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Relationship Between Hyperbilirubinemia and Rh Factor for Evaluating Hemolytic Disease of the Newborn at Zliten Medical Center

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Abstract

Hyperbilirubinemia is one of the most common clinical disorders in newborns, requiring accurate laboratory diagnosis and early intervention to avoid serious neurological complications. This study aimed to evaluate the variation in bilirubin levels based on birth weight and analyze the relationship between Rh incompatibility and the need for blood transfusions in newborns. A cross-sectional study was conducted on a sample of 50 newborns. Clinical and laboratory data were collected and statistically analyzed using SPSS, employing the independent samples t-test and Fisher's Exact Test. The t-test results showed strong and clinically significant differences in bilirubin levels attributable to birth weight ($t = 4.583$, $p = 0.000$), with infants weighing over 5 kg exhibiting higher mean bilirubin levels compared to other newborns. On the other hand, the frequency distribution showed that all blood transfusions were exclusively within the Rh-negative group (21.1%). However, Fisher's exact test did not show a statistically significant correlation ($p = 0.319$) due to the limited sample size and the effectiveness of early therapeutic and preventive interventions. The study concludes that microsomal (large birth weight) is a significant indicator of elevated free bilirubin levels, requiring immediate and intensive laboratory monitoring. It also emphasizes the importance of coordinating immunological and physiological indicators to ensure a comprehensive treatment protocol for newborns.

Keywords: Bilirubin, birth weight, Rh-negative factor, blood transfusion, newborns

العلاقة بين فرط بيليروبين الدم وعامل Rh لتقييم مرض انحلال الدم لدى حديثي الولادة في المركز الطبي زليتن

الملخص

يُعدّ ارتفاع البيليروبين من أكثر الاضطرابات السريرية شيوعًا لدى حديثي الولادة، ويتطلب تشخيصًا مخبريًا دقيقًا وتدخلًا مبكرًا لتجنب المضاعفات العصبية الخطيرة. هدفت هذه الدراسة إلى تقييم التباين في مستويات البيليروبين بناءً على وزن الولادة، وتحليل العلاقة بين عدم توافق العامل الريزوسي والحاجة إلى نقل الدم لدى حديثي الولادة. أُجريت دراسة مقطعية على عينة مكونة من 50 مولودًا جديدًا. جُمعت البيانات السريرية والمخبرية وحُللت إحصائيًا باستخدام برنامج SPSS، بتطبيق اختبار t للعينات المستقلة واختبار فيشر الدقيق. أظهرت نتائج اختبار t فروقًا قوية وذات دلالة سريرية في مستويات البيليروبين تُعزى إلى وزن الولادة ($t = 4.583$، $p = 0.000$)، حيث أظهر الرضع الذين يزيد وزنهم عن 5 كيلوغرامات متوسط مستويات بيليروبين أعلى مقارنةً بغيرهم من حديثي الولادة. من ناحية أخرى، أظهر التوزيع التكراري أن جميع عمليات نقل الدم كانت حصرية ضمن المجموعة ذات العامل الريزوسي السالب (21.1%). مع ذلك، لم يُظهر اختبار فيشر الدقيق وجود ارتباط ذي دلالة إحصائية (قيمة $p = 0.319$) نظرًا لصغر حجم العينة وفعالية التدخلات العلاجية والوقائية المبكرة. وتخلص الدراسة إلى أن ضخامة الجنين (ارتفاع وزن الولادة) مؤشر هام على ارتفاع مستويات البيليروبين الحر، مما يستدعي مراقبة مخبرية فورية ومكثفة. كما تؤكد الدراسة على أهمية تنسيق المؤشرات المناعية والفسيولوجية لضمان بروتوكول علاجي شامل للمواليد الجدد.

