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The Role of Lactobacillus Reuteri DSM 17938 Reducing Indirect Bilirubin Levels in Neonatal Hyperbilirubinemia

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Abstract

Neonatal jaundice is a yellowish discoloration of the skin, conjunctiva, and sclera due to increased serum or plasma bilirubin in the neonatal period. This condition can cause parents' anxiety and increase the need to go to the hospital. In general, phototherapy was one of the therapeutic options to reduce neonatal hyperbilirubinemia with subsequent side effects such as dehydration, retinal damage, and bronze-baby syndrome. There is a need for further therapeutic options to treat neonatal hyperbilirubinemia with minimal side effects, and Lactobacillus was one of many options that have been proposed. This double-blind, randomized clinical trial was conducted to determine the role of Lactobacillus reuteri DSM 17938 to treat neonatal hyperbilirubinemia in conjunction with phototherapy. In conclusion, administration of Lactobacillus reuteri DSM 17938 can reduce indirect bilirubin levels in neonatal hyperbilirubinemia faster compared to the group without intervention (p-value 0.000).

Keywords: *Neonatal Hyperbilirubinemia, Probiotic, Lactobacillus.*

INTRODUCTION

Neonatal jaundice is a yellowish discoloration of the skin, conjunctiva, and sclera due to an increase in serum or plasma bilirubin in the neonatal period that can be bothersome in parents (Ullah et al., 2016). most jaundice in neonates results from increased breakdown of red blood cells and decreased excretion of bilirubin. Nonetheless, there are some causes that develop jaundice in neonates such as breastfeeding jaundice, hemolysis, and some metabolic and genetic abnormalities (Ansong-Assoku et al., 2018). Hyperbilirubinemia especially indirect hyperbilirubinemia can be neurotoxic, causing acute or chronic encephalopathy that can lead to cerebral palsy, hearing loss, and seizures (Woodgate & Jardine, 2015). In Indonesia, neonatal jaundice occurs in about 7% of total newborns (Sampurna et al., 2019). About 2% of these infants develop acute neonatal jaundice within first seven days of life.

Over many years, the main therapy of indirect hyperbilirubinemia is phototherapy or exchange transfusion for severe cases with total bilirubin levels greater than 20 mg/dl (Woodgate & Jardine, 2015). while the therapy itself had some common side effects such as heat exposure that could lead to dehydration, retinal damage or bronze-baby syndrome. Microbiota has already known offers many benefits to the host, including gastrointestinal system, allergy-immunology, and inflammation and so on through various physiological functions. In gastrointestinal tract, it has function such as strengthening gut integrity or

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forming intestinal epithelium, protect against pathogens and regulating the host immune system (Thursby & Juge, 2017) (Natividad & Verdu, 2013) (Gensollen et al., 2016) (Schroeder & Bäckhed, 2016) (Chang & Lin, 2016). However, there is potential for disruption of this microbiota mechanism as a result of altered microbial composition, known as dysbiosis. With increasingly sophisticated methods for profiling and characterizing complex ecosystems being developed, the role of microbiota in a large number of gastrointestinal and out of gastrointestinal diseases is becoming increasingly clear (Hills et al., 2019) (Zhou et al., 2019).

There has been some research on the relationship between jaundice in neonates and adverse long-term health profiles, such as childhood asthma, type 1 diabetes, and diarrhea nor constipation (Huang et al., 2013) (McNamee et al., 2012) (Tuzun et al., 2013). Recent research in understanding the microbiota reveals the role of bacteria in bilirubin metabolism. Several studies have been conducted on the possible relationship between the increase in direct bilirubin and bacteria, one of which is Lactobacillus (Tuzun et al., 2013) (Flint et al., 2015). It is known that probiotics can improve intestinal motility and prevent enterohepatic cycle, so it was expected that bilirubin secretion can occur faster (Chang & Lin, 2016) (Hills et al., 2019) (Zhou et al., 2019). Therefore, this study aim to evaluate and compare benefit of probiotics, especially Lactobacillus reuteri DSM 17938 on neonates with hyperbilirubinemia at RSUP Kandou Manado in reducing bilirubin levels in the blood.

