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Hematological abnormality and associated factors in newborns with hyperbilirubinemia before and after phototherapy at University of Gondar Comprehensive Specialized Hospital

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This study aimed to assess the magnitude of hematological toxicity and associated factors in newborns with hyperbilirubinemia. A cross-sectional study was conducted from April to December 2023. A total of 247 newborns were included. The data were collected using questionnaires and a data extraction sheet. Four 4 ml of blood was collected. A Sysmex KX-21 analyzer was used for blood analysis, and a Mindray BS-240 analyzer was used for bilirubin measurement. The data were entered into Epi-data and analyzed by SPSS. The logistic regression was used. The P value was set at 0.05. Before phototherapy, the hematological toxicities, such as anemia, leucopenia, and thrombocytopenia, were 45.7%, 22.2%, and 6.1%, respectively, whereas after phototherapy, anemia and thrombocytopenia, significantly increased, but the leucopenia, significantly decreased. The risk of developing anemia increased, 3.5, 2.7, and 2.1-fold among newborns with bilirubin > 18 mg/dl, with Rh blood group incompatibility, and treated with intensive phototherapy, respectively. Both low birth weight and intensive phototherapy increased the incidence of thrombocytopenia by 2 and 3.4-fold, respectively. Hematological toxicity was found to be a severe public health issue in newborns. Thus, strict follow-up and early detection of toxicity by considering aggravation factors are necessary.

Keywords Hyperbilirubinemia, Newborns, Phototherapy, Hematological parameters, Ethiopia

Abbreviations

AOR	Adjusted odds ratio
Hb	Hemoglobin

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CI	Confidence interval
COR	Crude odds ratio
G6PDH	Glucose-6-phosphate dehydrogenase
LBW	Low birth weight
MCV	Mean cell volume
PLT	Platelet
PT	Phototherapy
TSB	Total serum bilirubin
RBCs	Red blood cells
ROS	Reactive oxygen species
WBC	White blood cells
WHO	World Health Organization

Hyperbilirubinemia is a complication that occurs when the serum bilirubin concentration exceeds 5 mg per deciliter (mg/dl) within 24 h after birth[1]. It is characterized by yellowish discoloration of the skin, sclera, conjunctiva, and tongue[2]. Hyperbilirubinemia can be classified as physiological or pathological[3]. Physiologically, the bilirubin levels of newborns may increase up to one week after birth[4]. On the other hand, pathologically, bilirubin levels in newborns may precedes as increased after 1 week of birth[5].

Hyperbilirubinemia is a common complication in newborns. Globally, approximately 24 million newborns are at risk for developing hyperbilirubinemia each year[6]. It affects nearly 60% of term and 80% of preterm newborns[7] and frequently results in hospitalization[6]. More than 75% of affected newborns are born in low- and middle-income countries, with a high prevalence in South Asian regions and sub-Saharan Africa[8]. In Ethiopia, hyperbilirubinemia is the tenth most common cause of neonatal mortality and morbidity[9]. The common causes of hyperbilirubinemia in newborns are blood group incompatibility, glucose-6-phosphate dehydrogenase (G6PDH) deficiency, liver disease, treatment side effects, polycythemia, neonatal sepsis, maternal factors, excessive weight loss, and inappropriate feeding[10,11].

Moreover, pathological hyperbilirubinemia can result in various complications, such as lethargy, fever, irritability, oculomotor impairments, encephalopathy, neurotoxicity, and hematological toxicity[9,12]. To minimize these complications, treatment is needed. The main treatment options for pathological hyperbilirubinemia are phototherapy (PT), exchange transfusion[13], and pharmacological treatment[14]. These treatments are not safe and can also cause different side effects, such as hematological toxicity[15,16].

In newborns with hyperbilirubinemia, the cause of hematological abnormalities may be related to the initial cause of hyperbilirubinemia, the toxic effect of elevated bilirubin, and the side effects of treatment, especially PT[15,17,18]. The initial cause of hyperbilirubinemia can result in hematological abnormalities through decreasing the survival and production of blood cells. On the other hand, elevated bilirubin in serum affects hematological parameters by functioning as an antimutagenic agent, inducing antioxidant, immune-modulatory, and inducing antiproliferative and anti-apoptotic factors, degrading hemoglobin (Hb), and damaging DNA[19]. Phototherapy also induces hematological toxicity by directly damaging the cell membrane of blood cells and increasing blood cell vulnerability to apoptosis[20]. Besides, PT can affect hematological parameters through directly inducing the expression of cytokines and growth factors as well as the generation of reactive oxygen species (ROS)[21,22].

