Original Article

Exchange Transfusion Trends and Risk Factors for Extreme Neonatal Hyperbilirubinemia over 10 Years in Shiraz, Iran

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Abstract

Background: Exchange transfusion (ET) is an effective treatment for acute bilirubin encephalopathy and extreme neonatal hyperbilirubinemia (ENH). It can reduce mortality and morbidity. This study aimed to investigate the trends and risk factors of ENH requiring ET in hospitalized neonates in Iran.

Methods: A retrospective analysis of medical records of neonates who underwent ET due to ENH was conducted from 2011 to 2021, in Shiraz, Iran. Clinical records were used to gather demographic and laboratory data. The quantitative data were expressed as mean±SD, and qualitative data was presented as frequency and percentage. $P<0.05$ was considered statistically significant.

Results: During the study, 377 ETs were performed for 329 patients. The annual rate of ET decreased by 71.2% during the study period. The most common risk factor of ENH was glucose-6-phosphate dehydrogenase (G6PD) deficiency (35%), followed by prematurity (13.06%), ABO hemolytic disease (7.6%), sepsis (6.4%), Rh hemolytic disease (6.08%), and minor blood group incompatibility (3.34%). In 28.52% of the cases, the cause of ENH was not identified. 17 (5.1%) neonates had acute bilirubin encephalopathy, of whom 6 (35.29%) had G6PD deficiency, 6 (35.29%) had ABO incompatibility, and 2 (11.76%) had Rh incompatibility.

Conclusion: Although the rate of ET occurrence has decreased, it seems necessary to consider different risk factors and appropriate guidelines for early identification and management of neonates at risk of ENH should be developed. The findings of the study highlighted the important risk factors of ENH in southern Iran, allowing for the development of appropriate prevention strategies.

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Keywords ● Hyperbilirubinemia ● Neonatal ● Jaundice ● Kernicterus ● Risk factors

What's Known

- Extreme hyperbilirubinemia is a major concern. Despite its decline throughout the years, it remains an important cause of mortality and morbidity in neonates.

What's New

- G6PD deficiency is the most prevalent risk factor of extreme newborn hyperbilirubinemia that leads to exchange transfusion in southern Iran. Therefore, hyperbilirubinemia must be monitored closely in these infants. Moreover, screening for G6PD deficiency is essential.

Introduction

Hyperbilirubinemia is a common and challenging condition in neonates that affects approximately 50% of term and 80% of preterm newborns.[1] It is a prevalent cause of hospital readmission in the first week of life.[2] Hyperbilirubinemia is frequently benign. However, it can be harmful and cause

bilirubin encephalopathy or kernicterus with long-term irreversible morbidity and mortality.[3] According to Shapiro's definition, total serum bilirubin (TSB) concentration >20 mg/dL in the first 72 hours of life is considered severe hyperbilirubinemia, and TSB concentration>25 mg/dL during the first 28 days of life is labeled as critical or extreme neonatal hyperbilirubinemia.[4] The main predisposing factors for extreme neonatal hyperbilirubinemia (ENH) are hemolytic diseases such as ABO and Rh incompatibility, glucose-6-phosphate dehydrogenase (G6PD) deficiency, infections, prematurity, and dehydration due to inadequate breastfeeding.[5, 6] The preventive recommendations include risk assessment, increased duration and frequency of breastfeeding, predischarge bilirubin measurement, and early treatment if indicated.[7] The standard treatments for ENH are phototherapy and exchange transfusion.[8] ET is an effective treatment for acute bilirubin encephalopathy (ABE) if administered promptly.[9] An exchange transfusion was performed based on clinical signs and symptoms of ABE, such as decreased feeding, lethargy, hypotonia and/or hypertonia, high-pitched cry, retrocollis, opisthotonos position, fever, seizure, decreased level of consciousness, and death,or an increased level of TSB at or above the gestational and post-natal age thresholds.[4] This study aimed to evaluate the frequency of ET and its risk factors in a referral hospital in southern Iran over a 10-years period.

