Research Report

Antioxidant Milk Supplementation in Pregnancy: A Study of Retinol Binding Protein and Angiogenic Factors in Preeclampsia

Susu Antioksidan dalam Kehamilan: Kajian terhadap Retinol Binding Protein dan Faktor Angiogenik pada Preeklampsia

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Abstract

Objectives: To assess the role of retinol supplementation in the process of angiogenesis in preeclampsia.

Methods: The research was a double blind randomized clinical trial conducted Dr. Cipto Mangunkusumo Hospital outpatient clinic since January 2009 - December 2010. Samples were analyzed in Prodia Laboratory and Showa University in Tokyo, Japan. Subjects were tested in trimester 1, 2 and 2 weeks after delivery. During pregnancy subjects were given pregnancy milk supplements available on the market which had and that has been retinol fortified. Bivariate and multivariate analysis were performed of which retinol milk feeding, retinol binding protein (RBP) level, and level of angiogenic factors as independent variables and preeclampsia acted as the dependent one.

Results: One hundred four study subjects were divided into a control group of 55 and treatment of 49. Incidence of preeclampsia in this study was is 8.7% from which 8 persons (14.5%) came from the control group and 1 person (2%) from the treatment group. Characteristics of the two groups were equivalent. RBP levels obtained in the treatment group was lower than the control group, while in the subjects with preeclampsia the RBP level was higher than the group without preeclampsia. Correlation between the level of RBP with angiogenic factors showed no significant relationship except for the level of PlGF (p=0.036) which had a weak correlation (r=0.206). The level of angiogenic factors in the treatment group showed lower result than the control group whereas among the subjects with preeclampsia angiogenic factor levels were always higher than without preeclampsia. With multivariate analysis we found that significant factors associated with preeclampsia was sFlt-1 levels (p=0.004) and sFlt-1/PlGF ratio (p=0.017).

Conclusions: Providing milk supplements can reduce the levels of retinol (RBP), and antiangiogenic factors, but does not significantly increase proangiogenic factor level. Assuming there is no problem with the process of angiogenesis provision of retinol-fortified milk can reduce the incidence of preeclampsia. However, further research with a larger scale is needed.

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Keywords: retinol-fortified milk, RBP level, sFlt-1 level, sEng level, PlGF level, preeclampsia

Abstrak

***Tujuan**: Menilai peran suplementasi retinol terhadap proses angiogenesis pada preeklampsia.*

***Metode**: Penelitian ini merupakan uji klinis dengan disain acak tersamar ganda, dilakukan di RS Dr. Cipto Mangunkusumo Jakarta sejak Januari 2009 - Desember 2010. Dilakukan pemeriksaan sampel di Laboratorium Prodia dan Showa University Tokyo, Jepang. Pemeriksaan dilakukan pada trimester 1, 2 dan 2 minggu pascasalin. Selama kehamilan subjek penelitian diberikan susu suplementasi yang beredar di pasaran dan susu suplementasi kehamilan yang telah difortifikasi retinol. Analisis bivariat dan multivariat dilakukan dengan pemberian susu retinol, kadar RBP, kadar faktor angiogenik sebagai variabel bebas dan preeklampsia sebagai variabel tergantung.*

***Hasil**: Didapatkan 104 subjek penelitian dengan 55 orang kelompok kontrol dan 49 orang kelompok perlakuan. Kejadian preeklampsia pada penelitian ini sebesar 8,7% di mana 8 orang (14,5%) dari kelompok kontrol dan 1 orang (2%) berasal dari kelompok perlakuan. Karakteristik kedua kelompok perlakuan adalah setara. Didapatkan kadar RBP pada kelompok perlakuan lebih rendah dari kelompok kontrol. Sedangkan kadar RBP pada preeklampsia lebih tinggi daripada kelompok tidak preeklampsia. Korelasi kadar RBP dengan faktor angiogenik tidak menunjukkan hubungan bermakna kecuali kadar PlGF (p=0,036) tetapi memiliki korelasi lemah (r=0,206). Kadar faktor angiogenik pada kelompok perlakuan menunjukkan hasil lebih rendah dari kelompok kontrol sedangkan pada preeklampsia hasil kadar faktor angiogenik selalu lebih tinggi dibandingkan tidak preeklampsia. Dengan analisis multivariat didapatkan faktor yang berhubungan bermakna dengan preeklampsia adalah kadar sFlt-1 (p=0,004) dan rasio sFlt-1/PlGF (p=0,017).*