METHODS

The research method used was an experimental study with a double-blind, randomized clinical trial to assess the reduction rate of indirect bilirubin levels in neonates who received Lactobacillus reuteri DSM 17938 during phototherapy. This study compared two groups based on the results of measuring indirect bilirubin levels in the neonatal group without Lactobacillus reuteri DSM 17938 (only using placebo by maltodextrin-filled mixed fluid administration) as the unexposed group and the neonatal group with Lactobacillus reuteri DSM 17938 administration as the exposed group. Each of these two groups will be observed, and the level of indirect bilirubin will be so that the level of indirect hyperbilirubinemia levels between exposed and unexposed groups can be compared.

This experimental study was conducted in the Neonatology Intensive Care Unit of Prof. Dr. R. D. Kandou Manado Hospital for ten months, from November 2022 until August 2023, with a total sample of 104 neonates. The inclusion criteria include newborns aged 0 – 7 days with neonatal hyperbilirubinemia at 36-38 weeks of gestational age. On the other hand, the exclusion criteria include neonatal diagnosed with sepsis, hepatocellular disease, TORCH infection, HIV infection, Hepatitis B infection, multiple congenital anomalies, direct hyperbilirubinemia, and phototherapy before the experiment. Kandou Hospital Research and Ethics Committee approved this study, and informed consent was obtained from parents. Sampling was done by purposive sampling that met inclusion criteria.

Then, those samples were divided into two groups based on random sampling for 52 samples in the control group and 52 samples in the intervention group that were given Lactobacillus reuteri 5 drops for each day from the first day until the fifth day forward. Those samples still got phototherapy as standardized therapy to prevent indirect hyperbilirubinemia complications such as kernicterus. The two groups will be evaluated for indirect bilirubin concentration on the first day, third day, and fifth day as the last observation day.

Univariate analysis was present with a distributive table, and parametric data were present in median and standard deviation. Bivariate analysis using an independent t-test was used

to compare two groups for quantitative data, and the Mann-Whitney test was used in comparing two groups with the non-parametric distribution. The confidence interval was set to 95%, and the margin of error accepted was 5%, with the p-value considered significant if p-value < 0.05 and highly significant if p-value < 0.01.

RESULTS AND DISCUSSION

Indirect hyperbilirubinemia is a medical condition in which the level of indirect bilirubin in the blood increases leading to excessive bilirubin deposition of the mucosa. It usually appears on first week of life and present around 2/3 of all neonates. Most jaundice in neonates results from increased breakdown of red blood cells and decreased excretion of bilirubin. Breastfeeding, hemolysis, and some metabolic and genetic abnormalities also increase the risk of jaundice. Generally, this condition is usually harmless and will improve on its own within a few weeks. However, in some cases indirect hyperbilirubinemia can be neurotoxic and cross the blood-brain barrier leading to brain damage or kernicterus, which can lead to serious and even life-threatening health problems. For many years beyond some medications had been used to treat this hyperbilirubinemia neonatal such as phenobarbital, clofibrate nor metalloporphyrin with effect its safeties and its complications. Phototherapy itself can cause heat exposure, dehydration, electrolyte abnormalities, retinal damage and social issue cause by reduce bonding between mother and babies. Probiotic as live micro-organism that could imply benefit to the host if consumed in the right dosage. It has been known for many mechanisms of action for allergy, inflammation and so on. It was believed that probiotic especially Lactobacillus spp. could alter gut microbiota and effect the bilirubin metabolism. (Pan & Rivas, 2017)

Characteristics of Research Samples

This study was conducted on neonates with hyperbilirubinemia neonatorum who were treated at the Neonatology Intensive Care Unit of RSUP Prof. Dr. R. D. Kandou Manado within ten months from November 2022 until August 2023 with a total sample of 104 neonates. Those 104 neonates who were selected by random sampling were divided into two groups: the experimental group and the control group. A double-blind experimental study - a randomized clinical trial method in assessing the reduction of indirect bilirubin levels in neonates who received Lactobacillus reuteri DSM 17938 during phototherapy. At the beginning of the study, researchers wanted to conduct a study with a minimum sample size of 72 neonatal samples. However, in the end, 104 neonatal samples were collected during the data collection and sample collection period, with 52 control group samples and 52 case group samples for intervention with DSM 17938 Lactobacillus reuteri administration. Of the 104 respondents, 55 (53%) were female patients. Patients' average median birth weight ranged from 3015 grams. In contrast, the patient's birth length had an average median of 48 cm. 104 infants had high total bilirubin levels with an average total bilirubin level of 11.42 mg / dL with direct bilirubin having an average of 9.22 mg/ dL.

