O+	O+	32	64
O+	O-	3	6
O+	A+	1	2
O+	B+	1	2
O-	O+	1	2
O-	A-	1	2
A+	O+	1	2
A+	A+	3	6
A+	A-	2	4
A+	AB+	2	4
B+	O+	1	2
B+	B+	2	4
	TOTAL	50	100

TABLE 9: NUMBER AND PERCENTAGE DISTRIBUTION OF CASES WITH ABO COMPATIBILITY AND ABO INCOMPATIBILITY

GROUP	BABY	MOTHER	NO (%) OF CASES	TOTAL
ABO COMPATIBLE	O+	O+	32 (64)	
	A+	A+	3 (6)	
	B+	B+	2 (4)	
	A+	AB+	2 (4)	
				39 (78)
ABO INCOMPATIBLE	O+	A+	1 (2)	
	O+	B+	1 (2)	
	A+	O+	1 (2)	
	B+	O+	1 (2)	
	O-	A-	1 (2)	
				5 (10)
RHESUS INCOMPATIBLE	O+	O-	3 (6)	
	O-	O+	1 (2)	
	A+	A-	2 (4)	
				6 (12)

TABLE 11: ANALYSIS OF RETICULOCYTE, TOTAL BILIRUBIN AND PACKED CELL VOLUME (P.C.V.) IN COMPATIBLE GROUP

CODE NO	BLOOD GROUP Baby	Mother	RETICULOCYTE	BILIRUBIN LEVEL	Packed Cell Volume
MA1	O +	O+	6.9	18.8	46
MA2	B+	B+	5.2	16.8	42
MA3	O+	O+	3.4	9.4	48
MA5	O+	O+	4.6	18.8	40
MA6	O+	O+	1.4	15.0	48
MA7	O+	O+	1.8	17.1	46
MA9	O+	O+	3.6	_	_
MA10	O+	O+	3.0	8.4	_
MA11	A+	A+	3.2	9.4	_
MA12	O+	O+	1.8	26.9	_
MA13	O+	O+	3.9	18.8	_
AP14	O+	O+	3.4	10.3	52
AP15	O+	O+	2.6	_	48
AP16	O+	O+	2.1	_	42
AP17	O+	O+	3.2	15	60
AP18	O+	O+	3.9	_	36
AP19	A+	A+	1.4	15	46
AP22	O+	O+	1.8	17.8	52
AP24	O+	O+	3.7	8.4	38
AP26	O+	O+	2.7	_	41
AP27	B+	B+	1.6	16.8	38
AP28	O+	O+	3.1	10.3	42
MY30	A+	A+	2.6	10.3	48
MY31	O+	O+	1.0	15.0	52
MY34	A+	AB+	4.1	10.3	40
MY35	O+	O+	1.5	_	54
MY36	O+	O+	4.5	31.0	42
MY37	O+	O+	3.6	16.8	46
MY38	O+	O+	2.7	_	_
MY39	O+	O+	3.1	29	42
MY40	O+	O+	1.1	10.5	46
MY41	O+	O+	1.5	23.4	46
MY42	O+	O+	3.7	25.7	48
JN44	O+	O+	2.9	_	48
JN45	O+	O+	1.1	18.8	_
JN46	O+	O+	4.2	10.4	52
JN47	A+	AB+	2.9	10.5	46
JN48	O+	O+	3.1	27.1	44
JN50	O+	O+	4.2	15.0	46

TABLE 12: ANALYSIS OF RETICULOCYTE COUNT TOTAL BILIRUBIN, AND PACKED CELL VOLUME (P.C.V.) IN INCOMPATIBLE GROUP.

CODE NO	BLOOD GROUP Baby	Mother	RETICULOCYTE	BILIRUBIN	Packed Cell Volume
MA4	B+	O+	3.1	27.1	46
MA8	O-	O+	5.2	28.0	_
AP20	O+	O-	2.1	_	_
AP21	O+	A+	3.8	16.8	42
AP23	A+	A-	2.1	_	46
AP25	O-	A-	2.0	26.2	38
MY29	O+	B+	4.1	25.7	38
MY32	O+	O-	1.9	12.2	_
MY33	A+	A-	4.1	8.4	46
MY43	A+	O+	3.8	8.4	48
JN49	O+	O-	5.9	34.1	38

4.6. WHITE CELL COUNT (W.C.C.)

The mean W.C.C. for the compatible group was 12558.09 cells per cubic milimetre of blood, which is 12.558 x 10^9 cells per litre of blood while the count for the incompatible group was quite low with a mean of 8154.22 cells per cubic milimetre of blood, which is 8.154. X 10^9 cells per litre of blood. The details are presented in Tables 13 & 14 below.

4.7. DIFFERENTIAL LEUCOCYTE COUNT:

The count was evenly distributed showing no significant difference in the compatible and incompatible groups. The results are presented in Tables 15 & 16 below.

4.8. COOMB'S REACTION:

In the direct test carried out, a total of six samples were highly positive constituting 12% of the total samples analyzed while 35 neonates were weakly Coomb's positive which is 70% of the total samples. However, 6 were negative constituting 12% of the samples. The results are represented in Table 17.

4.8.1 Comparative Study Of Compatible Group Using Coomb's Reaction:

A total of 33 compatible neonates were positive (66%) while only six were negative (12%). The group O compatible had 27 positive cases constituting 54% while negative cases were 5(10%). Other showed that group A had 3 cases (6%), group B 2(4%) and group A/AB 1(2%). This is presented in Table 18.

4.8.2 Comparative Study Of Incompatible Group Using Coomb's Reaction:

All of the cases analyzed were positive with no negative case. This is represented in Table 18.

