Original Article

Association between the serum folate levels and tea consumption during pregnancy

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Summary **Folate is a vital nutrient during pregnancy for the prevention of neural tube defects, intrauterine fetal growth restriction and preeclampsia. Circulating folate levels might be negatively affected by (–)-epigallocatechin gallate, which is a tea catechin found in green tea and oolong tea. The aim of this study was to determine whether consumption of green tea or oolong tea was associated with circulating folate levels among pregnant women in Japan. Two hundred and fifty-four healthy women with a singleton pregnancy (age: 30.4 ± 4.7, gestational age: 27.5 ± 9.6 weeks) were recruited from a prenatal clinic in metropolitan Tokyo, Japan. The serum folate levels were measured. Nutrient intake was assessed using a self-administered diet history questionnaire. Information on lifestyle variables was obtained from the questionnaire. The high consumption of green tea or oolong tea was defined as consumption more than 57.3 mL per 1,000 kcal, which is the 75th percentile of participants. The serum folate levels of the participants with high consumption of green tea or oolong tea was significantly lower than those of others ($p = 0.027$). A multiple regression analysis revealed the high consumption of green tea or oolong tea to be associated with a low serum folate level during pregnancy, after adjusting for confounding variables including dietary folate intake and use of folic acid supplements or multivitamins ($\beta = -0.131$, $p = 0.016$). The association between folate and the consumption of green tea or oolong tea may be useful to clarify the mechanism which links adverse perinatal outcomes and tea consumption.**

Keywords: Folate, catechin, tea, pregnancy

1. Introduction

Folate is a water-soluble B vitamin, which is absolutely essential for DNA synthesis, DNA repair and cell proliferation (*1*). Folate is required for the prevention of neural tube defects (NTDs), intrauterine fetal growth restriction (IUGR) and preeclampsia (*2-4*) before and throughout pregnancy. Therefore, the recommended intake of folate during pregnancy is 440 μg per day, which is almost twice as that of non-pregnant women (*5*). However, the average folate intake among Japanese pregnant women was about half of the recommended amount (*6-8*). In addition, the rate of pregnant women taking folic acid supplements was less than 30% (*6-8*), although the Ministry of Health, Labour and Welfare of Japan has recommended taking folic acid supplements for the prevention of NTDs since 2000 (*9*).The average serum folate levels of Japanese pregnant women were low in previous studies due to the decreased intake from diet or supplements (*6,8*), in comparison to that of pregnant women in other developed countries, where some foods are already fortified with folic acid, or supplements during pregnancy are in widespread use (*10-12*). Not only increasing folate intake but also giving up bad habits which interfere with the

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action of folate, such as smoking (*13*), is important for maximizing the effectiveness of the ingested folate, especially among such populations as that of Japanese pregnant women, who for the most part tend to consume insufficient amounts of folate.

Recently, (−)-epigallocatechin gallate (EGCG), which is a kind of tea catechin found in green tea and oolong tea (*14*), has been found to interfere in folate metabolism by inhibiting dihydrofolate reductase (DHFR) *in vitro* (*15,16*). DHFR is an important enzyme that reduces ingested folate to its active form, before folate is used in the body (*17*).

Most Japanese people consume green tea or oolong tea quite naturally at meals or with snacks. A previous study showed that about 50% of the Japanese pregnant women consumed green tea four or more times per day (*18*). Although the effect of EGCG on folate metabolism has been demonstrated *in vitro* (*15,16*), the relationship between folate status and daily consumption of tea among healthy pregnant or non-pregnant populations is unclear (*8,19,20*).

Therefore, the aim of this study was to determine whether consumption of green tea or oolong tea was associated with the circulating folate levels among pregnant women in Japan.

2. Materials and Methods

2.1. *Participants*

The present cross-sectional study was conducted at a private obstetric hospital from June to December of 2008 in metropolitan Tokyo, Japan. The study was approved by the Ethical Committee of the Graduate School of Medicine, at The University of Tokyo. Healthy women with a singleton pregnancy and no major complications such as diabetes or pregnancy-induced hypertension were recruited during their first trimester (8-12 weeks' gestation), their second trimester (24-27 weeks' gestation), or their third trimester (34-38 weeks' gestation) at a time when they were having routine blood tests. All the women underwent an ultrasound scan at 8-12 weeks' gestation to allow accurate gestational dating. The participants were given detailed information on the study protocol and all participants gave their written informed consent.

