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EFFECT OF VITAMIN B12 SUPPLEMENT ON SERUM LEVELS OF VITAMIN B12 AND MEMORIZING ABILITY ON PRESCHOOL CHILDREN

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ABSTRACT: This research aimed to analyze the effect of vitamin B12 supplementation on vitamin B12 serum level and memorizing ability of preschool children. A community - randomized controlled trial of 29 preschool children (4-6 year) was conducted for six months. Subjects were divided into two groups, treatment group (received 10 mcg of vitamin B12 daily syrup) and control group (placebo). Serum vitamin B12 and memory levels of children were measured before and after the intervention. Result of research was after 6 months of vitamin B12 on supplementation prevalence of vitamin B12 deficiency decreased in treatment group from 26.7% to 0.0%, while in the control group increased from 21.4% to 28.6%. The mean of increasing serum vitamin B12 was significantly different among those groups (148 ± 110.9 pg/ml in the treatment group and 3.7 ± 12.8 pg/mL in the control group). Memorizing ability affected by vitamin B12 supplementation for overall children with relative risk (RR) was 19.5. Reviews of this research imply the important result of vitamin B12 supplementation on improving vitamin B12 and memory levels of preschool children.

INTRODUCTION: According to Ickowitz *et al.*, [1] state that Indonesia is facing a multitude of food security and nutrition challenges. Despite considerable progress over the last few decades in reducing child mortality and the prevalence of underweight and stunted children, there remains a long way to go before all Indonesian children are free of malnutrition. Over a single decade, from the period 1993-1997 to 2003-2007, child mortality in Indonesia dropped by 33% from 69 to 44 child deaths per 1,000 live births, yet the prevalence of stunting in children under 5 years of age remains high at 25.2% and 39.2%, in urban and rural areas respectively. Vitamin B12 deficiency can affect individuals at all ages, but most particularly elderly individuals.

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Infants, children, adolescents and women of reproductive age are also at high risk of deficiency in populations where dietary intake of B12-containing animal-derived foods is restricted. Deficiency is caused by either inadequate intake, inadequate bioavailability or malabsorption [2]. The Low vitamin B12 status is attributed to vegetarianism in these populations. It is not known whether low B12 status is associated with metabolic risk of the offspring in whites, where the childhood metabolic disorders are increasing rapidly [3].

Another study suggests that poor vitamin B12 status in infancy is linked to poor growth and neurodevelopment. Brain development starts from conception, and pregnancy is a period of rapid growth and development for the brain [4]. Vitamin B12 is only synthesized by certain bacteria and humans obtain it from animal source foods such as meat, dairy, eggs, and fish [5]; which explains why children who rarely eat these foods are expectedly at risk of vitamin B12 deficiency.

In a group which is allegedly at high risk of vitamin B12 deficiency are the children from poor families due to low purchasing ability for animal source food. Related to the important function of vitamin B12 in cognitive development, an experimental study is necessary as an alternative effort to improve vitamin B12 status and simultaneously improve cognitive development of children. This study aimed to analyze the effect of vitamin B12 supplementation on vitamin B12 serum and memory level in preschool children.

METHODS: This experimental study was conducted using 'Community - Randomized Control Trial" design study. The design includes an initial period in which no clusters are exposed to the intervention. Subsequently, at regular intervals (the "steps") one cluster (or a group of clusters) is randomized to cross from the control to the intervention under evaluation. This process continues until all clusters have crossed over to be exposed to the intervention. At the end of the study there will be a period when all clusters are exposed. Data collection continues throughout the study, so that each cluster contributes observations under both control and intervention observation periods [6].

Subjects were preschoolers (4-6 years) in Kindergarten (TK) Al-Zahra district of Bogor in 2007, 15 children were in the treatment group and 14 children were in the control group. The treatment group received vitamin B12 syrup (glucose syrup contains vitamin B12) at a dose of 10 mg/day in 2.5 ml syrup. The control group was given a placebo syrup made from the same ingredient with the glucose syrup but without vitamin B12). The intervention was given every day for 6 months, sample syrups were given at school from Monday to Friday, while on Saturday and Sunday they were being given at home.