TABLE 13: WHITE BLOOD CELL COUNT OF ABO COMPATIBLE GROUP

CODE NO (N = 27)	COUNT OF WHITE BLOOD CELLS	
	Cells /Cubic mm	Cells / Litre
MA1	11200	11.2×10^9
MA2	11600	11.6×10^9
MA3	13400	13.4×10^9
MA5	6200	6.2×10^9
MA6	13400	13.4×10^9
MA7	8800	8.8×10^9
MA9	13900	13.9×10^9
MA10	8300	8.3×10^9
MA11	11200	11.2×10^9
MA12	11500	11.5×10^9
MA13	8800	8.8×10^9
AP14	11600	11.6×10^9
AP15	8800	8.8×10^9
AP16	11200	11.2×10^9
AP17	11300	11.3×10^9
AP18	12600	12.6×10^9
AP19	7500	7.5×10^9
AP22	13200	13.2×10^9
AP24	8800	8.8×10^9
AP26	10400	10.4×10^9
AP27	11200	11.2×10^9
AP28	11500	11.5×10^9
MY30	4600	4.6×10^9
MY31	12320	12.32×10^9
MY34	21300*	21.3×10^9*
MY35	16900	16.9×10^9
MY36	10400	10.4×10^9
MY37	7900	7.9×10^9
MY38	8800	8.8×10^9
MY39	14800	14.8×10^9
MY40	6800	6.8×10^9
MY41	6000	6.0×10^9
MY42	4400	4.4×10^9
JN44	13200	13.2×10^9
JN45	7200	7.2×10^9
JN46	16900	16.9×10^9
JN48	6600	6.6×10^9
JN47	8800	8.8×10^9
JN50	7500	7.5×10^9
MEAN ± SD	12558	$12.558 \times 10^9 \pm 1.89 \times 10^9$

N = Number of data, SD = Standard deviation

TABLE 14: WHITE BLOOD CELL COUNT OF ABO INCOMPATIBLE GROUP

CODE NO (N = 9)	COUNT OF WHITE BLOOD CELLS	
	Cells / Cubic mm	*Cells / Litre*
MA4	9200	9.2×10^9
MA8	7288	7.288×10^9
AP20	6800	6.8×10^9
AP21	6500	6.5×10^9
AP23	9200	9.2×10^9
AP25	4400*	$4.4 \times 10^{9*}$
MY29	6000	6.0×10^9
MY32	--	--
MY33	8800	8.8×10^9
MY43	13200*	$13.2 \times 10^{9*}$
JN49	6400	6.4×10^9
MEAN ± SD	8154	$8.154 \times 10^9 \pm 2.267 \times 10^9$

N = Number of data, SD = Standard deviation

TABLE 15: DIFFERENTIAL LEUCOCYTE COUNT OFABO COMPATIBLE GROUP.

CODE NO	NEUTROPHIL	LYMPHOCYTE	MONOCYTE	EOSINOPHIL	BASOPHIL
MA1	28	36	16	14	4
MA2	20	53	16	9	2
MA3	18	50	23	8	1
MA5	44	30	22	4	0
MA6	36	38	14	12	0
MA7	31	46	12	10	0
MA9	33	47	12	8	0
MA10	29	45	15	11	0
MA11	26	44	12	15	0
MA12	46	37	10	5	0
MA13	20	54	15	8	1
AP14	41	43	7	8	0
AP15	48	25	12	15	0
AP16	45	35	8	13	0
AP17	40	30	16	14	0
AP18	50	30	12	8	0
AP19	36	47	10	7	0
AP22	45	36	10	9	1
AP24	41	36	12	11	0
AP26	37	42	9	11	0
AP27	35	37	12	13	1
AP28	45	35	9	11	0
MY30	40	37	10	13	0
MY31	29	52	13	8	0
MY34	30	44	15	11	0
MY35	20	56	11	12	1
MY36	11	59	14	15	1
MY37	27	41	20	12	0
MY38	30	46	13	11	0
MY39	20	55	12	11	1
MY40	21	55	11	12	2
MY41	29	54	11	8	0
MY42	30	45	14	11	0
JN44	35	40	12	13	0
JN45	30	59	4	6	0
JN46	35	40	11	12	1
JN47	30	60*	4	6	0
JN48	26	46	12	13	0
JN50	48	30	10	12	0
MEAN	33.1	43	12.87	10.62	0.43

TABLE 16: DIFFERENTIAL LEUCOCYTES COUNT OFABO INCOMPATIBLE GROUP.

CODE NO	NEUTROPHIL	LYMPHOCYTE	MONOCYTE	EOSINOPHIL	BASOPHIL
MA4	46	37	9	6	2
MA8	26	45	11	16	2
AP20	48	30	12	10	0
AP21	42	40	9	8	1
AP23	38	41	9	12	0
AP25	35	37	13	13	2
MY29	38	42	10	11	0
MY32	41	38	10	11	0
MY33	49	31	11	9	0
MY43	27	49	13	10	1
JN49	40	35	10	14	1
MEAN	37.69	40.69	10.46	10.54	0.69

TABLE 17: COOMB'S TEST RESULT

NO OF SAMPLES WITH POSITIVE COMBINATION	COOMB'S TEST	
	DIRECT	INDIRECT
6	++	++
2	++	+
1	+	++
35	+	+
6	-	-

TABLE 18: COMPARATIVE STUDY OF COOMB'S REACTION FOR ABO COMPATIBLE AND INCOMPATIBLE CASES

CASE	BLOOD GROUP	COOMB'S REACTION POSITIVE	NEGATIVE	TOTAL
COMPATIBLE	O+	27	5	32
	A+	3	0	3
	B+	2	0	2
	A/AB+	1	1	2
INCOMPATIBLE	B+ O+	1	0	1
	A+ O+	2	0	2
	O- A-	1	0	1
	O+ B+	1	0	1
	O+ O-	3	0	3
	O- O+	1	0	1
TOTAL		42	6	48

4.9. TITRATION OF MATERNAL SERA:

The result showed that 2 samples (4%)-MA4 & MA6 had an unusual high titer of 512 while 6 samples had a titer of 256. Among these, most were from the incompatible group. The other titers were within borderline reactions and could be attributed to non-specific response. The result is presented in Table 19.

4.10. HEMOLYSIN DETERMINATION:

Four samples gave a moderate positive reaction when reacted with un-inactivated serum (unheated serum) compared with 14 samples, which were negative in complement-inactivated serum. On the other hand, 15cases were positive for both unheated and heated serum. The complete result is represented in Table 20.

4.10.1. Comparative Study Of Compatibility And Hemolysin Reaction:

Tables 21 & 22 showed that in the unheated serum, 23(46%) were positive in ABO compatible cases and 14(28%) were negative, whereas in the ABO and Rhesus incompatible cases, all the 11(22%) samples gave positive reaction. The result was however different with the heated serum with only 11(22%) positive cases in the compatible group while 26(52%) samples gave negative reaction indicating that most of the positive response observed in the unheated serum was as a result of complement action probably as a result of activation of the properdin (alternate) pathway. However, in the incompatible ABO and Rhesus cases, 9(18%) samples gave a positive reaction while only

2(4%) were negative. This is indicative of the presence of hemolysin probably Immunoglobulins that resulted in the hemolysis of the neonate's red cells.