2.2. *Variables and their measurement*

Questionnaires were completed by each participant while waiting for a regular pregnancy checkup. Participants, who did not have sufficient time to complete the questionnaires at the hospital, filled out the questionnaires after returning home and then returned them by mail. All participants completed a questionnaire on their characteristics, including maternal age, gestational age, and pre-pregnancy body mass index (BMI). Information on lifestyle variables, such as smoking during pregnancy, the use of folic acid supplements or multivitamins including folic acid, and the frequency of supplement use (regular or irregular) was also obtained from the questionnaire. The regular use of folic acid supplements or multivitamins was defined as using such supplements four or more times per week.

The women's dietary intake over the last month was assessed with a validated self-administered diet history questionnaire (DHQ), which measures the daily intake of 150 foods and selected nutrients (*21,22*). Information on both the frequency and amount of consumption was collected. The folate intake and consumption of green tea or oolong tea were adjusted by energy to minimize the influence of dietary underreporting. Participants who had a severe under- or over-reported energy intake, namely, those whose reported energy intake was less than half the energy requirement for the lowest physical activity category, or those women whose reported energy intake was equal to or more than 1.5 times the energy requirement of moderate physical activity according to the "Dietary Reference Intakes for Japanese" (2005) were excluded from the present series (*5,23*).

Non-fasting blood samples were drawn at the clinic on the day of recruitment when the participants answered the questionnaires. Blood samples were centrifuged for 10 min at 3,000 rpm to separate the serum and then were stored at −80°C until the analyses were performed. The serum folate levels were measured using a chemiluminescent enzyme immunoassay (CLEIA). The assay was conducted by SRL, Inc., Tokyo, Japan.

2.3. *Statistical analysis*

The consumption of green tea or oolong tea was divided by the 75th percentile of participants into a high and low consumption group. The differences in characteristics between participants with high and low consumption of green tea or oolong tea were compared using the chi-square test, Student's *t*-test or the Mann-Whitney U test after normality was tested using the Shapiro-Wilk test.

A multiple regression analysis was used to determine whether consumption of green tea or oolong tea was associated with the serum folate levels. Dietary folate intake, the regular use of folic acid supplements or multivitamins, gestational age, pre-pregnancy BMI and smoking during pregnancy were adjusted as confounding variables. These variables were checked for multicollinearity. This multiple regression analysis was conducted after the logarithmic transformation of the serum folate levels because it showed skewed distribution.

Statistical analyses were carried out with the SPSS

software package for Windows, version 15.0 (SPSS Japan Inc.). All statistical tests were two-sided; a p value of less than 0.05 was considered to be statistically significant.

3. Results

A total of 321 pregnant women were recruited; of these, 292 (91.0%) gave their written informed consent, answered the questionnaire, and had blood samples drawn. Thirty-eight of the 292 participants were excluded from the analyses: 17 had missing data, 13 provided an inadequate amount of blood sample, 4 had pregnancy complications, and 4 had a severe under- or over-reported energy intake. The data from 254 pregnant women (79.1%) were analyzed; 38 were in the first trimester, 104 were in the second trimester and 112 were in the third trimester.

The characteristics of the participants are shown in Table 1. The 75th percentile of participants was used as the cutoff point for high consumption of green tea or oolong tea, 57.3 mL per 1,000 kcal. The cutoff point corresponded to consumption of 100-130 mL of tea per day.

Table 2 shows the median levels of serum folate, energy intake, folate intake and consumption of green tea or oolong tea, and the use of folic acid supplements or multivitamins. Some of the participants reported that they regularly consumed more than 1,000 mL of green tea or oolong tea per day. Serum folate levels among the participants with high consumption of green tea or oolong tea were significantly lower in comparison to others ($p = 0.027$). Meanwhile, the amount of black tea consumption, which contains only a small quantity of EGCG, was not associated with serum folate levels ($p = 0.704$).

There was a significant difference in the serum folate levels among each trimester (first trimester, 14.4 nmol/L; second trimester, 13.8 nmol/L; third trimester, 10.0 nmol/L; $p = 0.002$). No significant difference in the use of folic acid supplements or multivitamins was found among each trimester (first trimester, 28.9%; second trimester, 23.1%; third trimester, 16.1%; $p = 0.185$). In addition, there was no significant difference in the folate levels between smokers and non-smokers ($p = 0.184$).