The synergistic effect of the initial cause of hyperbilirubinemia, the toxic effect of hyperbilirubinemia and PT side effects strongly increase the magnitude of hematological toxicity in newborns with hyperbilirubinemia[23]. In addition, factors such as the age of the newborn, low birth weight, genetic variability, polycythemia, sex, drugs, race, altitude, oxytocin induction, maternal illness, use of breast milk, type of PT used, duration of PT and presence of hemolytic disease affect hematological parameters in newborns with hyperbilirubinemia[24].

Hematological toxicities such as anemia, thrombocytopenia and impaired immune function can aggravate morbidity and mortality and negatively affect quality of life in newborns with hyperbilirubinemia[25,26]. As a result, investigating hematological toxicity and associated factors before and after hyperbilirubinemia treatment is important to benefit from early detection, prevention, and treatment of this toxicity[27,28]. This may help newborns with hyperbilirubinemia receive timely healthcare. In addition, investigating hematological toxicity may help policy makers develop and implement new guidelines. However, there is limited information regarding hematological toxicity and associated factors before and after PT among newborns with hyperbilirubinemia in Ethiopia and in the study setting. Therefore, the current study was performed to fill this gap by providing information that can ultimately reduce the risk of complications and improve outcomes for affected newborns.

Methods and materials

Study area, design, and period

The cross-sectional study was carried out among newborns with hyperbilirubinemia in Northwest Ethiopia from April 1 to December 30, 2023. The hospital is located approximately 738 km northwest of Addis Ababa, the capital city of Ethiopia, with an elevation of 2133 m above sea level and latitude and longitude of 12° 36′ N and 37′ 28′ E, respectively. The hospital is a teaching hospital in Ethiopia that provides health care services for more than 7 million individuals in a Gondar town and catchment area. It has different units; the neonatal intensive care unit offers critical care services to neonates and has a capacity of approximately 40 beds at any given time[29].

Population

All newborns with hyperbilirubinemia who were treated with PT in northwest Ethiopia were considered source populations. Moreover, all newborns with hyperbilirubinemia who were treated with PT in northwest Ethiopia within the study period were considered as study population.

Eligibility criteria

All newborns aged up to 28 days who were confirmed to have hyperbilirubinemia by laboratory tests and who received PT during the study period were included in this study. Newborns who had severe comorbidities, who were critically ill, who took immunosuppressants, who received iron supplements, who had critically ill mothers and whose mothers were not around were excluded from the study.

Variables

Hematological toxicity was considered an outcome variable. On the other hand, sociodemographic characteristics and clinical characteristics of the newborns, such as age, sex, weight, height, gestational age, feeding status, blood group, cause of hyperbilirubinemia, length of hospital stay, total serum bilirubin (TSB) level, place of delivery, duration of PT received, and type of PT, were considered explanatory variables. In addition, the sociodemographic, clinical, and obstetric characteristics of mothers, such as mother's age, history of sibling, mode of delivery, mother's residence, mother's blood group status, birth order, mother's complications, and maternal iron and folate status, were considered explanatory variables.

Sample size and sampling techniques

In the present study, the sample size was calculated by following a single population proportion formula using the following formula: $Z\alpha/2 = 1.96$, margin of error (d) = 5%, and expected magnitude of hematological toxicity = 50%, since no similar previous study regarding hematological toxicity in newborns with hyperbilirubinemia during the study period has been conducted. Subsequently, the sample size was calculated to be 384. However, the estimated study population was less than 10,000 in the study area, and the sample size correction formula was used. After the correction, the final sample size used in the current study was 247. The study participants were selected through the application of a systematic random sampling technique. To obtain actual study participants, the Kth interval was calculated and found to be two. Then, by using the lottery method, the first newborn was selected, and subsequently, in every two intervals, all study participants were selected.

Definitions

Hematological abnormalities were defined based on the hematological reference range of newborns by considering gestational and postnatal ages. Exclusive breastfeeding was defined as the practice in which a newborn solely consumes breast milk and not other liquids or solids[30]. Exclusive formula feeding was also defined as consuming only formula for the entire preceding time period[31]. Mixed feeding was defined as the practice in which breastfeeding by the newborn is supplemented with infant formula[32].

Data collection and laboratory methods

The demographic and clinical information of the study participants was collected using a structured interview-based questionnaire and a data extraction sheet by two trained nurse professionals. The questionnaires were developed by using American Academy of Pediatrics Clinical Practice Guidelines and by reviewing different studies[5,12,33]. Four milliliters of venous blood were collected before and after PT for laboratory analysis using a syringe under aseptic conditions by two trained laboratory professionals from each newborn. After collection, 2 ml of blood was transferred to a di-potassium ethylenediaminetetraacetic acid test tube for complete blood count and blood grouping. The remaining blood was transferred to a serum separator tube for bilirubin measurement. The collected samples were then transported to the analysis unit.

