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Laboratory Assessment for the Diagnosis of Jaundice in Newborns

Amjad Mahmood Qadir[1], Ali Hasan Ali[2], Qaraman Salih Mahmood[3], Rebaz Anwar Omer[4], Dara Latif Saifaddin[5]

[1] Department of General Science, College of Basic Education, University of Halabja, 46018 Halabja, Kurdistan Region, IRAQ

[2,3] Maternity and Paediatric Hospital of Halabja, Ministry of Health, 46018 Halabja, Kurdistan Region, IRAQ

[4]Department of Chemistry, Faculty of Sciences and Health, Koya University, Koya 44023, Kurdistan Region – F.R. IRAQ

[5]Department of Sport Sciences for Health and Performance, College of Physical Education and Sport Sciences, University of Halabja, 46018 Halabja, Kurdistan Region, IRAQ

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ABSTRACT:

Background: Hyperbilirubinemia is a primary cause of jaundice in babies. To perform early risk assessments and provide effective therapy, you need to understand what factors affect total serum bilirubin (TSB) levels. Objective: This study aimed to investigate the relationship between TSB levels and various parameters, including mode of delivery, maternal and neonatal blood types, gender, weight, and packed cell volume (PCV) in infants. Methods: The 74 neonates, aged 1 to 30 days, were admitted to either a neonatal intensive care unit or an outpatient clinic for this cross-sectional study. One-way ANOVA and independent t-tests were performed to examine the relationships between TSB levels and the selected factors. Result: The findings indicated no significant correlations between TSB levels and the following variables: gender ($p = 0.640$), weight ($p = 0.652$), PCV ($p = 0.076$), caesarean birth ($p = 0.509$), maternal blood group ($p = 0.127$), and infant blood type ($p = 0.450$). The effect sizes were small (Cohen's $d < 0.2$) for the average TSB levels for vaginal and caesarean births, which were 11.80 ± 3.55 mg/dL and 12.36 ± 3.42 mg/dL, respectively. Conclusion: The study found that newborn hyperbilirubinemia is not affected by delivery mode, maternal or fetal blood type, gender, weight, or packed cell volume. These findings reveal that hepatic immaturity, hemolysis, nursing behaviors, and genetic vulnerability are among the many factors that may alter bilirubin metabolism. Clinical management should focus on established risk factors, including gestational age and hemolysis symptoms. The environmental and genetic determinants of neonatal jaundice outcomes require thorough longitudinal examination.

Keywords: Neonatal hyperbilirubinemia, Total serum bilirubin, Caesarean delivery, Blood group incompatibility, Packed cell volume

1 INTRODUCTION

Neonatal jaundice is marked by increased total serum bilirubin levels and is quite prevalent. Bilirubin can cause kernicterus and other neurological complications if not properly addressed [1]. Newborn hemolytic disorders, including

*Corresponding author: author@organization.edu.co

ABO and Rh incompatibility, which elevate bilirubin levels, have required meticulous examination of the blood types of both mother and infant [2]. These incompatibilities, called hemolysis, can speed up the loss of red blood cells and the production of bilirubin [3]. Some studies indicate that hyperbilirubinemia is more prevalent in male neonates [4]. As the liver's conjugation processes improve, bilirubin levels decrease from the high levels seen at birth. Age is one of the most essential factors [5]. Packed cell volume, which is a measure of how many red blood cells there are, affects both bilirubin levels and the amount of hemoglobin in the blood [6]. Many studies have examined the association between total serum bilirubin (TSB) and factors such as gender, age, packed cell volume, and blood type incompatibility [7]. ABO and Rh incompatibility are recognized as factors that may cause jaundice in newborns. When the mother's and infant's blood types don't match, the newborn's blood cells break down, and bilirubin levels rise [8]. Research indicates that babies whose ABOs were not compatible with those of their compatible counterparts had higher total blood bilirubin levels. Rh isoimmunization can lead to a significant increase in bilirubin levels in areas where anti-D immunoglobulin is not readily available [9]. A baby's gender affects the amount of bilirubin in their blood. Research by Singh and Sharma (2020) [10] shows that hyperbilirubinemia is more frequent in male babies. This imbalance is exacerbated by the fact that men and women metabolize bilirubin and liver enzymes differently, regardless of the underlying cause [11]. Bilirubin levels are greatest during the first few days of life. Age is thus an essential determinant as well [12]. Age-specific total serum bilirubin nomograms enhance clinical decision-making and avert needless medications, as indicated by Abiha et al. (2023) [13].

