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Original article

Review of Patients with ABO Incompatibility in Healthy Neonatal Jaundice in Kirkuk

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Abstract:

- ***Background*:** Neonatal jaundice is a common condition encountered in pediatric practice. ABO incompatibility is one of the known causes that may lead to significant neonatal morbidity. This study aimed to review the frequency and characteristics of ABO incompatibility among neonates presenting with jaundice in Kirkuk Pediatric Hospital.
- ***Methods:*** This prospective study included 63 neonates admitted to Kirkuk Pediatric Hospital between 15 May and 15 July 2008. All patients underwent clinical evaluation and laboratory investigations, including total serum bilirubin (TSB), complete blood picture (CBP), reticulocyte count, and direct Coombs test using both the antihuman globulin method and the older O-cell technique. Blood group typing was performed for both neonates and their mothers.
- ***Result:*** The most common cause of neonatal jaundice among the studied patients was physiological jaundice, followed by ABO incompatibility. Male neonates were more frequently affected. The most common neonatal blood group was A, followed by B, while the predominant maternal blood group was O. A significant number of affected neonates had low birth weights (<3 kg). Elevated TSB levels (>200 mmol/L) were observed among these patients.
- ***Conclusions:*** ABO incompatibility is a well-documented and preventable cause of neonatal morbidity in Kirkuk. Early screening and diagnosis through TSB measurement, CBP, reticulocyte count, and direct Coombs testing in neonates with blood group A or B born to mothers with blood group O is strongly recommended to reduce the risk of complications.
- **Keywords:** ABO incompatibility, neonatal jaundice, Kirkuk, neonate

INTRODUCTION

The term *jaundice* originates from the Old French word *jaunisse*, itself rooted in the Latin *galbinus*, meaning greenish-yellow, derived from *galbus* (1). Jaundice is the most common medical condition requiring attention in newborns (1,2). It manifests as a yellowish discoloration of the skin and sclera due to the accumulation of unconjugated bilirubin. While in most neonates this unconjugated hyperbilirubinemia represents a normal physiological transition, in some, serum bilirubin levels can rise to potentially dangerous levels. Elevated bilirubin is neurotoxic and may result in kernicterus, leading to death or permanent neurological damage in survivors (1,2). Consequently, neonatal jaundice often necessitates prompt diagnostic evaluation.

Historical accounts suggest that neonatal jaundice was first mentioned in Chinese medical texts over a thousand years ago. Literature from the 18th and 19th centuries discusses the causes, management, and occasionally fatal outcomes of neonatal jaundice, particularly in cases likely caused by Rh isoimmunization. In 1875, Orth was the first to document yellow staining of the brain, a hallmark of what is now known as kernicterus (1–3).

Pathophysiology

Physiological jaundice in neonates results from two concurrent phenomena:

1. **Increased bilirubin production**, owing to a high red blood cell (RBC) mass and shortened lifespan of fetal erythrocytes.

2. **Reduced hepatic clearance**, due to low levels of the hepatic binding protein ligandin and reduced activity of the conjugating enzyme glucuronyl transferase (2,3).

Bilirubin is generated in the reticuloendothelial system as the end product of heme catabolism. Around 75% originates from hemoglobin, with contributions from myoglobin, cytochromes, and catalase. Heme is converted to biliverdin by heme oxygenase, releasing iron and carbon monoxide (measurable via breath analysis), followed by reduction to bilirubin (2,3). The predominant bilirubin isomer, bilirubin IXα Z,Z, is water-insoluble and transported in plasma bound to albumin. Only the unbound fraction (free bilirubin) crosses cell membranes, including the blood-brain barrier, posing neurotoxic risks (3,4).

In fetal life, bilirubin is excreted via the maternal system through passive placental diffusion. After birth, the bilirubin-albumin complex enters hepatocytes via specific receptors and binds to ligandin. Ligandin levels are low at birth but increase postnatally and may be upregulated by drugs like phenobarbital (4). Conjugation with glucuronic

acid, catalyzed by uridine diphosphoglucuronyltransferase (UDPGT), transforms bilirubin into water-soluble monoconjugates and, later, diconjugates (4,5). This process is vital for biliary excretion and is initially inefficient due to low UDPGT activity, which gradually reaches adult levels by 4–8 weeks of age. Pharmacologic agents such as phenobarbital and dexamethasone may enhance this enzymatic activity (5).