Complete blood analysis was performed using the Sysmex KX-21 automated three-differential hematology analyzer by senior medical laboratory technologists. The analyzer used a combination of impedance and optical principles for blood cell counts. In addition, the analyzer uses a spectrophotometric principle to measure the Hb concentration. Total and direct serum bilirubin levels were analyzed using a Mindray BS-240 automated chemistry analyzer by a senior medical laboratory technologist. It operated on the principles of photometry and potentiometry. Blood group of each newborn was determined using commercially prepared reagents and the forward blood grouping technique by the principal investigators. All laboratory analyses were performed in strict accordance with the manufacturer's instructions and standard laboratory operating procedures. After analysis, all laboratory data were collected by data extraction sheets by two trained laboratory professionals.

Data management and quality control

In the present study, to ensure the consistency of the questionnaires, they were initially prepared in the English language, translated into the local language and then retranslated back to English. Following this process, a pretest was performed on 5% of the total sample, and then, based on feedback, relevant modifications were made. One-day training was given for the data collectors about the data collection process and protocol. The performance of the hematology and clinical chemistry analyzer was assured by running commercially prepared quality control reagents. All laboratory data were collected through strict adherence to standard operational procedures. Furthermore, the investigators closely followed the data collection process and provided timely feedback.

Data analysis and interpretation

The completeness of the collected data was checked manually, and the data were entered into Epi-data software (version 4.6.0). Subsequently, the data were exported to SPSS version 25.0 for analysis. The data were described using descriptive statistics and are presented in tables and figures. The paired chi-square test was used to determine the presence of significant differences in hematological toxicity before and after PT. Additionally, independent chi-square test was used to determine the presence of a significant association between the outcome and the

explanatory variable. The strength of the association between hematological toxicity and the explanatory variable was measured using bivariable and multivariable logistic regression models. Multivariable logistic regression analysis was used for explanatory variables with p values less than 0.25 in bivariable logistic regression analysis to control for possible confounders. All logistic regression assumptions were checked and fitted. The crude odds ratio (COR) and adjusted odds ratio (AOR) within the 95% confidence interval (CI) were used to measure the degree of association between the bivariable and multivariable logistic regression analyses, respectively. For all the statistical analyses, a p value < 0.05 was used to determine the level of statistical significance.

Ethical consideration

Ethical approval was obtained from the ethical review committee of the School of Biomedical and Laboratory Science, College of Medicine and Health Sciences, University of Gondar with the letter reference number SBMLS 510/2023. Additionally, a permission letter was obtained from the hospital Chief Clinical Director. After providing an explanation of the possible benefits and risks, informed written consent was obtained from the parents or legal guardians. The collected data were kept confidential. Any abnormal findings obtained were linked to physicians for appropriate patient management.

Consent to participate and ethical approval

All the procedures were performed in accordance with the relevant guidelines and regulations. Ethical approval was obtained from the Ethical Review Committee of the School of Biomedical and Laboratory Sciences, College of Medicine and Health Science, the University of Gondar (Ref number SBMLS 510/2023). The objective and purpose of the study were explained to the medical director, and a permission letter was obtained to collect the data. After providing an explanation of the possible benefits and risks, informed written consent was obtained from the parents or legal guardians. The collected data were kept confidential. Any abnormal findings obtained were linked to physicians for appropriate patient management.

Results

Sociodemographic and clinical characteristics of newborns with hyperbilirubinemia

A total of 247 newborns with hyperbilirubinemia were enrolled in the study, for a 100% response rate**.** The majority of these, were male (142, 57.5%), aged less than 7 years (64.8%), had a gestational age less than 37 weeks (152, 72.9%), and had a birth weight less than 2.5 kg (141, 57.1%). Before PT treatment, 178 (72.1%) newborns had TSB levels greater than 18 mg/dl, but after treatment, the TSB levels of all newborns were less than 18 mg/dl. About 66% of newborns with hyperbilirubinemia had taken intensive PT. Regarding the cause of hyperbilirubinemia, ABO incompatibility (109/44.1%) was found to be the main cause (Table 1).

Sociodemographic and clinical characteristics of the mothers

The sociodemographic and clinical characteristics of all mothers whose newborns developed hyperbilirubinemia were included in this study. The majority of mothers included were aged less than 25 years (49.0%), followed by those aged between 25 and 35 121 years (39.3%). Seventy-nine (32.0%) mothers who participated in the study had a previous history of hyperbilirubinemia in newborns. Concerning birth order, 179 (72.5%) mothers were multiparous. Of the mothers included, 53 (21.5%) had complications, and 150 (60.7%) had taken iron and folate supplements during pregnancy (Table 2).

