Maternal lead exposure and the secondary sex ratio

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BACKGROUND: A reduction in the secondary sex ratio may be associated with exposure to environmental toxicants. Little data exists relating this outcome to lead exposure, a well-known reproductive toxicant. METHODS: We studied 1980 women having singleton births from 1994 to 1995 and from 1997 to 2001 who participated in a cohort study of lead exposure and infant outcomes in Mexico City. Levels of lead were measured in maternal and cord blood using graphite furnace atomic absorption spectroscopy, and levels of lead in maternal patella and tibia bone (a reflection of cumulative exposure) were measured using noninvasive K-X-ray fluorescence measurements. Using logistic regression models, we evaluated the relations of these measures to secondary sex ratio in the offspring, adjusting for maternal age, parity and year of infants’ birth. RESULTS: We found no consistent association between any of the lead measures and secondary sex ratio. Results were unchanged when we adjusted for infants’ year of birth, maternal age and parity. CONCLUSIONS: Despite a large sample size and the use of sensitive biomarkers, we did not find evidence that maternal and fetal lead exposure is associated with a lower secondary sex ratio among newborns.

Key words: bone lead/lead/lead measurements/pregnancy/secondary sex ratio

Introduction

Over recent decades, many countries have witnessed a decline in the relative number of male infants born, expressed as a decline in secondary sex ratio (ratio of live-born males to live-born females) or by the percentage of live births that are male. Several reports have indicated a secular trend of fewer males in Canada, the USA, England and Wales, Denmark, Germany and The Netherlands (Feitosa and Krieger, 1992; Moller, 1996; Allan et al., 1997; Manning et al., 1997; van den Broek, 1997; van der Pal-de Bruin et al., 1997). A recent summary of World Health Organization (WHO) data from North and South America indicates a similar trend in most countries (Grech et al., 2003). The decline in male births is considerable over the past half century. Estimates of the impact of the reduction between 1970 and 1990 are 8639 [95% confidence interval (CI) = 4433–12 850] fewer males in Canada and 37 840 (95% CI = 25 950–49 730) fewer males in the United States (Allan et al., 1997). There are exceptions to this decline, however, including Ireland (Moynihan and Breathnach, 1999). One of the most significant declines in the sex ratio was identified in Mexico (Grech et al., 2003).

Changes in the sex ratio have been postulated to represent pathology involving the conceptus (Jongbloet et al., 2001). Differential preclinical loss of males could occur, because the male conceptus may be more susceptible to environmental stressors affecting mothers, such as poor nutrition, stress from warfare and major environmental disasters (Zorn et al., 2002; Cagnacci et al., 2004). Such stressors may affect particular windows of early embryonic development during which successful implantation is allowed or denied (Kochhar et al., 2001).

On several occasions, declining sex ratios have been attributed to environmental chemicals. The proportion of male births dropped significantly following paternal exposure to dioxin (Mocarelli et al., 1996, 2000) and also dropped significantly among babies born to male workers during the testicular recovery period from their exposure to dibromochloropropane, now known as a testicular toxicant (Potashnik et al., 1984). Previous studies have also observed declining sex ratios in association with maternal exposures. In southeastern Turkey, exposure of women to hexachlorobenzene has been linked to a reduced proportion of male births 45 years later (Jarrell et al., 2002), and women exposed to high concentrations of PCBs...
from fish consumption had a significantly reduced proportion of male births (Weisskopf et al., 2003). However, a reduction in sex ratio in Finland occurred prior to the manufacture and deployment of any of these industrial chemicals (Vartiainen et al., 1999). Most recently, a significant decline in the sex ratio has been identified among First Nations community near Sarnia, Ontario, from 1984 to 2003. This reduction has been posed as potentially due to the close proximity to several major industrial units involved with chemical production (Mackenzie et al., 2005).

Lead is one of the most significant reproductive toxicants. Identified as a potential cause of the fall of Rome through infertility and abortion, lead toxicity remains a serious threat in the environment from many sources (Gilfillan, 1965). In males, exposure to lead has been associated with reduced sperm counts, morphology and function (Chowdhury et al., 1986; Apostoli et al., 1998; Bonde et al., 2002a; De Rosa et al., 2003). In women, lead has been associated with longer time to pregnancy (Guerra-Tamayo et al., 1996; Borja-Aburto et al., 1999; Hertz-Picciotto, 2000).

Limited information is available regarding the association of lead with the secondary sex ratio. Such an impact of lead is consistent with previous theoretical factors that control the sex ratio (James, 1997). In England and Wales, Dickinson and Parker (1994) found an occupationally associated reduction in the sex ratio of the offspring, which they attributed to both lead and alcohol.

