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Determining Fetal Manganese Exposure from Mantle Dentine of Deciduous Teeth

Manish Arora^{1,2,3,4,*}, Asa Bradman⁵, Christine Austin⁴, Michelle Vedar⁵, Nina Holland⁵, Brenda Eskenazi⁵, and Donald R. Smith²

Manish Arora: marora@hsph.harvard.edu; Asa Bradman: abradman@berkeley.edu; Christine Austin: Christine.Austin-1@uts.edu.au; Michelle Vedar: mvedar@berkeley.edu; Nina Holland: ninah@berkeley.edu; Brenda Eskenazi: eskenazi@berkeley.edu; Donald R. Smith: drsmith@ucsc.edu

¹Environmental and Occupational Medicine and Epidemiology, Harvard School of Public Health, Boston, MA, United States

²Department of Microbiology and Environmental Toxicology, University of California, Santa Cruz, CA, United States

³Institute of Dental Research, and Oral Pathology and Oral Medicine, Faculty of Dentistry, University of Sydney, Sydney, NSW, Australia

⁴Elemental Bio-Imaging Facility, University of Technology, Sydney, NSW, Australia

⁵Center for Environmental Research and Children's Health (CERCH), School of Public Health, University of California, Berkeley, CA, United States

Abstract

Studies addressing health effects of manganese (Mn) excess or deficiency during prenatal development are hampered by a lack of biomarkers that can reconstruct fetal exposure. We propose a method using the neonatal line, a histological feature in deciduous teeth, to identify regions of mantle dentine formed at different prenatal periods. Micro-measurements of Mn in these regions may be used to reconstruct exposure at specific times in fetal development. To test our hypothesis, we recruited pregnant women before 20 weeks gestation from a cohort of farmworkers exposed to Mn-containing pesticides. We collected house floor dust samples and mother's blood during the second trimester; umbilical cord blood at birth; and shed deciduous incisors when the child was ~7 years of age. Mn levels in mantle dentine formed during the second trimester (as ⁵⁵Mn:⁴³Ca area under curve) were significantly associated with floor dust Mn loading ($r_{\text{spearman}}=0.40$; $p=0.0005$; $n=72$). Furthermore, ⁵⁵Mn:⁴³Ca in sampling points immediately adjacent the neonatal line were significantly associated to Mn concentrations in cord blood ($r_{\text{spearman}}=0.70$; $p=0.003$; $n=16$). Our results support that Mn levels in mantle dentine are useful in discerning perinatal Mn exposure, offering a potentially important biomarker for the study of health effects due to environmental Mn exposure.

*Corresponding Author: Manish Arora, Environmental and Occupational Medicine and Epidemiology, Harvard School of Public Health Landmark Ctr East 3-111-14, 401 Park Dr, Boston MA 02215, USA Tel: 617 384 8876, Fax: 617 384 8994, marora@hsph.harvard.edu.

Supporting Information Available

Typical operating conditions for laser ablation analysis (Table S1); Schematic of tooth development (Figure S1); Reproducibility of ⁵⁵Mn:⁴³Ca measurements in dentine (Figure S2); Comparison of LA-ICP-MS Mn measurements in dentine with in-solution ICP-MS analysis (Figure S3); Direct measurement of enamel growth rate (Figure S4). This information is available free of charge via the Internet at <http://pubs.acs.org/>.

Keywords

biomarker; dentine; enamel; manganese; prenatal exposure

Introduction

Manganese (Mn) is an essential nutrient and recent studies indicate that both deficiency and excess of Mn during the perinatal period increase the risk of lower birth weight infants and neuropsychological deficits in children [1, 2]. Food is the primary source of Mn exposure to the general population, with vegetarian diets containing Mn-rich nuts, grains, and legumes providing higher levels of Mn than non-vegetarian diets [3]. Mn intake from water is generally lower than from food. The main sources of Mn release to the air are industrial emissions, combustion of fossil fuels, and re-entrainment of Mn-containing soils [3].