Packed cell volume (PCV) is associated with hemoglobin concentration and bilirubin synthesis. High PCV values cause red blood cells to break down, and the amounts of substances to rise [14]. Recent research has focused on the influence of demographic, physiological, and genetic variables on TSB levels, building upon previous studies [15]. This study examines the complex relationships between biological and demographic variables, including the blood types of both the newborn and the mother, as well as gender, age, PCV, and TSB levels. The findings should help healthcare professionals improve infant care and reduce the burden of bilirubin-related issues.

2 PATIENTS AND METHODS

2.1 STUDY POPULATION

The study involved 74 neonates (aged 1 to 30 days) hospitalized in neonatal intensive care units or outpatient clinics, including both male and female subjects diagnosed with jaundice, sourced from the Shifah Nwe Laboratory at Halabja's Medical Building. Access to comprehensive maternal and newborn medical records, including blood type information. To keep the experiment focused on this specific illness, the exclusion criteria specified that individuals with jaundice were ineligible.

2.2 STUDY DESIGN

This study used a cross-sectional study to investigate correlations among children's and their mothers' blood groups, gender, infants' weight, age, PCV, and TSB. The cross-sectional study enables data collection at a specific point in time, allowing analysis of correlations among variables.

2.3 SAMPLE COLLECTION

Using three different heparinized capillary tubes, all participants had their blood collected. Two of the tubes were for the children, one was for the mothers, and one was for the children. The blood group and total serum bilirubin levels were also tested.

2.4 BIOCHEMICAL AND HAEMATOLOGICAL TESTS

The biochemical test included TSB, and the haematological tests included PCV and blood type determination. The TSB capillary tube was spun at 12,000 rpm for 5 minutes, and then an APEL bilirubin meter was used to determine how much bilirubin was in the blood. A hematocrit reader was used to manually check the PCV. The ABO blood group system categorizes blood types, enabling them to be correctly identified among individuals.

2.5 ETHICAL CONSIDERATIONS

The study protocol was approved by the University of Halabja's Ethics Committee (Approval No. 07/2024/8). In accordance with the Declaration of Helsinki, all participants provided signed informed consent.

2.6 STATISTICAL ANALYSIS

The statistical software SPSS version 26.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 10.04 (GraphPad Software Inc., San Diego, CA, USA) were used for data analysis. We used one-way analysis of variance (ANOVA) to compare total serum bilirubin with birth weight, PCV, and blood type. For the two groups, we used a t-test to compare

total serum bilirubin with infant gender and delivery method. The data are presented as the mean ± standard deviation (SD), with a significance level of $P < 0.05$.

3 RESULT

3.1 CORRELATION BETWEEN THE TYPE OF DELIVERY AND TOTAL SERUM BILIRUBIN LEVEL

The levels of TSB were compared between those who had caesarean sections using an independent samples t-test. Statistical tests ($t(72) = -0.664$, $p = 0.509$, Cohen's $d = 0.51$) did not reveal a significant difference in TSB between the two groups. In contrast to the 12.36 (SD=3.42) mg/dL seen in the non-caesarean section group, the mean bilirubin level in the caesarean section group was 11.80 (SD=3.55 mg/dL). With a 95% CI of [-2.27, 1.13] for the mean difference, see Figure 1.

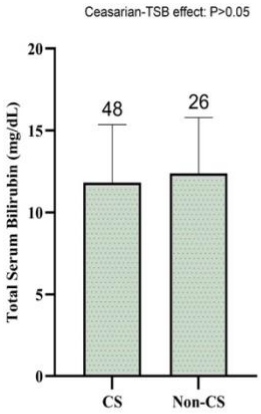

Figure 1. The effect of delivery type on total serum bilirubin (TSB) levels.
Data are represented as mean ± SD
Abbreviations: CS, Cesarean section; Non-CS, Non-cesarean section; TSB, Total Serum Bilirubin.

