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RESEARCH ARTICLE

A STUDY OF EFFECT OF ABO AND RH INCOMPATIBILITY ON ANEMIA AND JAUNDICE IN NEONATES BORN IN A TERTIARY CARE HOSPITAL

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Key Words:
(PT)-preterm, (AGA)-appropriate for gestational age, (NW)-Normal weight of baby-(2.5kg-3.99kg), (LBW)- low birth weight (1.5kg-2.499kg), (EONS)-early onset neonatal sepsis, (TSB)- Total Serum Bilirubin & (NNH)- Neonatal Hyperbilirubinemia.

ABSTRACT

In this study, the aim was to assess the effect of ABO and Rh incompatibility on anemia and jaundice in neonates born in a tertiary care hospital in Delhi, India. In our study of 516 neonates, approximately 34.10% of live births were at theoretical risk for immune mediated hemolysis based on ABO and Rh incompatibility, of which 28.29% neonates had mild physiologic jaundice and 0.39% neonates had mild anemia. However, clinical manifestation of pathologic jaundice developed in only 5.81% of at-risk infants with ABO incompatibility, of which 3.68% neonates had TSB between 12mg/dL to 17.99mg/dL and 2.13% neonates had TSB between 18mg/dL to 26mg/dL. All the neonates with TSB more than 18mg/dL were also associated with early onset neonatal sepsis. The incidence of ABO incompatibility with early onset neonatal sepsis with hyperbilirubinemia with severe anemia and severe hemolysis was about 0.2% in our study. Besides, no neonate had hydrops fetalis, kernicterus or cerebral palsy and the neonatal mortality rate was zero, in our study. Thus, in our study, the effect of ABO and Rh incompatibility on anemia and jaundice was mild in most neonates and the effect became severe in presence of risk factors, most commonly neonatal sepsis.

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INTRODUCTION

The word 'jaundice' comes from the French word 'jaune', meaning 'yellow' and 'jaunisse' meaning "yellow disease".[12] The medical term for jaundice is icterus. The word 'icterus' comes from the Greek word 'ikteros'.[8] The origin of the word icterus is quite bizarre, coming from an ancient belief that jaundice could be cured from looking at the yellow bird icteria.[12] The term icterus is sometimes incorrectly used to refer to jaundice specifically of sclera.[8][12] Neonatal jaundice is a yellowish discolouration of mucous membranes and skin in a neonate (infant under 28 days of age), due to high bilirubin levels.[19] Other symptoms may include excess sleepiness or poor feeding.[19] Complications may include seizures, cerebral palsy, or kernicterus.[19] Bilirubin was discovered by Rudolf Virchow in 1847.[17] The serum bilirubin level required to cause jaundice varies with skin tone and body region, but jaundice usually becomes visible on the sclera at a level of 2 to 3 mg/dL and on the face at about 4 to 5 mg/dL. With increasing bilirubin levels, jaundice seems to advance in a head-to-foot direction, appearing at the umbilicus at about 15 mg/dL and at the feet at about 20 mg/dL. [19]

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Under normal circumstance, the level of indirect bilirubin in umbilical cord serum is 1-3mg/dL and rises at a rate of <5mg/dL/24hr; thus, jaundice becomes visible on the 2nd or 3rd day, usually peaking between the 2nd and 4th days at 5-6mg/dL and decreasing to less than 2mg/dL between the 5th and 7th days after birth. Jaundice associated with these changes is designated *physiologic jaundice* (non-pathologic unconjugated hyperbilirubinemia) and is believed to be the result of increased bilirubin production from breakdown of fetal red blood cell breakdown combined with transient limitation in conjugation of bilirubin by the immature neonatal liver. In premature infants, the rise in serum bilirubin tends to be the same or somewhat slower but of longer duration than in term infants. Peak levels of 8-12mg/dL are not usually reached until the 4th-7th day, and jaundice is infrequently observed after the 10th day, corresponding to the maturation of mechanisms for bilirubin metabolism and excretion.[14] [16] [19]

In general, factors suggesting a *pathologic jaundice* a search to determine the cause of jaundice should be made if it appears in the 1st 24 hours after birth, serum bilirubin is rising at a rate faster than 5mg/dL/24hr, total serum bilirubin is >12mg/dL in a term infant (especially in the absence of risk factors) or 10-14mg/dL in a preterm infant, jaundice persists after 10-14 days after birth, or serum direct bilirubin fraction is >2mg/dL at any time. Other factors suggesting a *pathologic jaundice*

(unconjugated or conjugated hyperbilirubinemia) are family history of hemolytic disease, pallor, hepatomegaly, splenomegaly, vomiting, lethargy, poor feeding, excessive weight loss, apnea, bradycardia, hypothermia, light coloured stools, dark urine positive for bilirubin, bleeding disorder, failure of phototherapy to lower the bilirubin level and signs of kernicterus.[16][19] Persistent pathologic indirect hyperbilirubinemia - that is, jaundice persisting beyond the first 14 days- is also seen in neonates, more commonly in breastfed babies. [14]

Haemolytic disease of the fetus and newborn (HDFN), also known as eryhtroblastosis fetalis, is caused by transplacental passage of maternal antibodies directed against paternally derived red blood cell (RBC) antigens, which causes increased RBC destruction (hemolysis) in the infant. HDFN is an important cause of anemia and jaundice in newborn infants, and early recognition and diagnosis are crucial for proper management. Although more than 60 different RBC antigens are capable of eliciting a maternal antibody response, clinically significant disease is associated primarily with incompatibility of ABO blood groups and the RhD antigen.[15]

In young babies, unconjugated bilirubin (which is not carried by albumin) can penetrate the membrane that lies between the brain and the blood (the blood–brain barrier) because the blood–brain barrier has yet to develop fully, whereas more developed individuals with increased bilirubin in the blood are protected. Unconjugated bilirubin is potentially toxic to neural tissue (brain and spinal cord). The term kernicterus is used to denote the clinical features of acute or chronic bilirubin encephalopathy, as well as the yellow staining in the brain associated with the former. Kernicterus is also known to occur at lower levels of bilirubin in term babies who have risk factors, and in preterm babies.[14] [16] Kernicterus has been rising in recent years due to less time spent outdoors.[16] Haemolytic disease of the fetus and newborn and kernicterus are crucial global health issues, which must be addressed to reduce neonatal and child mortality globally and reach the sustainable development goals.

