Case-Control Study of Periconceptional Folic Acid Supplementation and Oral Clefts

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There is consistent evidence that the risk of neural tube defects is decreased by periconceptional supplementation with folic acid. A similar protective effect has been postulated for oral clefts. A case-control study was conducted in greater metropolitan Boston, Massachusetts; Philadelphia, Pennsylvania; and southeastern Ontario, Canada, from 1988 through 1991 to test the hypothesis that folic acid supplementation during the periconceptional period reduces the risk of oral clefts. Crude and multivariate-adjusted relative risks were calculated for all oral clefts (n = 303), cleft palate (n = 108), and cleft lip with or without cleft palate (n = 195). Controls (n = 1,167) were liveborn or stillborn infants less than age 6 months who had various congenital anomalies other than oral clefts, neural tube defects, or other "midline defects." Adjusted relative risks and 95 percent confidence intervals for daily folic acid supplementation during the periconceptional period were: oral clefts, 1.1 (95% Cl 0.8-1.7), cleft palate, 0.9 (95% Cl 0.5-1.6), and cleft lip with or without cleft palate, 1.3 (95% Cl 0.8-2.1). These findings do not support a protective association between the periconceptional use of folic acid supplements and the risk of oral clefts. Am J Epidemiol 1996;143:1229-34.

Folic acid supplementation during the periconceptional period has been well documented to result in a substantial reduction in the occurrence of neural tube defects (1–5). Whether supplementation with vitamins in general or folic acid in particular affects the risk of other congenital anomalies is inconclusive (6–8).

Oral clefts (cleft lip, cleft palate, and cleft lip with cleft palate) are among the most common congenital malformations, with an estimated prevalence of 1.5 per 1,000 births (9). Despite extensive study, the causes of most cases remain unknown. Oral clefts are believed to have a multifactorial etiology, with both genetic and environmental factors playing a role. Oral clefts occur as either isolated anomalies or with other congenital anomalies. Because of the heterogeneity of this class of defects, it has been difficult for researchers to elucidate information regarding risk factors.

Several lines of study have suggested that insufficient folic acid intake may be related to the etiology of clefts. First, oral clefts have been observed in the offspring of women who ingested folate antagonists during pregnancy, such as methotrexate, aminopterin (4-pteroylglutamic acid), and anticonvulsants (10–14). Second, neural tube defects and oral clefts may be embryologically related; facial and tooth tissue develop from neural crest cells that originate from the dorsolateral aspect of the forming neural tube (15). Further, neural tube defects and oral clefts may be both considered midline defects and occur together more frequently than would be expected by chance (16).

Studies of animal models have demonstrated a teratogenic effect of antifolate factors and folate deficiency on the risk of oral clefts (17–19), and a protective association between use of folic acid and/or multivitamins and the risk of oral clefts has been suggested in a number of human studies (20–25). However, most did not report information on timing of supplement use, compliance, or possible confounding factors; further, folic acid doses ranged from 0.5 to 10 mg. In contrast, a randomized controlled clinical trial of folic acid supplementation found no evidence of a protective effect associated with folic acid supplementation (1).

The purpose of this case-control study was to test the hypothesis that folic acid supplementation during
the periconceptional period reduces the risk of oral clefts.

MATERIALS AND METHODS
Identification of study participants

Study subjects were identified as part of an ongoing case-control surveillance study of environmental exposures and birth defects conducted by the Slone Epidemiology Unit Birth Defects Study at the Boston University School of Public Health (26). The Birth Defects Study has been under way since 1976 and is a multicenter “case-control surveillance” project; the present analysis used data collected in participating hospitals and clinics in metropolitan Boston, Massachusetts; Philadelphia, Pennsylvania; and southeastern Ontario, Canada, from 1988 to 1991 as part of a previous study of folate and neural tube defects (5). Cases and controls were ascertained by review of discharge diagnoses, contact with the newborn nursery, review of admission/discharge lists, and review of clinic and surgical logs. Permission to contact the mother was obtained from the infant’s primary physician. A letter introducing the study was sent to the mother, and it was followed by a telephone call to explain the study and arrange for an interview. An interview was conducted in person by a trained nurse-interviewer within 6 months of the delivery date.

Study population

Cases were mothers of liveborn or stillborn infants less than age 6 months with cleft lip (International Classification of Diseases, Ninth Revision (ICD-9) 749100–749199), cleft palate (ICD-9 codes 749000–749099), or cleft lip with cleft palate (ICD-9 codes 749200–749299). Oral clefts associated with a Mendelian inherited disorder or chromosomal anomaly were excluded from the case group (n = 45) since it is assumed that oral clefts associated with inherited anomalies (e.g., trisomy 13) have a different etiology than isolated or nonsyndromic clefts. The final case series was comprised of 303 mothers of infants with oral clefts.

