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Maternal nutritional status during pregnancy and surma use determine cord lead levels in Karachi Pakistan

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Abstract

Objectives—To estimate the umbilical cord blood lead levels (BLLs) of Pakistani neonates and to identify determinants for umbilical BLLs.

Methods—We conducted a cross-sectional study of mothers and infants at one of the two obstetric units of two tertiary care hospitals in Karachi during January to August 2005. Information from 540 mothers selected randomly from those registered for delivery was obtained about their pregnancy, diet, and current and past lead exposures. We collected umbilical cord blood for lead levels analyzed using graphite furnace atomic absorption spectrophotometry. We computed geometric and arithmetic means. We performed multiple linear regression analysis to identify factors associated with log transformed umbilical cord BLLs. We also performed logistic regression analysis to identify determinants of high lead cord BLLs ($\geq 10\mu\text{g/dl}$).

Results—The geometric mean cord BLLs of the neonates was $9.6\mu\text{g/dl}$; arithmetic mean (SD) was $10.8\mu\text{g/dl}$ (5.7) with a median of $9.7\mu\text{g/dl}$ and a range of $1.8\mu\text{g/dl}$ – $48.9\mu\text{g/dl}$. Women who reported intake of less than 58.5 mg of elemental iron supplement per day during pregnancy had cord BLL of $10.0\mu\text{g/dl}$; in comparison those women who had higher iron intake had lower cord BLL ($8.4\mu\text{g/dl}$). Those who used surma (an eye cosmetic) daily had higher cord BLL ($11.5\mu\text{g/dl}$) as compared to those who used it less frequently ($9.4\mu\text{g/dl}$). In multivariable linear regression model, higher iron intake, owning a car, and being in 2nd quartile of mid arm circumference were associated with low lead levels while father's occupation in lead based industry were associated with significantly higher umbilical cord blood lead levels. There was interaction of daily surma use and ethnicity. Geometric mean BLLs were varied among surma users by ethnicity.

Conclusions—Umbilical cord BLLs are high in Karachi, Pakistan in comparison to those in developed countries such as United States. Measures are needed to reduce fetal lead exposure to

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Institutional Review Board (IRB) approval

The study was approved both by the IRB of the University of Alabama at Birmingham and the Ethics Review Committee of the Aga Khan University in Karachi, Pakistan.

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prevent adverse affect on neurocognitive development. Association of low iron (below RDA of 60 mg per day) with high umbilical cord has implications for strengthening iron supplement intake during pregnancy. Umbilical cord BLLs of differed among surma users by ethnicity.

Keywords

Umbilical cord blood lead levels; iron; surma; socioeconomic status; Pakistan

Introduction

Lead toxicity is a major public health concern worldwide. Prenatal and early childhood lead exposure has long been found to be associated with low intelligence quotient (IQ) levels (Needleman et al. 1979). A recent pooled analysis of seven cohort studies showed that IQ declined by 6.9 points among children for an increase in blood lead level from 2.4 to 30 µg/dl (Lanphear et al. 2005). Lead exposure shifts the IQ distribution of an entire population towards lower values, leading to a decrease in overall intellectual level and productivity of the population (Grosse et al. 2002; Mendelsohn et al. 1998). Besides effect on IQ, prenatal lead exposure significantly affects other neurocognitive functions including fine motor movements (Baghurst et al. 1995; Dietrich et al. 1993; Ris et al. 2004; Shen et al. 1998). Some studies have reported that behavioral problems such as antisocial and delinquent behavior in childhood and adolescence are associated with prenatal lead exposure (Dietrich et al. 2001). Prenatal lead exposure also significantly increases the risk of adverse birth outcomes such as low birth weight, reduced head circumference and length (Gonzalez-Cossio et al. 1997; Hernandez-Avila et al. 2002). Furthermore, neurocognitive changes may be irreversible even if exposure to lead decreases or ceases after birth (Tong 1998; Tong et al. 1998). Therefore prevention of fetal lead exposure could yield enormous public health benefits from prevention in loss of health, productivity and adverse social impacts.

Like other developing countries leaded gasoline was major source of lead exposure in Pakistan (Rahbar et al. 2002). Unleaded gasoline was introduced in Pakistan during 2001–2002 (2002). Although there is decline in blood lead levels (BLL) over time from on average of 38.2 µg/dl in 1989 to 16.5 µg/dl in 2000 and 10.8 µg/dl in 2005, high BLL persists (Kadir et al. 2007). Investigators attribute the decline in BLL to declining levels of lead in gasoline over time. However, other sources of lead exposure still exist. Bones serve as the long term repository of lead. In an adult, about 95% of lead accumulates in bone (Barry and Mossman 1970). Lead competes with calcium for absorption and bone deposition. When there is an increased demand for calcium, for instance during pregnancy and lactation, lead is released from bones in the mother and gets transferred to fetus or nursing infant respectively (Johnson 2001; Tellez-Rojo et al. 2004). Thus, lead already deposited in maternal bones will continue to expose fetuses for decades to come even after decline in environmental exposures. Identification of factors that promote transfer of lead from mother to fetus during gestation could help in developing interventions for prevention of lead transfer. Studies have reported that low calcium intake, low iron intake and poor nutritional status are associated with high umbilical cord BLLs (Baghurst et al. 1987; Graziano et al. 1990; Johnson 2001; Schell et al. 2003). The distribution of determinants of prenatal lead exposure in Pakistan may be different from other settings because of differences in nutritional status, diet, environmental exposures and the cultural practices such as surma use (Akhter et al. 2004; Lone et al. 2004). The main purpose of this study was to determine if nutritional status in a Pakistani population where malnutrition is common is an important factor in fetal lead exposure. Further, it investigated the role of environmental factors other than gasoline that might be important determinants of high umbilical cord BLLs. In this study, we determined umbilical cord BLLs and their determinants in neonates born to mothers of low and middle socioeconomic status in Karachi, Pakistan.