AIMS AND OBJECTIVES

In this study, the aim was to assess the effect of ABO and Rh incompatibility on anemia and jaundice in a group of neonates, born in a tertiary care hospital in Delhi, India.

MATERIALS AND METHODS

Study Setting and Period of Study: The study was conducted in the Department of Paediatrics, Rockland Hospital in Delhi, India during the period of 01 January 2012 to 07 August 2014.

Study Design: The study was a Hospital Based Study, conducted at the Department of Paediatrics, Rockland Hospital in Delhi.

Sample Size: For the present study, 516 neonates (500 singleton neonates & 16 twin neonates) were recorded and studied in Paediatrics Department in Rockland Hospital. These neonates were born alive to 508 mothers in Obstetrics & Gynaecology Department in Rockland Hospital, during the period 01.01.2012 till 07.08.2014.

Sampling Design: The study was done as Random Sampling of the neonates noticed to have deep yellow discolouration of whole body that were born in Rockland Hospital, Delhi. In this study, all the venous blood samples of neonates for Total Serum Bilirubin (TSB) and Direct Serum Bilirubin were collected during first 9 days of life, and in one case of persistent pathologic hyperbilirubinemia, the sample was again collected on Day 21 of life. Besides, venous samples were collected for blood groups of all the 516 neonates as well as blood groups of their mothers were also done. These neonates and their mothers were further investigated for Direct Coombs Test, Haemoglobin estimation, Haematocrit estimation, Reticulocyte count estimation, Glucose-6-Phosphate Dehydrogenase (G6PD) activity and Peripheral blood smear to ascertain any hemolysis.

Study Variables: Gender of neonate, physiologic jaundice or pathologic jaundice, tests of neonate (blood group of neonate and mother, total serum bilirubin level, indirect serum bilirubin level and direct serum bilirubin level, Direct Coombs Test, Haemoglobin estimation, Haematocrit estimation, Reticulocyte count estimation, Glucose-6-Phosphate Dehydrogenase (G6PD) activity and Peripheral blood smear), maturity of neonate (term or preterm), morbidity (hydrops fetalis, kernicterus or cerebral palsy) and mortality rate in a group of neonates born in Rockland Hospital, Delhi.

Inclusion Criteria/ Selection Criteria: Participants in the study eligible for inclusion were neonates of either gender, born alive in Rockland Hospital during the period 01.01.2012 till 07.08.2014. The mothers of these neonates were also included as participants after obtaining written consent from them. Neonates were included after obtaining proper informed written consent from their parent/guardian. Intrauterine deaths were excluded from the study.

There were about 145 neonates with physiologic jaundice, whose blood groups were not done, so these 145 neonates were excluded from this study.

There were 7 neonates born in Rockland Hospital, Delhi and on Day 1 of life, these neonates were referred to the higher center. These 7 neonates were as follows:

- PT (28-29weeks) / Female /Very LBW
- PT (29-30weeks) /Male/ LBW
- PT(31-32weeks)/Male/Extreme LBW (920grams)
- PT (32 weeks) / Female / LBW
- Term / Male / AGA
- Term / Female / AGA
- Term / Female / AGA

The further outcome of these 7 neonates is not known. These 7 neonates were excluded from the study.

Study Characteristics: In this study, 516 neonates born alive in Rockland Hospital during the period 01.01.2012 till 07.08.2014, were recorded and studied. The demographic information, history, physical examination & investigations in the patient's questionnaire were recorded. Neonates that satisfied the inclusion criteria were selected and the neonates who did not meet the inclusion criteria were excluded.

Data Collection Methods and Tools: Neonates' history & investigations information was collected in questionnaires and the data was collected and reported, and then statistical

analysis of data was performed using SPSS software. Calculations of P values were done using QuickCalcs-Graphpad Software.

Statistical Methods and Statistical Interpretation: Chi-square test or Fisher's exact test was used to calculate the Two-tailed P values in our study. When presenting P values, it was helpful to use the asterisk rating system as well as quoting the P value:

P < 0.05* , it is statistically significant,
P < 0.01 ** , it is very statistically significant,
P <0.001*** , it is extremely statistically significant.

RESULTS AND OBSERVATIONS

For the present study, 516 neonates (500 singleton neonates & 16 twin neonates) were recorded and studied in Paediatrics Department in Rockland Hospital. These neonates were born alive to 508 mothers, in Obstetrics & Gynaecology Department in Rockland Hospital, during the period 01.01.2012 till 07.08.2014. [5] More than 70% neonates were observed in hospital for more than 72 hours before discharge, and the remaining healthy neonates with no risk factors were discharged after 24 hours of observation in Rockland Hospital. In our study, all the 516 neonates after discharge were followed up for progress of jaundice in Paediatrics Out-Patient Department at Rockland Hospital, Delhi.

The neonates and their mothers were investigated and the results were as follows:

Total Serum Bilirubin and Serum Indirect Bilirubin

Total Serum Bilirubin was calculated by adding Serum Indirect Bilirubin and Serum Direct Bilirubin.
Normal values of Bilirubin are as follows:

- Total Serum Bilirubin = 0.3 to 1.0 mg/dL
- Serum Direct Bilirubin = 0.1 to 0.3 mg/dL
- Serum Indirect Bilirubin = 0.2 to 0.7 mg/dL

Hyperbilirubinemia is a higher-than-normal level of bilirubin in the blood. In our study, the results showed that the Total Serum Bilirubin was more than 2 mg/dL and the Serum Direct Bilirubin level was less than 2 mg/dL, in all the 516 neonates. Thus, in our study of 516 neonates, the results showed that almost all neonates had transient unconjugated hyperbilirubinemia in the neonatal period.

