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Circulating folate levels and colorectal adenoma: a case-control study and a meta-analysis

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BACKGROUND/OBJECTIVES: The relationship between folate and colorectal neoplasia remains controversial. We examined the association between serum folate concentrations and colorectal adenomas in a case-control study of Korean adults and conducted a meta-analysis.
SUBJECTS/METHODS: Our case-control study included 113 pairs of case and control who underwent colonoscopy and provided blood samples. We used multivariable conditional logistic regression models to obtain the odds ratios and 95% confidence interval (CIs). For meta-analysis, we identified the relevant studies by searching the PubMed database up to February 2017, included our case-control study and combined the study-specific relative risks (RRs) using a random-effects model.
RESULTS: In this case-control study, we included 58 men and 55 women with colorectal adenomas and sex and fasting status matched the controls. We did not find any significant association between the serum folate levels and colorectal adenomas in either men or women. For meta-analysis, a total of eleven studies were included in our analysis and classified into two groups; polyp clearance group (PC) for the studies that included participants who underwent endoscopies and had their polyps removed at baseline; and no polyp clearance group (NPC) for the studies that included participants whose histories of endoscopies were unknown or who underwent their first endoscopies. Four PC (1,311 cases and 1,672 non-cases) and eight NPC studies (3,501 cases and 11,347 non-cases) were included. The combined RRs (95% CIs) comparing the bottom with the top categories of circulating folate levels were 1.07 (0.97-1.18) for the NPC group but 1.45 (1.16-1.74) for the PC group.
CONCLUSIONS: Low circulating folate levels were associated with new adenoma formation.

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INTRODUCTION

Colorectal cancer is the third most common cancer worldwide [1] as well as in Korea [2]. The incidence of colorectal cancer dramatically increased by 4.6% annually from 1999 to 2013 in Korea [2] partly due to the shift towards a Western lifestyle and diet such as high intake of meat, low intake of vegetables, or sedentary physical activity.

Fruits and vegetables are rich sources of micronutrients including folate and have been actively investigated as food groups that exert preventive effects against the development of colorectal cancer. Furthermore, folate has gained attention as a primary mediator of the anti-cancer effects of fruits and vegetables. Folate is one of the vitamins in the vitamin B series and is known for its role in deoxyribonucleic acid (DNA) methylation, synthesis and replication [3]. Folate may be a key epigenetic regulator because of its primary role as a methyl donor in one-carbon metabolism [4], which controls the transport of the one-carbon moiety (methyl group) to biological methylation reactions.

Given that colorectal adenomas are precursor lesions of colorectal cancer [5] and may share common etiological factors with colorectal cancer, it has been hypothesized that nutrients related to one-carbon metabolism may be involved in colorectal adenoma development. Several studies have examined the associations of folate intake [6-10] and circulating folate levels [11,12] with colorectal cancer and colorectal adenomas. In the pooled analyses of 13 prospective cohort studies, the dietary folate intake from food was not associated with colon cancer, while the dietary folate intake from food and supplements was associated with a 15% decreased risk of colon cancer [10]. A meta-analysis of intervention trial studies on folic acid supplementation and colorectal adenoma recurrence [13] and nested case-control studies on circulating folate levels and

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colorectal cancer [11] did not observe significant associations. However, the recent study on folate suggested that the timing of folate interventions relative to colorectal carcinogenesis may be important [14]. A pooled analysis of two observational studies suggested that folate has a role only in the early stage, not in the late stage of carcinogenesis [8].

Based on trials in which the participants had polyps removed and were subsequently followed, the development of recurrent adenomas may indicate the early occurrence of colorectal neoplasia within the sequence of colorectal carcinogenesis [15,16]. We hypothesized that folate may be inversely associated with newly developed colorectal adenomas once they are removed, but the association become less clear for advanced adenomas. Therefore, we categorized the studies by the histories of polyp clearance, including our case-control study, into two groups and conducted a meta-analysis of the circulating folate levels and colorectal adenomas. The two groups consisted of polyp clearance group (PC) and no polyp clearance group (NPC); PC was composed of studies that included participants who had undergone endoscopy and had polyps removed prior to the baseline, and NPC was composed of studies that included participants whose histories of endoscopy were unknown or patients who underwent their first endoscopies.

The aim was to examine 1) the association between circulating folate levels and colorectal adenomas in a matched case-control study in Korean adults and 2) whether the association between circulating folate levels and colorectal adenomas varied according to the clearance of previous polyps in a meta-analysis.

SUBJECTS AND METHODS

Case-control study

Study population

The study participants included 382 men and women aged 45-71 years who underwent a colonoscopy from August 2011 to September 2012 and provided blood samples at a university hospital in Daegu, Korea. We excluded participants who had any type of cancer (n=14). We matched the controls (n=113) with cases (n=113) by sex and fasting status. Written informed consent was obtained from all the participants. This study was approved by the Institutional Review Board of Daegu Catholic University Medical Center (No. CR-11-069-RES-003-R).

Ascertainment of colorectal adenomas

The subtype, size, and number of colorectal adenomas were determined by colonoscopy and histological examination. The colorectal polyps were classified into adenomatous, hyperplastic, and other nonadenomatous polyps. The adenomatous polyps were included as cases. A total of 113 (58 men and 55 women) adenoma cases were identified.

Measurement of the serum folate levels

Blood samples were collected from each participant between January and February 2013. The samples were centrifuged and sent on ice to the Neodin Medical Institute (Seoul, South Korea). The serum folate concentrations of the participants were measured with an electrochemiluminescence immunoassay at the Neodin Medical Institute. All laboratory technicians were blinded to the case status. The intra-assay coefficient of variation was 3.7-5.4%.

Information about potential risk factors for colorectal adenomas

The participants were asked about their sociodemographic characteristics, medical conditions, family histories of colorectal cancer, and lifestyles. Dietary information was assessed with a validated food frequency questionnaire [17]. The heights and weights of the participants were measured with the X-scan Plus II Professional (Jawon Medical, Gyeongsan, South Korea), and body mass index (BMI; kg/m^2) was calculated by dividing the weight in kilograms by the square of the height in meters.

Statistical analysis

The distributions of the characteristics were compared between the case group and control group with the Mantel-Haenszel test and paired t-test. The means and standard deviations were calculated for continuous variables, and the frequencies and percentages were calculated for categorical variables.