4.11. SALINE AGGLUTINATION;

In the compatible group, only two samples showed a Mild agglutination (2+) level while 13 cases were negative, without any visible agglutination. However, the incompatible group had higher levels with one sample highly positive (3+) and 4 with mild agglutination (2+). This is evident of the presence of immune agglutinins. The result is presented in Table 23.

TABLE 19: COMPARATIVE ANALYSIS OF MATERNAL ANTIBODIES IN THE NEONATE'S CIRCULATION FOR COMPATIBLE AND INCOMPATIBLE CASES

TITER	COMPATIBLE	INCOMPATIBLE	TOTAL
512	1	1	2
256	2	4	6
128	–	–	–
64	–	1	1
32	2	–	2
16	5	1	6
8	11	3	14
4	10	1	11
2	6	–	6
TOTAL	37	11	48

TABLE 20: HEMOLYSIN DETERMINATION OF MATERNAL ORIGIN DIRECTED AGAINST NEONATAL RED CELLS

NO. OF CASES	HEMOLYSIS IN UNHEATED SERUM	HEMOLYSIS IN HEATED SERUM
4	++	+
15	+	+
15	+	-
14	-	-

Key ++ Highly Positive

\+ Positive

\- Negative

TABLE 21: HEMOLYSIN REACTION FOR ABO COMPATIBLE GROUP

UNHEATED SERUM		TOTAL	HEATED SERUM		TOTAL	BLOOD GROUP
+	-		+	-		
17	13	30	9	21	30	O+
2	1	3	1	2	3	A+
2	0	2	1	1	2	B+
2	0	2	0	2	2	A+/AB+
23	14	37	11	26	37	TOTAL

TABLE 22: HEMOLYSIN REACTION FOR ABO INCOMPATIBLE

BLOOD GROUP B	M	UNHEATED SERUM +	-	TOTAL	HEATED SERUM +	-	TOTAL
B+	O+	1	0	1	1	0	1
A+	O+	2	0	2	2	0	2
O-	O-	1	0	1	1	0	1
O+	B+	1	0	1	0	1	1
O+	O-	3	0	3	3	0	3
O-	O+	1	0	1	0	1	1
A+	A-	2	0	2	2	0	2
TOTAL		11	0	11	9	2	11

TABLE 23: SALINE AGGLUTINATION RESULTS.

	1(-)	2(+)	3(++)	4(+++)	TOTAL
COMPATIBLE	13	22	2	-	37
INCOMPATIBLE	-	6	4	1	11
TOTAL	13	28	6	1	48

Key +++ Highly Positive

++ Mildly Positive

\+ Weakly Positive

CHAPTER FIVE

5.0 DISCUSSION.

All the neonates included in the study were jaundiced within the first week of life with 50% of them reporting to the hospital on the third day. Those admitted on the third day were 25 constituting 50% of the total subjects (Table 7). The actual time of the onset of the jaundice could not be accurately determined as this was a judgement of the mothers most of whom were illiterates as far as neonatal illnesses are concerned. However, the result correlate with the findings of many workers (Lawrence, 1979, Maisels, 1982, Kivlahan and James, 1984) that physiologic jaundice peaks at 72 hours and rapidly decreases thereafter. Similarly the figures compared with the compatible cases as presented in Table 9 in which 78% of the cases were of compatible relationship between mother and child indicating that most of the cases were physiologic and non hemolytic in nature. Whereas previous studies centered on babies of group A and B born to group O mothers (Rosenfield, 1955, and Risemberg, 1977) this study took into consideration all cases of jaundice admitted into the hospital within the period of the research. Of the 50 babies analyzed within the period, 39 (i.e. 78%) were compatible by the ABO grouping while the remaining 11 (22%) were incompatible (Table 9). A breakdown of the incompatible group showed that 5 cases constituting 10% were ABO incompatible while the remaining 6 cases constituting 12% were Rhesus incompatible (Table 9). This was in agreement with the findings of Risemberg *et al,* (1977).

The values obtained from the hematological findings showed a slight variation from the normal values. The mean reticulocyte count of 2.98 for the compatible group was slightly lower than the values of the incompatible group, which has a mean of 3.46. However the statistics showed no significance in the values P = 0.05 (refer to Appendix 8). Moreover, both figures were within the range indicated by Gupta (1978). The PCV values were also lower in the incompatible group with a mean of 42.75 as compared to the compatible group with a mean of 45.76. The analysis by the student's t' test, at P = 0.05, showed significant difference in the values (refer to Appendix 8). This reveals a mild degree of anemia in the incompatible group. Anemia is a known symptom associated with ABO incompatibility (Merck manual, 2003). The statistical analysis showed significance in the values thus confirming the existence of significant difference between the two values (refer to Appendix 8). The value of the compatible group was within the range stated by Gupta (1978), which was put at 54 $\pm$ 10.

Likewise, the bilirubin levels differ significantly in the two groups P =0.05 (refer to Appendix 8). The level in the incompatible group was higher. This is indicative of the severity of the jaundice (Hansen, 2002). The group has a means level of 20.77mg/100ml as compared to the compatible group with a mean of 16.348mg/100ml. These agree with other findings that jaundice resulting from incompatibility usually present higher levels of serum bilirubin above20mg/100ml (Hansen, 2002, Beeby, 2003). Physiologic jaundice is therefore likely in the case of most of the compatible group though some of them presented a positive

coomb's result. These antibodies so detected could be cross-reacting antibodies or other maternal immunoglobulin, which usually occur in the fetal circulation (Beard and Nathanielsz, 1976).

