Research Article

Journal of Neonatal Studies

A Prospective Study to Determine the Association between Serum Bilirubin and Early Developmental Milestones

Abstract

Introduction: Neonatal jaundice is the most common clinical condition requiring medical attention in newborns which is about 50% in term infants and 80% in preterm infants. One sequelae of hyperbilirubinemia is kernicterus which can result in possible fatality, or neurodevelopmental defect in survivors. Therefore, our study is designed specially to determine the relationship of serum bilirubin and developmental problems for infants in Malaysia.

Methods: This is a prospective cohort study conducted in a private community hospital in Malaysia. The participants were newborns born in 2021 and 2022 at the private community hospital who develop jaundice during the first 14 days of life. The sample selection was done by convenience sampling. A validated questionnaire adapted from the Dutch Developmental Instrument via phone calls or online platforms was used to collect data.

Results: The results concluded that there is association between serum bilirubin and the early developmental milestone.

Conclusion: Newborns that has serum bilirubin more than 15mg/dL has a lower D-score.

Keywords: Serum Bilirubin • Developmental Milestones • Developmental Problems • Neonatal Jaundice • Babies

Lyana Najwa Kamaruddin*, Faheezmal Ali Napiah, Muhammad Fahmi Mohd Fariz, Nurul Syahira Ab Rahman, Nor Azlina Mohammad Rashid

Faculty of Medicine, University of Cyberjaya, Persiaran Bestari, Cyber 11, 63000, Cyberjaya, Selangor, Malaysia

*Author for correspondence:
lyananajwa5@gmail.com

Received: 25-Jan-2023,
Manuscript No. jns-23-87884;
Editor assigned: 26-Jan-2023,
PreQC No. jns-22-87884(PQ);
Reviewed: 08-Feb-2023,
QC No. jns-22-87884;
Revised: 15-Feb-2023,
Manuscript No. jns-22-87884(R);
Published: 22-Feb-2023
DOI: 10.37532/jns.2023.6(1).17-22

Introduction

Neonatal jaundice is one of the most common medical conditions in newborn babies. In Malaysia, it is estimated to have about 301 000 newborns experiencing neonatal jaundice annually. This condition is associated with a state of hyperbilirubinemia which can affect brain development, which has been associated with neurodevelopmental delays in those affected. Therefore, this study aims to enlighten the association between serum bilirubin level and their role in causing developmental delay.

There was a statistically significant association between hyperbilirubinemia and participants with developmental delay found in a prospective cohort study. The researchers found that moderate hyperbilirubinemia (total serum bilirubin of 10-20 mg/dL) in term infants was associated with developmental delay in participants aged 2-4 years old. Similarly, another study done in Indonesia also found a significant correlation between healthy term infants with history of hyperbilirubinemia and developmental delay in gross motor at the age of 6 months (P=0.027; OR 5.97; 95%CI 1.22:29.12). Additionally, Wusthoff & Loe (2015) found a significantly increased risk of developmental delay among children with moderate hyperbilirubinemia as compared to no hyperbilirubinemia, with a relative risk (RR) of 1.6 (95% CI: 1.3-2.0).

To this day, Malaysia has not had any study that determines the relationship between serum bilirubin and developmental milestones. Hence, our study is designed specially to determine the relationship of serum bilirubin and developmental problems for infants in Malaysia, in hopes that once the relationship has been significantly established, an early detection of the underlying

cause behind a physiological or pathological jaundice may help physicians and clinicians to tackle the root of the problem before it leads to a neurodevelopmental issue related with Neonatal Jaundice [1].

Method and Materials

This is a prospective cohort study conducted in a private community hospital in Malaysia. Newborns born in 2021 and 2022 at the private community hospital who develop jaundice during the first 14 days of life were included in this study. The participants were healthy term infants, weighed more than or equal to 2.5 kg with no congenital conditions and had their serum bilirubin level measured. Exclusion criteria are newborns with congenital anomalies, neonatal sepsis, preterm and infants born with serious complications requiring NICU admission.

Non-respondents were the babies' parents who failed to respond. The sampling method that we used is convenience sampling. Based on our objectives, the sample size was calculated with an association and proportion formula. The highest sample size calculated was chosen to be the sample size of the study.