Table 3 shows the relationship between the serum folate levels and the consumption of green tea or oolong tea. High consumption of green tea or oolong tea was significantly associated with a low serum folate level during pregnancy, after adjusting for confounding variables ($\beta = -0.131$, $p = 0.016$).

4. Discussion

This cross-sectional study found an association between low serum folate levels and high consumption of green tea or oolong tea during pregnancy, after various potential confounding variables, such as dietary folate intake and smoking during pregnancy, were taken into

Table 1. Characteristics of participants

Participants	All participants ($n = 254$)	Consumption of green tea or oolong tea < 57.3 mL/1,000 kcal ($n = 190$)	Consumption of green tea or oolong tea ≥ 57.3 mL/1,000 kcal[a] ($n = 64$)	p[b]
Age (years)	30.4 ± 4.7	30.5 ± 4.6	29.8 ± 5.0	ns
Gestational age				0.049
First trimester	38 (15)	25 (66)	13 (34)	
Second trimester	104 (41)	73 (70)	31 (30)	
Third trimester	112 (44)	92 (82)	20 (18)	
Height (cm)	157.9 ± 5.4	157.8 ± 5.4	158.3 ± 5.5	ns
Pre-pregnancy body mass index (kg/m^2)	20.8 ± 2.8	20.7 ± 2.6	21.2 ± 3.4	ns
Parity				ns
Primipara	136 (54)	104 (77)	32 (23)	
Multipara	118 (46)	86 (73)	32 (27)	
Smoking during pregnancy				ns
Smoker	8 (3)	7 (88)	1 (12)	
Non-smoker	246 (97)	183 (74)	63 (26)	
Education level				ns
High school and below	97 (38)	77 (79)	20 (21)	
Junior college/Technical college	106 (42)	73 (69)	33 (31)	
College/University	51 (20)	40 (78)	11 (22)	

Data are mean ± S.D. or n (%). ns: not significant. [a] The high consumption of green tea or oolong tea was defined as consumption more than 57.3 mL per 1,000 kcal, which is the 75th percentile of participants. [b] Student's t-test or the chi-square test was conducted.

Table 2. Biological marker, dietary intake and use of supplements

Items	All participants (n = 254)	Consumption of green tea or oolong tea < 57.3 mL/1,000 kcal (n = 190)	Consumption of green tea or oolong tea ≥ 57.3 mL/1,000 kcal[a] (n = 64)	p[b]
Biological marker				
Serum folate (nmol/L)	11.2 (8.6-19.5)	13.0 (8.8-20.9)	10.2 (8.2-14.4)	0.027
Dietary intake[c]				
Energy (kcal/day)	1,863 (1,614-2,135)	1,868 (1,615-2,142)	1,837 (1,601-2,109)	ns
Folate (μg/1,000 kcal)	110 (91-139)	108 (89-138)	111 (97-150)	ns
Consumption of green tea or oolong tea (mL/1,000 kcal)	11.5 (0.0-57.3)	0.0 (0.0-19.1)	138.8 (76.9-255.4)	0.017
Supplementation				
Regular use of folic acid supplements or multivitamins (more than 4 times/week)	53 (21)	41 (22)	12 (19)	ns

Data are median (interquartile range) or n (%). ns: not significant. [a] The high consumption of green tea or oolong tea was defined as consumption more than 57.3 mL per 1,000 kcal, which is the 75th percentile of participants. [b] The Mann-Whitney U test or the chi-square test was conducted. [c] The dietary intake was assessed with a validated self-administered diet history questionnaire (DHQ).

Table 3. Relationship between the serum folate levels and tea consumption during pregnancy

Items	All participants (n = 254)	
	β	p
Consumption of green tea or oolong tea (1: ≥ 57.3 mL/1,000 kcal, 0: < 57.3 mL/1,000 kcal)	−0.131	0.016
Dietary folate intake (μg/1,000 kcal)	0.110	0.043
Regular use of folic acid supplements or multivitamins (1: Yes, 0: No)	0.502	< 0.001
R^2	0.313	
Adjusted R^2	0.296	< 0.001

Multiple regression analysis was conducted, adjusting for gestational age, pre-pregnancy body mass index and smoking during pregnancy. The analysis was conducted after logarithmic transformation of the serum folate levels (nmol/L).

consideration.

