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Original Article

Paediatrics Section

Cord Direct Antiglobulin Test for Predicting the Need for Phototherapy in Neonates with ABO Incompatibility: A Prospective Cohort Study

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ABSTRACT

Introduction: The Direct Antiglobulin Test (DAT) is a screening technique used to detect Immunoglobulin G (IgG) antierythrocyte antibodies on foetal Red Blood Cells (RBCs). Universal bilirubin screening decreases the incidence of severe hyperbilirubinemia. The diagnostic efficacy of DAT in cases of ABO incompatibility is not well established, in contrast to Rh incompatibility.

Aim: To evaluate the diagnostic ability of routine cord blood DAT in neonates ≥35 weeks of gestation with ABO incompatibility in predicting the requirement for phototherapy. Additionally, to compare the diagnostic ability between two methods of DAT estimation, namely the semiagglutination gel method and the conventional tube method.

Materials and Methods: This prospective cohort study was conducted from September 2022 to March 2024 at SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India. Following the acquisition of informed consent, cord blood samples were collected from 934 neonates delivered after 35 weeks of completed gestation to mothers with O blood type. In cases of ABO incompatibility (523 neonates), DAT was performed using both the manual tube method and the automated semiagglutination gel method in the blood bank. Serum bilirubin levels, complete blood counts with peripheral smear analysis and reticulocyte counts were performed on day 3 or earlier, as clinically indicated. The neonates were treated in accordance with the American Academy of Paediatrics (AAP) 2004 recommendations on phototherapy. Babies were monitored for jaundice until 14 days of age.

Results: Out of 3,092 eligible deliveries, 523 babies with ABO incompatibility were enrolled. The baseline characteristics were comparable. Bilirubin levels in DAT-positive and DAT-negative neonates were 14.60±3.58 mg% and 13.18±3.16 mg% (p-value <0.001), respectively. The adjusted odds ratio of positive DAT for both methods in predicting the requirement for phototherapy was 3.21 (95% CI 2.1-5.8). The sensitivity, specificity, positive likelihood ratio and negative likelihood ratio of the tube method were 37.5%, 85.16%, 2.53, and 0.73, respectively, while for the gel method they were 37.08%, 86.57%, 2.76, and 0.73, respectively.

Conclusion: In cases of ABO incompatibility, neonates with positive cord DAT had higher odds of requiring phototherapy; however, a high negative likelihood ratio indicates that additional factors may be involved in hyperbilirubinemia that necessitate phototherapy.

Keywords: Blood group incompatibility, Coomb's test, Neonatal hyperbilirubinemia, Predictive value

INTRODUCTION

Neonatal hyperbilirubinemia is a clinical manifestation of elevated total serum bilirubin; its characteristic features of which include yellowish skin, sclerae and mucosa [1]. The DAT, also known as the Coombs test, is a commonly utilised screening method that identifies the presence of transplacental IgG antierythrocyte antibodies on the surface of foetal RBCs [2]. The DAT serves to categorise haemolysis as either immune or non immune in origin. ABO incompatibility in the foetus may lead to the onset of haemolytic disease in the newborn. Following the implementation of Rh immunoprophylaxis, ABO incompatibility has emerged as the primary cause of Haemolytic Disease of the Newborn (HDN) in developed nations [3,4]. To prevent severe neonatal hyperbilirubinemia and kernicterus in newborns, universal bilirubin screening is implemented in numerous hospitals [5]. This screening aims to identify infants with bilirubin levels exceeding the 75th percentile for their age in hours, as well as those exhibiting a rapid bilirubin rise, defined as an increase greater than 0.2 mg per 100 mL per hour [5]. To prevent hyperbilirubinemia, routine DAT screening is optionally recommended in the AAP 2004 and AAP 2022 guidelines when the mother has O-positive blood [6,7].

