

The Potential of Probiotics from West Sumatra, Indonesia on Low-Density Lipoprotein and Interleukin-6 Levels with Dyslipidemia

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Abstract: Dyslipidemia is a lipid metabolism disorder characterized by abnormal elevations or reductions in one or more lipid fractions within the plasma. This condition is a critical component of both metabolic syndrome and cardiovascular disease (CVD). This study aims to see the potential of curd administration on Low-Density Lipoprotein (LDL) and Interleukin-6 (IL-6) levels in dyslipidemia patients. This study is an experimental study with a pre-post test control group design consisting of a control group and a treatment group totaling 42 people taken randomly at the DR Drs M Hatta Bukittinggi Brain Hospital in June and July 2024. In the three groups, LDL and IL-6 examinations were carried out and measured before and after curd for 14 days. Treatment group 1 was given curd as much as 150 grams, treatment group 2 was given curd as much as 200 grams, given 1x a day, and the control group was not given curd. Data analysis was performed using the Wilcoxon statistical test and paired t-test. Results: There was no significant difference in LDL ($p=0.360$) and IL-6 ($p=0.932$) levels after giving curd for 14 days. However, there was a decrease in LDL and IL-6 levels after giving curd for 14 days. Conclusion: The results showed that giving curd for 14 days in the treatment group 1 (150gram) and treatment 2 (200gram) can reduce LDL and IL-6 levels. Consuming probiotics for 14 days can reduce LDL and IL-6 levels.

Keywords: Dyslipidemia; interleukin-6; low-density lipoprotein; probiotics.

INTRODUCTION

In 2018, dyslipidemia prevalence was highest in Europe, recorded at 53.7%, followed by the Americas at 47.7% (Pirillo et al., 2021). In contrast, Southeast Asia reported a lower prevalence of 30.3% (Lin et al., 2018). According to WHO data, the prevalence of dyslipidemia in Indonesia, defined as a total cholesterol level of ≥ 160 mg/dL among adults over 25 years, was approximately 36%, with 33.1% in men and 38.2% in women (Lin et al., 2018). Further data from the 2023 Indonesian Health Survey (IHS) indicated that 27.8% of individuals over 15 years of age exhibited dyslipidemia, characterized by total cholesterol levels within the 200–239 mg/dL range (mildly elevated), triglyceride levels between 150–199 mg/dL (mildly elevated) affecting 17.8% of the population, LDL cholesterol levels of 130–159 mg/dL impacting 19.9%, and HDL cholesterol levels below 40 mg/dL observed in 87.0% of individuals. Comparing these findings with data from the 2018 Basic Health Research (Riskesdas) survey, total cholesterol levels decreased slightly from 28.8% to 27.8%. Triglyceride levels declined by 10.1%, from 27.9% to 17.8%, and LDL levels dropped by 5%, from 24.9% to 19.9%. However, HDL levels substantially increased by 62.7%, rising from 24.3% in 2018 to 87.0% in 2023.

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Dyslipidemia is a lipid metabolism disorder characterized by abnormal levels, either elevated or reduced, of one or more lipid fractions in plasma (Pappan & Rehman, 2022). It is often referred to as hyperlipidemia, describing elevated serum lipid levels that contribute as a significant risk factor for cardiovascular disease development (Liu et al., 2022).

Suboptimal cholesterol levels in circulation, particularly elevated LDL-C and remnant cholesterol associated with triglyceride-rich lipoproteins, represent a predominant form of dyslipidemia globally and constitute a significant risk factor for atherosclerotic cardiovascular disease (ASCVD). Atherosclerosis is causally associated with the retention of LDL within the intimal layer, which serves as the main conduit for cholesterol accumulation in localized regions of the arterial wall. Consequently, LDL-C has become the primary target for lipid-lowering interventions (Liu et al., 2022). The 2022 dyslipidemia management guidelines specifically designate LDL-C as the central focus of lipid-lowering therapy.

Plasma IL-6 levels indicate cardiovascular disease, with IL-6 playing a crucial role in plaque formation and instability and contributing to the progression of atherosclerosis (Su et al., 2021). Clinical studies have demonstrated a positive association between IL-6 levels in adipose tissue and LDL, suggesting that IL-6 may function as an early and specific marker in the pathogenesis and development of atherosclerosis (Bao et al., 2015).

Live Lactic Acid Bacteria (LAB), considered probiotics by the Food and Agriculture Organisation (FAO) and World Health Organisation (WHO), can be beneficial to the health of the host when administered in sufficient quantities (FAO/WHO, 2002). A variety of probiotics, such as *Lactobacillus rhamnosus* GG, *L. Reuteri*, *Bifidobacterium*, and many *L. Casei*, have been widely used in fermented milk (Susmiati et al., 2022).

