

Relation between Folate Levels of Maternal-Umbilical Cord Blood, Placenta Tissue and Pregnancy Outcomes

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ABSTRACT

The purpose of this study was to evaluate the folate nutritional status of Korean pregnant women and to investigate the relation between folate levels of maternal-umbilical cord blood, placenta tissue, and pregnancy outcomes. The study subjects consisted of 25 pregnant women who have had normal term deliveries. Dietary folate intakes of the pregnant women were estimated by semi quantitative frequency questionnaire and the serum and placenta tissue folate level was measured by microbiological analysis. The total folate intakes of the pregnant women was 655.6 μ g/d, which was 131.1% of the Korean RDA for pregnant women. Maternal serum folate level was 16.18ng/ml, which was significantly lower than that of umbilical cord blood (34.98ng/ml, $p < 0.05$). Mean folate concentration of the placental tissue was 998.0ng/ml, which was the highest compared to maternal and umbilical cord serum level. Umbilical cord serum folate level and placental tissue folate level were highly influenced by maternal serum folate level. The umbilical cord folate levels of the infant group whose birth weight was higher than 3500g were significantly higher than the group whose birth weight was less than 3500g ($p < 0.05$). The placental folate level was significantly higher in maternal group who showed desirable weight gain during pregnancy (11 - 14kg). In conclusion, the birth weight was related to the umbilical cord folate level and the maternal weight gain was affected by the placental folate level. (*J Community Nutrition* 6(2) : 91~96, 2004)

KEY WORDS : folate · maternal-umbilical cord blood · placenta · pregnancy outcome.

Introduction

Folate intake deficiency is a nutritional problem in pregnant women. It is the major cause of neurological damage in infants and could evoke hyperhomocysteinemia that results in abortion, premature birth, and increased birth rate of underweight babies (Scott et al. 1995). With the increasing importance of the relation between intake of folate and fecundity (Theresa et al. 1996 ; Barbara et al. 1995), the folate intake status of preconceptional young women (Lim et al. 2000 ; Kim et al. 1999), pregnant women, and breast-feeding women (Lim et al. 1999 ; Chang et al. 1993 ; Kang, Chang 1993) was evaluated in the late 1990s. The result showed a correlation between folate level in maternal blood during pregnancy and pregnancy outcomes. Since maternal nutritional status has an effect

on the growth and development of the fetus, it is necessary to comparatively analyze the nutrition level of placental tissue as well as the maternal and umbilical cord serum to explain the effect of maternal nutrition status on pregnancy outcomes and nutrient transportation mechanism. Baker et al. (1981) reported that folate level of the placenta was higher than that of maternal serum and the placental uptake of folate was determined by maternal folate nutritional status. However, folate level of placental tissue is not fully analyzed in any domestic and abroad studies and the folate transportation mechanism across the placenta was not accurately explained. Therefore the purpose of this study is to evaluate the folate nutrition status of Korean pregnant women and to investigate the correlation between folate levels of maternal and umbilical cord blood, and placenta tissue, and pregnancy outcome.

Subjects and Methods

1. Subjects

The subjects were 25 pregnant women who have had normal term from S obstetrics and gynecology in Seoul. Excluded

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from the study were patients with any metabolic disease and obstetrical disease. The baseline characteristics were reported in all subjects ; these included age, pregnancy weight and obtained blood pressure, hemoglobin concentration, hematocrit from medical examination. Pregnancy outcomes about maternal weight gain, gestational length, morning sickness, birth weight and Apgar score were obtained by medical examination and interview.

2. Dietary assessment

The maternal food-consumption surveys during pregnancy were conducted by trained interviewer using semiquantitative frequency questionnaire. Questionnaire were included high popularity among Koreans and seasonal food 100items and folate contents 90items. We referred the day of dietary standard which were used for the value of food exchange for diabetes. One serving size of the foods for eye measurement converted the intake. These food-consumption records were converted to nutrient intake using a computer-aided nutrient analysis program (CAN-pro by The Koran Nutrition Society). The folate intakes were evaluated on the basis of recommended dietary allowances (RDA) for the Korea pregnant. Folate intake was calculated from the folate content and supplements. Folate intake from supplements were obtained on the basis of type, application, intake period of supplements.