A survey of the white cell counts analysis did not reveal any unusual trend (Tables 13 &14). The count for both compatible and incompatible were low as compared to the normal value given by Gupta (1978). When the values were subjected to chi-square χ^2 analyses at P = 0.05, no significant difference was indicated in the compatible group and the normal accepted value while the incompatible group showed a remarked difference (refer to Appendix 8). The differential leukocyte counts obtained from both groups revealed a similar pattern (Tables 15 &16). The range of the counts was found to be within the range indicated by Lanzkousky (1980) as presented in Table 5. These figures also agree with the findings of Read, *et al* (1979), that blood counts in cases of hepatic and obstructive jaundice are usually normal unless there are cases of blood loss. The count was fairly low with the incompatible cases, which is indicative of mild anemia associated with hemolytic jaundice. Similarly the white cell counts (W.C.C.) for the compatible group fell within the range of that indicated as normal values for neonates in the first week of life, which was put as 5.0×10^9 to 12×10^9 cells per litre of blood. These values are useful indices in detecting cases of bacterial infections, which are known to result in neutrophilia (Baker, *et al.*, 2001).

The Coomb's reaction was positive in 44 cases constituting 88%. This figure includes the cases that have a weakly positive reaction (Table 17). A breakdown of the positive samples showed that 33 out of the 39 compatible subjects by the ABO system were positive representing 84.6%. The incompatible cases were all Coomb's positive (a case of 100% positivity). The findings of Rosenfield (1955) on the ABO incompatible cases, showed 88.4%cases of the total samples they analyzed as Coomb's negative. Risemberg *et al,* (1977) on the other hand reported that Coomb's reaction of infants with a bilirubin level at which hyperbilirubinemia is indicated (Wood *et al.*1979, Hansen 2002), was 60% positive and 40% negative in ABO incompatible cases. The present study showed that all the cases of ABO incompatibility were Coomb's positive thus agreeing with the use of Coomb's test for the establishment of cases of hemolytic disease due to incompatibility (Orzalesi *et al* 1973). This figure, however, included the neonates with Rh isoimmunization. When considered alone, the ABO incompatible cases constitute only 5 cases, which as stated earlier, represent 10% of the total cases analyzed.

The high level of cases with a positive coomb's result could be due to incompatibility in other blood group antigens erstwhile not given attention. The existences of incompatibility in these erstwhile rare antigens and the intending reactions have been documented (Zmijewski and Fletcher, 1972, Bryant. 1976, Blood book 2001). Findings from these reports reveal that incompatibility in these blood group antigens occurring between mother and child result in maternal antibodies which cross the placenta barrier into fetal circulation (Bloodbook,

2001). The hemolysin reaction is presented in Table 20 and the breakdown is indicated in Tables 21 and 22 for the compatible and incompatible groups. In all, 34 cases were positive in the presence of complement while only 19 were positive in the absence of complement constituting 70.8% and 39.9% respectively. It thus implies that out of the cases that present a positive hemolysin reaction, 55.9%were hemolytic while the remaining were as a result of complement action. The possible and common causes of hemolytic anemia as indicated in the Merck manual (2003) include Rh isoimmunization, ABO incompatibility and incompatibility due to other blood group antigens (Bloodbook, 2001, Hull, 2003).

Other factors that could have contributed to the incidence of jaundice as indicated in various literatures and routinely used in our society include the use of syntocin an oxytocic drug to induce labor in our maternity hospitals (Ghosh and Hudson, 1972, Sims and Neligan, 1975). This is commonly administered to women in the management of labor in most maternity hospitals today.

Similarly, the summary of the analysis in Table 7 showed that male children constituted 44% of the total infants included in the study. This brings to view the possibility of the Glucose-6-phosphate dehydrogenase deficiency for which Nigerians are noted as having a high prevalence rate (Bienzle, *et al,* 1976, Piomelli, 1987, Beutler, 1991). However, the G-6-P-D deficiency analysis was outside the scope of this work but would be desirable to note the contribution or otherwise of this deficiency to the incidence of neonatal jaundice in our country.

Finally, the campaign for exclusive breast-feeding, which has been embraced by many women in Nigeria, has its implication in the incidence of neonatal jaundice. It therefore calls for caution and monitoring in other to put in place measures to prevent or control jaundice in breast-fed infants. The exclusive use of breast milk could be a contributing factor to the incidence of jaundice, which has increased, in recent times.

5.1 CONCLUSION

ABO incompatibility was found to be responsible for 10% of the cases of infants with jaundice within the months covered by the study. The other 90% were of varying etiology which could include the use of oxytocic drugs, G-6-P-D deficiency, exclusive breast-feeding, prematurity, and neonatal sepsis. The Rhesus iso-immunization was responsible for 12% of the total cases. In all the cases, the mean bilirubin levels were above 205μmol/L (12mg/100ml), the levels for which hyperbilirubinemia is indicated. In 20 of the 39 compatible cases, the level was above the limit while 7 of the 11 incompatible cases had figures above this limit. The incompatible cases had an appreciably high level with a mean bilirubin value of 20.77± 9.48, indicating a more severe case of jaundice and associated with mild anemia PCV value of 42.75 ± 4.26. The mean bilirubin value of the compatible group was 16.35 ± 6.45 without any case of anemia PCV value of 45.76 ± 5.2. The others, generally referred to as physiologic jaundice, produced no appreciable hematological deviation from the normal values expected in normal infants.

5.2 RECOMMENDATION

This work has focused on the role of ABO incompatibility as a factor contributing to the incidence of neonatal jaundice. Many other factors have been documented to contribute to this phenomenon and are widely employed in Nigeria. These factors include the use of oxytocic drugs and the campaign for the introduction of exclusive breast-feeding, which has led to many hospitals being, designated Baby friendly Hospitals. Research into these areas is highly desirable and would help assert their contribution to neonatal jaundice in our society. Furthermore, the studies on G-6-P-D deficiency present an interesting area for exciting research. I would strongly recommend that a research work be initiated to determine the contribution of this phenomenon to the incidence of jaundice in Nigeria.

I would also recommend that all infants be screened for bilirubin levels before discharge especially those in which incompatibility is established between mother and child.

Finally the use of exclusive breast-feeding should be reviewed especially in mothers with a history of jaundiced infants.

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APPENDICES

APPENDIX 1

NAMES, CODE, AGE AND SEX DISTRIBUTION OF NEONATES INCLUDED IN THE STUDY.