Dadih, a traditional fermented milk from West Sumatra, Indonesia, holds promise as a functional food enriched with beneficial probiotics (Made & Hapsari, 2023). This fermented product, recognized for its probiotic potential, typically contains lactic acid bacteria (LAB) at concentrations of approximately 10^8 CFU/g (Helmizar et al., 2018). Numerous studies indicate that LAB in dadih exhibits various bioactive properties, including antimicrobial and antipathogenic effects (Harun et al., 2020), hypocholesterolemic activity, antimutagenic properties (Aritonang et al., 2022), antioxidant capabilities (Hapsari et al., 2019), immunomodulatory functions (Surono, 2015), anti-stress effects (Anggraini et al., 2019), and folate production (Purwandhani et al., 2018). The nutritional composition of dadih varies depending on its region of production, with typical values of 6.68% protein, 6.4% fat, 65% water, 2.12% acidity, and LAB concentrations reaching 21×10^9 CFU/g, which surpasses the FAO's probiotic standard of 2×10^6 CFU/g (Purwati, 2017).

Nutritional therapy to prevent dyslipidemia can include dietary modifications, such as incorporating functional foods. Probiotics, a prime example of functional foods, have been shown to reduce blood cholesterol levels and hypertension (Chen et al., 2023). Numerous studies support the cholesterol-lowering effects of probiotics; for instance, research by Ranasinghe et al. (2013) found that a two-week probiotic regimen lowered blood cholesterol levels by up to 30%. Similarly, a study by Mukhlisah and Irfan (2023) demonstrated a significant effect of probiotic dadih on total cholesterol levels in male white rats, with $p < 0.05$.

In previous research, curd was given to male mice model of hypercholesterolemia using a true experimental research method with a post-test design and only checking total cholesterol (Sa et al., 2022); the novelty of this research

was carried out on people living with dyslipidemia using experimental research methods with a pre-posttest control group design, the variables measured were LDL and IL-6. There is still limited research on the effect of probiotic supplementation on LDL and IL-6 levels in dyslipidemia patients, especially using typical *dadih* from West Sumatra with buffalo milk as the raw material. So this study aims to analyze the potential of giving typical *dadih* from West Sumatra on Low-Density Lipoprotein (LDL) and Interleukin-6 (IL-6) levels in dyslipidemia.

Based on health examination data (MCU) of employees at the Brain Hospital DR Drs M Hatta Bukittinggi in July - September 2023, out of 329 employees who carried out MCU, 60 employees were dyslipidemia based on doctor's diagnosis (LDL 130-159 mg/dl) at 21.2%, this data exceeds the prevalence of Dyslipidemia in Indonesia based on SKI 2023 which is 19.9%. Based on this, researchers are interested in examining the effect of curd on low-density lipoprotein (LDL) and IL-6 levels in dyslipidemia patients at Dr. Drs. M Hatta Bukittinggi Brain Hospital.

MATERIALS AND METHODS

Curd is obtained from Agam Regency, Kamang area of West Sumatera, Indonesia, one of the curd-making centers. The main ingredients for making curd are buffalo milk, bamboo tubes, and banana leaves. Buffalo milk has a high casein and fat content compared to other animals, affecting its creamy texture and consistency (Surono, 2015). Curd has a dense and thick structure, a yellowish-white color, and a sour taste that resembles a mixture of bamboo and buffalo milk that is squeezed, put into a bamboo tube, then covered with banana leaves, and left to ferment naturally at room temperature for 48 hours (Arnold et al., 2021). The bamboo tube used as a container during fermentation is because it is hygroscopic, keeping the curd from whey separation and the bitter taste present in the bamboo, and can also prevent contamination from ants (Surono, 2015). Using banana leaves aims to create an optimal facultative anaerobic condition for the fermentation process, and using banana leaves is also helpful in avoiding unwanted contamination (Arnold et al., 2021). LAB and native protease will perform lactic acid fermentation and proteolytic activity to produce curd with a thick consistency, dense texture, smooth surface, and good flavor. The nutritional value of curd used in this study is equivalent to the results of previous studies, namely Calories 162.48 kcal, Carbohydrates 5.65%, Protein 10.31%, Fat 10.96%, and probiotic content ranging from 10^8 CFU (Budiyatri et al., 2024).