To examine the associations between the serum folate levels and the prevalence of colorectal adenomas, we calculated the odds ratios (ORs) and two-sided 95% confidence interval (CIs) using multivariable conditional logistic regression models. Cases were categorized according to the tertiles based on the distribution among the controls. In the multivariable models, we adjusted for age (continuous; years), BMI (continuous; kg/m^2), family history of colorectal cancer (yes, no), history of colorectal polyps (yes, no), total energy intake (continuous; kcal/d), education level (less than or equal to elementary school graduate, middle school graduate, high school graduate, more than or equal to college graduate), marital status (spouse, spouseless), frequency of red meat intake (less than or equal to once per month, 2-4 times per month, more than or equal to 2 times per week), ethanol intake (0, 0-10, ≥10 g/d in men; 0, 0-5, ≥5 g/d in women), and smoking (0, 0-15, ≥15 cigarettes/d in men; never smoker, ever smoker in women). To test for trends, the participants were assigned the median value of their tertile, and this variable was used as a continuous term in the model. We also examined whether the associations between the serum folate concentrations and colorectal adenoma prevalence differed according to age (<60, ≥60 years), BMI (<25, ≥25 kg/m^2), ethanol intake (0, 0-10, ≥10 g/d), smoking status (never smoker, ever smoker), and frequency of red meat intake (less than or equal to once per month, 2-4 times per month, more than or equal to 2 times per week). We used the likelihood ratio test to examine the null hypothesis that there were no interactions by potential risk factors. All statistical analyses were done with SAS, version 9.3 (SAS Institute, Inc., Cary, NC, USA). Two-sided *P*-values of <0.05 were considered statistically significant.

Meta-analysis

Identification and selection of studies for the meta-analysis of circulating folate and colorectal adenomas

We performed a PubMed search for epidemiological studies that examined the association between circulating folate levels and colorectal adenomas for the period until February 28, 2017.

We used the following search term "((((((plasma folate) OR serum folate) OR red blood cell folate) OR erythrocyte folate) OR whole blood folate) AND colorectal adenoma". Among the total of fifty-nine extracted articles, we included the articles that met the following criteria: (1) the exposure of interest was circulating folate level, including plasma folate, serum folate, red blood cell folate, and whole blood folate; (2) the outcome of interest was either an initial or recurrent colorectal adenoma; (3) the association between circulating folate levels and colorectal adenomas was presented with either the risk ratio (RR) or OR estimates and the 95% CIs or we were able to calculate the OR or RR and 95% CI; and (4) the articles were human studies and were published in English. Two authors (Youn J and Lee JE) independently assessed the eligibility criteria. The circulating folate levels were measured at baseline or in cross-sectional or case-control design settings. Regarding the randomized double-bind clinical trial studies of supplements [12,18-20], if the supplement in the intervention group contained folic acid, we used the RRs and 95% CIs for colorectal adenoma recurrence only in the placebo group [18] or calculated the crude RRs and 95% CIs using the number of cases and non-cases in the placebo group [19,20]. One study combined a wheat bran fiber trial and ursodeoxycholic acid trial but reported the levels of folate at baseline; therefore, we included the RRs and 95% CIs of all the participants according to the reported baseline folate levels [12]. We did not include one randomized double-blind clinical trial of a folic acid supplement that did not report the estimates or numbers of cases and participants among the placebo group [21]. When the same data were reported in more than one study [22,23], we included the study with the greater number of participants [22]. When the results were presented alone [24] or in a pooled analysis of two datasets [12], we included the estimates from the pooled analysis [12]. For three studies, we calculated the crude ORs or RRs and 95% CIs using the numbers of cases and non-cases [19,20,25]. The following data were extracted from the selected articles: the first author, published year, country, study design, sex, the number of cases and controls or total, age at blood draw, specimen type, endpoint, type of endoscopy, the ORs or RRs and 95% CIs according to circulating folate level category, and adjusted covariates. We performed this meta-analysis according to the Meta-analysis of Observational Studies in Epidemiology guidelines [26].

Statistical analysis

We combined the ORs or RRs and 95% CIs in a random effects model developed by DerSimonian and Laird [27] and tested for heterogeneity using Q and I^2 statistics [28]. Weight was allotted to the individual studies based on the inverse proportion of their variances.

To construct dose-response models, we estimated the ORs or RRs per 10 nmol/L increase in circulating folate level using generalized least squares [29] or a variance-weighted least squares [30]. If units of ng/mL were used, we converted those values to nmol/L (1 ng/mL = 2.265 nmol/L). We used the median of each circulating folate level category and assumed that the level had the same amplitude as the neighboring categories if the category was open-ended. We did not include studies that did not report circulating folate levels in each category or that compared only two circulating folate level categories [29], which resulted in the inclusion of six studies for the dose-response models.

We conducted a meta-regression analysis to investigate whether the association between circulating folate levels and colorectal adenomas differed according to the PC or NPC groups, sex, geographic region (Europe and Americas, Asia), specimen type (plasma, serum), study design (observational study, clinical trial with observational data analysis), age at blood draw (mean or median <60, ≥60 years), and folic acid fortification at blood draw (yes, no). To assess the publication bias, we conducted an Egger's asymmetry test [31]. All statistical analyses were done with STATA11 (Stata Corp., College Station, TX, USA). Two-sided *P*-values of <0.05 were considered statistically significant.

RESULTS

Case-control study

Table 1 presents a comparison of the general characteristics between the case group and control group. The cases were more likely to drink alcohol compared with the colorectal adenoma-free controls. However, there were no statistically significant differences in the other variables between the case group and control group. The mean levels of circulating folate were 8.2 ng/mL among the men and 9.9 ng/mL among the women in our study. The minimum level of circulating folate was 3.1 ng/mL, in other words, there were no participants who were folate deficient (<3 ng/mL) in this population.

