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Pediatric germ cell tumors and maternal vitamin supplementation: A Children's Oncology Group Study

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Abstract

Maternal vitamin supplementation has been linked to a reduced risk of several pediatric malignancies. We examined this relationship in a study of childhood germ cell tumors (GCTs). Subjects included 278 GCT cases diagnosed <15 years during 1993-2001 at a United States or Canadian Children's Oncology Group Institution and 423 controls that were ascertained through random digit dialing matched to cases on sex, and age within one year. Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% Confidence Intervals (CIs) for the association between GCTs and maternal vitamin use at several time points during and around pregnancy. In models controlling for the child's age, sex, household income, and maternal education, any maternal vitamin use during the 6 months prior to conception through nursing was associated with a non-significant reduced risk of GCTs (OR=0.7; 95% CI 0.0.4-1.2). Inverse associations were observed for both extragonadal (OR=0.8; 95% CI 0.4-1.6) and gonadal (OR=0.6; 95% CI 0.3-1.1) tumors and for dysgerminoma/seminoma (OR=0.6; 95% CI 0.2-1.3) and teratoma (OR=0.5; 95% CI 0.2-0.9) but not yolk sac tumors (OR=1.1; 95% CI 0.5-2.3). No consistent patterns were found with respect to vitamin use during the periconceptional period (6 months before pregnancy and first trimester) or first trimester specifically. In conclusion, while our study suggests that maternal vitamin supplementation may reduce the risk or pediatric GCTs in the offspring, the small study size and limitations inherent to observational studies must be considered when interpreting these results.

Keywords

germ cell tumors; pediatrics; prenatal vitamins; folic acid; risk factors

Introduction

Pediatric germ cell tumors (GCTs) are rare malignancies affecting approximately 340 children less than 15 years of age every year in the United States (1,2)[1]. Incidence rates vary by sex and race with females more often affected than males, and whites, Asian/Pacific Islanders, and

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Spanish/Hispanic/Latinos more often affected than Black race/ethnicities (3). GCT incidence rates also show a bimodal distribution by age with peaks during infancy and adolescence in both sexes.

GCTs resemble tissues of normal early human development and are classified into two major histologic types, seminomas/dysgerminomas and nonseminomas, which can occur at either extragonadal or gonadal locations. Seminomas/dysgerminomas are rare before puberty and are thought to result from direct transformation of primordial germ cells (4,5). In contrast, nonseminomas, which are comprised of teratomas, yolk sac tumors, and choriocarcinomas, are most common during early childhood and are thought to arise from spontaneous differentiation of embryonal carcinomas that develop from primordial germ cells. Teratomas have differentiated along somatic cell lineages and often contain all three germ cell layers, while yolk sac tumors (endodermal sinus tumors) and choriocarcinomas have differentiated along extraembryonic cell lineages and contain cells resembling those of the endoderm and placental trophoblast lineage, respectively (4,6).

Molecular studies suggest that aberrant DNA methylation may be involved in the evolution of the various GCT histologic subtypes (4,7-11). The developmental time period may be especially vulnerable for abnormal DNA methylation to occur due to the epigenetic reprogramming of the genome that takes place (12). We hypothesized that maternal vitamin supplementation, a source of the methyl donor folic acid (13), during or around pregnancy may decrease the risk of GCTs in the offspring. In a case-control study in the Children's Oncology Group (COG), we evaluated whether maternal vitamin supplementation was related to GCT development in children.

Methods

Complete study details have been previously published (14) and are summarized briefly below. Individuals diagnosed with GCTs (n=278) from birth to 14 years during January 1st, 1993 to December 31st, 2001 were identified through participating COG institutions in the United States and Canada. Cases with GCTs at all sites except the brain and with the following histologies: dysgerminoma/seminoma/germinoma, embryonal carcinoma, yolk sac tumor, choriocarcinoma, mature teratoma, and mixed GCTs were eligible for inclusion. Controls (n=423) were sampled from the population through random digit dialing and frequency matched to cases on sex and birth year within one year, at ratios of approximately 1:2 for males and 1:1 for females. Both case and control subjects were excluded if they did not have a telephone in their residence, if their mother did not speak English, or if she was not available for interview. The response rates for case and control subjects were 81% and 66% respectively as reported previously (14). Institutional Review Boards at the University of Minnesota and each case's COG institution approved all protocols for this study.