For the association formula, the sample size was calculated based on the prevalence of neonatal jaundice in Malaysia of 60%. With a type I error probability of 0.05 and a power of 0.8, the calculated sample size was 47 including 10% dropout.

As for the proportion formula, the P1 is the percentage of pass scores among healthy babies, which is at about 90 percent. Jacobusse, Van Buuren (2006) and P2 is our expectation that the percentage of pass scores among hyperbilirubinemia is 20 percent less than P1. The calculated sample size was 64 including 10% dropout.

After comparison of sample sizes between both calculations, a sample size of 64 including 10% dropout was chosen as the sample size of this study [2].

Baseline data was collected from the hospital records and using google forms to potential participants on maternal blood grouping, date and time of birth, birth weight, APGAR score, G6PD status and serum bilirubin level.

Data was collected using a validated questionnaire adapted from the Dutch Developmental Instrument (DDI). Parents were required to answer the questionnaire via phone calls or online platforms (WhatsApp and email). The questionnaire consists of developmental milestones achieved by their child, from age 1 month until age 6 months. Parents answered either "Yes" or "No" for each item in the questionnaire. The items were then converted into Developmental score (D-score) by scoring 0 for "No" and 1 for "Yes". DDI was used as first-hand data used for constructing the D-score, where items found in the instrument were converted into D-score based on the participants ability to achieve the developmental outcome. The components were made up of items assessing the development of gross motor, fine motor, social skills and language milestone of the participants:

A. For 1 month old: 5 items

B. For 2 months old: 7 items

C. For 3 months old: 12 items

D. For 6 months old: 18 items

For this study, the data was analyzed with JASP software. First, descriptive statistics were used to describe the baseline characteristics of the participants and prevalence of serum bilirubin status. Subsequently, to test our primary hypothesis, a correlation study was done to determine the association between neonatal serum bilirubin level and their total D-score using Spearman's rank correlation. Lastly, to test our secondary hypothesis of finding the relationship of total D-score between two categories of serum bilirubin status, which are 'less than or equal to 15mg/dL' and 'more than 15mg/dL', an independent Welch's t-test was used. For all the tests performed, when the p value is less than 0.05, the data was to be interpreted as statistically significant.

Ethical Consideration

The research project was approved by the University of Cyberjaya Research Ethics Review Committee (CRERC) with CRERC Reference number: UOC/CRERC/AL-ER (32/2021).

Results

Baseline Data Analysis of the Participants

There were a total of 51 participants that agreed to participate in our study. The mothers are required to answer a questionnaire given by us to fill in their baseline data which are relevant to our study. After analyzing the baseline data

through Descriptive Statistics, we can recognize and calculate the frequencies of each baseline data and our two variables which are newborns with serum bilirubin (mg/dL) of less than 15 and more than 15. With that being said, we had 46 mothers with newborns that had less than 15 mg/dL of serum bilirubin and 5 others with newborns that had more than 15 mg/dL serum bilirubin.

Maternal Blood Group

It is found that within 51 mothers that participated in our study, in mothers that had a newborn with serum bilirubin less than 15 mg/dL, 41% of them had type O blood group. On the other hand, the other mothers in the same group had blood type A (33%), blood type B (11%) and blood type AB (15%).

In the other group of mothers that had newborns with serum bilirubin more than 15 mg/dL, it is found that majority of the mothers had blood type B (40%) with only a minority of blood group A, AB, and O (20% each respectively) [3].

Gender

In newborns that have less than 15mg/dL of serum bilirubin, majority of them are males, recorded at 61% and another 39% are females. Meanwhile, we saw a different outcome in newborns with more than 15mg/dL of serum bilirubin, where most of them are females (80%) and the remaining are males (20%).

G6PD Status

In terms of their G6PD status, newborns that had less than 15mg/dL serum bilirubin had a normal G6PD status (100%) while 60 % of newborns that had more than 15 mg/dL serum bilirubin are deficient in G6PD.

APGAR Score

In the APGAR score, where we categorized the score as excellent, moderately depressed, and severely depressed, it is found that in newborns that had serum bilirubin of more than 15mg/dL, about 20% of them had moderately depressed APGAR score while the other 80% had excellent APGAR score.