Materials and Methods

Study setting

The study was conducted in Karachi, the largest city, the main port and industrial and trade center of Pakistan. It has a population of more than 10 million, about 40% of whom live in squatter settlements. Karachi has the largest traffic fleet in the country. In May 2006, there were 1,508,215 registered vehicles in Karachi which constituted about 25% of total vehicles in Pakistan (2006). There are many industrial sites with automobile battery and car manufacturing plants. Besides a conventional industrial sector, there is a large informal sector such as backyard car repair and battery recycling workshops. Our study included neonates born at two tertiary care hospitals located in the inner city and receiving low and middle income patients from various parts of the city.

Study design

We conducted a cross-sectional study of mother-infant pairs in the obstetric units of two tertiary care hospitals in Karachi during January to August 2005 to assess the determinants of cord BLLs.

Study participants

Eligible subjects were women who were residents of Karachi for at least one year who were admitted for delivery in one of the two study hospitals, who were expected to deliver a singleton fetus at completed term (37–42 weeks of gestation) and who did not have had physician diagnoses of psychiatric morbidity, kidney or cardiac disease, history of repeated urinary tract infections, sickle cell anemia, thyrotoxicosis, autoimmune diseases as Crohn's or celiac disease, drug dependence, steroid intake during pregnancy, antepartum hemorrhage, placental abnormalities as abruptio placentae or fetus with congenital anomalies, or preeclampsia.

Subject selection

We randomly selected ten mothers from the registration records, who registered for delivery each day at the study hospitals. We invited selected mothers for eligibility screening and study participation. We screened the medical records of eligible mothers for exclusion criteria. Those found eligible at this stage were invited for participation in the study and written consent was obtained.

Exposure assessment

We used maternal interview, medical record review and maternal anthropometric examination to obtain information on possible sources of lead exposures and other exposures, behaviors and personal characteristics that might be related to cord BLL.

We used an Urdu translated questionnaire to collect information which was pretested before the start of data collection. Research assistants who were registered nurses interviewed mothers after receiving training in interviewing methods and field procedures. Before the start of data collection, we field tested study procedures. Maternal interview and measurements were conducted both before and after delivery depending on mother's condition.

The questionnaire elicited information on socio-demographic factors, obstetric history, diet during pregnancy, and sources of lead exposure; socio-demographic factors included maternal age, education, occupation, husband's education and occupation, household income, and possession of household assets. Since questionnaire based income information does not provide a good indication of socioeconomic status (SES) in developing countries, we constructed wealth index based on household possessions with proportionate weighting. Such indices have

been widely used in other developing countries including India (Gwatkin et al. 2000; Subramanian et al. 2006). Wealth indices based on household possessions have shown good reliability and validity for SES along with education and occupation for child death taken as an indicator for health status in Karachi, Pakistan (Durkin et al. 1994). In constructing the wealth index, the weight for each item was derived on the basis of the proportion of households owning the particular item. Thus, for example, if 40 households in a sample of 100 owned a TV, then a TV would get a weight of 60 (100–40). Weights for each item were summed into a linear index, and households were allocated a final score. Since the wealth index is a constructed composite measure, it does not have a direct interpretation. We divided the population into tertiles of the wealth index for our analysis.

Questions on lead exposure sources included current and past places of residence, total number of years spent at that residence in Karachi, location of house with respect to main road, frequency of keeping windows open and frequency of dust cleaning at home, maternal and paternal occupation in lead related industries elicited by asking about each potential occupation involving lead (laborer in bridge/tunnel/road construction, painter, auto battery manufacturing/repair, denting painting, radiator mechanic, soldering/welding, dyeing, cement factory, plastic manufacturing, pottery/ceramics maker, glass industry, brass/copper foundry, valve and pipe fitting, refinishing furniture, mining/sand blasting, jewelry making/polishing), condition of paint at home, remodeling of house in past 12 months, use of lead-containing utensils and use of surma (traditional eyeliner containing lead).

The questionnaire also included information on parity, pre-pregnancy weight, prenatal care (number and time of each visit), smoking during pregnancy, exposure to environmental tobacco smoke (ETS), anemia during pregnancy, use of calcium, iron and vitamin C supplement (with the duration of use and brand name) during pregnancy. Dietary intake of calcium, iron, and vitamin C during current pregnancy was assessed using a food frequency questionnaire (FFQ). FFQ elicited information on the intake of foods in the most recent month including the frequency per week and the quantity according to portion size. To help in recall, pictures and models of standard portion sizes applicable in the local settings were used.

Maternal weight, height, mid-upper arm circumference (MUAC), biceps and triceps skin fold thickness was measured after delivery. MUAC, biceps and triceps skin fold thickness was measured thrice on non-dominant arm. MUAC and the biceps skin fold thickness has been shown to predict the pre-pregnancy nutritional status as these indices change very little during pregnancy (Taggart et al. 1967).

Outcome assessment

The outcome of interest is umbilical cord BLLs. At delivery, umbilical cord blood was collected in trace metal BD Vacutainer™ glass sterile tube containing disodium ethylene diamine tetra acetate (Na₂EDTA). The laboratory analysis for BLL was performed at the Pakistan Institute of Scientific and Industrial Research laboratory, (PCSIR) Karachi, using graphite furnace atomic absorption spectrophotometer procedure with Zeeman background correction (Miller et al. 1987). The PCSIR has been participating in CDC's Blood Lead laboratory Reference System (BLLRS). To re-evaluate calibration and quality control in testing procedures, the PCSIR tested BLLRS bovine blood reference materials with known lead levels. Intra-class correlation coefficient was 0.999 mean (SD) of PCSIR: 29.7 (SD, 19.4) vs. mean of CDC target values: 30.4 (SD, 19.17).