NAME	CODE NO	SEX	AGE ADMITTED
REMI	MA1	F	3 DAYS OLD
ERIC	MA2	M	3 DAYS OLD
HANNATU	MA3	F	4 DAYS OLD
HADIZA	MA4	F	3 DAYS OLD
EKAETTE	MA5	F	3 DAYS OLD
CHIMECHETARA	MA6	M	7 DAYS OLD
ODION	MA7	M	5 DAYS OLD
HAUWA	MA8	F	3 DAYS OLD
AISHA	MA9	F	4 DAYS OLD
AMINU	MA10	M	3 DAYS OLD
REHINATU	MA11	F	3 DAYS OLD
UWAISU	MA12	M	4 DAYS OLD
ABUBAKAR	MA13	M	3 DAYS OLD
COMFORT	AP14	F	5 DAYS OLD
HADIZA	AP15	F	3 DAYS OLD
EDI	AP16	M	AT BIRTH
LUKA	AP17	M	4 DAYS OLD
HADIZATU	AP18	F	3 DAYS OLD
SADIKATU	AP19	F	3 DAYS OLD
NASIR	AP20	M	5 DAYS OLD
LATIFAT	AP21	F	3 DAYS OLD
PRAISE	AP22	M	3 DAYS OLD
ZALIHA	AP23	F	3 DAYS OLD
UWAISU	AP24	M	5 DAYS OLD
HELLEN	AP25	F	3 DAYS OLD
AARON	AP26	M	5 DAYS OLD
SHUAIBU	AP27	M	AT BIRTH
RAHILA	AP28	F	3 DAYS OLD
HAJARA	MY29	F	3 DAYS OLD
BILKISU	MY30	F	3 DAYS OLD
MICHAEL	MY31	M	3 DAYS OLD
AISHA	MY32	F	4 DAYS OLD
AARON	MY33	M	4 DAYS OLD
SARKI	MY34	M	AT BIRTH
IKECHUKWU	MY35	M	3 DAYS OLD
RITA	MY36	F	3 DAYS OLD
MUSA	MY37	M	3 DAYS OLD

GALI	MY38	M	5 DAYS OLD
YUSUF	MY39	M	AT BIRTH
AINA	MY40	F	4 DAYS OLD
HUSSAINA	MY41	F	AT BIRTH
HASSANA	MY42	F	AT BIRTH
MARIAM	JN43	F	3 DAYS OLD
AMINA	JN44	F	3 DAYS OLD
BILKISU	JN45	F	3 DAYS OLD
AISHA	JN46	F	4 DAYS OLD
RAHINAT	JN47	F	2 DAYS OLD
MUSA	JN48	M	4 DAYS OLD
RABIAT	JN49	F	4 DAYS OLD
MUKAILU	JN50	M	AT BIRTH

KEY MA- SAMPLES OBTAINED IN MARCH

AP- SAMPLES OBTAINED IN APRIL

MY- SAMPLES OBTAINED IN MAY

JN- SAMPLES OBTAINED IN JUNE

M-MALE [BOYS]

F-FEMALE [GIRLS]

SUMMARY-M = 22

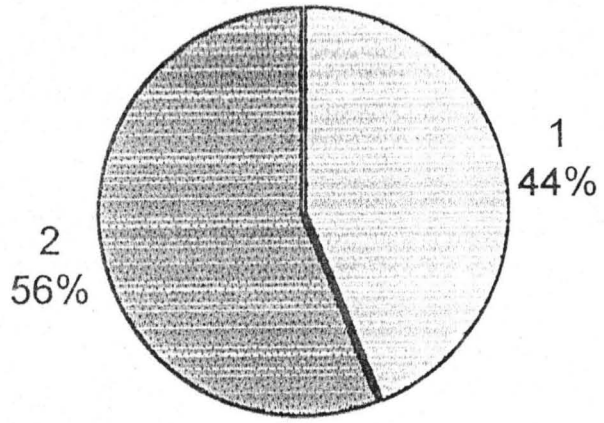
F = 28

AGE DISTRIBUTION:

AGE 0	=	AT BIRTH	=	7
AGE 1	=	ONE DAY OLD	=	0
AGE 2	=	TWO DAYS OLD	=	1
AGE 3	=	THREE DAYS OLD	=	25
AGE 4	=	FOUR DAYS OLD	=	10
AGE 5	=	FIVE DAYS OLD	=	6
AGE 6	=	SIX DAYS OLD	=	0
AGE 7	=	SEVEN DAYS OLD	=	1

TOTAL = 50

APPENDIX 2



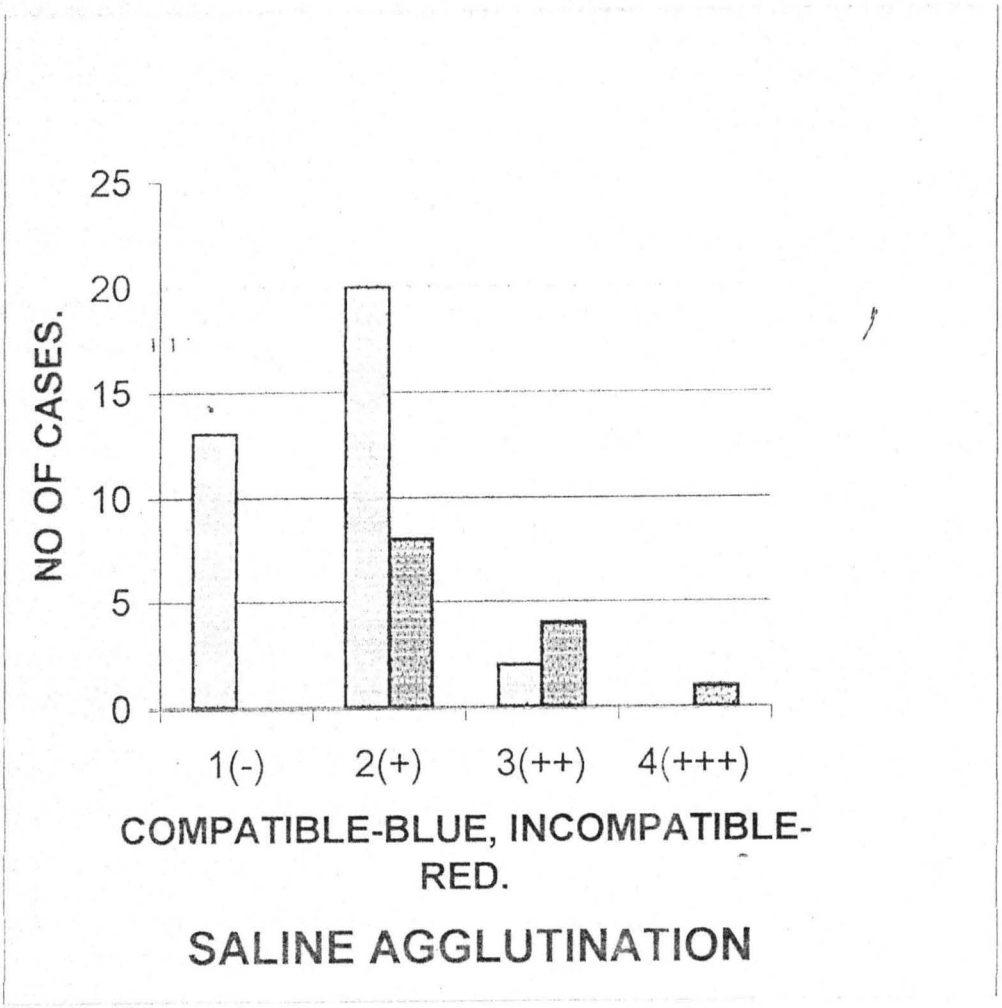
SEX DISTRIBUTION OF NEONATES.

