Clinical Nutrition xxx (2013) 1–7

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

# High homocysteine is associated with increased risk of colorectal cancer independently of oxidative stress and antioxidant capacities

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ARTICLE INFO

*Article history:*
Received 4 September 2013
Accepted 10 November 2013

*Keywords:*
Homocysteine
Cysteine
Pyridoxal 5′-phosphate
Oxidative stress
Antioxidant capacities
Colorectal cancer

SUMMARY

*Background & aims:* Increased homocysteine concentration and oxidative stress and decreased antioxidant capacities are thought to affect carcinogenesis. However, the associations of homocysteine, cysteine, pyridoxal 5′-phosphate (PLP) and folate with oxidative stress and antioxidant capacities in patients with colorectal cancer are unclear. The purpose of this study was to determine the associations of homocysteine, cysteine, PLP and folate with oxidative stress indicators and antioxidant capacities, and to further analyze their relationships with respect to risk for colorectal cancer.
*Methods:* One hundred and sixty-eight subjects with colorectal cancer (cases) and 188 healthy subjects (controls) were recruited.
*Results:* There were no significant associations of homocysteine, cysteine and folate with oxidative stress indicators and antioxidant capacities in cases; however, PLP positively correlated with glutathione *S*-transferase activities after adjusting for potential confounders in cases. Subjects with higher plasma homocysteine concentration exhibited significantly increased risk of colorectal cancer with or without adjustment for potential confounders. The associations of cysteine, PLP and folate with the risk of colorectal cancer were not observed when potential confounders were adjusted.
*Conclusions:* Increased homocysteine was strongly associated with the risk of colorectal cancer independently of oxidative stress indicators and antioxidant capacities. However, cysteine, PLP and folate were not found to be related to oxidative stress, antioxidant capacities and the risk of colorectal cancer.


## 1. Introduction

The mortality rate of colorectal cancer has risen over the past decade and is now the 3rd and 2nd most commonly diagnosed cancer in males and females worldwide, respectively.[1] Among risk factors related to colorectal cancer, increased homocysteine concentration,[2,3] higher levels of oxidative stress and increased or decreased antioxidant enzyme activities have been observed in patients with colorectal cancer compared with corresponding values found in healthy controls.[4–6] It is plausible that homocysteine and oxidative stress status or antioxidant capacities play a role in patients with colorectal cancer.

Hyperhomocysteinemia has been shown to be a new potential oxidative stress indicator via its impact on folate status.[7] However, the possibility that homocysteine might mediate oxidative stress in connection with the glutathione antioxidant system cannot be ruled out. When methionine is in negative balance, homocysteine is remethylated to form methionine by a methionine-conserving remethylation pathway, a process that requires methyltetrahydrofolate as a cosubstrate. When methionine is in excess, homocysteine is directed to the transsulphuration pathway. In the transsulphuration pathway, homocysteine is converted to cystathionine and then cysteine by the pyridoxal 5′-phosphate (PLP, the physiological active coenzyme form of vitamin B-6) dependent enzyme. Cysteine is the rate-limiting component in the synthesis of glutathione.[8] Glutathione is a key buffer of intercellular oxidative-reduction reaction, and its dependent antioxidant enzymes include glutathione *S*-transferase (GST) and glutathione peroxidase (GPx), which play a fundamental role in cellular defense against reactive free radical and other oxidant species.[9] Studies have shown

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0261-5614/$ – see front matter 
http://dx.doi.org/10.1016/j.clnu.2013.11.007

that high homocysteine concentration could cause oxidative damage to cells,[8,10] and cysteine may act as a pro-oxidant agent causing the overproduction of free radicals and hydrogen peroxide, and may further lead to gene mutation and subsequent development of cancer.[8–10] It would thus be reasonable to hypothesize that high homocysteine and lower PLP would affect cysteine and glutathione synthesis and, as a consequence, influence the entire glutathione-dependent antioxidant defense system, possibly triggering the development of colorectal cancer.

Although increased homocysteine and oxidative stress have been observed in patients with colorectal cancer, the associations of homocysteine, cysteine, PLP, and folate with oxidative stress are poorly understood and highly controversial. It is unclear whether homocysteine, cysteine, PLP and folate are independently related to risk for colorectal cancer or whether they mediate the risk of colorectal cancer in connection with high oxidative stress. The purpose of this case-control study was to determine the associations of homocysteine, cysteine, PLP and folate with oxidative stress indicators and antioxidant capacities, and to further analyze their relationships with respect to risk for colorectal cancer.

## 2. Subjects and methods

### 2.1. Subjects

Subjects with a diagnosis of colon or rectal cancer (cases) (International Classification of Diseases (ICD) 9, codes 153 and 154, respectively) were recruited from the division of colorectal surgery of Taichung Veterans General Hospital, Taiwan. Cases were excluded if they were pregnant, lactating, had attenuated adenomatous polyposis coli or inflammatory bowel disease or were taking any medication which could influence homocysteine, vitamin B-6, and folate status, such as $H_2$ blockers, proton pump inhibitors, metformin, phenytoin, or methotrexates. Patients' medical records were reviewed by two study oncologists for diagnostic confirmation and staging. Healthy subjects (controls) who exhibited normal blood biochemical values were recruited from the physical examination unit of Taichung Veterans General Hospital, Taiwan. Subjects in the control group who had history of gastrointestinal disorder, cardiovascular disease, liver or renal disease, diabetes, cancer, alcoholism or other metabolic disease were excluded. Informed consent was obtained from all subjects. The study was approved by the Institutional Review Board of Taichung Veterans General Hospital.