The existing literature includes retrospective studies from India [2,8-11]. In contrast, the present study is distinctive as it employs a prospective design, utilised both the gel and conventional tube methods, and implements a standardised approach for cord blood collection. Therefore, the present study was conducted to assess the diagnostic accuracy of the cord blood DAT in predicting the onset of neonatal hyperbilirubinemia necessitating phototherapy within the first week of life. Additionally, it aims to compare the diagnostic abilities between the two methods of DAT estimation, namely the semiagglutination gel method and the conventional tube method.

MATERIALS AND METHODS

This prospective cohort study was conducted following the procurement of informed consent from either parent and ethical approval from the Institutional Ethical Committee (IEC number: SRMIEC-ST0224-1361). It was performed at the SRM Medical College Hospital and Research Centre for a period of 18 months, from September 2022 to March 2024.

Inclusion criteria: Neonates with a gestational age of 35 weeks or more and ABO incompatibility, which was determined by analysing the blood type in the cord blood sample of each infant delivered to a mother with type O blood were included in the study.

Exclusion criteria: Neonates with Rh incompatibility, G6PD deficiency, a family history of haemolytic disorders, shock necessitating inotropes, culture-proven sepsis, severe congenital anomalies, severe birth asphyxia, an APGAR score of less than 3 at one minute, infants requiring assisted ventilation, and those requiring intravenous fluids for more than 72 hours were excluded from the study.

Sample size: The sample size was computed using Master 2.0 software. To achieve a specificity of 80% with a precision of 5% and a 95% confidence interval, the study required 246 infants necessitating phototherapy [8]. The sample size was calculated using the formula $n=Z^2_{\alpha/2}$ P (1-P)/d^2; where P=predetermined value of sensitivity or specificity and α=0.05. Consequently, a total of 523 babies were enrolled in the study.

Data collection: The technique for obtaining blood samples for the DAT entails clipping the umbilical cord approximately 15 centimeters from the infant's side. An umbilical cord clamp is positioned around 3-5 cm from the infant's side. A 2 mL blood sample was obtained from the umbilical vein using a 22-gauge luer lock butterfly needle connected to a 22-gauge needle in an EDTA vacutainer. Samples were dispatched for blood grouping and typing, and in the context of ABO incompatibility, the samples were then processed for DAT. The DAT was conducted using both the semiautomated gel agglutination method and the traditional tube approach, with findings documented as either negative or positive, indicating varied degrees of positivity (1+, 2+, 3+, 4+) [12]. DAT was deemed positive for study purposes if either technique yielded a positive result. The blood grouping and typing process employed the gel column agglutination method, utilising commercially available monoclonal antibodies for anti-A, anti-B, anti-AB, and anti-D (Rh) blood grouping and typing.

On the third day of life, the following tests were performed: universal screening for serum bilirubin using the Jendrassik-Grof method, complete haemogram, peripheral smear to detect evidence of haemolysis, reticulocyte count and thyroid function test, all of which were in accordance with hospital protocol. Routine screening for thyroid function tests is performed on the third day of life; hence, bilirubin testing was done concurrently. However, if the baby was icteric during routine examination, serum bilirubin was tested immediately.

The necessity for phototherapy and exchange transfusion was established by correlating the measured total serum bilirubin levels with the age-specific AAP phototherapy recommendations and AAP exchange transfusion charts from 2004, which were considered the gold standard in the present study. Bilirubin levels exceeding the threshold indicated the commencement of phototherapy. Exchange transfusion was contemplated in light of the presence of encephalopathy or values exceeding the exchange cut-off standards [5].

The AAP 2004 charts provide cut-offs for the management of hyperbilirubinemia based on gestational age and risk factors [6]. The AAP charts consist of three lines: the upper line serves as the cut-off for term neonates (38 completed weeks of gestational age) without risk factors; the middle line is used as the cut-off for neonates (38 completed weeks of gestation) with risk factors and for those (35 to 37+6 completed weeks of gestation) without risk factors. The lower line is the cut-off for neonates (35 completed weeks of gestation) with risk factors. Risk factors include isoimmune haemolytic anaemia, G6PD deficiency, asphyxia, significant lethargy, temperature instability, sepsis and acidosis.

