Original Article

No relation between folate and homocysteine levels and depression in early pregnant women

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Summary **The objective in this study was to evaluate the association between folate and homocysteine (Hcy) levels and depressive symptoms in early pregnancy. A cross-sectional study was conducted with 86 pregnant women in the first trimester. A Japanese version of the Center for Epidemiologic Studies Depression (CES-D) scale was used to screen for depression. Non-fasting blood samples were collected from the women to measure folate and Hcy levels. Fifty-three (61.6%) women scored at or above a clinical cut-off of 16, and were classified with depression. In logistic regression analyses, no significant associations were observed between the incidence of depression in the first trimester and elevated Hcy and deficiencies of serum folate, folate intake, vitamin B6 intake and vitamin B12 intake. Folate and Hcy concentrations, and folate consumption, may not be protective against depression in early pregnancy.**

Keywords: Folate, homocysteine (Hcy), pregnancy, depression, nutrition

1. Introduction

Proper nutrition during pregnancy is vital to the health of the woman and her fetus (*1,2*). Women are particularly vulnerable to the adverse effects of poor nutrition on mood because pregnancy and lactation increase nutrient requirements. It has been proposed that depletion of nutrient reserves throughout pregnancy and a lack of recovery postpartum may increase a women's risk for maternal depression (*3*). An association between depressive symptoms and low levels of several dietary B vitamins has been suggested, including folate, vitamin B2 (VB2), vitamin B2 (VB6), vitamin B12 (VB12), all possibly mediated by homocysteine (Hcy) (*4-6*).

Folate deficiency appears to be the most closely linked not only to neural tube defects (*7,8*), but also to depressive disorders. Evidence has been steadily mounting over the past several decades implicating folate in processes thought to underlie the regulation of mood (*9*). Folate and VB12 are essential for normal central nervous system function and may modulate mood through several mechanisms. Severe deficiencies of these vitamins cause loss of memory, mental dysfunction, and depression. Similarly, fatigue, confusion, dementia, and irritability are common clinical signs of folate deficiency. Zuckerman *et al.* (*10*) reported that depressed women were more likely to have poor nutrition. The active metabolite of folate is required for remethylation of Hcy in the production of methionine, which is involved in a number of biochemical processes involving the three aforementioned neurotransmitters (*11*). Thus, a deficiency in folate would impact the production and function of these neurotransmitters.

A deficiency of either folate or VB12 causes elevated Hcy concentrations, which may contribute to the pathogenesis of major depressive disorder (MDD) by mediating a vascular response (*12*). Patients

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diagnosed with MDD tend to have lower concentrations of serum or red-cell folate than healthy control subjects (*13*,*14*). Poor folate status has been associated with severity of depression and prolonged episodes of MDD. Women have roughly twice the risk of MDD as men. In addition, women of childbearing age are at high risk for MDD. The lifetime risk for MDD in community samples has varied from 10 to 25% for women, with peak prevalence between 25-44 years of age (*15*). A similar proportion of women are affected by MDD in pregnancy and the postpartum period (*16*).

Numerous researches have explored the relationship between dietary deficiency and depression among women in middle pregnancy and postpartum, but not in early pregnancy. Heron *et al.* (*5*) reported that women with antenatal depression have a 6.5-fold increased risk of the more widely known postpartum depression. Of the 10 to 15% of women who develop postpartum depression, up to 0.2% will develop postpartum psychosis, a serious illness associated with suicide, infanticide and homicide (*17*). Depression is a significant disease that has potentially deleterious effects on the woman and her infant, such as uterine irritability, pregnancy-induced hypertension, antepartum bleeding, decreased uterine artery blood flow and preterm delivery (*18*-*21*). However, the association between folate deficiency and prevalence of depression in the early pregnancy is not well known. The objective in this study was to evaluate the association between folate and Hcy levels and depressive symptoms in early pregnancy.

2. Materials and Methods

2.1. *Subjects*

A cross-sectional study on healthy women in early pregnancy who had antenatal care at the obstetrics and gynecology clinic of Narita Hospital or Hirowatari Ladies' Clinic, both of which are located in Aichi prefecture, Japan, was conducted from February to May 2009. Women were at 6-11 weeks of gestation, which was estimated from the first day of the last menstrual period, and fetal growth was confirmed by ultrasound examination. Women with pregestational diabetes of multiple deliveries were excluded from the study. The study was approved by the Ethics Committees of Kyoto University. Written consent was obtained from all participants, after obstetricians explained the purpose, significance, and protocol of the study.