3.2 CORRELATION BETWEEN THE MOTHER'S BLOOD GROUP AND TOTAL SERUM BILIRUBIN LEVEL

To test for statistical significance, we used a one-way analysis of variance to compare TSB levels among the six blood types: B-, B+, A+, AB+, O+, and O-. No statistically significant differences were found between the groups in the investigation, as shown in Figure 2 ($F(5,68) = 1.788$, $p = 0.127$).

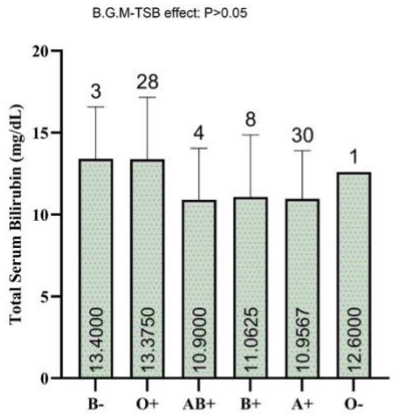

Figure 2. The effect of Blood Group Mother on total serum bilirubin (TSB) levels.
Data are represented as mean ± SD
Abbreviations: B.G.M, Blood Group Mother.

3.3 CORRELATION BETWEEN A CHILD'S BLOOD GROUP AND TOTAL SERUM BILIRUBIN LEVELS

A one-way NOVA was used to examine serum total bilirubin levels across blood types B+, A+, AB+, and O+, to detect statistically significant differences. No statistically significant differences were identified between the groups, as shown

by the study. Figure 3 indicates that the result is statistically not significant, with an F-value of 0.891 and a p-value of 0.450.

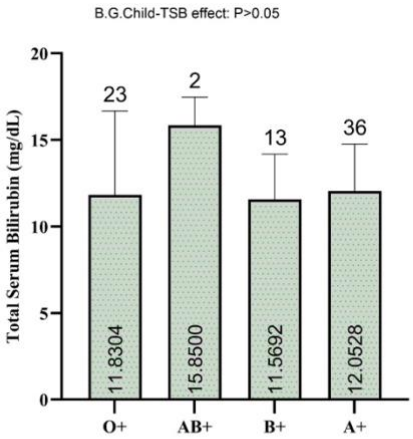

Figure 3. The effect of Blood Group Child on total serum bilirubin (TSB) levels.
Data are represented as mean ± SD
Abbreviations: B.G.Child, Blood Group Child; TSB, Total Serum Bilirubin.

3.4 CORRELATION BETWEEN AN INFANT'S WEIGHT AND TOTAL SERUM BILIRUBIN LEVELS

Substantial variations in TSB levels were identified among the five weight categories by a one-way ANOVA. Figure 4 shows that no statistically significant differences in mean blood bilirubin levels were observed across the weight groups, as evidenced by $F(4, 69) = 0.617$ and $p = 0.652$.

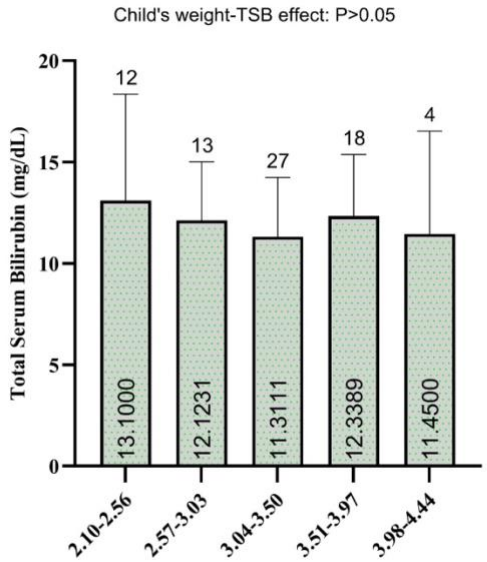

Figure 4. The effect of Child's weight on total serum bilirubin (TSB) levels.
Data are represented as mean ± SD

3.5 CORRELATION BETWEEN PACKED CELL VOLUME AND TOTAL SERUM BILIRUBIN LEVELS

We used a one-way NOVA to compare the mean TSB levels among the five PCV groups. Figure 5 indicates that there were no statistically significant differences in the mean TSB levels across the PCV groups. This indicates that there was no statistically significant difference in the average TSB levels among the PCV groups.