***Kesimpulan**: Pemberian susu suplementasi retinol dapat menurunkan kadar RBP, menurunkan kadar faktor antiangiogenik tetapi tidak meningkatkan kadar proangiogenik secara bermakna. Dengan asumsi proses angiogenesis berjalan baik maka pemberian susu fortifikasi retinol dapat mengurangi kejadian preeklampsia. Walaupun demikian diperlukan penelitian lanjutan dengan skala lebih besar.*

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***Kata Kunci**: susu fortifikasi retinol, kadar RBP, kadar sFlt-1, kadar sEng, kadar PlGF, preeklampsia*

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INTRODUCTION

Preeclampsia is a pregnancy specific syndrome, characterized by decreased perfusion of secondary organs and generalized vasospasm. It is a cause of maternal and fetal morbidity as wel as mortality, with an incidence of 5-8% of all pregnancies.[1] Success of pregnancy is determined by the formation of a fetal-maternal complex, in this case, placentation process.[2] Abnormalities of placental implantation will lead to a series of processes due to an increase of free radicals, inflammatory mediators, and an imbalance of pro and antiangiogenic factors that will lead to endothelial dysfunction.[3]

Angiogenesis is the process of blood vessel formation that involves a lot of factors, one of which is the angiogenic factors.[4-7] Angiogenic factors that play a role in angiogenesis are antiangiogenic factors soluble Endoglin (sEng), soluble Fms-like Tyrosine Kinase Receptor-1 (sFlt-1), and proangiogenic factor Placental Growth Factor (PlGF).[8] Levine et al stated that preeclamptic patients had increased antiangiogenic factor sFlt-1 and sEng, with a decrease of PlGF proangiogenic factor compared with normal.[8]

Angiogenesis is a complex process in which the process is largely determined by nutritional status.[9-10] One nutrient that associates with cellular processes is the micronutrient vitamin A, one of antioxidants that play a role in protein synthesis, cellular differentiation, and intercellular communication.[11-2] Retinol itself affects angiogenesis during differentiation of vascular smooth muscle cells (VSMC) as components of blood vessels and neointimal formation so blood vessels become larger in caliber.[13] Other researchers claim that milk has antioxidant capacity and is negatively correlated with risk of hypertension.[14-5]

Based on this background one question arises whether retinol-fortified milk as one of the antioxidants may be used in the process of angiogenesis in relation to prevention of preeclampsia, which requires a study of retinol effects in the process of angiogenesis that occurs in the first trimester with the incidence of preeclampsia in the second and third trimesters.

METHODS

This study was a stage II phase 3 clinical trial with a randomized, double blind design. Research was conducted at Dr. Cipto Mangunkusumo Hospital outpatient clinic since January 2009 - December 2010. Sample were analyzed in Prodia Laboratory and the Laboratory of Showa University in Tokyo, Japan.

All pregnant women with gestational age less than 13 weeks and were willing to participate in research, were measured for height, weight, and blood pressure before laboratory tested for FRAP (Ferric Reducing Ability of Plasma) to measure the antioxidant status with a reference value of less than 900 nmol/mg protein.[16]

When subjects fulfilled the inclusion criteria they were tested for angiogenic factors sFlt-1, PlGF, sEng and Retinol Binding Protein (RBP), which is the marker of retinol metabolism and pro-inflammatory cytokines. Physical and laboratory examinations were performed in each trimester of pregnancy and during pregnancy subjects received supplementation of milk available on the market, which has been fortified with retinol, with a randomized distribution of subjects. If there had been complications before reaching 20 weeks gestational age, the corresponding subject would have been excluded from the study. Results of this study was incidence of preeclampsia and infant birth weight.

Collected data were analyzed with bivariate and multivariate analysis and processed using SPSS 11.5.

