



High serum folate is associated with reduced biochemical recurrence after radical prostatectomy: Results from the SEARCH Database

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ABSTRACT

Introduction: To analyze the association between serum levels of folate and risk of biochemical recurrence after radical prostatectomy among men from the Shared Equal Access Regional Cancer Hospital (SEARCH) database.

Materials and Methods: Retrospective analysis of 135 subjects from the SEARCH database treated between 1991-2009 with available preoperative serum folate levels. Patients' characteristics at the time of the surgery were analyzed with ranksum and linear regression. Uni- and multivariable analyses of folate levels (log-transformed) and time to biochemical recurrence were performed with Cox proportional hazards.

Results: The median preoperative folate level was 11.6ng/mL (reference = 1.5-20.0ng/mL). Folate levels were significantly lower among African-American men than Caucasians (P = 0.003). In univariable analysis, higher folate levels were associated with more recent year of surgery (P < 0.001) and lower preoperative PSA (P = 0.003). In univariable analysis, there was a trend towards lower risk of biochemical recurrence among men with high folate levels (HR = 0.61, 95%CI = 0.37-1.03, P = 0.064). After adjustments for patients characteristics' and pre- and post-operative clinical and pathological findings, higher serum levels of folate were independently associated with lower risk for biochemical recurrence (HR = 0.42, 95%CI = 0.20-0.89, P = 0.023).

Conclusion: In a cohort of men undergoing radical prostatectomy at several VAs across the country, higher serum folate levels were associated with lower PSA and lower risk for biochemical failure. While the source of the folate in the serum in this study is unknown (i.e. diet vs. supplement), these findings, if confirmed, suggest a potential role of folic acid supplementation or increased consumption of folate rich foods to reduce the risk of recurrence.

ARTICLE INFO

Key words:

Prostatic Neoplasms;
Prostatectomy;
Prostate-Specific Antigen;
Disease-Free Survival

Int Braz J Urol. 2013; 39: 312-9

Submitted for publication:
January 01, 2011

Accepted after revision:
November 09, 2011

INTRODUCTION

Folate is a water-soluble B vitamin essential to innumerable bodily functions including nucleotide synthesis. Folate, the natural occurring form, can be found in green leafy vegetables such as spinach, broccoli and turnip greens. Folic acid is the synthetic form of folate used in vitamin supplements and in fortified foods. Since 1998, many countries, including the United States, have implemented mandatory folic acid fortification of flour and grain products to reduce the risk of neural-tube birth defects.

The human body needs folate to synthesize, repair and methylate DNA (1). Impairment to any of these functions may contribute to carcinogenesis. Indeed, folate deficiency has been implicated in the development of several tumors, including pancreatic, cervical and colon cancers (2). However, the role of folate in prostate cancer carcinogenesis is very controversial (3). There is evidence suggesting dietary folate may be protective against prostate cancer while folic acid supplementation may promote cancer (4). To date, no studies have examined the role of folate in prostate cancer recurrence after primary treatment. Therefore, we sought to analyze the association between serum folate levels and risk of biochemical recurrence after radical prostatectomy among men from the Shared Equal Access Regional Cancer Hospital (SEARCH) database.

MATERIALS AND METHODS

Study population

After obtaining Institutional Review Board approval from each institution, data from patients undergoing radical prostatectomy between 1991 and 2009 at 3 Veteran Affairs Medical Center (West Los Angeles and Palo Alto, CA and Durham, NC) were combined into the SEARCH database (5). The database includes information on patient age at surgery, race, height, weight, clinical stage, cancer grade on diagnostic biopsies, preoperative PSA, surgical specimen pathology (specimen weight, tumor grade, stage and surgical margin status) and follow-up PSA. Patients treated with preoperative hormonal therapy or radiotherapy were

excluded from the study. Of 1,596 patients in SEARCH from these 3 sites, 162 (10%) had preoperative folate levels available. We excluded 6 (4%) patients due to missing follow-up, 19 (12%) due to missing covariates and 2 (1%) due to very high folate levels ($> 60\text{ng/mL}$). This resulted in a study population of 135 subjects. Folate levels were determined by retrospective chart review. No banked sera were available to measure these levels on the other men. Only folate levels obtained within one year prior to surgery were considered. All patients were followed with serial PSA determinations and clinical visits at intervals according to attending physician discretion. Biochemical recurrence was defined as a single PSA above 0.2ng/mL , 2 concentrations at 0.2ng/mL or secondary treatment for an elevated PSA. Additional treatment after surgery was at the judgment of the patient and treating physician.