Neonates requiring phototherapy were treated with light-emitting diode light sources in the newborn nursery unit or the neonatal critical care unit. Serum bilirubin levels were assessed every six hours in infants requiring exchange transfusion and every 12 hours if elevated beyond the normal range. Phototherapy was discontinued when the bilirubin levels were ≤2 mg/dL below the starting cut-off value [6]. Infants were assessed every third day until day 14 of life, and the necessity for readmission was determined by clinical examination for jaundice and blood bilirubin levels in the presence of icterus.

Maternal complications were noted during the delivery process. The neonatal variables collected included gestational age determined via first-trimester ultrasound or the last menstrual period, classification as small, large, or appropriate for gestational age according to Intergrowth 21 charts, delayed cord clamping for a minimum of one minute postdelivery, presence of cephalhaematoma or other haematomas, feeding patterns, percentage of weight loss on day three, thyroid function tests, and family history of jaundice.

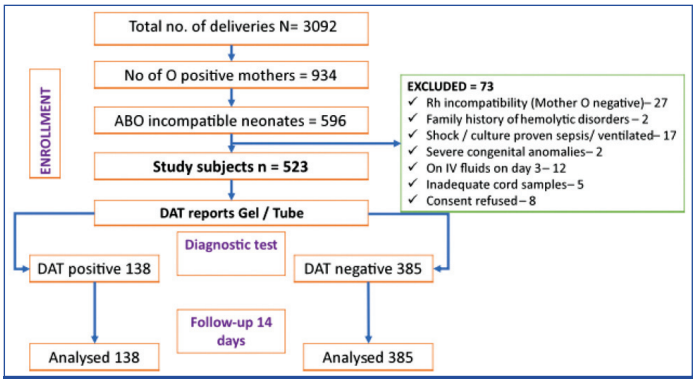
STATISTICAL ANALYSIS

The statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 19.0 for Windows. The Chi-square test or Fisher's exact test was employed to assess dichotomous data, while the t-test or Mann-Whitney U test was utilised to investigate continuous data. The odds ratio and adjusted odds ratio were determined through regression analysis. The calculated metrics include specificity, sensitivity, negative predictive value and positive predictive value.

RESULTS

During the research period, there were 3,092 births, of which 934 women had O-positive blood type, and 596 newborns had ABO incompatibility. A total of 523 babies with ABO incompatibility were recruited for the research [Table/Fig-1]. The mean birth weight and gestational age were 2.92±0.38 kg and 37.8±1.1 weeks, respectively (n=523). No substantial differences in baseline parameters, such as gender distribution, gestational age, birth weight, presence of cephalhaematoma, maternal diabetes/hypertension, mode of delivery, and APGAR score, were observed between the DAT positive and DAT negative groups. However, a statistically significant higher prevalence of OA setting was observed in neonates with DAT positivity compared to the OB setting, with 71 (51.5%) versus 67 (48.5%; p-value=0.026) [Table/Fig-2].

Of the 138 babies who tested positive for DAT, 94 (68.1%) required phototherapy. Conversely, among the 385 children who tested



[Table/Fig-1]: Study flow chart.

Variables	DAT positive N=138	DAT negative N=385	p-value
Gender			
Boy n (%)	70 (50.7)	194 (50.4)	0.95
Females n (%)	68 (49.3)	191 (49.6)	
Gestational age weeks Mean±SD	37.9±1.1	37.8±1	0.59
Birth weight (kg) Mean±SD	2.95±0.38	2.9±0.35	0.31
Blood group of neonate			
A n (%)	71 (51.5)	156 (40.5)	0.026
B n (%)	67 (48.5)	229 (59.5)	
AGA n (%)	122 (88.4)	334 (86.7)	0.735
SGA n (%)	12 (8.7)	34 (8.8)	
LGA n (%)	4 (2.9)	17 (4.4)	
Cephalhaematoma n (%)	6 (4.3)	22 (5.7)	0.5
Sibling history of jaundice n (%)	6 (7.9)	19 (9.8)	0.54
Maternal GDM n (%)	21 (15.2)	48 (12.5)	0.425
Maternal PIH n (%)	8 (5.8)	24 (6.2)	0.86