Patients and Methods

This retrospective study was conducted at Namazi Hospital, a Referral and Educational Medical Center in Shiraz (Iran), from March 2011 to March 2021. The medical reports and laboratory data of all neonates who underwent exchange transfusion during this period were reviewed by the authors and assessed for eligibility. The exclusion criteria were ET caused by diseases rather than hyperbilirubinemia, such as life-threatening infections, postnatal age of more than 30 days at the time of ET, and incomplete essential data including clinical and laboratory data (missing more than three parameters in one medical chart considered incomplete). Data was collected from the patients' hospital records, which included their maternal history, demographic information, clinical laboratory results, and hospital course. The Patient's characteristics, including the gestational age (GA), sex, age at admission, patient's origin, type of delivery, onset time of jaundice (day), age at the time of ET (day), type of feeding, maternal history of diabetic mellitus, hypothyroidism, parental consanguinity, family history of ET, signs of bilirubin encephalopathy, total and direct serum bilirubin levels at the time of ET (mg/dL), hemoglobin (HB) (g/dL), mean corpuscular volume (MCV) of the red blood cells (fl), reticulocyte count (%), maternal and neonatal blood group (ABO) and Rh (+/-), direct Coombs' test (DCT) (+/-), and G6PD deficiency(+/-) were extracted from the medical reports and recorded in data collecting sheets. Moreover, the frequency of ET and the presence of sepsis before ET were documented.

Neonates with GA≥37 weeks were classified as term, 35-36 weeks late preterm, and ≤34 weeks early and moderate preterm. Indications of phototherapy and ET were determined according to Maisels and others and AAP guidelines for neonates with GA≥35 and <35 weeks, respectively.[10, 11] Double volume ET was performed for neonates with clinical signs of bilirubin encephalopathy or bilirubin levels≥ the GA and postnatal age threshold. Then, according to ET guidelines, their bilirubin levels were considered extreme or critical. Rh incompatibility was defined as an Rh-positive neonate born from an Rh-negative mother. Rh hemolytic disease was defined as DCT positivity, anemia, and/or reticulocytosis. Anemia was defined as Hb<13 gr/dL, and reticulocytosis was defined as a reticulocyte count of more than 5%. ABO incompatibility was defined as having a mother with blood group O and a newborn with blood group A or B. ABO hemolytic disease was defined as jaundice on the first day of life, DCT positive, spherocytosis, anemia, and/or reticulocytosis. Neonates with positive DCT, anemia, and no ABO or Rh incompatibility were labeled as minor blood group incompatibility. According to Fanaroff and Martin's neonatology textbook,[12] "Conjugated hyperbilirubinemia was considered as direct bilirubin more than 1.5 mg/dL provided it comprised more than 10% of the total bilirubin". The G6PD was checked qualitatively in neonates admitted due to hyperbilirubinemia using the ultraviolet (UV) method. The UV method uses ultraviolet light to measure the G6PD enzyme in the blood sample. It is based on the principle that G6PD catalyzes the reduction of nicotinamide-adenine dinucleotide phosphate ($NADP^+$) to nicotinamide adenine dinucleotide phosphate (NADPH), which absorbs UV light at 340 nm. The amount of produced NADPH depends on the existed amount of G6PD enzyme.

This study was approved by the Ethics Committee of Shiraz University of Medical Sciences (code: IR.SUMS.REC.1400.142).

Since the study was performed retrospectively, the patient's consent was not required. To assure the patient's privacy, only the authors of this study reviewed the medical reports, and it was conducted in the records office.

Statistical Analysis

The collected data was analyzed using SPSS software, version 21 (IBM Statistics, Chicago, USA). The quantitative, such as jaundice onset age, ET age, duration of hospitalization, GA, Hb level, and bilirubin level, were presented as mean±SD. Qualitative data, such as type of delivery, feeding, maternal hypothyroidism, gestational DM, and risk factors of hyperbilirubinemia, were presented as percentages and frequencies. The Mann-Whitney, Chi square, Kruskal-Wallis tests, and Bonferroni *Post hoc* tests were used to determine and compare the significant differences between the groups. $P<0.05$ was considered statistically significant.