2.2. *Sociodemographic and anthropometric information*

Maternal body mass index (BMI) was calculated using prepregnancy weight and height (kg/m^2). A self-administered questionnaire was used to gather information on age, parity, medical history, number of episodes of nausea/d, number of vomits/d, and current smoking status. Smokers were defined as women who had smoked any number of cigarettes, regardless of smoking status before conception.

2.3. *Dietary assessment*

Dietary habits during the last month of gestation were assessed with a brief self-administered diet-history questionnaire (BDHQ), which was completed by each woman while waiting for the medical examination. The BDHQ took approximately 10 min to complete. The BDHQ is a four-page structured questionnaire, consisting of the following seven sections: general dietary behaviors, major cooking methods, consumption frequency and portion size of six alcoholic beverages, semiquantitative frequency of intake of 56 selected food and nonalcoholic beverage items, dietary supplements, amount and consumption frequency of 19 staple foods and open-ended items for foods consumed regularly (≥ 1 time/week). The food and beverage items and portion sizes in the diet-history questionnaire (DHQ) were derived primarily from the National Nutrition Survey of Japan data and several recipe books for Japanese dishes (*22*). Measures of dietary intake for 147 food and beverage items, energy, fat, total carbohydrates, alcohol, and dietary fiber were calculated by using an ad hoc computer algorithm developed for the DHQ, which was based on the Standard Tables of Food Composition in Japan (*23*). Values of dietary intake were energy-adjusted.

2.4. *Depressive symptom*

A Japanese version (*24*) of the Center for Epidemiologic Studies Depression (CES-D) scale was used to screen for depression. The CES-D is a widely used instrument for assessing general depressive symptom in Western (*24*) and Japanese (*25*) subjects. It is a short self-report scale designed to measure depressive symptoms associated with depression and has been validated against longer scales (*25*). Participants were asked to score the prevalence of salient symptoms of depression (*e.g.* sadness, hopelessness, fatigue, crying, low self-esteem, and changes in sleep or appetite) within the past seven days on a four-point response scales (0: 'less than 1 day', 1: '1-2 days', 2: '3-4 days', and 3: '5-7 days'). The total CES-D scores range from 0 to 60. Participants scoring more than 16 were categorized as having CES-D depression.

2.5. *Blood collection and analysis*

Blood samples for nutritional analyses and hematology were obtained at 4-11 weeks of gestation. Non-fasting blood samples were collected from pregnant women for measurement of folate, Hcy, hemoglobin, hematocrit,

serum iron level, albumin, and total protein. Blood samples were drawn from the antecubital vein into potassium ethylenediaminetetra-acetic acid and serum separator tubes. Serum folate was measured by chemiluminescent immunoassay method (Kemirumi ACS folate II: Siemens Healthcare Diagnostics, Deerfield, IL, USA). Plasma Hcy was measured by high performance liquid chromatography technique (YMC-Rack Pro C18: YMC Co., Ltd., Kyoto, Japan). The intra-assay coefficient of variation (CV) was 7.6% for folate and 10.6% for Hcy, respectively. Serum albumin was measured by Bromcresol green method (Albumin HR II: Wako Pure Chemical Industries, Osaka, Japan) and CV was less than 2.0%. Serum total protein was measured by Biuret method (Total protein HR II: Wako Pure Chemical Industries) and CV was less than 1.5%.

2.6. *Statistical analysis*

All statistical analyses were performed with SPSS for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Nutritional intake and biomarkers values were compared between the two groups. Differences in continuous variables were determined by independent *t*-test or the Mann-Whitney-Wilcoxon test for non-normally distributed data. Significant differences between the proportion of the depression group and the non-depression group based on biomarkers and nutrition intake were evaluated by using Pearson's chi-square analyses. Crude and adjusted odds ratios for depression were calculated *via* median. The depression model was adjusted for parity, gestational weeks, and number of vomits/d. Statistical significance was defined as $p < 0.05$.

3. Results

3.1. *Demographic data*

Of 94 pregnant women initially recruited, 86 were finally analyzed. Subjects' scores on the CES-D ranged from 1 to 38, with a mean of 17.0. Fifty-three (61.6%) women scored at or above a clinical cut-off of 16, and were classified with depression. The mean CES-D scores were 22.4 ± 5.6 for the depression group and 8.5 ± 3.7 for the non-depression group, respectively (Table 1). The gestational weeks of the subjects ranged from 6 to 11. No significant differences in age, height, prepregnancy weight, prepregnancy BMI, or gestational weeks were observed between the two groups. Around 20% of women reported using vitamin supplements of any kind. The proportion of primipara was significantly higher in the depression group than that in the non-depression group ($p = 0.04$). Episodes of nausea/d and number of vomits/d in the first trimester were not different in the two groups.