3. Sample preparation and analysis

Maternal blood samples were collected from brachial veins and umbilical cord blood samples were collected from placenta After blood samples left incubate 1hr room temperature. Serum samples were separated following centrifugation within 3000rpm for 15min after collection, transferred to polyethylene tubes and kept at -70°C until analyzed. Serum and placental tissue folate concentration were measured by microbiological method using *Lactobacillus casei* (ATCC 7469) (Leary, Sheehy 2001 ; Buehring et al. 1974 ; Waters, Mollin 1961) . Medium for folate analysis using Bacto folic acid casei.

4. Statistical analysis

All the data was analyzed using a SAS package program. Measurements were expressed as averages, standard deviations, and percentages. In all statistical testing performed, the null hypothesis was rejected at $p < 0.05$. Analysis of variance (ANOVA ; Turkey test) was used to test the folate concentration of placenta tissue and serum according to pregnancy outcomes.

Results and Discussion

1. General characteristics of subjects

Table 1 shows that the average age of pregnant women enrolled in the study was 30 years old and the average weight and height before pregnancy were 54kg and 160.6cm, respectively. BMI before pregnancy was 28.5, which is in normal range. The average systole and diastole blood pressure were 121.03mmHg and 77.45mmHg, respectively. Hemoglobin level and hematocrit value were 12.06g/dl and 36.06%, respectively, which were in relatively normal range compared to the iron nutritional criteria (Leary, Sheehy 2001) . All the subject took supplements and the average intake period was 16.27 weeks.

2. Folate intake

Table 2 presents the amount of calories and folate intakes of pregnant women in this study. The average amount of daily calorie intake was 2191.6 kcal, which is 93% of total recommended amount. Total daily folate intake was $655.6 \mu\text{g}$ of

Table 1. General characteristics of the subjects

Age (yrs)	30.00 \pm 2.4 ¹⁾
Height (cm)	160.60 \pm 4.3
Pre-pregnancy weigh (kg)	54.00 \pm 6.2
Pre-pregnancy BMI (kg/m ²)	20.85 \pm 2.1
Blood pressure (mmHg)	
Systolic BP	121.03 \pm 12.3
Diastolic BP	77.45 \pm 8.5
Hemoglobin (g/dl)	12.06 \pm 1.8
Hematocrit (%)	36.06 \pm 3.7
Nutrient supplements	25(100.00) ²⁾
Folate + iron	19(76)
Multivitamins	2(8)
Mineral and vitamins	4(16)
Duration of supplements (wks)	16.27 \pm 6.9

1) Mean \pm SD

2) Number of subjects (%)

Table 2. Intakes of energy and folate of the pregnant

	Intake	Range
Energy (kcal/d)	2191.63 \pm 569.7 ¹⁾	1347.7 – 3277.8
Food folate (μg /d)	171.62 \pm 97.7	28.6 – 437.9
Supplement folate (μg /d)	550.00 \pm 266.3	350.0 – 1050.0
Total folate (μg /d)	655.62 \pm 336.7	117.1 – 1240.6

1) Mean \pm SD

which 171.6 μ g is from food and 550.0 μ g from supplements. The amount is 131% of recommended amount. Even though folate intake from food was lower (24 – 31% of Korean recommended amount), all pregnant women took sufficient folate by taking supplements. The amount of folate intake in our study was higher than the results of a 24-hour retrospective study in which the amount of folate intake of pregnant women in Kwang-ju province was 580.6 μ g (Lim et al. 1999) and 326.9 μ g (Kang, Chang 1993) in Chon-an province. Not only were there differences in the subjects enrolled in each study but there was also the possibility that measuring the frequency of food intake could have over-measured (Hunter et al. 1988) the amount of nutritional intake compared to the 24-hour recall method or actual measurement method. The amount of folate intake only from food in subjects enrolled in this study was higher than those in subjects in Kwangju (Lim et al. 1999) and Chonan (Kang, Chang 1993) provinces (137 μ g and 139.8 μ g, respectively) but was lower than 185.7 μ g presented by Lim et al. (1999) The amount of folate intake from supplements in this study was lower than 612.5 μ g in a report by Kang et al. (1993) but higher than 406.7 μ g reported by Lim et al. (1999) and 480.5 μ g reported Ahn et al. (2000) The amount of folate intake from food in the study on pregnant women in United Kingdom (Anderson, Lean 1986) ranged from 143 to 184 μ g while that of European women was 247 μ g (Bree et al. 1997), a little higher than that of Korean pregnant women.