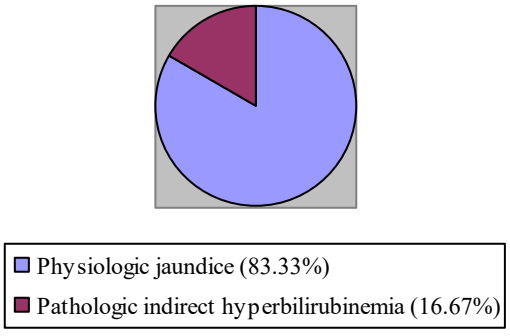

Figure 1: Pie diagram showing prevalence of jaundice in a group of 516 neonates born in Rockland Hospital, Delhi.

In our study of 516 neonates, the results showed that about 83.33% (430) neonates had physiologic jaundice (non-pathologic indirect hyperbilirubinemia) and 16.67% (86) neonates had pathologic indirect hyperbilirubinemia. In the present study, a late preterm neonate was again admitted on Day 21 of life, for persistent pathologic indirect hyperbilirubinemia. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant. In our study, hyperbilirubinemia was the main laboratory abnormality. Among the 5.81% (30) neonates with pathologic indirect hyperbilirubinemia and ABO incompatibility, about 3.68% (19) neonates had TSB between 12mg/dL to 17.99mg/dL & 2.13% (11) neonates had TSB between 18mg/dL to 26mg/dL. All the neonates with TSB more than 18mg/dL were also associated with early onset neonatal sepsis.

Table 1: Table showing that the venous blood samples of 86 neonates with pathologic jaundice for Serum Bilirubin were collected during first 9 days of life, and in one case of persistent pathologic jaundice, the sample was again collected on Day 21 of life.

Age of baby	Pathologic jaundice with compatible blood groups	Pathologic jaundice with ABO Incompatibility
Day 1	00	1
Day 2	01	4
Day 3	16	4
Day 4	12	0
Day 5	10	7
Day 6	07	8
Day 7	05	4
Day 8	04	1
Day 9	01	1
Day 21	01 Repeat neonate	--
Total	56 neonates	30 neonates

In this study, it is evident that almost all neonates with physiologic jaundice and about 90.70% (78) neonates with pathologic jaundice were diagnosed from Day 2 till Day 7 of life. Hence neonates should be routinely followed for progress of jaundice in the first week of life. Indirect-reacting bilirubin content did not rise rapidly to high levels in most neonates in the 1st 6-12 hour of life, except in one case , a healthy term B+ female neonate who was born to a primigravida O+ mother, developed jaundice on Day1 with TSB-14.34 mg/dL. Thus, the incidence of indirect-reacting bilirubin content rise rapidly to high levels in the 1st 6-12 hour of life was reduced to 0.19% in our study. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

Blood Groups of Neonates and their Mothers: The prevalence of various blood groups in 516 neonates was as follows: A 23.64% (122) (100+22), B 35.66% (184) (148+36), AB 9.88% (51) (46+5) & O 30.82% (159) (136+23). The prevalence of various blood groups in 508 mothers was as follows: A 22.64% (115) (98+18-1), B 33.47% (170) (150+21-1), AB 12.20% (62) (51+11) & O 31.69% (161) (131+36-6). In our study, approximately 93.02% of neonates expressed RhD antigen (Rh positive), and 90.75% of mothers were Rh-positive. In the tables 2 and 3, the blood groups of 8 mothers of twins were counted twice (A+ 1, B+1, O+5 and O-1), so the corresponding correction was done in the results. The prevalence of Rh negatives in 516 neonates was about 6.98% (36) (32+4). The prevalence of Rh negatives in 508 mothers was about 9.25% (47) (46+2-1). Overall, the prevalence of Rh negatives in both 508 mothers and 516 neonates was about 8.11% (83) (47+36), in our study.

Table 2: Table showing prevalence of ABO & Rhesus Blood Groups in a group of 430 neonates with physiologic jaundice, born in Rockland Hospital, Delhi.

BBG	A+	A-	B+	B-	AB+	AB-	O+	O-	Same BG	Other BG Compatible	ABO Inc	ABO & Rh Inc	Rh Inc	Total MBG	P value
MBG A+	42	2	11	1	13	0	21	1	42	24	25	0	0	091	<0.0001
MBG A-	03	0	00	0	01	1	02	0	00	00	01	1	5	007	<0.0001
MBG B+	12	2	69	1	15	2	30	2	69	33	31	0	0	133	<0.0001
MBG B-	01	0	08	3	2	0	1	2	03	02	00	3	9	017	<0.0001
MBG AB+	15	1	16	1	5	2	0	0	05	35	00	0	0	040	0.3112
MBG AB-	04	0	03	0	2	2	0	0	02	00	00	0	9	011	<0.0001
MBG O+	16	1	32	2	1	0	64	4	64	04	52	0	0	120	<0.0001
MBG O-	01	0	00	1	0	0	8	1	01	00	01	1	8	011	<0.0001
Total BBG	94	6	139	9	39	7	126	10	186	98	110	5	31	430	<0.0001

Table 3: Table showing prevalence of ABO & Rhesus Blood Groups in a group of 86 neonates with pathologic jaundice, born in Rockland Hospital, Delhi.

BBG	A+	A-	B+	B-	AB+	AB-	O+	O-	Same blood group	Other BG Compatible	ABO Incompatibility	Rh Incompatibility	Total MBG	P value
MBG A+	08	0	03	0	2	0	5	0	08	5	5	0	18	<0.0001
MBG B+	00	0	13	1	1	0	5	0	13	6	1	0	20	<0.0001
MBG B-	00	0	00	0	0	0	0	1	00	1	0	0	01	0.0027
MBG AB+	03	0	06	0	2	0	0	0	02	9	0	0	11	<0.0001
MBG O+	11	0	13	0	0	0	10	1	10	1	24	0	35	<0.0001
MBG O-	00	0	00	0	0	0	0	1	01	0	00	0	01	0.0027
Total BBG	22	0	35	1	5	0	20	3	34	22	30	0	86	<0.0001

Table 4: Table showing prevalence of compatible & incompatible blood groups in a group of 516 neonates with jaundice, born in Rockland Hospital, Delhi.