Sample Size

Sample size estimation was performed for high cord lead level, determinants of high lead level, and association of cord BLL and low birth weight (LBW). A sample size of 388 mother infant

pairs was required to estimate prevalence of high cord BLL ($\geq 10 \mu\text{g/dl}$). A sample of 396 mother and infants were required for determinants of cord BLL. A sample size of 533 mother-infant pair was needed to evaluate the association of high BLL and LBW. Thus we enrolled 540 mothers and infants. We assumed that about 80% of women will have blood lead level $\geq 10 \mu\text{g/dl}$ as in a previous study on children (Rahbar et al. 2002) and umbilical cord lead level will be 75%–90% of the maternal levels (Ernhart 1992). Sample size for examining the relationship between lead and LBW was based on the assumption that the 64% of normal weight infants would have $\text{BLL} \geq 10 \mu\text{g/dl}$. With 5% significance and 80% power and assuming 1:3 ratio of normal and LBW babies and to detect an odds ratio of 2.0 and 10% non-response rate we would require a sample size of 533 newborns.

Statistical Analyses

The data were double-entered and reconciled with cross-checks using Epi Info 2002. Analyses were performed using SAS version 9.103.

To perform statistical methods requiring normality we log transformed umbilical cord lead levels because distribution was skewed. We computed geometric mean, arithmetic mean, standard deviation, median and proportion of those who had levels $\geq 10 \mu\text{g/dl}$. We compared log transformed mean umbilical cord BLLs using t-test and ANOVA and back transformed lead levels for presentation purposes.

We performed two set of analyses to identify determinants of umbilical cord BLL: multiple linear regression modeling by using log transformed umbilical cord BLL as continuous variable, and logistic regression by using a dichotomous outcome, high lead ($\geq 10 \mu\text{g/dl}$) and low lead ($< 10 \mu\text{g/dl}$).

We evaluated socio-demographic variables, nutritional status and nutrient intake and environmental exposure for their association with both of the BLL outcome variables. Socio-demographic variables included mother's age categorized into decades; mother's and father's education categorized into < 5 , 6–12 and > 12 years of education; father's occupation classified into 4 occupational categories based on international occupational classification including (1) manual minimal skilled laborers, (2) skilled trades occupations, (3) administrative workers and (4) professionals and executives (Hoffmann and Scott 1993). The wealth index constructed for socio-economic position was categorized into tertiles. Income was dichotomized at the median. Ownership of car was also used as an indicator of SES (Table 1).

Nutritional status measured with MUAC and biceps skin fold thickness was converted into quartiles. Calcium intake was computed from data on supplements and diet. For supplements, quantity of calcium was determined based on manufacturer information provided in drug index (2006). Quantity from each supplement taken each day was summed to obtain daily calcium intake from supplements. Dietary calcium intake was derived from the quantity of calcium contained in each food item reported on FFQ. Calcium estimates were obtained from Pakistani food tables. If a food was not available in Pakistani food tables, the calcium quantity was derived from Indian or United States food tables. The amount of calcium consumed was computed by multiplying calcium contained in a serving of food with servings of food consumed each day. Total calcium intake per day was the sum of calcium from diet and supplements. The same approach was used to compute elemental iron and Vitamin C intake from supplements, diet, and daily total intake (Table 2).

Exposure to automobile lead was measured using the location of house from the main road, and the number of houses between the index house and the main road. Residence in Karachi was derived by classifying residents into those who had been living somewhere else vs. those who were living in Karachi since birth. Years of residence in Karachi was derived by computing

the time between the year the participant shifted to Karachi and the interview date or age for those born in Karachi (Table 2).

For multivariable linear regression analyses, we started model building with assessment of the association of each variable with umbilical cord lead levels. Variables that were significant at $P < 0.2$ or were of main interest based on literature were selected for evaluation in multivariable models. We started with most significant variables and continued adding variables till there was no additional change in R^2 and variables were not significant. After selecting the main effects model we tested biologically plausible interactions. Model goodness of fit was evaluated for outliers, normality, linearity and constant variance (Table 3).

In logistic regression analysis, the outcome variable was dichotomous: high lead ($\geq 10 \mu\text{g/dl}$) and low lead ($< 10 \mu\text{g/dl}$). We started with univariable analysis in which crude odds ratio and 95% confidence interval was computed for each variable to select variable for evaluation in multivariable model. Variables that were significant at $P < 0.2$ or were of interest (calcium intake) based on literature were selected for evaluation in multivariable model. We started multivariable model building with the most significant variable and continued adding variables while assessing their significance and change in odds ratio estimate. A variable that was not significant and did not produce change in effect estimate of $> 10\%$ was removed from the model. Interactions of variable included in main effect model or that were biologically relevant were evaluated. Goodness of fit was assessed using Hosmer and Lemshaw test.

Institutional Review Board (IRB) approval

The study was approved both by the IRB of the University of Alabama at Birmingham and the Ethics Review Committee of the Aga Khan University in Karachi, Pakistan.

Results

Study participants

Of 807 mothers initially screened for eligibility, 565 were eligible. All consented to participate in the study. Data on outcome or major portion of interview was missing for 25 participants. Thus, 540 subjects were available for analysis which was the planned target for enrollment (Figure 1).

The gender distribution of infants was approximately equal (male, 54%; female, 46%). The median birth weight was 3.0 Kg and head circumference was 34.5 cm. Mothers were relatively young with a mean and median age of 25 years. A higher proportion of fathers completed 12th grade in comparison to mothers (14% vs. 6.5%). Only 2% of mothers were employed. About 59% of fathers were manual or skilled laborers. Median monthly household income was Rs. 7000 (US\$ 117) and about 4% of the household owned a car. Mean maternal BMI was 24.5 while 4 % had BMI below 18 and 12% had BMI ≥ 30 . Mean daily calcium, vitamin C and iron intake were 973.0 mg, 801.5 mg and 70.0 mg, respectively.