Results

During the study period, 377 exchange transfusions were performed for all neonates, who were referred to this referral hospital, due to hyperbilirubinemia and required exchange transfusion. 324 newborns had once ET, 25 newborns had twice ET, and one newborn had thrice ET. Indeed, 6.88% of the patients who received phototherapy underwent exchange transfusion due to ENH or symptoms of bilirubin encephalopathy. The annual number of ET during these 10 years is shown in figure 1. During 2011-2012, 59 exchange transfusions were done in this center. The rate of exchange transfusion decreased annually, reaching 17 in 2020-2021, which resulted in a total decrease of 71.2% over the years.

21 medical charts of exchange transfusion were excluded due to incomplete essential data. Therefore, the characteristics of 329 neonates, who underwent ET, were evaluated in this study. Tables 1 and 2 present the demographic, laboratory, and hospital data.

166 (50.5%) of the neonates who underwent ET were female, and the majority of them, 261 (79.3%), were term. The ET was done at a mean TSB level of 27.54±6.91 mg/dL. About 108 (33%) of the newborns had bilirubin levels of 25-30 mg/dL, and 89 (27%) had TSB levels more than 30 mg/dL. 17 (5.16%) newborns had signs and symptoms of ABE at the time of admission. The patients' characteristics are presented in table 3. The mortality rate among the study population was 8 patients (2.4%).

Out of the 329 neonates, 115 (34.9%) neonates had G6PD deficiency, of whom 18 (15.8%) had anemia, and 14 (12.17%) had reticulocytosis. The G6PD-deficient neonates had a mean TSB of 28.17±6.80 mg/dL, while it was 27.59±7.09 in G6PD-sufficient ones. 85 (73.9%) G6PD-deficient neonates were male, and male-to-female ratio was 2.8:1. ABO incompatibility was found in 84 (25.5%) neonates, of whom 6 (7%) developed jaundice in the first 24 hours of life, 21 (25.3%) had anemia, and 25 (29.7%) had reticulocytosis. Only 2 (2.38%) of them had positive DCT. Rh incompatibility was found in 37 (11.2%) neonates, 20 (54.1%) of whom had anemia and reticulocytosis, and 9 (24.3%) had positive DCT. One neonate had both ABO and Rh incompatibility with positive DCT and hemolysis. 21 (6.38%) neonates had positive DCT, of whom 16 (76.1%) had anemia, and 11 (52.38%) had reticulocytosis.

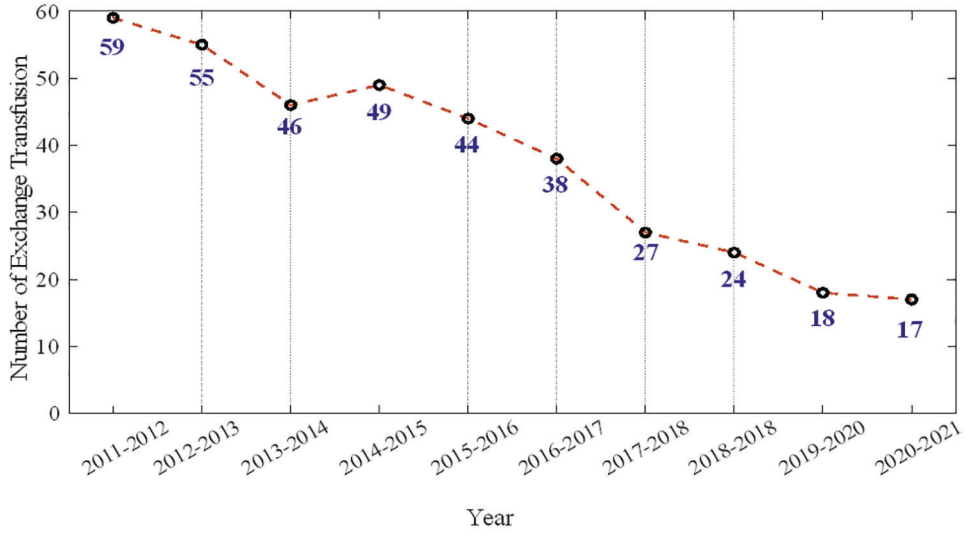

Figure 1: The trend of the annual rate of exchange transfusion during the study period illustrates a decreasing pattern.