3. Folate level in maternal-umbilical cord serum and placental tissues

Table 3 shows folate level of maternal and umbilical cord serum and placental tissue at the end of pregnancy. The average folate level was 16.18ng/ml in maternal serum, 34.98ng/ml in umbilical cord serum, and 998.00ng/g in placental tissue. The folate level of umbilical cord serum is two times higher than that of maternal serum. The folate level of placental tissue was significantly higher than those of maternal and umbi-

Table 3. Mean folate level in maternal, umbilical cord and placenta

	Folate level	
	Mean \pm SD	Range
Maternal (ng/ml)	16.18 \pm 8.08 ^a	3.4 – 39.8
Umbilical cord (ng/ml)	34.98 \pm 11.46 ^b	12.0 – 63.6
Placenta (ng/g)	998.00 \pm 176.08 ^c	680.24 – 1306.03

abc : Means with superscript not sharing the same letter are significantly different with Duncan's multiple range test ($p < 0.05$)

lical cord serum.

The folate level in this study is similar to 16.3ng/ml (Lim et al. 1999) in the microbiologic study of pregnant women in Kwangju province and higher than 5.62ng/ml (Kang, Chang 1993) of maternal serum in pregnant women in Chonan province. Folate level of maternal serum of pregnant women in Netherlands was reported to be 4.02ng/ml (Bartels et al. 1989) Folate levels of the study by Economides et al. (1992) and other study on pregnant women in Boston areas (Huber et al. 1988) were 9.8ng/ml and 10.0ng/ml, respectively, which are relatively lower than that of this study. Butte et al. (1981) reported 17.6ng/ml, similar to the result of this study. Folate level of umbilical cord of newborn baby in the study by Lim et al. (1999) was 36.3ng/ml, similar to the result of this study and relatively higher than 18.6ng/ml (Huber et al. 1988) of the US study that analyzed folate levels of both maternal and umbilical cord serum. This microbiologic study by using *Lactobacillus casei* had different folate level from those of RIA (radioimmunoassay) studies by Ahn et al. (2000) (folate level of maternal serum : 6.1ng/ml and folate level of umbilical cord : 14.2ng/ml) and other RIA study on English pregnant women (Economides et al. 1992) (folate serum of maternal serum : 9.8ng/ml and folate level of umbilical cord : 15.3ng/ml). Baker et al. (1981) calculated the folate level of umbilical cord by the concentration level per dried weight and reported that the average folate level of umbilical cord was 54.5pg/g. The results of this study were similar to those of the study by Ahn et al. (2000), who measured the folate levels of both maternal and umbilical cord to evaluate the effect of nutrition status of pregnant women on nutritional status of their fetus, and the study on English pregnant women (Economides et al. 1992), which had 2 – 2.4times higher folate level of umbilical cord serum than that of maternal serum. To detect the correlation of maternal serum, placental tissue, and umbilical cord serum, we divided the folate levels of maternal serum into three groups (below 11ng/ml, between 11 – 20ng/ml, and over 20ng/ml). Table 4 shows the comparison of folate levels of maternal and umbilical cord serum. Both the placental tissue and umbilical cord serum folate levels were the highest when the maternal serum folate level was higher than 20ng/ml.

According to the result of a foreign study that analyzed the nutritional levels of umbilical cord, transportation of soluble vitamins through umbilical cord has an essential effect on the nutritional status of fetus. The study proposed that folate, one

Table 4. Relationship between maternal serum folate level and umbilical cord serum level and placenta folate level

	Placenta folate (ng/g)	Umbilical cord serum folate (ng/ml)
Maternal serum folate (ng/ml)		
< 11 (n = 6)	808.91 ± 122.24 ^{1)a}	30.00 ± 14.19 ^a
≤ 11 < 20 (n = 11)	980.96 ± 114.99 ^{ab}	31.05 ± 7.37 ^a
≥ 20 (n = 8)	1163.25 ± 120.13 ^b	44.14 ± 9.35 ^b
	p-value	0.0001
		0.02

