

Frequency of ABO incompatibility in neonatal jaundice

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ABSTRACT

Background: ABO incompatibility is one of the leading causes of immune-mediated hemolytic disease in newborns and contributes substantially to the development of neonatal hyperbilirubinemia. Early identification of affected neonates is essential to prevent bilirubin-induced neurological complications. Our objective is to determine the prevalence of ABO incompatibility among term neonates with neonatal jaundice and evaluate its association with immune hemolysis and clinical outcomes.

Methods: This descriptive cross-sectional study included 152 term neonates diagnosed with neonatal jaundice at the Department of Pediatric Medicine, Children Hospital, Balochistan Institute of Child Health Services. Neonates with conjugated hyperbilirubinemia, congenital anomalies, syndromic disorders, or metabolic storage diseases were excluded. Clinical and demographic data were collected, and laboratory investigations included maternal and neonatal ABO blood grouping, total, direct, and indirect serum bilirubin measurements, and Direct Coombs Test (DCT). Statistical analyses were performed to determine the association between ABO incompatibility, immune hemolysis, and the requirement for exchange transfusion.

Results: The mean age at presentation was 7.55 ± 4.01 days, and the mean gestational age was 38.93 ± 1.22 weeks. The mean total serum bilirubin concentration was 15.93 ± 3.50 mg/dL, with predominantly indirect hyperbilirubinemia (15.06 ± 3.54 mg/dL). ABO incompatibility was identified in 22 of 152 neonates (14.5%). Overall, 78 neonates (51.3%) were DCT positive, and all ABO-incompatible neonates demonstrated positive DCT results, indicating a significant association between ABO incompatibility and immune-mediated hemolysis ($p < 0.001$). Exchange transfusion was performed in 88 neonates (57.9%), including 16 (72.7%) with ABO incompatibility and 72 (55.4%) without incompatibility; however, this difference did not reach statistical significance ($p = 0.197$).

Conclusion: Early immune-hematological screening using ABO blood grouping and DCT may facilitate timely diagnosis and optimize the clinical management of neonatal jaundice.

Keywords: ABO Incompatibility, Direct Coombs Test, Hyperbilirubinemia, Neonatal Jaundice

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Introduction

Neonatal jaundice is a common disorder across the globe, with considerable percentage of infants and especially in the first week of their life (1). It is marked by yellowing of the skin and sclera because of the high concentration of bilirubin in the blood a condition that is also known as hyperbilirubinemia. This is caused by

the fact that the immature liver cannot effectively process and excrete bilirubin which is a product of the normal breakdown of the red blood cells (2). Even though in many cases, neonatal jaundice is a mild and self-limiting disorder, in other cases, it can progress into serious neurological impairment, such as kernicterus, unless an effective treatment is undertaken within the required time (3). ABO incompatibility has been noted as one of the major and avoidable etiologies of neonatal jaundice. ABO incompatibility occurs when the mother and newborn have an incompatible blood group with the mother being of type O blood and the newborn being of type A or B blood (4). This incompatibility results in the maternal IgG antibodies attacking the red blood cells of the newborn and destroying them hence elevating the levels of bilirubin. ABO incompatibility has a clinical importance that is supported by the fact that this incompatibility has the potential of causing hemolytic disease of the newborn (HDN) that, when untreated or improperly treated, may result in severe morbidity and mortality (5). Although ABO incompatibility has been reported among neonates with jaundice in Pakistan, available evidence remains limited across different populations and healthcare settings, particularly regarding its clinical association with neonatal jaundice. In Pakistan, neonatal jaundice is a major problem in the general population, with high birth rates and the scarcity of healthcare resources (6). Although ABO incompatibility has been documented in Pakistani neonates, its frequency and clinical significance may vary across populations and healthcare settings and remain insufficiently characterized in some local populations. To enhance the detection, intervention, and treatment of neonatal jaundice, research is necessary to measure ABO incompatibility. This will contribute to the minimization of

complications and neonatal outcomes. A better level of awareness and more uniform guidelines can also positively influence care and decrease the rate of neonatal jaundice in Pakistan. A previous study by Ilyas et al. reported an incidence of 26.4% among neonates with jaundice (8,9,10,11). However, findings from a single study may not adequately represent the frequency and clinical characteristics of ABO incompatibility across different Pakistani populations and healthcare settings. The incompatibility of ABO takes place when a mother has a different blood type as the infant, and this causes the production of antibodies that potentially assault red blood cells of the baby leading to hemolysis and consequently to jaundice. This is among the most common causes of neonatal jaundice, and its occurrence may differ with maternal blood types of distribution and population of interest. Evaluating the frequency will assist health care providers to detect at-risk newborns better, initiate timely newborn interventions, including phototherapy, and monitor the bilirubin levels with greater attentiveness.