Table 1. Characteristic samples

Variable	Total n=104
Sex (%)	
Male	49 (47.1)
Female	55 (52.9)
Birth weight, mean (SD)	3015.67 (342.55)

Birth length, median (IQR)	48 (47-48.7)
Total bilirubin concentration, mean (SD)	11.42 (8.85)
Indirect bilirubin concentration, mean (SD)	9.22 (6.97)

On table 2 it was explained further in experimental group, mean birth weight was 3,069 gram and mean birth weight on control group was 2,962 grams. Between experimental and control group, median birth length was still the same approximately 48 cm.

Table 2. Characteristic samples between control group and experimental group

Variable	Group	
	Experimental	Control
	n=52	n=52
Sex (%)		
Male	27 (51.9)	22 (42.3)
Female	25 (48.1)	30 (57.7)
Birth weight, mean (SD)	3069 (392.65)	2962.31 (277.48)
Birth length, median (IQR)	48 (47-49)	48 (46-49)

Bivariate Analysis of Research Variables

The results showed that in both the experimental and control groups, there were no significant differences between the sexes of infants where the proportion of indirect hyperbilirubinemia between male or female babies were almost the same. Previous research has shown that male babies are more likely to develop indirect hyperbilirubinemia than girls. However, the results of this factor are considered not strongly significant. This could be due to placental dysfunction that is more common in male babies and metabolic rate caused by the theory that blastocyte XY grows faster than blastocyte XX (Norman et al., 2015) (Norman et al., 2015), However, several other studies also suggest that there is no difference in cases of indirect hyperbilirubinemia in both male or female babies. (Garosi et al., 2016) (Gupta et al., 2016)

Furthermore, in this study, there was no significant difference between birth weight in the experimental and control groups. Several studies have shown a significant positive association between birth weight and the incidence of jaundice in infants (Devi & Vijaykumar, 2017) (Farhat et al., 2016). This may be because low birth weight tends to be associated with premature birth, resulting in immature liver function and shorter erythrocyte life span to only 90 days (Farhat et al., 2016). In babies born prematurely, there are several pathways of both pre-hepatic and intrahepatic outcomes. From the pre-hepatic pathway there is an increase in bilirubin load on hepatocyte cells due to decreased erythrocyte age, increased erythrocyte volume, and increased enterohepatic circulation (Slaughter et al., 2022) (Anand et al., 2020). Whereas in intrahepatic pathway, reduced plasma bilirubin uptake by the liver or impaired bilirubin conjugation often occurred in preterm babies (Slaughter et al., 2022). However, Mitra et al report that approximately 60-80% of newborns will experience elevated levels of bilirubin in the blood during their first week of life regardless of their weight (Mitra & Rennie, 2017)

Based on birth length, there was no significant difference between the experimental and control groups. The median birth length in both the experimental and control groups was 48 cm or categorized as normal. Until now, there have been no studies that specifically show an association between body length and indirect hyperbilirubinemia in newborns.

Table 3. Bivariate Analysis

Variable	Total n=104	Group Experimental n=52	Kontrol n=52	P value
Sex (%)				
Male	49 (47.1)	27 (51.9)	22 (42.3)	0.326
Female	55 (52.9)	25 (48.1)	30 (57.7)	
Birth weight, mean (SD)	3015.67 (342.55)	3069 (392.65)	2962.31 (277.48)	0.113
Birth length, median (IQR)	48 (47-48.75)	48 (47-49)	48 (46-49)	0.497
Experimental group (%)		0	52 (100)	0.000

Below Figure 1 is a boxplot of bilirubin levels on days 1, 3 and 5. Based on the boxplot, the range of bilirubin levels in the control group was wider than the levels in the experimental group. On day 1, the results of the Mann Whiney U Test analysis showed that there were no significant differences between the experimental and control groups. On days 3 and 5, the results of the analysis showed that there was a significant difference in the bilirubin levels of the experimental and control groups where the median bilirubin levels in the experimental group were lower than those in the control group.