Table 1. General characteristics of participants according to colorectal adenoma status

	Case (n=113)	Control (n=113)	*P*-value[1)]
Age (yrs, mean ± SD)[2)]	60.29 ± 5.28	59.83 ± 5.40	0.52
Sex, n (%)			(Matched)
Men	58 (51.33)	58 (51.33)	
Women	55 (48.67)	55 (48.67)	
Education level, n (%)[3)]			0.42
Less than or equal to elementary school graduate	16 (14.16)	12 (10.81)	
Middle school graduate	25 (22.12)	37 (33.33)	
High school graduate	51 (45.13)	42 (37.84)	
More than or equal to college graduate	21 (18.58)	20 (18.02)	
Marital status, n (%)[3)]			0.44
Spouseless	6 (5.36)	9 (8.04)	

Table 1. continued

	Case (n=113)	Control (n=113)	*P*-value[1]
Spouse	106 (94.64)	103 (91.96)	
Family history of colorectal cancer, n (%)			0.32
Yes	6 (5.31)	3 (2.65)	
No	107 (94.69)	110 (97.35)	
History of colorectal polyps, n (%)			0.56
Yes	6 (5.31)	8 (7.08)	
No	107 (94.69)	105 (92.92)	
Frequency of red meat intake, n (%)			0.87
Less than or equal to once per month	12 (10.62)	12 (10.62)	
2-4 times per month	80 (70.80)	83 (73.45)	
More than or equal to 2 times per week	21 (18.58)	18 (15.93)	
Aspirin use, n (%)			0.25
Yes	7 (6.19)	12 (10.62)	
No	106 (93.81)	101 (89.38)	
Supplement use, n (%)[3]			0.12
Yes	43 (38.39)	56 (50.91)	
No	69 (61.61)	54 (49.09)	
Energy intake (kcal/day, mean ± SD)	1728.7 ± 718.1	1646.1 ± 499.5	0.31
BMI (kg/m^2, mean ± SD)	24.53 ± 2.66	24.16 ± 2.43	0.23
Smoking, n (%)			0.15
Never smoker	63 (55.75)	73 (64.60)	
Former smoker	31 (27.43)	28 (24.78)	
Current smoker	19 (16.81)	12 (10.62)	
Alcohol intake, n (%)			0.009
Never drinker	41 (36.28)	60 (53.10)	
Former drinker	8 (7.08)	4 (3.54)	
Current drinker	64 (56.64)	49 (43.36)	

BMI, body mass index; SD, standard deviation.
[1] *P*-values were obtained by using paired t-test for continuous variables or Mantel-Haenszel test for categorical variables.
[2] Mean ± SD for continuous variables and numbers (percentage) for categorical variables
[3] The total number of participants in each category were not equal to the total number of participants because some participants did not provide the relevant information.

Table 2. Odds ratios and 95% confidence intervals for colorectal adenoma according to serum folate levels

	Serum folate levels			*P* for trend
	Tertile 1	Tertile 2	Tertile 3	
Total				
Median levels (ng/mL)[1]	6.20	9.05	12.70	
No. of cases/controls	51/38	30/36	32/39	
OR (95% CI)[2]	1.00	0.63 (0.25-1.62)	0.66 (0.25-1.70)	0.10
OR (95% CI)[3]	1.00	0.82 (0.21-3.22)	1.20 (0.32-4.46)	0.92
Men				
Median levels (ng/mL)	5.50	8.00	11.55	
No. of cases/controls	28/19	17/20	13/19	
OR (95% CI)[2]	1.00	0.55 (0.23-1.31)	0.47 (0.19-1.17)	0.13
OR (95% CI)[3]	1.00	0.77 (0.18-3.33)	0.89 (0.19-4.13)	0.98
Women				
Median levels (ng/mL)	6.90	10.30	13.00	
No. of cases/controls	23/18	15/18	17/19	
OR (95% CI)[2]	1.00	0.63 (0.25-1.62)	0.66 (0.25-1.70)	0.36
OR (95% CI)[3]	1.00	0.82 (0.21-3.22)	1.20 (0.32-4.46)	0.78

OR, odds ratio; CI, confidence interval.
[1] None of the study participants were deficient (< 3 ng/mL) in circulating folate levels.
[2] Models were adjusted for age (continuous, years).
[3] Multivariable models were adjusted for age (continuous, years), body mass index (continuous, kg/m^2), family history of colorectal cancer (yes, no), history of colorectal polyps (yes, no), total energy intake (continuous, kcal per day), education level (less than or equal to elementary graduate, middle school graduate, high school graduate, more than or equal to college graduate), marital status (spouse, spouseless), frequency of red meat intake (less than or equal to once per month, 2-4 times per month, more than or equal to 2 times per week), ethanol intake (grams per day; 0, 0-10, ≥10 in men; 0, 0-5, ≥5 in women), and smoking (cigarettes per day; 0, 0-15, ≥15 in men; never smoker, ever smoker in women)