RESULTS

This study managed to collect 104 subjects who were divided into 2 groups: base on randomization the control group of 55 subjects and the treatment group of 49. Intervention was done to all subjects, the control group received milk supplements available on the market while the treatment group received milk ones that had been enriched with antioxidants. Characteristics of study subjects were as follows: subjects had an average age of 28.9 years with 90% having middle education level or higher. Subjects who worked only 49%. Most subjects had good nutritional status, only 14% who did not. The average gestational age reached 38 weeks, with 5% subjects having hypertension. Fetal birth weight had an average of 3138 grams with a minimum of 1500 grams and a maximum of 4200 grams. Results can be seen in Table 1.

Table 1. Characteristics of study subjects

	Control		Treatment	
	n	**%**	**n**	**%**
Research Subject	55	52.9	49	47.1
Education				
Low	3	5.5	8	16.3
Middle	24	43.6	21	42.9
High	28	50.9	20	40.8
Job				
Employed	30	54.5	21	42.9
Unemployed	25	45.5	28	57.1
Nutritional Status				
Poor	9	16.4	6	12.2
Normal	35	63.6	27	55.1
Over	11	20.0	16	32.7
Hypertension History				
Yes	4	7.3	1	2.0
No	51	92.7	48	98.0
Preeclampsia				
Yes	8	14.5	1	2.0
No	47	85.5	48	98.0
Age (year)	30±5		28±5	
Gestation age (week)	39±2		39±2	
Birth weight (gram)	3148±521		3127±451	

Distribution of subjects based on control and treatment groups showed no difference in characteristics between the two groups (Chi square test $p > 0.05$), which means that both groups are equal. In the end, there were nine subjects who experienced preeclampsia (8.7%), of which eight (14.5%) were in the control group, and 1 (2%) in the treatment group. All numerical data have a normal distribution.

To assess changes in RBP concentration ratio we used a general linear model for repeated measures (GLM). Results showed an increase of RBP in both groups from the first and second trimester, with the highest number being in the puerperium. RBP values are under constant treatment group and control group difference in the furthest contained in the third trimester. Although RBP elevation was significantly different for each semester in each group ($p < 0.05$) but the tendency of increasing ratio between control and

treatment groups did not differ significantly (p = 0.138). Results can be seen in Figure 1.

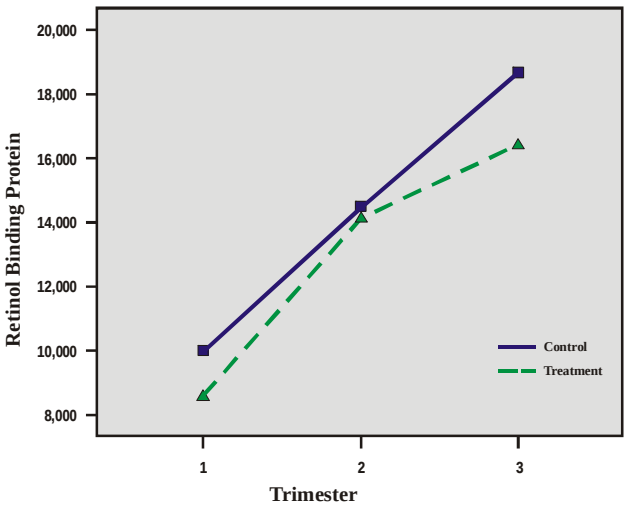

Figure 1. Trend changes in RBP each trimester in both groups based on treatment

Analysis of the relationship between RBP changes with changes in angiogenic factors was done with the parametric Pearson correlation test because the data had a normal distribution. The result was that only PlGF in both groups showed significant correlation with RBP (p=0.036), although the correlation was very weak (r=0.206).

Result analysis of changes in angiogenic factor from each trimester using GLM analysis showed a significant increase in each trimester consecutively.