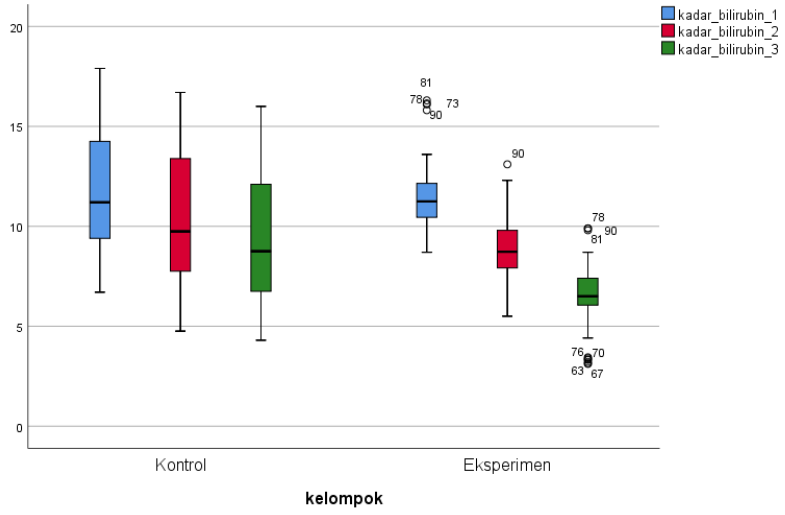

Figure 1. Boxplot Bilirubin Concentration on Day 1, Day 3 and Day 5 after administration of Lactobacillus reuteri DSM 17938

On the other hand, table 4 below describes the bilirubin levels on day 1, day 3 and day 5 after the intervention by giving Lactobacillus reuteri DSM 17938. From the table it is explained that giving Lactobacillus reuteri DSM 17938 has a significant relationship in accelerate the reduction of indirect bilirubin in blood levels on day 3 (p value 0.019) and day 5 (p value 0.000). However, on the first day, the intervention by giving Lactobacillus reuteri DSM 17938 did not give significant results in reducing bilirubin levels.

Table 4. Bivariate Analysis on Day 1, Day 3, Day 5

Bilirubin Level	Group Experiment	Control	P value
Day 1, median (IQR)	11.25 (10.42-14.27)	11.2 (9.40-14.27)	0.777
Day 3, median (IQR)	8.73 (7.8-9.8)	9.74 (7.73-13.45)	0.019
Day 5, median (IQR)	6.5 (6.04-7.40)	8.75 (6.72-12.10)	0.000

Figure 2 below is a Line Plot visualization which shows that in both the control and experimental groups there was a reduction in bilirubin levels on days 3 and 5. However, the bilirubin levels in the experimental group decreased significantly after administration of Lactobacillus reuteri 17938. The results of the analysis showed that administration of Lactobacillus reuteri 17938 helps accelerate the reduction of indirect bilirubin levels in neonatal hyperbilirubinemia.

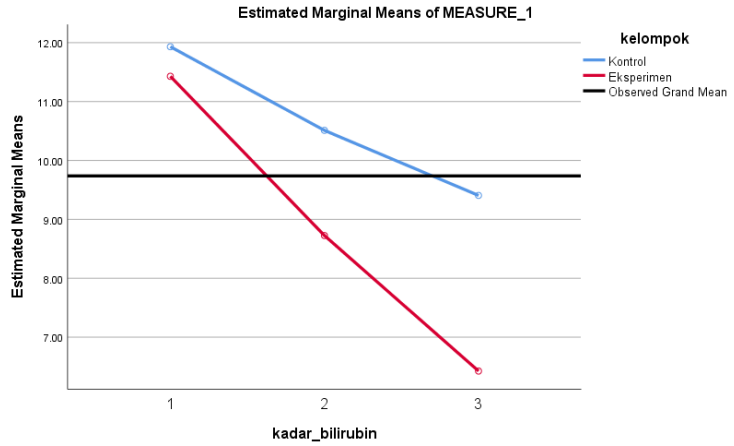

Figure 2. Line Plot Changes in Bilirubin Levels Day 1, day 3, day 5 after administration of Lactobacillus reuteri DSM 17938

After the third day of life, homeostasis between production, conjugation, and secretion in bilirubin metabolism begins to restore. Starting from the third day, there was a balance between maternal and neonatal aspects to accelerate the restoration of bilirubin metabolism. The enterohepatic cycle will decrease in the neonatal aspect, allowing more stable bilirubin direct. Microbiota growth begins normalizing gut flora on the third day to facilitate bilirubin metabolism. Sucking and swallowing reflexes in neonates begin to revolve to facilitate breastfeeding consumption. From the maternal aspect, lactogenesis II also maintains and highly produces breastfed milk for babies, increasing their intake production. Those factors facilitate a significant reduction of bilirubin levels starting from the third day of life.

