Chapter

Neonatal Hyperbilirubinemia in Newborns of the Republic of North Macedonia

Anet Papazovska Cherepnalkovski, Natasha Najdanovska Aluloska, Nikolina Zdraveska, Katica Piperkova and Vjekoslav Krzelj

Abstract

Neonatal indirect hyperbilirubinemia is one of the most frequent neonatal problems that affect almost two thirds of term infants. Although etiology of jaundice has been widely studied, identification of pathological causes presents constant clinical challenge. Our study group performed an extensive retrospective study of etiology of neonatal hyperbilirubinemia and showed high frequency (44.37%) of jaundice of undefined etiology. The group included exaggerated physiological jaundice, early- and late-onset breast-milk jaundice, and no identifiable etiology. Other etiologies were neonatal infection, prematurity, birth trauma, and hemolysis represented with 15%. We described hematological parameters in both non-hemolytic and hemolytic type of jaundice; a significant correlation of relevant laboratory findings with etiology was established. In this chapter we will present our own data and perform a data-relevant literature review. Furthermore, investigation and management plan of neonatal indirect hyperbilirubinemia will be presented in accordance with own data and available literature.

Keywords: neonatal indirect hyperbilirubinemia, etiology, undefined jaundice, hemolysis, hematological parameters

1. Introduction

Neonatal hyperbilirubinemia is defined as a total serum bilirubin level >5 mg/dL (86 μmol/L). This is a frequently encountered problem during the first week of life that affects approximately 60% of term and 80% of preterm babies [1, 2]. About 10% of breastfed babies are still jaundiced at 1 month of age [1]. The yellowish coloration results from deposition of unconjugated bilirubin pigment into the skin and mucous membranes [2]. Generally, neonatal jaundice is considered a transitional phenomenon without noticeable clinical impact, related to hepatic, red cell, and gastrointestinal immaturity [1, 3]. However, hyperbilirubinemia in the newborn period can be associated with severe illnesses such as hemolytic disease, metabolic and endocrine disorders, anatomic abnormalities of the liver, and infections [2]. Acute bilirubin-associated neuropathy caused by a dangerous rise of the total serum bilirubin level can often progress into a chronic neurologic condition characterized as kernicterus.

The latter is characterized by a severe athetoid cerebral palsy, auditory and visual problems, dental enamel dysplasia, and, less frequently, intellectual and other dysfunctions [1, 2, 4, 5]. Neonatal hyperbilirubinemia develops as an interaction between environmental and genetic factors; however, growing attention is turned to the genetically determined conditions. Gene variants related with neonatal hyperbilirubinemia are those that encode the erythrocyte enzyme glucose-6-phosphate dehydrogenase (G6PD), the hepatic isoenzyme uridine diphosphate (UDP) glucuronosyl transferase 1A1 (UGT-1A1), as well as the hepatic solute carrier organic anion transporter 1B1 [6–8].

1.1 Pathophysiology of jaundice

Neonatal hyperbilirubinemia results from a predisposition to a higher production of bilirubin in newborn infants and their limited ability of bilirubin excretion [9].

Newborns, especially preterm newborns, have higher rates of bilirubin production than adults, because they have a higher red cell turnover and a shorter life span. Newborns produce bilirubin at a rate of approximately 6–8 mg per kg per day which is more than twice the production rate in adults [2].

Other limitations that are evident in newborn infants are decreased hepatic uptake of bilirubin from plasma due to decreased ligandin and limited ability to conjugate bilirubin due to decreased activity of the hepatic conjugating enzyme UDP glucuronosyl transferase (UGT-1A1) [9, 10]. The products of the conjugation reaction are transferred via the bile into the intestines. In the newborns' intestines, considerable amount of the conjugated bilirubin is hydrolyzed back to unconjugated bilirubin. This reaction is catalyzed by the enzyme beta glucuronidase. The unconjugated bilirubin is reabsorbed back into the bloodstream by means of the enterohepatic circulation, thus adding an additional bilirubin load to the already-overstretched liver. Hence, enterohepatic circulation of bilirubin represents an important contributor to neonatal jaundice [10].

All the abovementioned features in the newborn infants' bilirubin metabolism contribute concurrently to the appearance of physiologic neonatal jaundice.

Physiologic jaundice refers to the transient increase of the serum bilirubin in term infants during the first week of life, followed by a constant decrease over the next few weeks to normal levels found in adults. Average peak serum bilirubin levels (TSB) found in physiologic jaundice vary between 5 and 6 mg/dL (86 and 103 μmol/L). Exaggerated form of physiologic jaundice is considered when levels of TSB extend to values of 7–17 mg/dL (104–291 μmol/L) [9]. And, when serum bilirubin levels increase above 17 mg/dL (291 μmol/L) in term infants, a pathologic cause of jaundice should be pursued [2, 9].