Epidemiologic studies on the health effects of Mn rely on prospective collection of exposure biomarkers, often blood Mn measurements, and several years of follow-up before health or developmental effects become evident in childhood. Furthermore, measures of Mn in maternal blood and urine during pregnancy may not reliably reflect fetal exposure due to homeostatic regulation of Mn in adults, the short half-life of Mn in these media, and complex kinetics that obscure the relationship of these biomarkers with environmental exposures [4, 5]. A retrospective biomarker that accurately estimates exposure during prenatal development would substantially aid epidemiologic investigations, particularly case-control studies addressing health outcomes of fetal exposure to Mn and other metals.

Deciduous teeth accumulate metals and their mineralization proceeds in an incremental pattern (akin to growth rings) spanning the prenatal and early postnatal periods (commencing gestational week 13–16 for incisors and concluding postnatal age 10–11 months for molars) [6]. Therefore, the distribution of Mn in deciduous teeth may provide information on environmental Mn exposure during fetal development and early childhood. Previous attempts to use deciduous enamel to estimate Mn exposure were hampered by insufficient consideration of the protracted maturation of enamel [7]. Initial incremental deposits of enamel matrix are only ~30% mineralized by weight, with the bulk of mineral (and metals) entering after the entire thickness of enamel matrix is deposited in that part of the tooth [8, 9]. Measurements of Mn at any single location in enamel, therefore, cannot be directly linked to the developmental timing of exposure.

The use of dentine to estimate metal exposure during specific prenatal periods has received limited attention, with most studies using fragments of circumpulpal dentine to estimate cumulative exposure (for example Fergusson et al. [10], Needleman et al. [11]). Dentine, unlike enamel, mineralizes to nearly its final state (70% inorganic by weight) almost immediately after matrix deposition [8] and may, therefore, be useful for obtaining temporal information of Mn exposure during the pre- and postnatal periods. To test this hypothesis, we examined, in a prospective birth cohort study spanning approximately eight years, detailed environmental and biological matrices collected coincident with tooth development and determined if Mn levels in mantle dentine, a structurally distinct region of teeth that undergoes incremental mineralization [12, 13], reflects environmental Mn exposure over time. In the present study, we provide a detailed description of the methodology and validate the proposed biomarker using data from a cohort of farmworkers at risk of Mn exposure.

Methods

Study Participants and Collection of Environmental and Biological Matrices

In keeping with the primary aim of the study (to validate the proposed biomarker), we used biological matrices and environmental data from a subsample of ~80 mother-child pairs currently enrolled in the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) study based in Monterey County, California, USA (additional details below and in Eskenazi et al. 2004 [14]). In this cohort, pregnant women were recruited before 20 weeks gestation and during the second trimester we collected blood from the mothers and house floor dust samples from which we calculated Mn loading ($\mu\text{g Mn/m}^2$ floor area) and concentration ($\mu\text{g Mn/g dust}$). At birth, we collected umbilical cord blood and at ~7 years of age, we collected naturally shed deciduous incisors. The parent study collected other biological samples at different times, but those are not part of the analyses presented here.

The CHAMACOS study women were enrolled between October 1999 and 2000. All women attending first prenatal care visits at a network of six community clinics serving a primarily low-income farmworker population were screened for eligibility. Women were eligible to participate if they were: Spanish or English speaking; 20 weeks pregnant; 18 years old; MediCal eligible; and planning to deliver at Natividad Medical Center, a hospital owned and operated by Monterey County. Of 601 women who enrolled in the CHAMACOS study, 536 had a live infant followed to delivery. Mothers in the CHAMACOS cohort were young (25.6 ± 5.3 years), married, low-income, Mexican-born, Spanish-speaking women who were farmworkers themselves or lived with farmworkers. We have previously documented environmental exposure to pesticides in this cohort [14], including the Mn-containing fungicides maneb and mancozeb [15]. Maneb and mancozeb are among the most heavily used pesticides in California, with 1.8 million pounds applied annually statewide. In the Salinas Valley where the participating children resided, approximately 345,000 pounds of these fungicides, which are approximately 21% Mn by weight, are used each year, resulting in over 72,000 pounds of Mn being released per annum [16]. Preliminary results from the parent study have shown a significant relationship between floor dust Mn loading ($\mu\text{g Mn/m}^2$ floor area) as well as Mn concentration ($\mu\text{g Mn/g dust}$) with proximity to use of Mn-containing pesticides and presence of an agriculture worker in the home [15]. Children participating in the CHAMACOS study have been followed from before birth until 10.5 years of age. All procedures were reviewed by the University of California, Berkeley Committee for the Protection of Human Subjects. Written informed consent was obtained from parents of all participating children.