Method of Delivery

The method of delivery also was taken into consideration where most of the mothers in both groups had the majority of undergoing a Lower Segment Cesarean Section (LSCS) upon delivering their newborn (61% for mothers with newborns less than 15mg/dL and 60% for mothers with newborns more than 15mg/dL).

Gestational Age

In this baseline data, we measured the mean standard deviation for both groups where mothers with newborns that had less than 15mg/dL has a mean standard deviation of 0.000, while mothers with newborns that had more than 15mg/dL has a mean standard deviation of 0.447 [4].

Birth Weight

For birthweight, we measured the mean standard deviation for both groups where mothers with newborns that had less than 15mg/dL has a mean standard deviation of 0.226, while mothers with newborns that had more than 15mg/dL has a mean standard deviation of 0.354.

The association between neonatal serum bilirubin level and their early d score

By virtue of the total D-score, which is a sum of multiple distributions, a Shapiro-Wilks test was performed and showed that the distribution of neonatal serum bilirubin (< 15) departed significantly from normality ($W = 0.266$, $p < .001$). However, the distribution of neonatal serum bilirubin (> 15) did not show evidence of non-normality ($W = 0.795$, $p = 0.073$). Hence, it is best to approach our objectives with non-parametric statistics and a Spearman's rank correlation was conducted to examine the relationships between neonatal serum bilirubin level and their early D- score.

Based on neonatal serum bilirubin level was strongly negatively related to D-score, $\rho (49) = -.499$, $p < 0.001$. A scatterplot of neonatal serum bilirubin and d score is seen.

The comparison of D-score of infants with more than 15mg/dL of serum bilirubin and less than 15mg/dL of serum bilirubin

An independent t-test was conducted to determine whether neonatal serum bilirubin (> 15) is more associated with a lower D-score compared to neonatal serum bilirubin (< 15). We recruited 51 babies and grouped them accordingly. The first group had a neonatal serum bilirubin of more than 15 mg/dL, and the second group had a neonatal serum bilirubin of less than 15 mg/dL. Due to the non-normal distribution data as mentioned per above, Welch's independent t-test was used to analyze data.

Based on the groups differed significantly, t(49) = 5.801, p = .007, 95% C.I [4.582, 15.601], d = 3.2. The mean for the neonatal serum bilirubin of more than 15 mg/d (M = 49.891, SD = 0.434) was statistically significantly higher than neonatal serum bilirubin of less than 15 mg/dL (M = 31.800, SD = 4.438) and the effect size was large (d = 3.2). These findings support the idea that neonatal serum bilirubin of more than 15 mg/dL (> 15) is more associated with a lower D-score compared to neonatal serum bilirubin of less than 15 mg/dL (<15) [5].

Discussion

In this study, we decided to observe the baseline data collected and compare their frequencies with the serum bilirubin level. The data chosen in this study were shown to be risk factors for hyperbilirubinemia according to the CPG Management of Neonatal Jaundice (2014). This is further justified as early prediction of jaundice has become increasingly important for identifying those babies at risk of neonatal hyperbilirubinemia considering the severe neurological morbidities caused by bilirubin toxicity.

From the results of our study, mothers that had a newborn with serum bilirubin less than 15 mg/dL, 41% of them had type O blood group. On the other hand, another group of mothers that had newborns with serum bilirubin more than 15 mg/dL, it is found that majority of the mothers had blood type B (40%). This finding was not consistent with literature, where it was established that newborns with blood O mothers have more risk of hyperbilirubinemia. However, this study had a large sample size in comparison to our current study. Therefore, having a small sample of participants with more than 15 mg/dL might explain this. One improvement that can be done is to relate maternal blood grouping with neonates' blood grouping while recording their serum bilirubin level [6].