KEY.

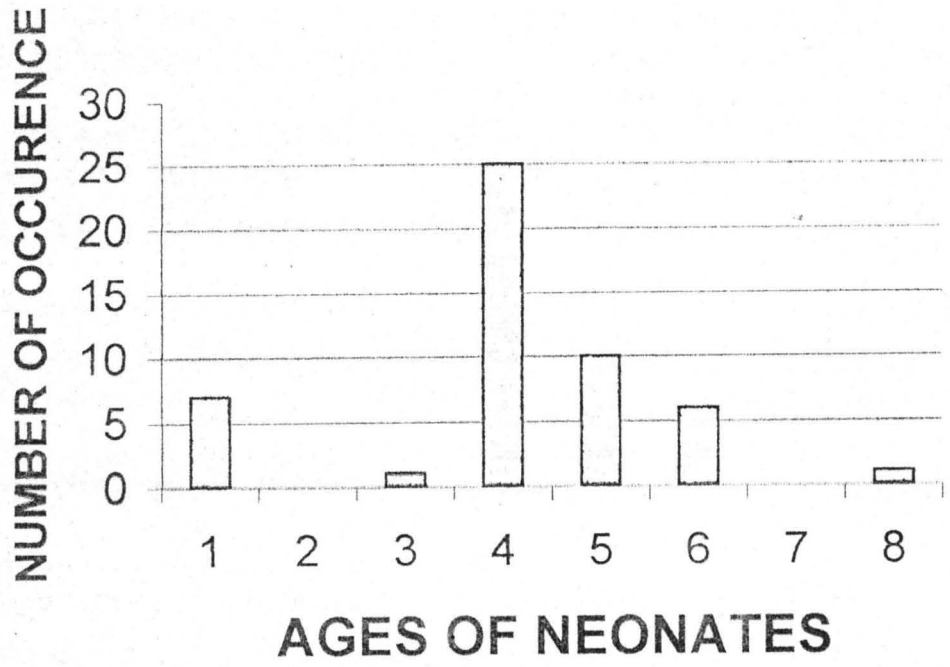
1 – M =MALE.

2 – F = FEMALE

APPENDIX 3


25
20
15
10
5
0
NO OF CASES.
1(-)
2(+)
3(++)
4(+++)
COMPATIBLE-BLUE, INCOMPATIBLE-RED.
SALINE AGGLUTINATION

APPENDIX 4



AGE DISTRIBUTION

KEYS: 1 –AGE 0 (at birth)

2- AGE 1 (one day old)

3- AGE 2 (two days old)

4- AGE 3 (three days old)

5- AGE 4(four days old)

6- AGE 5(five days old)

7- AGE 6(six days old)

8- AGE 7(seven days old)

