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ORIGINAL PAPER

Plasma Folate, Vitamin B6 and B12 in Their Relationship to the Presence of Probiotic Strain *Bifidobacterium animalis* subsp. *Lactis* HNO19 (DR10™) Among Indonesian Pregnant Women in Their Third Semester

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Abstract

Introduction Vitamin B12 plays a role during pregnancy in maintaining folate metabolism. Together with folate and vitamin B6, B12 is involved as a coenzyme in DNA synthesis and various methylation reactions in developing embryos. This study aims to compare plasma folate, vitamin B6 and B12 blood levels with respect to the presence of probiotic strain *Bifidobacterium animalis* subsp. *lactis* HNO19 (DR10™) among pregnant women in Indonesia. **Methods:** This study is part of a larger study entitled "Effects of *Bifidobacterium animalis* subsp. *lactis* HNO19 (DR10™), inulin, and micronutrient fortified milk on fecal DR10™, immune markers, and maternal micronutrients among Indonesian pregnant women." Further analyses were performed using independent-t test or Mann Whitney test, GLM-repeated measures and chi-square test, to compare folate, vitamin B6 and B12 intake and blood concentration during pregnancy with presence (n=22) and absence (n=55) subjects of fecal DR10™ at third trimester. **Results:** At the first trimesters there was no difference in plasma vitamin B6 and vitamin B12 levels between the two groups, based on the presence or absence of fecal DR10™. However, at the second and third trimester, vitamin B6 blood concentration (p=0.034 and p=0.001) and vitamin B12 blood concentrations at the third trimester (p=0.035) were significantly higher in the fecal DR10™ positive group, while having a similar vitamin B6 and B12 intake. **Conclusion:** Consumption of pre- and probiotics during the periconceptional period may be a useful strategy for improving maternal vitamin B's vitamins, especially vitamin B6 and B12 status and therefore provide benefits for the offspring's quality of life.

Keywords Folate, Indonesia, pregnancy, probiotic DR10™, vitamin B6, vitamin B12

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Introduction

Until recently, attentions are given more to several important nutrients for maternal nutrition, which play roles for fetal growth and development, i.e. iron and folic acid, but less for other vitamin B's, i.e. vitamin B6 and B12. Vitamin B12 also has a

potential role during pregnancy, to maintain folate metabolism, and preventing the production of homocystein, which otherwise increases the risk for cardiovascular diseases. While together with folate and vitamin B6, it plays role as coenzyme in DNA synthesis and many methylation reactions in developing embryos.[1] Study done in the UK found that maternal B12 levels are associated with BMI, risk of GDM, and additionally may have an independent effect on macrosomia.[2]

Folate rich foods are frequently consumed as it derives from variety of green leafy and colorful vegetables (i.e. broccoli, tomato, etc) and fortified staple foods (i.e. bread). Dietary folate is absorbed in the intestine and/or liver and metabolized primarily to 5-methyl tetrahydrofolate (THF) and subsequently gets polyglutamated for cellular retention. THF is then converted to 5,10-methyleneTHF by vitamin B6 dependent serine hydroxymethyltransferase and will be reduced irreversibly to 5-methyl-THF by methylene-THF reductase. 5-Methyl-THF acts as a primary methyl donor for the remethylation of homocystein to methionine, thus preventing homocysteinemia.[3]

The richest sources of vitamin B6 include fish, beef liver and other organ meats, potatoes and other starchy vegetables, and fruit (other than citrus). Maternal B6 adequacy is crucial at conception and throughout pregnancy to ensure healthy pregnancy outcomes. Although there is a crucial role of B6 in health, however, while it was reported that some women did not meet the estimated average requirement, there was no biochemical measurement of B6 status. Based on the US National Health and Nutrition Examination Survey (NHANES), over 40% of adult women (21–44 years) had B6 deficiency (defined as having plasma PLP concentration of<20nmol/L).[4]