Tooth Sampling Strategy

Mothers were asked to bring in at the time of the 7-year assessment a tooth the child had shed. In the present study, we used incisors that were free of obvious defects (caries, hypoplasias, fluorosis, cracks, extensive attrition) and prepared ~100–150 μm sections in an axial labio-lingual plane. Using light microscopy, we identified the neonatal line (NL), a histological feature formed in deciduous teeth at birth (see Supporting Information, Figure S1). With the NL as a reference point, we measured Mn levels (as $^{55}\text{Mn}:$ ^{43}Ca) in mantle dentine immediately adjacent to the enamel-dentine junction (EDJ) with laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) (Figure 1, Supporting Information Table S1, and Arora et al. 2011 [17]). We normalized Mn measurements to ^{43}Ca to account for intra- and inter-sample variations in mineralization. We confirmed that our sampling points were in mantle dentine by examining sections in polarized light where mantle dentine is clearly demarcated from circumpulpal dentine (Figure 1d).

We sampled mantle dentine at 30 equally spaced points adjacent to the EDJ from cusp tip to the cemento-enamel junction and calculated the area under the curve (AUC) of Mn levels across all the sampling points in prenatally formed mantle dentine to estimate cumulative Mn exposure in the prenatal period. Because floor dust and blood samples were collected during the second trimester, we also undertook analyses restricted to Mn levels in sampling points located in mantle dentine formed in the second trimester.

Identification of Sampling Points Located in Mantle Dentine Formed in the Second Trimester

To identify sampling points formed in the second trimester, we used established formulae on rate of enamel formation in primary teeth to locate on the EDJ (and in the adjacent mantle dentine) a point that demarcated the second and third trimesters. In transmission light micrographs of ~100–150 μm thick tooth sections, we identified the EDJ and the NL (Figure 1). Using direct measurements of cross-striations (daily growth rings) in enamel and formulae on the incremental formation of enamel, developed by Dean [18] and Birch and Dean [19] (shown below), we calculated along the enamel prism path the thickness of the enamel formed during the third trimester and, to indicate this on our micrographs, we drew a line parallel to the NL. The intersection of this line and the EDJ indicated the location where enamel/dentine formation commenced in the third trimester. Sampling points placed incisal (towards tooth tip) to this location were classified as those indicating exposure in the second trimester. In these analyses, we calculated the duration of pregnancy for each mother using data from their medical records abstracted by a registered nurse.

$$\begin{aligned}\text{Days of enamel formation} &= 9.848 + 0.275 \times (\text{distance from EDJ } (\mu\text{m})) \\ \text{Lower 95\% limit} &= 7.749 + 0.271 \times (\text{distance from EDJ } (\mu\text{m})) \\ \text{Upper 95\% limit} &= 11.946 + 0.279 \times (\text{distance from EDJ } (\mu\text{m}))\end{aligned}$$

In a similar manner, it is possible to identify numerous locations in mantle dentine formed at other times during tooth development. We choose to restrict our analyses to the second trimester because we had only collected other biomarkers (house dust, maternal blood) at that time. We validated these formulae for identifying prenatal parts of mantle dentine in teeth from our population by direct measures of enamel growth determined from enamel cross-striations (see Supporting Information, Figure S4, for details).

