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Original Research Article

Study of clinical spectrum of hyperbilirubinemia with frequency of glucose six phosphate dehydrogenase deficiency in neonates

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ABSTRACT

Background: Jaundice is defined as visible reflexion of serum hyperbilirubinemia on mucous membranes and skin as yellowish discoloration. The frequency of icterus among neonates is about 1 in 2500-5000 live births. When there is disparity between the production of bilirubin, conversion from unconjugated to conjugated bilirubin and excretion of bilirubin results in jaundice. Unconjugated bilirubin is usually harmless but it can also cross blood-brain barrier causing neurotoxicity or kernicterus.
Methods: A hospital based prospective observational study which is carried out in the department of paediatrics of Teerthanker Mahaveer Medical College, Moradabad, Uttar Pradesh from December 2019 to November 2020 on 74 neonates who required admission for hyperbilirubinemia.
Results: The most common jaundice occurred in neonates were idiopathic or breastfeeding jaundice as the neonates were breast fed (47.29%). The second most common cause was ABO incompatibility leading to jaundice in 27 (36.48%) neonates. Incidence of neonatal Sepsis, G6PD deficiency, hypothyroidism and cephalhematoma was 22.9%, 4.1%, 2.70% and 4.1% respectively. Polycythemia contributed to 1.35% and the frequency of hyperbilirubinemia in infants of diabetic mother's or GDM was 10.8%. Rh incompatibility was seen in 13.5%.
Conclusions: G6PD deficiency is a significant cause for NNHB and the cases with pathological jaundice if left untreated may lead to severe neurological deficits and lifelong disabilities, hearing impairment, mental retardation, seizures and movement disorders. Hence we recommend G6PD screening in every newborn with significant hyperbilirubinemia to reduce morbidity and mortality.

Keywords: Neonatal hyerbilirubinemia, Glucose 6 phosphate dehydrogenase deficiency, Neonatal sepsis, Breastfeeding, Polycythemia, Hypothyroidism

INTRODUCTION

Jaundice is defined as visible reflexion of serum hyperbilirubinemia on mucous membranes and skin as yellowish discoloration and is a commonly occurring benign issue after birth till one month of age, usually physiological; unconjugated hyperbilirubinemia and generally in most cases no action is required in full term and healthy infants. It is not an unusual phenomenon in neonates and in about 60% full term neonates and 80% of preterm neonates physiological jaundice is observed in first seven days of life.[1] The frequency of icterus among neonates is about 1 in 2500-5000 live births.[2] In India 5% newborns had significant jaundice with TSB>15 mg/dl according to NNPD 1995 survey. Neonatal hyperbilirubinemia accounts for 3.3% among all in house live births, and causes morbidity in 22.1% according to NNPD.[3] Also total bilirubin levels of>12.9 mg/dl and >15

mg/dl was found in 6.1% and 3% well term neonates respectively.[4]

When there is disparity between the production of bilirubin, conversion from unconjugated to conjugated bilirubin and excretion of bilirubin results in jaundice. Unconjugated bilirubin is usually harmless but it can also cross blood-brain barrier causing neurotoxicity or Kernicterus.[5] Identification of clinical spectrum and etiological factors for jaundice helps in good prevention strategies of complications and better outcomes. Significant hyperbilirubinemia seen in usually 5-10% of newborns; was defined by Bhutani et al. It is defined as patient's TSB≥95th percentile for age in hours of life in the first seven days of life in which the sample was taken.[4]

Despite of harmful effects of unconjugated bilirubin in causing severe neurological deficits some amount of unconjugated bilirubin(uric acid) is considered as an antioxidant for neonates as it prevents them from the oxidative stress.[6] Hyperbilirubinemia in neonates can be classified as physiological and pathological jaundice. Pathological jaundice occurs due to either increase the absorption of bilirubin or delay the process of metabolism of bilirubin (prematurity) or increase the breakdown of RBCs (hemolysis, polycythemia) due to collection of blood in extravascular spaces which results as a complication of tough deliveries (cephalhematoma) endocrine disorders and infections.[7]

ABO and Rh blood group incompatibilities are the most common causes responsible for hyperbilirubinemia among neonates. ABO is more common than Rh incompatibility and also the former is less severe but sometimes it causes severe hemolysis.[8] The incidence of Rh disease has been lowered down due to introduction of Rh immunoglobulin hence so far now ABO incompatibility is the most common cause of hemolytic disease in newborns. Breastfeeding causes neonatal jaundice in early days of neonatal period due to calorie deficiency or insufficiency but it does not serves as a factor for kernicterus.[9] Sepsis is also one of the most common causes responsible for neonatal hyperbilirubinemia.[10] Other common causes are GDM, hypothyroidism, prematurity and G6PD deficiency. Premature and preterm infants are also at increased risk of hyperbilirubinemia because of gut and hepatic immaturity.[11] Hyperbilirubinemia is seen in around 10% of neonates with hypothyroidism. Due to congenital hypothyroidism (CHT) the maturation of (UDPG-T) hepatic uridinediphosphate-glucoronyl transferase is slowed down and results in neonatal hyperbilirubinemia.[12]