Cases were classified into the following groups: mothers of all infants with oral clefts; cleft palate; and cleft lip with or without cleft palate. (Cleft lip and cleft lip with cleft palate share a common development process.) Controls were mothers of liveborn or stillborn infants less than age 6 months who had another congenital anomaly, but did not have an oral cleft. Because of the known association between folic acid and neural tube defects (1–5), mothers of infants with neural tube defects were excluded (n = 443), as were infants with other “midline” defects. The final control group included 1,167 subjects. The largest diagnostic subgroups among them were renal anomalies (n = 177), gastrointestinal anomalies (n = 156), limb defects (n = 139), craniosynostosis (n = 115), and chromosomal anomalies (n = 90). The participation rates for cases and controls were 80 and 72 percent, respectively.

Exposure measurements

The history of folic acid supplement and multivitamin use was obtained by questioning the mother regarding her use during the period 6 months prior to the last menstrual period through the end of the pregnancy. Each subject was given a calendar that highlighted the dates of the last menstrual period, date of birth of the child, and the 6 months prior to the last menstrual period. Dates when vitamin use started and stopped were recorded, as was the frequency of use. Medication bottles were examined to determine folic acid content and dose; when bottles were not available, information regarding brand name was recorded to permit identification of the folic acid content and dose. Supplementation was defined as use of a folic acid-containing multivitamin or a folic acid supplement alone. The periconceptional period was defined as 1 month prior to the last menstrual period through the fourth lunar month of pregnancy; the latter corresponds to the twelfth week of embryogenesis, at which time the palatal shelves are believed to close (15).

Information was also obtained on other variables, including reproductive history, parental age, parental education, family income, race, religion, ancestry, family history of congenital malformations, mother’s medical history, history of prenatal care, and medication use before and during pregnancy. A food frequency questionnaire was administered to permit estimation of dietary folate intake during the 6-month interval prior to the last menstrual period (5).

Exposure variable

Users of folic acid supplements during the periconceptional period were classified as either daily (i.e., women who reported their use as daily) or less-than-daily users; the latter group included those who reported less than daily use or daily use that stopped during the periconceptional period. For daily users, we created mutually exclusive categories according to timing of first supplementation relative to the last menstrual period: “Months 1–4” users were women who began before or during the month preceding the last menstrual period and continued through the first 4 months of pregnancy (i.e., they used supplements daily throughout months 1–4). “Month 3” users were
women who began use prior to the third lunar month (but after the month preceding the last menstrual period) and continued through the fourth month of pregnancy; "month 4" users were women who began use prior to the fourth lunar month (but after the beginning of the third lunar month). Women in the reference category were those who did not use folic acid supplements during the periconceptional period (those who began using multivitamins in months 5–10 plus those who did not use any multivitamins during pregnancy).

Data analysis

Cross-tabulations were examined for all oral clefts, cleft palate, and cleft lip with or without cleft palate in relation to daily use of folic acid categorized by timing of use. Crude and Mantel-Haenszel relative risks estimated by odds ratios were calculated; the latter were adjusted for the separate effects of smoking, alcohol, race, education, income, maternal age, paternal age, previous miscarriages, parity, and gravidity. Maternal education was the only factor found to alter the crude estimate and therefore was included in the multivariate analysis. Terms for dietary folate and caloric intake were also included in the multivariate model (5). Tertiles of dietary intake were based on the folate distribution among controls.

Relative risks for oral clefts were calculated for each tertile of dietary folate intake, with and without use of daily folic acid supplements, with nonsupplementers in the lowest tertile as the reference category.

RESULTS

The distribution of folic acid supplement use among control subgroups is shown in table 1. The distribution of exposure among cases and controls is shown in table 2. Crude and multivariate-adjusted relative risks revealed no significant association between use of folic acid supplements during the periconceptional period and the overall risk of oral clefts. The relative risk for all oral clefts and any folic acid supplementation during the periconceptional period compared with nonusers was 1.4 (95 percent confidence interval (CI) 0.8–1.7). Relative risks for all oral clefts, cleft palate, and cleft lip with or without cleft palate were close to the null for first use of folic acid in each of the time categories. Relative to controls, supplementation was slightly more common among cleft lip with or without cleft palate cases and slightly less common among cleft palate cases. For daily use throughout palatal embryogenesis, relative risks for cleft lip with or without cleft palate and cleft palate were 1.2 (95 percent CI 0.7–2.0) and 0.9 (95 percent CI 0.5–1.7), respectively.

