Is There any Alteration in Erythrocyte Folate Status in Patients with Thyroid and Breast Cancers?

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Abstract

The aim of the study was to determine folate reserve in patients with thyroid and breast diseases. Erythrocyte folate concentrations of ninety-eight patients and thirty healthy subjects were determined by a modified microbiological assay.

An increase in folate levels was observed in of both thyroid (n = 15) and breast (n = 22) cancer patients compared to controls, while a decrease was observed in benign breast disease patients (n = 24) compared to both control group and breast cancer patients. Patients with benign thyroid diseases (n = 37) had significantly lower folate concentrations as compared to both control and thyroid cancer groups.

The results pointed out the elevated erythrocyte folate levels in the patients with malignant tumors.

Key words: folate, erythrocyte, breast cancer, thyroid cancer

Introduction

Folate coenzymes or the related major pterins are present in virtually every known organism and cell type (1). One-carbon metabolism is a network of interrelated biochemical reactions that involve the transfer of one-carbon groups from one site to another (2). Folate and its co-factors are involved in the transfer of methyl intermediaries: these include formation of purines, interconversions between amino acids, formate metabolism and pyrimidine biosynthesis, and thymidine (3, 4). Animal studies revealed that folate may decrease or increase cancer risk depending on its dosage and timing. Also it may be postulated that the dietary intake or blood levels does not reflect the cellular level in cancer tissue (5).

Human epidemiological studies pertaining to the tissue have typically assessed folate status either by estimating the habitual dietary intake of folate or measuring blood folate concentrations directly (6). It is for example recognized that food folate composition data provide inexact estimations of folate intake and that there is considerable variation within and across methods of the analysis of serum and whole blood folate. It is not clear whether such measurements accurately assess the concentration of folate in the cells of cancer origin, which is likely to be more critical. Tissue-specific susceptibility to folate deficiency has for example been shown in smokers; buccal mucosal cells were low in folate while systemic folate concentrations were normal. Furthermore, it was described the lack of association between erythrocyte folate levels and colonic biopsy specimens in healthy individuals, indicating the potential difficulty in predicting localized folate deficiency. Epithelial cell folate depletion was reported in neoplastic but not in adjacent normal colonic mucosa. Conversely, in patients with polyps, the folate content of colon biopsy samples was significantly correlated with blood folate concentrations (5).

Generally, serum and erythrocyte concentrations of folate were the two most commonly used indicators of folate status (2, 4). In human, serum folate tends to be a reflection of short-term folate balance during the preceding 1-2 day. The folate that is packaged into a developing blood cell is no longer metabolized after maturation of that red cell occurs. Erythrocyte folate concentration therefore represents an integration of dietary folate intake during the preceding 120 d, the
half-life of a red cell. It is therefore not surprising that erythrocyte folate tends to be a more accurate reflection of tissue folate status, because the latter is also largely determined by habitual intake over a prolonged period. Although normative values vary somewhat from laboratory to laboratory, two standard deviations below the mean for serum and red blood cell folate concentrations are typically 3 ng/mL and 160 ng/mL, respectively (2). Depletion of folate does not occur in a symmetric fashion among the various tissues of the body. Animal models of folate depletion clearly demonstrate that some tissues are more susceptible to depletion than others. Therefore, the measurement of the folate content in the tissue of interest may give additional important information (2). The aim of the present study was to determine the alterations in the primary folate reserves in patients with common malignant diseases like breast cancer and thyroid cancer.

Subjects and Methods

The demographic characteristics of the patients and control groups are shown in Table 1. Ninety-eight patients were included in the present study, 52 of them were operated for various thyroid disorders. Fifteen of the thyroid patients were papillary thyroid cancer while the others were benign thyroid disorders (including thyroiditis and multinodular goiter). The 6th edition of the AJCC Cancer Staging system was used for staging (7). The patients were diagnosed with fine needle aspiration (FNA). The excluding criteria were; preoperative indetermined diagnosis of malignancy, history of chemotherapy or radiotherapy, patients with familial pattern of breast diseases, patients who had a history of breast or other organ malignancies and presence of active infection at the time of sample collection.