Table 3. Included studies of circulating levels of folate and colorectal adenoma

Author (year)	Group	Country	Study design	Sex	Number of cases/controls or total[1)]	Age at blood draw (yrs)	Endpoint	Type of endoscopy	Specimen type	Circulating folate category	Relative risks (95% CIs)	Adjusted variables
de Vogel S *et al.* (2011) [32]	NPC (unknown history of endoscopy)	Norway	Cross-sectional	C	High-risk adenomas 421/10,601[1)]	57.2, mean	First adenoma or recurrent adenoma	Sigmoidoscopy or colonoscopy	Serum	Q1: < 10.10 nmol/L Q2: 10.10-< 13.74 nmol/L Q3: 13.74-< 20.33 nmol/L Q4: ≥ 20.33 nmol/L	ORs 1.00 1.00 (0.76-1.32) 0.98 (0.74-1.30) 1.04 (0.78-1.39) *P* for trend=0.82	Age, sex, study center, smoking habits, and alcohol consumption
					Low-risk adenomas 1,380/10,601[1)]	56.7, mean				Q1: < 10.10 nmol/L Q2: 10.10-< 13.74 nmol/L Q3: 13.74-< 20.33 nmol/L Q4: ≥ 20.33 nmol/L	ORs 1.00 1.01 (0.86-1.19) 1.05 (0.89-1.23) 0.94 (0.79-1.11) *P* for trend=0.60	
Fujimori S *et al.* (2011) [33]	NPC (only first time endoscopy)	Japan	Cross-sectional	M	183/258[1)]	58.2 for cases, 56.2 for controls, mean	First adenoma	Colonoscopy	Serum	< 8 ng/mL ≥ 8 ng/mL	ORs 1.00 0.49 (0.32-0.66)	-
				W	104/200[1)]	59.2 for cases, 56.9 for controls, mean				< 8 ng/mL ≥ 8 ng/mL	ORs 1.00 0.77 (0.50-1.05)	
Ding H *et al.* (2016) [34]	NPC (only first time endoscopy)	China	Cross-sectional	C	422/888[1)]	58.8 for cases, 56.8 for controls, mean	First adenoma	Colonoscopy	Serum	≤ 4.55 ng/mL > 4.55 ng/mL	ORs 1.00 0.99 (0.92-1.07)	Age, BMI, family history of colorectal disease, and history of gastroenterology drug use
	PC (baseline polyps resection)		Cohort	C	87/175[1)]	62.2 for cases, 60.8 for controls, mean	Recurrent adenoma			≤ 4.55 ng/mL > 4.55 ng/mL	RRs 1.00 0.75 (0.32-1.74)	
Bird C L *et al.* (1995) [22]	NPC (only first time endoscopy)	USA	Case-control	C	332/350	50-75, range	First adenoma	Sigmoidoscopy	Red blood cell	Q1: < 165 ng/mL Q2: 165-228 ng/mL Q3: 229-314 ng/mL Q4: 315+ ng/mL	ORs 1.00 0.85 (0.54-1.33) 0.69 (0.43-1.08) 0.77 (0.49-1.21) *P* for trend=0.15	Age, sex, date of sigmoidoscopy, and study center
					332/339				Plasma	Q1: 3 ng/mL Q2: 5.7 ng/mL Q3: 9.5 ng/mL Q4: 16.9 ng/mL	ORs 1.00 0.96 (0.92-1.01) 0.91 (0.81-1.07) 0.74 (0.51-1.06) *P* for trend=0.10	Age, sex, date of sigmoidoscopy, study center, smoking, alcohol, calories, dietary fiber, and fat
Marugame T *et al.* (2003) [25]	NPC (only first time endoscopy)	Japan	Case-control	M	177/192	47-55, range	First adenoma or recurrent adenoma	Colonoscopy	Plasma	≤ 5.5 ng/mL > 5.5 ng/mL	ORs[2)] 1.00 0.71 (0.47-1.09)	-
Le Marchand L *et al.* (2011) [35]	NPC (only first time endoscopy)	USA (Caucasian, American, Japanese, Native Hawaiian)	Case-control	C	241/280	66 for cases, 65 for controls, median	First adenoma	Sigmoidoscopy	Plasma	Median 6.7 ng/mL 11.2 ng/mL 17.5 ng/mL	RRs 1.00 1.10 (0.70-1.74) 0.81 (0.49-1.34) *P* for trend=0.35	Age, sex, race/ethnicity, screening center, BMI, pack-years of smoking, intakes of alcohol, daily energy intake, life time hours of physical activity, plasma B_6, and plasma B_{12}

Table 3. continued

Author (year)	Group	Country	Study design	Sex	Number of cases/controls or total[1)]	Age at blood draw (yrs)	Endpoint	Type of endoscopy	Specimen type	Circulating folate category	Relative risks (95% CIs)	Adjusted variables
Martinez M E *et al.* (2006) [12]	PC (baseline polyps resection)	USA	Clinical trial	C	965/2,125[1)]	65.3 for WBF, 66.2 for UDCA, mean	Recurrent adenoma	Colonoscopy	Plasma	Q1: < 7.20 nmol/L Q2: 7.20-11.21 nmol/L Q3: 11.22-15.38 nmol/L Q4: > 15.38 nmol/L	RRs 1.00 0.83 (0.64-1.09) 0.85 (0.65-1.12) 0.74 (0.56-0.98) *P* for trend < 0.01	Age, sex, number of colonoscopies, and the year of randomization
Figueiredo J C *et al.* (2008) [18]	PC (baseline polyps resection)	USA Canada	Clinical trial	C	205/484[1)]	57.4, mean	Recurrent adenoma	Colonoscopy	Red blood cell	Q1: 64.9-338.0 ng/mL Q2: 339.0-449.0 ng/mL Q3: 450.0-1,133.0 ng/mL	RRs 1.00 0.88 (0.68-1.14) 0.84 (0.63-1.11) *P* for trend=0.21	Age, sex, center, duration of follow-up, aspirin treatment group, and multivitamin use
					187/446[1)]				Plasma	Q1: 2.4-13.6 nmol/L Q2: 13.6-26.7 nmol/L Q3: 26.7-159.9 nmol/L	RRs 1.00 0.86 (0.66-1.12) 0.72 (0.54-0.97) *P* for trend=0.03	
Wu K *et al.* (2009) [20]	PC (baseline polyps resection)	USA	Clinical trial	C	72/237[1)]	65.4, mean	Recurrent adenoma	Sigmoidoscopy (2%) or colonoscopy	Plasma	Q1: ≤ 7.5 ng/mL Q2: > 7.5 ng/mL	RRs[3)] 1.00 0.54 (0.31-0.95)	-
Song Y *et al.* (2012) [19]	NPC (unknown history of endoscopy)	USA	Clinical trial	W	128/725[1)]	61.8, mean	First adenoma or recurrent adenoma	Colonoscopy	Plasma	Q1: ≤ 10.6 ng/mL Q2: > 10.6 ng/mL	RRs[3)] 1.00 0.97 (0.71-1.31)	-
Our study	NPC (mostly first time endoscopy)	Korea	Case-control	M	58/58	60.29 for case, 59.83 for control, mean	First adenoma or recurrent adenoma (4.89%)	Colonoscopy	Serum	Median Q1: 5.50 ng/mL Q2: 8.00 ng/mL Q3: 11.55 ng/mL	ORs 1.00 0.77 (0.18-3.33) 0.89 (0.19-4.13) *P* for trend=0.94	Age, fasting status, BMI, smoking habit, ethanol intake, family history of colorectal cancer, history of colorectal polyps, total energy intake, education level, marital status, and frequency of red meat intake.
				W	55/55					Median Q1: 6.90 ng/mL Q2: 10.30 ng/mL Q3: 13.00 ng/mL	ORs 1.00 0.82 (0.21-3.22) 1.20 (0.32-4.46) *P* for trend=0.75	

NPC, no polyp clearance; PC, polyp clearance; OR, odds ratio; RR, risk ratio; C, men and women; M, men; W, women; Q, quantile; BMI, body mass index; UDCA, ursodeoxycholic acid trial; WBF, wheat bran fiber trial.