This study showed that the administration of Lactobacillus reuteri DSM 17938 showed a significant reduction in the level of directed hyperbilirubinemia. In the next multivariate analysis using linear regression, the intervention was obtained in the form of adding Lactobacillus reuteri DSM 17938 in the experimental group to be the main variable accelerating the reduction in indirect bilirubin levels in neonatal hyperbilirubinemia. Results showed effectiveness in the experimental group after day 3 (decrease ≥2mg/dl) of 92.3%, much higher than in the control group. Effectiveness after day 5 (decrease ≥4mg/dl) decreased to 85.5% compared to the first 3 days, as shown in Table 5.

Table 5. Effective Rate after Administration of Lactobacillus reuteri DSM 17938

Group	3 days In-effective	Effective	Effective rate	5 days In-effective	Effective	Effective rate
Experiment (n=52)	4	48	92.3%	7	45	85.5%

Control (n=52)	41	11	21.2%	49	3	5.8%

This is in line with a previous study conducted by Makhoul et al. conducted on 69 infants diagnosed with neonatal jaundice. These babies were then randomly divided into two groups. The first group received the probiotic Lactobacillus reuteri orally for 3 days, while the second group received exclusive phototherapy without probiotics. The results of the study showed that the group that receiving the probiotic Lactobacillus reuteri had a significant reduction in serum bilirubin levels compared to the untreated control group on days 1, 2 and 3. (Makhoul et al., 2018)

Another study conducted by (Teran et al., 2021) on the role of probiotics in preventing neonatal hyperbilirubinemia where this study compared the effectiveness of administration of Lactobacillus reuteri, S Bouardii and the control group (without treatment) in 98 subjects (consisting of a control group of 36 subjects and an experimental group of 31 subjects each). The study found that the group receiving Lactobacillus reuteri had lower average bilirubin levels than the other groups. However, in this study, bilirubin levels were measured only once, on day 4 after treatment (Teran et al., 2021). Lactobacillus reuteri is a commonly used microbiota because it has diverse functions such as promoting colonization of normal flora, modulating the immune system, preventing infections, and preventing the incidence of allergies. Until now it is known that there are other potential mechanisms owned by Lactobacillus reuteri, one of which is in bilirubin metabolism.

Table 6 explains the changes in indirect hyperbilirubinemia levels on days 1 to 3 showing a β coefficient of 1.305 in the experimental group which explain that when other variables are constant, the change in indirect hyperbilirubinemia levels in individuals with the addition of Lactobacillus reuteri DSM 17938 is 1.305 in accelerating the reduction. Furthermore, changes in indirect hyperbilirubinemia levels on days 3 to 5 showed a β coefficient of 1,169 in the experimental group and a β coefficient of 2,474 on days 1 to 5 with the administration of Lactobacillus reuteri DSM 17938.

Table 6. Clinical Outcomes

Variable	Day 1 – 3 β (95% CI)	p	Day 3 – 5 β (95% CI)	p	Day 1 – 5 β (95% CI)	p
Experimental group	1.305 (0.619-0.9)	0.000	1.169 (0.96-1.38)	0.000	2.474 (2.09-2.85)	0.000
Sex	0.134 (0.447-0.58)	0.400	0.045 (0.165-0.22)	0.665	0.179 (0.19-0.55)	0.347
Birth weight	0.000 (0.00-0.001)	0.402	0.000 (0.00-0.01)	0.195	0.000 (0.00-0.12)	0.979
Birth length	0.061 (0.061-0.18)	0.323	0.029 (0.05-0.11)	0.488	0.090 (0.05-0.23)	0.226