In India a neonate with hyperbilirubinemia evaluated most commonly for sepsis screen, Hb, blood group including mother's blood group and serum bilirubin levels to rule out the common causes. However jaundice in neonates can be due many other serious conditions like genetic and endocrine causes and enzymopathies. G6PD deficiency is one of the common causes of jaundice or hyperbilirubinemia in neonates. G6PD is a major enzyme found in humans present in RBCs and many other cells of body and a very crucial enzyme in HMP shunt and helps in the reduction of oxidized glutathione. This preserves the red cells from oxidative damage and prevents hemolysis.G6PD enzyme insufficiency is a leading issue in around 400 million population all over world.[13] Since it is an X linked enzyme deficiency hence mostly seen in males however some heterozygous females can also present with hemolysis if G6PD deficient.[14] Asian, Mediterranean and African. Middle Eastern population are mostly affected by this disease.[15] It presents clinically in various conditions or triggers like hemolysis, jaundice, certain bacterial and viral infections, exposure to harmful chemicals, drugs, oxidative stress to body or ingestion of fava beans and early diagnosis of this deficiency can be done in screening in neonatal hyperbilirubinemia.[16]

Many studies done before has proven that G6PD deficiency is a important cause for neonatal hyperbilirubinemia. The exact incidence of G6PD deficiency in India is unknown, a range of 2-27% incidence has been reported by various studies done in India.[17] Current study is framed to see the clinical spectrum of unconjugated hyperbilirubinemia and try to analyze the risk factors and causes of hyperbilirubinemia and to study the relation between the factors like birth weight, period of gestation and clinical conditions responsible for unconjugated hyperbilirubinemia in neonates like ABO and Rh incompatibility, breastfeeding, neonatal sepsis, gestational diabetes mellitus, hypothyroidism, cephalhematoma and G6PD deficiency and also to find out the frequency of G6PD deficiency in India and to evaluate the severity of hyperbilirubinemia in neonates admitted in NICU.

METHODS

Current study was a hospital based prospective observational study which is carried out in the department of paediatrics of Teerthanker Mahaveer medical college, Moradabad, Uttar Pradesh from December 2019 to November 2020 after taking ethical approval from research committee on 74 neonates who required admission for hyperbilirubinemia.

Sample size

Minimum sample size of 74 patients was taken as calculated from formula:

$$n=Z^2PQ/E^2$$

Where Z^2 is standard normal variable taken as 1.96, P=prevalence taken 26.6, Q=(100-P) and E=absolute error is taken as 10%.

Inclusion criteria

Term neonates with total serum bilirubin >12 mg/dl and Preterm (28-37 weeks) neonates with total serum bilirubin>10 mg/dl with pathological jaundice were included in the study.

Exclusion criteria

All neonates with nonsignificant hyperbilirubinemia and with direct hyperbilirubinemia (≥20% of total). Neonates with congenital deformities and syndromic babies and preterm newborns<28 weeks of gestational age were excluded.

This is prospective observational study in which neonates admitted to NICU at TMMC&RC and satisfying any of the criteria for abnormal jaundice were enrolled in the study and subjected to detailed neonatal history including birth history, day of onset of jaundice, gender, period of gestation, birth weight, mode and place of delivery, type of feeding given and maternal history of blood group and Rh typing, gestational diabetes mellitus were recorded.

Data was collected in one year with a minimum sample size of 74 patients. With written informed consent from mother/father/legally acceptable caregiver the neonates were subjected to physical examination to find out the level of jaundice according to Kramer's rule, any signs for sepsis, maturity and cephalhematoma and the relevant investigations were done under all aseptic conditions which included total serum bilirubin (indirect and direct), complete blood count, reticulocyte count, direct Coomb's test, thyroid screening, blood group and Rh typing and G6PD analysis. Serum bilirubin was measured by colorimetric diazo method. G6PD was done using G6PD quantitative test in which a lysing agent was used which releases G6PD in the RBC's and patient was labelled as G6PD deficient if had value <6.5. After obtaining all data compilation was done and analyzed statistically using SPSS 20.

RESULTS

Out of 74 neonates enrolled in the study 50 (67.6%) were males and 24 (32.4%) were females and majority of the patients i.e. 38 (51.4) presented at DOL 3 out of 74 neonates 60 (81.1%) are delivered in hospitals and 18.9% were home delivered (Table 1). The most common mother's blood group observed was O positive in 30 (40.5%) cases followed by B positive 15 (20.3%). 41 (55.4%) neonates were having blood group B positive.

Out of total 74 neonates 39 (52.7%) were between 1.5-2.5 kg birth weight (Table 2). Among all the babies who mostly presented with hyperbilirubinemia are SGA babies 38 (51.35) and also 47.7% of the neonates with maturity of SGA had 15-20 mg/dl of total serum bilirubin, rest were AGA and LGA babies. Majority of the patients i.e. 38 (51.4%) presented at DOL 3 which was followed closely by day of life 2 -29 (39.2%) than on DOL 4, 5 and 6 which is significant (p=0.001).

Table 1: Frequency and percentage distribution of background characteristics of mothers and neonates (n=74).