[1)] The number of cases/total participants

[2)] Crude odds ratio was calculated.

[3)] Crude risk ratio was calculated.

Table 2 shows the associations with the serum folate levels and colorectal adenomas for men and women. We found no significant associations in men or women. In the age-adjusted model, the ORs (95% CIs) for the highest tertile compared with the lowest tertile were 0.47 (0.19-1.17; *P* for trend=0.13) in men and 0.66 (0.25-1.70; *P* for trend=0.36) in women. In the multivariable model with adjustments for additional potential confounding factors, the ORs (95% CIs) for the highest tertile compared with the lowest tertile were 0.89 (0.19-4.13; *P* for trend=0.98) for men and 1.20 (0.32-4.46; *P* for trend=0.78) for women. When we examined whether the associations were modified by age, BMI, daily ethanol intake, smoking status, or the frequency of red meat intake, there were no significant interactions according to these factors (data not shown; *P* for the interactions ≥0.22).

Meta-analysis

A total of eleven articles reporting 4,812 colorectal adenomas and 13,019 non-cases were included in the meta-analysis (Table 3). Of the fifty-nine identified articles, twenty-four articles did not examine the association between circulating folate levels and colorectal adenomas; two articles were reported in non-English languages, and thirteen articles were not human studies. Out of twenty articles that examined the association between circulating folate levels and colorectal adenomas, ten articles were excluded because of the absence of estimates (n=7) and data overlap (n=3), and our study was included (Fig. 1). Consequently, three cross-sectional [32-34], three case-control [22,25,35], and four clinical trial studies [12,18-20] plus our study were included in the present meta-analysis. Among these four trial studies, the major endpoint of three of the trials was recurrent colorectal adenomas [12,18,20]. Folic acid was the treatment reagent for three trials [18-20]. Three trials included participants who underwent colonoscopies or sigmoidoscopies and had their adenomatous polyps removed [12,18,20], and the other study followed the participants without polyp removal [19].

We observed a statistically significant inverse association between circulating folate levels and colorectal adenomas in the meta-analysis (Fig. 2). The combined RR (95% CI) of the comparison of the bottom and top categories of the circulating folate levels was 1.15 (95% CI: 1.03-1.27). When we separated the PC and NPC groups, the RRs (95% CIs) when comparing the bottom and top categories of the circulating folate levels were 1.07 (0.97-1.18) and 1.45 (1.16-1.74) for the NPC and PC groups, respectively. We found that the estimates were different between the NPC and PC groups (*P* for difference = 0.06). When we examined the publication bias, *P* values for the publication bias were 0.097 and 0.988 for the NPC and PC groups, respectively.

When we examined the dose-response relationship in the

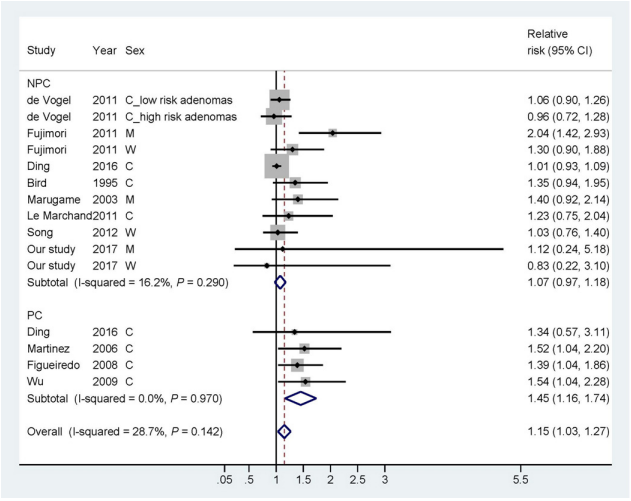

Fig. 2. Forest plot for bottom versus top categories of circulating folate levels in relation to colorectal adenoma by PC or NPC groups. NPC indicated no polyp clearance group and PC indicated polyp clearance group. M, W, and C represented men, women, and combined sex, respectively. The black circles indicate the study specific relative risks; the horizontal lines indicate the 95% confidence intervals. The gray squares represent the study specific weights, which are inverse of the variance. The dash line indicates the overall combined RR and the diamonds indicate the 95% CIs for the combined RRs. P for difference between NPC and PC groups was 0.06.

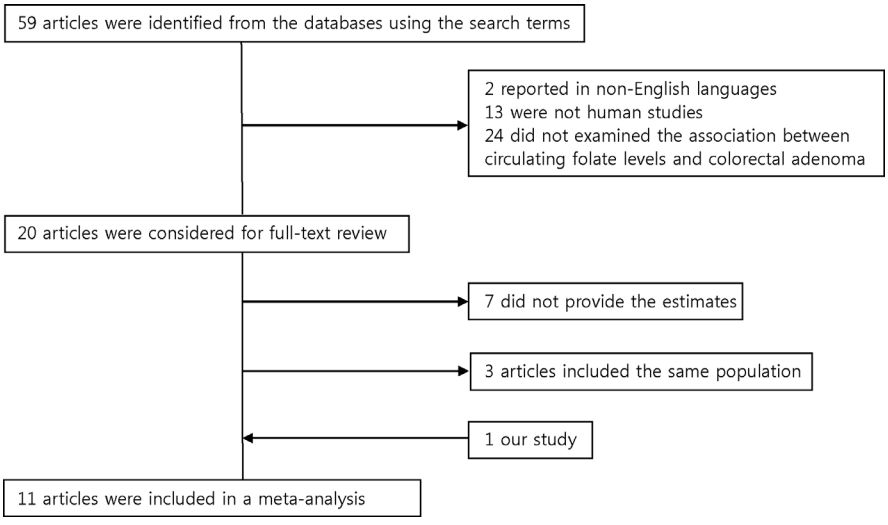

Fig. 1. Flow chart of publication selection for the meta-analysis of the association between circulating folate levels and colorectal adenoma

