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Serum concentrations of folate vitamers in patients with a newly diagnosed prostate cancer or hyperplasia

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ABSTRACT

Background: Folate is required for synthesis of methyl groups and DNA in growing cells. The association between folate and prostate cancer (PCa) is not conclusive.
Methods: We investigated concentrations of folate vitamers, S-adenosylhomocysteine (SAH) and S-adenosylmethionine (SAM) in blood of men with PCa (n = 129) or benign prostatic hyperplasia (BPH) (n = 73) who were recruited just after the first diagnosis.
Results: In younger subjects < 65 years, concentrations of (6S)-5-CH_3-H_4folate (15.3 vs. 17.7 nmol/L) or total folate (UPLC-MS/MS) (18.7 vs. 23.0 nmol/L) did not differ between men with BPH and those with PCa, while SAM was higher in the controls (128 vs. 116 nmol/L). Younger patients with low- and high grade cancer did not differ in (6S)-5-CH_3-H_4folate (17.8 vs. 17.3 nmol/L) or total folate (UPLC-MS/MS) (22.9 vs. 23.3 nmol/L), but SAM was lower in patients with low grade PCa (111 vs. 126 nmol/L). In the older group ≥65 years, (6S)-5-CH_3-H_4folate (18.4 vs. 18.2 nmol/L) and total folate (UPLC-MS/MS) (22.5 vs. 22.1 nmol/L) did not differ between BPH and PCa. Older patients with advanced tumors had lower (6S)-5-CH_3-H_4folate compared with those with low grade tumor (12.8 vs. 20.0 nmol/L: p = 0.013). Plasma SAM was not different between older patients and controls and was not related to PCa grade.
Conclusions: Lowered serum methyl folate measured at the time of diagnosis in older patients with advanced PCa, and lowered plasma SAM in younger patients with low grade PCa suggest differential folate metabolism that may have mechanistic, prognostic or predictive values.

1. Introduction

Prostate cancer (PCa) is one of the most common cancers in men. Tumor cells are postulated to have high requirements for the nutrient folate to provide methyl groups and synthesize purines and pyrimidines. The relationship between cancer and intake or concentrations of serum or red blood cell (RBC) folate has been controversially discussed over the last two decades, in particular because of the potential harms of mandatory folic acid fortification programs.

The results on the association between folate intake (including folic acid) or markers and the risk of future PCa are not consistent [5,6,11,21–23,29]. A meta-analysis of randomized controlled trials using folic acid supplementation has shown a borderline increased risk for incident cancer (all types) with folic acid supplementation [33]. Another meta-analysis of 10 prospective studies suggested that higher serum concentrations of folate, but not folate intake, were associated with a higher risk of PCa [32]. Inconsistencies in the results is discussed to be due to a dual role of folate in carcinogenesis [23] or to folate being an effect modifier in the relationship between other causes (i.e., smoking) and cancer. Observational and interventional studies in humans have suggested that high folate could be protective against mutagenesis, while it may promote the growth of preexisting tumors [2,13]. Folate intake is expected to be stable in populations without strong seasonal variations in food availability. Theoretically, folate demands increase over the course of cancer. Therefore, blood folate biomarkers may vary during the progression of cancer and according to tumor stage. Independent on the question of causality, plasma folate markers at diagnosis and their relation to the tumor severity could

Abbreviations: BPH, benign prostatic hyperplasia; NHANES, National Health and Nutrition Examination Survey; PCa, prostate cancer; PSA, prostate specific antigen; RBC-folate, red blood cell folate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; tHcy, total homocysteine; UPLC-MS/MS, ultra-performance liquid chromatography tandem mass spectrometry

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provide clue to folate homeostasis in patients with established cancer.

Mixed results were also reported in studies from countries without mandatory fortification with folic acid although serum folate levels were low (10–15 nmol/L) compared to countries applying fortification [21]. The value of serum folate as a predictor for future cancer is limited by several sources of biological and experimental variations [34]. The majority of the studies did not distinguish between different folate forms or free folic acid that has been suggested to increase the risk of cancer. Data on folate species just at diagnosis of cancer are not available. Plasma concentrations of methylation markers such as S-adenosylhomocysteine (SAH) and S-adenosylmethionine (SAM) that are assumed to play a mechanistic role in folate and cancer relationship have not been studied in patients with PCa.

The aim of the present study was to investigate concentrations of main forms of serum folate [(6S)-5-CH_3-H_4folate, (6S)-H_4folate, (6S)-5-HCO-H_4folate, (6R)-5,10-CH^+-H_4folate, and free folic acid] in addition to plasma SAH and SAM in samples collected prior to prostatectomy and to relate these concentrations to tumor grade.

2. Subjects and methods

Between July 2012 and March 2013, 253 consecutive newly admitted cases were recruited from the Department of Urology, Saarland University Hospital, Germany. The participants (age ≥ 50 years) had either a primary PCa or a benign prostatic hyperplasia (BPH). Exclusion criteria were renal failure, liver disease, chronic alcohol consumption, metastases, vitamin B supplementation, and methotrexate- or hormonal therapy. The current study included 129 patients with PCa and 73 controls with BPH. Fifty-one men were later excluded because of exclusion criteria: 12 patients had glomerular filtration rate (GFR) < 50 mL/min, 2 patients had liver cirrhosis, 2 patients had metastases, 2 used B-vitamins, 17 had other co-existing cancers, one patient had methotrexate therapy, and 15 had hormonal therapy.

One day before the prostatectomy upon admission to the hospital, blood samples were collected using tubes containing K^+ EDTA and in those without anticoagulant. The blood specimens were immediately placed on ice, centrifuged and separated within 30 min. For the measurement of SAH and SAM, 500 µL of the EDTA plasma was stabilized using 50 µL of 1 N acetic acid. All samples were stored at −70 °C until analysis. The blood measurements were performed using aliquots that were not thawed before. The results of the routine blood tests at admission and the pathology results of the prostatectomy specimens (Gleason score) were collected.