Umbilical cord BLLs

The geometric mean umbilical cord BLL was $9.59 \mu\text{g/dl}$. The cord BLL ranged from $1.78 \mu\text{g/dl}$ to $48.93 \mu\text{g/dl}$, with an arithmetic mean of $10.84 \mu\text{g/dl}$ (SD, 5.7), median of $9.73 \mu\text{g/dl}$ and interquartile range of $6.5 \mu\text{g/dl}$. About half (259/540, 48%) of the newborns had umbilical cord BLLs $\geq 10 \mu\text{g/dl}$ (Figure 2). The geometric mean BLL was higher for fathers working in lead based occupations (9.31 vs. 10.9, $P = 0.011$). The geometric mean BLL was significantly lower if the mother had completed 12th grade as compared to those who completed 5th grade or less (8.09 vs. 10.16, $P = 0.037$). The newborns of women who took ≥ 58.5 mg of elemental iron supplement per day during pregnancy had lower umbilical cord BLLs than of those who took

less (9.95 vs. 8.35, $P < 0.001$). Geometric umbilical cord mean BLLs were higher if the mother used surma daily during pregnancy than if she used surma less frequently (9.40 $\mu\text{g/dl}$ vs. 11.47 $\mu\text{g/dl}$, $P = 0.006$, Table 2). Calcium consumption during pregnancy was not significantly associated with lead level.

Determinants of umbilical cord BLLs

In univariable linear regression ownership of a car, monthly income above median, mother's education, mother's ethnicity, MUAC, intake of ≥ 58.9 mg of elemental iron supplement per day, having had used calcium before pregnancy, having had used calcium during pregnancy, vitamin C supplement intake, fathers occupation in lead related industry and daily surma use were significantly associated with lead levels.

Multivariable linear regression model showed that intake of equal to and more than 58.5 mg of elemental iron supplement per day during pregnancy, MUAC in 2nd and 3rd quartile, ownership of car or income above median was associated with low lead levels. Father's occupation in lead related industry was associated with high lead level. With increasing parity, umbilical cord lead level also increased (Table 3). There was a significant multiplicative interaction of daily surma use and mother's ethnicity ($P = 0.0085$). Predicted geometric mean umbilical cord BLLs were highest among Punjabi if they were also using surma daily (21.37 $\mu\text{g/dl}$) and lowest among Sindhi if they were using surma (6.69 $\mu\text{g/dl}$, Figure 3).

Multivariable logistic regression model of factors associated with high lead levels (≥ 10 $\mu\text{g/dl}$) revealed that umbilical cord BLL were higher for mothers living in houses which kept windows open most of the time (proxy for ambient air lead) than those who kept them closed most of the time (adjusted OR: 1.53, 95% CI: 1.03–2.26). Odds of high umbilical cord BLLs were higher if mothers used surma daily as compared to those who used surma less frequently (adjusted OR: 2.10, 95% CI: 1.13–3.91). If mothers did not take calcium supplement before pregnancy, odds of high lead level were higher than if mother used calcium before pregnancy (adjusted OR: 3.39, 95% CI: 1.18–9.70). Umbilical cord lead levels were higher if mother took iron supplement < 58.5 mg/day as compared to 58.6–387 mg per day of elemental iron (adjusted OR: 1.92, 95% CI: 1.23–3.00, Table 4).

We also performed multivariable logistic regression analyses to determine factors associated with high cord lead levels (4th quartile [≥ 13.43 $\mu\text{g/dl}$] vs. 1st quartile [≤ 6.91 $\mu\text{g/dl}$]). Results were similar to those reported above.

Discussion

This is the first systematic study of umbilical cord BLL in Pakistan. We investigated various socio-demographic, environmental, life style and biologic determinants of cord BLL among neonates born to low and middle socioeconomic mothers in hospitals in Karachi. Overall umbilical cord BLLs in Karachi were very high in comparison to those reported from developed countries like USA (Rothenberg et al. 1999). About half of the neonates had levels ≥ 10 $\mu\text{g/dl}$. Low iron intake, poor nutritional status, surma use among certain ethnic groups and father's occupation in lead related industry, determined higher cord BLLs. Lead levels were lower if mother had been taking calcium supplement before pregnancy. These factors suggest that along with the environmental and occupational exposures which were previously known nutritional status and cultural practices like surma use determine umbilical cord lead levels.

Umbilical cord BLLs in current study was high. There are no previous data on umbilical cord BLLs in Pakistan. However, our findings of high cord BLLs are consistent with high BLLs among preschool children in Karachi conducted in 2000 (Rahbar et al. 2002). However, proportion of BLLs above 10 $\mu\text{g/dl}$ in this study was higher (84%) than found in current study

(48%)(Rahbar et al. 2002). Despite phase-out of leaded gasoline in 2001–2002, BLL among young children remain high, though declining (Kadir et al. 2007). This has serious public health implications for child behavior and cognitive functioning of Pakistani children. Studies from other parts of the world have shown that IQ declined by 6.9 points for an increase in BLLs from 2.4 to 30 µg/dl (Lanphear et al. 2005). Applying these findings in the Pakistani context, we hypothesize that neonates in the current cohort found to have high cord BLL will be at a high risk for development of cognition/behavior problems.

In our study, we found that maternal intake of less than 58.9 mg of elemental iron supplement per day was associated with high cord BLLs. Some studies of children and pregnant women have reported similar findings (Baghurst et al. 1987; Bradman et al. 2001; Choi and Kim 2003; Hammad et al. 1996; Lanphear et al. 2002; Schell et al. 2003; Wright et al. 2003), whereas other studies including a randomized trial among children do not support this association (Brown et al. 2000; Rosado et al. 2006; Serwint et al. 1999). In the latter trial, the investigators suggest that the low iron-low BLLs association may be more pronounced in iron-deficient children, and that only 10% children were anemic in this study (Rosado et al. 2006). Studies of pregnant women have reported a relationship of low maternal iron intake/iron deficiency and elevated cord BLLs (Baghurst et al. 1987; Graziano et al. 1990; Schell et al. 2003). Studies in animals also have reported that iron deficiency created by feeding animals an iron-deficient diet increases absorption and retention of lead and its toxicity (Ragan 1977). It has been found that iron and lead compete for receptors in intestine for absorption (Bannon et al. 2002; Barton et al. 1978). Iron deficiency is common in Pakistan and iron intake is low. Studies have reported that if mothers are anemic or had low iron intake during pregnancy, cord BLLs are likely to be elevated (Graziano et al. 1990). Thus, although effect of iron supplement of mostly replete children on lead has been assessed through a randomized trial and it was not significant (Rosado et al. 2006), such studies are indicated among pregnant women in settings where iron deficiency is common like in Pakistan. Recommended daily iron intake during pregnancy is 60 mg/day (Stoltzfus and Dreyfuss 1998). Our results suggest that following current recommendations of daily iron intake during pregnancy can reduce lead transfer to fetus. This has pragmatic implications for enhancing efforts for increasing iron intake during pregnancy by public health programs.