Blood groups of neonates	Pathlogic indirect hyperbilirubinemia	Physiologic Jaundice	Total	P value
ABO /Rh Incompatibility	030 (05.814%)	146 (28.294%)	176 (34.108%)	<0.0001
Compatible Blood groups	056 (10.853%)	284 (55.039%)	340 (65.892%)	<0.0001
Total	086 (16.667%)	430 (83.333%)	516 (100%)	<0.0001

Key words for tables: (MBG)-Mother Blood Group, (BBG)-Baby Blood Group, (BG) - Blood Group, (Inc)-Incompatibility

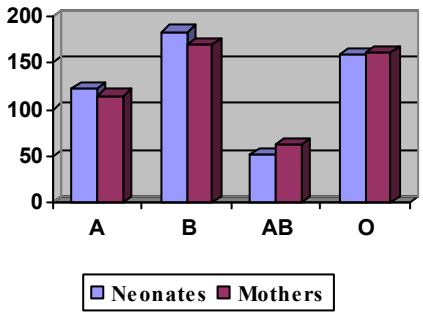

Figure 2: Bar diagram showing a comparison of prevalence of Blood Groups in a group of 516 neonates born to 508 mothers in Rockland Hospital, Delhi.

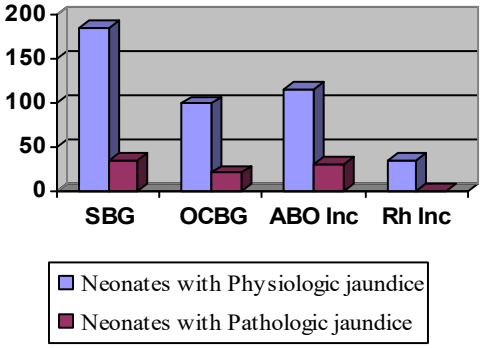

Figure 3: Bar diagram showing a comparison of prevalence of Same Blood Group (SBG), Other Compatible Blood Groups (OCBG), ABO Incompatibility (ABO Inc) and Rh Incompatibility (Rh Inc) in a group of 516 neonates, born in Rockland Hospital, Delhi.

The prevalence of same blood group in mother and baby was about 42.63% (220) (186 neonates with physiologic jaundice and 34 neonates with pathologic indirect hyperbilirubinemia) & prevalence of other compatible blood groups was about 23.26% (120) (98 neonates with physiologic jaundice and 22 neonates with pathologic indirect hyperbilirubinemia).

The prevalence of ABO incompatibility was 22.28% (110+5) and the prevalence of Rh incompatibility was 6.98% (36) (31+5) in neonates with physiologic jaundice. The prevalence of ABO incompatibility was 5.81% (30) and there was no case of Rh incompatibility in neonates with pathologic indirect hyperbilirubinemia. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

In the Table 4, the two-tailed P value equals 0.9011, in the Fisher's exact test. The association between rows (Compatible and Incompatible blood groups) and columns (Physiologic Jaundice & Pathologic Indirect Hyperbilirubinemia) was considered to be not statistically significant. Thus, in our study, ABO/Rh incompatibility did not show association with pathologic indirect hyperbilirubinemia.

Approximately 28.10% (110+5+30) of live births were at theoretical risk for immune mediated hemolysis based on ABO mismatch, most often the mother being group O (15.12%) (78) (54+24) and the infant either group A or B. Less often, the mother was group B (6.78%) (35) (34+1) and the infant group

A and least often, the mother was group A (6.39%) (33) (27+5) and the infant group B. However, clinical manifestation of pathologic jaundice developed in only 5.81% of at-risk infants with ABO incompatibility, most often the mother being group O (4.65%) (24), and the infant either group A or B. Less often, the mother was group A (0.97%) (5), and the infant group B and least often, the mother was group B (0.19%) (1) and the infant group A. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

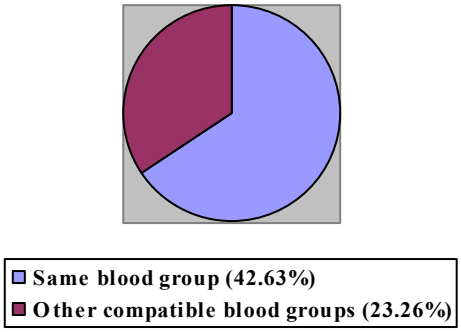

Figure 4: Pie diagram showing prevalence of Same Blood Group and Other Compatible Blood Groups in a group of 340 neonates, born in Rockland Hospital, Delhi.

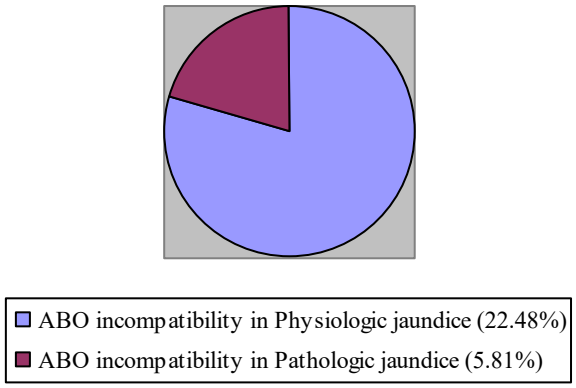

Figure 5: Pie diagram showing a comparison of prevalence of ABO Incompatibility in a group of 146 neonates, born in Rockland Hospital, Delhi.

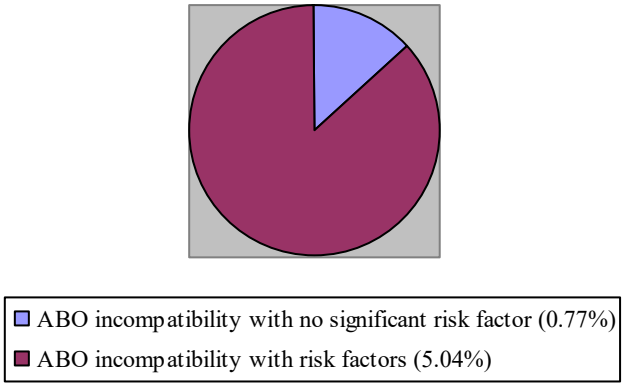

Figure 6: Pie diagram showing a comparison of prevalence of ABO incompatibility with known risk factors and with no significant risk factor in a group of 30 neonates in Rockland Hospital, Delhi.

