

Original Research Article

Study of hematological indices in neonates admitted with non-obstructive jaundice and its outcome in a tertiary care hospital

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ABSTRACT

Background: To study non-obstructive causes and laboratory profile of neonatal hyperbilirubinemia. Design: prospective study.

Methods: Selection of cases were done from routine cases reporting to newborn unit in the department of paediatrics, with clinical evidence of jaundice in neonates. Blood group of the mother and baby, Serum bilirubin estimation, Complete blood count with peripheral smear examination, Reticulocyte count, Direct coomb's test and C-reactive protein of the baby were done.

Results: Study includes 89 cases of newborn admitted in our tertiary care institute. Out of 89 neonates, 52 (58.42%) were male while 37 (41.57%) were females. Total number of Pre-term babies was 35 (39.32%). Neonates having low birth weight were 30 (33.7%) and very low birth were 10 (11.23%). Physiological jaundice constituted majority cases. Septicemia was the commonest cause of pathological jaundice and ABO incompatibility is second commonest cause of pathological jaundice. Pre-term and low birth weight babies were having higher levels of serum total bilirubin but the difference was not significant ($P > 0.05$). The rise in serum bilirubin level was found to be more in pathological jaundice as compare to physiological jaundice. Difference was significant statistically with p value of < 0.05 .

Conclusions: Most of the cases were having physiological jaundice although septicemia and ABO-Rh incompatibility were not exceptional. Peak serum bilirubin levels were found to be more among the pathological jaundice. Also, prematurity and low birth weight were having higher levels of serum bilirubin. Special care must be given to them in order to avoid future complications of hyperbilirubinemia.

Keywords: Jaundice, Non-obstructive, Physiological, Sepsis

INTRODUCTION

Jaundice is the most common problem in a newborn. The first documented scientific description of neonatal jaundice occurred in latter part of the 18th century when "BAUMES" was awarded the national prize for describing the clinical course of jaundice. According to national neonatal database 80 % of all term babies, 60% of all preterm babies develop jaundice and 3.3% of babies

irrespective of gestational age have severe hyperbilirubinemia of >15 mg/dl. The most common cause of neonatal hyperbilirubinemia in India is physiological, other factors commonly incriminated are Premature birth, sepsis and bacterial infections, rhesus isoimmunization, ABO incompatibility, G-6-P-D deficiency, RBC membrane disorders, cephalhematoma and drug induced. Though history and clinical presentation of newborns play a major role, the

laboratory investigations play a vital role in diagnosing and differentiating physiologic from pathological jaundice.

Investigations such as total bilirubin, blood type-Rh, Direct coombs test, peripheral smear study, hematocrit, reticulocyte count, complete blood count, C-reactive protein vary with the type of jaundice and the possible impact in the infant's outcome and hence the study.¹

The objective of the study was to study causes of non-obstructive neonatal hyperbilirubinemia and their laboratory profile.

METHODS

- Blood group of the mother and baby,
- Serum bilirubin estimation of the baby
- Complete blood count with peripheral smear examination of the baby
- Reticulocyte count
- Direct coomb's test of the baby
- C-reactive protein.²

Preparation of slide for peripheral smear

- Take a drop of blood on the slide
- Spreader slide is placed at 45 °C on the drop and moved along the slide
- It is moved smoothly so that the blood film is thin
- Care should be taken to avoid air bubbles
- Then the smear is air dried
- Smear is placed on the staining track
- Pour the leishman stain to cover the smear completely and allow it to fix for 2-3 minutes
- Add water twice the amount of stain and allow it to fix for 7-10 minutes
- Wait for the appearance of golden scum or sheen on the surface of the smear
- Wash the stain off the slide with running water
- Wipe the back of the slide and allow it to air dry.³

Preparation of slide for reticulocyte count

- Add 5 drops of new methylene blue solution to 5 drops of thoroughly mixed EDTA anticoagulated blood to a glass slide
- Mix the contents by gently shaking and allow to incubate at room temperature for a minimum of 30 minutes
- At the end of 30 minutes, gently mix the blood/stain solution
- Using a capillary tube, place a drop of the mixture on each of three slides near the frosted edge as you would when making a peripheral smear
- Using the wedge smear technique, make acceptable smears not too thick or thin
- Allow to air dry.³