Certain infants, especially those with Gilbert syndrome or UDPGT1A1 mutations, are at increased risk of significant hyperbilirubinemia. The interaction of such genetic factors with hemolytic conditions (e.g., G6PD deficiency, ABO hemolytic disease, hereditary spherocytosis) further elevates this risk (2–5).

Once excreted into bile, bilirubin undergoes bacterial reduction to colorless tetrapyrroles in the colon. However, β-glucuronidase activity in the proximal small intestine can deconjugate bilirubin, enabling its reabsorption and prolonging hyperbilirubinemia through the **enterohepatic circulation** (5,6). This mechanism is more prominent in neonates due to limited early feeding and prolonged intestinal transit. Components in some breast milk may exacerbate this cycle, contributing to **breast milk jaundice**, particularly in genetically predisposed infants (e.g., with UDPGT1A1 or OATP2 polymorphisms).

Etiology of Indirect Hyperbilirubinemia (6,7)

- **Hemolytic disorders**:
    - *Feto-maternal blood group incompatibility* (ABO, Rh)
    - *Genetic hemolysis*: hereditary spherocytosis, G6PD deficiency, thalassemia, galactosemia
    - *Drug-induced hemolysis* (e.g., vitamin K)
- **Other causes**:
    - *Extravascular blood* (hematomas, hemorrhage)
    - *Polycythemia* (due to hypoxia or placental transfusions)
    - *Exaggerated enterohepatic circulation* (e.g., due to obstruction or poor peristalsis)
    - *Reduced hepatic uptake* (e.g., persistent ductus venosus)
    - *Decreased bilirubin conjugation* (e.g., congenital enzyme deficiencies like Gilbert syndrome)

Differentiating Physiological from Pathological Jaundice (7,8)

- **Physiological jaundice**:
    - Appears after 2–3 days of life
    - Peaks at 4–6 days
    - TSB <12 mg/dL (term), <15 mg/dL (preterm)
    - Resolves by 2–4 weeks
- **Pathological jaundice**:
    - Appears within first 24 hours
    - TSB rises >5 mg/dL/day or exceeds 15 mg/dL
    - Elevated direct bilirubin (>1.5–2 mg/dL)
    - Associated with anemia, prolonged or recurrent jaundice

- **ABO Incompatibility vs. Rh Disease** (8,9)

Feature	ABO Incompatibility	Rh Disease
Frequency	Common	Less common
Pallor	Minimal	Marked
Jaundice	Moderate	Marked
Hydrops	Rare	Common
Hepatosplenomegaly	Rare	Marked
Direct Coombs Test	Often negative	Positive
Indirect Coombs Test	Positive	Positive
RBC Morphology	Spherocytes	Nucleated RBCs

ABO Hemolytic Disease of the Newborn (ABO HDN) (9–11)

In ABO HDN, maternal IgG antibodies specific to fetal ABO antigens cross the placenta and cause hemolysis. Unlike Rh disease, ABO HDN often affects firstborns and does not worsen in subsequent pregnancies. It occurs primarily in mothers with blood group O carrying fetuses with group A or B, due to the presence of naturally occurring IgG anti-A or anti-B antibodies.

- **Sensitization mechanisms:**
    - Natural exposure to environmental A/B antigens

- Fetal-maternal transfusion
- Rarely, incompatible blood transfusion

- **Moderating factors:**
  - Limited fetal antigen expression
  - Antigen distribution on non-RBC fetal tissues
  - Mild clinical course in most cases

Routine antenatal antibody screening does not detect ABO HDN. Diagnosis is typically made postnatally in neonates with early-onset jaundice and a positive Coombs test.

PATIENT and METHOD

This prospective study was conducted at Kirkuk Pediatric General Hospital over a two-month period, from May 15 to July 15, 2008. The study included all otherwise healthy neonates presenting with jaundice, whether admitted to the hospital or referred for evaluation. A total of 63 neonates were enrolled.

To ensure comprehensive data collection, a predesigned data collection form was distributed to all medical residents in the hospital. For each neonate, detailed history

taking and physical examination were performed. Blood samples (5 mL) were collected for the following laboratory investigations:

- Total serum bilirubin (TSB) and its fractionation,
- Blood group typing for the neonate,
- Complete blood picture (including red cell morphology and reticulocyte count),
- Direct Coombs test.

