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Blood levels of folate at birth and risk of childhood leukemia

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Abstract

Background—A role for folate in cancer etiology has long been suspected due to folate's function as a cofactor in DNA methylation and maintenance of DNA synthesis. Previous case-control studies examining the association between risk of childhood acute lymphoblastic leukemia (ALL) and mothers' self-reported folate intake and supplementation have been inconclusive.

Materials and Methods—We utilized a quantitative microbiologic assay to measure newborn folate concentrations in archived dried bloodspots collected at birth from 313 incident ALL cases, 44 incident acute myeloid leukemia (AML) cases, and 405 matched population-based controls.

Results—Overall, we found no difference in hemoglobin-normalized newborn folate concentrations (HbFol, nmol/g) between ALL cases and controls (2.76 vs. 2.77, p=0.97) or between AML cases and controls (2.93 vs. 2.76, p=0.32). Null results persisted after stratification by both birth period (1982-94, 1995-98, and 1999-2002) to account for the start of folate fortification of grain products in the US, and by self-reported maternal pre-pregnancy supplement use. Similarly, no association was observed for major ALL subgroups.

Conclusions—Our results do not support an association between birth folate concentrations and risk of childhood AML or major ALL subgroups.

Impact—However, they do not rule out a role for folate through exposures after birth or in early stages of fetal development.

Keywords

childhood leukemia; dried blood spot specimens; California; folate

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Introduction

Leukemia is the most common cancer among children under 15 years of age, comprising 31% of all childhood cancers diagnosed in the U.S. (1). The etiology of childhood leukemia, however, is poorly understood. Due to the disease's early age of onset, prenatal and early life exposures are thought to play a major role in leukemogenesis.

Folate is an important micronutrient involved in both maintenance of DNA replication fidelity and provision of methyl groups for epigenetic control of DNA expression. Recent epidemiologic studies have examined the role of folate in the etiology of childhood acute lymphoblastic leukemia (ALL), the most common form of childhood leukemia (2-4). Some studies of genetic susceptibility to ALL provide evidence of association for folate pathway genes in both the child (5) and the mother (6). However, no associations were reported for risk of ALL in both a recent meta-analysis of folate supplementation during pregnancy (4) and our previous study of pre-pregnancy maternal dietary folate intake (2), although there does appear to be limited evidence of an inverse association between risk of ALL and maternal folate supplementation prior to pregnancy (4). No studies have reported on the potential association of folate with childhood acute myeloid leukemia (AML), which accounts for 15% of childhood leukemia cases.

The folate measures used in previous epidemiologic studies are indirect or incomplete measures of the folate exposure experienced by the developing fetus. Exposure of the fetus is influenced by (a) the mother's intake from both dietary sources and supplements, (b) the mother's folate transport and metabolism capacity, and (c) the fetus's own transport and metabolism capacity. It is possible that elucidating the etiologic role of folate in childhood ALL risk will require that these factors be accounted for concomitantly. This has not been done in the previous epidemiologic studies that have examined dietary factors and genes, and may have contributed to the current lack of clarity in the literature.

Additionally, mandatory fortification of flour and grains over the last two decades in the US (7) and elsewhere may have complicated efforts to study the potential effects of folate intake and/or supplementation on childhood leukemia risk.

To better address these concerns and directly assess the role of folate in childhood leukemia, we conducted a population-based case-control study in California examining levels of folate at birth in neonatal dried blood spot (DBS) specimens.

Materials and Methods

Study subjects and specimens

Cases of childhood ALL and AML were participants in the Northern California Childhood Leukemia Study (NCCLS), a previously described population-based case-control study (2, 8). Briefly, cases of leukemia diagnosed in participating hospitals in a defined California catchment area (17 California counties during 1995-1999, and later expanded to 35 counties during 2000-2002) were considered for inclusion. Case children were eligible for inclusion to the study if the child was under age 15 at the time of diagnosis, had an English- or Spanish-speaking parent, resided in the catchment area, and had no prior cancer diagnosis. Control children free of leukemia had to meet the same criteria as cases; controls were identified from birth certificate records maintained by the Office of Vital Records at the California Department of Public Health (CDPH), and matched to cases based on age, gender, child's Hispanic status (one or more parents reporting Hispanic ethnicity), and maternal race. The match ratio was approximately 1:1 during 1995-1999, and 1:2 during 2000-2002.