Also, an appreciation of the extent of ABO incompatibility as a risk factor of jaundice assists in narrowing down prenatal care, such as blood type testing and the possible preventive strategy, ultimately eliminating the risks of such adverse effects as kernicterus a not very common but severe outcome of untreated jaundice.

Methods

This descriptive cross-sectional study was conducted in the Department of Paediatric Medicine, Children Hospital, Balochistan Institute of Child Health Services, Quetta, from February to August 2025. The study was approved by the Ethical Review Committee of the hospital under ethical approval of BICHQ/14425/26, before commencement and

was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from a parent or guardian of every neonate enrolled.

The sample size was calculated with the WHO sample size calculator using the formula $n = Z^2 \times P(1 - P) / d^2$, taking a 95% confidence level ($Z = 1.96$), an anticipated prevalence of ABO incompatibility among jaundiced neonates of 26.4% (9) and an absolute precision of 7%. This yielded a required sample of 152 neonates. Participants were enrolled by consecutive non-probability sampling until the calculated sample size was reached.

Neonates of either sex, aged 28 days or less and of gestational age 37 weeks or more, who presented with yellow discoloration of the skin or sclerae and in whom jaundice was confirmed biochemically, were eligible for inclusion. Neonates with conjugated (direct) hyperbilirubinaemia, congenital anomalies, syndromic disorders or metabolic storage disease were excluded, as these conditions act as effect modifiers on the severity of jaundice and on the need for exchange transfusion. ABO incompatibility was defined as a maternal blood group O with a neonatal blood group of A or B. Immune hemolysis was taken to be the combination of an incompatible maternal-neonatal pairing with a positive direct Coombs test.

ABO incompatibility was defined based on maternal-neonatal ABO blood-group mismatch, particularly O-mother/A-infant and O-mother/B-infant pairings; other ABO pairings were classified according to the predefined study criteria. The diagnosis was assessed alongside the Direct Coombs Test. Phototherapy and exchange transfusion were performed according to the 2022 American Academy of Pediatrics guideline, based on gestational age, postnatal age in hours, total

serum bilirubin level, and neurotoxicity risk factors, using the corresponding hour-specific treatment thresholds.

Each neonate underwent a detailed history and complete clinical examination. The age at onset of jaundice, family history, previous sibling history of phototherapy or exchange transfusion, and obstetric history were recorded. Weight, length, and occipito-frontal circumference were measured using standardized techniques. Body weight was measured using a calibrated neonatal weighing scale with a precision of 10 g, while length and occipito-frontal circumference were measured using standard neonatal measuring equipment. Gestational age was recorded in completed weeks, and neonatal anthropometric measurements were interpreted using appropriate newborn growth references. Laboratory investigations comprised maternal and neonatal ABO blood grouping, measurement of total, direct and indirect serum bilirubin, and the Direct Coombs Test. Peripheral venous blood was collected from each neonate under aseptic conditions, with 3 mL in ethylenediaminetetraacetic acid for the Direct Coombs Test. Samples were transported and stored at 1–8 °C. Specimens that were inadequately labelled, hemolyzed, collected in an inappropriate container, insufficient in volume, or unmatched to patient identity were rejected.

Maternal and neonatal ABO and Rh(D) blood groups were determined by standard tube agglutination using commercial anti-A, anti-B, and anti-D antisera, with both forward and reverse ABO grouping. Total and direct serum bilirubin concentrations were measured using a standard automated biochemical assay, and indirect bilirubin was calculated as the difference between total and direct bilirubin. The Direct Coombs Test (DCT) was performed

by the standard tube method using a polyspecific antihuman globulin reagent containing anti-IgG and anti-C3d. Results were interpreted according to standard laboratory procedures by trained laboratory personnel and validated before reporting. Maternal and neonatal Rh(D) typing was performed concurrently to assess possible Rh incompatibility and haemolytic disease.

