Mid-Pregnancy Cotinine and Risks of Orofacial Clefts and Neural Tube Defects

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Objective   Past studies of cigarette smoking as a contributor to orofacial clefts and neural tube defects (NTDs) used self-reports of smoke exposures. We have correlated measurements of cotinine (a nicotine metabolite) in mid-pregnancy sera with clefts and NTDs.

Study design   From a repository of >180 000 mid-pregnancy serum specimens collected in California from 2003 to 2005 and linked to delivery outcome information, we identified 89 orofacial cleft-associated pregnancies, 80 NTD-affected pregnancies, and randomly selected 409 pregnancy specimens that corresponded to infants without malformations as control subjects. Cotinine was measured by liquid chromatography-mass spectrometry. No smoke exposure was defined as cotinine values <2 ng/mL, and any exposure was defined as ≥2 ng/mL.

Results   We observed odds ratios of 2.1 (95% CI, 1.0-4.4) for clefts and 0.4 (95% CI, 0.1-1.7) for NTDs associated with exposure. After adjusting for race/ethnicity, age, and serum folate levels, odds ratios were 2.4 (95% CI, 1.1-5.3) and 0.6 (95% CI, 0.1-2.5). We explored 2 cotinine levels, 2 to 10 ng/mL and >10 ng/mL for clefts (data were too sparse for NTDs). Odds ratios for these levels were 3.3 (95% CI, 0.9-11.9) and 1.7 (95% CI, 0.7-4.2), respectively.

Conclusion   Smoking exposures, as measured with cotinine levels during mid-pregnancy, were associated with increased risks of clefts and possibly reduced risks of NTDs. *(J Pediatr 2009;154:17-9)*

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Numerous studies have explored potential teratogenic effects of periconceptional cigarette smoking. The evidence associated specifically with orofacial clefts, and smoking tends to support modest effects of approximately 1.5-fold for any versus no smoking in early pregnancy and evidence to indicate an exposure-response effect.[1] The evidence associated with NTDs is far less clear. Findings of reduced risks,[2,3] elevated risks,[4-6] and no risks[7,8] have appeared in the literature.

Past studies of cigarette smoking as a contributor to etiologies of orofacial clefts and neural tube defects (NTDs) have involved self-reports of active smoking and secondhand smoke exposures. In this prospective study, we attempt to improve on the challenges of collecting such exposures gathered study retrospectively by measuring cotinine (a nicotine metabolite) in mid-pregnancy sera from a large population in California.

METHODS

This study included data from a repository of mid-pregnancy sera. Specimens were collected from approximately 70% of women during the 15th to 18th week of pregnancy in selected regions of California (Orange, San Diego, and Central Valley counties) as part of the California Expanded AFP (alpha fetoprotein) program that screens for NTDs and cytogenetic abnormalities. The collection and processing of specimens was as follows: 1) samples were taken at draw stations with BD Vacutainer 3.5-mL serum separator tubes with no anticoagulants or preservatives and centrifuged; 2) samples were received by designated clinical laboratories from draw stations at room temperature, on average 3.0 days after draw; 3) AFP assays were run on samples, usually on the day received; 4) samples were refrigerated as long

AFP	Alpha fetoprotein		OR	Odds ratio
NTD	Neural tube defect			

Table I. Characteristics of cleft lip/palate-affected, neural tube defect-affected, and unaffected (control subjects without malformations) deliveries, California 2003 to 2005

	Cleft lip/ palate cases (n = 89)		NTD cases (n = 80)		Controls (n = 409)	
	n	%*	n	%*	n	%*
Race/Ethnicity						
Hispanic	40	44.9	53	66.3	196	47.9
White non-Hispanic	31	34.8	14	17.5	143	35.0
Asian	7	7.9	7	8.8	37	9.1
Black	2	2.3	1	1.3	12	2.9
Other	9	10.1	5	6.3	20	4.9
Age (years)						
<25	29	32.6	28	35.0	132	32.3
25-29	26	29.2	27	33.8	91	22.3
30-34	24	27.0	15	18.8	137	33.5
≥34	10	11.2	10	12.5	49	12.0

*Percentages may not equal 100 because of missing data or rounding.