Hematological toxicities in newborns with hyperbilirubinemia before and after PT initiation

According current study, median value (IQR) of white blood cell, hemoglobin, platelet and MCV before PT were 14.5 (12.6–16.2), 10.9 (9.1–12.6), and 241 (187–241), whereas median value of white blood cell, hemoglobin, platelet and MCV after PT were 13.5 (11.1–15.1), 11.3 (9.4, 13.2), and 233 (129–284), respectively. Before the initiation of PT, the magnitude hematological toxicity, such as anemia, leucopenia, and thrombocytopenia, were 115/46.6% (95% CI 40.1, 52.6), 52/21.1% (95% CI 16.2, 26.3), and 18/7.3% (95% CI 4.5, 10.5), respectively. After PT, the magnitudes of hematological toxicity, such as anemia and thrombocytopenia, significantly increased ($p < 0.05$) to 133/53.8% (95% CI 47.8, 60.3) and 78/33.6% (95% CI 25.9, 37.2), respectively. In contrast, after PT treatment, the magnitude of leucopenia significantly decreased (p value < 0.05) to 21/8.5% (95% CI 5.3, 12.1) (Fig. 1).

Among the patients who experienced anemia before PT treatment, 101/87.8% had normocytic normochromic anemia, whereas 10/8% and 4/3.6% had microcytic hypochromic and macrocytic normochromic anemia, respectively. On the other hand, after PT, only normocytic normochromic anemia (125/94%) and microcytic hypochromic anemia (8/6.1%) were observed. Of anemic newborns before PT, 60 (52.2%), 36 (31.3%), and 19 (16.5) were found as mild, moderate and severe, respectively. Similarly, out of anemia newborns after PT, 73 (54.9%), 39 (29.3%), and 21 (15.8%) were found as mild, moderate and severe, respectively). Furthermore, among anemic newborns, 87.7% was preterm, while among thrombocytopenic and leucopenia newborns 63.5% and 82.0%, respectively were preterm newborns (Fig. 2). Regarding WBC toxicity, lymphopenia was the most common toxicity observed before and after treatment [22/8.9% (95% CI 5.7, 12.6) and 16/6.9% (95% CI 4.0, 10.5), respectively] (Supplementary Table S1).

Factors associated with hematological toxicity

In current study, in bivariable analysis variable, age of newborn, birth weight, gestation age, concentration of TSB, mother's residence, cause of hyperbilirubinemia, and height of neonate were significantly associated with hematological toxicities with p value of less than 0.25 before PT. After adjusting potential confounders,

Variables	Categories	Frequency (n)	Percentage (%)
Sex	Male	142	57.5
	Female	105	42.5
Age of newborns at admission (days)	< 7	160	64.8
	≥ 7	87	35.2
Birth weight at admission (kg)	< 2.5	141	57.1
	≥ 2.5	106	42.9
Birth weight after PT (kg)	< 2.5	152	61.5
	≥ 2.5	95	38.5
Gestation age	< 37	180	72.9
	≥ 37	67	27.1
Height of newborns	Low	102	41.3
	High	145	58.7
Concentration of TSB before PT (mg/dl)	< 18	69	27.9
	≥ 18	178	72.1
Concentration of TSB after PT (mg/dl)	< 18	247	100
	≥ 18	0	0
Type of PT received	Conventional	85	34.4
	Intensive	162	65.6
Duration of PT (h)	< 48	124	50.2
	≥ 48	123	49.8
Place of delivery	Hospital	172	69..6
	Health center	69	27.9
	Home	6	2.4
Newborns feeding status	Exclusively breastfed	182	73.7
	Exclusively formula fed	36	14.6
	Mixed	29	11.7
Cause of hyperbilirubinemia	ABO related	109	44.1
	Rh related	38	15.4
	Both ABO and Rh related	7	2.8
	Non-ABO and Rh related	98	39.7
Hospital stays (days)	<7	88	35.6
	≥ 7	159	64.4
ABO status of the newborn's	A	83	33.6
	B	119	48.2
	AB	4	1.6
	O	41	16.6
Rh status of the newborn's	Positive	233	95.5
	Negative	14	4.5

Table 1. Sociodemographic and clinical characteristics of newborns with hyperbilirubinemia who received PT treatment in Northwest Ethiopia, 2023. *n* frequency, *mg/dl* milligram per deciliter, *kg* kilogram, *PT* phototherapy, % percentage, *TSB* total serum bilirubin.

multivariate logistic regression analysis confirmed that, age of newborn, birth weight, concentration of TSB, mother's residence, cause of hyperbilirubinemia, and height of neonate were found to be significant determinants for hematological toxicities before PT.

Newborns with hyperbilirubinemia before PT who were younger than seven days (AOR 2.5, 95% CI (1.4, 4.6) and whose TSB level was greater than 18 mg/dl (AOR 3.5, 95% CI (1.7– 7.2)) were more likely to develop anemia than were those aged less than seven days and whose TSB level was less than 18 mg/dl, respectively. The occurrence of anemia was about 3 times greater in newborns with Rh blood group incompatibility (AOR 2.7, 95% CI 1.7, 7.2) than in those with other causes of hyperbilirubinemia. Moreover, being a newborn of a rural resident mother increased the risk of developing anemia by 2.6 times (AOR 2.6, 95% CI (1.4, 4.7)) compared with being a newborn of an urban resident's mother. The odds of thrombocytopenia were two times greater among newborns with birth weights less than 2.5 kg (AOR 2.0, 95% CI (1.0, 3.2)). An increase in the TSB decreased the risk of developing leucopenia by 60% (AOR 0.4, 95% CI (0.2, 0.8)) in contrast to a decrease in the TSB before PT (Table 4).

