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The Relationship Between Bilirubin and Interleukin 4 Levels in the Jaundice Preterm Neonates

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ABSTRACT

Background: Preterm neonates, particularly infants ≤ 35 weeks gestation, has a greater risk of severe hyperbilirubinemia. The accumulation of bilirubin levels can inhibit the response of T helper 1 (Th1) cells making disruption of the balance towards T helper 2 (Th2). Th2 produces interleukin 4 (IL-4) as the most abundant cytokine and plays a major role in the process of allergy which in the future can trigger allergic diseases in childhood.
Aim: a cross-sectional study was conducted between August and December 2021. Subjects were 37 preterm neonates (≤ 35 weeks of gestational age) with jaundice who were admitted to Dr. M. Djamil Hospital Padang to evaluate level of total bilirubin and IL-4.
Results: The results of this study showed the mean level of total bilirubin was 10,92±2,73 mg/dl. The median IL-4 level was 90,72±0,21 pg/ml. The relationship between bilirubin and IL-4 level in the jaundice preterm neonates was statistically significant (p=0.033).

Keywords: allergy, bilirubin, interleukin 4, jaundice, preterm

INTRODUCTION

In the recent decade, the prevalence and incidence of allergy illnesses have increased. This rise is linked to a number of causes, one of which is neonatal hyperbilirubinemia, which can lead to allergies later in life. The immune response influences allergic illnesses, with the immune system in neonates responding to T helper 2 (Th2), which can trigger allergies. After birth, the T helper 2 (Th2) response mechanism must be switched to T helper 1 (Th1) to prevent allergies.[1,2]
Bilirubin inhibits the response of Th1 cells, which has an immunomodulatory impact.[3] Increased bilirubin levels in newborns can compromise the immune system. The findings of Haga et al investigation's revealed that bilirubin can decrease T helper 1 (Th1) cell responses, particularly the buildup of unconjugated bilirubin, which can disturb the Th1-Th2 balance, raising the risk of allergic illness.[4] Clinical symptoms of allergies range from mild skin rashes (atopic dermatitis), allergic rhinitis, and asthma. Cytokines and chemokines govern this inflammatory process precisely. Chemokines serve a function in attracting inflammatory cells to areas of inflammation, whereas cytokines regulate cellular responses at the transcriptional level. One of the cytokines that regulate the process of allergy is interleukin 4 (IL-4).[5]
Premature babies have a higher risk of having allergy disorders than full-term babies. In term newborns with an average interleukin 4 level of 0.300.21 pg/ml, Andriani's research discovered a link between IL-4 levels and bilirubin levels. [6] Because the high incidence of hyperbilirubinemia in preterm infants can promote allergy illnesses in childhood, this study aims to evaluate the link between interleukin 4 levels and bilirubin levels in neonates.

METHODS

The goal of this study is to see if there is a link between interleukin 4 levels and bilirubin levels in preterm newborns. It is an observational quantitative study with a cross sectional study design. Based on the sample calculation, the minimum sample size is 37 respondents in the perinatology ward Dr. M Djamil Hospital who was treated from August to December 2021.

The inclusion criteria for this study were all neonates with neonatal jaundice with gestational age 35 weeks who were treated at Dr. M.Djamil Hospital, Padang and were willing to participate in the study. The exclusion criteria for this study were: (1) Having a family history of atopy; (2) Proven sepsis or with clinical signs of severe sepsis; (3) Neonates with a maternal history of HIV or SLE; (4) Neonates with maternal history of using corticosteroids 2 weeks; (5) Neonates who have undergone phototherapy or previous exchange transfusion.

Sampling was done by consecutive sampling. Univariate analysis in the form of interleukin 4 and bilirubin levels in preterm neonates presented in the form of central tendency, namely the mean, standard deviation, minimum and maximum values. Analysis of interleukin 4 levels with bilirubin levels in premature neonates with jaundice was first tested for normality using the Shapiro Wilk (n < 50). This study used one way ANOVA test to see the relationship between respondent characteristics with interleukin 4 levels and bilirubin levels in preterm neonates in normally distributed data, while the data were not normally distributed using Kruskal Wallis test. While the relationship between levels of interleukin 4 and bilirubin levels in preterm neonates used Pearson correlation on normally distributed data, while Spearman correlation. All data analysis using the SPSS program. The research flow can be seen in Figure 1.