Biological mothers of cases and controls were interviewed in person to elicit information on demographic characteristics as well as exposures, including dietary intake using the Block food frequency questionnaire (FFQ) (9, 10). Based on the preference of the respondents, interviews were conducted in English or Spanish by trained bilingual interviewers; Spanish versions of the interview included culturally appropriate translations of the English interview, plus 7 additional food items important in the diets of the Spanish-speaking population (2). From the 76-item FFQ, levels of folate intake from both supplemental and dietary sources were determined and quantified in dietary folate equivalents (DFEs). Dietary folate included folate from natural and fortified dietary sources. Mothers were asked to report their usual diet and use of dietary supplements in the 12 months prior to pregnancy. This 12-month period was used because it represents the probable state of nutritional adequacy at the time of conception and during early pregnancy (i.e. the first trimester).

Participation rates for the interview among eligible cases and controls in the NCCLS were 87% and 86%, respectively. Of interviewed cases and controls, 90.4% and 100% were California-born, reflecting the selection of controls from birth records. Of these California-born subjects, neonatal dried blood spot (DBS) specimens archived by the Genetic Disease Screening Program at the CDPH were retrieved for 94.8% of cases and 89.1% of controls. Cases and controls included in the current study were those enrolled during 1995-2002 and had available DBS specimens. This resulted in 357 case subjects (313 ALL and 44 AML) and 405 control subjects.

This study was reviewed and approved by Institutional Review Boards at UC Berkeley, CDPH, and at participating hospitals. Informed consent was obtained from parents of participating children.

DBS folate assays

For each subject, a sample of a DBS specimen, corresponding to approximately 10-12 μl of whole blood, was extracted for folate analysis by the *Lactobacillus casei* microbiologic growth assay (11). Hemoglobin (Hb) concentration was measured in the same DBS extract using a sodium lauryl sulfate assay (12), and results were used to calculate HbFol (folate levels normalized to Hb, nmol/g), which is independent of the blood volume or blood dilution in the DBS. This metric can be converted to red blood cell (RBC) folate (nmol/L), the measure typically used to provide clinical interpretation of folate status, by multiplying HbFol with the mean corpuscular hemoglobin concentration (MCHC) of 345 g/L. The accuracy of the whole blood folate method has been established by confirming that the major folate form 5-methyltetrahydrofolic acid was nearly completely recovered (97%) when added to whole blood (13). The long-term stability of the method has been ensured by periodic measurement of a reference standard sample (National Institute for Biological Standards and Control 1st International Standard for Whole Blood Folate 95/528)(11). The validity of the DBS folate method has been established by comparing paired whole blood and DBS samples: good correlation (r^2=0.85) and agreement (HbFol concentrations in DBS were on average 6% lower than in whole blood) were obtained (14). The DBS folate assay work was all conducted in the same lab by staff masked to case-control status. To ensure the quality of folate and Hb measurements, three quality control (QC) pools of whole blood folate (8.6-11.6% inter-assay CV) and Hb (3.4-3.9% inter-assay CV) were analyzed in duplicate with each batch. To ensure the quality of the DBS extraction, two DBS QC pools were analyzed in duplicate with each batch (9.5-10.5% inter-assay CV). HbFol results for subject samples were only reported when all three sets of bench QC passed the predefined QC rules (15).

Statistical analysis

We compared means of log-transformed neonatal HbFol concentrations in cases with those in controls after adjusting for age, gender, race/ethnicity, year of birth, and income. We used analysis of variance methods as implemented in PROC GLM in SAS 9.2, estimating adjusted means using the LSMEANS statement and testing for significance of differences between cases and controls using T-tests. Presented means and standard errors were back-transformed to HbFol. For ALL, we also calculated means by major subgroups defined previously (16), including total B-cell ALL, B-cell ALL with high hyperdiploidy (>51 chromosomes), and total T-cell ALL. In addition, because of the low prevalence of most individual structural abnormalities (translocations, inversions, and deletions), we examined the broad category of B-cell ALL with “any structural abnormalities”, as structural abnormalities overall may be due to decreased fidelity of DNA replication. Mixed lineage leukemias (N=4) were excluded from subgroup analysis due to their rarity. To account for potential differences due to status of folate fortification programs in place at birth, we stratified by birth year: 1982-1994 (pre-fortification), 1995-1998 (fortification transition), and 1999-2002 (post-fortification). For similar reasons, we also stratified by maternal pre-pregnancy supplement use (yes/no).