In our study, it is evident that the clinical manifestation of pathologic jaundice developed in only 5.81% of at-risk infants, of which 5.04% neonates were associated with other risk factors (namely, early onset neonatal sepsis, preterm birth, low birthweight, very low birthweight and diabetic mother). The most common risk factor was early onset neonatal sepsis (EONS), which was seen in 24 neonates (4.65%). About (0.39%) 2 neonates were infants of diabetic mothers with no neonatal sepsis. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

About 4 (0.77%) neonates had ABO incompatibility with pathologic jaundice but were not associated with any significant risk factor. These 4 neonates were as follows:

- Term/Female (B+)/AGA/NNH (Day1 TSB-14.34mg/dL)/ (born to Primigravida O+ mother by LSCS for foetal distress with scanty liquor)
- Term/Male (B+)/AGA/NNH (Day2 TSB-12.8mg)/dL/ (Day1-S.TSH-11.19µIU/mL)/(born to Multigravida Hypothyroid O+ mother by LSCS for foetal distress)
- Term/Male (A+)/AGA/NNH (Day 2 TSB-15.50mg/dL)/ (born to Multigravida O+ mother by LSCS for foetal distress with scanty liquor)
- Term/Male (B+)/AGA/NNH (Day 5 TSB-16.0mg/dL)/ (born to Primigravida O+ mother by LSCS for foetal distress)

ABO incompatibility neonates associated other risk factors	Neonates with Pathlogic jaundice
Term/ EONS	14
Term/EONS/Infant of diabetic mother	03
Term/Infant of diabetic mother	02
PT/NW/EONS/Infant of diabetic mother	01
PT/NW/EONS	02
PT/LBW/EONS	04
Total	26 (5.04%)

Table 5: Table showing ABO incompatibility associated with other risk factors in a group of 26 neonates with pathologic jaundice in Rockland Hospital, Delhi.

Neonates	Compatible blood groups with Pathologic jaundice	ABO incompatibility with Pathologic Jaundice	Total
Term	41	23	64
Preterm	15	07	22
Total	56	30	86

Table 6: Table showing ABO incompatibility associated with preterm neonates in a group of 86 neonates with pathologic jaundice in Rockland Hospital, Delhi.

In the Table 6, the two-tailed P value equals 0.8000, in the Fisher's exact test. The association between rows (Term & Preterm groups) and columns was considered to be not statistically significant. In our study, the preterm gestation did not show any association with ABO incompatibility with pathologic indirect hyperbilirubinemia. This means ABO incompatibility has no effect on prematurity.

Direct Coombs Test

- Direct Coombs test was negative in all the cases, in ABO & Rh incompatibility. Indirect Coombs test was negative in all the mothers of the neonates. This

means, that there was mild haemolysis, in our study. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

Reticulocyte Count Estimation

- In present study, one (0.2%) neonate with pathologic jaundice had increased reticulocyte count (about 10%) in presence of aggravating factors like early onset neonatal sepsis, remaining all neonates had normal range of reticulocyte count (less than 5%). This shows that the incidence of severe hemolysis due to ABO incompatibility was reduced to 0.2%, in our study. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

Haemoglobin Estimation and Haematocrit Estimation

- In the present study, one neonate with ABO incompatibility with pathologic jaundice and early onset neonatal sepsis had severe anemia (Hemoglobin 4.9gm/dL and hematocrit of 15%). A male twin preterm (35 week+) neonate with early onset neonatal sepsis with ABO incompatibility and physiologic jaundice had mild anemia with haemoglobin level of 12.4 gm/dL and hematocrit of 36%. Besides, a term female neonate with ABO incompatibility with TSB-18mg/dL on Day8 had mild anemia (Hb-13.4gm/dL and hematocrit 39%). So the incidence of mild anemia was 0.39%, in our study. About 5 neonates with no ABO or Rh incompatibility had polycythemia with haemoglobin level at birth more than 22gm/dL and hematocrit more than 66%. Remaining all neonates had range of hemoglobin between 14 gm% to 22 gm% and haematocrit was normal in all these neonates. The majority of infants with theoretical risk of ABO or Rh mismatch were not associated with anemia, in our study. This indicates that the incidence of severe anemia due to ABO incompatibility was reduced to 0.2%, in our study. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

Peripheral Blood Smear Analysis

- Peripheral blood smear was normal in all the cases, except in one case of pathologic jaundice which was associated with severe anemia and early onset neonatal sepsis, in which the peripheral blood smear showed presence of numerous nucleated RBC's, increased leukocytes, no evidence of toxic changes and decreased platelets. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

Glucose-6-Phosphate Dehydrogenase (G6PD) activity

- Glucose-6-Phosphate Dehydrogenase (G6PD) activity was normal in all the neonates. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

Treatment

- Most cases of ABO compatibility were mild, with jaundice the only clinical manifestation. Hyperbilirubinemia due to ABO incompatibility, resolved naturally in most cases, as there was very mild hemolysis leading to physiologic jaundice. About 16.67% (86) neonates of pathologic indirect hyperbilirubinemia, who required treatment, were cured by phototherapy. None was treated by exchange transfusion. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

- Thus, most cases of ABO compatibility were mild, with jaundice the only clinical manifestation. Pallor and severe anemia were present in one case of ABO mismatch with pathologic jaundice, which was also associated with early onset neonatal sepsis. It also shows that hemolytic disease due to ABO incompatibility becomes severe in presence of aggravating conditions or with risk factor. Otherwise, hyperbilirubinemia due to ABO incompatibility was usually very mild and resolved naturally in most cases.

- In our study, no neonate had hydrops fetalis, kernicterus or cerebral palsy. Besides, the Neonatal Mortality Rate was zero, in our study. The two-tailed P value was less than 0.0001***, in the Chi-square test. By conventional criteria, this difference was considered to be extremely statistically significant.

