EFFECT OF VITAMIN B₁₂ AND FOLATE ON HOMOCYSTEINE LEVELS IN COLORECTAL CANCER

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ABSTRACT

Folate and cobalamin (Vitamin B₁₂) are two essential micronutrients involved in one-carbon metabolism, which affects heart disease, neural tube defects and cancer. Methylenetetrahydrofolate reductase, the key enzyme involved in one carbon metabolism produces methyl tetrahydrofolate from methylene tetrahydrofolate, which in turn donates methyl group to homocysteine to generate methionine. There exist two common low function polymorphic variants of the methylenetetrahydrofolate reductase gene involving nucleotides 677 C→T and 1298 A→C, which are associated with hyperhomocysteinemia. These polymorphisms are also linked with increased risk for certain cancers such as breast cancer and at the same time providing a protective effect on colorectal cancer. In this case control study, we have evaluated levels of folic acid, vitamin B₁₂ and homocysteine in patients with colorectal cancer. Folate and homocysteine levels did not differ significantly between the two groups; however an increasing trend was noticed with increase in homocysteine levels. Vitamin B₁₂ levels were increased in cases compared to control group.

KEY WORDS

Colorectal cancer, Folate, Vitamin B₁₂, Homocysteine

INTRODUCTION

The role of nutrition in affecting gene expression through interaction with genetic polymorphism and modulation of DNA methylation has received considerable attention recently. The disruption of homeostasis in the vitamin-dependent, one-carbon metabolism affects the risk of heart disease, neural tube defects and cancer. Such disruption can occur as a result of deficiencies of the two essential micronutrients involved in this metabolism; folate and vitamin B₁₂ (1).

One-carbon metabolism is divided into two main branches: one branch consists of reactions involving purine and thymidine synthesis and the other involves synthesis of methionine and S-adenosyl methionine for protein and polyamine synthesis

and methylation reactions. An enzyme that shunts methyl groups from the first of these branches to the second is methylenetetrahydrofolate reductase (MTHFR). MTHFR irreversibly converts 5, 10 methylenetetrahydrofolate to 5-methyl tetrahydrofolate, which then donates a methyl group to homocysteine to produce methionine.

Individuals who are severely deficient in MTHFR activity because of a germline mutation have excessive amount of homocysteine in the blood and urine. The concentration of total homocysteine in plasma or serum is an established marker of common diseases and a sensitive marker of cobalamin and folate deficiencies (2). There exist two common low function polymorphic variants of MTHFR, 677 C→T and 1298 A→C. The first of these variants 677 C→T has been shown to be associated with high baseline homocysteine levels in serum. This polymorphism is linked to an increased risk for certain cancers, such as endometrial cancer, breast cancer, ovarian cancer and decreased risk for leukemia and colorectal cancer (3-6). The cancer risk associated with MTHFR polymorphism may be modulated by folate.

In this study, we have evaluated folate, vitamin B₁₂ and homocysteine levels in patients with colorectal cancer to

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analyze the effects of vitamin B₁₂ and folate on plasma homocysteine levels.

MATERIALS AND METHODS

30 patients with a histologically confirmed diagnosis of colorectal cancer, irrespective of stage of disease and prior to treatment formed the subject group. 30 age and gender matched healthy volunteers belonging to the same socioeconomic group formed the control group. Subjects with altered renal function, hepatic dysfunction and hypothyroidism were excluded from the study. The study protocol was approved by the Institutional Ethical Committee and informed consent was obtained from the subject's for the study.

Homocysteine estimation: Blood samples were collected from all subjects after an overnight fast into tubes with EDTA as anticoagulant. The tubes were immediately placed on crushed ice, protected from light and centrifuged within 20 minutes at 2000xg for 10 minutes. Plasma samples were then stored at -80° C until analysis. Total homocysteine in plasma was estimated by Enzyme conversion immunoassay (7). A reference value of 5-15 micromoles /L was considered for comparison of test results.

Vitamin B₁₂ and Folate estimation: Vitamin B₁₂ and folate were simultaneously analyzed using a solid phase no boil dual count radioassay kit, procured from Diagnostic Product Corporation- DPC, CA, USA (8). A reference value of 180-900 pg/ml for vitamin B₁₂ and 3-17 ng/ml for folic acid were considered for comparison of cases and controls.

Statistical analysis : Biochemical levels were expressed as Mean $\pm$ SD. Student's 't' test (two tailed, independent) has been used to find the significance of study parameters between control and cases. Multivariate (Hottelling T²) analysis has been carried out keeping all three study parameters as dependent set and the study parameters were compared between cases and controls. The Discriminate Function analysis was used to determine the percentage of correct classification of study parameters between cases and control. Pearson correlation has been used to find the degree of relationship between study parameters in cases and controls individually.

RESULTS AND DISCUSSION

The mean age was 40 years for the healthy controls and 39 years for patients with colorectal cancer. There were 12 patients with colon cancer and 18 patients with rectal cancer.

Of the 30 patients taken for the study 19 were males and 11 were females.

Table 1 shows levels of folic acid, Vitamin B₁₂ and homocysteine among cases and controls. Folic acid levels did not differ significantly among the two groups while vitamin B₁₂ was significantly elevated among the patient group. Homocysteine levels though elevated in cases did not differ significantly from the control group. In a multivariate analysis of all three parameters, 75.6% of correct classification of case and control was obtained (using discriminate function analysis).