Vitamin B12-rich foods are derived only from animals, i.e. meat, fish, and dairy. Although the body storage of vitamin B12 is relatively high (i.e. approximately for 1–5 mg), however vitamin B12 deficiency is emerging as a growing public health problem, which is commonly seen in pregnancy.[1] There are several common causes of vitamin B12 deficiency. Besides of the increased requirements, such as in pregnancy, impaired intestinal absorption is also one of the causes.[5] Noroyono et al. shows that there are decreasing of serum B12 levels by trimesters during pregnancy regardless to the prebiotic and probiotic supplementation provided during the pregnancy.[6] However, it has been known that commensal bacteria produce vitamins, particularly B vitamins, thus it plays a major role in meeting the needs, as some studies have documented that some probiotic strains can synthesize vitamin K, folate, vitamin B2 and B12.[7] This study aims to compare plasma folate, vitamin B6 and B12 levels in relation to the existence of fecal probiotic strain *Bifidobacterium animalis* subsp. *lactis* HNO19 (DR10™) among pregnant women in Indonesia.

Methods

Study design

This study is part of a main study entitled "Effects of *Bifidobacterium animalis* subsp. *lactis* HNO19 (DR10™), inulin, and micronutrient fortified milk on fecal DR10™, immune markers, and maternal micronutrients among Indonesian pregnant women" by Noroyono et al.[4] We applied a repeated cross-sectional study design (i.e. at first, second and third trimester) among two comparative population (i.e. those having positive fecal DR10™ as compared to those with negative fecal DR10™). From the total population of the previous study, we purposively selected the subjects, i.e. pregnant women aged 18–35 years, were residents of Jakarta and likely to remain in the city for one year, had singleton pregnancy at 8–12 weeks of gestation, were apparently healthy, had uncomplicated pregnancy, provided written informed consent, and who had fecal DR10™ at their third trimester of pregnancy. The eligible subjects were allocated into those having positive or negative result as the comparative groups. Exclusion criteria were those subjects with incomplete data on dietary and plasma folate, vitamin B6 and B12.

From the previous study, we only had 22 subjects and 55 subjects of pregnant women with positive and negative fecal DR10™, respectively in their third trimester, as our quota sampling. This limited sample size might be a limitation of this study, because we should calculate minimal sample size for the equal number of each group to detect differences between two means. All the subjects were requested to undergo several measurements, including baseline weight and height measures, energy and nutrient intake by using repeated 24-hour food recall (i.e. at first, second and third trimester), which were then converted to folate, vitamin B6 and

B12 intake, by using the free Nutrisurvey 2007 food-processor software. The Indonesian RDA for dietary intake of folate is 600 μg, vitamin B6 is 1.7 mg, and vitamin B12 during pregnancy is 2.6 mcg.

Study overview

As mentioned in the main study, this study was done at Cipto-Mangunkusumo and Budi Kemuliaan hospital in Jakarta during 2013 until 2014. The study protocol was approved by the Ethics Committee of the University of Indonesia (71/H2.F1/ETIK/2013) and registered at clinicaltrials.gov. Written informed consent were provided from the subjects before doing subjects recruitment.

Data collection

Plasma folate, vitamin B6 and B12 was collected from the whole blood sample and analysed by using reversed-phase high-performance liquid chromatographic tandem mass spectrometry (RP-HPLC-MS/MS) procedure. Data collection was done at first, second and third trimester. The reference range for plasma folate level is 4.5–45.3 nmol/L (2–20 ng/mL), and its insufficiency is defined as <4.5 pmol/L (2.0 pg/mL). The reference range for serum B6 (pyridoxal phosphate) is 5–50 mcg/L), and its insufficiency is defined as <5 mcg/L). The reference range for serum B12 is 150–489 pmol/L (203.3–662.8 pg/mL), and its insufficiency is defined as <150 pmol/L (203.3 pg/mL).

Statistical analyses

All data was presented accordingly based on its normality distribution. Statistical analyses were done by using independent-t test or Mann Whitney test and chi-square (Fisher-exact) test, to compare dietary and blood folate, vitamin B_6 and B_{12} by pregnancy trimester among the two groups, i.e. having fecal DR10^{TM} positive versus having fecal DR10^{TM} negative at third trimester, respectively.