Background characteristics		N	(%)
Age of baby	DOL2	29	39.2
	DOL3	38	51.4
	DOL4	3	4.1
	DOL5	2	2.7
	DOL6	2	2.7
Baby gender	Male	50	67.6
	Female	24	32.4
Mothers age (years)	< 21	12	16.2
	21-30	45	60.8
	> 30	17	23.0
Mode of delivery	NVD	31	41.9
	LSCS	43	58.1
Place of delivery	Home delivery	14	18.9
	Institutional delivery	60	81.1
Period of Gestation (weeks)	Very preterm (28-32)	4	5.4
	Late preterm neonate (32-37)	40	54.1
	Term neonate (37-42)	28	37.8
	Post term neonate (>42)	2	2.7
Mothers Blood group	A Negative	2	2.7
	A Positive	7	9.5
	AB Negative	2	2.7
	AB Positive	12	16.2
	B Negative	3	4.1
	B Positive	15	20.3
	O Negative	3	4.1
	O Positive	30	40.5
Baby's blood group	A Positive	16	21.6
	B Positive	41	55.4
	O Positive	17	23.0
Birth weight (grams)	<1000	1	1.4
	1000-1500	4	5.4
	1500-2500	39	52.7
	>2500	30	40.5

Most of the neonates i.e. 70 (94.5%) who developed jaundice were breast fed or given mixed feeds and 35 (47.2%) were given exclusively breast feed (Table 3). Out of 74 neonates 17 (22.9%) had sepsis screen positive and 16 (21.6%) out of 74 had shown evidence of hemolysis i.e. reticulocyte count>4% and 58 (78.3%) out of 74 presented with hyperbilirubinemia without hemolysis.

Table 2: Frequency and percentage distribution of sample clinical characteristics (maternal characteristics and neonatal characteristics) according to TSB levels.

Sample characteristics	Total serum bilirubin levels mg/dl								P value
	10-15		15-20		20-25		>25		
	N	%	N	%	N	%	N	%	
Birth weight (grams)									
<1000	1	5.9	0	0	0	0	0	0	
1000-1500	0	0	4	9.1	0	0	0	0	
1500-2500	12	70.6	19	43.2	6	75.0	2	40.0	0.24
>2500	4	23.5	21	47.7	2	25.0	3	60.0	
Maturity									
AGA	7	41.2	17	38.6	1	12.5	4	80.0	
SGA	10	58.8	21	47.7	6	75.0	1	20.0	0.19
LGA	0	0	6	13.6	1	12.5	0	0	
Day of life on presentation									
DOL2	10	58.8	17	38.6	1	12.5	1	20.0	
DOL3	7	41.2	26	59.1	2	25.0	3	60.0	0.001*
DOL4	0	0	1	2.3	2	25.0	0	0	
DOL5	0	0	0	0	2	25.0	0	0	
DOL6	0	0	0	0	1	12.5	0	0	
Level of jaundice									
Face and neck	0	0	0	0	0	0	0	0	
Neck to umbilicus	0	0	0	0	0	0	0	0	
Umbilicus to knees	4	23.5	0	0	0	0	0	0	0.001*
Knees to ankles	8	47.1	4	9.1	0	0	0	0	
Palms and soles	5	29.4	40	90.9	8	100	5	100	

*(p<0.05 significant)

Most common jaundice occurred in neonates was idiopathic or breastfeeding jaundice as the neonates were breast fed and no other cause was found except this in these 47.29% neonates (Table 4). The second most common cause was ABO incompatibility leading to jaundice in 27 (36.48%) neonates. Incidence of neonatal sepsis, G6PD deficiency, hypothyroidism and cephalhematoma was 22.9%, 4.1%, 2.70% and 4.1% respectively.

Polycythemia contributed to 1.35% and the frequency of hyperbilirubinemia in infants of diabetic mother's or GDM was 10.8%. Rh incompatibility was seen as the fourth common cause and the frequency was 13.5%. 10 neonates had jaundice due to Rh incompatibility and only 3 (30%) had undergone exchange transfusion rest 7 (70%) were managed by phototherapy alone (Table 5). 95.9% of the neonates had >6.5 glucose 6 phosphate dehydrogenase level and only 4.1% of the neonates had <6.5 of the glucose 6 phosphate dehydrogenase level with G6PD deficiency (Table 6).

Out of 3 G6PD deficient neonates if we look for ethnicity all 3 belongs to muslim group as the cause may be consanguinity and all 3 neonates are males which also predisposes towards genetic inheritance (Table 7-8). It was also observed that out of 3 G6PD deficient neonates 2 (66.6%) were preterm and 1 (33.3%) was term and 1 was LBW rest 2 were normal birth weight. Out of 16 neonates with hemolysis 2 (12.5%) had hemolysis with G6PD deficiency and rest 14 (87.5%) had hemolysis without G6PD deficiency due to other causes but didn't find it statistically significant (Table 9).

DISCUSSION

The current study was an observational study done on 74 neonates with hyperbilirubinemia admitted in NICU at TMMC and RC. In this study we aimed to find out the clinical spectrum of hyperbilirubinemia and we proved statistically significant correlations in some pertinent parameters with G6PD deficiency and hyperbilirubinemia. The observations made are discussed here.