Measurement of Mn in Teeth by LA-ICP-MS

The laser ablation unit used was a New Wave Research UP-213 system (Kennelec Technologies, Mitcham, Victoria, Australia) equipped with a Nd:YAG laser emitting a nanosecond laser pulse in the fifth harmonic with a wavelength of 213 nm. The standard ablation cell was replaced with a Large Format Cell (LFC). The LFC has a large volume chamber capable of holding samples up to 15.2 cm^2 in area. The x - y - z stage of the LFC employs a small volume 'roving' sampling cup that traverses the sample while the laser beam remains stationary. An approximately 40 cm length of Tygon® tubing (i.d. 3 mm) connected the laser ablation unit to an Agilent Technologies 7500cx (Agilent Technologies Australia, Forrest Hill, Victoria, Australia) ICP-MS. The instrument was fitted with a 'cs' lens system for enhanced sensitivity. The system was tuned daily for sensitivity using NIST SRM 612 (trace elements in glass). Polyatomic oxide interference was evaluated and minimized by monitoring the Th^+/ThO^+ (m/z 232/248) ratio. Typical oxide formation was consistently under 0.3%. Operating conditions for the optimized LA-ICP-MS system are given in Supporting Information, Table S1.

Up to sixty ablations (30 each in enamel and dentine) were made using a 30 μm laser spot size along the incremental/extension zones of teeth adjacent to the EDJ (Figure 1). Ablations were done in pure helium and the tooth plus aerosol was mixed with argon before being introduced to the ICP. All measurements were made in time-resolved analysis mode allowing signals to be monitored during the ablation. A gas background was measured for 30 s at the start of each analysis and subtracted from the sample signal. Signal intensities for ^{55}Mn , ^{43}Ca and ^{44}Ca were measured for 30 s after commencement of ablation. Data were analysed as $^{55}\text{Mn}:^{43}\text{Ca}$ ratios to control for any variations in mineral content within a tooth and between samples.

To confirm the reproducibility of our $^{55}\text{Mn}:^{43}\text{Ca}$ measurements, we analyzed dentine of five teeth on three different days using LA-ICP-MS. Seven points were sampled in coronal dentine of each tooth per day (for results see Supporting Information, Figure S2). Furthermore, to provide an estimate of Mn concentration in dentine (as $\mu\text{g Mn/g dentine}$) corresponding to the $^{55}\text{Mn}:^{43}\text{Ca}$ ratios observed in our study, we dissected fragments of coronal dentine from four deciduous incisors, analyzed them with LA-ICP-MS and then digested in acid and undertook in-solution analyses. LA-ICP-MS analysis consisted of ten sampling points ablated across each fragment. The fragments were then weighed, placed in acid washed plastic vials and digested in 100 μg of HNO_3 and 400 μg of Milli-Q water to ensure the fragment was completely submerged. Fragments were sonicated and heated on a hot plate ($\sim 80^\circ\text{C}$) until digested. Once fully digested, Milli-Q water was added to make 5 g. Standards were prepared over 0–1000 $\mu\text{g/kg}$ (ppb) with the same acid content (1.5%). Digests and standards were then analysed by solution nebulisation (SN)-ICP-MS (for results see Supporting Information, Figure S3).

Measurement of Mn in Blood

Cord (fetal) blood was collected at delivery, and maternal blood was collected at the 26th gestational week by venipuncture. For the present study, these samples were available from a smaller number of participants (see Table 1 for details). Blood samples were shipped immediately to the biorepository at the School of Public Health, University of California-Berkeley and processed upon arrival (24 to 48 hours post-collection). Samples were collected in vacutainers with heparin and banked in low-temperature freezers (-80°C) and liquid nitrogen tanks in multiple aliquots. Whole blood Mn levels were analyzed using trace metal clean techniques and high resolution ICP-MS, as reported previously [20, 5]. Briefly, aliquots of whole blood (0.25 mL) were weighed and digested overnight at room temperature with 0.5 mL of 16 N HNO_3 (Optima grade, Fisher Scientific). Digestion was complete after addition of 0.25 mL 30% H_2O_2 and dilution to 2.5 mL final volume with Milli-Q water. Digestates were centrifuged (15,000g for 15 min), and the supernatant collected for Mn analysis. Samples were analyzed using a Finnigan XR magnetic sector ICP-MS. Rhodium was added to all samples as an internal standard; external standardization was via certified standards (Spex Industries, Inc., Edison, NJ). The analytical detection limit for Mn in blood by ICP-MS was 0.01 ng/mL. Standard reference materials (NIST SRM 1577b, bovine liver) and sample spike-recoveries were used to confirm analytical accuracy.