After PT variables such as type of PT received, duration of PT and birth weight after PT were significantly associated with hematological toxicities with p value of less than 0.25. After adjusting potential confounders,

Variables	Categories	Frequency	Percentage
Mother's age	< 25	121	49.0
	25–35	97	39.3
	> 35	29	11.7
Mode of delivery	CS	113	45.7
	VD	134	54.3
Birth order	Primiparous	68	27.5
	Multiparous	179	72.5
Sibling who had hyperbilirubinemia	Yes	79	32.0
	No	168	68.0
Mother's residence	Rural	160	64.8
	Urban	87	35.2
ABO status of the mother's	A	32	13.0
	B	44	17.8
	AB	24	9.7
	O	147	59.5
Rh status of the mother's	Positive	208	84.2
	Negative	38	15.2
Presence of any complications during pregnancy	Yes	53	21.5
	No	194	78.5
Mother's took iron and folate supplement	Yes	150	60.7
	No	97	39.3

Table 2. Sociodemographic and clinical characteristics of the mothers of newborns with hyperbilirubinemia who received PT treatment in Northwest Ethiopia, 2023. *CS* Cesarean section, *VD* Vaginal delivery, *n* frequency, % percentage.

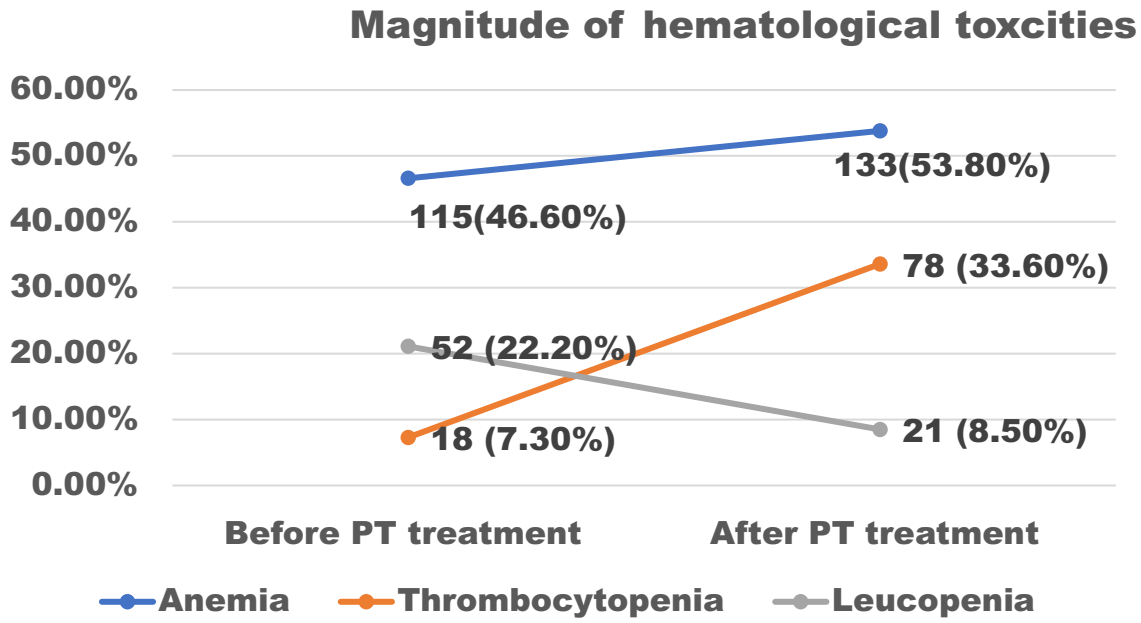

Figure 1. Magnitude of hematological abnormalities in newborns with hyperbilirubinemia before and after PT treatment in Northwest Ethiopia, 2023.

multivariate logistic regression analysis also affirmed that, all variables were found to be significant determinants for hematological toxicities.