Sex distribution

Out of 74 neonates 50 (67.6%) were males and 24 (32.4%) were females constituting a M:F ratio of 2:1, similarly the number of male patients were also more in studies performed by Sujaya et al, Ishanul et al, Keshwani et al, Singhal et al, Effiong et al, Narang et al and Korejo et al.[18-24] The probable explanation that can be given to this is social bias nature, medical attention is also more given to males and also males are more prone for genetic causes of pathological jaundice.

Table 3: Frequency and percentage distribution of sample clinical characteristics (maternal characteristics and neonatal characteristics) according to total serum bilirubin levels.

Sample characteristics	Total serum bilirubin levels mg/dl								P value
	10-15		15-20		20-25		>25		
	N	%	N	%	N	%	N	%	
Type of feeding									
Breastfeeding	8	47.1	22	50.0	3	37.5	2	40.0	0.77
Mixed feeding	8	47.1	20	45.5	5	62.5	2	40.0	
Top feeding	1	5.9	2	4.5	0	0	1	20.0	
Sepsis screen									
Positive	3	17.6	9	20.5	2	25.0	3	60.0	0.22
Negative	14	82.4	35	79.5	6	75.0	2	40.0	
Retic count (%)									
<2	1	5.9	0	0	1	12.5	0	0	0.01
2-4	14	82.4	37	84.1	3	37.5	2	40.0	
>4	2	11.8	7	15.9	4	50.0	3	60.0	
TRBC count (cells/mm^3)									
>6.5*10^6	1	5.9	0	0	0	0	0	0	0.65
3.7-6.5*10^6	15	88.2	41	93.2	7	87.5	5	100	
<3.7*10^6	1	5.9	3	6.8	1	12.5	0	0	
T3 (ng/dl)									
<75	0	0	1	2.3	0	0	0	0	0.96
75-260	17	100	42	95.5	8	100	5	100	
>260	0	0	1	2.3	0	0	0	0	
FT4 (ng/dl)									
<2.0	1	5.9	1	4.5	0	0	0	0	0.86
2.0-4.9	16	94.1	43	95.5	8	100	5	100	
>5.0	0	0	0	0	0	0	0	0	
TSH (µIU/l)									
<1.0	0	0	0	0	0	0	0	0	0.88
1.0-17.6	16	88.2	41	88.6	7	87.5	5	100	
>17.6	1	11.8	3	11.4	1	12.5	0	0	
GDM									
Yes	2	11.8	6	13.6	0	0	0	0	0.58
No	15	88.2	38	86.4	8	100.0	5	100	
Rh incompatibility									
Yes	1	5.9	4	9.1	1	12.5	4	80.0	0.001
No	16	94.1	40	90.9	7	87.5	1	20.0	
ABO incompatibility									
Yes	4	23.5	19	43.2	3	37.5	1	20.0	0.44
No	13	76.5	25	56.8	5	62.5	4	80.0	
Cephalhematoma									
Present	0	0	1	2.3	1	12.5	1	20.0	0.12
Absent	17	100	43	97.7	7	87.5	4	80.0	

Gestational age

In current study 44 (59.4%) neonates were preterm (<37 weeks), 28 (37.8%) had GA of 37-42 weeks (term) and only 2 (2.7%) were >42 weeks (post term). Among them maximum 38 (51.35%) neonates were SGA, 29 (39.1%) were AGA and only 7 (9.4%) were LGA. Similar to our study Ali et al also had more preterm neonates 128 (51%) whereas in contrast of that in a study done by Sujaya at al, Keshwani et al the number of term neonates were more compared to preterm 57.7% and 59.1% respectively.[25] Sujaya et al had 90% AGA neonates, 7.60% SGA and 1.90% were LGA. Singhal et al also found Prematurity as the second most common reason for jaundice seen in 16.7% cases in their study. In current study the number of preterm neonates (59.45%) who had jaundice is more as compared to term neonates 40.54% which signifies that preterm neonates are at more risk and vulnerable for development of jaundice. Bhutani et al also concluded that prematurity as a risk factor for the

occurrence of jaundice and bilirubin induced neurotoxicity.[26]

Table 4: Frequency and percentage distribution of etiological factors for hyperbilirubinemia (n=74).

Etiology	N	%
Idiopathic or breastfeeding jaundice	35	47.29
ABO incompatibility	27	36.48
Neonatal sepsis	17	22.9
Rh Incompatibility	10	13.5
GDM	8	10.8
G6PD deficiency	3	4.1
Hypothyroidism	2	2.70
Cephalhematoma	3	4.1
Polycythemia	1	1.35

Table 5: Comparison of Rh incompatibility with exchange transfusion.

Variables		Exchange transfusion				P value
		Yes		No		
		N	%	N	%	
Rh Incom-patibility	Yes	3	30	7	70	0.02
	No	3	4.7	61	95.3	

Table 6: Frequency and percentage distribution of glucose 6 phosphate dehydrogenase deficiency among neonates.

Glucose 6 phosphate dehydrogenase levels	N	%
<6.5	3	4.1
>6.5	71	95.9

In study done by Ali et al, according to gestational age 128 (51%) were preterm and 122 (49%) were term newborns. Bajpai et al had revealed the incidence of physiological jaundice with prematurity of 14% and Onyearugha et al also proved that prematurity was the second major cause of neonatal jaundice.[27] Probably due to physiological immaturity in preterm neonates Singhal et al also found an incidence of 16.7% of NNJ due to prematurity.