Comparison of trend changes in treatment and control groups also showed significant differences. The sFlt-1 and sEng in the treatment group were always lower than the control group, and the concentration of PlGF in the treatment group was lower in the first trimester, while higher in the second and third trimester. Results can be seen in Figure 2. Changes can also be seen in angiogenic factors level based on preeclampsia. In each trimester which showed a significant increase respectively. By using the same analysis, trend changes in preeclamptic and non preeclamptic group also showed significant differences, which were the higher level of sFlt-1 and sEng in preeclamptic group and the changes of PlGF which was lower in the preeclampsia group at the beginning, but higher in the third trimester. The results can be seen in Figure 3.

The changing relationship of RBP to preeclampsia showed a significant difference of RBP in preeclampsia and nor preeclamptic group, which can be seen in Figure 4.

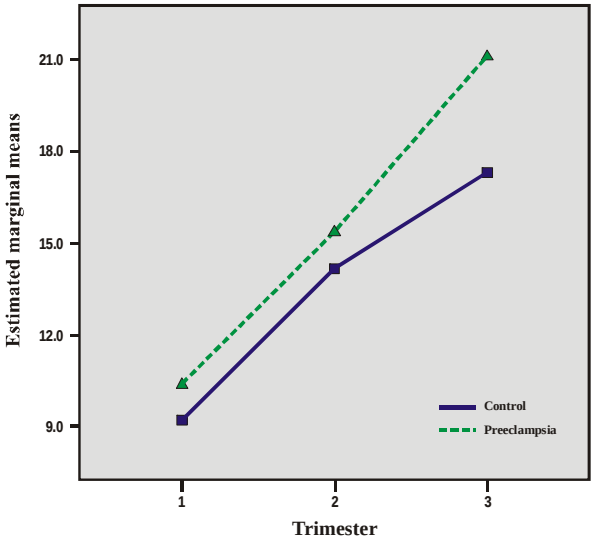

Figure 4. RBP changes trends based on the incidence of preeclampsia in each trimester

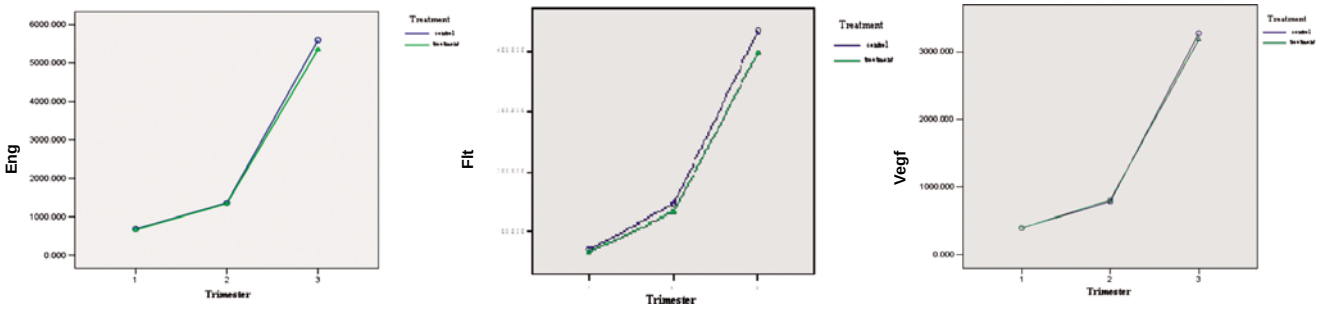

Figure 2. Trend changes in angiogenic factor sEng, sFlt-1 and P1GF in the control and treatment groups in each trimester