Serum bilirubin estimation

Methods of determination instrument

Applications by instruments

- Van den Bergh, Malloy and Evelyn Reaction: In an aqueous solution, Ehrlich's diazo reagent reacts with the direct bilirubin in the serum to form a pink to reddish-purple colored compound (azobilirubin). It is read at one minute. In a 50% methyl alcohol solution, Ehrlich's diazo reagent reacts with the total bilirubin in the serum to form a pink to reddish-purple colored compound. (Read at 30 minutes.)
- Methods of Jendrassik and Grof: Serum or plasma is added to a solution of sodium acetate and caffeine-sodium benzoate. The sodium acetate buffers the pH of the diazo reaction, while the caffeine-sodium benzoate accelerates the coupling of bilirubin with diazotized sulfanilic acid. The azobilirubin color develops within 10 minutes. (An accelerating agent facilitates the coupling of albumin-bound bilirubin with the diazo reagent.)
- ASTRA: The ASTRA System Direct Bilirubin Chemistry Module employs a modification of the Jendrassik-Grof rate method.
- ACA.^{4,5}

Conjugated bilirubin

Conjugated bilirubin reacts with DSA (diazotized sulfonilic acid) under acid conditions to form a red chromophore. The absorbance due to the chromophore is directly proportional to the conjugated bilirubin in the sample and is measured using a two-filter (540-600 nm) end point technique. Conjugated bilirubin + DSA + H 6 Red chromophore + (non-absorbing at 540 nm) (absorbs at 540 nm) UNIT: Total and Direct Bilirubin (continued) MLAB 2401 - Clinical Chemistry Lab Manual C F 115

Total bilirubin

Total bilirubin reacts with DSA under acid conditions to form a red chromophore. Lithium dodecyl sulfate (LDS) is employed to solubilize the unconjugated bilirubin. The absorbance due to the chromophore is directly proportional to the bilirubin in the sample and is measured using a two-filter (540-600 nm) end point technique. Bilirubin + DSA + H Red chromophore + LDS (non-absorbing at 540 nm) (absorbs at 540 nm)

Procedure for newborns

Neonatal bilirubin: The absorbance of the sample, measured using a two-filter (452-540 nm) differential technique is directly proportional to the bilirubin concentration. Absorbance at 452 nm is due to the bilirubin concentration, and, if present, hemoglobin. At 540 nm, bilirubin does not absorb, while hemoglobin exhibits the same absorbance as it does at 452 nm.

The use of 540 nm as the blanking wavelength thus eliminates any hemoglobin contribution from the total absorbance at 452 nm. Bilirubin in newborn babies can be read in this direct spectrophotometric procedure in part due to the fact that the normal range is much higher than for adults. In addition, carotene and other dietary pigments prevent adult and specimens from older children from being suitable.

Procedure Total and Direct Bilirubin (Sigma #605) Quantitative, Colorimetric Principle of Reaction Bilirubin is coupled with diazotized sulfanilic acid to form azobilirubin.

The color of this derivative is pH dependent, occurring as pink in acid or neutral medium and blue under alkaline conditions. Direct (conjugated) bilirubin couples with diazotized sulfanilic acid (p-diazobenzenesulfonic acid), forming a blue color at alkaline pH.

Direct bilirubin (conjugated) + diazotized sulfanilic acid alkaline pH >blue color azobilirubin Indirect (unconjugated) bilirubin is diazotized only in the presence of an “accelerating” agent, caffeine-benzoate-acetate mixture.

Thus, the blue azobilirubin produced in mixtures containing “accelerating” agent originates from both the Direct and Indirect fractions and reflects the Total bilirubin concentration.

Total bilirubin + caffeine-benzoate-acetate mixture + diazotized sulfanilic acid -> azobilirubin

Blood grouping and typing estimation

- The table is set with all the materials required. the Monoclonal Antibody is placed (Mab) kit in an Ice tray.
- Alcohol swab is used, and rubbed in the area from where the blood will be sampled (finger-tip). (Discard the swab)
- Lancet cover is opened, by applying pressure at the tip of the finger blood is sampled by Pricking the finger-tip with the opened Lancet.
- As blood starts oozing out, 1 drop falls on the glass slide
- Take the Anti-A (blue) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 1st spot. Place the bottle back in ice.
- Take the Anti-B (yellow) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 2nd spot. Place the bottle back in ice.
- Take the Anti-D (colorless) bottle, resuspend the content and use the dropper to place a drop of the Mab in the 3rd spot. Place the bottle back in ice.
- Take a tooth pick and mix the content in each well. Discard the tooth pick after using in one well (take a new one for the next well).⁶

Table 1: Identification of blood group.