Information on sociodemographic characteristics, family history, and prenatal and postnatal medical conditions and exposures was collected, from participant mothers through both a self-administered questionnaire and telephone interview. Less than 2% of individuals had missing data on all of the variables included in this analysis. Mothers provided the following information about vitamin supplementation during pregnancy on the self-administered questionnaire: 1) whether they took vitamins in the 6 months before pregnancy, during pregnancy or while nursing (yes, no, don't know), 2) the specific time point when they were taken (6 months before pregnancy, trimester 1, trimester 2, trimester 3, and/or while nursing),

[1]This statistic was calculated using data obtained using SEER*stat software available at www.seer.cancer.gov and from United States population data obtained at http://factfinder.census.gov/servlet/DatasetMainPageServlet?_program=PEP&_submenuId=datasets_3&_lang=en

3) the type of vitamin, and 4) whether a doctor prescribed them. Since the majority of participants who took vitamins took prenatal vitamins that were prescribed by a physician or multivitamins (99%), we did not consider specific vitamin formulations separately in our analyses.

Statistical analyses

We used SAS version 9.1 (Cary, NC) to conduct all analyses. The association between maternal vitamin use and GCTs was modeled using unconditional logistic regression. Statistical differences between cases and control characteristics were assessed using Pearson's chi-square test and one-way analysis of variance for categorical and continuous variables respectively. Potential confounders of the association between maternal vitamin use and pediatric GCTs were examined using a forward selection process (15) where each potential confounder (maternal education: < high school; highschool; college, no degree; college degree and/or graduate school, annual household income: <20,000; 20,000-30,000; 30,001-50,000; >50,000, maternal race: white; other, maternal age, and child's birth year) was added to models that included the matching variables sex and child's age one at a time to determine their effect on the parameter estimate. If they changed the parameter estimate by more than 10% they were retained in the model.

Results

The majority (58%) of male cases had tumors located in the gonads, while in females the percentage of tumors located in the ovaries (51%) was similar to those located at extragonadal locations (47%). The most common histology in both sexes was yolk sac tumor. Most male cases were diagnosed before the age of 5 years, while the majority of female cases were diagnosed after the age of 5 years (Table 1).

Slightly more cases than controls were born prior to 37 weeks of gestation (Table 2). Cases had a slightly lower mean birth weight than controls, although more cases than controls had birth weights above 4000 grams. The mean maternal age was similar in cases and controls, while control mothers were more likely to report having a college degree (37.7% vs. 31.1%), white race (84.4% vs. 76.9%), and household income ≥20,000 year (80.2% vs. 69.0%).

Any vitamin use during pregnancy was associated with a slight reduction in the risk of GCTs (OR=0.7; 95% CI 0.4-1.2) in models adjusted for sex, child's age, maternal education, and household income (Table 3). Maternal vitamin use during the six months prior to conception and first trimester (periconception), or first trimester specifically, was not associated with GCTs. In analyses that excluded subjects who did not take any vitamins before or during their pregnancy or while nursing; no associations were observed with respect to timing (data not shown).

We assessed whether the association between any maternal vitamin use and GCTs varied by sex, age at diagnosis, tumor location, and tumor histology (data not shown). Non-significant reduced risks were observed with any maternal vitamin use in males (OR=0.6; 95% CI 0.2-1.7) and females (OR=0.8; 95% CI 0.4-1.4) and in children ≤ 2 (OR=0.8; 95% CI 0.4-2.0) and >2 years of age (OR=0.7; 95% CI 0.4-1.4). Any maternal vitamin use was also associated with a reduced risk for both gonadal (OR=0.6; 95% CI 0.3-1.1) and extragonadal (OR=0.8; 95% CI 0.4-1.6) tumors. Directions of associations between maternal vitamin use and GCTs varied by histology with inverse associations for dysgerminoma/seminoma (OR=0.5; 95% CI 0.2-1.3) and teratomas (OR=0.5; 95% CI 0.2-0.9) but not yolk sac tumors (OR=1.1; 95% CI 0.5-2.3). Neither periconception nor first trimester use was significantly associated with GCTs in any of these subgroup analyses (data not shown).