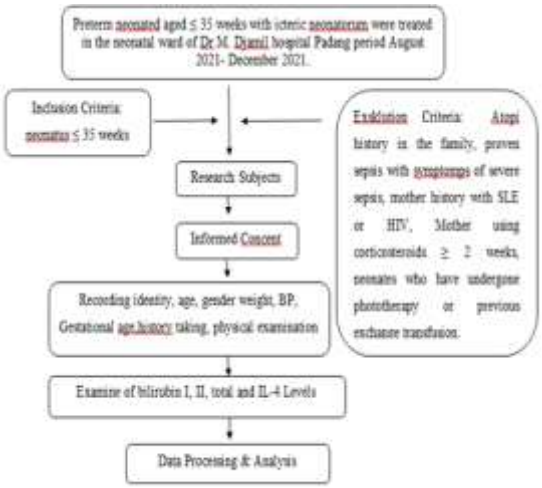

Figure 1. The research flow

RESULTS

This study was conducted to determine the relationship between interleukin 4 levels and bilirubin levels in preterm neonates in 37 research samples based on a cross sectional study approach. Characteristics of research subjects can be seen in table 1.

Table 1. Characteristics of Research Subjects

Variable	Value (n=37)
Clinical characteristics	
Gender (f/%)	
Boys	18 (48,6)
Girls	19 (51,4)
Gestational age (weeks), (f/%)	
< 28	2 (5,4)
28-32	16 (43,2)
> 32	19 (51,4)
Birth weight	
BBLR	18 (48,6)
BBLSR	11 (29,7)
BBLASR	8 (21,6)
Method of delivery	
Spontaneous	6 (16,2)
Vacum/ forceps	0
Sectio cesarean	31 (83,8)
Laboratory characteristics	
Interleukin 4 level (pg/ml), median(min-max)	90,72 (36,39-790,44)
Total bilirubin level (mg/dL), mean ± SD	10,92± 2,73

From table 1, it is known that more than half of the subjects (51.4%) had gestational age > 32 weeks and less than half of the subjects (43.2%) had gestational age of 28-32 weeks and a small proportion of subjects (5.4%) had a gestational age of <28 weeks. More than half of the subjects (51.4%) were female. Less than half of the subjects (48.6%) were LBW. Most of the subjects (83.8%) gave birth by cesarean section. Less

than half of the subjects had a total bilirubin level of 8-11 mg/dL (43.2%).

Table 2. Levels of IL-4 in Preterm Neonates

Variable	Median (Min-Max)
Levels of IL-4 (pg/ml)	90,72 (36,39-790,44)

From table 2, it is known that the median IL-4 in neonates is less months, namely 90.72 pg/ml with the lowest IL-4 level 36.39 pg/ml and the highest 790.44 pg/ml.

Table 3. Levels of bilirubin in preterm neonates

Variable	Mean±SD
Levels of Bilirubin (mg/dl)	10,92±2,73

From table 3, it is known that the average bilirubin level in preterm neonates is 10.92 mg/dl with a standard deviation of 2.73 mg/dl.

Table 4. Relationship between gestational age and IL-4 levels

Gestational age	Levels of IL-4 (pg/ml) (Median, min-max)	p-value
< 28 week	184,97 (167,39-202,54)	0,268
28-32 weeks	97,11 (36,39-490,10)	
32-35 weeks	81,13 (42,79-790,44)	

From table 4, it is known that the level of IL-4 at <28 weeks' gestation, which is 184.97 pg/ml, is higher than 28-32 weeks' gestation, which is 97.11 pg/ml and 32-35 weeks' gestation, which is 81.13 pg/ml. Based on the results of statistical tests using the Kruskal Wallis test, it was found that there was no relationship between gestational age and IL-4 levels (p>0.05).

Table 5. Relationship of Gestational Age with Bilirubin Levels

Gestational Age	Bilirubin levels (mg/dl) (Mean±SD)	p-value
< 28 weeks	8,55±0,21	0,059
28-32weeks	10 ,06±2,96	
32-35weeks	11,90±2,30	

From table 5, it is known that the average bilirubin level at <28 weeks' gestation is 8.55±0.21 mg/dl lower than 28-32 weeks' gestation, which is 10.06±2.96 mg/dl and 32-35 weeks' gestation. namely 11.90±2.30 mg/dl. Based on the results of statistical tests using the One-way ANOVA test, it is known that there is no relationship between gestational age and bilirubin levels (p>0.05).

Table 6. Relationship of Bilirubin Levels with Interleukin 4 Levels in Preterm Neonates

Variable	R	P
Levels of Bilirubin (mg/dL)	0,352	0,033*
Levels of IL-4 (pg/mL)		

*Spearman correlation test

From table 6, it is known that there is a positive relationship between IL-levels 4 with bilirubin levels in preterm neonates (p<0.05).