Table 4. Combined relative risk (RR)s and 95% confidence interval (CI)s for bottom versus top categories of circulating folate levels in relation to colorectal adenoma according to sex, geographic region of study, specimen type, study design, age at blood draw, and folic acid fortification at blood draw

	Number of studies	RR (95% CI)	*P* for difference[1)]
Sex			0.02
Men[2)]	3 [25,33]	1.63 (1.17-2.10)	
Women[2)]	3 [19,33]	1.10 (0.84-1.36)	
Geographic region of study			0.70
Europe and Americas	7 [12,22,32,35]	1.14 (1.01-1.28)	
Asia[2)]	4 [25,33,34]	1.26 (0.96-1.56)	
Specimen type			0.10
Plasma	7 [12,18-20,22,25,35]	1.28 (1.10-1.46)	
Serum[2)]	4 [32-34]	1.06 (0.94-1.18)	
Study design			0.35
Observational study[2)]	7 [22,25,32-35]	1.09 (0.98-1.20)	
Clinical trial with observational data analysis	4 [12,18-20]	1.29 (1.03-1.55)	
Age (mean or median) at blood draw			0.44
<60 yrs[2)]	6 [18,25,32-34]	1.12 (0,98-1.26)	
≥60 yrs	6 [12,19,20,22,34,35]	1.24 (1.03-1.45)	
Folic acid fortification at blood draw[3),4)]			0.95
Yes	3 [12,20,35]	1.14 (0.69-1.59)	
No[2)]	9 [18,19,22,25,32-35]	1.09 (0.99-1.20)	

RR, relative risk; CI, confidence interval.
[1)] A meta-regression analysis was used to estimate *P* value for difference.
[2)] Our study was included.
[3)] We included each of odds ratios of ursodeoxycholic acid trial and wheat bran fiber trial in Martinez study according to folic acid fortification at blood draw.
[4)] We categorized studies into two groups (yes, no) based on the presence of mandatory folic acid fortification legislation.

meta-analysis of six studies [12,18,22,32,35] including our data, we also observed a statistically significant inverse association between the circulating folate levels and colorectal adenomas. Based on a 10 nmol/L increase in the circulating folate levels, the combined RRs (95% CIs) for colorectal adenomas were 0.94 (0.91-0.98) for all, 0.95 (0.89-1.01) for the NPC group, and 0.94 (0.89-0.98) for the PC group (Supplementary Fig. S1).

We conducted a meta-regression to examine whether the association between the circulating folate levels and colorectal adenomas differed according to potential interactions (Table 4). Regarding the sex-specific association, we included four studies, including ours [19,25,33], and we observed a more pronounced association among men than among women. The associations did not vary according to the other factors.

DISCUSSION

We found that low circulating folate levels were associated with new colorectal adenoma formation. A higher colorectal adenoma occurrence was observed in those whose polyps were removed (PC group), but the association was not significant in the NPC group. Overall, low circulating folate levels were associated with a higher prevalence or occurrence of adenomas in the meta-analysis of 11 epidemiologic studies. To our knowledge, only a few epidemiologic studies have investigated the association between folate and colorectal cancer in Korea [36], and no Korean studies have examined the association of colorectal adenomas with circulating folate levels. Although we found no association in a case-control study of Korean adults, our meta-analysis supports the potential protection of folate against the new development of colorectal adenomas.

The circulating folate levels increased with increasing intake of folate from food and supplements. A recent review of 17 articles suggested that the correlation was stronger when folate from supplement use was included than when only dietary folate from foods was considered [37]. A recent meta-analysis of eight nested case-control studies found no associations between circulating folate levels and colorectal cancer [11]. A combined study of three large intervention trials suggested that folic acid supplementation may reduce colorectal adenoma recurrence among those with lower folate circulating levels but did not find any overall effect of folic acid supplementation on colorectal adenoma recurrence [7].

Evidence from experimental and epidemiological studies conducted in the 1990s and early 2000s suggests that folate deficiency might lead to carcinogenesis possibly by the misincorporation of uracil into DNA [38], decreased DNA methylation [39], and impaired DNA repair processes [40]. However, recent studies have provided evidence indicating that the effect of folate on colorectal carcinogenesis might not be this simple. A large randomized clinical trial involving the intake of 1 mg of folic acid per day reported an increase in the number of advanced colorectal adenomas [41]. The results regarding the relation between circulating folate levels and colorectal neoplasia are not consistent [12,42-46]. Some studies have found positive associations between circulating folate levels and colorectal cancer [46], whereas other studies have found inverse [47] or no associations [7,42,45,48]. These inconsistent findings across studies may reflect heterogeneities between the studies including differences in the baseline folate levels, which could

partially be due to folic acid fortification and supplement use, study design, and influences from confounding factors.

The findings from our meta-analysis suggest the potential benefit of folic acid for colorectal adenomas. We observed a stronger association when we combined the studies that included participants who had undergone endoscopies and had their adenomatous polyps removed at baseline. After the clearance of polyps at baseline, we observed a greater recurrence with low folate levels, which may support the hypothesis that early intervention with folate is beneficial in the prevention of colorectal carcinogenesis. Although folic acid supplementation increased or did not affect the recurrence in the trials [20,41,49], the low baseline folate levels in the non-supplementation group resulted in a higher rate of colorectal adenoma recurrence in our meta-analysis of three studies [12,18,20]. The pooled analysis of the three trials of folic acid supplementation suggested a potential interaction according to the baseline folate status, i.e., a possible benefit for those with low folate levels and a lack of effect from folate supplementation in those with high folate levels [7]. A recent pooled analysis of the Nurses' Health Study and the Health Professionals Follow-Up Study found that folate intake 12-16 years before colorectal cancer diagnosis was inversely associated with colorectal cancer risk, but intake in the recent past was not associated with colorectal cancer risk, suggesting the importance of timing of folate intake in relation to colorectal carcinogenesis [8].

In our case-control study of Korean adults, we did not find a statistically significant association between the serum folate levels and the prevalence of colorectal adenomas. The possible explanations for our findings include the small sample size, the small variance in the folate levels, a potential reverse causation and the potentially minimal influence of the folate levels compared with other risk factors such as alcohol consumption and smoking. Among men, we observed a suggestive inverse association in the age-adjusted model, but this association was attenuated after adjusting for confounding factors. Due to the high prevalence of smoking and alcohol consumption among Korean men, it is possible that the effect of folate by itself might not have been sufficiently large to detect in our study.