In terms of their G6PD status, newborns that had less than 15mg/dL serum bilirubin had a normal G6PD status (100%) while 60 % of newborns that had more than 15 mg/dL serum bilirubin are deficient in G6PD. This is consistent with Isa (2017), which reported G6PD-deficient neonates had significantly higher serum total bilirubin level as compared to G6PD-normal neonates in a retrospective done in Bahrain with 1129 participants (p < 0.0001). In addition, we found that newborns with serum bilirubin of more than 15mg/dL had moderately depressed APGAR scores (20%). Johnsen (1999) found that the highest incidence of abnormal neurologic findings was in babies with low APGAR Scores and high serum bilirubin level. This study suggested that consumption of bilirubin by oxidant radicals generated during hypoxia and reperfusion may lead to neurological problems.

In this study, we investigated the association between neonatal serum bilirubin level and development of the infants using D-score. A study by Buuren (2021) supported the usefulness and validity of D-score as an informative summary of child development during their first two years of life. Currently, our study is the first one to find the association between serum bilirubin level and development of the infants using D-score. Our findings revealed an association between neonatal serum bilirubin level and early D-score (p < 0.001). Literature has shown the association between hyperbilirubinemia and neurodevelopmental problems. A systematic review reported that neonatal jaundice was one of the major causes leading to life-long neurodevelopmental impairment. The result was also consistent within which they reported a statistically significant association between hyperbilirubinemia and participants with developmental delay found in a retrospective cohort study. In this study, they went through the medical records of 51 infants born with or without hyperbilirubinemia and identified the occurrence of developing developmental delay at age two to four years old.

Similarly, another study done in Indonesia also found a significant correlation between 112 healthy term infants with history of hyperbilirubinemia and developmental delay in gross motor at the age of 6 months. They found that at age 3 months, it was statistically significant for delay in fine motor while at age 6 months it was significant for gross motor skills in infants with history of hyperbilirubinemia. In addition, a prospective cohort study done in India reported that in 6-month-old full term healthy infants who had neonatal hyperbilirubinemia, there was a significant association between peak serum bilirubin levels and neurodevelopmental sequelae. They found that in 77 participants, their serum bilirubin level was linearly associated with neurodevelopmental delay measured using the Denver Developmental Screening Test II [7].

Wusthoff & Loe (2015) proposed a mechanism

behind developmental delay in infants with severe hyperbilirubinemia. Deposition of unconjugated bilirubin in brain cells may lead to neuronal damage in the brain and cause a syndrome of Bilirubin-Induced Neurologic Dysfunction (BIND). Developmental delay is one of the associated manifestations of BIND alongside issues with cognition, speech, executive function as well as neurobehavioral disorders. Overall, this significant finding of our study provided the opportunity to investigate the causative effect of hyperbilirubinemia on developmental delay and focus on efforts to elucidate this effect thus improving its long-term outcome. Another potential improvement in future research might be to focus on specific developmental domains such as fine motor, gross motor, cognitive and language as well as their association with serum bilirubin level.

The findings of our study revealed that infants with serum bilirubin level of more than 15 mg/dL are associated with a lower D-score compared to infants with serum bilirubin level of less than 15 mg/dL. Although there has never been a specific study done regarding the association of bilirubin level and D-score of infants, there have been several studies published which have shown that hyperbilirubinemia is associated with higher risk of developmental delay. For starters, our discovery corresponds to a previous retrospective cohort study which revealed that infants with total serum bilirubin level ranging from 10 to 20 mg/dL (moderate hyperbilirubinemia) are associated with neurodevelopmental delay in 51 children age ranging from 2 to 4 years old. This is also supported by another study which found significantly increased risk of developmental delay among children with moderate hyperbilirubinemia as compared to no hyperbilirubinemia.

On the contrary, one study done in 2006 reported that there was little evidence for infants with total serum bilirubin level higher than 25 mg/dL up to 30 mg/dL to develop neurodevelopmental problems. In the same study, no cases of kernicterus were reported despite using 30 mg/dL as the cut off value. This study could also be supported by another literature done by Hansen (2011) regarding prevention of neurodevelopmental sequelae of jaundice in the newborn which stated that infants may be unharmed despite high peak total serum bilirubin level up to 36 mg/dL. However, these positive outcomes may be attributable to early emergency treatment given for these patients [8].