Table 1: Qualitative clinical and laboratory data of neonates who underwent ET (N=329)

Variables		n (%)
Gestational Age (weeks)	≥37	261 (79.3)
	34-37	35 (10.6)
	<34	8 (2.4)
	Undetermined*	25 (7.6)
Sex	Male	163 (49.5)
	Female	166 (50.5)
Type of delivery	Normal vaginal	197 (59.9)
	Cesarean section	99 (30.1)
	Undetermined*	33 (10)
Feeding	Breast milk	243 (73.9)
	Formula	13 (4)
	Both	62 (18.8)
	Undetermined*	11 (3.3)
Patient's origination	Capital of the province	164 (49.84)
	In the province	154 (46.8)
	Out of the province	11 (3.34)
G6PD deficient		115 (35)
G6PD deficiency with anemia		18 (5.47)
Maternal blood group	A	89 (27.1)
	B	57 (17.3)
	AB	11 (3.3)
	O	155 (47.1)
	Undetermined*	17 (5.2)
Neonatal blood group	A	98 (29.8)
	B	89 (27.1)
	AB	18 (5.5)
	O	122 (37.1)
	Undetermined*	2 (0.6)
ABO incompatibility		84 (25.5)
ABO hemolytic disease		25 (7.6)
Maternal Rh	Positive	270 (82.1)
	Negative	42 (12.8)
	Undetermined*	17 (5.2)
Neonatal Rh	Positive	299 (90.9)
	Negative	28 (8.5)
	Undetermined*	2 (0.6)
Rh incompatibility		37 (11.2)
Rh hemolytic disease		20 (6.08)
Positive direct Coombs test		21 (6.4)

*It refers to missing data.

Table 2: Quantitative clinical and laboratory data of neonates who underwent ET (N=329)

Variable	Mean±SD	Range
Gestational age (week)	37.82±1.78	26-42
Time of onset of jaundice (day)	1.95±0.95	1-9
Age on admission (day)	5.00±2.98	1-27
Age of ET (day)	5.47±3.02	1-27
Duration of hospitalization(day)	5.0±2.98	1-27
Total serum bilirubin (mg/dl)	27.54±6.91	10.4-53.4
Direct bilirubin (mg/dl)	1.13±1.85	0.3-21
Hemoglobin (gr/dl)	15.09±3.33	2.3-24.4
Reticulocyte count (%)	3.83±4.59	0.1-35
MCV (fl)	102.98±11.28	92-156

ET: Exchange transfusion; MCV: mean corpuscular volume

Table 3: Characteristics of patients with acute Bilirubin encephalopathy (n=17)

Variable		N (%)	Variable	Mean±SD	Range
Sex	Male	9 (52.9)	Exchange time (day)	5.6±2.4	3-12
	Female	8 (47.1)			
Vaginal delivery		12 (70.58)	Onset time of jaundice (day)	2±1.03	1-4
Feeding	Breast milk	7 (41.17)	TSB (mg/dL)	34.12±8.46	20.5-50.8
	Formula	2 (11.76)			
	Both	8 (47.05)			
Sepsis		5 (29.4)	Direct bilirubin (mg/dL)	3.19±5.22	0.5-21
Etiology	G6PD deficiency	6 (35.29)	Hemoglobin (gr/dL)	13.65±3.56	7.5-20.7
	ABO incompatibility	6 (35.29)			
	Rh incompatibility	2 (11.76)			
Exchange in siblings		1 (5.88)	Reticulocyte count (%)	2.33±1.68	0.5-6.5
Direct hyperbilirubinemia		4 (23.52)	MCV (fl)	104.40±8.62	85.4-117

Table 4: Comparison of TSB, age of the onset of jaundice, and age of ET in three gestational age groups (n=304)

Variable	Group			P value*	P value		
	Very preterm (n=8)	Late preterm (n=35)	Term (n=261)		P1	P2	P3
Total serum bilirubin (mg/dl)	22.16±10.45	26.26±7.11	27.95±6.67	0.06	0.278	0.360	0.05
Onset time of jaundice (day)	2.67±1.36	2.26±0.85	1.92±0.95	0.022	0.563	0.023	0.093
Age of ET (day)	12.75±7.57	6.17±1.94	5.12±2.27	<0.001	0.166	0.003	0.003