Results

Table 1 shows that during baseline, at the first trimester, there is no difference in general characteristics of the two comparison groups based on the existence of fecal probiotic (DR10^{TM}), except for having daily food supplement in the form of prebiotic (FOS/inulin 2.5g) and probiotic (*Bifidobacteria animalis* subsp. *lactis* 5x10^{6}cfu) enriched-milk, consumed twice a day, as the intervention product as compared to the placebo, in the main study. Among the 36 subjects supplemented, 22 subjects (61.1%) revealed having

Table 1. Baseline characteristics of the subjects at first semester

Variables	Fecal DR10^{TM} 3rd Trimester		P-value
	Positive (n=22)	Negative (n=55)	
Socio-demographic:			
Age, y	31 (4)^	28 (7)*	0.157 ^{MW}
Gestational age, week by USG	10.6 (2.5)*	10.7 (2.8)*	0.897^{T}
Nutritional status:			
Height, cm	155.1 (4.7)*	157.3 (5.1)*	0.084^{T}
Weight, kg	58.6 (9.8)*	60.4 (11.6)*	0.514^{T}
BMI, kg/m^2	24.3 (3.9)*	24.3 (4.2)*	0.998^{T}
Haemoglobin, mg/dL	12.3 (0.8)*	12.4 (1.0)*	0.613^{T}
Total energy intake, kcal	1308 (418)*	1353 (317)*	0.611^{T}
Protein intake, g	48 (16)^	51 (14)*	0.710 ^{MW}
Iron intake, mg	10.7 (5.3)^	10.3 (10.3)^	0.946^{MW}
Folate intake, mcg	284 (418)^	422 (363)^	0.189^{MW}
Supplemented with pre- and probiotic enriched-milk, n(%)	22 (100)	14 (25.5)	**<0.001^{chi}**

^in median and interquartile range; *in mean and standard deviation, ^{MW}Mann-Whitney U test; ^{T}independent-t test; ^{chi}Pearson chi-square test

positive fecal DR10™, while none of the non-supplemented subjects had a positive result.

Focusing on folate, vitamin B_6 and B_{12} status during the pregnancy, Table 2 shows that during the pregnancy there were no significant differences in the folate and vitamin B_6 intake, while there was a significant difference in vitamin B_{12} intake (p=0.048) at first trimester. There was no difference in folate blood concentrations throughout the pregnancy. For vitamin B_6 blood concentration, there were significant higher concentrations in the presence of fecal DR10™ at first and second trimesters (P=0.035 and P=0.001, respectively). It was similar with vitamin B_{12}, in which there were higher vitamin B_{12} blood concentrations in the presence of fecal DR10 ™ at third trimesters (P=0.035), and by the third trimester the blood concentration changes were different between

Table 2. Folate, vitamin B_6 and B_{12} status during pregnancy by the existence of fecal DR10™ at the third trimester

Variables	Fecal DR10™ 3rd Trimester		P-value
	Positive (n=22)	Negative (n=55)	
Folate:			
Dietary intake, in mcg			
Trimester-1	284.2 (417.9)^	419.9 (366.2)^	0.189[MW]
Trimester-2	470.3 (68.5)^	470.6 (97.7)^	0.577 [MW]
Trimester-3	449.2 (39.7)^	471.1 (185.2)^	0.311 [MW]
Blood concentration, ng/mL			
Trimester-1	25.6 (11.5)^	19.6 (10.8)^	0.410 [MW]
Trimester-2	22.8 (5.9)*	25.8 (11.8)^	0.391 [MW]
Trimester-3	23.8 (8.8)*	19.5 (12.7)^	0.814 [MW]
Changes at trimester-3	-0.04 (9.16)*	1.04 (8.79)*	0.643[T]
Vitamin B_6:			
Dietary intake, in mg			
Trimester-1	0.3 (0.5)^	0.34 (0.3)^	0.450 [MW]
Trimester-2	0.5 (0.3)*	0.3 (0.6)^	0.168 [MW]
Trimester-3	0.4 (0.3)^	0.4 (0.4)^	0.819 [MW]
Blood concentration, mcg/mL			
Trimester-1	30.0 (39.7)^	22.6 (24.5)^	0.051 [MW]
Trimester-2	25.8 (12.1)^	20.1 (12.5)^	**0.035 [MW]**
Trimester-3	28.9 (25.1)^	19.7 (12.6)^	**0.001 [MW]**
Changes at trimester-3	-9.66 (35.04)*	-4.2 (20.9)^	0.527 [MW]
Vitamin B_{12}:			
Dietary intake, in mcg			
Trimester-1	0.8 (2.2)^	1.8 (1.9)^	**0.048[MW]**
Trimester-2	3.8 (14.4)^	3.6 (2.5)^	0.661[MW]
Trimester-3	3.8 (2.4)^	4.8 (3.2)^	0.855[MW]
Blood concentration, pg/mL			
Trimester-1	466.3 (189.8)*	489.3 (154.8)*	0.605[T]
Trimester-2	362.2 (95.4)*	312.5 (126.2)^	0.467[MW]
Trimester-3	326.3 (83.5)*	275.5 (112.5)^	**0.035[MW]**
Changes at trimester-3	-140.05 (149.66)*	-196 (164)^	**0.046 [MW]**