Our current study proposed that 15 mg/dL is the limit of serum bilirubin level that may predispose to developmental delay. This is supported by the significant result found in our study. This value was chosen in our study because a study reported that phototherapy should be initiated as a management in infants with total serum bilirubin level at or above 15 mg/dL in infants 25 to 48 hours old. In addition, Singh & Jialal (2020) proposed that phototherapy should be initiated as an early intervention in neonates with serum bilirubin level of 15 mg/dL or more. Therefore, this value of serum bilirubin level might provide an insight for medical professionals in managing neonatal hyperbilirubinemia, bearing in mind the potential complication of developmental delay if no intervention was initiated. Nonetheless, this finding provided a foundation for future research to investigate other potential values of serum bilirubin that might alter the developmental score.

The study that we have conducted has rooms for improvement. The first one would be to include a variety of places to obtain our data, not making it limited to only private hospitals. For example, government hospitals should be targeted as well. In addition to that, a larger sample size would be more beneficial as it would give more conclusive findings. Furthermore, different questionnaires or tools could be used to assess the neurodevelopmental stages in the infants, instead of being constrained to one tool and for our case it was the Dutch Development Instrument. Aside from the suggested improvements that could be made, our study also faced a few limitations. The most notable one was the lack of commitment from the mothers during the recruitment process. It is also highly understandable as they are required to communicate with our team during their postpartum period which could be an inconvenience for them. Moreover, another limitation would be that this study could be biased since the questionnaires used are limited to our subjective understanding based on how we explained it to the mothers involved [9].

Conclusion

Our study was done in hopes of finding an association between serum bilirubin level and early developmental milestones and we could conclude that there is indeed an association proven by Spearman's rank correlation ($p < 0.001$). In addition to that, we could also conclude that a serum bilirubin of more than 15 mg/dL is associated with a lower D-score ($p=0.007$).

Improvements that could be done are to use a larger sample size and to include government hospitals as well for data collection. This study was also limited by a few factors, the most prominent one being the lack of commitment from mothers during the recruitment process [10].

Acknowledgement

None

Conflict of Interest

None

References

1. Muenzer J. Early initiation of enzyme replacement therapy for the mucopolysaccharidoses. *Mol Genet Metab.* 111, 63-72 (2014).
2. Concolino D, Federica Deodato F, Parin R. Enzyme replacement therapy: Efficacy and limitations. *Ital J Pediatr*. 44, 120 (2018).
3. Tomatsu S, Alméciga Díaz CJ, Montaño AM *et al.* Therapies for the bone in mucopolysaccharidoses. *Mol Genet Metab.* 114, 94-109 (2015).
4. Al-Sannaa NA, Bay L, Barbouth DS *et al.* Early treatment with laronidase improves clinical outcomes in patients with attenuated MPS I: A retrospective case series analysis of nine sibships. *Orphanet J Rare Dis*. 10, 131 (2015).
5. Chuang CK, Lin HY, Wang TJ *et al.* Status of newborn screening and follow up investigations for Mucopolysaccharidoses I and II in Taiwan. *Orphanet J Rare Dis*. 13, 84 (2018).
6. Harrison SM, Heidi L, Rehm HL. Is 'likely pathogenic' really 90% likely? Reclassification data in ClinVar. *Genome Med.*11, 72 (2019).
7. Lin HY, Tu RY, Chern SR *et al.* Identification and functional characterization of IDS gene mutations underlying Taiwanese Hunter Syndrome (mucopolysaccharidosis type II). *Int J Mol Sci*. 21, 114 (2020).
8. Chuang CK, Lin HY, Wang TJ *et al.* A modified liquid chromatography/tandem mass spectrometry method for predominant disaccharide units of urinary glycosaminoglycans in patients with mucopolysaccharidoses. *Orphanet J Rare Dis*. 9, 135 (2014).
9. Lin HY, Lo YT, Wang TJ *et al.* Normalization of glycosaminoglycan-derived disaccharides detected by tandem mass spectrometry assay for the diagnosis of mucopolysaccharidosis. *Sci Rep*. 9, 10755 (2019).
10. Chuang CK, Lin SP, Chung SF. Diagnostic Screening for Mucopolysaccharidoses by the Dimethylmethylene Blue Method and Two Dimensional Electrophoresis. *Chin Med J.* 64, 15-22 (2001).