Forty-six of the study patients were operated for malignant diseases like breast cancer and thyroid cancer while 24 had benign breast tumors. The benign tumor group consisted of fibroadenoma and fibrocystic disease patients. The preoperative diagnoses of all breast cancer patients were confirmed by core biopsy. The exclusion criteria of the study were; obscure perioperative diagnosis, history of chemotherapy or radiotherapy, patients with familial pattern of breast diseases, patients who had a history of breast or other organ malignancies and presence of active infection at the time of sample collection.

The control group consisted of 30 healthy subjects who worked as personnel in the faculty. All of them answered an anamnestic questionnaire to confirm that they were systemically healthy and that they had not received any medication during this period. The principles of the University Ethical Committee according to the Helsinki Declaration were followed during the whole study.

Each blood sample was collected into glass tubes containing an anticoagulant and centrifuged (Heraeus Sepatech, Labofuge Ae, Germany) at 3,000 rpm for 15 min at room temperature. Reached erythrocyte samples were stored at -20°C until analysis.

All chemicals used in the study were analytical grade. Folinic acid and 2-mercaptoethanol were obtained from Sigma Chemical Co. (St Louis, MO). Both of agars and broth of Lactobacilli and Lactobacilli MRS were purchased from Difco (Becton Dickinson France S.A.). Folic acid casei medium (FACM) was obtained from Difco, Becton, Dickinson and Co. (Sparks, MD21152). Standard bacterial strain, Lactobacillus rhamnosus (L. rhamnosus) American Type Culture Collection (ATCC) 7469a was obtained from LGC Prochem. A modified microbiological method was used to determine the erythrocyte folate levels (8). Each stored sample was allowed for thawing to room temperature. They were diluted with 0.2M ME solution at 0.5 McFarland and 105 bacterial suspension per tube at 600 nm by spectrophotometer (Shimadzu UV1601, Japan).

The erythrocyte folate concentrations of the samples are presented as ng/ml and all of the results are expressed as the mean ± standard error of mean (SEM). The differences among the groups were evaluated with Kruskal-Wallis analysis of variance, and comparisons between two independent groups were made with the Mann-Whitney U-test. P-values <0.05 were regarded to indicate statistical significant differences.
Results

Erythrocyte folate levels in both the control group and the patients are shown in Figure 1. The breast cancer patients were found to have higher folate levels when compared to the control group, but the difference did not reach statistical significance (117 % of mean value in controls; p = 0.188) while the folate levels in the group with benign breast disorders were lower than the control (98 % of mean value in controls, not significant). In addition, the patients with thyroid cancer were found to have higher folate levels when compared to control group (113 % of mean value in controls, not significant). By contrast, the folate levels of the benign thyroid patients were significantly lower than the control group (33 % of mean value in controls; p <0.001). The mean folate levels in each benign group were compared with their malignant counterpart and a significant difference was found only among the patients with thyroid disorders (p <0.001). The mean folate levels in each benign group were compared with their malignant counterpart and a significant difference was found only among the patients with thyroid disorders (p <0.001). In patients with breast diseases; there was a difference among benign and malignant disease but did not reach statistical significance (p = 0.180).

Discussion

Folate coenzymes play essential roles in many major cellular processes, including nucleic acid biosynthesis, mitochondrial and chloroplast protein biosynthesis, amino acid metabolism, methyl group biogenesis, and vitamin metabolism (1). Additionally, folate metabolism is the target of two major and the oldest anticancer drug groups such as folate antagonists and thymidylate synthase inhibitors (9). Over the past two decades, evidence from several different types of research-epidemiologic, animal models and clinical intervention studies has increasingly supported the concept that diminished folate status predisposes to the development of several common cancers. Conversely, this body of literature also suggests that habitual ingestion of folate at concentrations that are somehow above present recommendations is preventive (10). Cancer cells frequently up-regulate folate receptors, most likely to meet their accelerated need for nucleotides to support DNA synthesis and cell growth (3). It is known that folate plays a key role in DNA replication and cell division. Consequently, a deficiency of folate in tissues with rapidly replicating cells results in ineffective DNA synthesis, resulting in reduced cell proliferation, impaired cellular physiology, and abnormal cytologic morphology. This biochemical function of folate has been utilized in the area of how folate modulates cell proliferation in the process of carcinogenesis. Intuitively, interruption of folate metabolism in neoplastic cells would be expected to lead to ineffective DNA synthesis, resulting in the inhibition of tumor growth. Indeed, this has been the basis for antitumor therapy utilizing a number of antifolate agents, including methotrexate and 5-fluo-

Figure 1. Erythrocyte folate levels of the groups.

