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Folate and Cancer: Is There Any Association?

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Abstract

Folate plays a central role in DNA synthesis and methylation, which are essential for DNA integrity maintenance and gene expression. Folate deficiency may lead to the incorporation of uracil into DNA and chromosome breaks, increasing the risk of cancer. However, conflicting evidence has been observed depending on the type of epidemiological study, dietary or circulating folate, and the type of cancer. New concern has arisen after a mandatory folic acid (FA) fortification program adopted for the prevention of neural tube defects in the United States, which suggested an increase in the incidence of colorectal cancer. In the present article, folate status and cancer are under review, and a discussion of the challenge of assessing folate status through food intake, supplement use, FA fortification programs, circulating folate, and the interaction of diet and the polymorphism of the enzyme involved in folate metabolism will be presented.

Keywords

folate, folic acid, unmetabolized folic acid, cancer, dysplasia

Introduction

In the 1980s, Doll and Peto evidenced for the first time that many common types of cancer were avoidable. They guesstimated that 35% of all cancer deaths among Americans were attributable to environmental and lifestyle factors such as tobacco smoking and diet. These epidemiologists estimated that dietary change could potentially avoid 10% to 70% of cancer.[1] Many food groups, nutrients, and dietary patterns have been investigated since then including folate. Folate is the generic term used for a group of water-soluble B9 vitamin essential for humans and naturally available from leafy vegetables, offals (eg, liver and kidney), yeast, and legumes or synthetic folic acid (FA) from a supplement or food fortification.[2]

Folic acid (pteroylmonoglutamic acid) is a fully oxidized folate, with chemical structure consisted of pteridine, p-aminobenzoic acid, and glutamic acid. Food folates contain additional glutamate residues, making them polyglutamates. When consumed, food folates are hydrolyzed to the monoglutamate form in the gut prior to absorption by active transport across the intestinal mucosa. Passive diffusion occurs when pharmacological doses of FA are consumed. The monoglutamate form is reduced to tetrahydrofolate (THF) and converted to methyl or formyl forms. The circulating folate form is 5-methyl-THF. Unaltered FA can also be found in the blood, known as unmetabolized folic acid (UFA).[3]

Folate plays a central role in DNA synthesis and methylation, which are essential for DNA integrity maintenance and gene expression, and also required for amino acid metabolism, including the remethylation of homocysteine to methionine.[4] During pregnancy, extra folate is required for nucleic acid synthesis, and dietary intake recommendation is increased to prevent neural tube defects. The recommended intake is difficult for many women to achieve through diet alone, and vitamin supplement is necessary. However, the use of folate supplement before and during pregnancy has been an unsuccessful public health program since many women do not notice gestation in the first month when the neural tube closes completely.[5] Therefore, in 1998, the United States and Canada established the mandatory FA fortification program. This program has been effective in increasing FA intakes, blood levels of folate, and reducing prevalence of neural tube defects. But,

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concerns of excessive intake of FA were raised: (1) the risk of nerve damage and anemia among vitamin B12-deficient persons, like elderly population and (2) an increase in the incidence of CRC suggested after mandatory FA fortification program in the United States.[6]

In the present review of the literature, results from a meta-analysis conducted since 2013 searched in the MEDLINE, PubMed, and Cochrane Library using the following terms: ("folic acid" OR "folate") AND ("cancer") AND ("meta-analysis" OR "systematic review") in well-designed studies with the best evidence, such as randomized clinical trial (RCT) and cohort studies, and in case of their absence, case–control studies will be presented.