Measurement of Mn Concentrations and Loading in House Dust

Carpet dust samples were collected from one square meter of the living area using a High Volume Small Surface Sampler (HVS3; CS3, Inc., Bend, Ore.). Samples were sieved to $<150 \mu\text{m}$ and digested in 7.5N HNO_3 (Trace Metal grade, Fisher Scientific) at 80°C for 2 hrs and analyzed by inductively coupled plasma optical emission spectrometry [21]. We found detectable levels of Mn in all homes.

Statistical Analysis

Spearman's correlation analysis was used to measure the association of $^{55}\text{Mn}:$ ^{43}Ca in teeth with Mn loading in house dust. Tooth Mn levels were also compared to Mn concentrations in mother's blood collected during the second trimester and cord (fetal) blood. For these analyses we used the AUC of the $^{55}\text{Mn}:$ ^{43}Ca distribution across the evenly spaced sampling points in dentine (calculated using the trapezoid method). We used the AUC to integrate our measurements of Mn in teeth because, in our LA-ICP-MS analyses, the $^{55}\text{Mn}:$ ^{43}Ca at different sampling points were along a continuum of tooth development from the EDJ at the tooth tip (start of tooth development) towards the cervix of the tooth (completion of primary dentine of tooth crown).

For our main analysis, we extracted time-specific data from our tooth-Mn measurements. Specifically, $^{55}\text{Mn}:$ ^{43}Ca levels in ablation spots in dentine formed during the second trimester were compared with Mn loading in house dust and mother's blood also collected at that time. Furthermore, $^{55}\text{Mn}:$ ^{43}Ca levels in ablation spots immediately adjacent to the NL (which according to our hypothesis are representative of exposure near the time of birth) were compared to Mn concentrations in cord blood.

Because the distance, along the EDJ, of the NL from the dentine at the incisal tip can vary, the number of sampling points in the prenatal vs postnatal zones differed between samples. To account for this, we standardised the AUC of $^{55}\text{Mn}:$ ^{43}Ca within any zone to the number of sampling points in that part of the tooth. When displaying bivariate associations as scatter plots with regression lines, we $\log_e$ transformed our data to achieve normally distributed variables. In some of our data analyses, we multiplied our $^{55}\text{Mn}:$ ^{43}Ca measurements with 10^4 to avoid negative values during $\log_e$ transformation (and have indicated in the results when this was done). Results were considered statistically significant at $p < 0.05$. All analyses were undertaken in STATA 10.0 (StataCorp, College Station, Texas).

Results

Association of Mn Levels in Mantle Dentine with Mn Loading and Concentration in Floor Dust

For our first key analysis, we compared Mn levels in mantle dentine with Mn loading in floor dust ($\mu\text{g Mn}/\text{m}^2$ floor area). The AUC of $^{55}\text{Mn}:$ ^{43}Ca across sampling points in mantle dentine formed in the second trimester was significantly associated with Mn loading in house dust collected in the second trimester ($r_{\text{spearman}} = 0.40$; $p = 0.0005$; $n = 72$) (Table 2, Figure 2a). When we undertook similar comparisons of $^{55}\text{Mn}:$ ^{43}Ca AUC of the third trimester dentine sampling points, the association with Mn loading in dust was weaker than that observed with second trimester sampling points ($r_{\text{spearman}} = 0.24$; $p = 0.04$; $n = 74$), although this difference was not statistically significant (Table 2, Figure 2b). Similarly, the association of $^{55}\text{Mn}:$ ^{43}Ca AUC in postnatal mantle dentine with Mn loading in dust was weaker than that observed for Mn levels in second trimester dentine ($r_{\text{spearman}} = 0.21$; $p = 0.07$; $n = 76$). We also calculated the $^{55}\text{Mn}:$ ^{43}Ca AUC of all sampling points in prenatal mantle dentine, an estimate of cumulative prenatal Mn exposure, and observed a significant positive association with floor dust Mn loading (Table 2). Similarly, we also found significant positive associations of dentine Mn levels with Mn *concentration* in dust samples (r_{spearman} range: 0.27 to 0.34; p values: 0.02 to 0.003) (see Table 2 for details).