The **Direct Coombs test** was performed by mixing 50 microliters of human anti-globulin reagent with 50 microliters of the patient's serum. The mixture was incubated at 37°C for 24 hours. The presence of agglutination was considered a positive result.

To confirm positive results, the **standard O-cell agglutination method** was also employed. Blood group O cells were washed three times with normal saline. A mixture of 2–3 drops of O-cell suspension and patient serum (in 2:1 or 4:1 ratios) was incubated for 45 minutes. Agglutination was then visually assessed. Titering was performed in positive cases.

Additionally, the blood group of the mother was determined and recorded. The neonate's birth order and weight were also documented in the format.

Diagnostic categorization was based on clinical and laboratory findings as follows (12):

1. **Physiological or breast milk jaundice**: Elevated indirect bilirubin with normal reticulocyte count and negative Coombs test.

2. **Hemolytic jaundice (e.g., ABO or Rh incompatibility)**: Elevated indirect bilirubin with increased reticulocyte count and positive Coombs test.

3. **Pathological jaundice**: Simultaneous elevation of both direct and indirect bilirubin with normal reticulocyte count and negative Coombs test.

Statistical Analysis

Statistical analysis was performed using SPSS (Probability Test) software obtained from an online platform. The analysis was conducted by a specialist in medical statistics.

RESULTS

A total of 63 neonates were enrolled in this prospective study. Among them, 17 cases (26.9%) were diagnosed with physiological jaundice, 5 (7.9%) with pathological jaundice, 14 (22.2%) with Rh incompatibility, 18 (28.5%) with ABO incompatibility, 5 (7.9%) with hemolysis, and 4 (6.3%) with breastfeeding-associated jaundice (Table 1).

Table 1. Causes of Neonatal Jaundice

Cause	Number (%)
Physiological	17 (26.9%)
Pathological	5 (7.9%)
Rh Incompatibility	14 (22.2%)
ABO Incompatibility	18 (28.5%)
Hemolysis	5 (7.9%)
Breastfeeding	4 (6.3%)

Among the 18 cases of ABO incompatibility, a male predominance was observed with 10 males (60%) and 8 females (40%), which was statistically significant ($p = 0.04$) (Table 2).

Table 2. Sex Distribution Among ABO Incompatibility Cases

Total No.	Male Patients	Female Patients	P value
18	10 (60%)	8 (40%)	0.04

The association between maternal and neonatal blood groups showed a statistically significant relationship when the mother had blood group O and the neonate had blood group A or B ($p < 0.04$) (Table 3).

Table 3. Association Between Mother and Child Blood Groups in ABO Incompatibility

Mother Blood Group	Child A+ve (%)	Child B+ve (%)	P value
O+ve	10 (62.5%)	6 (37.5%)	0.02
A+ve	0 (0%)	2 (100%)	0.1

Weight was identified as an important factor in the management of neonatal jaundice. Among neonates with ABO incompatibility, 12 (66.6%) weighed less than 2.5 kg, while 6 (33.4%) weighed more than 2.5 kg, with a significant p-value of 0.05 (Table 4).

Table 4. Association Between Weight and ABO Incompatibility

Total No.	Wt < 2.5 Kg	Wt > 2.5 Kg	P value
18	12 (66.6%)	6 (33.4%)	0.05

Total serum bilirubin (TSB) levels were elevated (>200 mmol/L) in 14 (77.7%) of the ABO incompatibility cases, while 4 (22.2%) had TSB levels <200 mmol/L (p = 0.04) (Table 5).

Table 5. Total Serum Bilirubin (TSB) Levels in ABO Incompatibility Cases

Total No.	TSB > 200	TSB < 200	P value
18	14 (77.7%)	4 (22.2%)	0.04

Regarding the type of hyperbilirubinemia, indirect hyperbilirubinemia was observed in 60 patients, whereas direct hyperbilirubinemia was present in only 3 patients (Table 6).

Table 6. Type of Hyperbilirubinemia

Total Patients	Indirect Hyperbilirubinemia	Direct Hyperbilirubinemia
63	60	3

DISCUSSION

This study aimed to identify the common causes of neonatal jaundice in the Kirkuk region, with a particular focus on ABO incompatibility in otherwise healthy neonates.