*Assessed by using the Kruskal-Wallis Test; P1: Very preterm versus late preterm; P2: Late preterm versus term; P3: Very preterm versus term assessed by using Bonferroni *Post hoc* test

Eleven of them did not have ABO and Rh incompatibility. Consequently, they were categorized as minor blood group incompatibility. A peripheral blood smear was normal in 313 (95.1%) neonates, and only 16 (4.86%) newborns had nucleated red blood cells (RBC) and evidence of hemolysis. 21 (6.4%) out of 329 were admitted with sepsis. The study population included 261 term (GA≥37 weeks), 35 late preterm (GA 34≥GA<37 weeks), and 8 early and moderately preterm (GA<34 weeks) neonates. The comparison of TSB, age of jaundice onset, and ET age is shown in table 4.

The onset time of jaundice in the study population is shown in figure 2. About 80% of patients developed jaundice in the first 48 hours of life. Exchange transfusion was performed in the first week of life at 282 (85.8%) of patients, followed by 41 (12.4%), 4 (1.2%), and 2 (0.6%) patients in the second, third, and fourth weeks of life, respectively. On the day of admission to the hospital, 200 (60.56%) neonates underwent ET. Twelve (3.7%) patients had a family history of ET. A history of maternal diabetes mellitus was observed in 3 (0.9%) neonates, and 10 (3%) had a history of maternal hypothyroidism. Moreover, 10 (3%) parents had consanguinity. More than 50% of the patients were discharged 2-3 days after admission.

Discussion

Over the study duration, a decline in the annual rate of ET utilization was observed in the studied

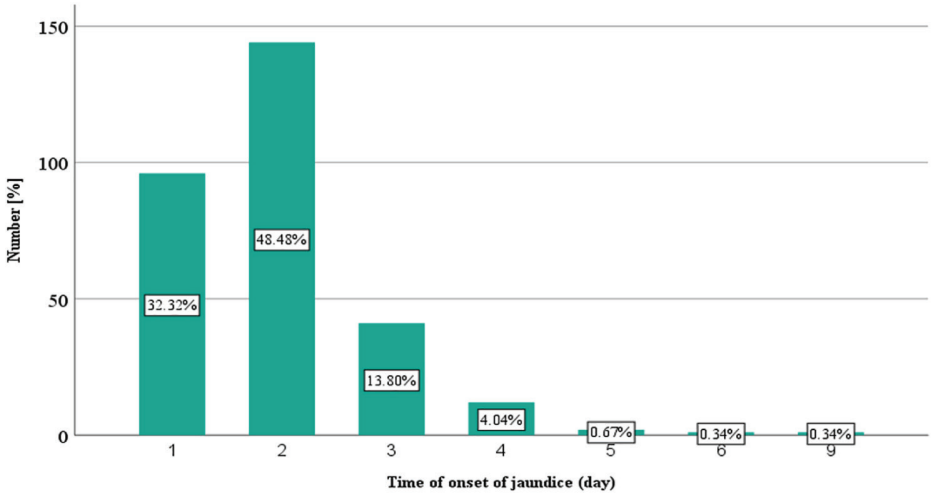

Figure 2: The number (%) of neonates based on the day of jaundice onset in the studied population is shown.

center. Remarkably, about half of the neonates were transferred from other cities. G6PD deficiency emerged as the prominent risk factor among patients undergoing ET. Subsequently, other risk factors were prematurity, ABO hemolytic disease, sepsis, Rh hemolytic disease, and minor blood group incompatibility. Among the hemolytic causes, ABO hemolytic disease was the primary risk factor. Furthermore, late prematurity, on its own, contributed to 35 (10.6%) cases.