Mn Levels in Mantle Dentine and Mn Concentration in Cord Blood

Our second key analysis was to explore the relationship of Mn levels in mantle dentine with cord blood, a direct measure of Mn levels in fetal circulation at the time of birth. There was no significant association between $^{55}\text{Mn}:$ ^{43}Ca AUC of all prenatal sampling points and cord

blood Mn in a subset of subjects with available cord blood ($r_{\text{spearman}}=-0.01$; $p=0.99$; $n=18$) (Figure 2d). Importantly, however, $^{55}\text{Mn}:$ ^{43}Ca in the sampling point in prenatal dentine immediately adjacent to the neonatal line in each tooth showed a significant positive correlation with cord blood Mn ($r_{\text{spearman}}=0.70$; $p=0.003$; $n=16$) (Figure 2e).

Exploratory Analyses of Mn in Mantle Dentine and Maternal Blood

While it remains uncertain if blood Mn adequately estimates environmental Mn exposure [5], we nonetheless undertook exploratory analyses in a subsample of subjects, comparing Mn levels in dentine formed during the second trimester with maternal blood collected during the second trimester. There was no significant association of $^{55}\text{Mn}:$ ^{43}Ca AUC in dentine with maternal blood collected during pregnancy ($r_{\text{spearman}}=-0.22$; $p=0.36$; $n=20$). There was also no significant correlation between mother's blood and Mn dust loading from the second trimester of pregnancy ($r_{\text{spearman}}=-0.16$; $p=0.50$; $n=19$).

Association of Mn Levels in Enamel with Mn in Dust and Cord Blood

Similar to our analysis of dentine, we also undertook measurements of Mn in enamel adjacent to the EDJ. $^{55}\text{Mn}:$ ^{43}Ca AUC of all 30 sampling points in prenatal enamel showed no significant association with floor dust Mn loading ($r_{\text{spearman}}=0.03$; $p=0.72$; $n=72$). When we restricted our analysis to sampling points located in enamel formed during the second trimester, the association with Mn dust loading remained non-significant ($r_{\text{spearman}}=-0.07$; $p=0.55$; $n=67$) (Figure 3a). Mn levels in enamel also showed no significant association with Mn concentrations in cord blood (Figure 3d, e).

Discussion

Manganese is an essential nutrient but a potent neurotoxicant at high exposures. The complex kinetics and regulation of Mn in human blood, urine and other media make it difficult to determine exposure histories [4,5]. In adults, Mn uptake and distribution are under strict homeostatic control which is not present in early life, resulting in increased susceptibility to this metal during the prenatal and early childhood periods. Epidemiologic studies examining health outcomes of Mn excess or deficiency are therefore significantly hampered since it is not feasible to obtain fetal blood at different stages of pregnancy and, while maternal blood may be readily sampled, it is not necessarily a valid marker of fetal Mn exposure. The aim of this study was to validate an analytical methodology that uses Mn levels in mantle dentine to distinguish children who we believe experienced contrasting levels of Mn exposure during prenatal development. Our results support that mantle dentine Mn measurements were useful in retrospectively discerning Mn levels in the developing fetus close to the time of birth and in estimating Mn exposure during the second and third trimesters and also cumulative exposure over the perinatal period.

Earlier studies have shown that Mn in indoor dust can be an important source of exposure, for example Röllin et al observed positive associations between Mn levels in classroom dust and schoolchildren's blood [22]. Since Mn loading in floor dust reflects both the concentration of Mn in dust and the amount of dust per floor area, we believe that Mn loading is a better indicator for potential risk of environmental Mn exposure than Mn concentrations in floor dust ($\mu\text{g Mn/g floor dust}$), as has been shown in studies on Pb exposure [23]. We, therefore, used Mn loading in floor dust as our primary environmental measure of exposure. We observed that prenatal mantle dentine Mn levels correlated significantly with Mn loading in floor dust, suggesting that variations in dentine Mn between children are likely due to differences in environmental Mn exposure experienced by the mothers during pregnancy. Compared to its association with Mn in second trimester dentine, house dust Mn loading showed a weaker association with Mn in dentine formed