Discussion

The results from this study provide a pattern, albeit weak, that is consistent with the *a priori* hypothesis that maternal vitamin supplementation during the perigestational period reduces the risk of GCTs. We observed some variation by histology but many of the observed inverse associations were modest and failed to reach statistical significance. Moreover, analyses evaluating the timing of vitamin exposure before and during pregnancy did not indicate any relationship to GCT risk.

One previous case-control study that included 105 pediatric GCTs cases and 639 controls reported no association with maternal vitamin use (OR=1.1; 95% CI 0.6-2.0) (16). Case-control studies of several other childhood tumors have generally indicated that prenatal vitamin use and/or folate supplementation reduces risk. A recent review by Goh and Koren reported reduced risks of childhood cancers in association with maternal vitamin supplementation in 6/7 studies of brain tumors, 4/4 studies of acute lymphoblastic leukemia, 2/2 studies of neuroblastoma, and 1/1 study of retinoblastoma. Some of the reviewed studies suggested that vitamin supplemention during the periconception period specifically is associated with a reduction in risk (17). However, we did not find any evidence for a relation between timing of maternal vitamin use and GCTs.

Molecular abnormalities in GCTs vary by sex, diagnosis age, and histologic subtype and include aneuploidy, chromosome abnormalities (most commonly involving 12p), and abnormal DNA methylation patterns (4,7,9,10). Aberrant methylation patterns have commonly been detected in pediatric GCTs in several genes, including the imprinted genes IGF2 and H19, testis/cancer-associated genes, and tumor suppressor genes (4,7-9,11). In addition, expression of the p40 protein of the long interspersed nucleotide element-1 (Line-1) transposable element, which is normally silenced through epigenetic mechanisms (18), has been reported in pediatric malignant GCTS (9). A biologically plausible hypothesis is that maternal vitamin deficiency during and around pregnancy alters GCT risk in offspring by affecting DNA methylation patterns in germ cells. The developmental time period may have increased susceptibility to DNA methylation errors because of the two major waves of epigenetic reprogramming that occur in the blastocyt and germ cells (12).

A major strength of this study is the relatively larger number of subjects compared to previous studies. To our knowledge, only five previous case-control studies of childhood GCTs (case numbers ranging from 41-105) have investigated etiological factors in GCT development (16,19-22). The size of our study also allowed for a more detailed examination than previous studies of the association between GCTs and etiologic factors by subject and tumor characteristics.

Our study had several limitations. Although our study included more cases than prior studies, all studies of this type of tumor, including ours, have limited statistical power to detect modest effect sizes. Another potential limitation is selection bias. Selection bias could have occurred if control participants were not a representative sample of the nondiseased population from which cases arose (15). Given that the response rate among potential controls was 66% (23) and more control than case mothers reported being of white race and having higher levels of education, characteristics associated with vitamin supplementation during pregnancy (24), we cannot exclude that selection bias affected our risk estimates. In addition, recall bias, where a mother's ability to accurately recall her exposures during pregnancy depends on whether her child is a case or control, may lead to differential exposure misclassification and consequent bias in the risk estimate (15).

In conclusion, these data are consistent with results from studies of other childhood cancers suggesting a reduced risk of malignancy in association with maternal vitamin supplementation.

However, limitations regarding statistical power and biases that affect observational studies preclude firm conclusions.

Acknowledgments

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Table 1

Tumor characteristics and diagnosis ages of pediatric GCT cases (n=278).