1.2 Etiology of pathologic jaundice

According to the mechanism of accumulation of bilirubin, causes of neonatal indirect hyperbilirubinemia are classified into three categories (**Table 1**).

1. *Bilirubin overproduction* ensues with hemolytic causes of disease such as Coombs-positive blood group incompatibilities in the ABO, rhesus, or minor blood group systems. On the other side of the hemolytic spectrum are the Coombs-negative disease causes such as erythrocyte membrane or enzyme defects, defects of hemoglobin (Hb) synthesis, sepsis, and some drugs. Bilirubin overproduction is also a mechanism of bilirubin accumulation in non-hemolytic disease causes such as cephalohematoma, bruising, central nervous system (CNS) hemorrhage, polycythemia, and exaggerated enterohepatic circulation [2, 9, 10].

Increased bilirubin load	Decreased bilirubin conjugation	Impaired bilirubin excretion
Hemolytic causes • Positive Coombs test ABO incompatibility, rhesus incompatibility, other blood group incompatibilities • Negative Coombs test Red blood cell membrane defects (spherocytosis, elliptocytosis, pyropoikilocytosis, stomatocytosis) Red blood cell enzyme defects (G6PD deficiency, pyruvate kinase deficiency, other deficiencies) Hemoglobinopathies (alpha thalassemia, beta thalassemia) Unstable hemoglobins: congenital Heinz body hemolytic anemia Drugs (vitamin K) Sepsis	Physiologic jaundice Crigler-Najjar syndrome types 1 and 2 Gilbert syndrome Hypothyroidism Breast-milk jaundice G6PD deficiency	• Biliary obstruction Biliary atresia, choledochal cyst, primary sclerosing cholangitis, gallstones, neoplasm, Dubin-Johnson syndrome, Rotor's syndrome • Infection Sepsis, urinary tract infection, syphilis, toxoplasmosis, tuberculosis, hepatitis, rubella, herpes • Metabolic disorder Alpha-1-antitrypsin deficiency, cystic fibrosis, galactosemia, tyrosinemia, glycogen storage disease, Gaucher's disease, hypothyroidism, Wilson's disease, Niemann-Pick disease • Chromosomal abnormality Turner's syndrome, trisomy 18 and 21 syndromes • Drugs Aspirin, acetaminophen, sulfonamides, alcohol, rifampin, erythromycin, corticosteroids, tetracycline
Non-hemolytic causes • Extravascular sources Cephalohematoma, bruising, CNS hemorrhage, swallowed blood • Polycythemia Fetal-maternal transfusion, delayed cord clamping, twin-twin transfusion • Exaggerated enterohepatic circulation Cystic fibrosis, intestinal atresia, pyloric stenosis, Hirschsprung's disease, breast-milk jaundice		

Information from Refs. [2, 10].

Table 1.
Classification of neonatal jaundice based on the mechanism of accumulation.

2. *Decreased bilirubin conjugation* is present in etiologies such as in physiologic jaundice, breast-milk jaundice, Crigler-Najjar syndrome types 1 and 2, hypothyroidism, Gilbert syndrome, and glucose-6-phosphate dehydrogenase deficiency (G6PD deficiency). Hemolysis was traditionally considered the pathophysiological mechanism of jaundice in G6PD deficiency, and indeed some known hemolysis triggers such as menthol or Chinese remedies applied to the umbilicus for antisepsis have been described in association with this etiology of jaundice. Moreover, other assumed triggers of hemolysis, such as fava transmitted through human breast milk, chemical cleansers, bacterial and viral infections, and henna applied to the newborn's skin in some cultures of the Middle East, have been described into association with G6PD deficiency-linked neonatal hyperbilirubinemia. However, the hematological markers of hemolysis such as hemoglobin and hematocrit (Hct) values and reticulocyte count have only occasionally been reduced in infants with G6PD

deficiency-associated hyperbilirubinemia [11, 12]. It has been shown that inadequate conjugation capacity of the liver mainly contributes to development of neonatal jaundice in G6PD-deficient infants [12–14]. This has been proven by significantly lower total serum bilirubin level as well as mono- and di-conjugated bilirubin fractions in G6PD-deficient newborns that developed hyperbilirubinemia than the non-hyperbilirubinemic G6PD-deficient newborns [13]. Research that further supports the report that the decreased bilirubin conjugation is the main element of jaundice in G6PD-deficient newborns has shown varying shortening of red cell life span, which could only partially contribute to the jaundice [11, 13]. Moreover, the problem of jaundice is potentiated in cases of inherited UDP glucuronosyltransferase promoter polymorphism associated with Gilbert syndrome [14, 15]. The combination of the two gene mutations has been shown to significantly increase the incidence of hyperbilirubinemia in a dose-dependent manner [15].

3. And finally, the third mechanism of jaundice marked by *impaired bilirubin excretion* causes direct (conjugated) hyperbilirubinemia [2, 9, 10, 16]. Neonatal sepsis can be featured by both indirect and direct hyperbilirubinemia [2, 16].