Challenges in Folate Assessment

Dietary Folate Assessment

Natural folate is found in leafy green vegetables (especially in broccoli and Brussels sprouts), yeast, poultry and meat (especially in liver and kidney), dairy products, and legumes, including beans. Folic acid (pteroylmonoglutamate) is added to dietary supplements and to fortified foods.[2]

In the case of folate intake, availability from several types of sources should be considered. Folic acid taken as a supplement has an availability of 100%, being rapidly absorbed across the intestine.[7] However, in its naturally occurring form, it has an absorption rate of no more than 50% due to the lack of stability during food storage and preparation. Fortified food has an intermediate availability of 85%. For these reasons, the average daily total folate intake or dietary folate equivalents are used to account for the difference in the bioavailability of naturally occurring food folate and FA.[7]

In epidemiology, dietary intake of folate can be assessed by participant reporting foods and beverages eaten in the (1) previous 24 hours, (2) recording during the meal time to minimize reliance on memories, or (3) using a questionnaire composed of food lists and answer about frequency and amount of intake during the previous weeks, months, or years.[8] These methods are used in epidemiology to estimate individual daily nutrient intake and to classify individuals according to ranges of intake (ie, higher or lower than median, tertile, or quartile).

Circulating Folate Assessment

Folate can be measured in serum or red blood cells. The latter reflects long-term folate status, as folate enters the red blood cells only during their development in the bone marrow, reflecting the average folate status over a life span of around 120 days.[9] Serum folate reflects both long-term and recent folate intake. Due to the high variability of diet and seasonality, serum folate should be measured repeatedly. Lack of specificity occurs when assessing folate status using serum or red blood cells, since it depends on other B vitamins and polymorphism in genes that encode folate-dependent enzymes.[9,10] Combination of measurements of serum or erythrocyte concentrations and the homocysteine concentration in tissue or plasma, which is an indicator of metabolic function of folate, or uracil content in nuclear DNA is suggested for assessing folate status.[4,9,11]

After an FA fortification program, increased FA intake was followed by elevated blood concentrations of folate but also by elevated levels of UFA. This is because the synthetic form needs to be reduced to THF by dihydrofolate reductase, before incorporation into the active cellular folate pool. The reduction has limited capacity, and when there is excessive intake (ie, >200 μg), the FA appears in the circulation as UFA.[12-14] Increases in the incidence of CRC in the United States[15] after FA fortification programs, and the possibility of the dual effect of FA for cancer (protection and risk of colon, breast, and prostate),[16] challenged the scientific community and suggested that the potential risk of FA may come from the accumulation of the UFA, leading to the initiation of tumors.[17]

Site-Specific Evidence for Folate and Cancer

Epidemiological studies have shown that raised intakes or concentrations of folate protect against the development of different types of cancer.[6,18] Evidence from in vitro, animal, and human studies has shown that low folate status is associated with DNA strand breaks, impaired DNA repair, increased mutations, and aberrant DNA methylation.[19]

In the next section, evidence from the meta-analysis conducted by RCTs, cohort studies, and case–control studies concerning dietary folate, UFA, and circulating folate will be presented in relation to the most incident cancers in the world. According to the estimated cancer incidence in 2012, the 3 most incident for men are lung, prostate, and CRC and for women are breast, colorectal, and cervical cancer, in that order.[20]

Colorectal Cancer

Colorectal cancer is the third most commonly diagnosed cancer and the fourth leading cause of cancer death.[20] A long induction period is required to progress from normal cells of the colon and rectum to small adenomas, to large adenomas, and finally to adenocarcinoma, which can take up to 30 to 40 years.[21] Established risk factors are red meat, processed meat, alcohol, increased weight, and increased abdominal fatness, and protective factors are physical activity and a diet rich in fiber.[22] However, results from studies investigating circulating folate, UFA, and diet are controversial.