Data was entered and analyzed using SPSS version 26.0. Quantitative variables such as age, weight, length, occipital-frontal circumference, gestational age, family income, and length of hospital stay were reported as mean $\pm$ standard deviation. Normality of data was assessed using the Shapiro-Wilk test. Qualitative variables, including gender, residence, mode of delivery, presence of infection, need for exchange transfusion, and socioeconomic status, were presented as frequencies and percentages. Effect modifiers such as age, gender, residence, and socioeconomic status were controlled through stratification. Post-stratification Chi-square or Fisher's exact test (where cell frequency ≤ 5) was applied to assess associations. A p-value ≤ 0.05 was considered statistically significant.

Results

Data was collected from 152 neonates. The mean age at presentation was 7.55 ± 4.01 days, and the mean gestational age was 38.93 ± 1.22 weeks, consistent with the inclusion of term neonates. The mean birth weight was 3.17 ± 0.44 kg, with a mean length of 49.71 ± 2.68 cm and head circumference of 33.87 ± 1.18 cm. The mean total serum bilirubin level was 15.93 ± 3.50 mg/dL, comprising predominantly indirect bilirubin (15.06 ± 3.54 mg/dL) and direct bilirubin (0.87 ± 0.38 mg/dL). The mean hospital stay was 4.30 ± 1.71 days. ABO incompatibility was identified in 22 (14.5%) neonates, while 130 (85.5%) had no ABO incompatibility.

Table 1. Baseline Characteristics of Neonates (n = 152)

Variable	Value
Age at presentation (days)	7.55 ± 4.01
Gestational age (weeks)	38.93 ± 1.22
Birth weight (kg)	3.17 ± 0.44
Length (cm)	49.71 ± 2.68
Head circumference (cm)	33.87 ± 1.18
Total bilirubin (mg/dL)	15.93 ± 3.50
Indirect bilirubin (mg/dL)	15.06 ± 3.54
Direct bilirubin (mg/dL)	0.87 ± 0.38
Hospital stays (days)	4.30 ± 1.71
ABO incompatibility - Yes	22 (14.5%)
ABO incompatibility - No	130 (85.5%)
Total	152 (100%)

Among the neonates, 75 (49.3%) were male and 77 (50.7%) were female. Rural residence was reported in 82 (53.9%) cases, compared with 70 (46.1%) from urban areas. Cesarean section was the mode of delivery in 84 (55.3%) neonates, while 68 (44.7%) were delivered by normal vaginal delivery. The Direct Coombs Test was positive in 78 (51.3%) neonates and negative in 74 (48.7%).

Table 2. Demographic and Clinical Characteristics

Variable	Value
Male	75 (49.3%)
Female	77 (50.7%)
Rural residence	82 (53.9%)
Urban residence	70 (46.1%)
Cesarean section	84 (55.3%)
Normal vaginal delivery	68 (44.7%)
Direct Coombs Test - Positive	78 (51.3%)
Direct Coombs Test - Negative	74 (48.7%)
Exchange transfusion - Yes	88 (57.9%)
Exchange transfusion - No	64 (42.1%)
Total	152 (100%)

Among neonates with ABO incompatibility, 16 (72.7%) required exchange transfusion, whereas 6 (27.3%) did not. Among those without ABO incompatibility, 72 (55.4%) required exchange transfusion, while 58 (44.6%) did not. Although exchange transfusion was more frequent among ABO-

incompatible neonates, the association was not statistically significant ($p = 0.197$).

Table 3: Association Between ABO Incompatibility and Exchange Transfusion

ABO Incompatibility	Exchange Transfusion - Yes	Exchange Transfusion - No	Total	p-value
Yes	16 (72.7%)	6 (27.3%)	22	0.19
No	72 (55.4%)	58 (44.6%)	130	
Total	88 (57.9%)	64 (42.1%)	152	

Chi-square test applied.

All neonates with ABO incompatibility (100%) were Direct Coombs Test positive, while none were negative. In contrast, among neonates without ABO incompatibility, 56 (43.1%) were DCT positive and 74 (56.9%) were DCT negative.

Table 4: Association Between ABO Incompatibility and Direct Coombs Test

ABO Incompatibility	DCT Positive	DCT Negative	Total	p-value
Yes	22 (100%)	0 (0%)	22	<0.001
No	56 (43.1%)	74 (56.9%)	130	
Total	78 (51.3%)	74 (48.7%)	152	

Chi-square test applied.