We examined supplement use and tertiles of dietary folate intake together. Relative to nonsupplementers in the lowest tertile, risk estimates for oral clefts were: 0.8 (95 percent CI 0.5–1.3) for supplementers in the highest tertile, 1.1 (95 percent CI 0.7–1.7) for supplementers in the middle tertile, 0.9 (95 percent CI 0.6–1.5) for supplementers in the lowest tertile, 0.9 (95 percent CI 0.5–1.6) for nonsupplementers in the highest tertile, and 1.1 (95 percent CI 0.6–1.9) for nonsupplementers in the middle tertile (data not shown).

DISCUSSION

We found no significant protective effect for folic acid supplementation in relation to the risk of all oral clefts, cleft palate, or cleft lip with or without cleft palate. For all oral clefts and any use of folic acid supplements, the 95 percent confidence interval excludes effects more extreme than a 20 percent reduction or a 70 percent increase in risk. In the subgroup cleft lip with or without cleft palate, either a halving or doubling of risk can be ruled out, irrespective of timing of exposure. In addition, consideration of dietary folate intake had no appreciable effect on these findings. The lowest observed risk was for cleft palate in association with daily use that began in the fourth month (relative risk = 0.7, 95 percent CI 0.3–1.8). In addition to the fact that this risk was not statistically significant, the association has little biologic plausibility since it would be difficult to explain why folic

| TABLE 1. Prevalence (%) of periconceptional folic acid supplementation among control subgroups, Boston, Massachusetts; Philadelphia, Pennsylvania; and Ontario, Canada, 1988–1991 |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Subgroup                    | Folic acid supplementation | Less than daily | Daily | None |
|                             | Prevalence | % | Prevalence | % | Prevalence | % |
| Renal anomalies (n = 177)    | 26        | 14.7 | 120       | 67.8 | 31        | 17.5 |
| Gastrointestinal anomalies (n = 156) | 29 | 18.6 | 108       | 69.2 | 19        | 2.2 |
| Limb defects (n = 139)       | 22        | 15.8 | 98        | 70.5 | 19        | 13.7 |
| Craniosynostosis (n = 115)   | 15        | 13.0 | 87        | 75.7 | 13        | 11.3 |
| Chromosomal anomalies (n = 90) | 20 | 22.2 | 52        | 57.8 | 18        | 20.0 |

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acid supplementation that began close to the time of palatal closure would provide a greater protective effect than use beginning at an earlier time and continuing throughout that period.

Tolarova and Harris recently identified a 65 percent decrease in the expected number of cleft lip with or without cleft palate cases in a prospective study of women at risk of a child with cleft lip with or without cleft palate for a 10-mg dose of folic acid (24). Our study did not examine dose, but the most common level of folic acid is likely to be closer 0.4 mg. Therefore, we would be unlikely to identify effects associated with doses substantially higher than 0.4 mg. Further, over 95 percent of folic acid supplementation was in the form of a multivitamin. Thus, we cannot separate the effects of folic acid from those of other nutrients.

There may be limitations in our study. With respect to selection bias, cases and controls were ascertained in the same manner as described previously (5), and participation rates were not appreciably different for cases and controls. Since both cases and controls were mothers of malformed infants, selection bias related to presence of a malformation is unlikely. If vitamin or folic acid supplement use reduced the risk of all or several of the most common malformations among the controls, as has been suggested (6–8), the exposure prevalence for our control group would be lower than that for normal subjects; in that setting, our findings would be biased toward the null. However, exclusion of each of the largest defect subgroups did not appreciably change the risk estimates. In addition, a previous analysis of the association between folic acid supplementation and neural tube defects, drawn from the current study population (5), identified a reduction in risk that was comparable in magnitude with those observed in other studies that used either malformed or nonmalformed infants as comparison subjects (1–4). Therefore, a reduction in oral cleft risk at least as great as that observed for neural tube defects would likely be identified here. Finally, we believe our ascertainment of cases is unbiased because the distribution of type of cleft by sex was consistent with those in the general population; 67 percent of cleft palate cases were females, while 64 percent of cleft lip and 58 percent of cleft lip and palate cases were males.

This study used a control group comprised of infants with other congenital malformations in an attempt to decrease the potential for biased recall of exposures, illnesses, and events during pregnancy. However, there is no consensus regarding the existence of recall bias in the study of congenital malformations. Some have found no significant differences in the reporting accuracy of women of healthy or malformed children.
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REFERENCES