In conclusion, we observed that low folate levels were associated with a higher prevalence or occurrence of colorectal adenomas in a meta-analysis of eleven epidemiologic studies, and this association was apparent in a meta-analysis of studies in which participants were followed after polyp removal at baseline. Our study suggests that the adverse effect of low folate may be relevant to the early stage of colorectal cancer.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interests.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7-30.
2. Oh C-M, Won Y-J, Jung K-W, Kong H-J, Cho H, Lee J-K, Lee DH, Lee KH. Cancer Statistics in Korea: Incidence, Mortality, Survival, and Prevalence in 2013. Cancer Res Treat 2016;48:436-50.
3. Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer 2003;3:601-14.
4. Park LK, Friso S, Choi SW. Nutritional influences on epigenetics and age-related disease. Proc Nutr Soc 2012;71:75-83.
5. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525-32.
6. Boyapati SM, Bostick RM, McGlynn KA, Fina MF, Roufail WM, Geisinger KR, Hebert JR, Coker A, Wargovich M. Folate intake, MTHFR C677T polymorphism, alcohol consumption, and risk for sporadic colorectal adenoma (United States). Cancer Causes Control 2004;15:493-501.
7. Figueiredo JC, Mott LA, Giovannucci E, Wu K, Cole B, Grainge MJ, Logan RF, Baron JA. Folic acid and prevention of colorectal adenomas: a combined analysis of randomized clinical trials. Int J Cancer 2011;129:192-203.
8. Lee JE, Willett WC, Fuchs CS, Smith-Warner SA, Wu K, Ma J, Giovannucci E. Folate intake and risk of colorectal cancer and adenoma: modification by time. Am J Clin Nutr 2011;93:817-25.
9. Sanjoaquin MA, Allen N, Couto E, Roddam AW, Key TJ. Folate intake and colorectal cancer risk: a meta-analytical approach. Int J Cancer 2005;113:825-8.
10. Kim DH, Smith-Warner SA, Spiegelman D, Yaun SS, Colditz GA, Freudenheim JL, Giovannucci E, Goldbohm RA, Graham S, Harnack L, Jacobs EJ, Leitzmann M, Mannisto S, Miller AB, Potter JD, Rohan TE, Schatzkin A, Speizer FE, Stevens VL, Stolzenberg-Solomon R, Terry P, Toniolo P, Weijenberg MP, Willett WC, Wolk A, Zeleniuch-Jacquotte A, Hunter DJ. Pooled analyses of 13 prospective cohort studies on folate intake and colon cancer. Cancer Causes Control 2010;21:1919-30.
11. Chuang SC, Rota M, Gunter MJ, Zeleniuch-Jacquotte A, Eussen SJ, Vollset SE, Ueland PM, Norat T, Ziegler RG, Vineis P. Quantifying the dose-response relationship between circulating folate concentrations and colorectal cancer in cohort studies: a meta-analysis based on a flexible meta-regression model. Am J Epidemiol 2013;178:1028-37.
12. Martinez ME, Giovannucci E, Jiang R, Henning SM, Jacobs ET, Thompson P, Smith-Warner SA, Alberts DS. Folate fortification, plasma folate, homocysteine and colorectal adenoma recurrence. Int J Cancer 2006;119:1440-6.
13. Ibrahim EM, Zekri JM. Folic acid supplementation for the prevention of recurrence of colorectal adenomas: metaanalysis of interventional trials. Med Oncol 2010;27:915-8.
14. Ulrich CM, Potter JD. Folate and cancer--timing is everything. JAMA 2007;297:2408-9.
15. Peipins LA, Sandler RS. Epidemiology of colorectal adenomas. Epidemiol Rev 1994;16:273-97.
16. Neugut AI, Jacobson JS, De Vivo I. Epidemiology of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 1993;2:159-76.
17. Ahn Y, Kwon E, Shim JE, Park MK, Joo Y, Kimm K, Park C, Kim DH. Validation and reproducibility of food frequency questionnaire for Korean genome epidemiologic study. Eur J Clin Nutr 2007;61:1435-41.
18. Figueiredo JC, Levine AJ, Grau MV, Barry EL, Ueland PM, Ahnen DJ, Byers T, Bresalier RS, Summers RW, Bond J, McKeown-Eyssen