Birth weight

In terms of birth weight our results were similar to results obtained by Keshwani et al. They also found maximum neonates with birth weight 2001-2500 g. Singhal et al concluded that one fourth of LBW babies (29.16%) developed jaundice. Narang et al also observed similar results in their study and stated that the chances of jaundice in LBW babies were three times more compared to those who had birth weight >2.5 kg.[28]

Mode of delivery

Regarding the mode of delivery 81.1% newborns were delivered institutionally and among them nearly more than half (58.1%) of the neonates were born by cesearean section, similar to our study was seen in a study by Moktaderet al (71.9% neonates were delivered by LSCS) but opposite to that was observed by Keshwani et al.[29] Sujaya et al too in their study observed more number of vaginal deliveries (55.80%) compared to cesearean section.

Day of onset of jaundice

In current study majority of the patients, 38 (51.4%) presented with jaundice at DOL 3 which was followed closely by day of life 2-29 (39.2%) than three neonates presented on DOL 4 only two on DOL-5 and on DOL 6 only 1 neonate presented with hyperbilirubinemia, we found it statistically significant with p=0.001. Very close results were obtained by Sujaya et al with maximum number of neonates (32.60%) presented with jaundice on DOL-3 followed by DOL-4. Similarly Anand et al spotted maximum neonates with onset of jaundice on 3rd day (45%) of life followed by 4th day (35.5%) whereas slightly different from this Bhatia et al declared maximum neonates presented with jaundice on DOL-2 (67%) followed by DOL-3 (14%).[30,31]

Feeding

If we talk about nutrition in our study maximum neonates (94.59%) who developed jaundice were given mixed feeding, 47.29% were breast fed and only 5.40% were top fed. Hence breast fed neonates were at higher risk for the development of jaundice. Similar results were noticed by Sujaya et al in their study 63.5% neonates were beast fed who developed jaundice.

Maternal characteristics

The most common mother's blood group observed in our study was O Positive in 30 (40.5%) followed by B positive 15 (20.3%) which was similar to what was observed by Suvitha et al. 41 (55.4%) neonates were having blood group B positive resulting in ABO Incompatibility.

Neonatal characteristics

Total serum bilirubin level which was similar to results obtained by Keshwani et al maximum neonates with TSB 15-18 mg/dl. Similar results were obtained by Chhetri et al.

Aetiology

According to our study the most common aetiology of jaundice occurring in neonates was idiopathic or breastfeeding jaundice as the neonates who were breast

fed, no other cause was found except this in these 47.29% neonates. Hence it was probably breast feeding jaundice or might be idiopathic. In a study done by Narang et al 73.6% neonates had idiopathic jaundice which was comparatively higher to our study whereas Bahl et al and Shao et al reported lower incidence of idiopathic cause of NNJ 11.4% and 12.9% respectively.[32,33]

Table 7: Frequency and percentage distribution of sample clinical characteristics (maternal characteristics and neonatal characteristics) according to glucose 6 phosphate dehydrogenase level.

Sample characteristics		Glucose 6 phosphate dehydrogenase level				P value
		<6.5		>6.5		
		N	%	N	%	
Religion	Hindu	0	0	28	39.4	0.23
	Muslim	3	100	43	60.6	
	Other	0	0	0	0	
Gender of baby	Male	3	100	50	70.4	0.03
	Female	0	0	21	29.6	
POG (weeks)	Very preterm (28-32)	0	0	4	5.6	0.94
	Late preterm neonate (32-37)	2	66.7	38	53.5	
	Term Neonate (37-42)	1	33.3	27	38.0	
	Postterm neonate (>42)	0	0	2	2.8	
Birth weight (grams)	<1000	0	0	1	1.4	0.81
	1000-1500	0	0	4	5.6	
	1500-2500	1	33.3	38	53.5	
	>2500	2	66.7	28	39.4	

Table 8: Frequency and percentage distribution of sample clinical characteristics (maternal characteristics and neonatal characteristics) according to glucose 6 phosphate dehydrogenase level continued.

Sample characteristics		Glucose 6 phosphate dehydrogenase level				P value
		<6.5		>6.5		
		N	%	N	%	
Reticulocyte Count	<2%	1	33.3	1	1.4	0.003
	2-4%	0	0	55	77.5	
	>4%	2	66.6	15	21.1	
TRBC (cells/mm^3)	>6.5*10^6	0	0	1	1.4	0.48
	3.7-6.5*10^6	2	66.7	66	93.0	
	<3.7*10^6	1	33.3	4	5.6	
Total serum bilirubin levels (mg/dl)	10-15	0	0	17	23.9	0.48
	15-20	3	100	42	59.2	
	20-25	0	0	7	9.9	
	>25	0	0	5	7.0	