Table II. Tobacco smoke exposures as estimated with cotinine levels among cleft lip/palate-affected, neural tube defect-affected, and unaffected (control subjects without malformations) deliveries, California 2003 to 2005

	Cleft lip/palate cases (n = 89)	NTD cases (n = 80)	Controls (n = 409)
Smoke exposure*			
No	78	77	383
Yes	11	2	26
OR	2.1	0.4	Ref
95% CI	1.0-4.4	0.1-1.7	—
Adjusted OR†	2.4	0.6	Ref
95% CI	1.1-5.3	0.1-2.5	—

Ref, Reference.
*Refers to detection of cotinine in serum specimens. No exposure defined as a cotinine measure <2 ng/mL, and exposure defined as cotinine measures ≥2 ng/mL. One NTD case specimen had a missing value for cotinine.
†Adjusted for maternal age, race/ethnicity, and serum folate levels.

as 7 days when further testing was necessary; 5) samples were sent on cold packs via overnight mail to the serum storage bank; and 6) samples were aliquoted, labeled with barcodes, and frozen at −70° C within an average of 3.5 days of receipt at the serum repository.

Each woman's serum specimen was linked with delivery outcome information to determine whether her fetus had an orofacial cleft, NTD, or another structural malformation as defined elsewhere[9] or was born without malformation. The study included deliveries that were live-born, stillborn (fetal deaths at >20 weeks gestation), or electively terminated on the basis of prenatal diagnoses. Among the >180 000 pregnancy specimens collected for testing in the period 2003 to 2005, we identified 89 orofacial cleft-associated pregnancies and 80 NTD-affected pregnancies. All the 89 cases with clefts had cleft lip with or without cleft palate. Of the 80 NTD cases, 31 had spina bifida, and 49 had anencephaly. To serve as a referent group (control subjects), we randomly selected pregnancy specimens among the total number of available specimens at a ratio of approximately 5 control subjects/case. These potential control subjects were further reviewed for presence of a structural malformation in the delivered infant. Infants with malformations or infants identified with insufficient specimen to analyze were excluded. This sampling scheme resulted in 409 control specimens. Thus, this was analyzed as a nested case-control study. The collection of samples and the study protocol were approved by the California Health and Welfare Agency Committee for the Protection of Human Subjects.

Serum specimens for the 578 cases and control subjects were sent on cold packs to the University of Bergen for analyte measurements. To assess cigarette smoking exposures, the metabolite cotinine was measured with LC-MS/MS. No smoke exposure was defined as values <2 ng/mL, and any smoke exposure was defined as ≥2 ng/mL. Similar cutoff points have been used in earlier studies of reproductive outcomes.[10,11] That is, cotinine levels <2 ng/mL indicate no exposure, whereas levels of 2 to 10 ng/mL are indicative of environmental tobacco smoke exposure, and levels >10 ng/mL are indicative of active smoking. Serum folate was also measured. Details about the laboratory assays can be found elsewhere.[12] All analyte measurements were done with researchers blind to case or control status.

We estimated risks with odds ratios (ORs) and 95% CIs (SAS software version 9.1). Models were constructed to assess effects associated with the 2 defined categories of cotinine. Covariates in some analyses included maternal race/ethnicity (Hispanic; white, non-Hispanic; Asian; black; other), age (<25 years; 25-29 years; 30-34 years; and >34 years), and serum folate levels (nmol/L). Self-reported information on cigarette smoking was not available.

RESULTS

Table I provides background characteristics of the study population showing an expected greater frequency of Hispanic subjects in the NTD case group. NTD and cleft cases tended to be slightly more represented in the 25- to 29-year age group and slightly less represented in the 30- to 34-year age group.

Table II shows a 2.1-fold increased risk for cleft lip with/without cleft palate associated with smoke exposures, and a 2.4-fold risk when adjusted for race/ethnicity, age, and serum folate levels. Although data were limited, we attempted to estimate the effects associated with higher cotinine levels. We explored 2 cotinine levels, 2 to 10 ng/mL and >10 ng/mL, for cleft lip with/without cleft palate. The ORs for these levels were 3.3 (95% CI, 0.9-11.9) and 1.7 (95% CI, 0.7-4.2), respectively. We also explored whether observed effects differed by the phenotypic distinction of unilateral versus bilateral cleft lip. Of the 89 cases, 74% were unilateral,

18% bilateral, and 8% unknown. The ORs associated with smoke exposures were 1.7 (95% CI, 0.7-4.2) for unilateral clefts and 3.4 (95% CI, 0.9-12.7) for bilateral clefts.