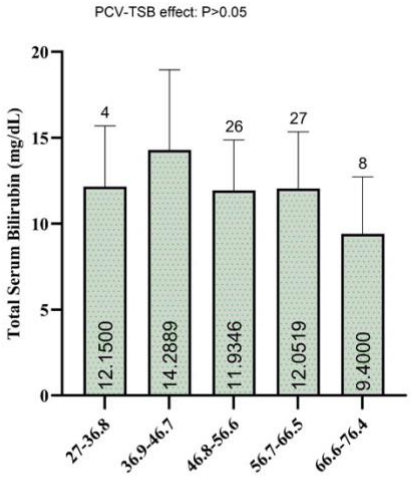

Figure 5. The effect of Packed cell volume (PCV) on total serum bilirubin (TSB) levels.
Data are represented as mean ± SD
Abbreviations: PCV, Packed Cell Volume; TSB, Total Serum Bilirubin.

3.6 CORRELATION BETWEEN THE INFANT'S GENDER AND THE TOTAL SERUM BILIRUBIN LEVEL

To compare TSB levels between males and females, an independent-samples t-test was performed. According to the data, there was no statistically significant difference: t(72) = 0.221, p = 0.640, and Cohen's d = 0.52. At 11.97 ± 3.34 mg/dL, the average total soluble fiber TSB content was found in men, whereas in females it was 12.03 ± 3.70 mg/dL. As seen in Figure 6, the 95% confidence interval for the mean difference was [-1.69, 1.57]. In this sample, there was no statistically significant difference in TSB levels between the sexes.

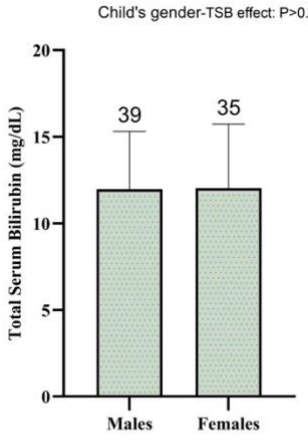

Figure 6. The effect of Child's gender on total serum bilirubin (TSB) levels.
Data are represented as mean ± SD
Abbreviations: TSB, Total Serum Bilirubin.

4 DISCUSSION

The analysis of the association between the administration approach and TSB levels revealed no significant link (Figure 1). This study suggests that the delivery method (vaginal or caesarean) does not affect newborn bilirubin levels. It appears that factors such as the infant's underdeveloped liver, hemolysis, and feeding habits have a greater impact on TSB levels

than the mode of birth [13]. Previous research has yielded inconsistent results on the impact of delivery methods on neonatal jaundice. There is contradictory information about the association between caesarean births and elevated total blood bilirubin levels. Some studies suggest that this may be attributed to variations in neonatal stress responses or a delay in the onset of breastfeeding; however, other studies have found no such association [16]. The findings indicate that the delivery method does not significantly affect TSB levels in babies, supporting the second perspective. Various environmental, genetic, and physiological factors converge to influence TSB levels [17]. Gestational age, birth weight, and maternal health issues, such as diabetes and blood type incompatibility, have a big effect on bilirubin levels [18]. Breastfeeding practices, which may vary according to the birth method, might affect neonatal jaundice [19].