Although prospective and retrospective studies have shown that low dietary folate intake was associated with increased risk of CRC, investigation between blood folate levels and CRC from prospective studies is inconsistent.[23-30] In a big, nested, case–control study of women and men in 3 prospective studies (from the Nurse's Health Study, the Health Professional Follow-up study, and the Physicians Health Study), lower plasma folate levels collected before FA fortification program in the United States (median plasma folate ranging from 3.8 to 15.7 ng/mL) suggested an association with an increased risk of CRC (adjusted odds ratio [OR]: 1.27, 95% confidence of

interval [CI]: 0.90-1.78), after adjusting for many confounding variables.[31] Different risk factors were estimated according to the period of analysis in the transition of fortification period (adjusted OR: 1.02, 95% CI: 0.48-2.20) to after-fortification period (adjusted OR: 2.56, 95% CI, 1.09-6.02).[31] However, in a more recent meta-analysis of case–control study nested within 10 prospective studies, circulating folate (ranging from 4.8 to 70.9 nmol/L) was not associated with CRC (adjusted OR: 0.96, 95% CI, 0.91-1.02).[32] Results from animal studies on CRC suggest that folate plays a dual role: it may protect against the initiation of cancer but enhances the growth and progression of preneoplastic cells.[19]

The scientific community became concerned about FA fortification as a public health program after observing an increase in CRC incidence rates in the United States after a nationwide FA fortification program.[15] The hypothesis was that this was caused by the potential carcinogenic effect of UFA.[15] To test this hypothesis, many clinical trials and analyses based on data from the American Surveillance, Epidemiology, and End Results were conducted. In the last investigation, in which time trends in the American CRC incidence rates and death rates were plotted considering (1) a long induction period between improved folate status after FA fortification program, (2) change in the FA supplement limit by the Food and Drug Administration (FDA), and (3) the differential impacts of regulatory changes in the mandatory FA fortification programs in the 1970s to the 1990s, it was found that the increase in CRC incidence rates in the latter 1990s was not attributed to FA fortification, and it was suggested that folate could have a protector effect, explaining the downward trend of CRC incidence rates in the United States.[21]

Investigation regarding UFA, a nested case–control study from the Nurse's Health Study and Health Professional follow-up studies, authors assessed UFA collected prior to the 1998 FDA-mandated fortification. No association was observed for the risk of CRC (pooled OR [95% CI]) for a concentration of less than 0.5 nmol/L of UFA (pooled OR: 1.03, 95% CI: 0.73–1.46) and (pooled OR: 1.12, 95% CI: 0.81–1.55) when compared with undetectable levels.[33] Different risks were observed when stratified by sex and the presence of folate metabolism-related enzyme polymorphism, the methylenetetrahydrofolate reductase (MTHFR 677). Increased risk was observed among men carrying the polymorphism of MTHFR 677CT/TT genotype. But the authors suggest that this observation could be attributed to chance, since the association was in the opposite direction for women with the MTHFR 677CC genotype[33] (Table 1).

Lung Cancer

Lung cancer is the leading cause of cancer-related deaths worldwide for both men and women, and the established risk factor is smoking. Evidence for the consumption of fruit and vegetables being a protective factor has been observed, with a reduction of 8% to 14% for higher intakes of fruits and vegetables compared to lower intakes.[39]

A meta-analysis conducted in population-based RCTs observed no association of total folate (FA) with lung cancer (Table 1).[34,37] The durations of the clinical trials were between 27 and 80 months, the daily dose of FA supplementation varying from 0.5 to 2.5 mg, and designed for the prevention of cardiovascular disease or the occurrence or recurrence of CRC.[37] A similar effect was observed in a meta-analysis investigating the risk of overall first cancer incidence (Table 1).[35]

For lung cancer, no association was observed with folate intake, circulating folate, or FA RCT. The follow-up periods were 5.3 to 16.4 years. No dose–response effect was observed (Table 1).[37,40]

Cervical Cancer

Latin America and the Caribbean have one of the highest incidence and mortality rates for cervical cancer in the world. Age-adjusted incidence rates range from 20 to 80 per 100 000 women per year, with extremely high mortality rates, in spite of the availability of Pap screening. Established risk factors are repetitive infection by oncogenic human papillomavirus (HPV), smoking, oral contraceptive use, multiparity, low immunity, lower socioeconomic status, lower educational level, being unaware of cervical cancer screening, or having no access to it.[43,44]

Results from a meta-analysis of 6 case-control studies showed no association between circulating folate and cervical cancer.[41] These results should be interpreted with caution because they were not properly adjusted by types of HPV infection and the results are prone to residual confounding.