DISCUSSION

For the present study, 516 neonates (500 singleton neonates & 16 twin neonates) were recorded and studied in Paediatrics Department in Rockland Hospital. These neonates were born alive to 508 mothers, in Obstetrics & Gynaecology Department in Rockland Hospital, during the period 01.01.2012 till 07.08.2014. [5] In our study, the prevalence of various blood groups in 516 neonates was as follows: A 23.64%, B 35.66%, AB 9.88% & O 30.82%. The prevalence of various blood groups in 508 mothers was as follows: A 22.64%, B 33.47%, AB 12.20% & O 31.69%. The prevalence of Rh negatives in 516 neonates was about 6.98% and the prevalence of Rh negatives in 508 mothers was about 9.25%. Overall, the prevalence of Rh negatives in both mothers and neonates was about 8.11%, in our study. The prevalence of same blood group in mother and baby was about 42.63% & prevalence of other compatible blood groups was about 23.26%. In our study, hyperbilirubinemia was the main laboratory abnormality. In our study, the results showed that almost all neonates had transient unconjugated hyperbilirubinemia in the neonatal period, with more than ninety percent affected during their first week of life. Hence neonates should be routinely followed for progress of jaundice in the first week of life. The incidence of indirect-reacting bilirubin content rise rapidly to high levels in the 1st 6-12 hour of life was reduced to 0.2% in our study. In our study, about 83.33% neonates had physiologic jaundice and 16.67% neonates had pathologic indirect hyperbilirubinemia. In our study of 516 neonates, approximately 34.10% of live births were at theoretical risk for

immune mediated hemolysis based on ABO and Rh incompatibility, of which 28.29% neonates had mild physiologic jaundice and 0.39% neonates had mild anemia. However, clinical manifestation of pathologic jaundice developed in only 5.81% of at-risk infants with ABO incompatibility, most often the mother being group O (4.65%) and the infant either group A or B. Less often, the mother was group A (0.97%) and the infant group B and least often, the mother was group B (0.19%) and the infant group A. Among these 5.81% of at-risk infants with ABO incompatibility, about 3.68% neonates had TSB between 12mg/dL to 17.99mg/dL and 2.13% neonates had TSB between 18mg/dL to 26mg/dL. All the neonates with TSB more than 18mg/dL were also associated with early onset neonatal sepsis. The incidence of ABO incompatibility with hyperbilirubinemia with severe anemia and severe hemolysis was about 0.2%, and this one neonate was also associated with early onset neonatal sepsis, in our study. The prevalence of Rh incompatibility was 6.98% in neonates with physiologic jaundice and there was no case of Rh incompatibility in neonates with pathologic indirect hyperbilirubinemia. Thus, in our study, the risk of initial sensitization of Rh-negative mothers was reduced to almost zero by the routine administration of Rh-immunoglobulin (RhoGAM) to all mothers at risk for Rh alloimmunization. In our study, no neonate had hydrops fetalis, kernicterus or cerebral palsy. Besides, the neonatal mortality rate was zero, in our study. The two-tailed P value was less than 0.0001***, in the Chi-square test, in all the above results. By conventional criteria, this difference was considered to be extremely statistically significant.

Following references support our observations:

- In our study, the prevalence of different blood groups among one group of 458 neonates was approximately: B+ 34%, O+ 27%, A+ 24%, AB+ 8% and Rh negative- 7 %. It was also concluded that the prevalence of different blood groups among the 458 mothers of the group of neonates was approximately as follows: O+ 30%, B+ 30%, A+ 21%, AB+ 10% and Rh negative- 9 %. Thus, there was similar trend of blood groups in both mothers and neonates. [4]

- A blood type incompatibility between the mother and baby is also a reason to track the newborn's jaundice more closely. This exists when a mother has the blood type O (and therefore has antibodies against A and B cells) and her newborn is of blood type A or B. This *may* cause the newborn's red blood cells to break down more quickly due to maternal antibodies that have leaked into the baby's bloodstream. A blood type incompatibility also exists if the mother has a Rh (Rhesus) factor negative blood type and the newborn is Rh factor positive. This had been a common cause of severe neonatal jaundice, but is now very uncommon because Rh immune globulin (Rhogham) is given to mothers at risk before delivery. [9]

- Alloimune hemolytic disease from RhD antigen incompatibility is approximately 3 times more common among whites than among blacks, because of differences in Rh allele frequency. Approximately 85% of caucasians express RhD antigen (Rh positive), whereas 99% of persons from Africa or Asia are Rh-positive. Before treatment, the direct antiglobulin test (DAT) or Coombs test is positive, and anemia is generally present. The initial reticulocyte count is increased, another abnormal finding at birth, and the peripheral blood smear typically shows polychromasia with a marked increase in nucleated RBCs. Indirect-reacting bilirubin content rises rapidly to high levels in the 1st 6-12 hour of life. The risk of initial sensitization of Rh-negative mothers has been reduced to less than 0.1% by the routine administration of Rh-immunoglobulin (RhoGAM) to all mothers at risk for Rh alloimmunization.[15]

- In about a third of all ABO incompatible pregnancies maternal IgG anti-A or IgG anti-B antibodies pass through the placenta to the fetal circulation leading to a weakly positive direct Coombs test for the neonate's blood. However, ABO HDN is generally mild and short-lived and only occasionally severe because: (1) IgG anti-A (or IgG anti-B) antibodies that enter the fetal circulation from the mother find A (or B) antigens on many different fetal cell types, leaving fewer antibodies available for binding onto fetal red blood cells. (2) Fetal RBC surface A and B antigens are not fully developed during gestation and so there are a smaller number of antigenic sites on fetal RBCs.[1]

- Almost all hyperbilirubinemia in the immediate neonatal period is unconjugated. Physiologic hyperbilirubinemia occurs in almost all neonates. Shorter neonatal RBC life span increases bilirubin production; deficient conjugation due to the deficiency of UGT decreases clearance; and low bacterial levels in the intestine combined with increased hydrolysis of conjugated bilirubin increase enterohepatic circulation. Bilirubin levels can rise up to 18 mg/dL by 3 to 4 days of life (7 days in Asian infants) and fall thereafter. Physiologic jaundice generally lasts less than seven days. The condition affects over half of babies in the first week of life. Of babies that are born early about 80% are affected.[19]

- Transient neonatal jaundice is one of the most common conditions occurring in newborns (children under 28 days of age) with more than eighty percent affected during their first week of life.[17]