Lactobacillus reuteri has a mechanism in reducing bilirubin levels by increasing glucoronyltransferase activity in elimination bilirubin and increasing gastrointestinal motility so that bilirubin transit time is reduced and inhibits reabsorption into enterohepaticcirculation (Teran et al., 2021). One study conducted by Tan et al. showed that Lactobacillus reuteri would increase the activity of the enzyme glucuroniltransferase, which converts unbound bilirubin into a bound form that is easily excreted by the liver. (Teran et al., 2021)

Another mechanism is the administration of Lactobacillus reuteri microbiota will increase anaerobic bacteria in the gastrointestinal tract which creates a balance of

intestinal microflora, creates an alkaline atmosphere or alkaline environment thereby reducing the effectiveness of the work of beta glucoronidase, preventing bilirubin binding and making bilirubin hydrolyzed. This process increases excretion of conjugated bilirubin (Khoman et al., 2020). Other research by Jiang et al showed that Lactobacillus reuteri can reduce inflammation and oxidative stress in rats with hyperbilirubinemia. The study showed that administration of Lactobacillus reuteri DSM 17938 can decrease levels of interleukin-6 (IL-6) and malondialdehyde (MDA), which are indicators of inflammation and oxidative stress. (Khoman et al., 2020)

As we know, jaundice in neonatal can occur caused by imbalance between production, conjugation, and secretion. Pathway increase or decrease those factors to build balance among them were required to eliminate jaundice in neonatal. Through this study researchers found that Lactobacillus reuteri DSM 17938 is believed to alter both conjugation and secretion pathway. In conjugation pathway, Lactobacillus reuteri increase oxidation of bilirubin direct so it will eliminate collection of indirect bilirubin. It also produces as a buffer to create alkaline environment that will alter activity of β-glucoronidase to inhibit enzymatic hydrolysis of bilirubin, thus decrease enterohepatic circulation. On secretion pathway, Lactobacillus reuteri DSM 17938 increase gut motility, enterocyte production and stabilize direct bilirubin to prevent to degradation.

A study conducted by Zhou Wang et al[16] showed that supplementation of Lactobacillus Plantarum can activate the protein kinase C pathway that can inhibit tight junction protein dysfunction in the intestinal epithelium induced by unconjugated bilirubin. With the administration of Lactobacillus spp.ini will tighten tight junction cells in the intestine so that it cannot be penetrated by indirect bilirubin which will activate the activity of β-glucoronidase. One physiological neonatal jaundice has an etiology in the form of breastmilk jaundice where the theory states the mechanism of jaundice due to increased enterohepatic circulation, although it may be caused by several other factors such as increased activity of β-glucuronidase and pregnane-3a, 20β-diol contained in breast milk, enzyme activity UGT 1A1 enzyme activity and intestinal microbiota. As we know, breast milk contains a large number of live microorganisms, including Lactobacillus, Staphylococcus, Streptococcus, Bifidobacterium, Proteobacteria, which can regulate microbiome development and affect functional metabolites of the gastrointestinal tract in newborns (Parada Venegas et al., 2019) (Schirmer et al., 2016). Research has also found that metabolic gene expression in the microbiota contained in breast milk is associated with galactose metabolism, which increases UDP-glucose production, increases UDPGA, and influences bilirubin transformation. Lactobacillus spp is known to be directly involved in the use of galactooligosaccharides (GOSs), and converts GOSs into galactose and UDP-glucosa through galactose metabolism, while UDP-glucose which is a galactose product is an ingredient in making UDPGA. (Maurice et al., 2013)

In accordance with previous studies, it was found that Lactobacillus spp. is related to bilirubin metabolism by galactose which affects the galactose metabolic pathway. A decrease in microbiota diversity, one of which is Lactobacillus, will cause a decrease in the amount of galactose which also depends on a decrease in the number of genes related to the galactose metabolic pathway, causing intestinal UDP-glucose deficiency and affecting the glucuronic acid pathway to form conjugated bilirubin. Decreased abundance of gut microbiota of glucokinase producing species; has exacerbated the decrease in UDP-glucose in the pentose phosphate pathway. A decrease in the abundance of phosphogluconate dehydrogenase-associated gut microbiota (PGM) may indicate abnormalities in the ATP-associated glycolysis pathway and affect the glucuronic acid pathway requiring ATP.