Delivery mode			
LSCS n (%)	72 (52.2)	185 (48.1)	0.35
Assisted vaginal delivery n (%)	2 (1.5)	15 (3.9)	
Normal vaginal delivery n (%)	64 (46.3)	185 (48)	
APGAR 1 median (IQR)	8 (4-8)	8 (6-8)	0.06
APGAR 5 median (IQR)	9 (8-9)	9 (8-9)	0.73
Exclusive breastfeeding n (%)	132 (95.6)	373 (96.8)	0.73
Reticulocyte count (%) Mean±SD	5.1±2.6	4.3±2.5	0.02
Peripheral smear suggestive of haemolysis n (%)	44 (31.8)	67 (17.4)	0.001
TSH mU/L Mean±SD	5.9±3.42	5.8±3.7	0.72
Free T4 ng/dL Mean±SD	2.9±0.73	2.8±0.69	0.82

[Table/Fig-2]: Baseline characteristics of study subjects.
AGA: Apropriate for gestational age; SGA: Small for gestational age; LGA: Large for gestational age; PIH: Pregnancy-induced hypertension; LSCS: Lower segment caesarean section; TSH: Thyroid stimulating hormaone

negative for DAT, only 146 (37.9%) infants needed phototherapy. Among 71 neonates with A blood type and positive DAT, 46 (64.8%) required phototherapy, with a peak serum bilirubin level of 14.6±3.9 mg/dL. Only 66 (42.3%) of the 156 neonates with A blood type and negative DAT required phototherapy, with a peak serum bilirubin level of 13.3±3.1 mg/dL. Among 67 neonates exhibiting DAT positivity and harboring a B-positive blood type, 48 (71.6%) required phototherapy, with a peak serum bilirubin level of 14.7±3.1 mg/dL. Only 80 (34.9%) of the 229 neonates with B blood type and negative DAT required phototherapy, with a peak serum bilirubin level of 12.9±3.2 mg/dL. The calculated odds ratio for a DAT positive neonate requiring phototherapy was 3.44 (95% CI 2.26-5.24) [Table/Fig-3]. The adjusted odds ratio calculated by regression analysis after adjusting for variables including birth weight, gestation, blood type, sibling history, haemolysis in peripheral smear, and cephalhematoma was 3.21 (95% CI 2.1 - 5.8). None of the infants needed exchange transfusion.

The tube method demonstrated a sensitivity of 37.5%, specificity of 85.16%, a positive likelihood ratio of 2.53, and a negative likelihood ratio of 0.73. In the gel technique, the results were 37.08%, 86.57%, 2.76, and 0.73, respectively [Table/Fig-4].

Outcome	DCT positive n=138	DCT negative n=385	Unadjusted odds ratio/mean difference	95% CI	p-value
Need for phototherapy n (%)	94 (68.1)	146 (37.9)	3.44[a]	2.26-5.24	<0.0001
Peak bilirubin levels Mean±SD (mg/dL)	14.60±3.58	13.18±3.16	1.54[b]	0.91-2.2	<0.001
Readmission for phototherapy N (%)	4 (2.9)	8 (2.1)	1.41[a]	0.42-4.74	0.51
Duration of phototherapy in days* Median (Range)	1 (1-5)	1 (1-3)	0.12[b]	-0.5-1.3	0.51
Age of starting phototherapy in hours* Median (Range)	65 (32-90)	70 (44-88)	-6.8[b]	-10.5-2.3	<0.001
Blood group A outcomes	**DAT positive n=71**	**DAT negative n=156**	**Unadjusted odds ratio/mean difference**	**95% CI**	**p-value**
Need for phototherapy n (%)	46 (64.8)	66 (42.3)	2.51[a]	1.4-4.48	0.002
Peak serum bilirubin Mean±SD (mg%)	14.6±3.9	13.3±3.1	1.3[b]	0.3-2.24	0.01