It is particularly important for clinical practice, as there is no strong link between the way a baby is delivered and TSB levels. This underscores the necessity of addressing modifiable risk factors for newborn hyperbilirubinemia, including promoting effective and timely breastfeeding, monitoring at-risk infants, and taking appropriate actions. When trying to connect TSB levels to delivery style, doctors should exercise caution and consider a broader range of important factors. Figure 2 shows no statistically significant association between maternal blood type and TSB levels in the neonates studied. Prior studies have shown that maternal blood type incompatibility, including ABO or Rh mismatches, may trigger hemolysis, leading to elevated TSB levels in neonates [20]. Prior research has indicated that maternal blood type does not predict neonatal hyperbilirubinemia [22], and our results corroborate these findings. Incompatible blood types increase the risk of hemolytic disorder; it is also plausible that the mother's blood type exerts minimal influence on bilirubin metabolism independently, particularly given the baby's blood type and specific antigen interactions [2].

The absence of a result may be attributed to the study's focus on the mother's blood type rather than the infants. In current maternity care, such as the use of anti-D immunoglobulin for Rh-negative women, prophylactic interventions and early clinical monitoring can help reduce the risk of blood type incompatibility [21]. Gestational age, birth weight, breastfeeding, genetic predispositions (e.g., Gilbert syndrome), and ethnic differences in bilirubin metabolism influence total blood bilirubin levels in newborns [22]. These factors may have a greater impact than the mother's blood type. The likelihood of identifying a correlation may have been diminished because the research group had limited diversity of blood types or a significant degree of incompatibility. Research shows that a baby's blood type should not be the only factor that decides if they have hyperbilirubinemia. Clinicians should priorities newborn blood types, hemolytic signs (such as the Coombs test), jaundice symptoms, and other relevant risk factors. The previously listed demographic parameters must be comprehensively assessed in compliance with the established criteria [23].

The study's cross-sectional approach and limited sample size may have missed weak correlations. In the future, researchers should examine how maternal-neonatal blood group interactions affect larger populations or areas where untreated blood group incompatibility is more prevalent. Long-term genetic and environmental research may clarify some of the intricate causes of newborn jaundice. In conclusion, maternal blood type incompatibility poses a significant problem in certain therapeutic scenarios; however, our results suggest it exerts minimal to no effect on total serum beta levels in infants undergoing current therapy. It is essential to conduct a comprehensive evaluation and categorization of hazards when caring for newborns. This study found no correlation between TSB levels and a child's ABO or Rh blood group, even after accounting for maternal-fetal incompatibility (Figure 3). Numerous investigations employing multivariate regression analysis identified birth weight, gestational age, genetic predispositions (notably UGT1A1 polymorphisms), and hemolytic conditions as significant predictors of this outcome. Blood type incompatibility, which includes ABO or Rh isoimmunization, is a known risk factor for hyperbilirubinemia because it speeds up the breakdown of red blood cells [24]. It seems that TSB levels have no connection to the child's natural blood type [25]. This result is supported by methodological factors. Cohort studies lacking maternal alloimmunization revealed no correlation between the newborn's blood type and bilirubin levels [26]. The hemolytic effects of the child's blood type antigens seem to be minor when compared to the effectiveness of hepatic conjugation or genetic variations influencing enzyme activity [27]. Population-based research shows that TSB variability is more affected by demographic and environmental factors (such as preterm nursing practices) than by blood type categorization. Although a child's blood type may not predict TSB levels, blood group incompatibility remains a therapeutically significant issue [28].

Physiological, genetic, and environmental factors often exert a greater influence on hyperbilirubinemia than blood type [29]. Blood type may reduce bilirubin fluctuations; however, its association with comorbidities warrants further investigation [30]. Neonatal jaundice is a prevalent clinical issue, with TSB serving as a vital diagnostic indicator [31]. Gestational age, hemolytic diseases, and dietary habits can predict hyperbilirubinemia; however, the relative significance of total serum bilirubin and body mass index remains under debate [32]. Figure 4 indicates that larger infants do not exhibit altered bilirubin dynamics, contrary to prior assumptions, as recent studies have shown no significant association between these variables [33]. Numerous studies have examined the relationship between breastfeeding status and gestational maturity, employing various criteria for analysis [34]. A 2020 cohort study (n = 1,500) revealed no independent link ($p = 0.34$) between birth weight and peak total blood bilirubin levels after controlling for gestational

age and maternal variables [35]. These findings support physiological studies indicating that bilirubin metabolism is regulated by genetic polymorphisms (e.g., UGT1A1 enzyme activity) and hepatic conjugation capacity, rather than body mass [36]. Larger babies may have more red blood cells, but liver function, not weight, controls how quickly they break down and how well they are cleared [37].