Statistical analysis

As folate levels were not normally distributed, folate was examined after logarithmic transformation. The association of folate levels with patients' characteristics at the time of the surgery and tumor features, such as race (Caucasian, African American, other), body-mass index (BMI, continuous, log-transformed), age at surgery (continuous), year of surgery (continuous), surgical center (1-3), preoperative PSA (continuous, log-transformed), surgical margin status (positive or negative), extracapsular extension (present or absent), seminal vesicle invasion (yes or no) and pathological Gleason score (2-6, 3+4, 4+3 and 8-10), were all analyzed using linear regression with folate being the outcome variable and the various patient characteristics being the predictor variable. Uni- and multivariable analyses of time to biochemical recurrence were performed with Cox proportional hazards. We adjusted our multivariable models for patient demographics, clinical and pathological findings (as described above). All statistical analyses were two-tailed and performed using Stata 10.1 (StataCorp, College Station, TX) and R 2.11.1 (R Foundation for Statistical Computing, Vienna, Austria). A $P < 0.05$ was considered to indicate statistical significance.

RESULTS

In the study population, the median (interquartile range [IQR]) of preoperative folate levels was 11.6ng/mL (7.3-18.5). The reference values for folate levels were 1.5-20.0ng/mL. The mean age was 62.4 years. There was a similar proportion of white (45%) and black men (49%; Table-1).

Preoperative folate levels were significantly higher among white men than black men ($P = 0.003$). In addition, higher folate levels were asso-

ciated with more recent year of surgery ($P < 0.001$) and lower preoperative PSA ($P = 0.003$). Given that year and PSA track together, we adjusted the results for year and found a trend towards lower PSA in patients with higher serum folate levels ($P = 0.069$). Age and BMI were unrelated to folate levels. Also, no significant associations were observed between folate levels and pathological features such as Gleason score, positive surgical margins, extracapsular extension or seminal vesicle invasion (Table-2).

The median follow-up was 36 months. During this time, 42 (31%) patients develop a biochemical recurrence. In univariable analysis, there was a trend towards lower risk of biochemical recurrence among men with high folate levels (HR = 0.61, 95%CI = 0.37-1.03, $P = 0.064$). After adjustments for patients characteristics' and pre- and post-operative clinical and pathological findings, higher serum levels of folate were independently associated with lower risk for biochemical recurrence (HR = 0.42, 95%CI 0.20-0.89, $P = 0.023$, Table-3).

Table 1 - Patient demographics and tumor characteristics.

Variable	Mean±SD or N (%)
Folate (ng/mL)	12.9 ± 7.1
Age at surgery (years)	62.4 ± 6.3
Race	
White	61 (45%)
Black	66 (49%)
Others	8 (6%)
BMI (kg/m ²)	27.0 ± 4.5
Preoperative PSA (ng/mL)	8.5 ± 6.1
Year of surgery (years)	2002 ± 4.6
Pathological Gleason score	
2-6	48 (35%)
3+4	59 (44%)
4+3	17 (13%)
8-10	11 (8%)
Positive surgical margins	59 (44%)
Extracapsular extension	24 (18%)
Seminal vesicle invasion	14 (10%)

BMI: Body mass index, **PSA:** prostate-specific antigen, **SD:** Standard deviation.

DISCUSSION

Data concerning the association between folate and prostate cancer is limited, dispersed and controversial (6,7). Several studies examined the association between folate and prostate cancer risk with mixed results. For example, in a small case-control study by Hultdin et al., higher levels of folate were statistically significantly associated with increased prostate cancer risk (OR = 1.60; $P = 0.02$) (8). Results from a large randomized trial comparing folic acid supplementation versus placebo for reduction of colon adenoma development showed a secondary outcome of higher risk of prostate cancer in men receiving folic acid (4). Conversely, an Italian case-control study found lower folate intake (measure by questionnaire) was associated with higher risk of prostate cancer (9). Findings from the American Cancer Society Cancer Prevention Study II Nutrition Cohort demonstrated that neither dietary nor total folate intake were associated with incidence of prostate cancer (10). Moreover, higher folate serum levels were associated with a nonsignificant decrease risk of advanced prostate cancer. Data from the European Prospective Investigation into

Table 2 - Association of serum folate levels with patient and tumor characteristics.