High calcium intake during pregnancy has been reported to be associated with low lead levels, though results are not consistent (Brown et al. 2000; Ettinger et al. 2007; Graziano et al. 1990; Gulson et al. 2004; Hernandez-Avila et al. 1997; Hertz-Picciotto et al. 2000; Janakiraman et al. 2003; Kawata et al. 2006; Morris et al. 1990). It has been suggested that lead compete with calcium during absorption from intestine, deposition in bone and release from bone during bone resorption as in pregnancy (Fullmer 1991; Gulson et al. 1997; Gulson et al. 1999; Tellez-Rojo et al. 2004). In our study, calcium intake during pregnancy was not associated with lead level. Mean calcium intake from diet and supplement was 974.0 mg per day and many women had adequate calcium intake as recommended (1000 mg per day during pregnancy)(Picciano 2003). However, calcium supplement intake before pregnancy was associated with low umbilical cord BLLs. This may suggest that long term calcium intake may prevent lead transfer in this population. Since lead competes with calcium for absorption and then deposition, (Fullmer 1991; Gulson et al. 1997; Gulson et al. 1999; Tellez-Rojo et al. 2004), if women are taking calcium before pregnancy, the amount of lead absorbed and deposited may be less thus, reducing the release of lead from bone and its transfer to fetus. Calcium supplement intake before pregnancy may be confounded by the high socioeconomic status, as women from higher socioeconomic strata are more likely to have low lead level and more likely to take calcium supplement (Shahar et al. 2005). The reported association is adjusted for socioeconomic status; hence association of prepregnancy calcium intake cannot be explained by the confounding by socioeconomic status.

Poor nutritional status during pregnancy has been reported to be associated with high lead levels. In our study poor nutritional status measured by MUAC was associated with high lead level. A study from USA also reported association of poor nutritional status measured by MUAC with high cord BLLs (Schell et al. 2003). Our findings suggest that if women have a poor nutritional status to start with and then their iron intake is low, cord BLL of their neonates will be high. Since nutritional status is a modifiable factor, supplementation of women with poor nutritional status during pregnancy could reduce transfer of lead from mother to fetus.

This is the first study of prenatal lead exposure to report an association between surma use and cord BLLs. Furthermore, cord BLLs varied by ethnicity among surma users. Surma is an eye cosmetic which is mined and then ground to make powder which is applied to eyes. Surma is mainly used in Africa, Arab and many South Asian countries. Studies of South Asian immigrants in United Kingdom (UK) (Ali et al. 1978) and US (Parry and Eaton 1991; Sprinkle 1995) and of children in Nigeria, Saudi Arabia, (Al-Ashban et al. 2004; Al-Saleh et al. 1999) and Pakistan (Rahbar et al. 2002; Wright et al. 2005) have reported associations between surma use and elevated BLLs among preschool and school age children. Previous studies conducted in the US, the UK, Saudi Arabia and Oman found that Pakistan-produced surma generally contains a large amount of lead varying from 16% to 70% (Al-Ashban et al. 2004; Ali et al. 1978; Birch 2006; Hardy et al. 1998; Parry and Eaton 1991). A popular surma brand from Pakistan that has been shown to contain high lead levels in Saudi Arabian and American studies was found to contain many other ingredients besides surma ore (Al-Ashban et al. 2004). The above finding suggests that other ingredients in surma besides the surma ore may also contain lead. This fact is further substantiated by our finding of low lead levels in Sindhis using surma daily compared to all other ethnic groups. Preliminary discussion with the local shopkeepers and our project staff indicated that Sindhis usually prepare surma from surma ore without adding any other ingredients, whereas the surma used by Punjabis contains other ingredients. Thus possible reasons for differences in cord BLLs among surma users by ethnicity may include difference in sources of surma, its preparation, addition of other constituents during preparation that may contain lead and method of its application. Therefore, there is a need to investigate the lead levels of surma used by various ethnic groups. Furthermore, behaviors and beliefs of various ethnic groups about surma use need to be studied to develop educational and behavior change interventions for reducing lead level from this source. At the national level, regulations for lead in surma need to be considered.

We found that father's occupation in lead related industry to be associated with high umbilical cord BLLs. Association of prenatal lead exposure and fathers occupation in lead based industry have been reported from Yugoslavia (Graziano et al. 1990). Father's occupation was also associated with child BLL Karachi (Rahbar et al. 2002). Carrying of lead dust on clothing, skin and hair from workplace to homes has been reported to be associated with high lead level among children of lead workers (Baker et al. 1977). Pregnant women also get exposed from spouses who work in lead-related jobs. There is a need to institute regulatory hygiene measures for lead workers as well as education to protect their families from lead exposure.

Low SES is associated with higher BLLs (Gump et al. 2007; Lidsky and Schneider 2003; Malcoe et al. 2002; Mathee et al. 2006; Wright et al. 2005). Low SES affects cord BLLs through several pathways: nutritional deprivation leading to higher lead absorption and then release during pregnancy (Cheng et al. 1998), living in highly polluted area; high prevalence of smoking; and occupational lead exposure (Lidsky and Schneider 2003; Malcoe et al. 2002). In current study, low SES was associated with higher cord BLL even after adjusting for iron intake and MUAC. We assessed SES using two different indicators of SES, ownership of car and income, and results were same. Thus, our results indicate that neonates born in low socioeconomic families are at the risk of high lead exposure. Furthermore, studies also suggest that children from disadvantaged socioeconomic background are more sensitive to the adverse

effects of lead exposure (Bellinger 2000; Tong et al. 2000). Thus, pregnant women from low socioeconomic background should receive greater attention in prevention efforts.