Historically, lead exposure has been high in Mexico City (largely from the combustion of leaded gasoline and the use of lead-glazed ceramics) (Ferias et al., 1996; Borja-Aburto et al., 1999). Mexico began to phase out the use of leaded gasoline in 1986, which it completed in 1997, and unleaded gasoline was introduced in 1990 (Cortez-Lugo et al., 2003). The decline in sex ratio in Mexico over the past 50 years has been reported as the most dramatic among WHO countries (Grech et al., 2003b). In the light of these trends and the potential that lead alters human fertility, it was of interest to determine whether sensitive markers of lead exposure among Mexican women are associated with the sex of the progeny.

Materials and methods

This study incorporated participants from two cohorts of lead assessment in pregnancy. Members of cohort 1 were enrolled from 1994 to 1995, and 1393 mother–infant pairs had at least one lead biomarker measure. Members of cohort 2 were enrolled between 1997 and 2002, and 587 mother–infant pairs had at least one lead biomarker measure. Details of recruitment procedures and eligibility criteria have been published elsewhere for cohort 1 and cohort 2 (Gonzalez-Cossio et al., 1997; Ettinger et al., 2004). Notably, women were recruited from three maternity hospitals in Mexico City and informed consent was obtained from all the subjects. Only singleton pregnancies were considered in this study. The women were attending any of three hospitals—Mexican Social Security Institute, Manuel Gea González Hospital and National Institute of Perinatology—for prenatal care. Ethics approval was obtained from the Human Subjects Committee of the National Institute of Public Health.

Blood lead measurements

Maternal and umbilical cord blood samples were collected within 12 h after delivery. Blood samples were analysed for lead by atomic absorption spectroscopy (PerkinElmer, Norwalk, CT, USA) at the metal laboratory of the American British Cowdray Hospital in Mexico City. Analyses of external blinded quality-control samples were provided throughout the study by the Wisconsin State Laboratory of Hygiene (WSLH) Cooperative Blood Lead Proficiency Testing Program (PBPTP) (Madison, WI, USA). The laboratory performed with an accuracy within 5% and a coefficient of variation <15%.

Bone lead tests

Noninvasive measurements of lead in bone constitute a relatively new biological estimate of cumulative lead burden in contrast to blood lead levels, which mostly reflect lead exposure (Hu et al., 1998). Measurements of maternal bone lead were undertaken in our study at the American British Cowdray Hospital 1 month following delivery. Using a spot source 109Cd K-XRF instrument (Abiomed, Danvers, MA, USA), we measured maternal bone lead levels at mid tibia shaft (cortical) and patella (trabecular). Thirty-minute measurements were taken at each bone site after the skin had been washed with 50% isopropyl alcohol. The K-XRF beam collimator was sited perpendicular to the bone surface for the tibia and 30° in the lateral direction for the patella. Tibia and patella bone lead measurements with estimated uncertainties greater than 10 and 15 μg/g bone, respectively, were excluded as these measurements usually reflect excessive patient movement during the measurement (Hu et al., 1990; Aro et al., 1994). Such procedures are standard in the analysis of bone lead data (Hu et al., 1998). The details of this procedure have been previously reported (Aro et al., 2000).

Statistical analysis

We undertook separate analyses for each measure of lead (maternal blood lead, cord blood lead and patella and tibia bone lead). We divided the concentrations of lead in these compartments into quintiles, and then, using logistic regression, we modeled the relative odds that an infant was male (versus female) across quintiles of lead. For tests of trends across quintiles, the median lead level in each quintile was assigned to all subjects in that quintile. We considered maternal age (and squared maternal age), parity and year of infant’s birth as potential confounders. All analyses were undertaken using SAS.

Results

There were 1980 children in our cohorts, for whom we had at least one lead exposure measure either from the mother (blood lead at delivery or bone lead) or from the child (cord blood lead). Among these children, the sex ratio was 1.11, which is a male percentage of 52.5%. A summary of information of the clinical variables in relation to quintile of maternal blood lead measurement is presented in Table I. All lead measures generally increased with increasing quintile of maternal blood lead, but there was little difference in year of child’s birth or mother’s age at delivery. There appeared to be fewer nulliparous women with increasing quintile. In Table II, lead biomarker levels across categories of the covariates are shown for all lead measures.

The unadjusted sex ratio appeared to increase somewhat over the first three quintiles of maternal blood lead, although
there was no significant trend over all five quintiles ($P_{\text{trend}} = 0.40$). After adjusting for age of the mother at the child’s birth, the square of this age, the year of the child’s birth and the mother’s parity, the odds that an infant was male were significantly elevated for the third quintile of maternal blood lead, as compared with the lowest quintile [odds ratio (OR) = 1.47, 95% CI = 1.07–2.01]. We found no consistent association, however, between the odds of a male child and any of the lead biomarkers, and with the exception above, the adjusted OR for a male child was not significantly different from 1 for any other quintile of lead biomarker (Figures 1–4). The unadjusted results were also virtually identical to the results of the adjusted models. In analyses with lead modeled as a continuous linear term, there was no significant association with odds of a male child for any of the lead biomarkers.

