EFFECT OF PROBIOTIC *LACTOBACILLUS REUTERII* ON SERUM BILIRUBIN LEVELS IN PRETERM INFANTS

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Protocol

Responsible Personnel

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Summary:

This study aims to assess the effect of probiotic Lactobacillus reuteri administration on serum bilirubin levels in preterm infants. It was a randomized controlled trial conducted on 40 preterm infants (32 to <37 weeks gestation, 1000-2499g birth weight) admitted to the NICU at Dr. Wahidin Sudirohusodo Hospital in Makassar, Indonesia. The infants were randomly divided into two groups of 20 each - one receiving L. reuteri probiotic and a control group. Serum bilirubin levels were measured at baseline and after 7 days of intervention, the need for phototherapy following hyperbilirubinemia will also be assessed.

Introduction:

The global neonatal mortality rate stands at 18 deaths per 1,000 live births, with Indonesia ranking fifth highest among Southeast Asian countries. Prematurity is a leading cause of neonatal death in Indonesia, which ranks fifth worldwide for the number of premature births. One significant complication of premature birth is hyperbilirubinemia, occurring in 80% of premature babies in developing countries like Indonesia. This condition can lead to severe neurological complications if left untreated.

Probiotics have emerged as a potential management strategy for hyperbilirubinemia in preterm infants. These live microorganisms can improve intestinal microbial colonization and regulate gastrointestinal motility. Several studies have shown promising results in reducing serum bilirubin levels and the need for phototherapy in newborns through probiotic administration. However, research findings have been mixed, with some studies showing significant reductions in bilirubin levels while others found no difference.

Given the potential benefits of probiotics and the lack of comprehensive research on their effect on bilirubin levels in premature infants, particularly in Indonesia, further investigation is warranted. This study aims to explore the impact of probiotic Lactobacillus reuteri on serum bilirubin levels in premature infants, with the goal of providing an alternative approach to prevent and manage hyperbilirubinemia in this vulnerable population. As the first study of its kind in South Sulawesi and Indonesia, it is expected to contribute valuable knowledge for future clinical applications.

Objectives of Trial:

1. **Primary Objective**
   a. To assess the effect of probiotic *lactobacillus reuteri* on serum bilirubin levels in preterm infants.
2. **Secondary Objective**

   a. To assess serum bilirubin levels of preterm infants before and after receiving probiotic *lactobacillus reuteri* in the probiotic group and the non-probiotic group.
   b. To compare serum bilirubin levels in preterm infants before and after receiving probiotic *lactobacillus reuteri* in the probiotic group with the non-probiotic group
   c. To compare changes in serum bilirubin levels of preterm infants the probiotic group with the non-probiotic group.
   d. To assess the percentage of hyperbilirubinemia requiring phototherapy in the probiotic group
   e. To assess the percentage of hyperbilirubinemic events requiring phototherapy in the non-probiotic group
   f. To compare changes in serum bilirubin levels of preterm infants requiring phototherapy in the probiotic group with the non-probiotic group.

Hypothesis:

H0: Bilirubin levels in preterm infants in the probiotic group are lower compared to those in the non-probiotic group

H1: The incidence of hyperbilirubinemia requiring phototherapy in the probiotic group is less than in the non-probiotic group

Study Design:

Interventional study

Study Population:

Number of samples:

40 subjects

Inclusion criteria:

- Premature Infants (32 - < 37 weeks gestation)
- Baby Weight 1000 - 2499 grams
- Sick babies who are treated in the NICU
- Preterm infants who received enteral feeding ≥ 30 cc/kgBB less than 72 hours after birth, without jaundice or jaundice kremer 1,2, and 3
- Parents have signed their child's consent to participate in the study

Exclusion criteria:

- Infants with multiple congenital abnormalities

- Infants with jaundice before 24 hours or having jaundice cremer $\geq$ 4 less than 72 hours from birth
- Infants with Sepsis, Metabolic acidosis

Product Description:

Lactobacillus reuterii in the form of Interlac, contains: Lactobacillus reuteri DSM 17938 100.000.000 CFU

Outcomes:

The main outcome being measured is serum bilirubin levels in preterm infants. Specifically, serum bilirubin screening will be conducted by taking blood samples from the radial or femoral artery of the infants, collecting approximately 1 cc of blood to measure total and direct bilirubin levels. Samples will be taken at two time points: after infants receive enteral feeding >30 cc/kg body weight and after 7 days of intervention or when Kramer scale reaches IV/V.

The study will compare bilirubin levels between the probiotic group and control group, looking at initial and final levels within each group as well as the change (delta) in levels from initial to final measurement between groups. Additionally, the percentage of infants requiring phototherapy will be compared between the probiotic and control groups. Statistical analysis will utilize paired and unpaired t-tests to analyze differences in bilirubin levels between and within groups, while a chi-square test will compare phototherapy requirements. The primary outcome is the difference in serum bilirubin levels between the probiotic and control groups after 7 days of intervention, with secondary outcomes including the need for phototherapy and the rate of bilirubin decline between groups. Overall, this methodology aims to comprehensively evaluate the effect of probiotic supplementation on bilirubin levels and management in preterm infants.