either during the third trimester or postnatally. This is likely due to the fact that dust measures undertaken in the second trimester may not accurately reflect household dust levels at other times. We did not observe any significant association between Mn concentrations in maternal blood collected during pregnancy and prenatal dentine Mn. While the transfer of environmental Mn to developing fetal teeth is likely to be mediated via maternal blood, the short half-life and complex kinetics of Mn in blood may obscure any relationship between maternal blood Mn concentrations and fetal/child dentine Mn levels. The importance of dentine Mn as a potential exposure biomarker is further underscored by the ongoing uncertainty over the suitability of blood and urine Mn concentrations in estimating environmental exposure to Mn.

Our proposition that mantle dentine retains the original signature of Mn exposure experienced during dentine formation requires that deposits of Mn in mantle dentine are relatively stable over time. This is supported by our finding that Mn measurements in prenatally formed mantle dentine immediately adjacent to the NL were significantly associated with cord blood Mn, while mantle dentine Mn levels at points away from the NL were not. Given the short half-life of Mn in blood (~4 days) [4] and the marked changes in circulating levels during pregnancy [24], the lack of an association between cord blood Mn and the cumulative measure of prenatal Mn in dentine ($^{55}\text{Mn}:\text{}^{43}\text{Ca}$ AUC of all prenatal dentine sampling points) is not surprising. Furthermore, it is likely that only mantle dentine closest to the NL reflects Mn levels in fetal circulation at birth, as measured by cord blood Mn.

While the stability of elemental deposits in dentine has been investigated [25, 26], it has been done so in large sections of circumpulpal dentine and not exclusively in mantle dentine, which is developmentally and structurally distinct from other parts of dentine. Importantly, in fully developed deciduous teeth, it is unlikely that odontoblastic processes extend up to the EDJ [27, 28], and mantle dentine has very few dentinal tubules and these are significantly narrower than those found near the pulp, which limits peritubular dentine accumulation. Furthermore, the greater distance of separation from the pulpal blood supply makes it likely that Mn deposits in mantle dentine are more stable than those in circumpulpal dentine adjacent to the pulp, although further evidence on the kinetics of Mn in mantle dentine is needed to confirm this.

Sampling Mn in enamel has been suggested by others as a potential biomarker of prenatal environmental Mn exposure [7], but those studies did not include environmental and biological markers collected during pregnancy and at birth. Our data suggest that it is not possible to use Mn distribution in enamel to obtain information on timing of perinatal environmental Mn exposure using the method we have proposed here (see Figure 3). This absence of temporal exposure information is likely due to the prolonged maturation phase of enamel whereby minerals and metals are acquired into the developing enamel at times different than the initial incremental deposition of the protein matrix, which is when the growth rings/incremental lines are formed [29]. While our data do support that Mn measurements in mantle dentine are more useful than enamel to estimate environmental exposure, these results cannot be directly extrapolated to other metals. In any such investigation, it is important to consider the differences in uptake and distribution of elements in developing teeth.

The results presented here are strengthened by the prospective design of our study and collection of environmental and biological matrices at times coincident with tooth development. Importantly, our preliminary data support that the use of Mn-containing pesticides may be an important source of exposure to our participants and our primary exposure metric, Mn loading in floor dust, was significantly associated with Mn exposure

risk factors (proximity to use of Mn-containing pesticides and presence of an agricultural worker at home) [15]. Our study is limited by the absence of other potential Mn biomarkers, such as hair or toenails, which may be of value in estimating exposure to Mn [30–32]. We have also not considered dietary sources of Mn.