APPENDIX 5

SUMMARY OF ANALYSIS CARRIED AND RESULTS OBTAINED

CODE	ABO	RH	RET	DIR.COOMBS TEST	COOMBS TEST INDIR.	WBC $\times 10^9$	MAT. BL.GP	TOTAL BIL.µmol/L	CON. BIL.	TITRE	HEM UNH.	SAL. AG G.	HE HD	PC V
MA1	0	POS	6.9%	++	+	11.2	O+	320	32	1/8	+	+	--	46
MA2	B	POS	5.2%	++	+	11.6	B+	288	16	1/256	++	++	+	42
MA3	O	POS	3.4%	++	++	13.4	O+	160	16	1/8	--	+	--	48
MA4	B	POS	3.1%	+	++	9.2	O+	464	288	1/512	+	+++	+	46
MA5	O	POS	4.6%	++	++	6.2	O+	320	16	1/8	--	--	--	40
MA6	O	POS	1.4%	++	++	13.4	O+	256	8	1/512	++	+	+	48
MA7	O	POS	1.8%	+	+	8.8	O+	293	32	1/8				46
MA8	O	NEG	5.2%	++	++	7.288	O+	480	16	1/256	+	+	--	
MA9	O	POS	3.6%	--	--	13.9	O+	752	112	1/32	--	--	--	
MA10	O	POS	3.0%	--	--	8.3	O+	144	16					
MA11	A	POS	3.2%	+	+	11.2	A+	160	32	1/8	+	+	--	
MA12	O	POS	1.8%	+	+	11.5	O+	460	16	1/16	+	+	+	
MA13	O	POS	3.9%	+	+	8.8	O+	320	8	1/8	--	--	--	
AP14	O	POS	3.4%	+	+	11.6	O+	176	8	1/8	+	+	--	52
AP15	O	POS	2.6%	+	+	8.8	O+	116	32	1/4	--	--	--	48
AP16	O	POS	2.1%	+	+	11.2	O+	1152	88	1/4	++	++	+	42
AP17	O	POS	3.2%	+	+	13.2	O+	256	24	1/16	--	--	--	60
AP18	O	POS	3.9%	+	+	12.6	O+	96	16	1/4	--	--	--	36
AP19	A	POS	1.4%	+	+	7.5	A+	256	24	1/256	+	+	+	46
AP20	O	POS	2.1%	++	++	6.8	O--	32	16	1/256	++	++	+	
AP21	O	POS	3.8%	+	+	6.5	A+	288	8	1/64	+	+	+	42
AP22	O	POS	1.8%	+	+	13.2	O+	304	24	1/4	+	+	--	52
AP23	A	POS	2.1%	+	+	9.2	A--	114	33	1/8	+	++	+	46
AP24	O	POS	3/7%	+	+	8.8	O+	144	16	1/4	--	+	--	38
AP25	O	NEG	2.0%	+	+	4.4	A--	448	24	1/8	+	++	+	38
AP26	O	POS	2.7%	+	+	10.4	O+	64	8	1/4	+	--	--	41
AP27	B	POS	1.6%	+	+	11.2	B+	288	32	1/4	+	--	--	38
AP28	O	POS	3.1%	+	+	11.5	O+	176	120	1/4	+	+	+	42
MY29	O	POS	4.1%	+	+	6.0	B+	440	33	1/8	+	+	--	38
MY30	A	POS	2.6%	+	+	4.6	A+	176	17	1/2	--	+	--	48
MY31	O	POS	1.0%	--	--	12.32	O+	256	16	1/2	--	+	--	52
MY32	O	POS	1.9%	+	+	--	O--	208	16	1/16	+	+	+	
MY33	A	POS	4.1%	+	+	8.8	A---	144	16	1/4	+	+	+	46
MY34	A	POS	4.1%	+	+	21.3	AB+	176	120	1/16	+	+	--	40
MY35	O	POS	1.5%	+	+	16.9	O+	64	8	1/2	--	+	--	54
MY36	O	POS	4.5%	+	+	10.4	O+	528	24	1/4	--	+	--	42
MY37	O	POS	3.6%	+	+	7.9	O+	288	32	1/2	+	--	--	46
MY38	0	POS	2.7%	--	--	8.8	O+			1/8	+	+	+	
MY39	O	POS	3.1%	+	+	14.8	O+	496	44	1/2	+	--	--	42
MY40	O	POS	1.1%	+	+	6.8	O+	180	58	1/4	+	--	--	46
MY41	O	POS	1.5%	+	+	6.0	O+	400	40	1/8	+	+	+	46
MY42	O	POS	3.7%	--	--	4.4	O+	440	32	1/8	+	+	--	48
MY43	A	POS	1.8%	++	++	13.2	O+	144	16	1/256	+	+	+	48
JN44	O	POS	2.9%	+	+	13.2	O+			1/2	--	--	--	48
JN45	O	POS	1.1%	+	+	7.2	O+	320	16					
JN46	O	POS	4.2%	+	+	16.9	O+	178	58	1/8	+	+	+	52
JN47	A	POS	2.9%	--	--	8.8	AB+	180	34	1/16	+	+	--	46
JN48	O	POS	3.1%	+	+	6.6	O+	464	88	1/16	+	+	--	44
JN49	O	POS	5.9%	+	+	6.4	O--	583	32	1/256	+	++	+	38
JN50	O	POS	4.2%	+	+	7.5	O+	256	16	1/32	+	+	+	46

KEY:	MA -	MARCH
	AP -	APRIL
	MY -	MAY
	JN -	JUNE
	ABO -	BLOOD GROUPS (A, B, &O)
	RH -	RHESUS BLOOD GROUP
	RET -	RETICULOCYTE COUNT
	INDIR.COOMB'S TEST -	INDIRECT COOMB'S TEST
	DIR.COOMB'S TEST -	DIRECT COOMB'S TEST
	WBC-	WHITE BLOOD COUNT (WHITE CELL COUNT)
	MAT.BL. GP. -	MATERNAL BLOOD GROUP
	TOTAL BIL. -	TOTAL BILIRUBIN LEVEL
	CON BIL. -	CONJUGATED BILIRUBIN
	HEMO. UNHT. -	HEMOLYSIS TEST IN UNHEATED SERUM.
	HEMO. HTED. -	HEMOLYSIS IN HEATED SERUM
	SAL. AGGL. -	SALINE AGGLUTINATION
	PCV	PACKED CELL VOLUME
	+ -	POSITIVE
	- -	NEGATIVE

APPENDIX 6

CONVERSION OF BILIRUBIN VALUES IN μmol/L TO mg/100ml.

CODE	TOTAL BIL.μmol/L	TOTAL BIL. Mg/100ml
MA1	320	18.8
MA2	288	16.8
MA3	160	9.4
MA4	464	27.1
MA5	320	18.8
MA6	256	15.0
MA7	293	17.1
MA8	480	28.0
MA9	752	--
MA10	144	8.4
MA11	160	9.4
MA12	460	26.9
MA13	320	18.8
AP14	176	10.3
AP15	116	--
AP16	1152	--
AP17	256	15.0
AP18	96	--
AP19	256	15.0
AP20	32	--
AP21	288	16.8
AP22	304	17.8
AP23	114	--
AP24	144	8.4
AP25	448	26.2
AP26	64	--
AP27	288	16.8
AP28	176	10.3
MY29	440	25.7
MY30	176	10.3
MY31	256	15.0
MY32	208	12.2
MY33	144	8.4
MY34	176	10.3
MY35	64	--
MY36	528	31.0
MY37	288	16.8
MY38		
MY39	496	29.0
MY40	180	10.5
MY41	400	23.4
MY42	440	25.7
MY43	144	8.4
JN44		--
JN45	320	18.8
JN46	178	10.4
JN47	180	10.5
JN48	464	27.1
JN49	583	34.1
JN50	256	15.0

Calculation based on >=1mg/dL (100ml) >= 17 μmol/L. (Merck manual, 2003)