Variable	Coefficient	P
Age at surgery (years)	0.00	0.857
Race		
White	ref	-
Black	-0.27	0.004
Others	-0.01	0.969
BMI (log[kg/m ²])	0.32	0.264
Preoperative PSA (log[ng/mL])	-0.17	0.007
Year of surgery (years)	0.04	< 0.001
Pathological Gleason score		
2-6	ref	-
3+4	-0.05	0.609
4+3	-0.14	0.369
8-10	0.16	0.376
Positive surgical margins	0.12	0.213
Extracapsular extension	0.03	0.784
Seminal vesicle invasion	-0.24	0.118

BMI: Body mass index, **PSA:** prostate-specific antigen.

Cancer and Nutrition Study, where 869 cases and 1,174 controls had serum folate level analyzed, showed no significant association between serum folate and prostate cancer risk (11). Additionally, in a U.K. population-based matched case-control study with nearly 3,000 subjects, serum folate levels were not associated with prostate cancer (12). In the same manuscript, they performed a meta-analysis of 7 studies and also found no correlation between serum folate and risk of prostate cancer. Thus, while the association between folate and prostate cancer risk is controversial and remains the subject of intense research, no studies

examined the association between folate and disease recurrence after primary treatment for localized prostate cancer. Therefore, in the present study we analyzed the association between serum folate levels and biochemical recurrence after radical prostatectomy.

In a cohort of men undergoing radical prostatectomy at several Veteran Affairs Medical Centers across the country, we found serum folate levels were associated with more recent year of surgery. This finding can be explained by the fortification of the US diet with folate since 1998 (13). We also observed that higher folate levels were associated with trends toward lower PSA levels even after adjusting for the more recent year of surgery. This result is in agreement with data from Stevens et al. who found a trend between higher dietary folate and lower risk of advanced prostate cancer at diagnosis (10). Finally, we found higher serum folate levels were independently associated with decreased risk of biochemical recurrence. As no prior study specifically examined this question, further validation of our findings is necessary. However, these findings are consistent with data from the American Cancer Society Cancer Prevention Study II Nutrition Cohort, which suggested that higher folate intake was associated with lower risk of advanced prostate cancer, though they did not look at outcomes after diagnosis in that study (10). In the U.K. population-based matched case-control study with nearly 3,000 subjects opposite results were found (12). Specifically, in that study, higher folate levels were associated with increased PSA velocity after diagnosis of prostate cancer suggesting folate was associated with faster prostate cancer progression. Again, disease progression such as recurrence after treatment, metastasis or death were not evaluated. Thus, the currently available evidence on folate and prostate cancer progression is limited and somewhat controversial. Studies looking at the association of folate and cancer progression including metastasis and disease-specific mortality are needed. Nevertheless, our results, if confirmed in larger studies, support the potential role of folic acid supplementation or increased dietary intake of folate rich foods in patients with prostate cancer undergoing radical prostatectomy to reduce the risk of recurrence.

Table 3 - Univariable and multivariable predictors of biochemical recurrence.

Variable	Univariable analysis			Multivariable analysis [†]		
	HR	95%CI	P	HR	95%CI	P
Folate (log[ng/mL])	0.61	0.37-1.03	0.064	0.42	0.20-0.89	0.023
Age at surgery (years)	1.04	0.99-1.09	0.132	1.10	1.02-1.76	0.011
Race						
White	ref.	-	-	ref.	-	-
Black	1.64	0.84-3.19	0.148	1.36	0.63-2.96	0.431
Others	0.74	0.16-3.40	0.695	2.88	0.52-15.99	0.228
BMI (log[kg/m ²])	1.47	0.25-8.55	0.667	11.32	0.95-134.89	0.055
Preoperative PSA (log[ng/mL])	2.06	1.30-3.27	0.002	1.57	0.82-3.01	0.172
Year of surgery (years)	1.03	0.95-1.11	0.445	1.17	1.06-1.29	0.003
Pathological Gleason score						
2-6	ref.	-	-	ref.	-	-
3+4	4.99	1.89-13.22	0.001	4.19	1.30-13.55	0.017
4+3	6.96	2.27-21.38	0.001	3.81	1.01-14.42	0.049
8-10	9.09	2.74-30.08	<0.001	5.87	1.37-24.99	0.017
Positive surgical margins	2.90	1.52-5.51	0.001	3.84	1.71-8.68	0.001
Extracapsular extension	2.85	1.48-5.47	0.002	2.06	0.83-5.10	0.117
Seminal vesicle invasion	2.97	1.40-6.28	0.019	3.30	1.15-9.45	0.026

[†]Adjusted for center

BMI: body mass index, **CI:** confidence interval, **PSA:** prostate-specific antigen.