Not only that, it is known that Lactobacillus spp. bacteria have a role in enterohepatic circulation by reducing degradation of direct bilirubin, and stimulating intestinal peristalsis movements that will increase the excretion of bilirubin from the body. Lactobacillus also increases fecal moisture and fecal volume thereby accelerating the

excretion of bilirubin that has been broken down into stercobilin, increasing the formation of the lining / barrier of the gastrointestinal tract coated by mucin, regulating the tight junction of the gastrointestinal tract so as to maintain epithelial integrity, and decreasing the absorption of direct bilirubin. Lactobacillus spp also reduces the activity of β-glucuronidase78, thereby inhibiting the enterohepatic circulation of bilirubin and creating an alkaline or alkaline atmosphere that will affect the effectiveness of the work of β-glucuronidase.

The underlying risk of probiotic strategies is that newborns are susceptible to infections caused by live microbes. Therefore, the use of probiotics in neonates with hyperbilirubinemia should weigh the pros and cons, especially in infants with low birth weight or comorbidities such as neonatal sepsis, and closer monitoring needs to be done when administering probiotics. (Devi & Vijaykumar, 2017)

Researchers found that therapeutic probiotic supplementation in newborns was beneficial for promoting gut colonization and inhibiting pathogens, and different probiotics exhibited strain-specific functions. Research states that in vitro, Lactobacillus can increase intestinal epithelial tight junction protein, after high bilirubin concentrations increase intestinal permeability (Makhoul et al., 2018).

Some other potential mechanisms of Lactobacillus are 1) Promoting colonization of good bacteria in the gut; 2) Suppress pathogens by competitively attaching to the intestinal edge; 3) Stimulates intestinal peristalsis and increases the frequency of bowel movements which reduces enterohepatic circulation and inhibits the activity of the enzyme β-glucoronidase which reduces the degradation of bound bilirubin; 4) Improve tight junction tight junction; 5) Increase polyamines in the gut to improve intestinal maturity (Flint et al., 2015) (Pan & Rivas, 2017) [19]. Stimulation of intestinal peristalsis in neonates is not only beneficial for lowering bilirubin levels, but intestinal motility also affects nutrient absorption and one of the main influencing factors is propionic acid levels. Propionic acid is a short-chain fat that has been studied as a potential factor for autism. Elevated levels of propionic acid have been shown to impair brain function in mouse models.

Some research still believes that microbiota will affect the gut-brain axis which is an integrated communication system between neuroprotective, hormonal, and immunological signaling between the gut and brain[15]. Although the exact mechanism by which gut microbiota communicates with the brain is not yet known, some studies have found that infants given probiotics have better neurological development outcomes[16-18].

In this study, of the 104 newborns who received probiotics for a total of five days, none experienced complications, although the administration was limited to full-term infants without complications such as neonatal sepsis.

RESEARCH LIMITATIONS

Researchers are aware of shortcomings in this study. Among others, characteristics in the selection of patients in the control group are not known further, such as the mother's medical history during pregnancy, which can be a factor that affects the baby's bilirubin levels, such as hypertension, diabetes, maternal blood, or rhesus type. Furthermore, the sample in this study was babies with normal birth weight, so the results were generalized only in the group of babies with normal birth weight. More research is needed on babies with low birth weight or preterm. Blood type in infants is also not further checked to rule out the possibility of neonatal jaundice caused by ABO blood group incompatibility.

Experimental research design is one of the main advantages of this study. In this study, confounding factors such as sex and birth weight were controlled, showing no significant difference in bilirubin levels at baseline measurement, sex, and birth weight

in the case and control groups. In addition, conditions that affect bilirubin levels have been excluded at the beginning of the study with the control of these variables. The results of this study are reliable. Furthermore, randomization, where sampling is carried out randomly, reduces bias in determining experimental and control groups. This study was conducted for 5 days, where the measurement of bilirubin levels was carried out more than 1 time, namely on days 3 and 5. Previous research showed that bilirubin concentrations began to decrease on day 5, so it could be interpreted that the administration of Lactobacillus reuteri DSM.77 indeed caused the acceleration of the decline in bilirubin levels in the study

CONCLUSION

This study concluded that administration of Lactobacillus reuteri DSM 17938 can help accelerate reduction of indirect bilirubin levels in hyperbilirubinemia neonatorum compared to the group without intervention.

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