In Iran, ET research was conducted in different cities. In Isfahan, among 68 patients who underwent ET, the most prevalent risk factors were ABO incompatibility and G6PD deficiency,[13] as in Guilan.[14] In Tabriz, the primary risk factors were ABO incompatibility, and Rh incompatibility, followed by idiopathic cases. Besides, G6PD deficiency accounted for only 2% of their patients.[15] In Tehran, among 94 patients, prematurity emerged as the prominent risk factor, followed by ABO incompatibility and G6PD deficiency.[16] In Mashhad, the most common causes were unknown, followed by ABO incompatibility, Rh incompatibility, and G6PD deficiency.[17] The difference in ABO incompatibility prevalence within the present center and other studied centers could be attributed to the larger sample size in the present study and the ABO incompatibility criteria, which included hemolysis in addition to the maternal O blood group and neonatal A/B blood group. Additionally, the frequency of G6PD deficiency was different among different races and regions.

Delays in neonatal transfers can lead to missing the ideal time for ET.[18] In recent years, Fars Province reduced transfer rates by enhancing primary care facilities and expertise in different centers. Recently, providing more effective intensive phototherapy devices, disseminating information, screening for Rh isoimmunization during pregnancy, neonatal G6PD screening, predischarge bilirubin measurement, risk assessment, and providing earlier and continuous visit facilities for high-risk neonates have all contributed to a decrease in severe hyperbilirubinemia and the rate of ET requirement. Using IVIG transfusion when indicated was the other decreasing factor.[19] Despite successful prevention strategies, kernicterus cases continued to be reported around the world.[20]

Recent reports highlighted a strong correlation between G6PD deficiency, ENH, ABE, neonatal mortality, and neurodevelopmental disorders, emphasizing its importance as a neonatal health concern.[21] The rate of G6PD deficiency in the Iranian population and among Iranian neonates with jaundice was 6.7% and 7%, respectively.[22, 23] However, in the present study, 35% of 329 neonates with ENH and 35.29% of 17 patients with ABE were G6PD deficient, emphasizing the causal link between G6PD deficiency and ENH and ABE. Since G6PD deficiency is an X-linked state, males might have normal or deficient traits, whereas females might be either normal, deficient, or heterozygotes. A previous study reported fatal kernicterus in a heterozygote.[24] According to the findings of the present study, 73.9% of 115 G6PD deficient neonates were male, resulting in a male-to-female ratio of 2.8:1, which was consistent with Javadi and others' findings on neonates with jaundice.[23] Therefore, more ET in G6PD-deficient males was due to the higher prevalence in males. The G6PD enzyme plays a main role in the stabilization of the RBC membrane against oxidative damage. In neonates with G6PD deficiency, ENH may develop suddenly, often without a recognizable reason, with a drop in hemoglobin and hematocrit values, reticulocytosis, and an explanation for the increase in TSB to dangerous levels. In other words, hematologic indices are not accurate predictors of hemolysis. In the present study, only 12.18% and 15.8% of G6PD-deficient neonates had reticulocytosis and a drop in hemoglobin, respectively. A previous study used endogenous CO formation, an accurate index of heme catabolism, and demonstrated an increased hemolysis in these newborns. [12] Hence, the term "nonhemolytic jaundice" should be used cautiously, because hemolysis potentiates bilirubin neurotoxicity could be prevented by lowering TSB concentration.[25, 26] Furthermore, G6PD-deficient neonates had lower bilirubin conjugation. Gene interaction between G6PD deficiency and the gene of Gilbert syndrome was reported to influence the incidence of hyperbilirubinemia.[27] Therefore, G6PD deficiency was one of the important causes that kernicterus elimination might become impossible, leaving ET as the only option.[12]

According to the findings of the present study, prematurity was the second risk factor of ENH. Preterm neonates had a delay in the maturation of hepatic UDP glucuronosyltransferase family 1 member A1 (UGT1A1) activity, making them more sensitive to ABE at a lower TSB level. Late preterm neonates, who were frequently discharged early with their mothers, were at risk for ENH. In a US survey, late prematurity increased the risk for neonatal hyperbilirubinemia more than fivefold.[28] Immature hepatic conjugative capacity and suck-swallow immaturity were two factors that contribute to ENH and ABE incidence in these infants.[29]

