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Effect of Probiotic Milk containing *L. casei* Shirota on Immunoglobulin G Levels among Anemic Adolescent Girls

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ABSTRACT

Probiotic play important role on immune systems. This study was to investigate effect of probiotic milk containing *L. casei* Shirota on immunoglobulin G levels among anemic adolescent girls. A total of 22 adolescent girls aged 13-18 years with anemia were included on this study. The subjects were separated into 2 groups i.e. a probiotic group and a control group by simple random sampling. During 4 weeks, a probiotic group received 1 tablet of 60 Fe elemental + 250 μg folate acid once a week and probiotic milk once daily. While a control group received 1 tablet of 60 Fe elemental + 250 μg folate acid once a week. Blood samples were collected at 0 and 4 weeks and assayed for immunoglobulin G levels by ELISA. Immunoglobulin G levels were not significantly increase after intervention. Compared with control group, probiotic milk administration in probiotic group did not significantly increased immunoglobulin G levels ($p>0.05$). The probiotic milk containing *L. casei* Shirota did not improve immunoglobulin G levels on anemic adolescent girls.

Keywords: Probiotic, Immunoglobulin G levels, Anemia, Adolescent girls

INTRODUCTION

Anemia is the major serious health problems among adolescent girls[(1)]. Risk of anemia on adolescent due to period of rapid growth and higher requirement as result of menstruation cycle for a month[(2)]. In addition, bad food pattern worsen high risk of anemia among adolescent girls[(1)]. Anemia can cause a decrease in the immune system. Previous studies had found the declining of immune system on individu with anemia[(3-7)].

Probiotic are bacteria which, when administered in adequate amounts, can to promote health benefit on humans[(8)]. One of health benefit as result of consuming probiotic was the increasing of immunoglobin levels, especially immunoglobulin G levels. Previous studies had found the effect of probiotic administration on immunoglobulin G levels, but the results were controversial[(9-18)]. Clinical trials were needed to clearly investigate the effect of probiotic bacteria on immunologlobulin G levels.

L. casei Shirota was probiotic strain can improve gastrointestinal tract. Previous clinical studies had found the effect of L. casei shirota on immune systems by inducing levels of NK cells production[(19)], inducing IL-2 and IFNγ production[(20)], activation on T cells[(17)], and inducing immunoglobulin secretion[(21-22)]. However, effect of *L. casei* Shirota on immunoglobulin G levels has not been established. Therefore, the study was to investigate effect of probiotic milk containing *L. casei* Shirota on immunoglobulin G levels among anemic adolescent girls.

METHODS

This study was undertaken at Jabon's Community Health Centers in Sidoarjo District during Agustus 2017 until Oktober 2017. All of the participant had written informed consent before recruitment this study. This study protocol was approved by the Health Research Ethics Committee, Faculty of Public Health Airlangga University

(No. 418-KEPK). This study was double blind, randomized, pretest posttest control group design. A total of 22 adolescent girls aged 13-18 years who had low hemoglobin levels (Hb < 12 g/dl) were taken as subjects. The subjects were separated into 2 groups by simple random sampling, i.e. a probiotic group and a control group. During 4 weeks, a probiotic group received 1 tablet of 60 Fe elemental+250 μg folate acid once a week and 65 ml portion probiotic milk containing 6.5 x 109 cfu/ml *L.casei* Shirota once daily. While a control group received only 1 tablet of 60 Fe elemental+250 μg folate acid once a week. Data of baseline characteristics were obtained by interview. Data of weight and height body were obtained by anthropometric measurenment. Blood samples were collected at 0 and 4 weeks and assayed for immunoglobulin G levels by ELISA. data was analysed by independent t test with confidence interval of 95%.

RESULTS

Baseline characteristics of anemic adolescent girls were based on age distribution, family size, maternal education, maternal occupation, BMI-for-age, and frequency of morbidity 1 month before study (Table 1). Subjects were higher on early adolescent age with small family size in both group. Subjects had mother with high levels of education and worked as a housewife in both group. Base on BMI-for-age, subjects had normal nutrition status. Compared with control group, probiotic group had higher frequency of sick 1 month before study.