This study is experimental with a pre-post-test control group design (Akbar et al., 2023). The study used 1 control and 2 treatment groups by taking measurements before and after treatment. The number of samples for each group was 14, with a total sample of 54. Samples were randomly selected. This study provides treatment to 2 treatment groups, namely treatment group 1, as many as 14 people with curd given as much as 150 grams; treatment group 2, as many as 14 people with curd given as much as 200 grams; and 14 people in the control group without being given curd only consumption of drugs. The participants were randomly selected. This study was conducted at Dr. Drs M Hatta Bukittinggi Brain Hospital from June to July 2024 and involved a hospital patient with dyslipidemia.

Employees who participated in this study met the inclusion and exclusion criteria. The inclusion criteria of the research subjects were patients with dyslipidemia based on a doctor's diagnosis with LDL criteria of 130 - 159 mg/dl based on The Cholesterol Education Program Adult Treatment Panel 2001 (NCEP - ATP III) and employees at the Drs M Hatta Bukittinggi Brain Hospital Indonesia.

Exclusion criteria for pregnant subjects and diarrhea. The criteria for dropping out are resigning to continue the study, leaving at the time of the study, and illness.

Blood samples were taken to assess LDL and IL-6 levels before the intervention. On day 15, blood samples will be retaken to assess LDL and IL-6 levels after the intervention. This study involved Prodia Bukittinggi's laboratory for blood samples of research subjects. Blood samples of 2-3 cc were taken and put into a vacuum tube. LDL and IL-6 levels were examined using the TMS 24i/50i and Respons 910 tools; the sample examination was carried out after the blood samples reached room temperature of 15 - 30°C. This study was approved by the Research Ethics Committee of DR Drs M Hatta Bukittinggi Brain Hospital (No: 000165/KEP.RSOMH Bukittinggi/2024).

Data analysis will begin by testing the data for normal distribution using the Shapiro-Wilk statistical test. The Wilcoxon test and paired t-test were used to determine the difference in LDL and IL-6 levels before and after the intervention in each group. The effect of curd on LDL and IL-6 levels was identified using the Kruskal-Wallis and one-way ANOVA tests.

RESULTS AND DISCUSSION

The general characteristics of the employees who participated in this study will be presented in Table 1, where the gender distribution of the samples in this study was primarily female, including in the control group (64.3%), treatment group 1 (100.0%), and treatment group 2 (57.1%). The Body Mass Index (BMI) of the samples varied in the control group, primarily normal (64.3%); treatment group 1, mostly overweight (42.9%); and treatment group 2, mostly obese (50.0%).

Table 1. General Characteristics

Characteristics	Control (n=14) (%)	Treatment 1 (n=14) (%)	Treatment 2 (n=14) (%)
Gender			
Male	5 (35,7)	0 (0,0)	6 (42,9)
Women	9 (64,3)	14 (100,0)	8 (57,1)
Body Mass Index			
Normal	9 (64,3)	4 (28,6)	2 (14,3)
Overweight	3 (21,4)	6 (42,9)	5 (35,7)
Obesity	2 (14,3)	4 (28,6)	7 (50,0)
Total	14 (100,0)	14 (100,0)	14 (100,0)

LDL and IL-6 levels were measured twice, before giving curd (H0) and after giving curd (H14), the average LDL levels at H0 and H14 can be seen in Table 2 and Table 3.

In Table 2, the Wilcoxon statistical test of LDL levels at H0 (before giving curd) and H14 (after giving curd) shows no significant difference in each group, and the one-way ANOVA test also shows no significant difference. However, Figure 1 shows a decrease in LDL levels after 14 days in group 1 (giving curd 150 grams) and group 2 (giving curd 200 grams).

Table 2. Differences in Mean LDL Levels Before and After Treatment

Group	Mean \pm SD LDL level (mg/dl)		Δ	pa
	H0	H14		
K	163,07 \pm 16,03	165,00 \pm 21,66	1,93 \pm 19,28	0,145
P1	163,71 \pm 29,99	151,64 \pm 32,73	-12,07 \pm 20,32	
P2	166,86 \pm 21,91	162,57 \pm 22,00	-4,29 \pm 22,35	
pb	0,663	0,360	0,213	

Description: K: Normal group; P1: 150 grams of curd; P2: 200 grams of curd; pa : Statistical test with *Wilcoxon*; pb : Statistical test with one-way ANOVA.