3.2. *Biological markers*

Logarithmically transformed serum folate concentrations were inversely correlated with plasma Hcy concentrations ($r = -0.227$, $p < 0.05$) (Table 2). We also assessed the degree of correlation between CES-D scores and blood biomarkers. A small, significant correlation was observed between CES-D scores and total protein ($r = 0.263$, $p < 0.05$) and hemoglobin concentrations ($r = 0.222$, $p < 0.05$), but CES-D scores were not significantly related to serum folate, plasma Hcy, albumin, iron, hematocrit, or hematocrit concentrations.

Table 1. Demographic characteristics

Items	All ($n = 86$)	Non-depression ($n = 33$, 38.4%)	Depression ($n = 53$, 61.6%)	p value*
Age (year)	30.9 ± 4.6 (20-41)	30.6 ± 4.5 (24-39)	30.8 ± 4.7 (20-41)	0.86
Height (cm)	159.4 ± 5.5 (148-172)	159.8 ± 5.9 (148-172)	159.1 ± 5.3 (150-170)	0.56
Prepregnancy weight (kg)	51.1 ± 5.9 (40-66)	52.0 ± 5.9 (42-66)	50.5 ± 5.5 (40-63)	0.24
Prepregnancy BMI (kg/m^2)	20.1 ± 1.8 (17.0-25.0)	20.3 ± 1.7 (17.0-23.4)	19.9 ± 1.9 (17.0-25.0)	0.34
Gestational week	8.2 ± 1.2 (6-11)	8.6 ± 1.0 (6-10)	8.1 ± 1.3 (6-11)	0.07
CES-D scores**	17.0 ± 8.4 (1-38)	8.5 ± 3.7 (1-15)	22.4 ± 5.6 (16-38)	< 0.001
Primipara (%)	54.4	39.4	62.3	0.04
Smoking (%)	21.1	18.2	23.1	0.59
Anemia (%)***	7.8	15.2	3.8	0.54
Vitamin supplements	20.2	18.8	21.2	0.79
Episodes of nausea/d				0.54
none (%)	19.8	24.2	17.0	
1-2 times (%)	33.7	36.4	32.1	
≥ 3 times (%)	46.5	39.4	50.9	
Number of vomits/d				0.19
none (%)	77.9	75.8	77.4	
1-2 times (%)	15.1	9.1	18.9	
≥ 3 times (%)	7.0	12.1	3.8	

Values are presented as mean ± S.D. (Range) or %. * p values: depression *vs.* non-depression pregnant women (Mann-Whitney U test). ** CES-D: Center for Epidemiologic Studies Depression Scale. Depression: CES-D ≥ 16. *** Anemia: Hemoglobin less than 11.0 g/dL.

Median (interquartile range) serum folate concentrations were 8.0 (5.5) ng/mL in the non-depression group and 8.2 (4.5) ng/mL in the depression group, respectively. The median (interquartile range) plasma Hcy concentrations were 6.0 (2.4) nmol/mL in the non-depression group and 6.1 (1.4) nmol/mL in the depression group, respectively. The median of serum folate, plasma Hcy, total protein and albumin, and the mean of serum iron, hemoglobin, and hematocrit concentrations were not significantly different between the non-depression and depression groups (Table 3).

3.3. *Dietary intake*

The mean folate intake was 197.1 ± 59.4 μg/1,000 kcal in the non-depression group, and 179.9 ± 52.4 μg/1,000 kcal in the depression group, respectively. Total energy intake in the first trimester was 1,519.6 ± 395.9 kcal/d in the non-depression group and 1,413.9 ± 470.9 kcal/d in the depression group, respectively (Table 4). The mean of energy intakes, and all other nutrient intakes per 1,000 kcal, was not significantly different between two groups.

3.4. *Risk factors of depression in the first trimester*

The risks of depression in early pregnancy, according to the biomarker concentrations and nutrient intakes which were used by each median, are shown in Table 5. In logistic regression analyses, no significant associations were observed between the incidence of depression in the first trimester and elevated Hcy or lower serum folate, folate intake, VB6 intake, or VB12 intake, even adjusted for parity, number of vomits/d, and gestational weeks (Table 5).