Serum vitamin B12 levels were measured by the AxSYM method (Abbott Laboratories, USA, 2005) performed two times at the beginning and the end of the intervention at the Laboratory Pondok Indah Hospital in Jakarta. Vitamin B12 status is determined by vitamin B12 serum levels. The deficiency was a condition when serum vitamin B12 <300 pg/mL, normal was when vitamin B12 serum levels ≥ 300 pg/mL.

Memorizing each child was measured by 'memorizing picture' method. The child was showed a picture for 0, 5 min. Every picture has its score. This method was performed at the beginning and at the end of the intervention. The score was based on the picture size, namely: score for large size image was 3, for the medium size was 5, for the small size was 7, and for extra small size was 9. Data analysis was performed with different test Independent, Sample T Test, to determine the difference in level of vitamin B12 serum and the difference in scores of memorizing ability in both groups. Furthermore, to analyze the effect of vitamin B12 supplementation on vitamin B12 serum levels and memorizing ability of children, logistic regression test was performed.

RESULT: Serum levels of vitamin B12 and Vitamin B12 Status: The average serum vitamin B12 levels in the beginning of intervention was almost the same in both groups: 337 ± 62.8 pg/ml in the treatment group and 350.4 ± 83.2 pg/ml in the control group **Table 1**. Statistical analysis showed that there was no significant difference between the two groups at the start of the intervention (p>0.5). But after six months, the average vitamin B12 serum levels of the treatment group increased by 148.4 ± 110.9 pg/ml.

While the average vitamin B12 serum level in the control group increased by 3.7 ± 12.8 pg/ml. Statistical analysis showed a significant difference in the levels of vitamin B12 serum between the treatment group and the control group (p = 0.009). The difference was even more pronounced with the test of difference, which showed that difference or change of serum vitamin B12 levels was significant with p = 0.0001.

TABLE 1: AVERAGES SERUM LEVELS OF VITAMIN B12

Time measurement	Vitamin B12 levels (pg/mL)			Sig.
	Treatment (n=15)	Control (n=14)	Total (n=29)	
Beginning	337.9 ± 62.8	350.4 ± 83.2	343.9 ± 72.3	0.965
End	486.3 ± 131.8	354.1 ± 82.3	422.5 ± 127.8	0.009[a]
Difference	148.4 ± 110.9	3.7 ± 12.8	78.6 ± 107.9	0.000[a]

[a] = significantly different (p<0.05)

The increase in serum levels of vitamin B12 in the treatment group was followed by the improvement of the status of vitamin B12. At the beginning of the study, there were 26.7% of the children have a Vitamin B12 deficiency in the treatment group and 21.4% in the control group. After 6 months of Vitamin B12 supplementation, the prevalence of Vitamin B12 deficiency in the treatment group decreased to 0.0%, which means there were no children with vitamin B12 deficiency in the treatment group at the end of the intervention **Fig. 1**.

Whereas in the control group, the prevalence of vitamin B12 deficiency increased from 21.4% to 28.6% at the end of the intervention.

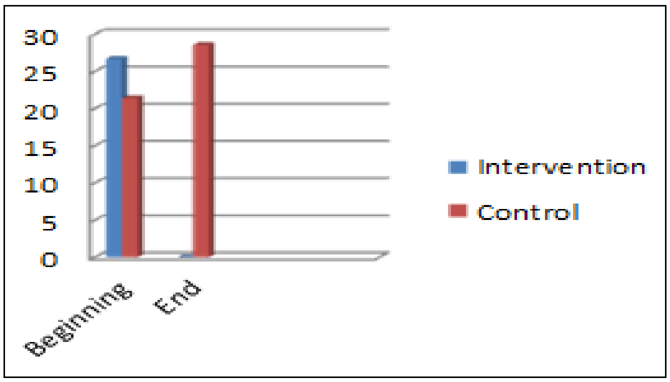

FIG. 1: PERCENTAGE OF CHILDREN WITH VITAMIN B12 DEFICIENCY

The Effect of vitamin B12 supplementation on serum vitamin B12 levels was analyzed by linear regression test **Table 2**. The results indicated that vitamin B12 supplementation has a significant influence over serum levels of vitamin B12 (p = 0:03). This suggested that the increase of level vitamin B12 levels in the treatment group occurred due to vitamin B12 supplements given for 6 months. R Square rate of 0.276 means that 28% of the variance of serum levels of vitamin B12 occurred due to 6 months vitamin B12 supplementation.