Classification of prostate cancer grade according to Gleason system [9] was used. Prostatectomy specimens are examined and scored (from 1 to 5) according to cell pattern. The grade increases with increasing malignancy level and therefore cancer aggressiveness [7]. The Gleason score represents the sum of the most and second most predominant Gleason grades present in the tissue section. Revised Gleason groups were recently introduced [8]. We stratified the patients according to Gleason score into low- (Gleason score ≤3 + 4), and high-grade (Gleason score ≥4 + 3).

The study was conducted according to the ethical principles for medical research involving human subjects stated in Helsinki Declaration. The study protocol was reviewed and approved by the medical ethics commission of the Saarland region and all participants provided a signed consent to the study.

The quantification of total serum folate was performed using ARCHITECT immunoassay (Abbott, Wiesbaden, Germany). The between-day coefficient of variation (CV) for the ARCHITECT folate assay was ≤6.0%. The individual folate forms were measured in serum samples using an Acquity Ultra Performance LC system coupled to a MicroMass Quattro Premier XE tandem quadrupole mass spectrometer (UPLC-MS/MS) (Waters Corporation, Milford, MA, USA) as described earlier [15,18]. The method enables the quantification of key folate forms [(6S)-5-CH_3-H_4folate, (6S)-H_4folate, (6S)-5-HCO-H_4folate, (6R)-5,10-CH^+-H_4folate, and folic acid]. The between-day CVs for (6S)-5-CH_3-H_4folate ranged between 2%-in pool serum (at 13.1 nmol/L) and 6% or 11.2% in quality control samples of pure substance (at 80 and 0.6 nmol/L, respectively). The lower limits for detection are: ≥0.91 nmol/L for (6S)-H_4folate, ≥0.10 nmol/L for (6S)-5-HCO-H_4folate, ≥0.16 nmol/L for (6R)-5,10-CH^+-H_4folate, and ≥0.21 nmol/L for folic acid. The mathematical sum of the individual forms was calculated and expressed as total folate (UPLC-MS/MS).

The concentrations of SAM and SAH were measured in acidified EDTA-plasma samples by using an established UPLC-MS/MS method [14]. Serum total homocysteine (tHcy) was determined by gas chromatography–mass spectrometry [26]. Concentrations of plasma creatinine and serum total prostate specific antigen (PSA) were determined by using automated methods on Hitachi Cobas® 8000 modular device (Roche Diagnostics, Mannheim, Germany). The between day CVs for the SAH, SAM, tHcy, creatinine and PSA assays are < 10%.

Statistical analyses were performed using SPSS (Statistical Package for the Social Sciences, version 22.0). We observed age-dependency of several markers of one-carbon metabolism (Table 2). Therefore, we analyzed the data after adjusting for age or after separation according to age (< 65 years or ≥65 years) (Tables 2–3). To assess the relationship between the tumor grade and the metabolic biomarker, patients with PCa were divided according to Gleason score into two groups; low-grade (≤3 + 4) and high-grade (≥4 + 3). All variables were not normally distributed and were log-transformed before applying the statistical analyses. The results are presented as mean ± SD. *t*-test for independent samples was used to study differences in continuous variables between independent groups. Chi-square test was used to compare categorical variables. Spearman Rho test was applied to investigate correlations between continuous variables. *t*-test for paired samples was used to compare serum folate concentrations using the two indicated methods. The difference in folate forms has not been reported before in patients with PCa or according to tumor grade. Therefore, the sample size was not estimated prior to the study and the present study is considered exploratory in nature. All tests were 2-sided and p values < 0.05 were considered to be statistically significant.

3. Results

Table 1 shows the main characteristics of the participants with BPH or PCa, as well as the concentrations of folate biomarkers. The controls were older than the cases (mean age 70 vs. 65 years; $p < 0.001$). Serum concentrations of PSA were higher (11.4 vs. 4.6 ng/mL) and those of creatinine were lower (85.2 vs. 91.5 µmol/L; $p = 0.018$) in patients with PCa compared with the controls.

Plasma concentrations of tHcy tended to be lower in the patients (15.7 vs. 17.1 µmol/L; $p = 0.092$), plasma SAM was lower in the patients (125 vs. 138 nmol/L; $p = 0.002$), while none of the folate forms or the total folate (ARCHITECT or UPLC-MS/MS) differed between patients and controls. The differences in concentrations of tHcy, SAM and folate between the patients and the controls were not significant after adjusting for age and creatinine (Table 1). Moreover, the percentages of subjects with detectable amounts of (6S)-5-HCO-H_4folate, (6R)-5,10-CH^+-H_4folate, (6S)-H_4folate, or free folic acid in serum (> the corresponding detection limits) were not different between the patients and the controls (Supplemental Fig. S1).

Thirty nine of the PCa patients (30%) had high grade cancer (Gleason score ≥4 + 3) (Table 1). Serum concentrations of (6S)-5-CH_3-H_4folate (15.3 vs. 19.3 nmol/L; $p = 0.024$), total folate (ARCHITECT) (19.7 vs. 22.8 nmol/L; $p = 0.047$), and total folate (UPLC-MS/MS) (19.7 vs. 24.1 nmol/L; $p = 0.046$) were lower in patients with high grade tumor compared with those with low grade tumor.

The methylation and folate biomarkers were studied according to age (Table 2). The mean plasma concentrations of tHcy did not differ between old and young control men (17.4 vs. 16.2 µmol/L, $p = 0.203$), but were significantly higher in older than in younger PCa patients