Compared with conventional PT treatment, intensive PT treatment increased the risk of developing anemia (AOR 2.1, 95% CI (1.1, 4.2)) and thrombocytopenia (AOR 3.4, 95% CI (1.6, 6.9)) in newborns with hyperbilirubinemia. Newborns who received PT treatment for more than 48 h were approximately twice as likely to have anemia (AOR 1.8, 95% CI (1.1, 3.3)) and thrombocytopenia (AOR 2.1, 95% CI (1.6, 3.8)) than those who received

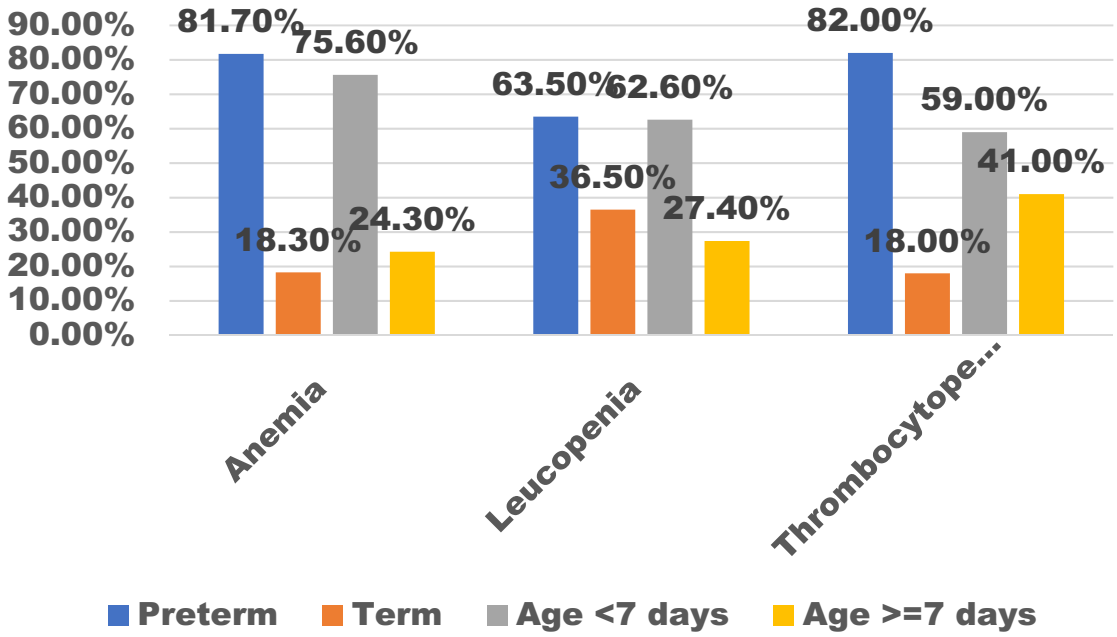

Figure 2. Distribution of hematological abnormalities based gestational and postnatal ages in newborns with hyperbilirubinemia before PT treatment in Northwest Ethiopia, 2023.

PT treatment for less than 48 h. All factors were assessed for leucopenia after PT treatment, but no statistically significant associations were observed (Table 5).

Discussion

The synergistic effect of the initial cause of hyperbilirubinemia, the toxic effect of bilirubin, and PT can aggravate hematological toxicity and result in morbidity and mortality in newborns with hyperbilirubinemia[27]. This study aimed to assess hematological toxicity and associated factors among newborns with hyperbilirubinemia before and after the initiation of PT. This study revealed various hematological toxicities.

Before PT, the incidence of anemia among newborns with hyperbilirubinemia was 46.6% (95% CI 40.1, 52.6). This figure shows anemia as a severe public health problem among newborns with hyperbilirubinemia in the study area according to the WHO public health significance classification of anemia[34]. A possible explanation for the high magnitude of anemia could be an increase in RBC distraction as a result of the initial cause of hyperbilirubinemia, such as blood group incompatibilities, G6PDH deficiency, liver disease, sepsis, and maternal factors[10,11]. Besides, hemolysis of RBCs by the toxic effect of bilirubin may increase the magnitude of anemia[35–37]. Furthermore, frequent blood sampling to assess different blood parameters in newborns may contribute to increased anemia[25]. The magnitude of anemia is greater than that reported in studies conducted in different area in Ethiopia[38–40], Cameroon[41], Afghanistan[42], and the United States[36]. This discrepancy could be explained by variations in sample size, study populations, and operational definitions.

On the other hand, after PT treatment, the magnitude of anemia significantly increased. This agrees with studies conducted in Turkey[43,44] and Egypt[28,45–47]. The possible reasons could be that PT may induce hemolysis in RBCs, increase oxidative stress in RBCs[48,49] and increase the osmotic fragility of RBCs[50]. Additionally, PT can directly damage DNA in immature RBCs and induce RBC eryptosis in the early stage. This may increase anemia after PT[26].