1) Mean ± SD

ab : Means with superscript not sharing the same letter are significantly different with Duncan's multiple range test (p < 0.05)

Table 5. Pregnancy outcomes of the subjects

Maternal	
Gestational age (wks)	39.00 ± 1.15 ¹⁾
Weight gain (kg)	11.85 ± 3.91
Morning sickness	
Yes	16 (64) ²⁾
No	9 (36)
Anemia	
Hb ≤ 11g/dl, Hct ≤ 33%	5 (20)
Hb > 11g/dl, Hct > 33%	20 (80)
Newborn	
Sex	
Boy	10 (40)
Girl	15 (60)
Birth weight (g)	3318.24 ± 381.21
Apgar score	
1min	8.72 ± 0.45
5min	9.72 ± 0.53

1) Mean ± SD

2) Number of subjects (%)

of soluble vitamin, was sufficiently accumulated to the certain amount in the umbilical cord, and then, is transported to the fetus serum (Tamura et al. 1992 ; Baker et al. 1981). Folate level of umbilical cord changes on the basis of maternal nutrition status. However, even though maternal folate level was low, folate level of fetus is always higher than that of maternal serum. This implies that the umbilical cord might accumulate a certain amount of folate from maternal body and then transport it to the fetus serum.

4. Relationships between pregnancy outcomes and folate level in maternal-umbilical cord serum and placental tissues

Table 5 shows the gestational age, weight gain during pregnancy, existence of morning sickness and anemia, sex and birth weight of newborn babies, and Apgar score. The average gestational age was 39 weeks, desirable period ; weight gain was 11.8 kg, which is in reasonable range of 11.5 – 16.0 kg (Institute of Medicine 1990) of the women with normal pre-pregnancy BMI. Sixty-four percent (16 of 25) pregnant wo-

men experienced morning sickness. Apgar score of 1 and 5 minutes after birth were 8.72 and 9.72, respectively, implying that the newborn babies were in good condition.

Table 6 shows the correlation between maternal and umbilical cord serum, placental tissue, and pregnancy outcomes. The gestational age was divided into three groups (less than 38 weeks, between 38 – 40 weeks, and more than 40 weeks) to compare the folate levels of maternal and umbilical cord and placental tissue of three groups. There was no significant difference between the folate levels of three different gestational age.

When the correlation between maternal weight increase during pregnancy and folate level was compared, there was no significant difference between pregnancy weight increase (less than 11 kg increase : 8 women, between 11 – 14 kg increase : 12 women, and more than 14 kg : 5 women) and folate level of umbilical cord. However, folate of placental tissue has significantly higher when the desirable weight gain during pregnancy was between 11 – 14 kg (p < 0.05). While pregnant women with normal maternal weight gain between 11 – 14 kg had higher folate level of umbilical cord serum, those who failed to keep the desirable weight gain range had lower folate level of umbilical cord serum. When maternal weight increase was much higher, folate levels of both maternal and umbilical cord serum tended to decrease. When birth weight of newborn babies was divided into three groups (less than 3200g, between 3200 – 3500g, and more than 3500g) to compare the folate levels of both serum and umbilical cord serum, there was a significant correlation between birth weight and folate level of umbilical cord (p < 0.05). The babies with much higher birth weight had higher folate level of umbilical cord serum. There was no significant correlation between the existence of morning sickness and anemia, and Apgar score correlation between folate level of umbilical cord serum and birth weight of newborn baby was reported (Lim et al. 1999). A study in 1990 on Western women reported that good maternal nutrition status had some good aspects such as increase in Apgar score of newborn babies and decrease of maternal

Table 6. Relationship between pregnancy outcomes and serum in maternal and umbilical cord blood and placenta folate level