Studies have reported association of proximity of house to the road,/highways distance of house from road and residence in the city from Karachi and other places(Chuang et al. 2001; Rahbar et al. 2002; Shen et al. 1997). In our study, these markers of automobile lead exposure were not significant. Lead from gasoline has been phased out in 2002 and exposure from leaded gasoline no longer exists. Although lead already released into environment and deposited in dust continues to be source of lead exposure. Furthermore, cord BLL is derived from the lead deposited in maternal bones which is influenced by other factors like nutritional status and nutrients intake such calcium and iron before and during pregnancy.

Strengths and Limitations

Major strengths of the study include reasonable sample size, no refusals, rigorous training and run-in phase, lab personnel blinded to exposure data and interviewers blinded to lead levels, and measurement and control for important confounders.

Many of the exposure measurements methods were based on self reports with possibility of imperfect recall and misclassification. Misclassification of exposure may have attenuated measures of effect. However, information bias is unlikely as women at the time of interview were not aware of their cord BLLs. More objective methods of exposure measurement like lead level in environmental sources like paint, dust etc should be used in future studies.

The study enrolled women who gave birth in hospitals. A significant proportion of women (30%) in Karachi, Pakistan deliver at home (2007). Hence, those delivering at hospital may be different from women delivering at homes in their cultural beliefs, medical conditions forcing them to deliver in hospital, or are more health conscious. Therefore, we caution about the generalizability of our findings.

Conclusion

Umbilical cord lead levels in Pakistan are very high in comparison to those in developed countries and large proportion of neonates had lead levels above the CDC levels of concern. Measures are needed to reduce fetal lead exposure to prevent adverse affect on neurocognitive development.

In post gasoline lead phase-out era, nutrient intake and nutritional status (low iron and calcium intake), and cultural factors (surma use), low socioeconomic status besides traditional factors like occupation in lead based industries, and dust lead determined umbilical cord BLL. Furthermore, there is difference in lead levels among ethnic groups by surma use. There is need to investigate the behaviors and beliefs about surma use, its preparation and application to understand the difference in BLL among surma users by ethnicity. Our findings have policy implications for regulating lead levels of commercially available surma preparation in Pakistan. Further, there is need to educate women about hazard of surma application as many women apply surma to newborns as well. Association of low iron (below RDA of 60 mg per day) with high umbilical cord has implications for strengthening iron supplement intake during pregnancy. There is also need to confirm the role of iron supplement intake during pregnancy and prevention of transfer of lead from mothers to fetuses through a randomized controlled trial. In the meanwhile public health program should stress the need for regular iron intake during pregnancy.

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Figure 1.
Selection of study participants

Figure 2.
Distribution of umbilical cord blood lead levels

Figure 3.
Predicted geometric mean umbilical cord blood lead level according to ethnicity and frequency of surma use based on multivariable linear regression model

Table 1

Selected socio-demographic characteristics of mother-infant pair and distribution of umbilical cord blood lead level across them

Variables	n	%	Geometric mean	Mean	SD	Inter quartile range	<i>p</i> ^a	% BLL≥10 µg/dl
Child characteristics								
Sex								
Male	289	54	9.6	10.8	5.5	6.5	0.9763	48
Female	251	46	9.6	10.9	6.0	6.6		48
Low birth weight								
Normal	440	81	9.7	11.0	5.8	6.5	0.3198	49
LBW	100	19	9.1	10.3	5.3	6.8		43
Parent's characteristics								
Mother age at delivery (years)								
<20	38	7	9.4	10.3	4.7	3.6	0.8175	13
21–30	401	74	9.5	10.9	6.0	6.6		18
>31	101	19	9.8	10.9	4.7	6.5		22
Mother's education								
<5 years of schooling	177	33	10.2	11.4	5.7	7.0	0.0368	20
6–12 years of schooling	328	61	9.5	10.7	5.8	6.5		18
>12 years of schooling	35	6	8.1	9.2	5.0	6.0		15
Father's education								
<5 years of schooling	125	23	9.9	11.4	6.1	8.0	0.0104	51
6–12 years of schooling	341	63	9.8	10.9	5.3	5.9		49
>12 years of schooling	74	14	8.1	9.6	6.7	5.9		35
Mother's employed								
No	469	87	9.5	10.8	5.7	6.5	0.5831	18
Yes	12	2	10.0	10.7	3.8	7.0		23
Unemployed	59	11	10.2	11.5	6.0	6.4		22
Wealth index^b								
1 st tertile	64	12	10.6	11.6	4.9	6.2	0.1869	22
2 nd tertile	307	57	9.5	10.8	5.5	6.3		18
3 rd tertile	169	31	9.3	10.7	6.4	6.9		17
Own a car								
No	517	96	9.7	10.9	5.7	6.5	0.0304	19
Yes	23	4	7.7	8.9	5.0	6.9		13
Mother's Ethnicity								
Urdu (Mohajir) ^c	368	68	9.5	10.6	5.2	6.4	0.0282	18
Sindhi	26	5	8.1	9.2	5.1	5.2		10
Punjabi	73	14	9.7	11.5	7.9	6.5		21
Pakhtoon	30	6	9.0	10.2	5.6	7.2		12
Baloch	43	8	11.8	12.9	5.6	7.5		24
Household Income								
Median and less (≤Rs. 7000)	289	54	10.1	11.3	5.4	6.4	0.0048	20
Above median (>Rs. 7000)	251	46	9.0	10.3	6.0	6.4		17
Father's occupation								
Manual minimal skilled laborers	320	59	9.7	10.9	5.5	6.1	0.3965	19
Skilled trades occupations	83	15	10.0	11.6	6.7	7.8		20

Variables	n	%	Geometric mean	Mean	SD	Inter quartile range	<i>p</i> ^a	% BLL≥10 µg/dl
Administrative and associate professionals	80	15	9.2	10.3	4.8	7.2		18
Professionals and executives	57	11	8.8	10.2	6.5	5.4		17
Parity							0.2499	
Primipara	213	39	9.5	10.7	5.7	5.7		44
1	137	25	9.2	10.7	5.7	5.7		45
2	99	18	10.5	10.3	4.9	6.6		59
≥3	91	17	9.6	12.2	7.4	8.0		51