Results

Characteristics of the 313 ALL cases, 44 AML cases, and 405 controls are presented in Table 1. As expected due to the matched design, cases and controls were comparable in terms of age, gender, and ethnicity. Control households tended to have higher income, a covariate that was adjusted for in the statistical analysis.

Tables 2 and 3 show the mean neonatal HbFol concentrations for ALL cases vs. controls and AML cases vs. controls, respectively, both overall, and stratified by birth period and maternal use of supplements prior to pregnancy. Overall, there was no difference in mean HbFol concentrations between either ALL or AML cases and controls. For both ALL and AML, this absence of effect persisted through all birth periods, and among groups defined by maternal pre-pregnancy supplement use, although the available sample size for these stratified analyses was limited for AML. Adjustment for birth weight and gestational age did not change the results appreciably (data not shown).

Table 4 presents results for the major studied ALL subgroups, specifically total B-cell ALL (n=282 cases), B-cell high hyperdiploid ALL (n=97 cases), B-cell ALL with structural changes (n=136 cases), and T-cell ALL (n=27 cases). The null effect observed for total ALL continued through all the B-cell subgroups. However, the mean HbFol concentration was significantly higher among the relatively small number of T-cell ALL cases vs. controls in our study population (3.41 vs. 2.79 nmol/g, p=0.005).

Separately, when we compared the mean HbFol among control children in the pre-fortification vs. post-fortification eras, we found no difference (p=0.985), indicating that folate fortification had little to no impact on neonatal folate levels.

Discussion

In this study, we aimed to assess whether a child’s folate concentration in neonatal DBS specimens, which we postulated was reflective of both the child’s folate exposure at the end of pregnancy (via maternal diet, supplementation, and other factors) and the child’s own folate metabolism, was associated with risk of childhood leukemia. We found no association of folate concentrations at birth with either total ALL or total AML. No significant differences in HbFol concentrations were observed between the controls and any of the major ALL subgroups, including total B-cell ALL, B-cell high hyperdiploid ALL, or B-cell ALL with structural abnormalities. Thus, our results indicate that folate concentrations at birth are not associated with childhood AML or major ALL subgroups. In the relatively small subgroup of T-cell ALL cases, however, we observed significantly higher folate levels than among controls. This provocative observation will need to be replicated in future studies with larger sample sizes.

Previous studies have examined self-reported maternal supplement use and/or maternal dietary folate intake in relation to childhood ALL risk (2-4, 17); while one found mild protective effects (17), others did not (2-4). The mixed findings of these dietary studies may be attributed to several factors, including population differences, different study settings and countries with different policies and practices regarding folate supplementation and fortification, as well as chance. A recent meta-analysis concluded that there is no effect of maternal folic acid supplementation during pregnancy on risk of childhood ALL (4). While the results of our study indicate that folate levels at birth are not associated with risk of subsequent childhood leukemia (with the possible exception of T-cell ALL), they do not rule out a role for maternal dietary folate or folate supplementation before or during early pregnancy (pre-conception or peri-conception), or potentially after birth.

When we converted HbFol to RBC folate concentrations using the MCHC, we found that all children but one (245 nmol/L) had RBC folate concentrations above the WHO recommended low folate threshold of 340 nmol/L, regardless whether the DBS samples were collected pre- or post-fortification (18). In contrast, prevalence estimates of low RBC folate among US adults during pre-fortification ranged from 2.1-4.5%, while post-fortification they were <1% (19). Indeed, it has been well established that newborns generally have high folate status (20), with transport of folate across the placenta being established early in pregnancy (21).

The meaning of our observation that T-cell ALL cases have slightly higher folate concentrations at birth than comparable controls is unclear. Although early high folate is typically viewed as a factor associated with either a reduced or null risk of various diseases, folate is also a possible promoter after cancer initiation due to the fast-growing cancer cell's need for folate (22), a fact that has been exploited with chemotherapeutic success for childhood leukemia and other cancers in the form of the folate poison methotrexate (23).

In our study, among cases and controls combined, we observed significant correlations of neonatal folate levels with the following surrogate folate measures: total maternal folate intake (supplements + food sources, Spearman correlation coefficient = 0.086, p=0.017) and maternal supplemental folic acid alone (Spearman correlation coefficient = 0.153, p<0.0001). Additionally, we observed that HbFol mean concentrations tended to be higher among children whose mothers reported using pre-pregnancy supplements than among those whose mothers did not, an effect that was true for all birth periods. These observations provide reassurance that the HbFol concentrations measured in neonatal blood are indeed affected by self-reported maternal intake and supplementation, and that these surrogate measures have some validity. That the correlation coefficients were very weak, however, suggests that other factors, including maternal genes, child's genes, and other perhaps unmeasured factors modulate neonatal folate concentrations. This is an area for future study.