Previous *in vitro* studies showed EGCG, which is an ester-bonded gallate catechin in green tea and oolong tea, to be an inhibitor of human DHFR activity (*15,16*). Folate drawn into the human body is absorbed from the small intestine. The absorbed folate is reduced by DHFR and changes into tetrahydrofolate (THF) (*17*). THF is methylated and changes into 5-methyl-THF (*17*). Folate is present in blood as 5-methyl-THF. Therefore, the inhibition of DHFR by EGCG decreases circulating folate levels. The current results might thus be explained by this mechanism.

Tea has been reported to have effects, such as anti-cancer, anti-oxidant effects, anti-inflammatory and promoting weight loss (*14,24-26*). This widely held positive belief may cause pregnant women to believe that tea is a healthy choice during pregnancy. In addition, advice by health care providers on tea consumption during pregnancy is inconsistent, even though teas have been generally acknowledged to contain caffeine that correlates with adverse pregnancy outcomes (*27*). Chu *et al*. showed that plasma EGCG levels in pregnant rats were about 1.5 times of those in non-pregnant rats when an equal amount of green tea was consumed (*28*). They have speculated that this difference may occur in association with changes in plasma protein composition and effective renal plasma flow. The folate status is likely affected by EGCG during pregnancy in comparison to non-pregnancy periods. No limits for the consumption of green tea or oolong tea can be established based on the results of the current study. However, health care providers might need to pay closer attention to the consumption of green tea or oolong tea in the future.

Correa *et al*. reported that maternal caffeinated tea consumption during the peri-conceptional period was associated with high rate of spina bifida, which is a kind of NTDs, although other caffeinated beverages were not associated with that risk (*29*). This result indicated that substance other than caffeine in tea, such as catechin, may affect the pathogenesis of NTDs. In the present study, consumption of green tea or oolong tea, which contains EGCG, was correlated with the level of serum folate, although black tea consumption, with little EGCG, did not correlate with serum folate levels. This result may suggest that substances in green tea or oolong tea, not black tea, are associated with folate status. Alternatively, because more than a half of the participants did not consume black tea, thus, the distribution of black tea consumption was more distorted than that of consumption of green tea or oolong tea, it might have been more difficult to show any statistical relationship between folate and black tea. Further studies are needed to investigate whether the circulating level of folate is affected differently by types of tea.

Folate has been identified as a nutrient required for fetal development, and prevention of NTDs and preeclampsia (*2-4*). In addition, folate plays a crucial role as an enzyme that prevents increasing homocysteine levels (*30-32*). A high tHcy level during

pregnancy is also a factor contributing to adverse perinatal outcomes, including NTDs, preterm birth, placental abruption, stillbirth and preeclampsia (*33-35*). Therefore, preventing low folate levels during pregnancy is important for both pregnant women and their fetuses. However, the folate intake among 75% of the current participants was far below 440 μg per day, which is the recommended intake for pregnant women (*5*). In addition, the rate of the participants taking folic acid supplements or multivitamins was only 21%, which was far lower than that other developed countries (*11,12,36*). Pregnant women need to be advised to take a sufficient amount of folate from dietary or take folic acid supplements in order to increase the circulating folate levels, as well as to cut down on excessive consumption of either green tea or oolong tea.

This study had several limitations. First, the number of participants was small, and this may have reduced the overall statistical power. Second, the effect of green tea or oolong tea on the serum folate levels could not be independently clarified, because the participants tea consumption was asked as a single question. Third, the questionnaire did not indicate the types of green tea or oolong tea and the brewing method, although the catechin content of tea varies depending on the types and the extraction time. Therefore, the results presented here should be examined by larger studies that include more detailed information on the types and brewing methods of various types of tea.

Despite these limitations, this study has useful clinical implications. A high consumption of green tea or oolong tea was associated with low serum folate levels among healthy pregnant women, after adjusting for several confounding variables. The association between folate and consumption of either green tea or oolong tea may therefore be useful for elucidating the mechanism which links tea consumption and adverse perinatal outcomes.

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