1.3 Neurotoxicity of bilirubin

1.3.1 Cellular toxic effects of bilirubin

Even though being of great clinical importance, hyperbilirubinemia neurotoxicity effects on the cellular level are not entirely understood. It has been established that the mitochondria could be the primary target of the bilirubin neurotoxicity as evidenced by uncoupling of oxidative phosphorylation. Additional effects expressed in neuronal tissue include inhibition of DNA synthesis, induction of DNA strand breakage, inhibition of protein synthesis, and changes in neurotransmitters' synthesis and function. Experiments in immature rats have shown association between hyperbilirubinemia and impaired cerebral glucose metabolism [9].

1.3.2 Neurotoxicity risk factors

Of specific clinical importance is to recognize the risk factors associated with brain damage in newborn infants with significant hyperbilirubinemia. According to the 2009 AAP recommendation, neurotoxicity risk factors are isoimmune hemolytic disease, G6PD deficiency, asphyxia, sepsis, acidosis, and albumin <3.0 mg/dL [17]. The neurotoxicity risk factors are used in making the decision when to initiate phototherapy or perform an exchange transfusion. These interventions are recommended at a lower bilirubin threshold level in the presence of any of the neurotoxicity risk factors [17].

Prematurity represents a well-recognized predisposition to development of jaundice. In premature newborns the rise of the total serum bilirubin tends to be slightly slower but of longer duration than term newborns [18]. There is still insufficient amount of evidence-based data to provide recommendations for treatment in this group of patients. Recommendations are mainly based on consensus agreement-based guidelines on the safe spectrum of thresholds [19, 20]. Bilirubin neurotoxicity has been associated with prematurity; however, birth weight and gestation are not the sole variables predictive of the neuronal damage. Other factors such as the presence of a concurrent neonatal disease, sepsis, cholestasis, drugs that alter the albumin-bilirubin binding, or the use of total parenteral nutrition have been found to enhance the risk of neurotoxicity. Moreover, premature newborns have similar

but often more subtle clinical manifestations of acute bilirubin encephalopathy than term infants [21–23]. For all the abovementioned reasons, it is reasonable to observe premature newborns as a distinct entity of neonatal jaundice and not assign them to an "undetermined etiology" group as done by certain authors [16].

1.3.3 Kernicterus

This term refers to the neurologic consequences of the deposition of unconjugated bilirubin in brain tissue with subsequent damage and scarring of the basal ganglia and brainstem nuclei. Determinants of the neurotoxic effect of bilirubin are the duration of exposure and the concentration of bilirubin in the brain. Poor correlation exists between serum bilirubin level and bilirubin encephalopathy in the absence of hemolysis [9]. Other important determinants of bilirubin influx in the brain are the bilirubin-binding capacity of albumin and the integrity of the blood-brain barrier. If the serum unconjugated bilirubin level exceeds the bilirubin-binding capacity of albumin, unbound lipid-soluble bilirubin crosses the blood-brain barrier. Conditions that alter the permeability of the blood-brain barrier such as sepsis, acidosis, hypoxia, hyperoxia, hypoperfusion, and hyperosmolality can potentiate bilirubin entry in the brain [2, 9]. Differentiating neurons are particularly sensitive to bilirubin-related injury; therefore, premature newborns are more susceptible to the effects of bilirubin deposition in the brain [9]. For the purpose of greater consistency when defining bilirubin-induced neurological damage, it has been recommended to separate the terms "acute bilirubin encephalopathy" and "kernicterus." The former is used to describe the acute manifestations of bilirubin toxicity in the first weeks of life, whereas the latter is reserved for the chronic and permanent clinical sequelae of bilirubin toxicity [4]. The exact bilirubin concentration associated with kernicterus in the healthy term infant is unpredictable. Toxicity levels may vary among ethnic groups, also with maturation of an infant, and in the presence of hemolytic disease. The clinician's concerns of possible bilirubin toxicity should rise in the presence of bilirubin >25 mg/dL (428 μmol/L) in the term newborn without hemolysis and > 20 mg/dL (342 μmol/L) in the term newborn with hemolysis [2]. The early phase of acute bilirubin encephalopathy is characterized by lethargy, hypotonia, and poor sucking. In the intermediate phase, irritability and hypertonia develop. The infant may develop a fever and high-pitched cry, which may alternate with drowsiness and hypotonia [4, 24]. The hypertonia is demonstrated by backward arching of the neck (retrocollis) and trunk (opisthotonos). The advanced phase is characterized by pronounced hypertonia, apnea, and fever, deep stupor to coma, sometimes seizures, and death. Features of chronic bilirubin encephalopathy (kernicterus) include athetoid cerebral palsy, hearing loss, visual and dental problems, and moreover intellectual and other handicaps [1, 2, 4, 5, 9, 10].