In a case–control study conducted in Brazil, polymorphism in genes involved in folate metabolism modified the association of dietary and circulating folate and the risk of precancerous lesions, cervical dysplasia grades 2 and 3 (CIN2 and CIN3). In this study, women with lower folate intake and carrying more than 4 risk alleles of folate-metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR C677 T, A1298C), 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR A2756G), methionine synthase reductase (MTRR A66G), or reduced folate carrier (RFC1 G80A) presented increased risk of CIN2 and CIN3 after adjusting for confounding variables.[45]

Breast Cancer

Breast cancer is the most incident cancer among women worldwide. Established risk factors are alcohol consumption, greater birth weight, and increased body fatness, and protective factors are lactation and weight control.[46]

In a meta-analysis comprising cohort studies of breast cancer and folate intake, the use of FA supplement was not associated with breast cancer compared with those who did not use supplements (pooled relative risk = 1.07; 95% CI: 0.95-1.21). Stratified by menopausal status, no association was observed.[38] Higher dietary folate intake was negatively associated with breast cancer among women who reported higher alcohol

Table 1. Summarized Results From Meta-Analysis.

Authors	Type of Study	Variable of Interest	Outcome	No. of Studies	Range of Folate (Dose for RCT, Mean ± SD, Median)	Sample (n)	Overall Results	95% CI
Qin et al[34]	RCT	FA supplement	Total cancer mortality	6	0.5-4.0 mg FA/day	31.930	1.02	0.90-1.15
Qin et al[34]	RCT	FA supplement	Total cancer incidence	13	0.5-4.0 mg FA/day	49.406	1.05	0.99-1.11
Vollset et al[35]	RCT	FA supplement	Total cancer incidence	3	0.6-40.0 mg FA/day	2.652 colorectal adenomas	1.33	0.98-1.80
Tio et al[36]	Case–control	Folate intake	Esophageal carcinoma	4	Mean (SD): 276 ± 98 μg folate/day	829 cases, 3.977 controls	0.63	0.44-0.89
Tio et al[36]	Case–control	Folate intake	Esophageal adenocarcinoma	3	Mean (SD): 276 ± 98 μg folate/day	1.019 cases	0.57	0.43-0.76
Tio et al[36]	Case–control	Folate intake	Gastric	13	124-275 μg folate/day	3.638 cases, 12.530 controls	0.87	0.70-1.09
Tio et al[36]	Cohort	Folate intake	Gastric	3	<258 vs >404 μg FA/day	776 cases, 197.159 total	1.19	0.92-1.54
Tio et al[36]	Case–control	Folate intake	Pancreatic	8	<247 vs >396 μg folate/day, <247 vs >738 μg FA/day	2.209 cases, 295.526 controls	0.66	0.49-0.89
Tio et al[36]	Cohort	Folate intake	Pancreatic	5	<176 vs >396 μg folate/day	1.247 cases, 291.950	0.65	0.38-1.11
Tio et al[36]	Case–control	Plasma	Pancreatic	4	<9 to >20 nmol/L	839 cases, 2.215 controls	0.73	0.47-1.13
Chuang et al[32]	Nested case–control	Plasma	Colorectal	8	4.8-70.9 nmol/L	3.477 cases, 1.039 controls	0.96	0.91-1.02
Qin et al[37]	RCT	FA supplement	Colorectal	8	0.5-2.5 mg FA/day		1.01	0.82-1.22
Qin et al[37]	RCT	FA supplement	Colorectal	6	0.5-2.5 mg FA/day	184 cases/16.926 treatment group, 183 cases/16.899 control	1.01	0.82-1.23
Qin et al[37]	RCT	FA supplement	Breast	4	<1 vs >1 mg FA/day	19.800	0.82	0.63-1.07
Chen et al[38]	Cohort	Folate intake	Breast	16	244 vs 422 μg folate/day	744.068	0.95	0.87-1.03
Chen et al[38]	Case–control	Folate intake	Breast	26	129-975 μg folate/day	16.826 cases, 21.820 controls	0.79	0.67-0.92
Qin et al[37]	RCT	FA supplement	Lung	5	0.5-4.0 mg FA/day	31.864	1.00	0.84-1.21
Vieira et al[39]	Cohort	Folate intake	Lung	9	244 vs 422 μg folate/day	566.921	0.92	0.84-1.01
Dai et al[40]	Case–control	Folate intake	Lung	6	Not available	4.390 cases, 6.138 controls	0.74	0.65-0.84
Dai et al[40]	Case–control	Serum folate	Lung	4	Not available	1.438 cases, 2.582 controls	0.78	0.60-1.02
Zhou et al[41]	Case–control	Serum folate	Cervix	6	Range of deficient criteria: <1.5 to <6.4 ng/mL	873 cases, 1.510 controls	0.97	0.65-1.45
Wang et al[42]	Cohort	Folate intake	Prostate	5	<177 vs 413 μg folate/day	192.702	1.02	0.95-1.09
Wang et al[42]	Cohort	Serum folate	Prostate	5	<4.8 vs >17.5 nmol/L	192.702	1.21	1.05-1.39