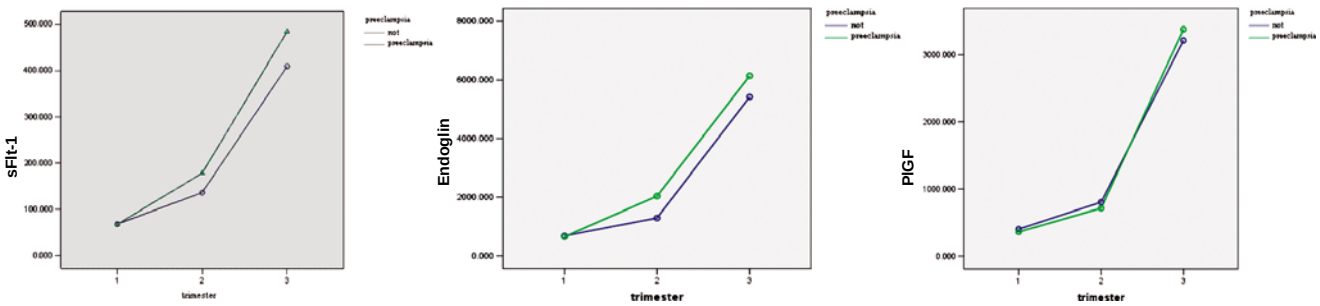

Figure 3. Graphic changes in angiogenic factor value based on the incidence of preeclampsia in each trimester

Multivariate analysis was performed to assess the relationship of all factors (antioxidants-milk intake, RBP changes, and changes in angiogenic factors) and preeclampsia by using a multiple logistic regression. Factors that influence preeclampsia were sFlt-1 and ratio of sFlt-1/PlGF. One unit increase in sFlt-1 biomarker would increase the risk of preeclampsia by 1.029, whereas one unit reduction of Flt-1/PlGF ratio would reduce the risk of preeclampsia, eventhough the Odd ratio was very small ($p<0.001$). The relationship between levels of RBP with preeclampsia did not show significant results and milk supplementation in this analysis also showed no significant results.

DISCUSSION

This study aims to assess the role of micronutrients, especially vitamin A nutrition in the prevention of preeclampsia. The results showed that there was no significantly different characteristics in both groups, which meant that characteristics of both groups was similar. In Table 1, it appears that most research subjects were on average reproductive age, had a level of middle and high education, mostly unemployed; and had normal nutritional status with no history of hypertension. All research subjects routenely underwent antenatal care, met the nutritional intake and reached an average gestational age of 38 with an average birth weight 3318 grams. From this data can be viewed as equal subjects of research. Results also showed that subjects with milk supplementation reach full term gestational age. Suggesting that good prenatal care, balanced lifestyle, and good pregnancy improve the course of pregnancy.

Same result were also shown in Table 1 in which among subjects who had provision of retinol-fortified milk, only 2% had preeclampsia, compared to 14.5% in subjects with additional food.

These results suggest that preeclampsia is a disease in pregnancy with an unknown cause, affected by various factors. It seems that preeclampsia can still occur even after milk supplementation. In the treatment group, which had vitamin A fortification, it can be seen that preeclampsia only occured in 2% of subjects; indicating initial hypothesis according to which retinol will improve the process of angiogenesis is the result of placental preeclampsia, 2%. Assuming there is no problem in the angiogenesis process. Based on the pathophysiology of angiogenesis, the results are consistent with the opinion of Miano et al about the role of retinol in vascular smooth muscle differentiation.[13]

Regarding the RBP concentration ratio based on the treatment as shown in Figure 1, indicating that retinol binding protein (RBP) was increased mainly in the control group, explained that with the fortification of retinol that would last series of the process from the metabolism of vitamin A, retinol will bind RBP and retinol transport will last well, where the end result can be assumed that the process of angiogenesis will be formed adequately. It seems that in addition of being a retinol transport protein, RBP also act as inflammatory marker as stated by Vaisbuch et al, thus enabling it has a possible biomarker for preeclampsia.[17] Combining many theories of preeclampsia, this study showed that with provisions of micronutrients and antioxidants, inflammation processes and endothelial dysfunction that occur in preeclampsia would be reduced and placentation process would go.

The correlation between RBP and angiogenic factors showed that RBP didn't strongly correlate with angiogenic factors. RBP only showed a significant association with proangiogenic factor PlGF, although it was weak, while its correlation with other angiogenic didn't show a significant relationship. Analysis of this study suggested that RBP didn't show any activity of angiogenesis instead it was a retinol transport that suspected to improve angiogenesis.

These results indirectly show the presence of potential angiogenesis of vitamin A. Because the metabolism of vitamin A in pregnant women is still not fully understood, these results still needs further research.[18] Although the results are not very satisfactory but from this process, we can see the role of vitamin A in angiogenesis.