^in median and interquartile range; *in mean and standard deviation, [MW]mann-whitney U test; [T]independent-t test

groups based on the presence of fecal DR10^{TM} (P=0.046).

Furthermore, to see the insufficiency status of folate, vitamin B_6 and B_{12}, Figure 1 clearly shows the increasing proportions of serum B_{12} insufficiency by trimesters among subjects having negative fecal DR10^{TM}, although they are not statistically significant by Fisher's exact test. While all subjects had no insufficiency for blood folate and vitamin B_6.

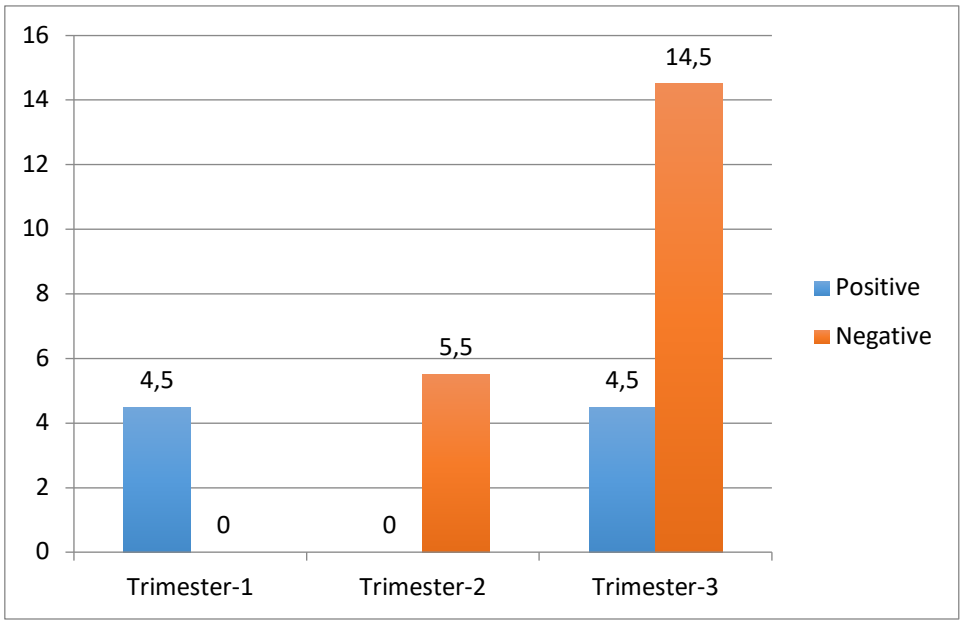

Figure 1
Proportions (%) of serum B_{12} insufficiency during pregnancy between groups by trimester and the presence of fecal DR10^{TM}
(no insufficiency status for folate and vitamin B_6 levels)

Discussion

This study found that there were no significant differences in the folate and vitamin B_6 intake during pregnancy, while there was a significant difference in vitamin B_{12} intake at first trimester only. The vitamin B's deficiency occurs primarily as a result of insufficient dietary intake and poor absorption. Folate is present in high concentrations in legumes, leafy vegetables and some fruits, and fortified wheat, maize or rice.[8] Indonesian pregnant mothers as part of Indonesian populations, commonly consume unfortified rice, however high consumption of leafy vegetables and folic acid supplementation program, would prevent them from having insufficient blood folate concentration. This was confirmed in this study in that no insufficiency for blood folate concentration was observed throughout the pregnancy.