Blood group B outcomes	DAT positive n=67	DAT negative n=229	Unadjusted odds ratio/mean difference	95% CI	p-value
Need for phototherapy n (%)	48 (71.6)	80 (34.9)	3.5[a]	2.3-5.3	<0.001
Peak serum bilirubin Mean±SD (mg%)	14.7±3.1	12.9±3.2	1.54[b]	0.91-2.17	<0.001

[Table/Fig-3]: Primary and secondary outcomes.
*Calculated for neonates requiring phototherapy n=240; a=Odd's ratio; b=Mean difference

	Tube method	Gel method
Sensitivity (95% CI)	37.5% (31.36%-43.96%)	37.08% (30.96%-43.53%)
Specificity (95% CI)	85.16% (80.47%-89.09%)	86.57% (82.04%-90.32%)
Positive likelihood ratio (95% CI)	2.53 (1.83-3.49)	2.76 (1.97-3.87)
Negative likelihood ratio (95% CI)	0.73 (0.66-0.82)	0.73 (0.65-0.81)
Positive predictive value (95% CI)	68.18% (60.80%-74.75%)	70.08% (62.54%-76.67%)
Negative predictive value (95% CI)	61.64% (59.02%-64.19%)	61.87% (59.30%-64.37%)
Accuracy (95% CI)	63.29% (59.00%-67.43%)	63.86% (59.58%-67.99%)

[Table/Fig-4]: Diagnostic ability of tube and gel method of DAT in predicting the need for phototherapy.

At a prevalence of 45% of neonates with ABO incompatibility requiring phototherapy, the diagnostic accuracy of DAT in forecasting the necessity for phototherapy has been estimated at 0.63 (0.59-0.67) for the tube method and 0.64 (0.6-0.68) for the gel method. The frequency and percentage for the sample distribution of neonates with hyperbilirubinemia tested positive by each method are tabulated in [Table/Fig-5].

Method	Result (N)	Serum bilirubin mg%	Phototherapy	
			Yes	No
DCT gel	Negative (396)	13.1±3.2	151	245
	1+ (69)	13.4±2.8	39	30
	2+ (42)	15.9±3.7	37	5
	3+ (16)	17±4.3	13	3
DCT tube	Negative (391)	14.6±3.5	150	241
	Positive (132)	13.2±3.2	90	42

[Table/Fig-5]: Frequency and percentage for sample distribution of neonates with hyperbilirubinemia tested positive by each method.

DISCUSSION

Haemolytic Disease of the Foetus/Newborn (HDFN) is characterised by a positive DAT in infants due to maternal IgG antibodies that bind to foetal RBC antigens, which traverse the placenta and trigger haemolysis [13]. The predominant aetiology of mild HDFN is ABO antibodies. Following the decline of maternal-foetal Rh incompatibility, ABO alloimmunisation has emerged as the primary cause of haemolysis in newborns. Approximately 15-25% of pregnancies are affected by ABO incompatibility [14].

This study examined the diagnostic accuracy of the DAT in predicting newborn hyperbilirubinemia in neonates with a gestational age beyond 35 completed weeks, specifically in cases of ABO incompatibility. In light of the universal screening for thyroid disorders conducted on day 3 of life, serum bilirubin levels were measured in the same sample (if the baby was not icteric). This prospective cohort study, which employed a standardised procedure for cord blood collection, demonstrated a sensitivity of 37.08% and a specificity of 86.57% for positive DAT in predicting the necessity for phototherapy.