In our study the second most common cause was ABO incompatibility leading to jaundice in 27 (36.48%) neonates whereas Narang et al reported only 1.95% cases with ABO incompatibility. Singhal et al, Ajay et al and Sujaya at al reported almost equal incidence of ABO incompatibility around 15% cases with ABO incompatibility. The 3rd common cause was Neonatal Sepsis seen in 22.9% cases in our study. Narang et al, Singhal et al, Ali et al reported very less incidence of NNJ due to Neonatal Sepsis 4.49%, 5.7% and 6% respectively. This might be due to lack of education in people of different geographical distribution along with practices like day handling which further leads to early onset neonatal sepsis. Similar to our results, Singh et al, Sujaya et al and Bedowara et al had 21%, 34.60% and 26.7% cases with neonatal sepsis.[32,33] Rh incompatibility was seen as the 4th common cause of NNJ in our study and the frequency was 13.5%. Singh et al, Ali et al, Ishanul et al reported almost similar results in their studies with frequency of 14%, 13.5%, 13.6% respectively responsible for NNJ. Narang et al (0.39%) and Bahl et al (1.9%) reported very low frequency of Rh incompatibility as a cause for NNJ which was slightly different from results obtained in our study.

In current study frequency of G6PD was found to be 4.05% whereas Narang et al had comparatively higher cases with G6PD deficiency. They reported a frequency

of 12.1% of G6PD deficient neonates with hyperbilirubinemia. Comparatively higher incidence was found by Keshwani et al, Singh et al, Ali et al and Elmoktader et al of 0.83%, 0.5%, 0.8% and 1.8% respectively.

Table 9: Frequency of hemolysis in G6PD deficient and normal group.

G6PD enzyme	Hemo-lysis N (%)	No hemolysis N (%)	Total N (%)	P value
Deficient	2 (12.5)	1 (1.73)	3 (4.1)	
Normal	14 (87.5)	57 (98.27)	71 (95.9)	0.47
Total	16 (100)	58 (100)	74 (100)	

In current study hypothyroidism and cephalhemtaoma were seen in 2.70% and 4.05% cases respectively. Only two studies had reported incidence of hypothyroidism as a cause for Shao et al and Singhal et al. They found very less incidence of hypothyroidism 0.7% and 0.2% respectively when compared to our study. Polycythemia contributes to 1.35% in our study which was quite similar to incidence reported by Chhetri et al but less than what was observed by Singhal et al. The frequency of hyperbilirubinemia in infants of diabetic mothers or GDM in our study was 10.8% and very close results were obtained by Singhal et al in their study.[21]

Comparison of frequency of G6PD deficiency

Our study involved 74 neonates with hyperbilirubinemia and all were subjected to G6PD evaluation. G6PD deficiency is an X-linked disorder which majorly affects males. Females neonates may be affected if they are homozygous, which is found in populations where the frequency of G6PD deficiency is high. Heterozygous females are primarily carriers and the clinical disease is expressed due to gene mosaicism, inactivation of X chromosome or hemizygosity.[34] In our study also, there was male preponderance of 3 male neonates with G6PD deficiency and we found this correlation of G6PD deficiency with sex distribution statistically significant (p=0.03). This was similar to a study done by Sinha et al and Moustafa et al who reported all males with G6PD deficiency with an incidence of 2.5% and 8.9% respectively, no females had G6PD deficiency in their study however Suvitha at al reported totally opposite results from this.[35,36] They found all 3 females were G6PD deficient with a frequency of 1%, no male was found G6PD deficient by them and they found it statistically significant (p=0.045).

We found no significant statistical correlation with birth weight, period of gestation, religion, birth order and Total Serum Bilirubin levels of G6PD deficient and non G6PD deficient neonates which was opposite to results obtained by Sinha et al. However Sinha et al and Moustafa et al found no significant difference in terms of onset of jaundice, day of life of presentation to hospital which was opposite to our study. We found significant statistical correlation of G6PD deficiency with gender of baby (p=0.03) and reticulocyte count (p=0.003). Sinha et al also found significant statistical correlation with reticulocyte count in their study. Suvithaet al found significant association with gender distribution.[37] We studied that all the three neonates with G6PD deficiency belongs to Muslim community although we didn't find it statistically significant. Studies too revealed such higher incidence. In a study by Rai et al G6PD deficiency in Muslim community.[38] The TSB of all 3 G6PD deficient neonates was between 15-20 mg/dl which was similar to results obtained by Moustafa et al with mean TSB of 17.2±4.46 mg/dl whereas Adil et al concluded high up levels of TSB in neonates with G6PD deficiency.[25]

The prevalence of G6PD deficiency in India is seen since 50 years from 2.3 to 27% approximately in different tribal group. We found the frequency of G6PD deficiency in neonates with hyperbilirubinemia to be 4.1%. 100% males were affected. No female was found to be G6PD deficient. Eghbalian et al Boksabadi et al reported similar incidence of 4.4% and 5.2% respectively. They too observed males were more affected compared to females. In studies done in India by Sinha et al at Jodhpur, Goyal et al at New Delhi and Suvitha et al at Karnataka reported comparatively less frequency than what was observed by us whereas Chhetri et al at West Bengal and Bisoi et al at Kolkata reported higher incidence than us 11.11% and 14.68% respectively.[35,37,39,40] In studies done outside India the incidence ranges from 2-16%. In a study done in Iraq, Adil et al observed an incidence of 16%. Abolghasemi et al in Tehran observed 2.1% and Boksabadi et al in Iran reported 5.2% incidence of G6PD deficiency in neonates with hyperbilirubinemia. In our study, the frequency of G6PD deficiency with hyperbilirubinemia was found to be 4.1%. Similarly in a study done by Karimi et al the frequency of G6PD deficiency was approximately 9 % whereas Frankool et al, Moustafa et al revealed the frequency of G6PD deficiency in neonates with hyperbilirubinemia was 10.65 %, 8.9% respectively whereas Castro et al reported an incidence of G6PD insufficiency was 7.9 % among neonates in Brazil.