There were no significant differences between probiotic and control group at baseline characteristics ($p>0.05$). Similarly, immunoglobulin G levels were similar between probiotic and control group at baseline ($p>0.05$).

Subjects had high compliance and similar in both group. On average, total compliance score of supplementation Fe+folat was 93.2% in both group. While total compliance score of probiotic milk drink was 95.8% in probiotic group (data not shown).

Table 1. Baseline characteristics of anemic adolescent girls

Characteristics	Group			
	Probiotic (n=11)		Control (n=11)	
	n	%	n	%
Age groups (years)				
• 13-15	7	63.6	7	63.6
• 16-18	4	36.4	4	36.4
Family size				
• ≤ 4	9	81.8	8	72.7
• >4	2	1.2	3	27.3
Maternal education				
• Low	5	45.5	5	45.5
• High	6	54.5	6	54.5
Maternal occupation				
• Proffesion	2	18.2	2	18.2
• Skilled worker	3	27.3	4	36.4
• Housewife	5	45.5	5	45.5
• Others	1	9.1	0	0.0
BMI-for-age-groups				
• Normal	10	90.9	11	100.0
• Overweight	1	9.1	0	0.0
Frequency of sick 1 month before study				
• ≤ 1				
• 2-3	1	9.1	0	0.0
• >3	3	27.3	6	54.5
	7	63.6	5	45.5

No significant between probiotic and control group were identified by mann whitney test, where $p>0.05$

Tabel 2 shows the average of immunoglobulin G levels before and after intervention in both group. On average, immunoglobulin G levels after intervention showed an increase in both group, but not significant. Compared with control group, probiotic group had higher increases immunoglobulin G levels.

Table 2. Immunoglobulin G levels before and after the treatment in both group

Variable	Group		*p-value*
	Probiotic	Control	
Immunoglobulin G (μg/ml)			
Baseline	64.8±9.9	66.2±6.6	0.688[b]
Endline	85.8±40.9	76.6±14.3	0.487[b]
Change	21.1±38.4	10.3±15.5	
p-value	0.099[a]	0.051[a]	

[a]paired t test, with p<0.05
[b]independent t test, with p<0.05

DISCUSSION

This present study investigated the effect of probiotic milk containing *L. casei* Shirota on immunoglobulin G levels among anemic adolescent girls. There were no increases significantly immunoglobulin G levels as a result consuming probiotic milk containing *L. casei* Shirota.

Similar to other clinical trials[9-12],[14],[17], present study found that probiotic milk had no effect on immunoglobulin G levels. However, contrast to previous studies, probiotic administration had significantly increased immunoglobulin G levels[13],[15-16],[18]. The present study strongly contrast with animal studies that clearly explained the effects of probiotic on immunoglobulin G levels[23-25].

The lack of the effect of probiotic milk containing *L. casei* Shirota on immunoglobulin G levels can be explained by several reasons. The effect of probiotic milk on immunoglobulin G levels may be specific bacteria strain dependent. Other studies with different strains had a higher effect[13],[15-16],[18]. Furthermore, the effect of probiotic may be dose dependent[26]. Other clinical trials with higher doses and combined several probiotic strains had the effect on immunoglobulin G levels[15],[25]. In addition, the selection of intervention period may be important. previous studies with longer intervention period had significantly increased immunoglobulin G levels[21].

Present study shows that no effect of probiotic milk on immunoglobulin G levels due to the selection of probiotic strains used, dose and intervention period. However, maybe there were others factors that becomes causes no effect of probiotic milk administration on immunoglobulin g levels. One such factor was the possibility of intestinal microflora under normal conditions so that the immune system was stable conditions[9].This condition had caused probiotic milk administration becomes undetectable.

CONCLUSION

The probiotic milk containing *L. casei* Shirota did not improve immunoglobulin G levels on anemic adolescent girls. Future study are needed by increasing of dose levels, selecting of specific strain used and selecting of treatment period, and recruiting of participants with low immune systems.

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