Table1: Comparison of study parameters between cases and controls (Result are presented as mean $\pm$ SD)

Study Parameters	Cases	Controls	P Values
Folic acid (ng/ml)	10.30 $\pm$ 7.05 (3.82-18.26)	10.53 $\pm$ 3.99 (2.57-32.10)	0.901
Vitamin B ₁₂ (pg/ml)	419.76 $\pm$ 281.70 (103.30-523.6)	239.41 $\pm$ 116.58 (126.50-1035)	0.014
Homocysteine (μ mol/L)	17.8 $\pm$ 14.75 (2.64-33.63)	12.73 $\pm$ 9.6 (2.99-83.08)	0.161
Multivariate Statistics (MANOVA)		F=3.306 P=0.021	
% correct classification		75.6%	

Table 2 shows the Pearson correlation of homocysteine versus folic acid and homocysteine versus vitamin B₁₂. The Pearson correlation coefficient between homocysteine and folic acid was -0.141 in subjects as compared to 0.09 in controls indicating that homocysteine levels increase with decrease in folic acid in subjects. An inverse correlation was observed between vitamin B₁₂ and homocysteine levels in both cases and controls.

Table 2: Pearson correlation of Homocysteine versus Folic acid and Vitamin B₁₂ in cases and controls

Pair	Subjects (CRC)		Controls	
	Pearson correlation (r)	P value	Pearson correlation (r)	P value
Homocysteine Vs Folic acid	-0.141	0.501	0.090	0.732
Homocysteine Vs Vitamin B ₁₂	-0.285	0.167	-0.248	0.355

Epidemiological studies implicated low folate status in the development of cancer in several organs, including the cervix, lung, brain, pancreas breast, colon and rectum (9). These observations may be explained by the crucial role of folate as the donor of one-carbon groups in both DNA methylation and nucleotide synthesis. In humans, folate deficiency induces

decreased DNA methylation, which is a nearly universal feature of early tumorigenesis (9, 10). Insufficient methylation of DNA may promote carcinogenesis by the derepression of proto-oncogenes or by the induction of genomic instability.

Evidence supports the key role of folate status in human health. An unbalanced diet with reduced uptake of folic acid was associated with increased risk of colon cancer (11). In studies on human donors, low levels of serum folic acid were associated with a higher misincorporation of uracil into DNA as well as with increased incidence of micronucleus in peripheral lymphocytes (12). Micronucleus was also positively correlated to serum homocysteine levels (13, 14).

In addition to dietary uptake of micronutrients such as folic acid and vitamin B₁₂, some genetic polymorphisms may affect folic acid metabolism, modulating its effect on human health. MTHFR is a key enzyme in folate metabolism and converts 5, 10 methylenetetrahydrofolate to 5 methyl THF. The latter form of folate is used for the remethylation of homocysteine to methionine. There exist two common low-function polymorphic variants of MTHFR, the T variant at nucleotide 677 (677 C → T) and C variant at nucleotide 1298 (1298 A → C). Lower colon cancer risk was associated with the MTHFR 677 TT genotype, and reduced risk of childhood and adult lymphoblastic leukemia was attributed to subjects with the MTHFR mutations 677 C→T or 1298 A→C (15-18). Such protection however only occurs with normal folate uptake, whereas unbalanced diet makes variant carriers as susceptible as wild type to cancer, maybe because the availability of 5-methyl THF for biological methylation becomes critical, balancing the beneficial effect of reduced uracil misincorporation (15). The regulation of homocysteine metabolism is complex and is dependent on multiple vitamin cofactors including folate and vitamin B₁₂. Folate deficiency is the leading cause of hyperhomocysteinemia, however MTHFR mutations also affect folate metabolism, resulting in increased homocysteine (19, 20).

In this case control study, we have evaluated levels of folic acid, vitamin B₁₂ and homocysteine in patients with colorectal cancer. The patients and healthy control belonged to the same socioeconomic group. Folic acid and total homocysteine levels were not statistically significant between the cases and controls. A number of case control studies have demonstrated an inverse association between folate intake and risk of colorectal cancer with an average reduction of about 30% for the highest versus the lowest folate intake (21). Van Guelpen et al suggested a decreased colorectal cancer risk in subjects with low folate status (22). In another study on breast cancer,

plasma folate level was inversely associated with breast cancer risk (23).

Results of studies examining associations between biochemical measures of folate status and risk of colorectal adenoma or cancer are inconsistent (24). Perhaps of particular relevance is the observation that folate concentration in the healthy mucosa of patients with colorectal adenoma are lower than in control subjects even though circulating folate concentration did not differ between these two groups (25). It would be helpful to establish whether folate concentrations in colonic mucosa are sensitive to changes in folate intakes.

Vitamin B₁₂ levels were increased in cases as compared to controls. A possible increased risk for prostate cancer in subjects with high Vitamin B₁₂ and possibly also folate status has been reported in one small case control study and the use of multivitamins has been reported to be associated with an increase in prostate cancer mortality (25, 26). In a case control study in a Turkish population, significantly (P=0.01) higher levels of vitamin B₁₂ were observed in cancer patients (27). In another study conducted on 300 Finnish patients, a not significant (p=0.014) positive trend between vitamin B₁₂ and cancer risk was reported, with a 41% additional risk in the high vitamin B₁₂ group (28). Due to the limited size of our study this association needs to be further investigated.

In conclusion, the present case control study did not observe any statistically significant levels of folic acid among cases and controls. A trend was noticed in the association between increasing plasma total homocysteine levels and colorectal cancer, although this was not statistically significant. Vitamin B₁₂ levels were significantly higher among the patients (P= 0.14).

The results need to be confirmed with a larger sample size. As an adjunct to the preliminary findings reported here, we propose to increase the sample size and to study the prevalence of the 677 C→T and 1298 A→C single nucleotide polymorphisms of the MTHFR gene.

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