A study revealed that the risk of developing anemia is 2.5 times greater in newborns ≥ 7 days of age than in newborns < 7 days of age. This finding was consistent with previous studies performed in Ethiopia[11], Cameroon[41], and Japan[51]. This could be due to physiological changes in the Hgb concentration as the age of the newborn changes[52]. In addition, newborns with a TSB ≥ 18 mg/dl were 3.5 times more likely to develop anemia than those with a TSB < 18 mg/dl. This was concurrent with studies performed in Egypt[28] and Germany[53]. The possible reason could be the toxic effect of bilirubin, since bilirubin is known to be an inducer of RBC apoptosis, and increasing anemia as the bilirubin level increases is expected[53]. The occurrence of anemia was 2.7 times greater among newborns who were born with Rh blood group incompatibility. These findings are supported by previous similar studies[11,27,54]. Since Rh alloimmunization is a well-known cause of severe anemia, this finding is expected[55]. Furthermore, newborns who underwent intensive PT and who were treated with PT for more than 48 h were approximately two times more likely to develop anemia. The possible reason could be that PT may induce and exacerbate RBC hemolysis[48,49].

Thrombocytopenia was identified in 7.3% (95% CI 4.5, 10.5) of the newborns before PT treatment. The occurrence of thrombocytopenia before PT may be related to the aggregation, swelling, and apoptosis of PLTs due to elevated bilirubin[56]. The finding of the current study is lower than that of studies conducted in Ethiopia[57], Iran[58,59], India[17], and Egypt[28]. The reason for this disparity may be related to variability in the cutoff point used to define thrombocytopenia since the current study used the hematological reference range of newborns by considering

Logistic regression for anemia							
Variables	Category	Anemia		COR (95% CI)	P value	AOR (95% CI)	P value
		Yes	No				
Age of newborn	< 7	86	76	2.29 (1.4–3.7)	0.04	2.5 (1.4–4.6)	0.003
	≥ 7	29	58	1*	1*	1*	1*
Birth weight (kg) at admission	< 2.5	75	68	1.8 (1.2–3.2)	0.029	1.5 (0.8–2.7)	0.17
	≥ 2.5	40	66	1*	1*	1*	1*
Gestation age	< 37	93	89	2.1 (1.2–3.7)	0.014	1.4 (0.7–2.7)	0.4
	> 37	22	45	1*	1*	1*	1*
Concentration of TSB before PT	< 18	23	46	1*	1*	1*	1*
	≥ 18	92	88	2.1 (1.1–3.6)	0.016	3.5(1.7–7.2)	0.001
Cause of Hyperbilirubinemia	ABO related	54	62	1.5 (0.8–2.6)	0.19	1.4 (0.7–2.6)	0.33
	Rh related	19	14	2.3 (1.0–5.3)	0.042	2.7 (1.1–6.88)	0.047
	Both ABO and Rh related	9	3	5.1 (1.3–20)	0.02	7 (1.5–21)	0.012
	Non-ABO and Rh related	33	55	1*	1*	1*	1*
Mother's residence	Rural	86	76	2.3 (1.3–3.8)	0.004	2.6 (1.4–4.7)	0.002
	Urban	29	58	1*	1*	1*	1*
Logistic regression for thrombocytopenia							
Variables	Category	Thrombocytopenia		COR (95% CI)	P value	AOR (95% CI)	P value
		Yes	No				
Birth weight (kg) at admission	< 2.5	49	128	2.1 (1.2–3.6)	0.013	2.0 (1.0–3.2)	0.049
	≥ 2.5	29	119	1*	1*	1*	1*
Gestation age	< 37	64	117	2.0 (1.0–3.9)	0.035	1.7 (0.9–3.5)	0.15
	≥ 37	14	53	1*	1*	1*	1*
Logistic regression for leucopenia							
Variables	Category	Leucopenia		COR (95% CI)	P value	AOR (95% CI)	P value
		Yes	No				
Concentration of TSB before PT	< 18	22	47	1*	1*	1*	1*
	≥ 18	30	150	0.4 (0.2–0.8)	0.05	0.4 (0.2–0.8)	0.006
Height of neonate	Low	14	88				
	High	38	109	2.1 (1.1–4.1)	0.035	2.1 (1.0–4.1)	0.038

Table 4. Logistic regression analysis of hematological toxicity and sociodemographic and clinical characteristics of newborns with hyperbilirubinemia before PT treatment at the UoG-CSH, 2023. *AOR* adjusted odds ratio, *COR* crude odds ratio, *CI* confidence interval, *1** reference, *PT* phototherapy, *TSB* total serum bilirubin.