* The mean folate level in the benign group is statistically different from the control group (p <0.05). ** The mean folate level in the benign group is statistically different from its malign group (p <0.05).
rouracil (11). Clinically, it has been observed that folate treatment of children with acute leukemia results in an "acceleration phenomenon" of the cancer. Experimentally, it has been shown that growth of a transplanted cancer is inhibited in folate-deficient rats, that folate deprivation reduces virally induced cancers, and that the time required for developing a neoplasm in transgenic mice, which are predisposed to developing nerve sheath tumors similar to human neurofibromatosis, is significantly delayed by restricting the level of folate in the diet (11). Kim et al. showed that folate levels in the normal rectosigmoid mucosa were significantly lower in patients with adenomatous polyps than in those with hyperplastic polyps (6). Breast cancer is a multifactorial disease that is triggered by gene-environment interactions. A great deal is known about the causes of breast cancer, but the best available findings account for less than fifty percent of the possible risk factors (12). According to the retrospective nature of the study performed by Graham et al., the observed lower dietary folate intake in patients with breast cancer does not establish folate deficiency as a cause of breast cancer (13). According to the results of the meta-analysis performed by Larsson et al. due to the prospective studies (including nested case-control studies) and case-control studies of blood folate levels and breast cancer risk, high blood levels versus low levels were not statistically significantly associated with the risk of breast cancer and there was statistically significant heterogeneity among the case-control studies but not among the prospective studies (14). Lin et al. reported that a higher circulating concentration of folate is associated with breast cancer risk (15). Our results were similar to these findings. It can be summarized that results from other epidemiologic investigations of folate intake and breast cancer risk have varied, reporting protective effects, increased risk, or no associations (3). Unfortunately, the relationship between blood levels of folate and the risk of thyroid cancer is less well defined. Green reported that folate may prevent initiation of cancer through DNA repair while in an established tumor; folate may also promote the growth of rapidly proliferating cells (16). Indeed, the efficacy of methotrexate and other antifolates depends on the interference of folate dependent nucleotide synthesis. Additionally, the epigenetic regulation of DNA through methylation can be influenced by folate availability and hence may affect the expression of critical oncogenes or tumor suppressor genes (16). According to our findings erythrocyte folate levels in the group with thyroid benign diseases were significantly lower than the control group, while the lower erythrocyte folate levels observed in the benign breast disorders was not significant. We do not know the reason of the reduction of the folate in the benign thyroid cases. However, it is known that thyroid is a metabolically active tissue and it has a high tissue oxygen tension. This may lead to oxidation of folate due to imbalance between oxidative stress and antioxidant mechanisms in thyroid tissue. As a result of this, if folate is depleted the DNA damage may be observed and/or the lack of DNA repair may occur. A decrease in folate levels may also lead to impairment of integrity in p53 gene (17). This may contribute development of malignancy. This may cause transformation of benign tumor to a malignant growth. Further studies with longer follow up periods are needed to confirm these hypotheses.

The results also show that there are enhanced erythrocyte folate levels in patients with malignant diseases. If the folate pathway influences generation of cancers, we may speculate that elevated blood levels of folate may be a biomarker for uncontrolled cell turnover and impaired DNA methylation. All cancers are a genetic disease; because they arise from and contain cells with genetic defects (18). Gene-specific DNA hypermethylation occurs during tumor development. It is known that folate status alters DNA stability. Carcinogenesis is affected by alterations in folate pathway, including some impaired critical enzymes such as MTHFR (17). Moreover, it has been suggested that folate causes a decrease in the activity of natural killer cells (18). Since most carcinogenic events occur at a cellular level, knowledge of the actual folate concentration within the tissue of interest, rather than in the blood, may be of considerable importance (6). It is unclear whether blood concentrations of folate accurately reflect folate status in the thyroid or breast tissue. That's why; we believe that further studies with folate measurements in cancer tissue are needed to confirm our results.

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