	Males n (%)	Females n (%)
Anatomic Location		
Testis/Ovary	47 (58)	97 (51)
Extragonadal	31 (38)	89 (47)
Metastatic	3 (3.7)	4 (2.1)
Histology		
Yolk sac tumor (Endodermal sinus tumor)	46 (55.4)	79 (40.5)
Teratoma	14 (16.9)	57 (29.2)
Seminoma	2 (2.4)	43 (22.1)
Other nonseminomas* (Embryonal carcinoma, Choriocarcinoma, Polyembryoma)	17 (20.5)	8 (4.1)
Other† (mixed germ cell tumor components and malignant tumor cells)	3 (3.6)	7 (3.6)
Not specified	1 (1.2)	1 (0.51)
Age at diagnosis (years)		
<1	22 (26.5)	35 (18.0)
1-4	42 (50.6)	49 (25.1)
5-9	4 (4.8)	37 (19.0)
10-14	15 (18.1)	74 (38.0)
Total	**83 (100)**	**195 (100)**

*Embryonal carcinoma, Choriocarcinoma, Polyembryoma

†Mixed germ cell tumor components and malignant tumor cells

Table 2

Birth and parental sociodemographic characteristics of pediatric germ cell tumor cases and controls.

Characteristic	Cases (n=278) n (%)*	Controls (n=423) n (%)*
Gestational age (weeks)		
< 37	35 (12.6)	44 (10.4)
37 - 42	236 (84.9)	367 (86.8)
> 42	7 (2.5)	12 (2.8)
Mean (sd)	39.5 (5.6)	39.6 (4.6)
Birth weight (grams)		
≤2500	22 (7.9)	26 (6.2)
2501-4000	214 (77.0)	351 (83.0)
>4000	42 (15.1)	46 (10.9)
Mean (sd)	3354 (681)	3374 (590)
Maternal age category (years)		
≤24	90 (32.4)	128 (30.3)
25-29	99 (35.6)	145 (34.3)
30-34	60 (21.6)	111 (26.2)
≥35	29 (10.4)	39 (9.2)
Mean (sd)	27.2 (5.5)	27.3 (5.4)
Maternal Education		
<High school	28 (10.1)	23 (5.4)
High school graduate	110 (39.6)	143 (33.8)
College, no degree	53 (19.1)	97 (22.6)
College degree and/or graduate school	86 (30.9)	159 (37.7)
Household Income (dollars)		
<20,000	85 (30.6)	83 (19.6)
20,000-30,000	60 (21.6)	109 (25.8)
30,001-50,000	63 (22.7)	124 (29.3)
>50,000	66 (23.7)	102 (24.1)
Maternal Race		
White, not of Hispanic origin	213 (76.6)	356 (84.2)
African-American or Black, not of Hispanic origin	25 (9.0)	25 (5.9)
Hispanic	27 (9.7)	23 (5.4)
Native American Indian or Alaskan Native	3 (1.2)	4 (0.9)
Asian, Asian-American, or Pacific Islander	9 (3.3)	9 (2.1)
Other	0 (0.0)	5 (1.2)

* All characteristics had <2.0% missing data. Numbers may not add to total number of cases and controls due to missing data. Percentages are based on all subjects (including those with missing data).

Table 3

Adjusted odds ratios and 95% CIs for pediatric GCTs according to maternal vitamin supplementation during periods in and around pregnancy.

	Cases (n=278) n (%)*	Controls (n=423) n (%)*	OR†	95% CI
Any use‡				
no	40 (14.6)	38 (9.1)	1.0	Ref.
yes	233 (85.0)	377 (90.4)	0.7	0.4-1.2
Periconception§				
no	230 (83.9)	349 (83.7)	1.0	Ref.
yes	42 (15.3)	66 (15.8)	1.1	0.7-1.7
First trimester use//				
no	65 (23.7)	87 (20.9)	1.0	Ref.
yes	207 (75.5)	328 (78.7)	0.9	0.6-1.4

*Numbers may not add to total number of cases or controls due to missing data. Percentages are based on all subjects (including those with missing data).

†Adjusted for child's age, sex, household income, and maternal education.

‡Any use during 6 months before pregnancy, 1st trimester, 2nd trimester, 3rd trimester, or while nursing

§Any use during the six months before pregnancy and first trimester

//Any use during the first trimester