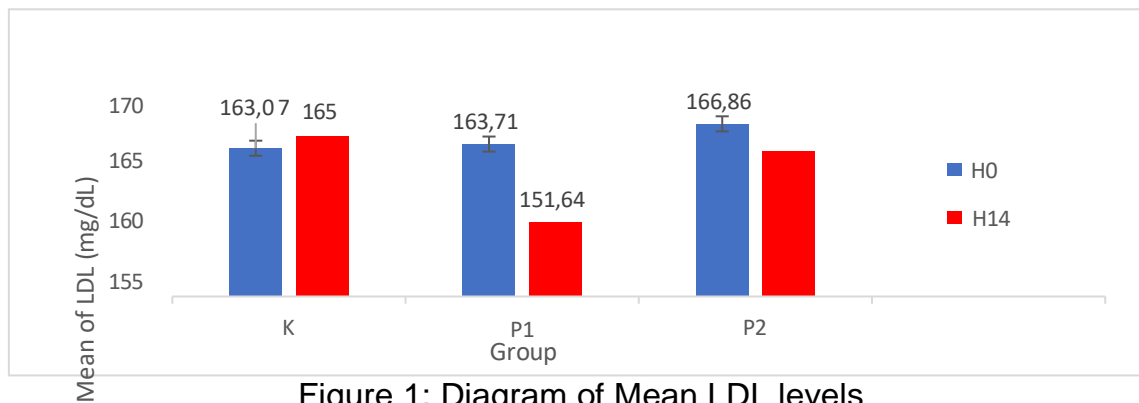


Figure 1: Diagram of Mean LDL levels

Table 3. Difference in Mean IL-6 Levels Before and After Treatment

Group	Mean \pm SD IL-6 (pq)		Δ	pa
	H0	H14		
K	3,45 \pm 4,04	2,71 \pm 1,57	-0,74 \pm 4,05	0,164
P1	3,73 \pm 2,24	3,53 \pm 3,46	-0,19 \pm 4,10	
P2	3,29 \pm 1,85	2,66 \pm 1,18	-0,62 \pm 1,87	
pb	0,139	0,932	0,487	

Description: K: Normal group; P1: 150 grams of curd; P2: 200 grams of curd; pa : Statistical test with *Wilcoxon*; pb : Statistical test with *Kruskal Wallis*

Interleukin-6 (IL-6) levels were measured twice, namely before giving curd (H0) and after giving curd (H14); the average IL-6 levels at H0 and H14 can be seen in Table 3. In the *Wilcoxon* statistical test, there was no significant difference, and in the *Kruskal-Wallis* test, there was no significant difference. Figure 2 shows a decrease in IL-6 after 14 days in group 1 (giving curd 150 grams) and group 2 (giving curd 200 grams).

The characteristics in Table 1 from the analysis results show that the average age of the sample is above 40 years; this is to the 2024 Indonesian Health Survey where 35 - 44 years old, 20.9% have LDL 130 -159 mg/dl, 45 - 54 years old 25.7% have LDL 130 -159 mg/dl. According to (Heeren & Scheja, 2021), with age, the function of the body's organs decreases, including a decrease in the activity of LDL receptors, resulting in increased fatty tissue in the body so that cholesterol levels are higher. The statistical analysis results in research (Lestari et al., 2018) obtained a value of $p < 0.005$, meaning that age has a significant relationship with the incidence of dyslipidemia.