Mean: Arithmetic mean, SD: standard deviation, BLL: cord blood lead level

^aTest of significance for comparison of log transformed cord BLL presented as geometric mean

^bWealth index: index based on proportionate weighted sum of household assets

^cMohajirs are group of people migrated from parts of India not included in Pakistan at the time of creation of Pakistan, mostly speak Urdu

Table 2
Selected nutritional and environmental characteristics and distribution of umbilical cord blood lead level across them

Variables	N	%	Geometric mean	mean	SD	Inter quartile range	<i>P</i> ^a	% BLL≥10 µg/dl
Nutritional factors								
Prepregnancy BMI								
Low (<18.5)	22	4	10.3	11.0	3.9	5.8	0.9024	59
Normal (18.5–24.9)	297	55	9.5	10.8	5.9	5.9		45
High (25–29.9)	158	29	9.5	10.8	5.3	7.2		50
Obese (≥30)	63	12	9.8	11.3	6.3	8.0		52
Anemia^b								
No (hemoglobin > 11g/dl)	152	30	9.2	10.5	6.2	6.6	0.2396	21
Yes (hemoglobin ≤ 11g/dl)	307	70	9.7	11.0	5.6	6.8		22
Mid arm circumference (cm)								
1st quartile (<26.0)	135	25	9.8	11.2	6.5	6.0	0.0281	19
2nd quartile (26.1–28.9)	135	25	8.6	9.6	4.4	4.9		15
3rd quartile (29.0–31.9)	135	25	9.7	11.0	5.7	7.2		20
4th quartile (>31.9)	135	25	10.2	11.6	5.9	7.1		21
Biceps skin-fold thickness (mm)								
1st quartile (≤7.0)	159	29	9.5	10.7	6.2	6.8	0.1099	17
2nd quartile (7.5–9.0)	113	21	9.7	11.2	6.1	6.7		20
3rd quartile (9.5–11.5)	136	25	8.9	9.9	4.5	5.9		16
4th quartile (> 11.5)	132	24	10.3	11.6	5.8	0.5		22
Daily iron supplement intake (mg)								
1st -3rd quartile (< 47)	426	79	9.9	11.3	6.0	6.6	0.0009	20
4th quartile (58.5–387.0)	114	21	8.4	9.3	4.4	4.8		14
Daily dietary iron intake (mg)								
1st quartile (≤17.4)	134	25	9.9	11.3	6.1	6.8	0.4210	19
2nd quartile (17.5–22.4)	136	25	9.1	10.0	4.2	5.9		18
3rd quartile (22.5–27.8)	135	25	9.4	11.2	7.4	8.0		18
4th quartile (>27.8)	135	25	10.0	10.9	4.6	6.0		20
Daily iron intake per day diet and supplements								
1st -3rd quartile (≤77.3)	405	75	9.9	11.3	6.0	6.6	0.0030	15
4th quartile (>77.3)	135	25	8.6	9.6	4.6	5.2		20
Used calcium supplement before pregnancy								
Yes	22	4	7.32	7.5	2.8	4.6	0.0096	20
No	518	96	9.70	10.9	5.8	6.6		49
Daily dietary calcium intake during pregnancy								
1st quartile (≤543.8)	135	25	9.1	10.1	4.5	6.4	0.5474	17
2nd quartile (543.9–710.7)	136	25	9.7	11.0	6.2	6.7		19
3rd quartile (710.8–899.4)	138	26	9.8	11.3	6.7	6.9		17
4th quartile (> 899.4)	131	24	9.7	10.9	5.2	6.7		21
Daily calcium supplement intake during pregnancy								
1st quartile (0)	164	30	10.0	11.0	5.0	5.9	0.3799	19
2nd & 3rd quartile (110–260)	289	54	9.5	11.0	6.2	7.1		19
4th quartile (268–1500)	87	16	9.1	10.2	5.4	6.8		17

Variables	N	%	Geometric mean	mean	SD	Inter quartile range	<i>p</i> ^d	% BLL ≥ 10 µg/dl
Daily total calcium intake during pregnancy								
1st quartile (≤724.9)	135	25	9.2	10.4	4.6	6.4	0.7975	18
2nd quartile (725–965.6)	135	25	9.8	11.3	6.7	6.4		18
3rd quartile (> 965.7–1181.1)	135	25	9.7	11.5	5.9	6.8		21
4th quartile (> 1181.1)	135	25	9.7	10.2	5.5	6.4		17
Daily dietary vitamin C intake during pregnancy								
1st quartile (≤130.2)	135	25	9.8	11.2	5.8	6.7	0.6794	20
2nd quartile (130.3–208.7)	135	25	9.7	11.0	5.7	7.3		29
3rd quartile (208.8–348.5)	135	25	9.7	10.8	5.4	6.0		13
4th quartile (> 348.5)	135	25	9.2	10.4	6.1	6.2		18
Daily vitamin C intake from supplement								
1st quartile (0)	134	25	10.3	11.3	5.2	5.6	0.0639	20
2nd quartile (50–325)	91	17	8.9	10.1	5.4	6.1		11
3rd quartile (326–550)	185	34	9.9	11.3	6.5	7.3		28
4th quartile (>550)	130	24	9.0	10.2	5.2	6.4		16
Total daily vitamin C intake during pregnancy (mg/day)								
1st quartile (≤297.9)	135	25	10.1	11.2	5.3	5.7	0.2499	20
2nd quartile (298.0–662.3)	135	25	9.1	10.4	5.5	6.7		17
3rd quartile (662.4–979.6)	135	25	9.8	11.3	6.7	6.6		20
4th quartile (>979.6)	135	25	9.3	10.5	5.3	6.8		18
Environmental Factors								
Father's occupation in lead based industry								
No	441	82	9.3	10.4	5.1	6.3	0.0114	18
Yes	99	18	10.9	12.7	7.5	9.2		21
Living outside of Karachi								
Outside Karachi	40	7	8.6	10.2	7.6	6.4	0.1367	15
Within Karachi	500	93	9.7	10.9	5.6	6.6		19
Location of house with respect to road								
On the main road	94	17	9.9	11.4	6.8	6.7	0.8127	18
In the street near road	286	53	9.5	10.7	5.8	6.4		17
In the street far from road	160	30	9.6	10.7	4.9	6.8		21
Keep windows of house open most of the time								
No	161	30	9.0	10.1	4.9	6.1	0.0782	16
Yes	379	70	9.8	11.2	6.0	6.9		20
Receive a lot of dust								
No	419	78	9.7	10.7	5.0	5.4	0.3486	19
Yes	121	22	9.2	10.2	4.9	5.8		17
Condition of paint in house								
No peeling	290	54	9.9	11.2	5.8	6.3	0.1264	20
Peeling at window, door or wall	250	46	9.3	10.5	5.6	6.4		17
Exposure to smoke at home or work								
							0.5334	