1.4 Laboratory evaluation of jaundice

Laboratory evaluation of jaundice is directed by the age of the newborn. The first step in evaluation, for a newborn jaundiced in the first 24 hours of life, is to perform total serum bilirubin (TSB) or transcutaneous bilirubin (TcB) measurement [2, 4, 10]. Transcutaneous bilirubin (TcB) can be a powerful and noninvasive screening tool for bilirubin estimation with reported close correlation to TSB measurement in different populations [10]. When jaundice appears excessive for newborn's age, a TSB should be obtained. In infants under phototherapy and TSB above the 75th percentile or rising rapidly (i.e., crossing percentiles), it is recommended to extend the diagnostic workout by performing additional tests such as complete blood count and smear, reticulocyte count, blood grouping, and Coombs

test as well as end-tidal carbon monoxide levels. In cases of specific ethnic origin or positive family history, analysis of G6PD and pyruvate kinase deficiencies is considered. Once direct (or conjugated) bilirubin level is elevated, urinalysis, urine culture, and evaluation for sepsis are recommended. As per jaundice persisting beyond the third week of life, a diagnostic protocol for identification of cholestasis causes should be followed [2, 4, 10].

1.5 Treatment of jaundice

1.5.1 Phototherapy

It is a standard method for treatment of hyperbilirubinemia that is applied when bilirubin levels exceed gestation and hour-specific treatment thresholds [1, 4, 9, 25–27]. It is effective through photoisomerization of bilirubin to a water-soluble product that is readily excreted via bile or urine. The efficacy depends on the wavelength and the dose of the delivered light, as well as on the illuminated skin surface area [9, 27]. Specific phototherapy treatment graphs have been developed to address the phototherapy needs in term and in preterm babies [10, 26, 27]. General measures are involved concurrently to phototherapy such as maintenance of fluids and treatment of underlying disease cause such as infection [27].

1.5.2 Exchange transfusion (ECT)

It is a method for rapid elimination of the bilirubin and the circulating antibodies from the circulation, therefore most beneficial in cases of ongoing hemolysis. Small amount of blood are removed through a central venous catheter and replaced with the same amount of donor red blood cells suspended in plasma. The procedure is repeated until twice the blood volume of the newborn is replaced with the donor blood. This procedure involves multiple complications among which most pronounced are graft-versus-host disease, necrotizing enterocolitis, and portal thrombosis [1, 9]. Although being the first therapy for severe jaundice, this intervention is becoming virtually obsolete and reserved only for cases of severe hyperbilirubinemia that could not be managed by intensive phototherapy. Likewise phototherapy, exchange transfusion treatment threshold graphs have been devised for term and preterm gestations to serve as clinical guidance for initiation of therapy [10, 26, 27]. Exchange transfusion should only be performed in highly developed neonatal intensive care units (NICU) adequately equipped for monitoring and resuscitation as well as with trained personnel [4].

1.5.3 Pharmacologic therapies

These drugs interfere with variable effectiveness at different stages of the bilirubin metabolism. For example, phenobarbital has been used to improve the conjugation and excretion of bilirubin. Tin mesoporphyrin inhibits heme oxygenase thereby acting on the production of bilirubin. Other drugs are involved with the enterohepatic circulation of bilirubin [9, 10]. Intravenous immunoglobulin has been shown to significantly reduce the need for exchange transfusion in Rh or ABO hemolytic disease [4].

2. Neonatal hyperbilirubinemia in the Republic of North Macedonia

Our study group performed an extensive retrospective study for the purpose of evaluation of the etiology and management of indirect hyperbilirubinemia at the

University Pediatric Clinic in Skopje (UPCS), now Republic of North Macedonia (RNM). The study group included 284 newborns who had been admitted to the neonatology department at the University Pediatric Clinic in Skopje with the diagnosis of neonatal indirect hyperbilirubinemia during the period of 2 years [28]. They represented one quarter of the total number of 1126 hospitalized patients during this period in a tertiary level university teaching clinical hospital setting. Relevant history, clinical data, laboratories, and the type of therapy applied were retrieved from the medical records, recorded on questionnaires, and statistically analyzed. Perinatal history data of relevance were birth parameters, Apgar scores, and delivery mode. Clinical presentations that could potentially influence duration and intensity of jaundice had been searched for such as hematomas, cephalohematoma, intracranial hemorrhages, hypothyroidism, impaired intestinal motility, and infection. All laboratories and investigations of relevance were recorded, as well as the therapies applied. Moreover, the day of the bilirubin peak was noted, as well as two subsequent bilirubin measurements. Standard techniques for analysis of blood count and smear, bilirubin and fractions, serum aminotransferases, and G6PD, as well as infants' and mothers' blood group and direct antiglobulin Coombs test (DAT), were applied as described elsewhere [12, 28]. Statistical Package for the Social Sciences (SPSS) for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Absolute numbers and percentages were used to present the categorical variables, whereas mean, standard deviation, minimum, maximum, median, and rang were used to present the quantitative variables. Testing of significance between groups was performed using Kruskal-Wallis test, student *t*-test, Mann–Whitney *U* test, and analysis of variance. The result was considered significant if probability value (p) was <0.05 and <0.01 for high significance. Nine types of jaundice had been identified and grouped as follows: (1) ABO incompatibility, (2) rhesus (Rh) incompatibility, (3) cephalohematoma and bruising, (4) sepsis, (5) prematurity, (6) intracranial hemorrhage, (7) hemolysis (neither ABO nor Rh incompatibility), (8) Down syndrome, and (9) undefined. The highest prevalence was found for jaundice of undefined etiology (44.37%), and the second most prevalent was etiology of neonatal infection represented with 19.37%. Hemolytic etiologies were represented with 8.45% for ABO incompatibility, 5.63% for Rh incompatibility, and 0.35% for hemolysis neither ABO nor Rh or in total 14.43%. Least prevalent were cephalohematoma and bruising due to birth trauma (2.82%), intracranial hemorrhage (2.46%), and Down syndrome (0.70%) (**Table 2**) [28].