Study	Country/Year	Type of study	Population DAT +ve/total population screened	Method of estimation	PPV	NPV	Sensitivity	Specificity
Meberg A and Johansen KB [10]	Chicago/1998	Prospective	100/2463	Conventional tube method	12	96	64	65
Herschel M et al., [11]	Jerusalem/2002	Prospective	23/660	Conventional tube method	53	89	15	98
Dinesh D [9]	New Jersey/2005	Prospective	94/1724	Not cited	23	92	15	95
Chowdhary S et al., [2]	India/2022	Retrospective	68/424	Semiautomated gel agglutination method	89.7	60.1	29.9	96.9
Das S et al., [8]	India/2020	Prospective	108/727	Both gel agglutination and conventional tube method	34	96	51	93
Present study	India/2025	Prospective	138/523	Both gel agglutination and conventional tube method	70.08	61.87	37.08	86.57

[Table/Fig-6]: Comparison of DAT diagnostic ability in studies [2,8-11].

The AAP indicates that selective cord blood donation from only O-positive mothers may be suitable, contingent upon ABO incompatibility and available follow-up resources [6]. Given the elevated number of deliveries, premature discharges and increased prevalence of neonatal hyperbilirubinemia in India, cord blood DAT for ABO incompatibility could facilitate meticulous monitoring of infants with positive results and mitigate the risk of severe hyperbilirubinemia. Limited retrospective investigations have explored selective DAT testing in babies with ABO incompatibility and its implications, yielding contradictory results, while prospective research remains limited [12,15].

The findings of the various studies are tabulated in [Table/Fig-6] [2,8-11]. The positive predictive value of the DAT in present study was higher due to the greater incidence of newborn jaundice in India compared to studies conducted overseas. The results are analogous to those of other research conducted in India [2,8]. No difference in the diagnostic capabilities was noted between the gel method and the tube method regarding sensitivity and specificity; nevertheless, research by Das S et al., indicated that the conventional tube method had a superior positive predictive value [8]. Additionally, research conducted by Chowdhary S et al., indicated that infants with blood group A had higher DAT positivity compared to those with blood group B, corroborating present study findings [2]. Contamination of cord blood can affect DAT results; thus, measures were implemented in the current study to prevent contamination [16]. Furthermore, research conducted by Lin ZX and Dong QS revealed that the positive ratio of the DAT for predicting the occurrence of ABO-HDN was 59.14% (110/186), facilitating early diagnosis and intervention [17].

AlKhater SA et al., concluded that, in addition to a good DAT test, several clinical factors were crucial in assessing the need for phototherapy in infants [18]. Sarici SU et al., proposed that, in full-term healthy infants with ABO incompatibility, elevated reticulocyte counts, a positive DAT, and a sibling history of neonatal jaundice are valid predictors of significant hyperbilirubinemia and severe HDN [19].

The highlight of present study was that a standardised method of DAT (both gel and tube methods) was employed, and infants were monitored after 14 days of treatment. The current investigation produced a positive likelihood ratio of 2.53 and 2.76 for the tube and gel methods, respectively, indicating that newborns with DAT positivity pose an increased risk for severe hyperbilirubinemia necessitating phototherapy. However, the low accuracy of DAT (0.63, 95% CI (0.59-0.67)) limits its utility in settings where routine monitoring for jaundice and follow-up is available. A prevalence of 45% of neonates requiring phototherapy due to ABO incompatibility would have decreased the accuracy of DAT, as prevailing literature states that the diagnostic accuracy of a test decreases with a rise in the prevalence of the disease, especially if the test has lower sensitivity and high specificity, as seen in present study [20].

Limitation(s)

- The AAP 2004 guidelines were utilised to determine the necessity for phototherapy.
- Serum bilirubin levels were evaluated pragmatically at intervals of 6 to 12 hours, with no more frequent assessments conducted.
- There was a lack of availability of transcutaneous bilirubinometry.

CONCLUSION(S)

In newborns with ABO incompatibility, a positive cord DAT significantly increases the likelihood of requiring phototherapy. However, the low accuracy and substantial negative likelihood ratio of the DAT for phototherapy requirements indicate that other variables influence hyperbilirubinemia in ABO incompatibility. Therefore, subsequent larger studies exploring other markers for haemolytic hyperbilirubinemia should be considered.

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