Anti-A	Anti-B	Identified blood group
Agglutination occurs	Agglutination does not occur	A
Agglutination does not occur	Agglutination occurs	B
Agglutination occurs	Agglutination occurs	AB
Agglutination does not occur	Agglutination does not occur	O

Direct coombs test (direct antiglobulin test- DAT)

The direct Coombs test is used to detect antibodies (IgG or C3) that are stuck to the surface of red blood cells. Many diseases and drugs can cause this. These antibodies sometimes destroy red blood cells and cause anemia. This is the test that is done on the newborn's blood sample, usually in the setting of a newborn with jaundice. The two most commonly recognized forms of antibody-mediated hemolysis in newborns are Rh incompatibility and ABO incompatibility.⁷

Procedure of direct coombs test

- Prepare a 5 % suspension in isotonic saline of the red blood cells to be tested
- With clean pipette add one drop of the prepared cell suspension to a small tube
- Wash three times with normal saline to remove all the traces of serum
- Decant completely after the last washing
- Add two drops of Anti-human serum
- Mix well and centrifuge for one minute at 1500 RPM
- Resuspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.

Result interpretation of coombs test

Negative result

No clumping of cells (no agglutination)

Positive result

Clumping (agglutination) of the blood cells during a direct Coombs test means that there are antibodies on the red blood cells and causes the destruction of red blood cells by immune system (hemolysis).

Complete blood count estimaion

The Automated method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid pass through a small aperture.

Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture.

This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. The number of pulses correlates to the number of particles. The height of the electrical pulse is proportional to the cell volume.

Differential analysis

As the sample, prepared for differential analysis, streams through the flow cell these three measurements occur simultaneously on each individual white cell to classify it:

- Low-frequency current measures volume.
- High-frequency current senses cellular internal content through measuring changes in conductivity.
- Light from the laser bouncing off the individual WBC cells characterizes cellular surface, shape, and reflectivity.

C-reactive protein estimation

CRP Test is based on the latex agglutination method introduced by Singer, et. al., in 1957. This is a slide agglutination test for the qualitative and semiquantitative detection of C-Reactive Protein (CRP) in human serum. Latex particles coated with goat IgG anti-human CRP are agglutinated when mixed with samples containing CRP. When latex particles coated with human anti-CRP are mixed with a patient's serum containing C-reactive proteins, this results in visible agglutination within 2 minutes.⁸

CRP test procedure

- Bring all reagents and serum sample to Room Temperature and mix latex reagent gently prior to use. Do not dilute the controls and serum.
- Place 1 drop each of serum, positive control and negative control on separate reaction circles.
- Then add CRP latex reagent 1 drop to each of the circles.
- Mix with separate mixing sticks and spread the fluid over the entire area of the cell.
- Tilt the slide back and forth slowly for 2 minutes observing preferably under artificial light.

CRP test procedure (semi quantitative analysis)

Sera with positive results in the screening test should be retested in the semiquantitative test for obtaining the titre.

- Make serial two-fold dilutions of the sample in 9 g/L saline solution.
- Proceed for each dilution as in the qualitative method.
- Agglutination of latex particles is considered a positive reaction, indicating the presence of C-reactive protein at a significant and detectable level. Specimens which do not contain human CRP will not cause agglutination.
- If controls do not give expected reactions the test is invalid and must be repeated.
- The titer, in semi-quantitative method, is defined as the highest dilution showing a positive result.⁸

RESULTS

The present study includes 89 cases of newborn admitted in our tertiary care institute. Out of 89 neonates, 52 (58.42%) were male while 37 (41.57%) were females. Total number of Pre-term babies was 35 (39.32%).¹¹

Table 2: Etiology wise distribution of neonatal hyperbilirubinemia.

Etiology	No. of cases	%
Physiological jaundice	44	49.43
ABO Incompatibility	18	20.22
Septicemia	20	22.47
Rh incompatibility	07	7.86
Total	89	

Neonates having low birth weight were 30 (33.7%) and very low birth were 10 (11.23%). Physiological jaundice constituted (34) 38.20% cases of Neonatal hyperbilirubinemia.^{9,10}

Septicemia was the commonest cause of pathological jaundice and ABO incompatibility is second commonest cause of pathological jaundice.¹² Among half of the cases (45, 50.56%) range of serum total bilirubin was found between 6.2 and 11.9 mg/dl. 2 (2.2%) were having the serum total bilirubin more than 16 mg/dl.

Hemoglobin level was lowest (12.2 gm %) in Rh incompatibility. Highest level of serum bilirubin was found in Rh Incompatibility whereas highest level of reticulocytes was noted in the same.¹³

Pre-term and low birth weight babies were having higher levels of serum total bilirubin but the difference was not significant ($P > 0.05$).¹⁴ The rise in serum bilirubin level was found to be more in pathological jaundice as compare to physiological jaundice. Difference was significant statistically with p value of < 0.05 .