Etiology of sepsis was assigned to newborns with a positive blood/cerebrospinal fluid culture or clinically relevant infection requiring antibiotic therapy. Subjects that had sepsis and elevated direct bilirubin were not included [2, 28]. Prematurity, defined as less than completed 37 weeks of gestational age, was considered a distinct etiology of jaundice. Our group of undefined etiology included cases of early- and late-onset breast-milk jaundice, exaggerated physiological jaundice, [2, 9, 28], and no identifiable etiology. Cephalohematoma and bruising were representatives of birth trauma.

2.1 Undefined etiology of jaundice

We found a high percentage of jaundice of undefined etiology (44.37%). Another study reported higher prevalence of undetermined etiology (75.8%) [16]. Clinical evaluation of severe neonatal hyperbilirubinemia in a resource-limited setting similarly showed highest prevalence of idiopathic jaundice (33.3%) [24]. No cause for the extreme hyperbilirubinemia of ≥25 mg per dL (428 μmol per L) could be identified in 65.6% of cases admitted for treatment at the NICU in Southern Turkey [29]. Etiology was unknown in 11 of the 79 ECT cases (13.9%) in the Eastern Mediterranean region of Turkey as reported by Davutoğlu et al. [30]. Dissimilarly,

Etiology	Number	Percentage (%)
Undefined etiology	126	44.37
Neonatal infection	55	19.37
Prematurity	45	15.85
ABO incompatibility	24	8.45
Rh incompatibility	16	5.63
Cephalohematoma and bruising due to birth trauma	8	2.82
Intracranial hemorrhage	7	2.46
Hemolysis (neither ABO nor Rh incompatibility)	1	0.35
Down syndrome	2	0.70
Total	284	100

Information from Ref. [28].

Table 2.
Causes of neonatal indirect hyperbilirubinemia in the republic of North Macedonia.

	Mean	Min	Max	*M*	Interquartile range
Age	4 ± 2.5	2	14	3	3–4
GW	39 ± 1.2	37	42	39	38–40
BW	3247.1 ± 437.4	2200	4500	3245	2980–3500
BL	50.2 ± 1.8	46	56	50	49–51
Mode of delivery	N	%			
Spontaneous	114	90.47			
CS	9	7.14			
Vacuum extraction	2	1.59			
Forceps	1	0.8			
Perinatal hypoxia	N	%			
No	109	86.51			
AS 7	16	12.7			
AS 4–6	1	0.79			

Min, minimum; Max, maximum; M, median; GW, gestation weeks; BW, birth weight; BL, birth length; CS, cesarean section; AS, Apgar score. Information from Ref. [28].

Table 3.
Basic characteristics of the undefined etiology group at UPCS, RNM.

we did not find undefined etiology among our ECT cases. It could be assumed that the variable prevalence of "undefined etiology" reported in different studies was a result of diverse classification of the causes of neonatal jaundice and also of different levels of TSB considered (pathologic or extreme). We described undefined etiology as such, where intensive workout could not provide an identifiable cause or contributing factor for jaundice. Through a careful selection process, a homogenous group of clinically stable patients was obtained that had normal birth parameters and required treatment with phototherapy (**Table 3**). We speculated that an imbalance between bilirubin production and conjugation was the primary concept of jaundice in this group since no history, clinical, and laboratory data existed to indicate another mechanism of jaundice [28, 31].

The basic characteristics of the undefined etiology group are presented in **Table 3**. Newborn infants of this group were generally delivered spontaneously (90.47%) with normal birth parameters [birth weight (BW) and birth length (BL)] and did not suffer major perinatal hypoxia. The median (interquartile range) age of presentation of jaundice was at day 3 (3–4) (**Table 3**). In the group of undefined etiology, the median (interquartile range) day at which bilirubin reached its peak was 9 (6–17). The median (interquartile range) of the peak TSB level was 324 (270–394) μmol/L, whereas the mean ± standard deviation (SD) peak serum bilirubin concentration was 333.4 ± 91.1 μmol/L.