4. Discussion

Nearly 62% of women were found to have depression symptoms in the first trimester, even though they were healthy and well-nourished women. The mean of the CES-D score was 17.0 ± 8.4 in these subjects, a little bit higher than the 16.0 threshold used to indicate a positive screen for depression, but lower than those of non-pregnant Japanese women aged 18-30 years old (*26*). Similar results were observed among 60 pregnant American women at an average of 15 weeks

Table 2. Correlation between biomarkers and CES-D scores in early pregnancy

Items	CES-D	Serum folate	Plasma Hcy	Total protein	Albumin	Serum iron level	Hemoglobin	Hematocrit
CES-D	1	0.098	0.036	0.263*	−0.091	0.001	0.222*	0.143
Serum folate			−0.227*	0.052	0.216	0.019	0.179	0.207
Plasma Hcy				0.144	−0.092	−0.113	−0.099	−0.187

CES-D: Center for Epidemiologic Studies Depression scale. * $p < 0.05$.

Table 3. Biomarkers in non-depression and depression groups in early pregnancy

Biomarkers	Non-Depression ($n = 33$)	Depression ($n = 53$)	p value
Serum folate (ng/mL)*	8.0 (5.5)	8.2 (4.5)	0.862[a]
Plasma Hcy (nmol/mL)*	6.0 (2.4)	6.1 (1.4)	0.964[a]
Total protein (g/dL)*	7.1 (0.5)	7.3 (0.6)	0.063[a]
Albumin (g/dL)*	4.3 (0.3)	4.3 (0.3)	0.074[a]
Serum iron (μg/dL)**	93.6 ± 41.8 (13.0-163.0)	92.6 ± 40.4 (19.0-201.0)	0.673[b]
Hemoglobin (g/dL)**	11.9 ± 1.2 (8.7-13.8)	12.4 ± 1.0 (9.3-15.7)	0.099[b]
Hematocrit (%)**	37.2 ± 2.9 (30.0-42.5)	38.1 ± 2.9 (28.7-47.2)	0.254[b]

Depression: Center for Epidemiologic Studies Depression (CES-D) scale ≥ 16. * Values are median (Interquartile range). ** Values are mean ± S.D. (Range). [a] Student's t-test [b] Mann-Whitney U test.

Table 4. Energy adjusted nutrient intake/d by BDHQ in non-depression and depression groups

Items	Non-depression ($n = 33$)	Depression ($n = 53$)	p value*
Energy (kcal)	1,519.6 ± 395.9 (814.1-2,258.6)	1,413.9 ± 470.9 (779.1-2,738.4)	0.159
% energy from protein (g)	14.2 ± 2.1 (8.8-19.4)	13.6 ± 2.2 (8.9-18.9)	0.119
% energy from total fat (%)	30.1 ± 4.2 (22.9-38.2)	28.8 ± 5.3 (16.1-39.4)	0.309
% energy from carbohydrate (%)	54.7 ± 5.4 (42.2-64.4)	56.4 ± 6.9 (42.5-70.5)	0.289
Calcium (mg/1,000 kcal)	304.1 ± 91.1 (169.6-613.2)	285.5 ± 84.9 (72.9-499.6)	0.549
Iron (mg/1,000 kcal)	4.3 ± 0.9 (2.7-6.8)	3.9 ± 0.8 (2.3-6.8)	0.063
Zinc (mg/1,000 kcal)	4.2 ± 0.5 (2.9-5.4)	4.0 ± 0.6 (2.3-5.1)	0.159
Folate (μg/1,000 kcal)	197.1 ± 59.4 (96.7-336.9)	179.9 ± 52.4 (63.9-362.4)	0.199
Vitamin B6 (mg/1,000 kcal)	0.7 ± 0.1 (0.3-1.0)	0.6 ± 0.1 (0.3-0.8)	0.289
Vitamin B12 (μg/1,000 kcal)	3.9 ± 1.5 (1.0-8.0)	3.7 ± 1.8 (0.5-10.1)	0.277
Vitamin C (mg/1,000 kcal)	78.1 ± 28.9 (30.5-132.2)	85.6 ± 43.3 (13.7-195.2)	0.746

Values are presented as mean ± S.D. (Range). BDHQ: brief self-administered diet-history questionnaire. Depression: Center for Epidemiologic Studies Depression (CES-D) scale ≥ 16. * p values: depression *vs.* non-depression pregnant women (Mann-Whitney U test).