Figure 5 illustrates the effect of Packed Cell Volume (PCV) on Total Serum Bilirubin (TSB) levels in a group of subjects [38]. Hyperbilirubinemia does not invariably arise from polycythaemia, characterized by elevated prothrombin time and PCV in infants [39]. The lack of correlation between PCV and TSB highlights the complex nature of bilirubin metabolism, which is influenced by several factors that affect the total blood bilirubin levels in infants. These include immature hepatic conjugation systems, genetic factors such as UGT1A1 mutations, environmental factors, and the enterohepatic circulation process [40, 41]. Even with an increase in red blood cell turnover, hepatic conjugation can help lower TSB levels in healthy livers [40]. Low PCV, indicative of iron-deficiency anaemia in the absence of hemolysis, can coexist with normal TSB levels; nevertheless, polycythaemia vera may not increase TSB in the absence of hepatobiliary disease [42]. This difference could be worsened by problems with the methods used. Changes in plasma volume, including dehydration, that don't impact bilirubin production, may have a short-term influence on RBC concentration [43]. In hemolytic diseases, RBC lysis is generally more closely associated with an elevation in TSB than with the total number of RBCs [44]. Investigating regulatory systems may elucidate these inconsistencies. Neonatal hyperbilirubinemia, characterized by elevated total blood bilirubin levels, is a common clinical concern in newborns [45]. The cause is linked to gestational age, birth weight, and eating patterns [46].

The research indicated that both male and female neonates had similar mean total blood bilirubin levels and instances of severe hyperbilirubinemia, with p-values exceeding the standard significance threshold ($p > 0.05$). At this period, sex hormones do not affect the development of infants (Figure 6), indicating that bilirubin metabolism, regulated by hepatic enzymes such as UDP-glucuronosyltransferase, is not sexually dimorphic [47]. A limited number of studies have indicated a potential rise in TSB levels in men [48]. These findings may lack practical significance and might result from random chance or other unmeasured factors, such as the incidence of birth trauma. The study has several limitations, including its cross-sectional design, which hinders causal inference, and its reliance on hospital-based populations, which may limit generalizability. Consequently, further research may investigate longitudinal designs to record temporal variations in bilirubin levels. This method aims to enhance comprehension of neonatal hyperbilirubinemia and its management by providing significant insights into the factors that influence total serum bilirubin concentrations.

CONCLUSION

This study shows that a range of physiological and environmental variables can alter TSB levels in newborns. These determinants encompass, but are not confined to, the method of delivery, the blood type of the mother or newborn, the infant's birth weight, the hematocrit PCV, and the gender of the infant. A recent study indicates that the primary variables influencing bilirubin metabolism include hepatic immaturity, hemolysis, genetic predispositions (namely, UGT1A1 polymorphisms), and nursing practices. However, an earlier investigation has revealed conflicting results. This study supports a comprehensive strategy to manage baby jaundice that emphasizes manageable factors, such as early breastfeeding support and focused monitoring, rather than irrelevant demographic or delivery-related considerations. Further research investigating patterns of blood type incompatibility across diverse cohorts is essential, given methodological constraints such as limited sample sizes and inadequate study designs. This research should focus on genetic-environmental interactions. To enhance the treatment of hyperbilirubinemia, evidence-based methodologies must incorporate thorough risk assessments.

Author's contributions

The authors contributed equally. Conceptualization: A.M.Q.; Data curation: A.H.A., D.L.S., A.M.Q.; Software and Resources: A.M.Q., D.L.S.; Sample collection: Q.S.M., A.H.A., A.M.Q.; Methodology: A.M.Q., A.H.A., Q.S.M., R.A.O.; Supervision: R.A.O.

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Not Applicable.

Ethical approval: The Ethics Committee at the University of Halabja authorized this study protocol (Approval No. 07/2024/8).

Informed consent: All participants provided written informed consent in accordance with the Declaration of Helsinki.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest associated with this study.

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