Meanwhile, it is also revealed in this study that there was no difference in folate blood concentrations in relation to the existence of fecal DR10^{TM}. This shows that there was no effect of *Bifidobacterium animalis* subsp. *lactis* in producing/release and/or increase folate in foods. Actually, probiotics have the ability to synthesize folate in fermented foods, however the ability varies considerably being a strain-dependent trait. For example, it is claimed that *Strep. thermophilus* normally produces folate, whereas *Lactobacillus delbrueckii* subsp. *bulgaricus* is a folate consumer.[9]

Dietary vitamin B_6 sources come from wide variety of foods, such as fruits, vegetables, dairy and organ meats. Thus, its consumption should fulfil the requirements, as shown in this study that there was no subject having insufficient blood vitamin B_6 concentrations throughout the pregnancy. However, it revealed in this study that there were significant higher concentrations in the presence of fecal DR10^{TM} at second and third trimesters. This finding shows that the benefit of probiotic *Bifidobacterium animalis* subsp. *lactis* provided throughout the pregnancy to the increased level of vitamin B_6. Thus it confirmed by the strain used in this study that fermentations had been carried out in the milk-based

intervention product.[9] It is also concluded that this is the first study on vitamin B6 related to supplementation of milk-based beverage fermented by *Bifidobacterium animalis* subsp. *lactis* HNO19 (DR10™).

Vitamin B_{12} rich food sources are mostly from animal foods that commonly not consumed frequently by Indonesian pregnant mothers because of the high price. Beside due to inadequate dietary intake, there is also a physiological decline of maternal vitamin B_{12} concentrations, i.e. the increased maternal metabolic rate and active transport by the placenta to the fetus.[10] In return the insufficiency of vitamin B_{12} is evidence especially among pregnant mothers. This study found that there were higher vitamin B_{12} blood concentrations in the presence of fecal DR10™ at third trimesters. So far, it is known that only bacteria and archaea are able to synthesize vitamin B_{12}, i.e. *Lact. reuteri* B_{12}-producing strain with enhanced capacity to produce this essential vitamin.[9] Thus this study also adds the evidence that milk-based beverage fermented by *Bifidobacterium animalis* subsp. *lactis* HNO19 (DR10™) also facilitates the production of vitamin B_{12}. This evidence is proven by the increasing proportions of serum B_{12} insufficiency throughout the pregnancy among subjects having negative fecal DR10™ found in this study.

After showing the benefits of *Bifidobacterium animalis* subsp. *lactis* HNO19 (DR10™) in improving the blood vitamin B_6 and B_{12} concentrations, it raises a question whether there is a need to provide probiotics along with vitamin supplements to pregnant mothers. We should then consider several potential benefits of probiotics. One of it is the widely known that commensal bacteria have the capacity to synthesize vitamins, particularly B vitamins, thus play a major role in meeting our needs for those essential vitamins.[10]. In addition, focusing on absorption of the vitamins, it is also known that most transporters for vitamins are in the duodenum and ileum, not in colon where most of the microbes are found. Thus, it is still unclear if they can be a source of the vitamins, and there is a need to study further to its bioavailability.

Despite the findings in this study, folate, vitamin B_6 and B_{12} especially for pregnant mothers, are potential and closely related as coenzyme in DNA synthesis and numerous methylation reactions that occur in developing embryos. Thus, it is essential for cell multiplication during pregnancy.[11]

Therefore, it can be concluded that there is a need to improve folate, vitamin B6 and B12 concentrations during pregnancy for the benefits of offspring's quality of life. Besides dietary vitamin B's intake, intra-partum maternal gut microbe improvement is also needed, i.e. by the consumption of pre- and probiotic during periconceptional period.

Conflict of Interest

Authors declared no conflict of interest regarding this study.

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