- Jaundice is observed during the 1st week after birth in approximately 60% of term infants and 80% of preterm infants.[14] [16]

- The prevalence of neonatal jaundice in healthy term babies at National District Hospital in Bloemfontein was 55.2%. Although 52% of sampled infants had jaundice on the Bilicheck® meter, only 17% appeared clinically jaundiced. The consequence of a missed diagnosis and delayed treatment may cause serious morbidity (kernicterus).[3]

- The incidence of neonatal hyperbilirubinemia in a retrospective study done in a tertiary care hospital was 13.47%. Preterm gestation showed a strong association with neonatal hyperbilirubinemia. [27]

- The neonatal morbidity was studied in 7015 neonates born at the All India Institute of Medical Sciences

Hospital, New Delhi. Neonatal hyperbilirubinemia occurred in 5.9 per cent, most of whom were premature. [28]

- Hyperbilirubinemia is a common and, in most cases, benign problem in neonates. Overall, 6-7% of full term infants have indirect bilirubin levels >13mg/dL, and <3% have levels >15mg/dL. [16]
- Approximately 20% of live births are at theoretical risk for immune mediated hemolysis based on ABO mismatch, most often the mother being group O and the infant either group A or B. Les often, the mother will be group A and the infant group B, or vice versa. However, clinical manifestations of hemolysis develop in only 1-10% of at-risk infants. Most cases of ABO compatibility are mild, with jaundice the only clinical manifestation. The infant is not generally affected at birth but will develop jaundice in the 1st 24 hour, which is always abnormal. Pallor and hepatosplenomegaly are not present and the development of hydrops fetalis or kernicterus is extremely rare. Hyperbilirubinemia is often the main laboratory abnormality. In 10-20% of affected infants, the unconjugated serum bilirubin level may reach 20mg/dL or more unless phototherapy is administered. Usually the infant has mild anemia and reticulocytosis; the peripheral blood smear may show polychromasia, nucleated RBCs and spherocytes. Direct antiglobulin test (DAT) or Coombs test is negative or weakly to moderately positive.[15]
- The tragedy of occurrence of kernicterus is compounded by the fact that, if newborn jaundice and neonatal hyperbilirubinemia are detected early, kernicterus is completely preventable. All newborn infants are at risk for newborn jaundice, which when unmonitored or untreated can progress to excessive bilirubin levels.[26]
- In a retrospective study on 1020 patients admitted at a hospital during one year period 1st January 2012–31st December 2012, in 260 there was a diagnosis of indirect hyperbilirubinemia, associated pathology consisted of urinary tract infection in 15 cases, piodermatitis in 12, otitis media in 7, acute diarrhea in 14 cases and severe dehydration in 9 cases. Only one case complicated with kernicterus.[10]
- In a study, majority of new-borns with ABO incompatibility, developed hyperbilirubinemia between 3-5 days. It shows, hemolytic disease due to ABO incompatibility, becomes severe in presence of aggravating conditions or with risk factor. Otherwise, hyperbilirubinemia due to ABO incompatibility is usually very mild and resolve by simple management. Direct Coombs test is weakly to moderately positive, in ABO incompatibility. Only 9% newborns had positive DCT. Indirect Coombs test was negative in all the mothers of the patients, and this means, that this test is a weak marker for haemolysis. Only 5(2.5%) new-born had >6% reticulocyte count, remaining all new-born had normal range of reticulocyte count. This shows that ABO incompatibility does not cause severe haemolysis. Mean value of hemoglobin was 16.941± 2.01. This indicates that anaemia due to hemolysis, in ABO incompatibility is not commonly found in affected babies as well. Hyperbilirubinemia due to ABO incompatibility resolves naturally in most cases (56%), as there is very mild hemolysis. In cases, who required treatment, most of them were cured only by phototherapy (43%).[22]
- Neurotoxicity is the major consequence of neonatal hyperbilirubinemia. An acute encephalopathy can be followed by a variety of neurologic impairments, including cerebral palsy and sensorimotor deficits; cognition is usually spared. Kernicterus is the most severe form of neurotoxicity. Although it is now rare, kernicterus still occurs and can nearly always be prevented.[19]
- Common complications of preterm birth are high rates of respiratory distress syndrome, sepsis, periventricular leucomalacia, seizures, intraventricular hemorrhage, cerebral palsy, infections, pathologic jaundice, kernicterus, hypoxic ischemic encephalopathy, and visual and hearing problems. Complications of preterm birth were the leading cause of death in children younger than 5 years of age globally in 2016, accounting for approximately 16% of all deaths, and 35% of deaths among newborn babies. Preterm neonates who survive are at greater risk of a range of short-term and long-term morbidities. [30]
- Preterm birth is the most common cause of death among infants worldwide. Complications from preterm births resulted in 0.81 million deaths in 2015 down from 1.57 million in 1990. Approximately 0.5% of births are extremely early periviable births, and these account for most of the deaths.[2]
- The chance of survival at 22 weeks is about 6%, while at 23 weeks it is 26%, 24 weeks 55% and 25 weeks about 72%.[6] The chances of survival without any long-term difficulties are lower.[14]
- By pathologic criteria, kernicterus develops in 30% of infants (all gestational ages) with untreated hemolytic disease and bilirubin levels >25-30 mg/dL. The incidence at autopsy in hyperbilirubinemic preterm infants is 2-16%. [16]

Following references don't support our observations:

- Globally over 100,000 late-preterm and term babies die each year as a result of jaundice.[21]
- Neonatal mortality rate of India fell gradually from 85.2 deaths per thousand live births in 1969 to 22.7 deaths per thousand live births in 2018. [13]
- In the present study, the neonatal mortality rate (NMR) was zero. [5]

SUMMARY

In this study, the aim was to assess the effect of ABO and Rh incompatibility on anemia and jaundice in neonates born in a tertiary care hospital in Delhi, India. For the present study, 516 neonates (500 singleton neonates & 16 twin neonates) were recorded and studied in Paediatrics Department in Rockland Hospital. These neonates were born alive to 508 mothers, in Obstetrics & Gynaecology Department in Rockland Hospital, during the period 01.01.2012 till 07.08.2014. The study was done as random sampling of the neonates noticed to have deep yellow discoloration of whole body that were born in Rockland Hospital, Delhi. In this study, all the venous blood samples of neonates for Total Serum Bilirubin (TSB) and Direct Serum