TABLE 2: REGRESSION TEST RESULTS SERUM LEVELS OF VITAMIN B12

Variables	R Square	B	T	Sig.
Constants	0.276	354.143	11.961	0.000
Vit. B12 supplementation (0=control; 1=treatment)		132.170	3.211	0.003[a]

[a] = significant effect ($p < 0.05$)

The Effect of vitamin B12 Supplements on Memorizing Ability: Children memorizing ability measured by the 'memorizing picture' method presented as memorizing scores **Table 3**. After 6 months vitamin B12 supplementation, the average of memorizing score improved, compared to score of memorizing before intervention to both the treatment group and the control group.

TABLE 3: MEAN SCORES ON THE CHILDREN MEMORIZING ABILITY

Time measurement	Memorizing Score			Sig.
	Treatment (n=15)	Control (n=14)	Total (n=29)	
Beginning	26.5 ± 5.8	25.4 ± 6.5	26.0 ± 6.0	0.609
End	40.2 ± 9.2	34.5 ± 5.8	37.5 ± 8.2	0.059
Difference	13.7 ± 4.5	9.1 ± 2.4	11.5 ± 4.2	0.002[a]

[a] = significantly different ($p < 0.05$)

The increases of memorizing scores in the treatment group were higher at 13.7 ± 4.5 points compared to the control group at 9.1 ± 2.4. The test of difference result showed that there were no significant differences on memorizing scores before and after the intervention. Also, there were no differences of the average of memorizing scores in both treatment group and control group before and after the intervention. But the test of difference result showed that there were differences (p = 0.002) of memorizing scores in the treatment group and the control group.

The effect of vitamin B12 supplementation on memorizing ability was analyzed by logistic regression test. The test result showed that there was a significant effect of vitamin B12 supplementation on children memorizing scores (p = 0.021). This indicated that 6 month vitamin B12 supplementation can improve children memorizing scores in **Table 4**.

TABLE 4: LOGISTIC REGRESSION TEST RESULTS EFFECT OF VITAMIN B12 SUPPLEMENTS AGAINST MEMORY

Variables	B	Error	Exp (B)	Sig.
Constants	-2.565	1.038	0.077	0.292
Vit. B12 supplementation (0=control; 1=treatment)	2.970	1.164	19.500	0.021[a]

[a] = significant effect ($p< 0.05$)

The Exp (B) was the value of the Relative Risk (RR) amounted to 19,500, which indicated that children who received vitamin B12 supplements would have a 19.5 times greater chance of having memorizing scores higher than the average, compared to children who did not receive Vitamin B12 supplements.

DISCUSSION: The test result at the beginning of intervention (before vitamin B12 supplementation) showed that 26.7% preschool children were deficient in vitamin B12 in the treatment group and 21.4% in the control group. The numbers were lower compared to a research in Kenya, which its prevalence of vitamin B12 deficiency 40 % of children in Kenya [7]. Poor Vitamin B12 status is common among young children in many low- to middle-income countries (LMIC) [8]. According to Shipton and Thachil [9] Vitamin B12 deficiency is a common condition which can present with non-specific clinical features, and in severe cases with neurological or haematological abnormalities. Although classically caused by pernicious anaemia, this condition now accounts for a minority of cases and vitamin B12 deficiency occurs most often due to food-bound cobalamin malabsorption.

The major risk factor for developing vitamin B12 deficiency is the well-characterized autoimmune disease pernicious anaemia, which is caused by a lack of production of intrinsic factor by gastric parietal cells that is needed for the intestinal absorption of vitamin B12 and eventually leads to the development of anaemia and/or severe neurological symptoms. Pernicious anaemia can affect people of all ages, but its incidence rises with age. Conservative estimates indicate that pernicious anaemia affects 2 - 3% of individual's ≥65 years of age. In addition, important risk factors are gastrointestinal surgery, such as gastric bypass or removal of the terminal ileum, which compromise the absorption of vitamin B12.