Table II shows an OR of 0.4 for NTDs associated with smoke exposures. This estimated effect was imprecise because of a small number of subjects exposed. This small sample size precluded further detailed analyses.

DISCUSSION

We observed that smoking exposures, as defined with measured cotinine levels during mid-pregnancy, were associated with increased risks of cleft lip with or without cleft palate and possibly reduced risks of NTDs. Adjustment for potential effects of race/ethnicity, age, and serum folate levels did not yield substantially different estimates. Although the number of cases considered here was relatively modest, it investigated the unbiased measure of smoking exposure (cotinine) on risks for NTDs and orofacial clefts. Indeed, a recent paper that quantified the reliability of smoking reporting in studies of orofacial clefts indicated that, to be informative, future studies will need to reduce the effects of exposure misclassification from self-reported information on smoking.[13]

Observing a positive relation between cotinine and risk of cleft lip with/without cleft palate extends the relatively consistent findings in the literature that periconceptional smoking increases clefting risk.[1,14,15] We observed limited evidence to suggest that the effect may be higher for bilateral clefts, an observation made earlier.[14] Several hypothesized mechanisms have been put forward to explain associations observed between cigarette smoke exposures and orofacial clefts. These include hypoxia from carbon monoxide,[16] teratogenic effects of nicotine, cadmium, or myriad amines,[17-19] and alterations to folate metabolism.[20,21] However, estimated effects were only minimally influenced by adjusting for serum folate levels in these data.

The reduced risk of NTDs with measurable cotinine levels adds to a literature that conveys mixed findings. Studies have shown reduced risks,[2,3,22] no risks,[7,8] or increased risks[4-6] associated with maternal smoking during the periconceptional period and NTDs. Our finding is quite imprecise, (ie, the confidence intervals were consistent with an interpretation of both increased risk and protective effects).

This study investigated cotinine levels measured during pregnancies that subsequently resulted in the delivery of infants/fetuses with cleft lip with/without cleft palate and NTDs. These data extend our inferential ability because of its prospective design and the sensitivity and specificity of cotinine as an index of tobacco smoke exposure. Cotinine measurements overcome the criticisms of biased exposure reporting.[23] This study, however, is not without its limitations. First, although the study involved nearly 200 000 women, the number of affected pregnancies was relatively small, resulting in a fairly imprecise estimation of effects. Second, the collection of women's specimens was on average 12 weeks after closure of the neural tube and 8 weeks after the closure of the lip and palate processes. The half-life of cotinine is short, 8 to 16 hours, in pregnancy.[24] Although it seems likely that bias caused by this single point-in-time measurement would tend to result in an underestimate of effects, we cannot be certain. Third, the results for cleft lip with/without cleft palate did not indicate a dose-response relationship. Our ability to observe such a relationship was hampered by the sample size. Thus, these observations should be replicated in other prospective designs in pregnant women who may become available in places such as Norway and Denmark.