### 2.2. Data collection

All subjects' age, gender, height, weight, smoking and drinking status, family history of colorectal cancer and medication uses were recorded. Body mass index (BMI) ($kg/m^2$) was calculated from height and weight measurements. Blood pressure [systolic and diastolic blood pressure (SBP and DBP)] was measured after a resting period of at least 5 min. Fasting venous blood samples were drawn and collected in vacutainer tubes (Becton Dickinson, Rutherford, NJ) containing an appropriate anticoagulant or no anticoagulant as required for hematological and biochemical measurements.

### 2.3. Hematological and biochemical measurements

Hematological measurements, including serum albumin creatinine, total cholesterol, high-density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglycerides, were assessed using an automated biochemical analyzer. Automated serum high sensitivity C-reactive protein (hs-CRP) measurement was performed using particle-enhanced immunonephelometry with an image analyzer.

Plasma homocysteine and cysteine were measured by high performance liquid chromatography (HPLC) as previously described.[11] The intraassay and interassay variabilities of plasma homocysteine were 3.19% ($n = 5$) and 5.52% ($n = 15$), respectively. The intraassay and interassay variabilities of plasma cysteine were 3.22% ($n = 5$) and 9.52% ($n = 15$), respectively. Hyperhomocysteinemia was defined as a plasma homocysteine concentration $\geq$14 μmol/L.[12] Plasma PLP was determined by HPLC according to the method of Talwar et al.[13] The intraassay and interassay variabilities of plasma PLP were 1.70% ($n = 5$) and 3.98% ($n = 28$), respectively. Vitamin B-6 deficiency was defined as plasma PLP concentration <20 nmol/L.[14] Homocysteine, cysteine and PLP measurements were carried out under yellow light to prevent photodestruction. Serum folate was analyzed using standard competitive immunochemiluminometric methods on a Chiron Diagnostics ACS:180 Automated Chemiluminescence Systems (Chiron Diagnostics Corporation, USA). Folate deficiency was defined as serum concentrations of <2.8 ng/mL.[14] Reduced glutathione concentration in plasma was determined using a glutathione commercial assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). All analyses were performed in duplicate.

Oxidative stress was estimated as the levels of plasma malondialdehyde (MDA) and oxidized-LDL (ox-LDL). Plasma MDA was measured by thiobarbituric acid reactive substances according to a method described by Lapenna et al.[15] The level of ox-LDL was measured using an ox-LDL commercial assay kit (Mercodia AB, Uppsala, Sweden). Among the methodologies used to evaluate total antioxidant capacity (TAC), the most widely used colorimetric method for serum and plasma samples are 2′-2′-azinobis-3-ethylbenzothiazoline-6-sulfonate-based methods. Therefore, TAC was measured according to a method described by Erel,[16] who developed a novel colorimetric and automated direct assay. Antioxidant enzyme activities, including those of GPx, GST and superoxide dismutase (SOD), were determined using GPx, GST and SOD commercial kits (Cayman Chemical Company, Ann Arbor, MI, USA), respectively.

### 2.4. Statistical analyses

Data were analyzed using the SAS statistical software package (version 9.2; Statistical Analysis System Institute Inc., Cary, NC, USA). A Kolmogorov–Smirnov test was performed to test the normal distribution. Demographic characteristics and biochemical data were compared for significance using Student's $t$ test or Mann–Whitney Rank Sum test. Chi-square test or Fisher's exact test were used for the analysis of categorical variables. Multiple linear regression (β) was used to analyze the association of plasma homocysteine, cysteine, PLP and folate with oxidative stress indicators and antioxidant capacities in the case and control group after adjusting for potential confounders. Adjusted odds ratio (OR) with 95% confidence intervals (CI) for colorectal cancer was calculated from the logistic regression model. Statistical results were considered to be significant at $p < 0.05$. Values presented in the text are means and standard deviation (SD).

## 3. Results

Table 1 shows the demographic characteristics and hematological measurements of cases and controls. One hundred and sixty-eight cases and 188 controls were recruited. Of 168 cases, 82 cases (49 women, 33 men) with colon cancer and 86 cases (53 women, 33 men) with rectal cancer were identified. Since subjects with colon cancer and rectal cancer had comparable demographic and clinical characteristics, as well as levels of homocysteine, cysteine, PLP, folate, glutathione, oxidative stress indicators and

Please cite this article in press as: Chiang F-F, et al., High homocysteine is associated with increased risk of colorectal cancer independently of oxidative stress and antioxidant capacities, Clinical Nutrition (2013), http://dx.doi.org/10.1016/j.clnu.2013.11.007