This study has several unique strengths. The microbiologic folate assay method used in our study is accurate; indeed the accuracy of other folate assay methods is evaluated against the microbiologic assay (24, 25). While the DBS assay has higher imprecision than the whole blood assay, it allows the use of neonatal DBS specimens, which to our knowledge, no other studies to date have done. This is important because of the timing of collection of these specimens: the levels of folate reported here represent the folate status of the child at the end of pregnancy, a more direct measure of the child's pre-diagnostic folate exposure than other surrogate measures used previously, such as maternal folate supplementation before or during pregnancy. In addition, the matched case-control design mitigates concerns regarding potential confounding by racial or ethnic differences, particularly as childhood ALL incidence rates are higher in Hispanics than non-Hispanics in California (26).

We acknowledge that by comparing each subtype of cases to the same control group, we have conducted non-independent statistical tests, which might increase the risk of false positive associations. Nonetheless, the observations were null except for a significant finding for T-cell ALL at a relatively robust p=0.003. This finding is based on a relatively small sample size (n=27 cases), however, and may be due to chance. Our null findings for total AML and ALL and for major ALL subgroups, including total B-cell ALL, B-cell ALL with structural changes, and B-cell high hyperdiploid ALL, are robust. It is possible that a study that includes much larger numbers of the subtypes might observe significant differences; however, the adjusted mean estimates were not markedly different from one another. In addition, the low prevalence of folate insufficiency (RBC folate <340 nmol/L) may have precluded observation of putative effects attributable to extremely low folate levels.

In conclusion, in this case-control study using a biomarker of folate status, we found that folate concentrations at birth were not significantly associated with childhood AML or major subgroups of childhood ALL. These null findings, taken with our observation that newborns do not have low folate status at birth, suggest that any role played by folate in childhood leukemia risk is likely to occur either very early in the pregnancy while the child is *in utero* (i.e., the peri-conceptional period), or during the post-natal period resulting from the child's own exposures after birth. Future studies should characterize the influence of maternal and child factors, including genetic factors, on neonatal folate levels, and examine the effects of post-natal exposures to folate on childhood leukemia risk.

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Table 1

Characteristics of ALL cases, AML cases, and Controls, Northern California Childhood Leukemia Study, 1995-2002

Total	**ALL cases 313**	**AML cases 44**	**Controls 405**
Age			
Mean (SD)	5.4 (3.4)	6.1 (4.7)	5.3 (3.5)
Sex			
Male, N (%)	164 (52.4%)	26 (59.1%)	214 (52.8%)
Ethnicity			
Hispanic, N (%)	132 (42.2%)	15 (34.1%)	164 (40.5%)
Non-Hispanic White, N (%)	129 (41.2%)	16 (36.4%)	177 (43.7%)
Non-Hispanic Other, N (%)	52 (16.6%)	13 (29.5%)	64 (15.8%)
Birth year			
1982-1994, N (%)	147 (47.0%)	24 (54.6%)	194 (47.9%)
1995-1998, N (%)	130 (41.5%)	10 (22.7%)	173 (42.7%)
1999-2002, N (%)	36 (11.5%)	10 (22.7%)	38 (9.4%)
Annual household income			
<$15,000, N (%)	44 (14.1%)	10 (22.7%)	35 (8.6%)
$15,000-$29,999, N (%)	61 (19.5%)	2 (4.5%)	60 (14.8%)
$30,000-$44,999, N (%)	48 (15.3%)	12 (27.3%)	51 (12.6%)
$45,000-$59,999, N (%)	53 (16.9%)	3 (6.8%)	57 (14.1%)
$60,000-$74,999, N (%)	36 (11.5%)	3 (6.8%)	55 (13.6%)
≥$75,000, N (%)	71 (22.7%)	14 (31.9%)	147 (36.3%)

Table 2

Adjusted mean neonatal folate levels (in nmol/g of hemoglobin) in ALL cases and controls, by pre-pregnancy supplement use and birth period