Our findings indicated that physiological jaundice was the most frequently encountered cause, followed by ABO incompatibility, Rh incompatibility, breastfeeding-associated jaundice, pathological jaundice (direct hyperbilirubinemia), and other hemolytic diseases (Table 1). These results align with a local study conducted in Baghdad at Al-Mansour Teaching Hospital in 2001, which also found physiological jaundice as the leading cause (10–13). However, that study reported Rh incompatibility as the second most common cause, followed by ABO incompatibility. The observed reduction in Rh-related cases in our study is likely due to the routine administration of anti-D immunoglobulin to Rh-negative mothers delivering Rh-positive infants (10,13).

In contrast, a study from India by Lalita Bahl et al. reported a similar pattern to ours—physiological jaundice was most common, followed by ABO incompatibility, with breast milk jaundice and other hemolytic conditions ranking third, and Rh incompatibility being the fourth cause. The difference in order between the studies was not statistically significant (13).

Michael Sgro and colleagues, in their Canadian study investigating causes of severe hyperbilirubinemia, identified ABO incompatibility as the most common cause, followed by G6PD deficiency—further supporting the importance of ABO-related hemolysis in the neonatal population (1,13).

In terms of gender distribution, our study showed a male predominance (60%) among affected neonates, consistent with a Baghdad study (59%) and other international data (1,10). This disparity may be attributed to the "male disadvantage theory," which suggests that XY embryos have higher metabolic rates and faster developmental growth than XX embryos, making them more susceptible to stressors such as jaundice. Moreover, a known inverse relationship exists between metabolic rate and lifespan, potentially influencing bilirubin metabolism (14).

We also noted a significant association between ABO incompatibility and blood group distribution, particularly between mothers with blood group O and neonates with group A or B (Table 3). Group A neonates were more commonly affected than group B, likely reflecting the general population distribution where blood group A is more prevalent (14,15). Interestingly, a small subset of cases involved mothers with blood group A and neonates with blood group B. This suggests that, although rare, maternal antibodies can form against fetal B antigens when the mother is type A, or vice versa (16).

Birth weight was another significant factor influencing jaundice severity. Neonates weighing less than 2.5 kg were more affected by ABO incompatibility (Table 4), which aligns with the known increased risk of hyperbilirubinemia in low birth weight (LBW) and preterm infants (17). These infants are particularly vulnerable due to higher red cell turnover and immature hepatic conjugation mechanisms (17,18). Additionally, delayed bowel motility and poor feeding in preterm neonates exacerbate enterohepatic circulation, leading to further accumulation of bilirubin (17,18).

These vulnerabilities put premature and LBW infants at higher risk for kernicterus, even at bilirubin levels that would be considered safe in term infants. Unfortunately, no precise threshold has been universally established to distinguish between safe and toxic bilirubin levels in this population (18,19).

Finally, the most critical determinant in managing ABO incompatibility-related jaundice remains the total serum bilirubin (TSB) level and its fractionation (Table 5). In our study, 77.7% of neonates with ABO incompatibility had TSB levels exceeding 200 mmol/L, highlighting the potential for severe jaundice in this group. These findings are in agreement with a study from India, which also found higher TSB levels in ABO-incompatibility than in other causes of neonatal jaundice (13,18).

CONCLUSION

ABO incompatibility represents a significant cause of neonatal morbidity among jaundiced infants in the Kirkuk region. It should be strongly considered in any neonate with blood group A or B born to a mother with blood group O, as these infants are at increased risk of developing hyperbilirubinemia that may necessitate prompt medical intervention.

Recommendation

Given the findings of this study, it is recommended that special attention be given to newborns with blood group A, as they appear to be more susceptible to developing ABO incompatibility. To support early diagnosis and effective management, blood group determination should be routinely performed for all neonates and their mothers who are referred to the hospital for the assessment and treatment of neonatal jaundice.

Ethical Clearance:

In accordance with the 2013 WMA Helsinki Declaration, all ethical aspects of this study were approved. Before enrolling the participants, an informed oral consent was obtained from their families as an ethical agreement. Additionally, approval from the hospital administrator was obtained.

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Conflicts of interest: There are no conflicts of interest**.**

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