GE, Sandler RS, Haile RW, Baron JA. Colorectal adenomas in a randomized folate trial: the role of baseline dietary and circulating folate levels. Cancer Epidemiol Biomarkers Prev 2008;17:2625-31.
19. Song Y, Manson JE, Lee IM, Cook NR, Paul L, Selhub J, Giovannucci E, Zhang SM. Effect of combined folic acid, vitamin B(6), and vitamin B(12) on colorectal adenoma. J Natl Cancer Inst 2012;104:1562-75.
20. Wu K, Platz EA, Willett WC, Fuchs CS, Selhub J, Rosner BA, Hunter DJ, Giovannucci E. A randomized trial on folic acid supplementation and risk of recurrent colorectal adenoma. Am J Clin Nutr 2009;90: 1623-31.
21. Gao QY, Chen HM, Chen YX, Wang YC, Wang ZH, Tang JT, Ge ZZ, Chen XY, Sheng JQ, Fang DC, Yu CG, Zheng P, Fang JY. Folic acid prevents the initial occurrence of sporadic colorectal adenoma in Chinese older than 50 years of age: a randomized clinical trial. Cancer Prev Res 2013;6:744-52.
22. Bird CL, Swendseid ME, Witte JS, Shikany JM, Hunt IF, Frankl HD, Lee ER, Longnecker MP, Haile RW. Red cell and plasma folate, folate consumption, and the risk of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 1995;4:709-14.
23. Levine AJ, Siegmund KD, Ervin CM, Diep A, Lee ER, Frankl HD, Haile RW. The methylenetetrahydrofolate reductase 677C-->T polymorphism and distal colorectal adenoma risk. Cancer Epidemiol Biomarkers Prev 2000;9:657-63.
24. Martinez ME, Henning SM, Alberts DS. Folate and colorectal neoplasia: relation between plasma and dietary markers of folate and adenoma recurrence. Am J Clin Nutr 2004;79:691-7.
25. Marugame T, Tsuji E, Kiyohara C, Eguchi H, Oda T, Shinchi K, Kono S. Relation of plasma folate and methylenetetrahydrofolate reductase C677T polymorphism to colorectal adenomas. Int J Epidemiol 2003;32:64-6.
26. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA 2000;283:2008-12.
27. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
28. Higgins J, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-58.
29. Orsini N, Bellocco R, Greenland S. Generalized least squares for trend estimation of summarized dose-response data. Stata J 2006; 6:40.
30. Grizzle JE, Starmer CF, Koch GG. Analysis of categorical data by linear models. Biometrics 1969;25:489-504.
31. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
32. de Vogel S, Schneede J, Ueland PM, Vollset SE, Meyer K, Fredriksen A, Midttun O, Bjorge T, Kampman E, Bretthauer M, Hoff G. Biomarkers related to one-carbon metabolism as potential risk factors for distal colorectal adenomas. Cancer Epidemiol Biomarkers Prev 2011;20:1726-35.
33. Fujimori S, Gudis K, Takahashi Y, Kotoyori M, Tatsuguchi A, Ohaki Y, Sakamoto C. Determination of the minimal essential serum folate concentration for reduced risk of colorectal adenoma. Clin Nutr 2011;30:653-8.
34. Ding H, Gao QY, Chen HM, Fang JY. People with low serum folate levels have higher risk of colorectal adenoma/advanced colorectal adenoma occurrence and recurrence in China. J Int Med Res 2016;44:767-78.
35. Le Marchand L, Wang H, Selhub J, Vogt TM, Yokochi L, Decker R. Association of plasma vitamin B6 with risk of colorectal adenoma in a multiethnic case-control study. Cancer Causes Control 2011; 22:929-36.
36. Kim J, Cho YA, Kim DH, Lee BH, Hwang DY, Jeong J, Lee HJ, Matsuo K, Tajima K, Ahn YO. Dietary intake of folate and alcohol, MTHFR C677T polymorphism, and colorectal cancer risk in Korea. Am J Clin Nutr 2012;95:405-12.
37. Park JY, Vollset SE, Melse-Boonstra A, Chajes V, Ueland PM, Slimani N. Dietary intake and biological measurement of folate: a qualitative review of validation studies. Mol Nutr Food Res 2013;57:562-81.
38. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, Wickramasinghe SN, Everson RB, Ames BN. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A 1997;94:3290-5.
39. Duthie SJ, Narayanan S, Blum S, Pirie L, Brand GM. Folate deficiency in vitro induces uracil misincorporation and DNA hypomethylation and inhibits DNA excision repair in immortalized normal human colon epithelial cells. Nutr Cancer 2000;37:245-51.
40. Choi SW, Kim YI, Weitzel JN, Mason JB. Folate depletion impairs DNA excision repair in the colon of the rat. Gut 1998;43:93-9.
41. Cole BF, Baron JA, Sandler RS, Haile RW, Ahnen DJ, Bresalier RS, McKeown-Eyssen G, Summers RW, Rothstein RI, Burke CA, Snover DC, Church TR, Allen JI, Robertson DJ, Beck GJ, Bond JH, Byers T, Mandel JS, Mott LA, Pearson LH, Barry EL, Rees JR, Marcon N, Saibil F, Ueland PM, Greenberg ER, Polyp Prevention Study G. Folic acid for the prevention of colorectal adenomas: a randomized clinical trial. JAMA 2007;297:2351-9.
42. Eussen SJ, Vollset SE, Igland J, Meyer K, Fredriksen A, Ueland PM, Jenab M, Slimani N, Boffetta P, Overvad K, Tjonneland A, Olsen A, Clavel-Chapelon F, Boutron-Ruault MC, Morois S, Weikert C, Pischon T, Linseisen J, Kaaks R, Trichopoulou A, Zilis D, Katsoulis M, Palli D, Berrino F, Vineis P, Tumino R, Panico S, Peeters PH, Bueno-de-Mesquita HB, van Duijnhoven FJ, Gram IT, Skeie G, Lund E, Gonzalez CA, Martinez C, Dorronsoro M, Ardanaz E, Navarro C, Rodriguez L, Van Guelpen B, Palmqvist R, Manjer J, Ericson U, Bingham S, Khaw KT, Norat T, Riboli E. Plasma folate, related genetic variants, and colorectal cancer risk in EPIC. Cancer Epidemiol Biomarkers Prev 2010;19:1328-40.
43. Le Marchand L, White KK, Nomura AM, Wilkens LR, Selhub JS, Tiirikainen M, Goodman MT, Murphy SP, Henderson BE, Kolonel LN. Plasma levels of B vitamins and colorectal cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev 2009;18:2195-201.
44. Lee JE, Wei EK, Fuchs CS, Hunter DJ, Lee IM, Selhub J, Stampfer MJ, Willett WC, Ma J, Giovannucci E. Plasma folate, methylenetetrahydrofolate reductase (MTHFR), and colorectal cancer risk in three large nested case-control studies. Cancer Causes Control 2012;23:537-45.
45. Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S, Japan Public Health Center-based Prospective Study G. Plasma folate and risk of colorectal cancer in a nested case-control study: the Japan Public Health Center-based prospective study. Cancer Causes Control 2008;19:67-74.
46. Van Guelpen B, Hultdin J, Johansson I, Hallmans G, Stenling R, Riboli E, Winkvist A, Palmqvist R. Low folate levels may protect against colorectal cancer. Gut 2006;55:1461-6.

47. Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Akhmedkhanov A, Zeleniuch-Jacquotte A, Riboli E. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. Br J Cancer 1999;79:1917-22.
48. Weinstein SJ, Albanes D, Selhub J, Graubard B, Lim U, Taylor PR, Virtamo J, Stolzenberg-Solomon R. One-carbon metabolism biomarkers and risk of colon and rectal cancers. Cancer Epidemiol Biomarkers Prev 2008;17:3233-40.
49. Logan RF, Grainge MJ, Shepherd VC, Armitage NC, Muir KR. Aspirin and folic acid for the prevention of recurrent colorectal adenomas. Gastroenterology 2008;134:29-38.