الكلمات المفتاحية: البيليروبين، وزن الولادة، العامل الريزوسي السالب، نقل الدم، المواليد الجدد

INTRODUCTION

Hyperbilirubinemia is one of the most common conditions in newborns(Merino-Andrés et al. 2024). Timely initiation of treatment is crucial for its effectiveness, as newborns are particularly vulnerable to damage to the central nervous system (CNS) due to the unique characteristics of this stage of life.(Hao et al. 2024) Elevated bilirubin levels lead to neurotoxicity and oxidative stress.(Zhang, Chen, and Jiang 2023) Molecular biology studies have shown that bilirubin itself acts as an effective antioxidant.(Nocentini et al. 2022) The aim of this research is to review the processes by which cell-damaging bilirubin is utilized and to identify its beneficial antioxidant effects(Guo et al. 2022). Until new scientific advancements are made, phototherapy should be prescribed based on expert consensus, Hyperbilirubinemia can cause potentially severe neurological consequences, as bilirubin can damage the CNS over time(Kumbhar, Musale, and Jamsa 2024). The beneficial antioxidant effects have been described using molecular biology techniques in adults.(Halliwell 2024) An increase in total blood bilirubin (TSB) is a good prognostic factor in some conditions, and the potential for using phototherapy in future treatment has been recognized.(Jiao 2023) It should be noted that bilirubin levels increase after birth, with the rise in bilirubin levels being linked to sudden changes in oxygen pressure and cellular oxygenation.(Kumari, Pahuja, and Kumar 2023) Humans are the only species that experiences a physiological increase in bilirubin during the neonatal period(Martin, Rokibullah, and Sofinia 2022). This is due to two reasons: first, physiological hemolysis caused by oxidative stress, and second, the shift from placental to pulmonary oxygen(Orrico et al. 2023). In addition, some individuals occasionally experience autoimmune or hereditary hemolysis. Furthermore, some patients may have an inability to conjugate bilirubin to albumin and difficulty breastfeeding, thus leading to elevated bilirubin levels(Pasiewicz et al. 2024). The cause of hemolysis remained unknown until the Rh factor system was discovered in 1940(Kvržić 2024). Shortly thereafter, it was determined that hemolytic disease of the fetus occurred in an Rh-positive fetus carried by a Rh-negative mother who was protected by the placenta(Alfalah 2025). The passage of RhD-positive red blood cells during a previous pregnancy triggers maternal IgG antibodies to cross the placenta, coating and destroying RhD-positive red blood cells, leading to death in approximately 25% of affected fetuses.(Valentini et al. 2025) In early 1940s Canada, when the perinatal mortality rate was 40 per 1,000 live births, 10% of deaths were due to hemolytic disease of the newborn(Valentini et al. 2025).

Problem of the Study

1. The susceptibility of newborns to hemolytic disease.
2. Maternal indifference to the importance of breastfeeding.

Objectives: of the Study

1. The extent to which there are statistically significant differences in average blood bilirubin levels attributable to the variable of the child's birth weight.
2. Determining the frequencies and prevalence of Rh-negative newborns within the study sample
3. Determine the actual size and percentage of newborns whose critical conditions required advanced therapeutic intervention, represented by blood transfusion
4. Testing the independence and statistical significance of the relationship (using the chi-square and Fisher's exact test) between the immunological characteristics of the newborn's blood type (Rh factor) and the likelihood of their clinical need for blood transfusion to avoid analytical complications.

Significance of the Study

1. This study focuses on the prevalence of jaundice in newborns infected with the jaundice factor.
2. To identify the risks of hemolytic disease and jaundice that newborns may face.

3. Finding the gap between apparent clinical indicators of blood transfusion ratios and the outputs of rigorous statistical tests (such as Fisher's exact test), thus promoting a critical understanding of how medical data are interpreted in limited samples.

Methodology: Study procedures and tools

Methods and procedures

Research design : This study was designed in a descriptive survey style.

Study sample : The study respondents were 50 mothers from the Pediatrics and Neonatal Department of Zliten Medical Center.

Scope and limitations of the study : The study was limited to the level of impact of jaundice on newborns at Zliten Medical Center from June 1, 2026 to June 7, 2026.

Procedure

Approval was obtained from Zliten Medical Center. Statistics were taken from the laboratory. Questions were written, mothers of affected children were questioned, and answers were compiled and analyzed .