DISCUSSION

In this study, the number of preterm neonates with jaundice was almost equal, namely 51.4% female and male (49.6%). According to research by Gupta et al, the ratio of male and female sex in neonates with jaundice is 1:1. [7] Hahn et al. reported that the prevalence of men and women was 50.3% women and 49.7% men.[8] Meanwhile, research by Stevenson et al. found that there were more males than females.[8] Arman et al, the prevalence of jaundice in neonates by sex was 40.4% in males and 59.6% in females.[10] Nurani et al, reported on the characteristics of neonates with hyperbilirubinemia at Dr Hasan Sadikin Hospital, Bandung. A higher prevalence of hyperbilirubinemia was reported in males than females, namely 56.9% cases.[11]

The incidence of hyperbilirubinemia in this study was mostly found in neonates with gestational age 32-35 weeks followed by the 28-32 weeks gestational age group. Morioka et al, the incidence of jaundice in neonates <30 weeks' gestation is 1.8 per 1000 live births.[12] Hahn et al, gestational age was significantly associated with the incidence of hyperbilirubinemia (P=0.014). The average gestational age for preterm neonates with hyperbilirubinemia is 28.4 weeks.[8] Arman et al. reported a mean gestational age of icteric neonates of 30.0 ± 2.64 weeks. The prevalence of jaundice based on the gestational age group was 38.4% of cases at the age of 29-31 weeks, 32.3% at the age of 32-34 weeks, and 29.3% at the age of 24-28 weeks.[10] Aynalem et al, the prevalence of hyperbilirubinemia was most commonly reported at 32-34 weeks' gestation.[13]

The mean total bilirubin level in the study subjects was 10.92±2.73 mg/dL. Hegyi et al, 580 samples of preterm neonates were measured for bilirubin levels. The average bilirubin level found in the first week of life is 13.6±9 mg/dL.[14] Rohsiswatmo et al, the average bilirubin level in preterm neonates was 10.18 ± 2.91 mg/dL.[15] Pendse et al, measured total bilirubin levels in preterm neonates prior to phototherapy. The average total bilirubin level obtained was 12.05 mg/dl.[16] Andriani reported the mean total bilirubin on icteric term neonates was 12,40 ±2,56 mg/dL. The icteric age's mean was 4,00 ±1,35 days.[6]

The mean level of interleukin 4 was 90.72 pg/ml with the lowest level of IL-4 was 36.39 pg/ml and the highest was 790.44 pg/ml. Anam et al, reported IL-4 levels in preterm neonates who received breast milk and formula milk. The average range of IL-4 levels reported in this study was between 513.83 ng/L to 734.55 ng/L. Andriani reported that mean level of IL-4 term baby was 0,30 ± 0,21 pg/ml.[6] Oshima et al studied serum IL-4 and soluble CD23 in children with allergic disorders, found that IL-4 levels in healthy neonates were 0.73 ± 0.68 pg/mL.[17] In this study, a normality test was carried out using the Shapiro Wilk test. interleukin 4 data is not normally distributed. It has been tested with Log10 and logn, the data is still not normally distributed, so this interleukin data is shown with the median result not the mean, but in this study it can be seen that IL-4 preterm neonates who are icteric are higher than IL-4 healthy neonates or term neonates.

Interleukin-4 is a cytokine that functions as an anti-inflammatory. Interleukin-4 will inhibit the release of proinflammatory cytokines. Low levels of IL-4 will be found in patients with necrotizing enterocolitis.[18]

Interleukin-4 is one of the anti-inflammatory cytokines that can prevent inflammation. Neonates who have an IL-4 Z score of less than -1 have the possibility for the development of neuronal disorders. In general, there was no significant difference between IL-4 levels in preterm and term neonates. However, several studies reported that the smaller the gestational age, the higher the IL-4 concentration. Preterm neonates accompanied by inflammation will cause increase systemic IL-4 levels.[18] Benkoe et al who evaluated 11 cytokines in premature infants with Necrotizing Enterocolitis found IL-4 was 1.4 times lower than the control group, while IL 6, IL 8 and IL 10 (inflammatory cytokines) increased.[19] In Lusyati et al study regarding levels 25 cytokines in the first 7 days of neonatal life ≥ 24-72 hours of age with a median value of 3 (1-5.5) pg/ml.[20]

In this study, a significant relationship was found between bilirubin levels and IL-4 levels. The mechanism for increasing IL-4 is due to bilirubin which indirectly causes a shift in the balance to Th2. In accordance with the study of Haga et al found that bilirubin can inhibit the response of Th1 cells, and the accumulation of unconjugated bilirubin so that the Th1-Th2 balance shifts towards Th2. Then Th2 releases cytokines, with IL-4 as the most cytokine.[4]

CONCLUSION

The average bilirubin level in preterm neonates is 10.92 mg/dl ± 2.73 mg/dl. The mean level of bilirubin in icteric preterm neonates was almost the same when compared with the results of similar studies.

The median IL-4 in preterm neonates was 90.72 pg/ml (36.39 pg/ml - 790.44 pg/ml).

Interleukin 4 levels in icteric preterm neonates were higher than icteric term neonates. There is a significant relationship between bilirubin levels and interleukin 4 levels in icteric preterm neonates.

Declaration by Authors

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