REFERENCES

1. Little J, Cardy A, Munger RG. Tobacco smoking and oral clefts: a meta-analysis. Bull World Health Organ 2004;82:213-8.
2. Shaw GM, Velie EM, Morland K. Parental recreational drug use and risk for neural tube defects. Am J Epidemiol 1996;144:1155-60.
3. Kallen K. Maternal smoking, body mass index, and neural tube defects. Am J Epidemiol 1998;147:1103-11.
4. Evans DR, Newcombe RG, Campbell H. Maternal smoking habits and congenital malformations: a population study. Br Med J 1979;2:171-3.
5. Kelsey JL, Dwyer T, Holford TR, Bracken MB. Maternal smoking and congenital malformations: an epidemiological study. J Epidemiol Community Health 1978;32:102-7.
6. Suarez L, Felkner M, Brender JD, Canfield M, Hendricks K. Maternal exposures to cigarette smoke, alcohol, and street drugs and neural tube defect occurrence in offspring. Matern Child Health J 2008;12:394-401.
7. McDonald AD, Armstrong BG, Sloan M. Cigarette, alcohol, and coffee consumption and congenital defects. Am J Public Health 1992;82:91-3.
8. Wasserman CR, Shaw GM, O'Malley CD, Tolarova MM, Lammer EJ. Parental cigarette smoking and risk for congenital anomalies of the heart, neural tube, or limb. Teratology 1996;53:261-7.
9. Hanke W, Sobala W, Kalinka J. Environmental tobacco smoke exposure among pregnant women: impact on fetal biometry at 20-24 weeks of gestation and newborn child's birth weight. Int Arch Occup Environ Health 2004;77:47-52.
10. Eskenazi B, Bergmann JJ. Passive and active maternal smoking during pregnancy, as measured by serum cotinine, and postnatal smoke exposure. I. Effects on physical growth at age 5 years. Am J Epidemiol 1995;142:S10-8.
11. Croen LA, Shaw GM, Jensvold NJ, Harris JA. Birth defects monitoring in California: a resource for epidemiologic research. Paediatr Perinat Epidemiol 1991;5:423-7.
12. Ueland PM, Midttun O, Windelberg A, Svardal A, Skalevik R, Hustad S. Review: quantitative profiling of folate and one-carbon metabolism in large-scale epidemiological studies by mass spectrometry. Clin Chem Lab Med 2007;45:1737-45.
13. MacLehose RF, Olshan AF, Herring A, Honein MA, Shaw GM, Romitti PA. Orofacial clefts and misclassification of maternal smoking. Epidemiology. In press 2008.
14. Honein MA, Rasmussen SA, Reefhuis J, Romitti PA, Lammer EJ, Sun L, et al. Maternal smoking and environmental tobacco smoke exposure and the risk of orofacial clefts. Epidemiology 2007;18:226-33.
15. Shi M, Christensen K, Weinberg CR, Romitti PA, Bathum L, Lozada A, et al. Orofacial cleft risk is increased with maternal smoking and specific detoxification-gene variants. Am J Hum Genet 2007;80:76-90.
16. Millicovsky G, Johnston MC. Hyperoxia and hypoxia in pregnancy: simple experimental manipulation alters the incidence of cleft lip and palate in CL/Fr mice. Proc Natl Acad Sci U S A 1981;78:5722-3.
17. Upshall DG. Correlation of chick embryo teratogenicity with the nicotinic activity of a series of tetrahydropyrimidines. Teratology 1972;5:287-93.
18. Carmichael NG, Backhouse BL, Winder C, Lewis PD. Teratogenicity, toxicity and perinatal effects of cadmium. Hum Toxicol 1982;1:159-86.
19. Lambert GH, Nebert DW. Genetically mediated induction of drug-metabolizing enzymes associated with congenital defects in the mouse. Teratology 1977;16:147-53.
20. Shaw GM, Nelson V, Carmichael SL, Lammer EJ, Finnell RH, Rosenquist TH. Maternal periconceptional vitamins: interactions with selected factors and congenital anomalies? Epidemiology 2002;13:625-30.
21. McDonald SD, Perkins SL, Jodouin CA, Walker MC. Folate levels in pregnant women who smoke: an important gene/environment interaction. Am J Obstet Gynecol 2002;187:620-5.
22. Grewal J, Carmichael SL, Ma C, Lammer EJ, Shaw GM. Maternal periconceptional smoking and alcohol consumption and risk for select congenital anomalies. Birth Defects Res A Clin Mol Teratol. In press 2008.
23. England LJ, Grauman A, Qian C, Wilkins DG, Shisterman EF, Yu KF. Misclassification of maternal smoking status and its effects on an epidemiologic study of pregnancy outcomes. Nicotine Tobacco Res 2007;9:1005-13.
24. Dempsey D, Jacob P III, Benowitz NL. Accelerated metabolism of nicotine and cotinine in pregnant smokers. J Pharmacol Exp Ther 2002;301:594-8.