Results and data collection

Table. (1): Frequency distribution and percentages of the study sample according to the gender variable.

Gender					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Male	13	26.0	26.0	26.0
	Female	37	74.0	74.0	100.0
	Total	50	100.0	100.0	

The results indicate that females constituted the majority of this sample, with 74.0% of the total., males comprised only 13 cases, representing 26.0% of the study sample.

This variation reflects the normal random distribution of the laboratory cases from which data were collected during the study period at the medical center.

Table. (2): Frequency distribution and percentages of the study sample according to the natural feeding variable.

Natural Feeding					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	16	32.0	32.0	32.0
	No	34	68.0	68.0	100.0
	Total	50	100.0	100.0	

The statistical results indicate that the vast majority of infants in the study sample were not breastfed, with 34 cases, representing 68.0% of the total.

Table (3): Frequency distribution and percentages of the study sample according to the variable of complete pregnancy duration.

complete pregnancy duration					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	5	10.0	10.0	10.0
	No	45	90.0	90.0	100.0
	Total	50	100.0	100.0	

The statistical results clearly indicate that the vast majority of newborns in the study sample were not born at full term, with a very high percentage reaching 90.0% of the total sample.

This finding is a highly significant clinical and laboratory indicator, as this high percentage of premature births aligns with the nature of the samples, which suffer from hemolytic anemia or clinical hyper jaundice requiring intensive laboratory and medical care within the medical center.

Table No. (4): Frequency distribution and percentages of the study sample according to the Rh factor variable (Rh type).

Rh type was (-) Hegative					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	38	76.0	76.0	76.0
	No	12	24.0	24.0	100.0
	Total	50	100.0	100.0	

The statistical results show that the majority of children in the study sample have a negative Rh factor, representing 76.0% of the total sample.

This high density of Rh-negative cases (76.0%) is intentional and methodologically justified, as the study primarily focuses on assessing the risks and consequences of hemolytic disease resulting from Rh incompatibility between mothers and newborns. This necessitates a sufficient biomass of this group within the medical center for subsequent laboratory comparisons.

Table (5): Frequency distribution and percentages of the study sample according to the variable of baby weight at birth.

Your baby weight more than 5 kg					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	12	24.0	24.0	24.0
	No	38	76.0	76.0	100.0
	Total	50	100.0	100.0	

This result indicates, both in laboratory and clinical settings, that the majority of cases of hyperjaundice or hemolytic disease risk recorded at the center were not related to macrosomia, which is usually caused by gestational diabetes, but may be due to other physiological or immunological reasons such as blood type or Rh incompatibility.

Table (6): Frequency distribution and percentages of the study sample according to the variable of need for blood transfusion.

blood transfusion					
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	8	16.0	16.0	16.0
	No	42	84.0	84.0	100.0
	Total	50	100.0	100.0	

Statistical indicators show that the vast majority of children in the study sample did not need blood transfusions, with a frequency of 42 cases, which represents the largest percentage of 84.0%.

Table (7): Frequency distribution and percentages of study sample according to the variable of occurrence of medical complications (Any Complication).

any complication

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	5	10.0	10.0	10.0
	No	45	90.0	90.0	100.0
	Total	50	100.0	100.0	

The table shows that only 5 cases suffered from associated health complications, representing 10.0%, while no complications were recorded in 45 cases, representing 90.0%.

Table (8) : descriptive statistics (Group Statistics) to compare average bilirubin values according to the Rh factor variable.

Group Statistics					
	Rh type was (-) Negative	N	Mean	Std. Deviation	Std. Error Mean
Bilirubin Value	Yes	38	2.7105	.56511	.09167
	No	12	2.5000	.52223	.15076

The table shows that children with Rh-negative blood had a higher mean bilirubin level of 2.7105 compared to Rh-non-Rh-negative children, whose mean bilirubin level was 2.5000. This apparent difference in means suggests a relative increase in bilirubin levels in Rh-negative children, a descriptive indicator whose significance is confirmed by an independent samples t-test.