Statistical analyses included comparison of laboratory parameters between five etiological groups: (1) hemolytic etiology of jaundice including ABO incompatibility, Rh incompatibility, and hemolysis (neither ABO nor Rh incompatibility), (2) neonatal infection/sepsis, (3) prematurity, (4) hematomas (cephalohematoma, bruising, intracranial hemorrhage), and (5) undefined etiology.

To summarize the analyzed laboratory parameters, mean peak bilirubin levels in newborns with hemolysis (group 1) were shown to be statistically significantly higher than levels in the groups with neonatal infection, prematurity, and hematomas (groups 2, 3, and 4). The first control serum bilirubin level was significantly higher in newborns with hemolysis (group 1) than prematurity and undefined etiology (groups 3 and 5). No statistically significant differences were found in the second control bilirubin measurement; also levels of hepatic transaminases (AST and ALT) were not found to depend significantly on the etiology of jaundice. Estimation of hepatic transaminases has not proven of substantial influence on jaundice workout and management.

2.2 Prematurity and jaundice

Premature newborns, due to physiological characteristics, associated risk factors, and proneness to development of pronounced jaundice, were assigned a separate etiological group contrary to assignment of these patients into the "undetermined etiology" performed by other authors [16]. We were able to show slower increase toward the peak bilirubin level in the group of premature newborns than in groups of hemolysis, hematomas, and infection. Levels of erythrocytes (Er), hemoglobin (Hb), and hematocrit (Hct) in premature newborns were statistically significantly lower than the groups of undefined etiology and infection.

2.3 G6PD deficiency

We did not find cases of G6PD deficiency in the studied group. Although no cases of G6PD deficiency were confirmed, a standard was set for a new quantitative spectrophotometric assay for G6PD detection, thereby overcoming the uncertainties connected with the previously used qualitative methods. Previous qualitative studies of the G6PD deficiency in Macedonia are those of Fraser et al. [32] and of Andreeva et al. [33]. The first group of authors assessed the average prevalence of the G6PD deficit in Yugoslavia from 1% [32] based on tests carried out on 144 samples from then Republic of South Macedonia and 512 samples from the region of Dalmatia. The second group of authors in 1974 examined the prevalence of the G6PD deficiency in 3263 male school children from the area of Southeastern Macedonia (territory of nowadays Republic of North Macedonia) and showed a frequency of 1–2% of the G6PD deficit in that part of the republic. In the second examination of the same group of authors, realized on samples of 1196 male school children from the territory of Skopje, when processing the enzyme, it was concluded that it was a Mediterranean variant and the prevalence of the

deficit of 1.02% was reported among the children of Macedonian nationality and 6.63% for Roma children [33]. Quantitative testing for G6PD deficiency has been recommended to be performed, thus avoiding partially G6PD-deficient patients such as heterozygous females to be missed [34, 35]. As much as 1/3 higher levels of G6PD in the neonatal period can be encountered due to the presence of physiologic polycythemia in this period [35]. Therefore, it is reasonable to schedule for another subsequent test in cases of borderline normal results and a specific ethnic origin.

In a subsequent neonatal jaundice study, we showed an incidence of 8.57% of G6PD-deficient infants in a strictly prospectively selected group of infants with jaundice of undetermined etiology (own unpublished results). From this study, a population-specific range of normal values for the G6PD quantitative spectrophotometric assay will be derived.

2.4 Extravasation of blood and jaundice

A separate group of patients with hematomas was developed, encompassing patient with extravascular collections of blood where an increased bilirubin load was presumed the fundamental mechanism of hyperbilirubinemia [2, 9, 16, 18, 28]. No statistically significant hematological correlations between this group and the other four groups of patients were found.

2.5 Infection-associated jaundice

Sepsis is a known perinatal risk factor for both unconjugated and conjugated jaundice [2, 9, 18] and is also listed as a risk factor for hyperbilirubinemia neurotoxicity [17]. Analysis of prevalence rates in different regions of the world showed varying importance of infection in connection with jaundice. Highest variability of prevalence rates was reported in Asia (from 9.7 to 31.2%). In Africa infection was related with over 13.9% of the hyperbilirubinemia or kernicterus cases, whereas in Europe and North America, infection was related with 14.3% of the kernicterus cases [1].

In our study, the group of infection-associated jaundice was represented with 19.37%. On the contrary, sepsis was found in almost twice as much (35.3%) severe hyperbilirubinemia cases in South East Nigeria [24]. Similar to the North Macedonian study, sepsis was present in 15.7% indirect hyperbilirubinemia cases at Zanjan Province of Iran [16]. We assumed our figure an overrepresentation due to the fact that not only culture positive cases were included but also newborns with clinical or biochemical markers of sepsis. Reliable discrimination between culture positive and culture negative cases was not possible due to the variety of processing of initial hemoculture between the tertiary level and the referral hospitals. Therefore, the term "infection" rather than "sepsis" was used for more accurate reflection on this group of patients. Statistically significant higher levels of hematological parameters (Er, Hb, and Hct) were shown for this group than the hemolytic group and the premature newborns.