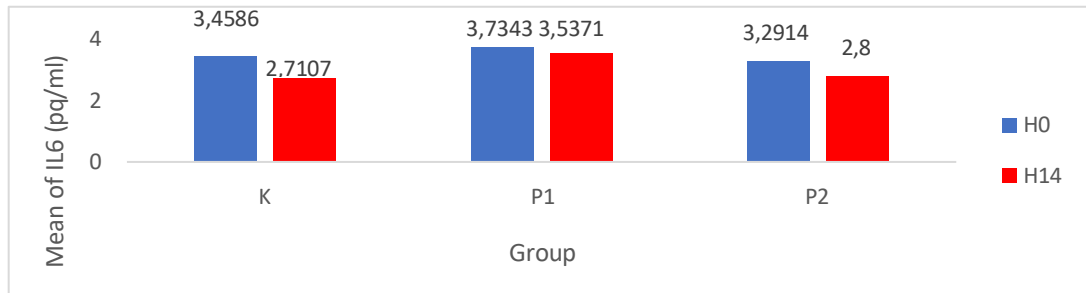


Figure 2: Diagram of Mean IL6 levels

Based on gender in this study, 73.8% of women while men were 26.1%; this is in line with SKI 2023; 20.3% of women have LDL 130 - 159 mg/dl, while in men, 19.5%. Based on theory, the risk of dyslipidemia in men is more significant than in women. This is because productive women have a protective effect from the hormone estrogen, which reduces plaque formation in blood vessels by preventing the oxidation of LDL cholesterol. However, the risk of developing dyslipidemia will be high if women have menopause (Ko & Kim, 2020). Body Mass Index In the subjects of this study, 14 people had overweight BMI (33%), and 13 people had obese BMI (31%); this is in line with research (Rahmawati & Dewi Sartika, 2020) which shows that respondents who are declared obese based on BMI have a 3.98-fold more significant risk of suffering from dyslipidemia than respondents who have normal BMI. According to (Kwon et al., 2023), obesity is one of the factors of dyslipidemia. Based on research (Tang et al., 2022), there is a significant relationship between the incidence of dyslipidemia with overweight and obesity.

The results of the study of LDL levels at H0 and H14 statistically showed that groups K, P1, and P2 were insignificant ($p \Rightarrow 0.05$). This is in line with research (Rahayu et al., 2021) that there is no significant difference in LDL changes before and after probiotic *Lactobacillus Plantarum* Dad 13 consumption. The length of time probiotics are given will affect changes in LDL because several probiotic groups have a longer time to oxidize LDL; it takes 10 weeks of research to reduce LDL cholesterol by 10.4% (Flaig et al., 2023). However, the average LDL level of the sample in treatment 1 with 150 grams of curd decreased by 12 mg/dl, and the sample LDL level also decreased by 4.3 mg/dl in treatment 2 after being given 200 grams of curd (Figure 1). Several studies mentioned that lactic acid bacteria contained in curd have hypocholesterolaemia properties Artonang et al., 2022). The results of this study are the same as research conducted by (Ranasinghe et al., 2013), which concluded that consuming probiotics for 2 weeks can reduce total cholesterol levels in the blood by 30%. This study is in line with research (Palani Kumar et al., 2021) that the administration of *Lactobacillus fermentum* MCC 2760, which is a probiotic strain from curd, can reduce total cholesterol, triglyceride, and LDL levels in rats given a high-cholesterol diet. This study is also in line with research (Sa et al., 2022) that the administration of probiotic curd can reduce total cholesterol in male white rats (*Rattus Norvegicus*) with $p < 0.05$.

The results of the study of IL-6 levels at H0 and H14 statistically showed that groups K, P1, and P2 were insignificant ($p \Rightarrow 0.05$). Based on previous research, the decrease in IL-6 was followed by a decrease in LDL levels, which took 10 weeks (Flaig et al., 2023)

This study also showed that the average IL-6 level of the sample decreased by 0.2 pg/dl after being given 150 curd, and the sample IL-6 level also

decreased by 0.6 pg/dl after being given 200 curd. This study aligns with research (Palani Kumar et al., 2021) that probiotic supplementation of curd strains can decrease bacterial translocations in mesenteric adipose tissue. The expression of inflammatory markers by qPCR showed a decrease in TNF- α , IL-6, and IL-12. Thus, the administration of probiotics plays a role in increasing the barrier or anti-inflammatory function. The decrease in IL-6 levels in this study sample could be due to the provision of curd as part of the diet, which may positively reduce IL-6 levels in patients with dyslipidemia through anti-inflammatory mechanisms and improve lipid profiles. IL-6, cortisol, and C-reaction protein levels increase along with a decrease in HDL and TG (Pietrzak et al., 2020). A clinical trial conducted showed that rheumatoid arthritis patients had higher cholesterol due to IL-6 blocking, which suggests that atherogenic lipid profiles may be caused by functional deficiency of IL-6 (Su et al., 2021). Changes in the gut microbiota will activate the Toll-like receptor (TLR) signaling pathway. Subsequently, this will increase intestinal permeability to endotoxins such as Lipopolysaccharides (LPS). As a result, LPS can enter the systemic circulation. When LPS and/or FFA are elevated, pro-inflammatory cytokines, including interleukin (IL)-1 β , IL-6, and Tumour Necrosis Factor (TNF)- α , will increase in the gastrointestinal tract (Jarukamjorn et al., 2016). In this study, it took a long time to obtain normal results for LDL (<130mg/dL) and IL-6 (<40pg/ml), and more specific examination is needed regarding the effect of probiotic consumption on intestinal microbiota, such as SCFA examination.

CONCLUSION

In treatment groups 1 (150 grams) and 2 (200 grams), curd administration over 14 days reduced LDL and IL-6 levels, although these changes were not statistically significant. Future research should explore varying dosages and extended durations of curd administration to achieve statistically significant outcomes. Curd supplementation has the potential to serve as an adjunctive therapy in dyslipidemia prevention.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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