G6PD deficiency with hemolysis

In our study the G6PD deficient neonates had Coomb's test negative and 2 patients fulfilled the criteria of hemolysis. The frequency of hemolysis in G6PD deficient neonates was 66.6%. Two neonates out of 3 with G6PD deficiency had hemolysis and we found it statistically significant (p=0.01%). Similar to our study Eghbalian et al also didn't report any neonate with positive Coomb's test among G6PD deficient neonates. The total frequency of G6PD deficiency with hemolysis found in our study was 12.5% comparable to Eghbalian et al who reported frequency of hemolysis with G6PD

deficiency in their study to be 16.7%. At last we concluded that the frequency of G6PD deficiency in Northern part of India is high and also comparable to other studies done in past. We found it similar or high to many other studies performed before and we recommend neonatal screening for G6PD deficiency and all relevant investigations to rule out the etiology for NNJ in significant hyperbilirubinemia to prevent further neurological sequelae and Kernicterus.

Limitations

Limitations of current study were; the study is time bound study of one year period so chances of study related positive patients being missed before and after the study time period is there. On the other hand the study is hospital based study and the male biased nature of the society. So there are chances of male patients being detected more as cases. In order to prevent these limitations there should be some screening test available for every newborn.

CONCLUSION

The current study was done to find out the clinical spectrum of hyperbilirubinemia and to assess the frequency of G6PD deficiency and after studying the clinical spectrum of jaundice in neonates we concluded that most of the neonatal jaundice cases are due to idiopathic cause or may be physiological. But in rest of the cases which are pathological if left untreated may lead to severe neurological deficits and lifelong disabilities, hearing impairment, mental retardation, seizures and movement disorders. So indentifying the pathology of jaundice and treating it may prevent the newborn from these deadly complications. Hence we recommend G6PD screening in every newborn with significant hyperbilirubinemia to reduce morbidity and mortality.

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REFERENCES

1. Bhutani V, Zipursky A, Blencowe H, Khanna R, Sgro M, Ebbesen F, et al. Neonatal hyperbilirubinemia and Rhesus disease of the newborn: incidence and impairment estimates for 2010 at regional and global levels. Pediatr Res. 2013;74(S1):86-100.
2. Suchy FJ. Neonatal cholestasis. Pediatr Rev. 2004; 25(11):388-96.
3. Investigators of National neonatal perinatal database. Morbidity and mortality among outborn neonates at 10 tertiary care institutions in India during the year 2000. J Trop Pediatr. 2004;50(3):170-4.
4. Euchenwald H, Stark M. Cloherty and Stark's manual of neonatal care. 8th ed. China: Wolters Kluwer; 2016:314-6.
5. Ramachandran A. Neonatal hyperbilirubinaemia. Paediatr Child Health. 2015;26(4):162-8.
6. Sedlak TW, Snyder SH. Bilirubin benefits: cellular protection by a biliverdinreductase antioxidant cycle. Pediatr. 2004;113:1776-82.
7. Watchko JF. Avery's Diseases of the Newborn. 10th ed. Elsevier: Saunders; 2012:325-8.
8. Weng YH, Chiu YW. Spectrum and outcome analysis of marked neonatal hyperbilirubinemia with blood group incompatibility. Chang Gung Med J. 2009; 32(4):400-8.
9. Maisels M, Bhutani V, Bogen D, Newman T, Stark A, Watchko J. Hyperbilirubinemia in the Newborn Infant >=35 Weeks' Gestation: An Update With Clarifications. Pediatr. 2009;124(4):1193-8.
10. Naveh Y, Friedman A. Urinary tract infection presenting with jaundice. Pediatr. 1978; 62: 524-5.
11. Cashore WJ. Bilirubin and jaundice in the micropremi. Clinperinatol. 2000;27:171-9.
12. Weldon AP, Danks DM. Congenital hypothyroidism and neonatal jaundice. Arch Dis Child. 1972;47(253): 469-71.
13. Nouri DMR, Hejazi SH, Yousefi A, Mohammad GSH, Soltanian S, Javadi KR. Identification of mutations in G6PD gene in patients in Hormozgan province of Iran. Med J Islam Repub Iran Fall. 2006; 17(4):313-6.
14. Glader B. Hereditary hemolytic anemias due to red blood cell enzymedisorders. In: Greer JP, Foerster J, Rodgers GM, eds. Wintrobe'sclinical hematology. 12th ed. Philadelphia: Lippincott Williams &Wilkins; 2009:933-4.
15. Kaplan M, Hammerman C. Neonatal screening for glucose-6-phosphatedehydrogenase deficiency: biochemical versus genetictechnologies. Semin Perinatol. 2011;35(3):155-61.
16. Gari MA, Chaudhary AG, Al-Qahtani MH, Abuzenadah AM, Waseem A, Banni H, et al. Frequency of Mediterranean mutation among a group of Saudi G6PD patients in Western region-Jeddah. Int J Lab Hematol. 2010;32:17-21.
17. Mohanty D, Mukherjee MB, Colah RB. Glucose-6-phosphate dehydrogenase deficiency in India. Indian J Pediatr. 2004;71:525-9.
18. Mukhopadhyay S, Hassan N. Etiology and clinico-hematological profile of neonates with pathological unconjugated hyperbilirubinemia: a tertiary care centre experience. Int J Contemp Pediatr. 2019;6(5): 1888-92.
19. Haq UI, Israrul H, Khan S, Sayed Z. Common aetiological spectrum of indirect hyperbilirubinemia in neonates. JSMC. 2017;7(2):112-6.
20. Keshwani A, Dolas A. Etiology, Risk Factors and morbidity profile associated with neonatal hyperbilirubinemia in a tertiary care hospital. New Indian J Pediatr. 2015;4.3:131-42.
21. Singhal PK, Singh M, Paul VK, Deorari AK, Ghorpade MG. Spectrum of neonatal hyperbilirubinemia: an analysis of 454 cases. Indian Pediatr. 1992;29(3):319-25.