Variables	Category	Anemia		COR (95% CI)	P value	AOR (95% CI)	P value
		Yes	No				
Type of PT received	Conventional	15	70	1*	1*	1*	1*
	Intensive	68	96	3.2 (1.7–6.1)	0.001	2.1 (1.1–4.2)	0.033
Duration of PT (hours)	< 48	30	94	1*	1*	1*	1*
	≥ 48	53	72	1.9 (1.3–3.8)	0.004	1.8 (1.1–3.3)	0.038
Birth weight after PT (kg	< 2.5	66	88	3.3 (1.8–6.2)	0.001	2.7 (1.4–5.1)	0.003
	≥ 2.5	17	78				
Variables	Category	Thrombocytopenia		COR (95% CI)	P value	AOR (95% CI)	P value
		Yes	No				
Type of PT received	Conventional	18	67	1*	1*	1*	1*
	Intensive	60	102	2.2 (1.2–4.0)	0.012	3.4 (1.6–6.9)	0.001
Duration of PT (h)	< 48	30	94	1*	1*	1*	1*
	≥ 48	51	72	2.1 (1.3–3.7)	0.006	2.1 (1.6–3.8)	0.015
Birth weight after PT (kg)	< 2.5	42	53	2.5 (1.5–4.4)	0.001	4.4 (2.3–8.5)	0.001
	≥ 2.5	36	116	1*	1*	1*	1*

Table 5. Logistic regression analysis of hematological toxicity and clinical characteristics of newborns with hyperbilirubinemia after PT in the northwest region of Ethiopia, 2023.

gestational and postnatal ages, which is much different than that used in the above studies. Regarding associated factors, the odds of thrombocytopenia were two times greater among newborns with low birth weight (LBW) than among those with high birth weight. This finding is supported by a study conducted in Egypt[60]. The possible mechanism could be that LBW newborns often have less mature bone marrow, which might be less efficient for the production of PLTs[61].

After PT, thrombocytopenia significantly increased. This finding is similar to those of other studies[28,62–64]. The potential mechanism for the increase in thrombocytopenia following PT could be the effect of white light on PLTs. Photosensitization of PLTs induces the formation of hematoporphyrin, functional impairment, aggregation and the release of essential substances, subsequently shortening the lifespan of PLTs[60,65,66]. Taking intensive PT and treating with PT for longer than 48 h increased the risk of developing thrombocytopenia 3.4 times and 2.1 times, respectively. These findings are supported by similar studies[64,66,67]. A potential explanation for this finding is that exposing newborns to double PT for a longer period can compromise the production of PLTs and accelerate deterioration compared with exposing newborns to a single PT[68]. Additionally, double PT affects DNA, RNA and protein synthesis and shortens the life span of immature PLTs[26].

Before PT, leucopenia occurred in 21.1% of newborns (95% CI 16.2 26.3), but after PT, the magnitude of leucopenia significantly decreased to 21/8.5% (95% CI 5.3, 12.1). These findings are supported by studies conducted in Serbia[69], Iran[21,70], and Egypt[47,62]. The occurrence of leucopenia before PT may be related to the immunosuppressive effect of hyperbilirubinemia[21,62]. However, the increase in the WBC count after PT may be attributed to stress from hospitalization and exposure to a new environment[18]. Besides, decrement WBC count after PT may be related to the generation of ROS, which can in turn activate the immune system and lead to an increase in the production of WBCs[71–73]. Furthermore, alterations in cytokine production following exposure to PT may increase the WBC count[74]. A lower likelihood of leucopenia was observed among newborns admitted with a TSB greater than 18 mg/dl in the present study. These findings are supported by several studies[75,76]. The possible reason could ultimately be the stimulation of WBC production by bilirubin[76] and the neutralization of ROS by free bilirubin in the serum[19].

As a strength, this was a primary study that assessed the magnitude and associated factors of hematological toxicity among newborns with hyperbilirubinemia before and after PT treatment. However, a limitation of this study was the use of data from medical charts for maternal blood groups, which could minimize the accuracy of the data. Besides, we did not perform reticulocyte count due to constraint of reagent during study period, even if it is essential in the anemia diagnostic algorithm for neonates.

Conclusion and recommendation

In the study area, hematological toxicity was found to be a severe public health concern. Various hematological toxicities were observed before and after PT among newborns with hyperbilirubinemia. Among the identified toxicities, anemia, thrombocytopenia and leucopenia were the most prevalent. All of the prevalent toxicities, except leucopenia, were significantly elevated following PT. Additionally, age less than seven days, a TSB level less than 18 mg/dl, Rh blood group incompatibility, and taking intensive PT for a long period were found to be the major causes of most toxicity. Therefore, strict follow-up and early detection of hematological toxicity before and after PT treatment are necessary for better patient management and improved quality of life in newborns. Additionally, physicians should consider hematological complications and their aggravating factors during PT treatment. Furthermore, it is better further study conducted with large sample size which included reticulocyte count.

Data availability

All the data supporting these findings are contained within the manuscript.

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Author contributions

D.M.B. and B.B.T. and B.W. and E.C. and Z.M. and A.K. contributed to the design and implementation of the study, collected the data, undertook the statistical analysis, performed the data interpretation, and drafted the manuscript. N.C. and M.T. and A.A.A. and A.Y. and E.A. contributed to the data interpretation and drafting. All authors reviewed the main manuscript.

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Competing interests

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Additional information

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