Bilirubin were collected during first 9 days of life, and in one case of persistent pathologic hyperbilirubinemia, the sample was again collected on Day 21 of life. Besides, venous samples were collected for blood groups of all the 516 neonates as well as blood groups of their 508 mothers were also done. These neonates and their mothers were further investigated for Direct Coombs Test, Haemoglobin estimation, Haematocrit estimation, Reticulocyte count estimation, Glucose-6-Phosphate Dehydrogenase (G6PD) activity and Peripheral blood smear to ascertain any hemolysis. Participants that satisfied the inclusion criteria were selected and the participants who did not meet the inclusion criteria were excluded. Neonates' history & investigations information was collected in questionnaires and the data was collected and reported, and then statistical analysis of data was performed using SPSS software. Calculations of P values were done using QuickCalcs-Graphpad Software. Chi-square test or Fisher's exact test was used to calculate the Two-tailed P values in our study.

In our study, the prevalence of various blood groups in 516 neonates was as follows: A 23.64%, B 35.66%, AB 9.88% & O 30.82%. The prevalence of various blood groups in 508 mothers was as follows: A 22.64%, B 33.47%, AB 12.20% & O 31.69%. The prevalence of Rh negatives in 516 neonates was about 6.98% and the prevalence of Rh negatives in 508 mothers was about 9.25%. Overall, the prevalence of Rh negatives in both mothers and neonates was about 8.11%, in our study. The prevalence of same blood group in mother and baby was about 42.63% & prevalence of other compatible blood groups was about 23.26%. In our study, hyperbilirubinemia was the main laboratory abnormality. In our study, the results showed that almost all neonates had transient unconjugated hyperbilirubinemia in the neonatal period, with more than ninety percent affected during their first week of life. Hence neonates should be routinely followed for progress of jaundice in the first week of life. The incidence of indirect-reacting bilirubin content rise rapidly to high levels in the 1st 6-12 hour of life was reduced to 0.2% in our study. In our study, about 83.33% neonates had physiologic jaundice and 16.67% neonates had pathologic indirect hyperbilirubinemia. In our study of 516 neonates, approximately 34.10% of live births were at theoretical risk for immune mediated hemolysis based on ABO and Rh incompatibility, of which 28.29% neonates had mild physiologic jaundice and 0.39% neonates had mild anemia. However, clinical manifestation of pathologic jaundice developed in only 5.81% of at-risk infants with ABO incompatibility, most often the mother being group O (4.65%) and the infant either group A or B. Less often, the mother was group A (0.97%) and the infant group B and least often, the mother was group B (0.19%) and the infant group A. Among these 5.81% of at-risk infants with ABO incompatibility, about 3.68% neonates had TSB between 12mg/dL to 17.99mg/dL and 2.13% neonates had TSB between 18mg/dL to 26mg/dL. All the neonates with TSB more than 18mg/dL were also associated with early onset neonatal sepsis. The incidence of ABO incompatibility with hyperbilirubinemia with severe anemia and severe hemolysis was about 0.2%, and this one neonate was also associated with early onset neonatal sepsis, in our study. The prevalence of Rh incompatibility was 6.98% in neonates with physiologic jaundice and there was no case of Rh incompatibility in neonates with pathologic indirect hyperbilirubinemia. Thus, in our study, the risk of initial sensitization of Rh-negative mothers was reduced to almost zero by the routine administration of Rh-immunoglobulin (RhoGAM) to all mothers at risk for Rh alloimmunization. In our study, no neonate had hydrops fetalis, kernicterus or cerebral palsy. Besides, the neonatal mortality rate was zero, in our study. The two-tailed P value was less than 0.0001***, in the Chi-square test, in all the above results. By conventional criteria, this difference was considered to be extremely statistically significant.

CONCLUSION

From this study of 516 neonates, it is concluded that almost all neonates had transient unconjugated hyperbilirubinemia in the neonatal period, with more than ninety percent affected during their first week of life. Hence neonates should be routinely followed for progress of jaundice in the first week of life. The incidence of indirect-reacting bilirubin content rise rapidly to high levels in the 1st 6-12 hour of life was reduced to 0.2% in our study. In our study, about 83.33% neonates had physiologic jaundice and 16.67% neonates had pathologic indirect hyperbilirubinemia. In our study of 516 neonates, approximately 34.10% of live births were at theoretical risk for immune mediated hemolysis based on ABO and Rh incompatibility, of which 28.29% neonates had mild physiologic jaundice and 0.39% neonates had mild anemia. However, clinical manifestation of pathologic jaundice developed in only 5.81% of at-risk infants with ABO incompatibility, most often the mother being group O (4.65%) and the infant either group A or B. Less often, the mother was group A (0.97%) and the infant group B and least often, the mother was group B (0.19%) and the infant group A. Among these 5.81% of at-risk infants with ABO incompatibility, about 3.68% neonates had TSB between 12mg/dL to 17.99mg/dL & 2.13% neonates had TSB between 18mg/dL to 26mg/dL. All the neonates with TSB more than 18mg/dL were also associated with early onset neonatal sepsis. The incidence of ABO incompatibility with hyperbilirubinemia with severe anemia and severe hemolysis was about 0.2%, and this one neonate was also associated with early onset neonatal sepsis, in our study. The prevalence of Rh incompatibility was 6.98% in neonates with physiologic jaundice and there was no case of Rh incompatibility in neonates with pathologic indirect hyperbilirubinemia. Thus, in our study, the risk of initial sensitization of Rh-negative mothers was reduced to almost zero by the routine administration of Rh-immunoglobulin (RhoGAM) to all mothers at risk for Rh alloimmunization. In our study, no neonate had hydrops fetalis, kernicterus or cerebral palsy. Besides, the neonatal mortality rate was zero, in our study. Thus, in our study, the effect of ABO and Rh incompatibility on anemia and jaundice was mild in most neonates and the effect became severe in presence of risk factors, most commonly neonatal sepsis. By conventional criteria, this difference was considered to be extremely statistically significant.

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