22. Boskabadi H, Omidian M, Mafinejad S. Prevalence and clinical manifestation of glucose-6-phosphate dehydrogenase deficiency in newborns with hyperbilirubinemia in Mashhad, Iran. Maced J Med Sci. 2010;3(4):383-7.
23. Narang A, Kumar P, Kumar R. Neonatal jaundice in very low birth weight babies. The Indian J Pediatr. 2001;68(4):307-9.
24. Korejo H. Risk facors for kernicterus in neonatal jaundice, Karachi, Pakistan. GJMS 2010; 8(1):34-6.
25. Ali A, Tomar A. Etiological profile of neonatal hyperbilirubinaemia in the rural area of Rajasthan. Indian J Basic App Med Res. 2015;4(2):223-2.
26. Bhutani VK. Evidence based issues regarding neonatal hyperbilirubinemia. Pediatr Rev. 2005;114: 130-53.
27. Onyearugha CN, Onyire BN, Ugboma HAA. Neonatal Jaundice: prevalence and associated factors as seen in Federal medical centre Abakaliki, Southeast Nigeria. J Clin Med Res. 2008;3(3):40-5.
28. Hussain BG. Risk factors for kernicterus in neonatal Jaundice. GJMS. 2010;8:12-4.
29. Elmoktader A, Hussein S, Boraik M. Hyperbilirubinemia in neonatal intensive care unit: incidence and etiology at fayoum university hospital. Fayoum Univ Med J. 2019;3(2):8-14.
30. Anand JS, Magotra ML. Neonatal jaundice-its incidence and etiology. Indian Paediatr. 1978;15:155-60.
31. Porter ML, Dennis BL. Hyperbilirubinemia in the term newborn. Am Fam Physician. 2002;65(4):599-606.
32. Singh S, Singh S, Kumar M, Tripathi S, Bhriguvanshi A, Chandra T, et al. Etiology and clinical profile of neonates with pathological unconjugated hyperbilirubinemia with special reference to Rhesus (Rh) D, C, and E incompatibilities: A tertiary care center experience. Clin Epidemiol Glob Health. 2016; 4(2):95-100.
33. Bedowra et al. Risk factors and outcome of neonatal jaundice in a tertiary hospital. Med J. 2010;4(2):70-3.
34. Minucci A, Giardina B, Zuppi C, Capoluongo E. Glucose 6 phosphate dehydrogenase laboratory assay: How, when and why? IUBMB Life. 2009;61(1):27-34.
35. Sinha R, Sachendra B, Syed V, Nair L, John B. To study the prevalence of glucose 6 phosphate dehydrogenase(G6PD) deficiency in neonates with neonatal hyperbilirubinemia and to compare the course of the neonatal jaundice in deficient versus non deficient neonates. J Clin Neonatol. 2017;6(2):71-3.
36. Abo-El FW, Rizk M. Prevalence of glucose-6-phosphate dehydrogenase deficiency in jaundiced Egyptian neonates. J Mat Fetal Neonat Med. 2016; 29(23):3834-7.
37. Thilakarajan S, Niveditha S.R, Keshavamurthy. G6PD screening in neonatal hyperbilirubinemia. Indian J Neonat Med Res. 2014;3(3):1-6.
38. Rai V, Kumar P. Glucose 6 phosphate dehydrogenase deficiency in Muslim community settled in Junapur district. Indian J Hum Genet. 2014;20(1):96-127.
39. Kapoor S, Ramji S, Goyal M, Garg A, Goyal M, Kumar S. Newborn screening for G6PD deficiency: A 2-year data from North India. Indian J Public Health. 2015;59(2):145-8.
40. Chhetri N, Chhetri A. Pattern of glucose 6 phosphate dehydrogenase deficiency in neonates with hyperbilirubinemia in a tertiary care center. Int J Med Health Res. 2017;3(9):61-5.

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