However, in low-income countries, vitamin B12 deficiency is largely due to a low intake of vitamin B12 rich foods of animal origin, but possibly also to gastrointestinal infections and infestations, and host-microbiota interactions [10]. Giving vitamin B12 for 6 months (24 weeks) to preschool children may increase serum levels of vitamin B12 of 148.4 ± 110.9 pg/mL). Increase levels of serum vitamin B12 followed by a reduction in the number of children with vitamin B12 deficiency, 26.7% of children could overcome the deficiency after receiving the intervention. While in the control group, the percentage of children with deficiency increased from 21.4% to 28.6% at the end of the intervention. Supplementary feeding for school children in the form of meat 60-85 g / h for one academic year was able to reduce the prevalence of vitamin B12 deficiency until 16 percent (80.7% at the beginning of the intervention to 64.1% at the end intervention). Likewise, the provision of milk to school children 200-250 ml/h reduced the prevalence of vitamin B12 deficiency about 26 percent (71.6% at the beginning of the intervention to 45.1% at the end of the intervention). A Research conducted by Kennedy [11], the many intervention studies that have involved administering just folic acid ± Vitamins B12 and/or B6, have generated equivocal results. Similarly, whilst we have some knowledge of the minimum levels of each B vitamin required in order to prevent explicit deficiency related diseases, we have a poor understanding of the negative effects of levels of consumption that lie above the minimum, but under the optimal level of consumption for these vitamins.

Another study by Duong [12] explained that emerging evidence from observational studies in adults has linked low vitamin B-12 intake with depression and mood disorders. Moreover, Finklestein [13] stated that A study in Turkey noted that DNA damage was increased in vitamin B-12-deficient (vitamin B-12: <200 pg/mL) children and their mothers; however, intramuscular cobalamin injections significantly decreased DNA damage in leukocytes of vitamin B-12-deficient children 9 d after treatment (P<0.001). The increase of memorizing scores of preschool children who received vitamin B12 supple-mentation at 13.7, 4.5 points is higher than children who did not receive the supplementation at 9.1, 2.4 points. The test of difference in children memorizing score before the intervention indicated that there is no significant difference. Likewise, at the end of the intervention the average memorizing scores were not significantly different between the two groups. But test of difference results indicated that there was no significant differences (p=0.002) between scores in the intervention group and the control group.

This was supported by research of Gaskins *et al.*, [10] which state that there is reason to believe that certain micronutrients such as folate and vitamin B-12 could positively influence reproductive success. Pretreatment intakes of folate and vitamin B-12 were related to a higher probability of live birth among women undergoing ART in the United States. On the other hand, elevated plasma homocysteine concentration is a sensitive marker to evaluate vitamin B12 and folate deficiency. Elevated circulating total homocysteine (tHcy) concentration is a risk factor for cardiovascular disease. In addition, HHcy in the elderly is strongly associated with depression, impaired cognitive function and dementia [14]. Plasma homocysteine and MMA were usually high due to a low vitamin B12 and folate. Furthermore, it was said that oral supplementation of vitamin B12 could lower plasma homocysteine and methylmalonic acid (MMA) but has not been able to improve cognitive function.

CONCLUSION: Vitamin B12 supplementation to preschool children made positive and significant effect on serum vitamin B12 levels, with an increase at 148.4, 110.9 pg/mL. The increase of vitamin B12 serum levels was also followed by improvement of preschool children memorizing ability (the average of score increase to 13.7, 4.5) Children who received vitamin B12 supplements for 6 months had the opportunity to have a score of memory above the average of 19.5 times greater than children who did not receive vitamin B12 supplements. Vitamin B12 supplementation is suggested as an alternative option to improve vitamin B12 status of preschool and school children since majority of children in Indonesia still lack of animal source foods (ASFs) as the source of vitamin B12.

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CONFLICT OF INTEREST: Nil

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