Correlation between levels of angiogenic factors in both groups in each trimester can be seen in Figure 2. The results of this study showed that levels of antiangiogenic factor, sFlt-1 and sEng in the treatment group were always lower than the control group at each trimester. On the countrary PlGF were always lower in the treatment group compared to control group. These results suggest that supplementation with retinol fortified milk reduce the concentration of antiangiogenic factors but not increase the levels of proangiogenic factor. From the results we can also see that fortified milk decreased the levels of antiangiogenic factors but did not increase the level of proangiogenic factors. Also two suggestion can be drawn from such result, first there are many other factors that affect the levels of angiogenic factors and second there's not enough of antioxidants used.

Another thing we are able to derive from the results is the association between angiogenic factor with preeclampsia, as shown in Figure 3. The results of this study indicate that the concentration of antiangiogenic factor, sFlt-1 and sEng in preeclampsia group were always higher than the non preeclampsia group on each trimester. Although the concentration of proangiogenic factor PlGF in early trimester was lower in preeclampsia, but in the third trimester PlGF showed an agreement a higher level than the non preeclampsia group. The results are concordant with studies conducted by Levine et al and Karumanchi et al who stated that there was an increased angiogenic factors sFlt-1 and sEng as well as a decrease PlGF.[5,8] These results show that the levels of pro and antiangiogenic factors have a role in the pathophysiology of preeclampsia, in which the concentration of antiangiogenic factor was higher in preeclampsia group while proangiogenic factor PlGF, was lower in the first trimester. This shows that preeclampsia is a disease that occurs due to a defect of placentation, where low proangiogenic factor showed that the process of angiogenesic was not adequate.[5,8]

The association between angiogenic factors and preeclampsia was shown by the levels of angiogenic factors that correlated with preeclampsia. It was shown that the levels of pro and antiangiogenic factors had a role in the pathophysiology preeclampsia.[4-7] Those

results also showed that preeclampsia was a disease which began since early gestational placentation.[17-19]

The association between RBP and preeclampsia (Figure 4) showed that in preeclampsia, RBP levels was higher than the non preeclamptic group. According to Sapin et al, diseases that are classified in the "great obstetrical syndrome" such as preeclampsia and fetal growth restriction can be associated with abnormal concentrations of retinol and retinol binding protein.[19] On another note, Vaisbuch et al stated that the RBP could be used as a marker of inflammation, a new biomarker in preeclampsia.[17] This research showed that preeclampsia was a disease of pregnancy accompanied by inflammatory response and systemic endothelial dysfunction, resulting in a series of symptoms usually found in preeclampsia.[20-2]

By using multivariate analysis, this study showed that antiangiogenic factors had a significant associations with preeclampsia (sFlt-1 p=0.004 and sFlt-1/PlGF ratio p=0.017). Milk supplementation and RBP levels didn't show a significant result. This was probably caused by failure to fulfill one of multivariate analysis statistical test requirements related to the "Rule of Thumb", one independent variable must be represented by 10 output. This study didn't have an adequate output to be used in multivariate analysis (only 9 people suffered preeclampsia). This means that the number of samples and outcomes are not adequate.

In a study conducted by A-Kholy et al in Egypt (2010) it was said that the RBP and angiogenic factors were associated with the pathogenesis and severity of preeclampsia.[18] If we take a look at the initial proportions the incidence of preeclampsia in the group with retinol fortified milk only 2% compared with the non retinol fortified milk that reached 14.5%, which suggested that provision of milk with antioxidants could reduce the incidence of preeclampsia, eventhough we are not able to fully explain the detailed process yet. Another weakness of this study was the number of subjects that were not adequate.

The final results of this study showed that preeclampsia was a disease of pregnancy that occurred since the beginning of placentation and influenced by both macronutrient and micronutrient. However, there is no denial that preeclampsia is a multifactorial disease with a cause that needs further studies.

CONCLUSION

This study can conclude and recommend that providing milk supplements can reduce levels of retinol (RBP), and antiangiogenic factors, but does not significantly increase proangiogenic levels. Assuming there is no problem with process of angiogenesis the provision of retinol-fortified milk can reduce the incidence of preeclampsia. However, further research with a larger scale is needed.

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