Conduct of Trial:

This study will be conducted as a randomized controlled trial (RCT) to evaluate the effect of probiotic Lactobacillus reuteri administration on serum bilirubin levels in preterm infants. The trial will take place in the Neonatal Intensive Care Units of Dr. Wahidin Sudirohusodo Hospital and Cahaya Medika Hospital in Makassar, South Sulawesi. Eligible participants will be preterm infants born at 32 to <37 weeks gestation with birth weights between 1000-2499 grams. Written informed consent will be obtained from the parents of eligible infants after providing a detailed explanation of the study purpose and procedures. Using consecutive sampling, 40 infants meeting the inclusion criteria will be randomly allocated into two groups of 20 each - an intervention group receiving probiotic L. reuteri and a control group. The intervention group will receive 5 drops of Interlac (containing 1x108 CFU L. reuteri) daily for 7 days, while the control group will receive standard care without probiotics. Serum bilirubin levels will be measured at baseline (after infants receive >30 cc/kg enteral feeding) and again after 7 days of intervention or when Kramer scale reaches IV/V. Blood samples of approximately 1 cc will be collected from the radial or femoral artery to measure total and direct bilirubin levels. The primary outcome will be the difference in serum bilirubin levels between groups after 7 days, with secondary outcomes including the need for phototherapy and rate of bilirubin decline. Statistical analysis will be performed to compare outcomes between the two groups.

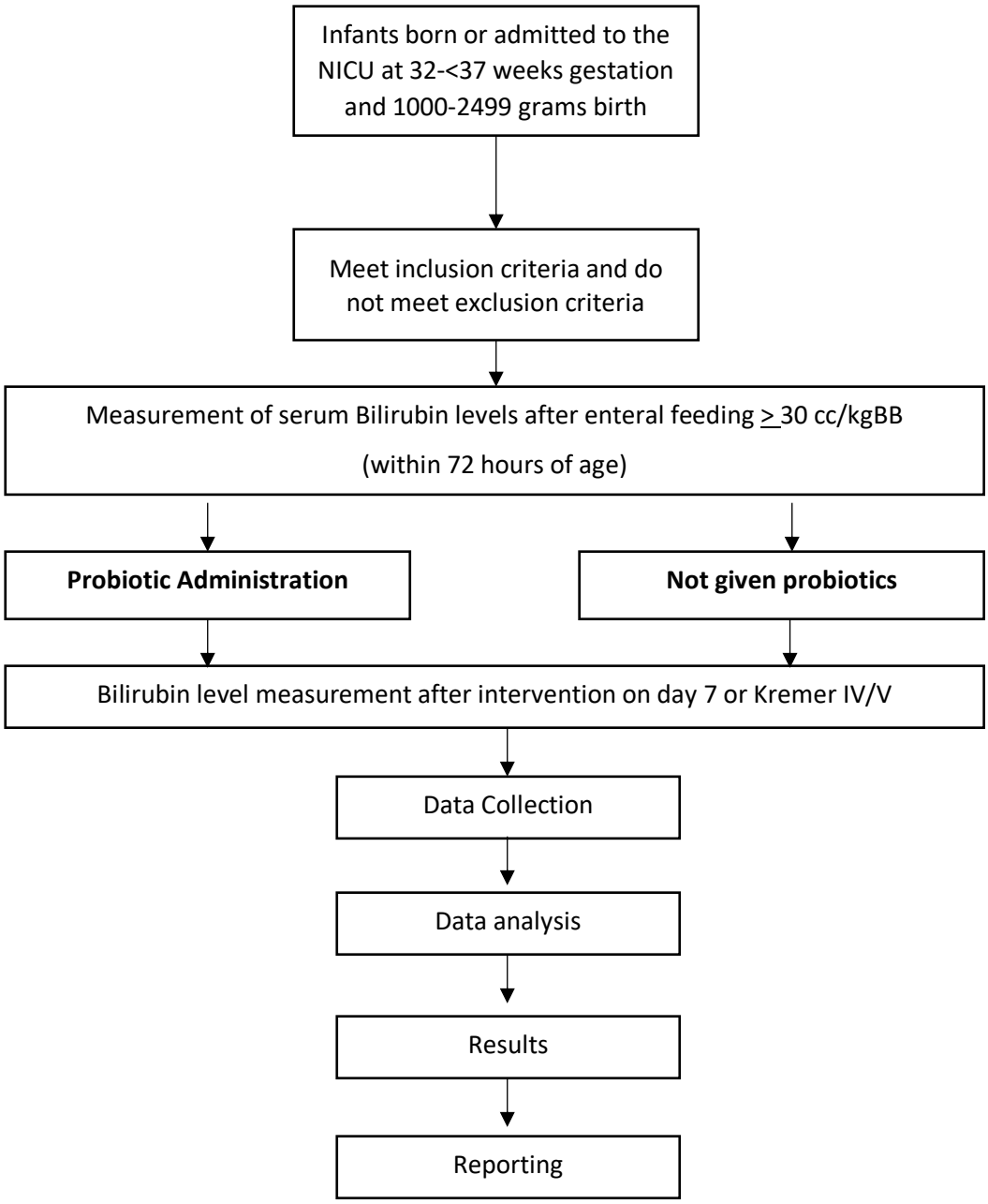

Figure 1. Study Procedure

Statistical Aspects and Considerations:

This study employs a randomized controlled trial (RCT) design to evaluate the effect of probiotic Lactobacillus reuteri on serum bilirubin levels in preterm infants. The sample size was calculated using a formula for a two-sided test, with a 99% confidence level (α=0.01) and 90% power (β=0.10). The standard deviation of bilirubin levels was estimated at 3.20 based on a previous study. Subjects meeting inclusion criteria will be randomly allocated to intervention and

control groups using consecutive sampling. Statistical analysis will include both univariate and bivariate methods. Univariate analysis will describe basic data characteristics using frequencies, means, standard deviations, and ranges. Bivariate analysis will employ paired and unpaired t-tests for normally distributed data, and Wilcoxon and Mann-Whitney tests for non-normally distributed data. Chi-square tests will be used for categorical variables. The significance level is set at $p \leq 0.05$ for all analyses. This comprehensive statistical approach aims to rigorously assess the efficacy of probiotic intervention on bilirubin levels in preterm infants while controlling for potential confounding factors.

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