APPENDIX 7

LEUKOCYTE DIFFERENTIAL COUNT

CODE	NEUTROPHIL	LYMPHOCYTE	MONOCYTE	EOSINOPHIL	BASOPHIL
MA1	28	36	16	14	4
MA2	20	53	16	9	2
MA3	18	50	23	8	1
MA4	46	37	9	6	2
MA5	44	30	22	4	0
MA6	36	38	14	12	0
MA7	31	46	12	10	0
MA8	26	45	11	16	2
MA9	33	47	12	8	0
MA10	29	45	15	11	0
MA11	26	44	12	15	0
MA12	46	37	10	5	0
MA13	20	54	15	8	1
AP14	41	43	7	8	0
AP15	48	25	12	15	0
AP16	45	35	8	13	0
AP17	40	30	16	14	0
AP18	50	30	12	8	0
AP19	36	47	10	7	0
AP20	48	30	12	10	0
AP21	42	40	9	8	1
AP22	45	36	10	9	1
AP23	38	41	9	12	0
AP24	41	36	12	11	0
AP25	35	37	13	13	2
AP26	37	42	9	11	0
AP27	35	37	12	13	1
AP28	45	35	9	11	0
MY29	38	42	10	11	0
MY30	40	37	10	13	0
MY31	29	52	13	8	0
MY32	41	38	10	11	0
MY33	49	31	11	9	0
MY34	30	44	15	11	0
MY35	20	56	11	12	1
MY36	11	59	14	15	1
MY37	27	41	20	12	0
MY38	30	46	13	11	0
MY39	20	55	12	11	1
MY40	21	55	11	12	2
MY41	29	54	11	8	0
MY42	30	45	14	11	0
MY43	27	49	13	10	1
JN44	35	40	12	13	0
JN45	30	59	4	6	0
JN46	35	40	11	12	1
JN47	30	60*	4	6	0
JN48	26	46	12	13	0
JN49	40	35	10	14	1
JN50	48	30	10	12	0

APPENDIX 8

THE STATISTICS:

A:- *t'* – test statistics.

The formula: $t' = \dfrac{X_1 - X_2}{\sqrt{\dfrac{S^2_1}{n_1} + \dfrac{S^2_2}{n_2}}}$

Degree of freedom = $(n_1 + n_2) - 2$

$\alpha = 0.05$

I. Reticulocyte count

Incompatible group: $n_1 = 11$; $X_1 = 3.4636$; $S^2_1 = 1.3574$

Compatible group: $n_2 = 39$; $X_2 = 2.9769$; $S^2_1 = 1.2519$

$$t' = \frac{(3.4636 - 2.9769)}{\sqrt{\dfrac{1.3574}{11} + \dfrac{1.2519}{39}}}$$

$$= \frac{0.4867}{\sqrt{01234 + 0.0321}}$$

$$= \frac{0.4867}{0.3943}$$

$t_{cal} = 1.234$

δ = degree of freedom = $(n_1 + n_2) - 2$

$= (11+39) - 2 = 48$

$t'_{table} = t'_{0.\,05,48} = 2.011.$

The null hypothesis is accepted meaning that there is no significant difference between the treatments.

II. **Bilirubin levels.**

Incompatible group: n = 9, $\bar{X}$ = 20.7667, S^2 = 9.4806

Compatible group: n = 31, $\bar{X}$ = 16.348, S^2 = 6.4506

$$t' = \frac{(20.7667 - 16.348)}{\sqrt{\frac{9.4806}{9} + \frac{6.4506}{31}}}$$

$$= \frac{4.4187}{\sqrt{1.0534 + 0.2081}}$$

$$= \frac{4.4187}{1.1232}$$

t'_{cal} = 3.9340

δ = degree of freedom = $(n_1 + n_2) - 2$

= (9 + 31) – 2 = 38

$t'_{table} = t'_{0.05,38} = 2.024$

The null hypothesis is rejected indicating that there is a significant difference between the two treatments.

III. P.C.V. Values

Incompatible group: $n = 8$, $\bar{X} = 42.75$, $S^2 = 4.2678$

Compatible group: $n = 32$, $\bar{X} = 45.7613$, $S^2 = 5.1914$

$$t' = \frac{(42.75 - 45.7613)}{\sqrt{\frac{4.2678}{8} + \frac{5.1914}{32}}}$$

$$= \frac{3.0114}{\sqrt{0.5335 + 0.1622}}$$

$$= \frac{3.0114}{0.8341}$$

$t'_{cal} = 3.61$

δ = degree of freedom = $(n_1 + n_2) - 2$

$= (8 + 32) - 2 = 38$

$t'_{table} = t'_{0.05,38} = 2.024$

The null hypothesis is rejected indicating that there is a significant difference between the treatments.

B:- The chi-square statistical analysis.

$\chi^2 = \Sigma\frac{(O-E)^2}{E}$ N.B. O = Observed, E = Expected

$\alpha = 0.05$

I. White cell count for compatible group.

Observed (o)	Expected (e)	O – E	$(O-E)^2$	$(O-E)^2/E$
11.2	17	-5.8	33.64	1.9788
11.6	17	-5.4	29.16	1.7153
13.4	17	-3.6	12.96	0.7624
13.4	17	-3.6	12.96	0.7624
13.9	17	-3.1	9.61	0.5653
11.2	17	-5.8	33.64	1.9788
11.5	17	-5.5	30.25	1.7794
11.6	17	-5.4	29.16	1.7153
11.2	17	-5.8	33.64	1.9788
11.3	17	-5.7	32.49	1.9112
12.6	17	-4.4	19.36	1.1388
13.2	17	-3.8	14.44	0.8494
10.4	17	-6.6	43.56	2.5624
11.2	17	-5.8	33.64	1.9788
11.5	17	-5.5	30.25	1.7794
12.32	17	-4.68	21.9024	1.2884
16.9	17	0.1	0.01	0.0006
10.4	17	-6.6	43.56	2.5624
14.8	17	-2.2	4.84	0.2847
13.2	17	-3.8	14.44	0.8494
16.9	17	0.1	.01	0.0006

$\chi^2_{cal} = 28.4426$

δ = degree of freedom = n – 1 = 21 – 1 = 20

$\chi^2_{table} = \chi^2_{0.05, 20} = 31.41.$

The null hypothesis is accepted indicating that there is no significant deviation.

II. White cell count for incompatible group.

Observed	Expected	O – E	$(O - E)^2$	$(O - E)^2/E$
9.2	17	-7.8	60.84	3.5788
7.288	17	-9.712	94.3229	5.5484
6.8	17	-10.2	104.04	6.12
6.5	17	-10.5	110.25	6.4853
9.2	17	-7.8	60.84	3.5788
6.0	17	-11	121	7.1176
8.8	17	-8.2	67.24	3.9553
13.2	17	-3.8	14.44	0.8494
6.4	17	-10.6	112.36	6.6094

$\chi^2_{cal} = 43.84$

δ = degree of freedom = $n - 1 = 9 - 1 = 8$

$\chi^2_{table} = \chi^2_{0.05, 8} = 15.508$.

The null hypothesis is rejected, as there is a significant difference in deviation