Elevated serum homocysteine levels and increased risk of invasive cervical cancer in US women

Stephanie J. Weinstein¹, Regina G. Ziegler¹*, Jacob Selhub², Thomas R. Fears¹, Howard D. Strickler³, Louise A. Brinton¹, Richard F. Hamman⁴, Robert S. Levine⁵, Katherine Mallin⁶ & Paul D. Stolley⁷

¹Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, 6120 Executive Blvd, MSC 7246, Bethesda, MD, 20892, USA. Ph.: 301/402-3372; Fax: 301/402-2623; E-mail: ziegler@exchange.nih.gov; ²Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA; ³Department of Epidemiology and Social Medicine, Albert Einstein College of Medicine, Bronx, NY, USA; ⁴Department of Preventive Medicine and Biometrics, University of Colorado School of Medicine, Denver, CO, USA; ⁵Meharry Medical College, School of Medicine, Occupational and Preventive Medicine, Nashville, TN, USA; ⁶Division of Epidemiology and Biostatistics, School of Public Health, University of Illinois at Chicago, Chicago, IL, USA; ⁷Department of Epidemiology and Preventive Medicine, University of Maryland School of Medicine, Baltimore, MD, USA (*Author for correspondence)

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Abstract

Objectives: To explore the relationship between serum homocysteine, a sensitive biomarker for folate inadequacy and problems in one-carbon metabolism, and invasive cervical cancer.

Methods: A large case–control study was conducted in five US areas with up to two community controls, obtained by random-digit dialing, individually matched to each case. Cervical cancer risk factors were assessed through at-home interview. Blood was drawn at least 6 months after completion of cancer treatment from 51% and 68% of interviewed cases and controls. Serum homocysteine was measured by high-performance liquid chromatography, and exposure to human papillomavirus (HPV) type 16, the most prevalent oncogenic type, was assessed using an enzyme-linked immunosorbent assay. Cases with advanced cancer and/or receiving chemotherapy were excluded, leaving 183 cases and 540 controls.

Results: Invasive cervical cancer risk was substantially elevated for women in the upper three homocysteine quartiles (> 6.31 μmol/L); multivariate-adjusted odds ratios ranged from 2.4 to 3.2 (all 95% CIs excluded 1.0). A trend was apparent and significant (p = 0.01). When cases were compared with HPV-16 seropositive controls only, odds ratios were comparable.

Conclusions: Serum homocysteine was strongly and significantly predictive of invasive cervical cancer risk. This association could reflect folate, B₁₂ and/or B₆ inadequacy, or genetic polymorphisms affecting one-carbon metabolism.

Introduction

For the past 25 years there has been credible speculation that folate inadequacy might be a risk factor for cervical neoplasia [1, 2]. However, observational studies of both preinvasive and invasive cervical neoplasia have produced conflicting results [3–14] and treatment trials of dysplasia with folate supplements have had mixed success [2, 15, 16].

Serum homocysteine is a sensitive indicator of folate status [17] and an emerging biomarker of folate inadequacy, as well as other problems in one-carbon metabolism [17–19]. Folate is essential for one-carbon metabolism, which encompasses amino acid metabolism, purine and pyrimidine synthesis, and formation of S-adenosylmethionine, the agent primarily responsible for methylation of DNA [20]. Disruption of one-carbon metabolism can interfere with DNA synthesis, repair,
and methylation and thus promote carcinogenesis [21]. In addition, chromosomal fragile sites, which can be caused by folate inadequacy, may also be involved in cancer [22]. The human papillomavirus (HPV), the major causal agent of cervical cancer [23, 24], may integrate into the host genome at or near fragile sites [25–27], providing another mechanism whereby folate and one-carbon metabolism may influence cervical carcinogenesis.

Efficient one-carbon metabolism requires not only folate, but also adequate levels of several other vitamins and optimal activity of several enzymes. In one-carbon metabolism, homocysteine accepts a one-carbon group from folate to form methionine in a vitamin B_{12}-requiring reaction or, alternatively, homocysteine is degraded in a vitamin B_{6}-requiring reaction [28]. Impairment of either pathway may result in the accumulation of homocysteine [28]. In human populations, elevated levels of homocysteine have been associated with low levels of folate, vitamin B_{12}, and vitamin B_{6} [19, 29, 30], and with a common polymorphism (677C→T) in the methylenetetrahydrofolate reductase (MTHFR) gene, which reduces enzyme activity [18]. Thus, serum homocysteine can be a sensitive, integrative biomarker of disruption in one-carbon metabolism [17–19, 28].

Because of continued interest in diet and cervical neoplasia, and in order to evaluate directly the importance of efficient one-carbon metabolism in the etiology of the disease, we examined the association between serum homocysteine levels and risk of invasive cervical cancer in a large, multicenter, community-based case–control study in US women.

Methods

Study design

Eligible subjects were all incident cases of histologically confirmed, primary invasive cervical cancer, aged 20–74 years, identified between April 1982 and January 1984 in five US areas reporting to the Comprehensive Cancer Patient Data System. The study sites were centered around Birmingham, AL; Chicago, IL; Denver, CO; Miami, FL; and Philadelphia, PA. Up to two potential controls, matched on age (±5 years), ethnicity (white, black, Hispanic), and neighborhood (first six digits of a 10-digit telephone exchange), were identified by random-digit dialing for each case. Trained staff conducted interviews in the subjects’ homes with structured questionnaires to obtain detailed information on demographic characteristics, sexual behavior, reproductive and menstrual history, exogenous hormone use, personal and familial medical history, smoking, and diet. The methodology for the overall study has been described [31, 32].

Blood samples were drawn at least 6 months after completion of any treatment for cervical cancer (days after treatment: 10th, 50th, 90th percentiles = 190, 342, 669 days, respectively). Treatment included surgery (44%), radiation (18%), or both (28%). A small percentage of subjects (4%) also received chemotherapy (6% of subjects were missing treatment information). The blood was allowed to clot at room temperature for 40–60 minutes before being centrifuged, and serum samples were stored at −70 °C.

All study participants provided informed written consent prior to study initiation. The study was approved by the Institutional Review Boards of the National Cancer Institute and the five participating study centers.

Participation rates

A total of 1281 women were interviewed (480 of 658 eligible cases and 801 of 1114 eligible controls). Blood was obtained from 245 cases and 545 controls (51% and 68% of those interviewed, respectively). Reasons for non-participation in the blood draw included death (17% of cases, 0.4% of controls), contact and scheduling difficulties (15%, 17%), subject refusal (9%, 13%), hospital refusal (6%, 0%), cases who were not yet 6 months post-treatment at the completion of the study (2%, 0%), and unsuccessful blood draws (2%, 1%).

Cases who received chemotherapy treatment (n = 11) and/or who had advanced (stage III or IV) cervical cancer (n = 17) were excluded from the epidemiologic analyses to minimize the possibility that advanced disease or poor health would influence the results. Also excluded were cases with non-squamous cell cervical cancer (n = 28), and women whose ethnicity was other than white, black, or Hispanic (seven cases, two controls). Three cases and one control had insufficient serum for the homocysteine assay, and serologic HPV data were unavailable for one case and one control. These exclusions were not mutually exclusive. Included in the epidemiologic analyses were 183 cases and 540 controls.

Laboratory methods

Homocysteine. Serum homocysteine analyses were conducted using a modification of a high-performance liquid chromatography method [33]. Cases and their matched controls were assayed consecutively within the