		Serum folate (ng/ml)		Placenta folate (ng/g)
		Maternal	Umbilical cord	
Gestational age (wks)	< 38 (n = 3)	11.97 ± 4.08 ¹⁾	34.53 ± 3.65	816.10 ± 122.29
	38 ≤ χ < 40 (n = 19)	16.24 ± 8.92	34.06 ± 2.55	1010.88 ± 169.60
	≥ 40 (n = 3)	20.00 ± 1.83	41.27 ± 8.74	1098.31 ± 178.63
	p-value	0.49	0.62	0.12
Weight gain (kg)	< 11 (n = 8)	14.85 ± 7.60	29.93 ± 12.55	940.96 ± 133.06 ^a
	11 ≤ χ < 14 (n = 12)	19.36 ± 7.99	39.96 ± 10.07	1095.16 ± 164.70 ^b
	≥ 14 (n = 5)	10.68 ± 6.60	31.14 ± 9.44	856.09 ± 143.04 ^c
	p-value	0.07	0.11	0.01
Birth weight (g)	< 3200 (n = 8)	11.35 ± 6.09	27.20 ± 9.96 ^a	958.08 ± 189.99
	3200 ≤ χ < 3500 (n = 11)	19.80 ± 8.68	33.82 ± 12.31 ^c	1040.98 ± 182.56
	≥ 3500 (n = 6)	15.98 ± 6.68	40.17 ± 6.42 ^b	972.44 ± 155.67
	p-value	0.07	0.04	0.57
Morning sickness	Yes (n = 16)	16.78 ± 6.08	34.58 ± 10.31	1017.17 ± 132.79
	No (n = 9)	15.12 ± 11.17	35.71 ± 13.91	963.93 ± 240.66
	p-value	0.63	0.82	0.48
Anemia	Hb ≤ 11g/dl, Hct ≤ 33% (n = 4)	11.65 ± 4.45	37.10 ± 8.34	858.64 ± 131.15
	Hb > 11g/dl, Hct > 33% (n = 21)	17.04 ± 8.40	34.58 ± 12.09	1024.55 ± 173.21
	p-value	0.23	0.70	0.08
Apgar score (1min)	≤ 8 (n = 7)	17.37 ± 11.77	37.94 ± 13.16	989.52 ± 197.35
	> 8 (n = 18)	15.72 ± 6.52	33.83 ± 10.93	1001.30 ± 173.16
	p-value	0.66	0.43	0.88

1) Mean ± SD

ab : Means with superscript not sharing the same letter are significantly different with Duncan's multiple range test ($p < 0.05$)

infection (Tamura et al. 1992 ; Iyengar, Rajalakshmi 1975). Other report on correlation between maternal serum folate level and pregnancy outcomes presented higher folate level brought about good effects such as higher birth weight of newborn babies and increase of Apgar score (Scholl, Johnson 2000). There were some reports that the supplement of folate decreased the rate of low birth weight babies in the areas (Iyengar, Rajalakshmi 1975) where they had deficient folate intake rate and higher rate of low birth weight babies. There were other reports that when the folate intake of mother rats increased, the birth weight their baby rats increased significantly (Heid 1992). However, the effects of slight or medium folate deficiency of pregnant women on pregnancy outcomes were different in each report.

Summary and Conclusion

The folate intake and folate levels of maternal and umbilical cord serum, and placental tissue of 25 women who had pre-delivery care as well as normal delivery in S General Hospital in Seoul were measured by using microbiologic method in this study. Correlation of folate levels of maternal and umbilical cord serum, and placental tissue was investigated and the

effect of nutritional status of maternal and umbilical cord serum and placental tissue on pregnancy outcomes like health conditions of newborn babies, gestational age, and maternal weight gain during pregnancy were investigated. The average amount of daily calorie intake of subjects in this study was 2191.6kcal, 93.2% of RDA. Folate intake from food and supplements was 655.6 μ g, much higher than RDA. The average maternal serum folate level was 16.18ng/ml. Folate level of umbilical cord serum was 34.98ng/ml, two times of maternal folate level. The average folate level of placental tissue was 998.00ng/g. Maternal folate level has a positive correlation with the levels of umbilical cord serum and placental tissue ($p < 0.001$ & $p < 0.05$). The placental folate levels of maternal group whose weight gain was 11 – 14kg were significantly high ($p < 0.01$). The umbilical cord folate levels of the group whose birth weight was higher than 3500g were significantly high ($p < 0.05$). According to the folate intake and serum folate level, folate nutritional status of the subjects was relatively favorable.

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