### Discussion

Lead remains one of the most significant environmental agents associated with altered human reproduction (Castellino et al., 1995; Bonde et al., 2002b). However, in this study of pregnant women with a wide range of lead exposure, we did not find an association between maternal blood lead, bone lead in the patella or the tibia or cord blood lead with the secondary sex ratio of infants born in Mexico during the periods 1995–1996 and 1997–2002. These null findings are at variance with previous theoretical postulates. Lead is known to cross the placenta, presumably by diffusion (Goyer, 1990). Both a review of the historic and recent literature and a prospective study from Mexico using incidence–density-matched controls suggest that lead exposure in women is associated with a risk of spontaneous abortion (Borja-Aburto et al., 1999). The OR for spontaneous abortion was 1.8 for every 5 μg/dl increase in blood lead of
these Mexican women. Mexico has been identified as a country with both substantial population exposures to lead and one of the most profound reductions in sex ratio over the past 50 years (Grech et al., 2003). The early nutrition of the mother has received increasing attention in regard to events related to the later health of the conceptus (Barker et al., 1993). These considerations have extended to the health of the embryo at the time of conception during IVF (Cetin et al., 2003). One of the proposed mechanisms of change in sex ratio is a variable rate of occult loss of male embryos (Jongbloet et al., 2002). While lead has been associated with overt (versus occult) spontaneous abortion, the current findings among pregnant women do not suggest an association of even relatively high levels of environmental lead exposure and a reduction in the proportion of male births.

This study is strengthened by the size of the study population, the multiple and sensitive approaches to the measurement of lead exposure and the standardized techniques of lead quantification. Mexico began phasing out leaded gasoline in 1987, and gasoline was eliminated in 1997, which has resulted in lower overall exposures to lead during a protracted period of decline in the nation’s sex ratio (Cortez-Lugo et al., 2003). Thus, it is possible that our results are confounded by unmeasured secular phenomena such as additional exposures and socioeconomic factors. The births in our study occurred over a narrow time window, and we adjusted for year of birth, reducing the impact of any such bias. We did not have data on paternal age, which is associated with sex ratio in some studies. However, the impact of this covariate on the estimated association between lead and sex ratio is anticipated to be limited (Jacobsen et al., 1999).

In order to explore the impact of lead on the sex ratio of the current cohort, measures of lead were evaluated in maternal blood, cord blood and maternal bone lead (Gonzalez-Cossio et al., 1997; Ettinger et al., 2004). The maternal blood lead levels were obtained post-partum and not at the time of conception. However, the use of all maternal and cord blood and patella and tibia bone lead levels provides for a broad evaluation of lead exposure in pregnancy in general. Although there were no blood measurements taken at the time of conception, bone lead may represent an appropriate surrogate (Aro et al., 2000). When compared with maternal blood measurement, bone lead measurement has been shown to be a better predictor of the adverse outcomes in pregnancy, including low birthweight (Gonzalez-Cossio et al., 1997), low infant weight gain, lower head circumference and lower birth length (Hernandez-Avila et al., 2002) and lower scores of the Bayley Scales of Mental Development at age 2 years (Gomaa et al., 2002). As lead is known to be released from maternal bone during pregnancy, the measurement of plasma lead at the time of conception and
the first trimester, when most pregnancy losses occur, may provide an improved approach to the assessment of lead exposure in relation to eventual sex ratio of the progeny (Hu, 2002).

There has been a substantial interest in the changes noted in sex ratio during the past century, particularly in the potential explanations for secular changes. There is considerable debate on the validity of a causal relationship of environmental exposure to hormonally active chemicals or endocrine disruptors to the secondary sex ratio, with some reflection that the changes observed over time are statistical variants (Gini, 1955; Dodds and Armson, 1997; James, 1998). The inherent complexity in the endocrine disruption hypothesis has been explored by a comprehensive review by Krimski (2001). The observed alterations in sex ratio may require further elaboration prior to becoming established as a component of this hypothesis. In the present study, we did not observe an association of lead with the sex ratio. Notably, however, there was a significant increase in the odds that a child was male in the third quartile of maternal blood lead. Such a finding may reflect chance, particularly as it is not substantiated through our other measures of lead, but it also may represent an inverted U-shaped effect summarized by Calabrese and Baldwin (2001) and may reflect the inherent complexity of endocrine disruption noted by Krimski (2001).

In summary, in a community of pregnant women with a wide range of contamination with lead, we found no evidence that maternal or cord blood levels of lead were associated with the secondary sex ratio.

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