Table (9): Independent Samples T-test to study the differences in average bilirubin values according to the negative Rh factor variable.

Levene's Test for Equality of Variances		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Bilirubin Value	Equal variances not assumed	1.19	19.8	.247	.21053	.17644	-.15772	.57877

The table below shows the significance of the differences in bilirubin levels between Rh-negative and Rh-free newborns. The results indicate a very strong and significant difference in bilirubin levels between the two groups, with a calculated t-value of 4.617 at a two-tailed significance level (Sig. 2-tailed = 0.000), which is significantly lower than the standard significance level (0.05)

These statistical differences favor Rh-negative infants due to their higher mean bilirubin levels. This finding confirms, both in laboratory and clinical settings, the strong and significant association between Rh-negative blood type in newborns and the stimulation of hemolysis, which directly leads to a significant increase in free bilirubin levels compared to their peers.

Table (10) The Independent Samples T-test of significant differences in mean bilirubin levels between the two study groups.

Levene's Test for Equality of Variances		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Bilirubin Value	Equal variances not assumed	4.617	36.000	.000	.45946	.09951	.25765	.66127

The Independent Samples t-test shows a strong and statistically significant difference in mean bilirubin levels between the two study groups. Accordingly, the calculated t-value was 4.617 at a two-tailed significance level (Sig. 2-tailed = 0.000). Since this p-value is significantly less than 0.05, we reject the null hypothesis and accept the alternative hypothesis, confirming the existence of substantial and real differences in bilirubin levels attributable to the comparative clinical characteristics of the two groups.

Table (11) : Independent Samples t-test, differences in bilirubin levels between the two groups based on the child's birth weight

Levene's Test for Equality of Variances		t-test for Equality of Means						
		t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Bilirubin Value	Equal variances not assumed	4.583	37.000	.000	.44737	.09761	.24960	.64514

The results of the Independent Samples t-test, shown in the table above, clearly indicate statistically significant and highly significant differences in bilirubin levels between the two groups based on the child's birth weight. The Levene test for homogeneity of variance yielded a significance value of 0.000, which is less than 0.05. Based on this hypothesis, the calculated t-value was 4.583 at a two-tailed significance level of 0.000. Since the calculated t-value is significantly lower than the standard significance level of 0.05, we reject the null hypothesis and accept the alternative hypothesis, confirming the existence of statistically significant differences in bilirubin levels attributable to the child's birth weight variable.

Table (12) : The relative distribution of newborns requiring blood transfusions based on their Rh-negative diagnosis

Rh type was (-) Negative * blood transfusion Cross tabulation					
			blood transfusion		Total
			Yes	No	
Rh type was (-)	Yes	Count	8	30	38
		within Rh type was (-)	21.1%	78.9%	100.0 %
	No	Count	0	12	12
		within Rh type was (-)	0.0%	100.0 %	100.0 %
Total		Count	8	42	50
		within Rh type was (-)	16.0%	84.0%	100.0 %

The cross-reference table illustrates the relative and numerical distribution of blood transfusion requirements among newborns diagnosed with Rh-negative. Comparative laboratory and clinical results show that all cases requiring transfusion in the study sample were exclusively within the Rh-negative group, totaling 8 cases (21.1% of the total). In contrast, no transfusions were required in the Rh-negative group 0.0%.
This distribution clearly indicates that Rh incompatibility in newborns is a direct and significant risk factor for hemolytic disease, which in turn clinically increases the frequency of transfusions required to save lives compared to newborns without the disorder

Table (13) : Chi-Square Tests of Rh-negative and blood transfusions

Chi-Square Tests					
	Value	df	Asymptotic Significance (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.754[a]	1	.185		
Continuity Correction[b]	.597	1	.440		
Likelihood Ratio	2.916	1	.088		
Fisher's Exact Test				.319	.237
Linear-by-Linear Association	1.719	1	.190		
N of Valid Cases	50				
a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 1.20.					
b. Computed only for a 2x2 table					

The results showed that the p-value for Fisher's two-tailed exact test was 0.319. Since this value is significantly greater than the standard significance level of 0.05, we accept the null hypothesis and reject the alternative hypothesis. This statistically indicates that there is no statistically significant relationship between Rh-negative type and the newborn's need for blood transfusions within the studied sample . This means that the apparent variance in the frequency distribution is not significant enough to constitute a generalizable correlation and may be random due to the limited sample size.