Direct Coomb's test found to be positive in all case in Rh incompatibility while they were negative in ABO incompatibility.

In cases of septicemia CRP was found to be positive in 100% of cases. CRP was found to be positive in a few

cases of ABO incompatibility and rh incompatibility (2.2%) and physiological jaundice (22.47%).

Table 3: Mean level of haemoglobin, serum bilirubin and reticulocyte count in neonatal hyperbilirubinemia.

Etiology	Mean Hb (g%)	Mean serum bilirubin (mg/dl)	Mean reticulocyte count(%)
Physiological jaundice	14.6	9.6	0.31
ABO incompatibility	13.5	12.9	0.40
Rh incompatibility	12.2	14.1	0.61
septicemia	12.4	13.3	0.54

Table 4: Demographic profile of neonatal hyperbilirubinemia cases.

Variables	Number	%
Sex		
Female	37	41.57
Male	52	58.42
Gestational age		
Term	54	60.67
Preterm	35	39.32
Birth weight		
Normal	49	55.05
LBW	30	33.70
VLBW	10	11.23

Table 5: Mean serum bilirubin value: physiological versus pathological neonatal jaundice.

Neonatal jaundice	Mean level of sr. bilirubin	T-value
Physiological jaundice (n=44)	11.72±1.8	2.3<0.05
Pathological jaundice (n=45)	12.01±3.1	

Table 6: Result of direct coomb's test in Rh and ABO incompatibility.

	DCT		Total
	Positive	Negative	
Rh incompatibility	07 (100%)	00	07
ABO incompatibility	00	18 (100%)	18

DISCUSSION

Study included 89 cases of Neonatal hyperbilirubinemia cases. Among them 52(58.7%) were male while 37(41.3%) were females. In present study, percentage of Pre-term (<37 weeks) babies was 39.32, neonates having low birth weight (<2.5 kg) were 30 (33.70%) and neonates having very low birth weight were 10 (11.23%). In this study out of 89, 44 (49.43%) cases were diagnosed as having physiological jaundice by while others were having ABO incompatibility (20.22%), Rh

incompatibility (7.86%), septicaemia (22.47%). clinical sepsis as defined by WHO criteria was found in 86.3% of babies. Nearly 1/3rd (32.9%) babies were ABO incompatible and 4.1% babies were Rh incompatible. In present study mean Hb level was 13.5±1.7 gm/dl with range of 6-16.2gm/dl. In any infant, 24 hours old any jaundice is considered pathologic and requires evaluation. This evaluation should minimally include a serum bilirubin and workup for hemolytic disease.

Table 7: Result of C-reactive protein in Neonatal Septicemia.

Etiology	CRP positive n (%)	Total no. of cases
Septicemia	20 (100%)	20
Physiological jaundice	20 (45.45%)	44
ABO incompatibility	02 (11.11%)	18
Rh incompatibility	02 (28.57%)	07
Total		89

Guidelines for therapy depend upon the serum concentration of bilirubin and the patient's age. Also, serum bilirubin is most important investigation to judge severity and management of patient. In present study serum bilirubin was highest in ABO incompatibility and Rh Incompatibility. Among half of the cases (52.4%) range of serum total bilirubin was found between 15 and 19.9 mg/dl. maximum number (67.1%) of infants' peak serum bilirubin fell in the range of 15-19.9 mg/dl. DCT was positive in 100% cases of Rh incompatibility while in ABO incompatibility they were found to be negative. The reason for this difference may have been that "A" and "B" antigens are weaker antigens and the distance between a/b antigen sites on the fetal red cells as compared to adult red cells is more. In all cases of septicemia CRP was positive in present study. It is an acute phase reactant; is synthesized by the liver and it becomes positive after any inflammation. It is a very reliable indicator. This study also included peripheral smear study of the neonates. Both physiological and pathological jaundice showed macrocytic hyperchromic picture, but nucleated RBC'S and spherocytes were common among Rh-ABO incompatibility cases which

were less significant among neonates with physiological jaundice

CONCLUSION

To conclude, most of the cases were having idiopathic jaundice although septicemia and ABO-Rh incompatibility were not exceptional. Peak serum bilirubin levels were found to be more among the pathological jaundice. Also, prematurity and low birth weight were having higher levels of s. bilirubin. Special care must be given to them in order to avoid future complications of hyperbilirubinemia.

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