	Regardless of supplement use			No supplement use (prepregnancy)			Supplement use (prepregnancy)		
	N[a]	Mean (SE)[b]	p-value[c]	N[a]	Mean (SE)[b]	p-value[c]	N[a]	Mean (SE)[b]	p-value[c]
All birth periods									
Case	313	2.76 (1.03)	0.969	224	2.62 (1.03)	0.891	87	3.10 (1.05)	0.980
Control	405	2.77 (1.02)		286	2.63 (1.03)		109	3.11 (1.04)	
1982-1994									
Case	147	2.73 (1.03)	0.818	106	2.64 (1.04)	0.763	39	3.06 (1.06)	0.709
Control	194	2.76 (1.03)		138	2.68 (1.03)		50	3.15 (1.06)	
1995-1998									
Case	130	3.00 (1.04)	0.320	93	2.89 (1.04)	0.713	37	3.40 (1.07)	0.309
Control	173	2.87 (1.03)		130	2.83 (1.04)		42	3.07 (1.07)	
1999-2002									
Case	36	2.65 (1.07)	0.353	25	2.44 (1.10)	0.755	11	2.79 (1.09)	0.166
Control	38	2.90 (2.05)		18	2.56 (1.14)		17	3.27 (1.07)	

[a]Data on supplement use was missing for 2 ALL cases and 10 controls

[b]Means and standard errors of hemoglobin-normalized folate levels (HbFol) in dried bloodspot specimens, adjusted for income, race/ethnicity (Hispanic, Non-Hispanic White, Non Hispanic Other), sex, age, and year of birth; back-transformed from log(HbFol)

[c]T-test p-values for pairwise differences in adjusted means

Table 3

Adjusted mean neonatal folate levels (in nmol/g of hemoglobin) in AML cases and controls, by pre-pregnancy supplement use and birth period

	Regardless of supplement use			No supplement use (prepregnancy)			Supplement use (prepregnancy)		
	N[a]	Mean (SE)[b]	p-value[c]	N[a]	Mean (SE)[b]	p-value[c]	N[a]	Mean (SE)[b]	p-value[c]
All birth periods									
Case	44	2.93 (1.06)	0.321	32	2.78 (1.07)	0.412	12	3.44 (1.05)	0.378
Control	405	2.76 (1.03)		286	2.63 (1.04)		109	3.14 (1.11)	
1982-1994									
Case	24	2.88 (1.08)	0.444	18	2.82 (1.09)	0.505	6	3.59 (1.18)	0.482
Control	194	2.72 (1.03)		138	2.66 (1.03)		50	3.21 (1.08)	
1995-1998									
Case	10	3.00 (1.13)	0.739	5	2.86 (1.19)	0.898	5	3.43 (1.21)	0.674
Control	173	2.88 (1.03)		130	2.80 (1.04)		42	3.15 (1.08)	
1999-2002									
Case	10	3.28 (1.12)	0.542	9	3.04 (1.15)	0.473	1	5.14 (1.23)	0.064
Control	38	3.02 (1.06)		18	2.65 (1.12)		17	3.26 (1.05)	

[a]Data on supplement use was missing for 10 controls

[b]Means and standard errors of hemoglobin-normalized folate levels (HbFol) in dried bloodspot specimens, adjusted for income, race/ethnicity (Hispanic, Non-Hispanic White, Non Hispanic Other), sex, age, and year of birth; back-transformed from log(HbFol)

[c]T-test p-values for pairwise differences in adjusted means

Table 4

Adjusted mean neonatal folate levels (in nmol/g of hemoglobin) in ALL cases and controls, by ALL subtype

	N	Mean (SE)[a]	p-value [b]
Total ALL			
Case	313	2.76 (1.03)	0.969
Control	405	2.77 (1.02)	
B-cell ALL			
Case	282	2.69 (1.03)	0.438
Control	405	2.75 (1.03)	
B-cell High Hyperdiploid ALL			
Case	97	2.87 (1.04)	0.534
Control	405	2.79 (1.03)	
B-cell Structural Change ALL			
Case	136	2.71 (1.03)	0.342
Control	405	2.81 (1.04)	
T-cell ALL			
Case	27	3.41 (1.07)	**0.005**
Control	405	2.79 (1.03)	

[a] Means and standard errors of hemoglobin-normalized folate levels (HbFol) in dried bloodspot specimens, adjusted for income, race/ethnicity (Hispanic, Non-Hispanic White, Non Hispanic Other), sex, age, and year of birth; back-transformed from log(HbFol)

[b] T-test p-values for pairwise differences in adjusted means