2.6 Hemolytic jaundice

We have established a group of hemolytic jaundice according to the mechanism of the hyperbilirubinemia employed in cases of ABO and Rh isoimmunization. According to the 2009 update on the management of newborn infants ≥35 weeks' gestation, isoimmune and other hemolytic diseases (e.g., G6PD deficiency) were included in two important of risk factors' categories: severe hyperbilirubinemia and hyperbilirubinemia-induced neurotoxicity [17]. Furthermore, it has been

postulated that DAT-positive isoimmune hemolytic disease and severe hyperbilirubinemia exert synergistic effect in potentiating the bilirubin-induced neurotoxicity [36]. Lower phototherapy and exchange transfusion threshold levels have been recommended in isoimmune hemolytic disease in order to prevent the acute manifestations of bilirubin toxicity that might evolve into chronic neurological condition, kernicterus, also a pre-discharge risk assessment and early post-discharge follow-up [4, 17, 25–27]. Tiker et al. report isoimmunization in 19 out of 93 (20.43%) patients admitted for treatment of extreme hyperbilirubinemia in Southern Turkey [29]. ABO isoimmunization was reported the most common cause of hyperbilirubinemia requiring ECT in two other studies performed in Turkey; the reported rates were 38% and 27.8%, respectively [30, 37]. ABO incompatibility was present in 8.45% of our study cases. Rh incompatibility was represented with 5.63% of all hyperbilirubinemia cases. We found one hemolysis positive patient who had neither ABO nor Rh incompatibility. The pooled prevalence of all hemolytic etiology cases in our study was 14.43%. When compared to the groups of neonatal infection, prematurity, and hematomas, the group of hemolytic etiology presented with significantly higher peak bilirubin levels. A statistically significant higher level of bilirubin in hemolytic etiology than prematurity and undefined etiology was also noted on the first control bilirubin level estimation. This observation pointed out a slower tendency of reduction of bilirubin under phototherapy in hemolysis than undefined etiology. However, the majority of cases with hemolytic etiology (97.89%) were managed conventionally by phototherapy using double-surface blue light phototherapy lamps at wavelength of 460 nm, and only 2.11% Coombs-positive ABO/Rh incompatibility patients were treated by exchange transfusion.

2.7 Laboratory analyses

A comparison of hematological and biochemical parameters was performed between groups of patients with undefined (unspecified) etiology (126 patients, 74.5%) and 41 patients with ABO or rhesus-type hemolytic disease of the newborn (24.6%).

The group of newborns with ABO/Rh incompatibility presented with significantly lower mean values of all analyzed hematological parameters than the group of jaundice with unspecific etiology [hemoglobin ($p = 0.038$), erythrocytes ($p = 0.0023$), and hematocrit ($p = 0.037$)] (**Table 4**) [38]. Mean reticulocyte count was significantly higher ($p = 0.000036$) in the group of ABO/Rh incompatibility (27.88 ± 26.4 vs. 11.94 ± 7.4). The group of hemolytic etiology was characterized by significantly earlier jaundice appearance ($p < 0.01$) than the group of jaundice of unspecified etiology. The mean ± standard deviation (SD) for hemolysis group was 2.63 ± 2.4 days versus 4.02 ± 2.5 days for the unspecified etiology group. The peak bilirubin level (mean ± SD) in hemolysis group of 379.76 ± 133.5 μmol/L was higher than unspecified etiology (333.44 ± 91.1 μmol/L) although the differences were not statistically significant ($p = 0.052$). Statistically insignificantly higher levels at the first ($p = 0.062$) and second ($p = 0.448$) control bilirubin measurements were registered for the hemolytic etiology group than the unspecific jaundice group.

On the other hand, duration of the bilirubin peak was significantly lengthier ($p = 0.036$) for the group of unspecific jaundice. The mean ± SD for this group was 15.03 ± 25.7 days versus 10.22 ± 9.02 days for the ABO/Rh incompatibility group (**Table 5**) [38].

Despite the fact that we did not show statistically significant higher peak levels of bilirubin in the hemolytic etiology group than the other group of jaundice, a propensity toward faster elevation of bilirubin and more pronounced level of jaundice was noted. The peak bilirubin level showed significantly longer duration

Groups	Descriptive statistics			
	N	**Mean ± SD**	**Min-max**	***t*-value, *p***
Hb (g/L)				
ABO/Rh	41	155.02 ± 30.3	74–218	$t = 2.09$ $p = 0.038^{*}$
Unspecified	126	165.36 ± 26.5	105–224	
Er ($\times 10^{12}$)				
ABO/Rh	41	4.29 ± 0.8	2.05–5.81	$t = 3.09$ $p = 0.0023^{*}$
Unspecified	126	4.67 ± 0.6	3.27–6.58	
Hct (%)				
ABO/Rh	41	41.35 ± 8.9	18.9–61.9	$t = 2.11$ $p = 0.037^{*}$
Unspecified	126	44.26 ± 7.2	28.4–64.6	

$^{*}p < 0.05$.
t, student t-test; N, number of patients; SD, standard deviation; p, probability value. Information from Ref. [38].