Abbreviations: CI, confidence of interval; FA: folic acid; RCT: randomized clinical trial; SD, standard deviation.

intake compared to nondrinkers, with a pooled OR of 0.60 (95% CI: 0.45-0.82).[38] However, when stratified by temporality of the study design, significant association was observed in a meta-analysis conducted with case–control studies for participants with the highest category of dietary folate intake compared with those of lowest category.[38]

Other Organs

Significant negative association was observed in a meta-analysis of dietary folate and esophageal carcinoma and adenocarcinoma but was available only in case–control studies.[47] For pancreatic cancer, controversial results were observed: negative association for dietary folate intake in case–control studies but no association in dietary and circulating folate in cohort studies.[36,47]

For esophageal cancer, previous studies reported that polymorphisms in folate metabolism genes resulted in lower circulating levels of folate and increased risk of esophageal cancer.[48] Regarding prostate and gastric cancer, no association was observed.[36,48]

Discussion

In the present review, results from a meta-analysis were presented to allow for a more objective view of the evidence as the original research presented conflicting results. Evidence from the meta-analysis considering studies that adjusted for multiple potential confounding factors, even from RCT that are studies with the best methodological strengths, presented no association. However, in trials, participants are mostly health conscious with healthy lifestyles, practice more physical activity, do not smoke, have a healthy diet, and may have a good folate status. If trials targeted individuals with suboptimal folate, a significant benefit of folate intake on the risk of CRC might be shown.[21]

Significant results of the meta-analysis conducted in case–control studies should be interpreted with caution. It is possible that these results were affected by recall bias and selection bias, when patients usually overestimate their lack of folate intake when they are subconsciously informed that folate could be a favorable nutrient, and both limitations of retrospective study design and the results should be interpreted with caution.

In the case of CRC cancer, it is possible that the studies were limited by their short duration of follow-up; the longest time was 15 years of follow-up, and researchers recommend an extended follow-up of clinical trials to allow for the long induction period. Pooled studies of participants with a history of adenomas recruited from long-established cohorts could be the key.[21]

In conclusion, no significant evidence was observed for circulating folate and colorectal, breast, cervical, and lung cancer. Investigating folate status is a lengthy, complex process, with interaction of many factors, due to its many chemical forms, polymorphisms in folate-related enzymes, the dose and timing of FA, folate intake, and carcinogenesis. More prospective studies and intervention studies are necessary for further evaluation of the effects of folate in the cancer process.

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