Table 5. Incidence of depression (CES-D ≥ 16) and odds ratio by maternal biomarkers and nutrition

Variables	Crude OR			Adjusted OR*		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Serum folate (ng/mL)**						
< 8.1	0.74	0.31-1.78	0.51	0.63	0.24-1.61	0.34
≥ 8.1	1.0			1.0		
Plasma Hcy (nmol/mL)**						
< 6.1	1.0			1.0		
≥ 6.1	1.04	0.43-2.49	0.93	0.62	0.23-1.72	0.36
Intake folate (μg/1,000 kcal)**						
< 187.9	1.19	0.50-2.84	0.69	1.12	0.44-2.83	0.82
≥ 187.9	1.0			1.0		
Intake VB6 (mg/1,000 kcal)**						
< 0.66	1.25	0.52-2.98	0.62	1.36	0.54-3.42	0.52
≥ 0.66	1.0			1.0		
Intake VB12 (μg/1,000 kcal)**						
< 3.3	1.96	0.80-4.78	0.14	1.79	0.70-4.56	0.22
≥ 3.3	1.0			1.0		

* Adjusted odds ratio from a logistic regression model controlling for parity, number of vomits/d and gestational weeks. ** Values are medians. OR, odds ratio; CI, confidence interval. Depression: Center for Epidemiologic Studies Depression (CES-D) scale ≥ 16.

(*27*). Several studies have linked nausea and vomiting of pregnancy with depression (*28,29*). Kelly *et al.* (*30*) reported that pregnant depressive women have significantly more somatic complaints, such as nausea, stomach ache, shortness of breath, and headache, than non-depressive women. Inconsistent with their findings, the prevalence of episodes of nausea and number of vomits in this study was not different between the depressive and non-depressive women. This may mean that the incidence of nausea/d and vomits/d in early pregnancy does not appear to affect the CES-D score.

Women in early pregnancy did not consume adequate amounts to meet the nutrient requirements for energy intake, calcium, iron, folate, and vitamin D, which are similar to the findings of Mito *et al.* (*31*). Nearly 90% of subjects had a folate intake below the recommended 400 μg/d (data not shown). Furthermore, only 20% of pregnant women took vitamin supplements, which is a little higher than the 12% reported by Kondo *et al.* (*32*). Thus, we strongly feel that awareness of the importance of adequate folate intake has not improved in Japanese women since 1998, the year the guidelines on folate intakes (*33*) were issued.

Considerable research has reported that folate deficiency, low folate status, or high Hcy levels have been linked in clinic studies to depression and persistent depressive symptoms (*34-36*), especially in elderly people. The Rotterdam study of older men and women found that hyperhomocysteinemia, VB12 deficiency, and folate deficiency were related to depressive disorders (*37*). However, Penninx *et al.* (*38*) found no association between folate/Hcy and depression. In addition, Morris *et al.* (*14*) observed that serum Hcy was not found to be associated with depression diagnoses even in young populations. Consistent with their findings, our study found that all biomarkers including folate, Hcy, total protein, and albumin were no difference between non-depressive and depressive women in early pregnancy. We also found that the intake of folate and other vitamins such as B6 and B12 showed no significant differences between them. CES-D score was significantly correlated only with total protein and hemoglobin, but not with serum folate and plasma Hcy concentrations. The association of their deficiency with depression is still unclear.

A few researchers have examined their association with depression throughout pregnancy. In a study among 865 Japanese postpartum women by Miyake *et al.* (*39*), 14% of women had postpartum depression at two to nine months postpartum. They reported that no significant association was observed between the intake of folate and the risk of postpartum depression by using the Edinburgh Postnatal Depression Scale. Cho and colleagues (*40*) evaluated the effect of prenatal multivitamins containing folic acid on reducing the incidence of depression among 1,277 pregnant Korean women using Goldberg's depression scale. They noted that intake of multivitamins containing folic acid was not associated with lower rates of depression at either less than or greater than 20 weeks' gestation. Regarding the antenatal and postpartum periods, their findings do not suggest an association between low folate and vitamin intakes and depressive disorders.

The major limitation of our study was that we were not able to examine the risk factors for depressive episodes including history of depression, lack of partner, lack of social support, poverty, family violence, and increased life stress (*18,41*). In addition, the study subjects were few and not randomly selected. The study was conducted in only two clinics. Therefore, our findings may not be generalizable to other pregnant Japanese women. Further research is needed.

In conclusion, nearly 62% of women were found to have depression symptoms in the first trimester. CES-D score was significantly correlated only with total protein

and hemoglobin, but not with serum folate, plasma Hcy concentrations, or intake of folate, VB6, or VB12. Our results suggest that consumption of these may not be protective against early pregnancy depression. Almost none of the pregnant women in this study were meeting recommended folate intakes. Pregnancy can be an opportune time to improve nutrition, and presents an ideal time for health promotion activities. Health care providers should encourage all women of reproductive age to have a well-balanced diet behavior which leads to improved birth outcomes.

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