Table 4.
Hematological parameters in neonatal jaundice, comparison between ABO/Rh incompatibility and unspecified etiology groups.

Groups	Descriptive statistics				
	N	**Mean ± SD**	**Median**	**Min-max**	***t*-value, *p***
Ret					
ABO/Rh	41	27.88 ± 26.4	22.0	2–121	$Z = 4.13$ $p = 0.000036^{**}$
Unspecified	126	11.94 ± 7.4	11.0	1–39	
Day of bilirubin peak					
ABO/Rh	41	2.63 ± 2.4	2.0	1–14	$Z = 5.78$ $p = 0.000^{**}$
Unspecified	126	4.02 ± 2.5	3.0	2–14	
Peak bilirubin level (μmol/L)					
ABO/Rh	41	379.76 ± 133.5	364.0	158–801	$Z = 1.95$ $p = 0.052$ NS
Unspecified	126	333.44 ± 91.1	324.0	107–598	
Duration of the bilirubin peak (days)					
ABO/Rh	41	10.22 ± 9.02	6.0	1–37	$Z = 2.09$ $p = 0.036^{*}$
Unspecified	126	15.03 ± 25.7	9.0	2–279	
First control bilirubin (μmol/L)					
ABO/Rh	40	274.2 ± 124.9	235.5	96–682	$Z = 1.87$ $p = 0.062$ NS
Unspecified	112	227.39 ± 80.7	211.5	60–473	
Second control bilirubin (μmol/L)					
ABO/Rh	24	227.46 ± 83.4	206.0	111–437	$Z = 0.76$ $p = 0.448$ NS
Unspecified	48	221.92 ± 48.3	228.5	51–314	

$^{*}p < 0.05$.
$^{**}p < 0.01$.
Z, (Mann-Whitney U test); N, number of patients; SD, standard deviation; p, probability value; NS, not significant. Information from Ref. [38].

Table 5.
Reticulocytes and bilirubin analyses in neonatal jaundice, comparison between ABO/Rh incompatibility and unspecified etiology groups.

in the group of unspecific jaundice. It remains speculative whether this was due to different mechanisms of jaundice involved, different responses to the phototherapy applied, or other influences such as diverse stringency to phototherapy.

According to an evidence-based review on neonatal hyperbilirubinemia, the majority of kernicterus cases occurred in infants with a bilirubin level higher than 20 mg/dL (342 μmol/L) [39]. It was obvious that our hemolysis cases with mean peak bilirubin levels of 379.8 ± 133.5 μmol/L were eligible for the neurotoxic effects of the hyperbilirubinemia, especially the ones toward the higher end of the spectrum and candidates for long-term neurodevelopmental follow-up. Therefore, clinicians' awareness of potential treats and harms that might be associated with isoimmunization is vital.

3. Conclusions

Neonatal indirect hyperbilirubinemia is a common phenomenon during the first week of postnatal life affecting almost two thirds of term newborns. The mechanism of neonatal jaundice is multifactorial, involving delicate balance between processes that potentiate bilirubin production and the ones that diminish bilirubin clearance. Although etiology of jaundice has been widely studied, identification of pathological causes presents constant clinical challenge.

Hyperbilirubinemia was found to be a common clinical presentation at the neonatology department of the University Pediatric Clinic in Skopje, Republic of North Macedonia, and encompassing one quarter of the hospitalized patients. Most cases suffered from a less severe jaundice of undefined etiology that had tendency to longer duration. Almost 15% of the hyperbilirubinemia cases presented with hemolytic causes of jaundice that had earlier and more severe peak of the bilirubin level. Those required immediate clinicians' attention and prompt management plan and were candidates for subsequent neurodevelopmental follow-up.

Conflict of interest

The authors declare no conflict of interest.

Author details

Anet Papazovska Cherepnalkovski[1*], Natasha Najdanovska Aluloska[2], Nikolina Zdraveska[2], Katica Piperkova[2] and Vjekoslav Krzelj[3]

1 University Clinic for Gynecology and Obstetrics, University Hospital Split and School of Medicine, University of Split, Croatia

2 University Children's Hospital, Medical Faculty, University Ss. Cyril and Methodius, Skopje, Republic of North Macedonia

3 University Clinic for Children's Diseases, University Hospital Split and School of Medicine, University of Split, Croatia

*Address all correspondence to: anet.cherepnalkovski@gmail.com

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