It is important to highlight that dietary folate manipulation is still very controversial (6). From a biochemical standpoint, reduction of folate by antifolate drugs (e.g. methotrexate) can decrease cancer cell proliferation but it may induce DNA damage which, in turn, can lead to carcinogenesis (14,15). Alternatively, folic acid supplementation may prevent malignant cell transformation on the

one hand, but maintain the high cell proliferation rate that is characteristic in neoplastic cells on the other (16-18). From an epidemiological perspective, the largest randomized trial comparing folic acid supplementation versus placebo showed a higher risk of prostate cancer in men receiving folic acid supplementation, though this was a secondary outcome (4). The same results were not

seen in large observational cohort studies where folate was not associated with prostate cancer (10,19). The current findings of lower recurrence risk among men with high serum folate levels, if confirmed in subsequent studies, would support future randomized trials, which are ultimately needed to determine the true effects of a folic acid supplementation in patients with prostate cancer.

Our study was limited by being retrospective in nature. As such, we could not prospectively measure folate levels, but rather relied on tests that had been done for some clinical reason. As such, folate was not available for the majority of patients undergoing radical prostatectomy. Thus, our sample represents only the selected few for which folate levels were obtained for other reasons (e.g. anemia work up), which may limit the generalizability of our results. This also led to a limited sample size (N = 162) which reduced the statistical power of the study. Also, given folate intake as supplement or in the diet is associated with other micronutrients, the association of folate levels and cancer outcomes may be confounded by these nutrients. Moreover, folate deficiency is associated with certain diseases such as alcoholism, malabsorption and chronic liver disease which could potentially act as confounding factors. Finally, as we assessed serum folate levels, we were unable to determine the source of the folate - dietary or via supplementation. Thus, it is possible that men who had higher folate levels, whether obtained from a high-folate diet or via supplementation, were more likely to have other behaviors associated with a healthy lifestyle (e.g. more exercise, healthier diet) which, in turn, may correlate with lower risk of disease progression after surgery. Therefore, we were not able to determine the causality in the association between folate with PSA and biochemical recurrence in the current study.

The strengths of the present study include the use of serum folate levels which reflect the total body folate better than folate intake alone. In addition, the detailed demographic and pathological data allowed us to control for these potential confounders.

In conclusion, among men undergoing radical prostatectomy at several Veteran Affairs Medical Centers across the country, higher serum fo-

late levels were associated with lower preoperative PSA and lower risk for biochemical failure. While the source of the folate in the serum in this study is unknown (i.e. diet vs. supplement), these findings if confirmed in future prospective studies, suggest a potential role of folic acid supplementation or increased intake of folate rich foods to reduce the risk of recurrence. However, further studies are required to determine whether folate supplementation or diet modification to increase folate intake can reduce prostate cancer progression.

CONFLICT OF INTEREST

None declared.

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EDITORIAL COMMENT

The authors present the correlation between serum folate levels and biochemical recurrence in a retrospective cohort of patients submitted to radical prostatectomy. The results suggest a potential beneficial effect of higher serum folate levels on reducing the risk of biochemical failure after surgery.

Folate, also named vitamin B9, is involved in synthesis, repair, and methylation of DNA. Folic acid is converted to folate in human body (1). Dietary factors, such as folate dietary intake, can trigger epigenetic mechanisms that could play a role in cancer development (1). Actually, a systematic review and meta-analysis of randomized controlled trials showed a significant, albeit border-

line, increase in incidence of overall cancer, and, especially, prostate cancer (1). On the other hand, the association between dietary or circulating levels of folate with prostate cancer risk was not demonstrated in other cohorts of patients (2,3).

Folate deficiency is implicated in developmental conditions such as neural tube defects in developing embryos (1). This is the reason why folic acid is added to foods in several countries, including Brazil. (RDC 344/02) Consequently, the potential role of folic acid supplementation in either promotion of or protection from the most incident male cancer, excluding skin cancer, is very important in terms of public health. Despite the limitations (a small, retrospective, and selected cohort of patients), the study represents a contribution to advance the knowledge of folate and prostate cancer relationship.

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