Variables	N	%	Geometric mean	mean	SD	Inter quartile range	<i>p</i> ^d	% BLL≥10 µg/dl
No	396	73	9.5	10.7	5.6	6.4		18
Yes	144	27	9.8	11.2	6.0	6.7		19

Frequency of surma use							0.0058	
Less than daily	487	90	9.4	10.6	5.2	6.4		18
Daily	53	27	11.5	13.5	9.1	7.6		23

Mean: Arithmetic mean, SD: standard deviation, BLL: cord blood lead level

^aTest of significance for comparison of log transformed cord BLL, presented as geometric mean

^bData for all subjects not available

^cDaily intake during pregnancy measured in mg

^dSurma is traditional eye cosmetic containing lead

Table 3
Multivariable linear regression models for determinants of umbilical cord BLLs^a

Variable	Model with car ownership ^b				Model with income ^b			
	P	β coefficient	95% CI	P	β coefficient	95% CI	P	95% CI
MUAC quartile^c								
>26.0 cm–28.9cm	0.068	–0.11	(–0.22–0.01)	0.060	–0.11	(–0.23–0.00)		
>28.9 cm–31.9 cm	0.832	–0.01	(–0.13–0.10)	0.891	–0.01	(–0.12–0.11)		
>31.9 cm	0.282	0.07	(–0.05–0.18)	0.376	0.05	(–0.07–0.17)		
Mothers ethnicity^d								
Sindhi	0.374	–0.09	(–0.28–0.11)	0.432	–0.08	(–0.27–0.12)		
Punjabi	0.852	–0.01	(–0.14–0.12)	0.891	–0.01	(–0.14–0.12)		
Pukhroon	0.703	–0.04	(–0.24–0.16)	0.693	–0.04	(–0.24–0.16)		
Baloch	0.004	0.24	(0.08–0.41)	0.004	0.25	(0.08–0.41)		
Surma use daily vs not daily^e	0.001	0.30	(0.13–0.47)	0.001	0.28	(0.11–0.45)		
Ethnicity and daily surma use								
Sindhi & daily surma use	0.120	–0.79	(–1.79–0.21)	0.062	–0.93	(–1.91–0.05)		
Punjabi & daily surma use	0.189	0.29	(–0.14–0.73)	0.144	0.33	(–0.11–0.77)		
Pukhroon & daily surma use	0.303	–0.24	(–0.71–0.22)	0.429	–0.19	(–0.65–0.28)		
Baloch & daily surma use	0.011	–0.55	(–0.98–0.13)	0.009	–0.56	(–0.99–0.14)		
Own a car/income	0.045	–0.21	(–0.42–0.00)	0.047	–0.09	(–0.17–0.00)		
Iron intake 4th quartile vs others^f	<0.001	–0.19	(–0.29–0.09)	<0.001	–0.18	(–0.28–0.08)		
Paternal occupation in lead-based industry vs others	0.002	0.16	(0.06–0.27)	0.004	0.16	(0.05–0.26)		
Parity (number)	0.047	0.04	(0.00–0.08)	0.088	0.03	(0.00–0.07)		
Mothers age (yrs)	0.063	–0.01	(–0.02–0.00)	0.048	–0.01	(–0.02–0.00)		
Intercept	<0.001	2.48	(2.222–75)	<0.001	2.54	(2.26–2.81)		
95%CI: 95% confidence interval;		Adj R-Sq=0.0932, F = 4.26, P <.0001			Adj R-Sq=0.0931, F = 4.25, P <.0001			

^aNatural log-transformed cord BLLs;

^bincome and car ownership as indicator of SES;

^c1st quartile as reference group,

^dUrdu/Mohajirs as reference group;

^eSurma is traditional eye cosmetic containing lead;

^f58.5–387 mg per day vs. <47 mg of elemental iron per day

Table 4Multivariable logistic regression model for factors associated with high umbilical cord blood lead levels ($\leq 10 \mu\text{g/dl}$)

Risk factors	n	adjOR	95% Confidence Limits
Mother's Ethnicity ^a			
Urdu (Mohajir)	361	1.00	
Sindhi	28	0.39	(0.16–0.99)
Punjabi	72	1.41	(0.84–2.38)
Pusho	33	0.45	(0.19–1.04)
Balochi	46	1.58	(0.80–3.14)
Iron supplement intake per day during pregnancy (mg)			
4th quartile (58.5–387)	135	1.00	
1st-3rd quartile (0–47.0)	405	1.92	(1.23–3.00)
Have been using calcium supplement before pregnancy			
Yes	22	1.00	
No	518	3.39	(1.18–9.70)
Frequency of surma use ^b			
Less than daily	487	1.00	
Daily	53	2.10	(1.13–3.91)
Keep windows of house open most of the time			
No	161	1.00	
Yes	379	1.53	(1.03–2.26)
Father's education (years)			
>12	48	1.00	
≤ 12	233	1.96	(1.14–3.35)
Parity	540	1.06	(0.91–1.24)
Age (years)	540	1.41	(0.95–2.09)

adjOR: adjusted odds ratio,

^aUrdu/Mohajirs as reference group: population group migrated from parts of India not included in Pakistan at the time of creation of Pakistan^bSurma is traditional eye cosmetic containing lead