Discussion

This paper assessed ABO incompatibility prevalence in neonates who came to the Department of Pediatric Medicine, Children's Hospital, Balochistan Institute of Child and Health Services, with complaints of jaundice. Among 152 term newborns, ABO incompatibility was identified in 22 (14.5%). This result, although smaller than the 26.4% prevalence rate in the parent study on which the sample size was calculated, nonetheless indicates that ABO incompatibility remains a clinical factor contributing to neonatal hyperbilirubinemia in our population. The average age at presentation was 7.55 ± 4.01 days, with an average gestational age of 38.93 ± 1.22 weeks, indicating that most

neonates were at term and presented in the early neonatal period. The median overall serum bilirubin was 15.93 ± 3.50 mg/dL, of which the majority was indirect (15.06 ± 3.54 mg/dL), consistent with a hemolytic jaundice pattern. The results are consistent with prior studies indicating that immune-mediated hemolysis due to ABO incompatibility is typically observed during the first week of life and is characterized primarily by unconjugated hyperbilirubinemia (12).

In the current study, 78 (51.3%) neonates tested positive for the Direct Coombs Test (DCT). Notably, 100% of the ABO incompatible (ABO) neonates were DCT positive, and the relationship between ABO incompatible and DCT positive was significant ($p < 0.001$). This close relationship confirms the immunological nature of hemolysis in ABO-incompatible neonates and aligns with previous studies showing that higher DCT levels are associated with immune-mediated neonatal jaundice (13). The post hoc analysis was not statistically significant ($p = 0.197$), although 88 (57.9) of the total neonates who received the transfusion required exchange transfusion. Though the percentage of 72.7 of ABO-incompatible neonates who needed exchange transfusion was higher than 55.4% of non-incompatible babies, the difference was not significant. This implies that ABO incompatibility is a contributing factor to severe hyperbilirubinemia, but other factors like sepsis, dehydration, or breastfeeding jaundice may also affect the intensity of bilirubin and the treatment needed. Past studies also show that not every ABO-incompatible neonate results in clinically significant hemolysis, which needs aggressive treatment (14,15,16). The gender distribution was also close to the equality point (50.7 percent female, 49.3 percent male), and the rural residence slightly

out-prepared (53.9 percent), which is possibly attributed to the referral patterns and healthcare access in the area. Operative delivery was found in 55.3 percent, which is in line with the trends of increasing the rate of operative deliveries although mode of delivery is not a direct risk factor in ABO-related hemolysis. Overall, the results indicate that ABO incompatibility remains a crucial immune hematological factor in neonatal jaundice, closely associated with DCT positivity but not predictive of exchange transfusion in this case (17). Early blood grouping and DCT screening of jaundiced neonates, especially those who are born to group O mothers, can assist in the early identification and treatment of this condition. The use of early neonatal surveillance can reduce the risk of bilirubin neurotoxicity and prevent permanent neurological outcomes (18,19,20).

Limitations of study:

There are several limitations of this study that should be considered when interpreting the findings. To begin with, the cross-sectional design does not permit establishing a temporal or causal relationship between ABO incompatibility and the severity of neonatal jaundice. Second, the research was carried out at one tertiary care facility, Children Hospital, Balochistan Institute of Child and Health Services, and this could be a limitation to the external validity of the findings to other territories or primary care areas. Third, the other possible causes of indirect hyperbilirubinemia as G6PD deficiency, minor blood group incompatibilities, or subtle hemolytic disorders were not thoroughly studied, and this might have affected the severity of jaundice and required exchange transfusion. Additionally, there may be a risk of selection bias due to the use of hospital-based cases only, as cases with mild jaundice

treated at home or in outpatient care were excluded. Lastly, although DCT was closely linked to ABO incompatibility, quantitative antibody titers and hemolysis markers were not measured, which limited immune hematological correlation.

Conclusion

It is concluded that ABO incompatibility remains an important cause of neonatal jaundice and is significantly associated with immune-mediated hemolysis. Although it contributes to the development of hyperbilirubinemia in a substantial proportion of neonates, it does not independently predict the need for advanced therapeutic interventions in all cases. Early identification through blood grouping and Direct Coombs testing is essential for timely diagnosis and appropriate management. Strengthening neonatal screening protocols and ensuring close clinical monitoring can help reduce the risk of severe hyperbilirubinemia and its associated complications, ultimately improving neonatal outcomes.

Conflict of Interest

The authors declare that they have no conflicts of interest regarding the publication of this study.

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All the authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed.	