In the present study, ABO incompatibility with hemolysis was the third risk factor that led to ET, while it was the most common cause of ABE or ENH reported in the United States, Canada, China, and Nigeria, affecting 19-55% of infants.[30-33] About 25.5% of the patients were ABO incompatible. ABO incompatibility is defined as the situation in which infants with blood group A or B are born to mothers with group O because anti-B or anti-A antibodies of blood group-O mothers are predominantly smaller IgG molecules that may cross the placenta, as opposed to the corresponding antibodies of blood groups A or B mothers, which are IgM molecules with limited ability to cross the placenta. Some or all of the following criteria are required to support the diagnosis of ABO hemolytic disease: hyperbilirubinemia on the first day of life, anemia, spherocytosis increased reticulocyte counts, and increased end-tidal CO measurements corrected for ambient CO (ETCOc). We did not assess. However, according to other criteria, 25 (7.6%) cases with ENH had ABO hemolytic disease. In ABO-incompatible newborns with negative DAT, a polymorphism for the (TA) 7 sequence in the promoter of the gene encoding UGT1A1 significantly increased the incidence of hyperbilirubinemia.[29] In Fars Province, the prevalence of Gilbert syndrome, detected using the rifampin test, was 25.6% and 12.8% in males and females, respectively.[34] Thus, ABO incompatibility might have increased the risk of ENH in our neonatal population, who had the Gilbert syndrome gene.

Sepsis occurred in 6.4% of neonates with ENH and 29.4% of patients with ABE. Sepsis causes hyperbilirubinemia through a variety of mechanisms, including hemolysis, impaired conjugation, and decreased bilirubin excretion. Neonatal RBCs are susceptible to injury due to oxidative stress, and sepsis-induced disseminated intravascular coagulation can lead to hemolysis.[35] Furthermore, oxidants can stimulate the heme oxygenase enzyme, resulting in enhanced heme catabolism to bilirubin. In addition, sepsis-induced hepatitis can cause conjugated hyperbilirubinemia. Acidosis and meningitis, which might be associated with sepsis, enhanced the risk of ABE with lower TSB levels.[36, 37] In the studied patients, sepsis was not the primary risk factor. However, it played a significant role in the increased incidence of ABE.

11 (3.34%) of our cases were classified as minor blood group incompatibility due to positive DCT in the absence of ABO and Rh incompatibility. The most reported RBC antibodies that cause hemolytic disease in the newborn were anti-C, anti-Kell, and anti-E.[38, 39]

The advantages of the present study were enrolling a large number of participants and considering the criteria for ABO hemolytic disease, which were overlooked in previous studies and might explain the reason for more prevalent ABO incompatibility in their results. The limitation of this study was its retrospective nature. Since the data were collected from medical records, some details were not documented, resulting in incomplete data. Furthermore, we did not have ETCOc and albumin levels, which could help in determining the pathogenesis of ENH and susceptibility to ABE, respectively. Pediatricians and other healthcare providers should be educated on the causes, prevention, and diagnosis of ENH. Therefore, further prospective studies with more detailed evaluation such as the determination of minor blood group antibodies, ETCOc, and albumin levels are recommended. Moreover, a follow-up study of patients who have undergone ET should also be considered.

Conclusion

Extreme hyperbilirubinemia is a serious neonatal health issue, and finding the risk factors of ENH in each region is crucial for developing prevention strategies. The annual rate of ET has decreased during the last decade. However, ABE and kernicterus should be eliminated while the rate of ET should be reduced as much as possible. This study highlights the key risk factors of ENH in southern Iran.

G6PD deficiency was the most common cause of ENH, and the prevalence of G6PD deficiency in ENH and ABE suggested a cause-and-effect relationship between them. Consequently, the screening program for G6PD in Iran seems feasible. Late prematurity was the second risk factor, and appropriate management of preterm neonates was crucial for preventing ENH. ABO hemolytic disease, Rh hemolytic disease, minor blood group incompatibility, and sepsis were other etiologies of ET in neonates which required appropriate preventative strategies.

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Authors' Contribution

FH and HB: Concept and design. SMM, MB,

AHA acquisition of data. FH, HB, SMM, MB, and AHA drafting the manuscript. FH and HB revised the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Conflict of Interest: None declared.

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