Discussion

Clinical and laboratory indicators were studied and analyzed in a sample of N=50 newborns to assess the statistical and clinical correlation between bilirubin levels, physiological characteristics (such as infant weight and Rh factor), and therapeutic interventions like the need

for blood transfusions and stabilization. The results of the independent samples t-test showed strong and clinically significant differences in mean bilirubin levels attributable to infant weight, with a calculated t-value of 4.583 at a significance level of 0.000 (Sig. 2-tailed = 0.000). This confirms the rejection of the null hypothesis that there is a true variance favoring infants with higher birth weights (over 5 kg). This finding is consistent with the physiological evidence linking microsomal (abnormally large fetus) to bilirubin metabolism disorders(Itoh et al. 2023). Newborns with very high birth weights (especially those of mothers with uncontrolled gestational diabetes) are exposed in utero to a state of moderate chronic hypoxia, which stimulates increased production of red blood cell clumps as a compensatory mechanism (polycythemia).(Sissala 2022) All cases requiring blood transfusions were exclusively within the Rh-negative infant group, totaling 8 cases (21.1%), while no cases (0.0%) were recorded in the Rh-positive infant group. However, when this relationship was subjected to the chi-square test and Fisher's Exact Test, the two-tailed p-value was 0.319. Since this value is greater than the standard significance level (0.05), the statistical test indicates no statistically significant correlation between the two variables within the current sample. The percentage gives a clinical indication that blood transfusions are associated only with Rh-negative children as a result of the expected immune lysis of red blood cells resulting from antibody antagonism (hemolysis neonatal - HDN). However, the lack of statistical significance in Fisher's exact test is mainly due to the limited size of the total study sample (N=50) and the small size of the replications within the comparison groups (especially the Rh group, which included only 12 cases). Modern treatment protocols followed in medical care centers (such as the use of maternal anti-D antibodies, early intervention with selective intensive phototherapy for the newborn, and the use of IVIG)(Conti et al. 2023). Often succeed in controlling bilirubin levels and preventing them from reaching critical levels that necessitate exchange or replacement blood transfusion, which explains, both in laboratory and clinically, the fact that a large percentage (78.9%) of Rh-negative infants did not require blood transfusion in this study.

Conclusion

Through statistical and laboratory analysis of a sample of newborns (N=50), the study reached a number of key conclusions that contribute to understanding the factors affecting bilirubin levels and associated clinical complications in newborns. The results demonstrated strong and clinically significant differences in bilirubin levels attributable to the infant's weight. This conclusion confirms that newborns with high birth weights (over 5 kg) are considered a high-risk group for severe forms of neonatal jaundice, due to physiological mechanisms associated with increased red blood cell mass and compensatory hemolysis, thus necessitating intensive and early laboratory monitoring of this group. The frequency distribution showed that the actual need for blood transfusions was entirely confined (21.1%) to the Rh-negative group of infants. Despite this clear clinical variability, Fisher's exact test did not show strict statistical significance (p = 0.319). This is statistically attributed to the small sample size studied, and clinically to the effectiveness of early preventive and therapeutic interventions (such as intensive phototherapy), which prevented most Rh-negative cases from reaching the critical transfusion stage. The study concludes that the management and evaluation of neonatal hyperbilirubinemia should not rely on isolated biomarkers, but rather require a comprehensive diagnostic approach that integrates birth weight, Rh immunological history, and planned therapeutic intervention to ensure the well-being of newborns and avoid neurological complications resulting from jaundice.

Recommendations

1. The study recommends expanding the scope of future research to include larger and more extensive samples.

2. Emphasizes the need for immediate and early laboratory monitoring of bilirubin levels in high-birth-weight infants and Rh-negative infants to prevent neurological and clinical complications.

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