Overall, the value of the technique proposed here lies in the efficient study of health outcomes in children and adolescents due to Mn exposure during critical developmental events in the prenatal and early childhood periods. This biomarker offers the advantage of providing temporal exposure information retrospectively, thus aiding epidemiologic studies by reducing the need for prospective biomarker collection and lengthy follow-ups to elicit health effects. This method could be used to measure exposure to other metals at various prenatal times, and be expanded to estimate postnatal exposure beyond childhood using permanent teeth, but such applications would require validation using metal concentrations in environmental and biological matrices collected at corresponding times, as we have done here for Mn.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Tooth analysis methodology. (a) Schematic of human primary central incisor showing the neonatal line (blue line), enamel (En), dentine (D), enamel-dentine junction (EDJ) and sampling points in dentine adjacent to the EDJ. Similar points were also sampled in enamel. (b) Light micrograph of NL in enamel. Sampling points in dentine are indicated by arrowheads. (c) Estimating the location of dentine formed during the second trimester. A line (indicated in red) is drawn parallel to the NL at a distance determined by formulae on incremental formation of enamel (see Methods). Intersection of this line with the EDJ indicates the boundary between dentine formed in the second and third trimesters. Dashed red lines indicate 95% confidence limits of this estimate; black circles indicate location of sampling points in mantle dentine. (d) Mantle (MD) is demarcated from circumpulpal dentine in polarized light. Laser ablation spots are visible in MD adjacent to the EDJ. Scale bar 50 μm . (e) Area highlighted in white box in Panel d. Laser ablation spot and few branched dentinal tubules in mantle dentine are visible. Scale bar 10 μm .

Figure 2.

The relationship of Mn levels ($^{55}\text{Mn}:^{43}\text{Ca} \times 10^4$) in pre- and postnatally formed dentine with Mn loading in house dust and Mn concentrations in cord blood. (a-c) Association of Mn loading in house dust ($\mu\text{g Mn}/\text{m}^2$ floor area) with area under curve (AUC) of Mn levels in sampling points in dentine formed during the second trimester (a), third trimester (b) and postnatally (c). (d) No significant association between cord blood Mn ($\mu\text{g/L}$) and AUC of Mn levels in all sampling points in prenatal dentine. (e) $^{55}\text{Mn}:^{43}\text{Ca}$ in sampling points in prenatal mantle dentine immediately adjacent to the neonatal line (NL) showed a significant association with cord blood Mn.

Figure 3.

The relationship of Mn levels ($^{55}\text{Mn}:^{43}\text{Ca} \times 10^4$) in pre-and postnatally formed enamel with Mn loading in house dust and Mn concentrations in cord blood. (a-c) Association of Mn loading in house dust ($\mu\text{g Mn/m}^2$ floor area) with area under curve (AUC) of Mn levels in sampling points in enamel formed during the second trimester (a), third trimester (b) and postnatally (c). (d) No significant association between cord blood Mn ($\mu\text{g/L}$) and AUC of Mn levels in all sampling points in prenatal enamel. (e) $^{55}\text{Mn}:^{43}\text{Ca}$ in sampling points in prenatal enamel immediately adjacent to the neonatal line (NL) did not show a significant association with cord blood Mn.

Table 1

Mn measurements in environmental and biological matrices.

Sample	Mn Levels		
	N	Median	Range
House dust ($\mu\text{g/g}$)	76	164	20.4 – 335
Mn loading in house dust ($\mu\text{g Mn/m}^2$ floor area)	76	375	2.5 – 8528
Prenatal dentine (AUC) ^a	83	0.49	0.13 – 1.3
Postnatal dentine (AUC)	81	0.14	0.02 – 1.1
Maternal blood at 26 th gestational week (ppb)	21	14.7	5.3 – 32.8
Cord (fetal) blood (ppb)	19	40.7	27.0 – 64.9

^a AUC = area under curve

Table 2

Association of Mn in dentine with Mn concentration and loading in house dust.

Area under curve of ^{55}Mn : ^{43}Ca in dentine	Spearman's ρ 95% CI P value	
	Mn loading in house dust ($\mu\text{g Mn/m}^2$ floor area)	Mn concentration in house dust ($\mu\text{g Mn/g}$ floor dust)
	0.36	0.29
All prenatal dentine sampling points (n=77)	0.14 – 0.54	0.06 – 0.49
	0.0013	0.01
	0.40	0.28
Second trimester dentine (n=72)	0.18 – 0.58	0.04 – 0.49
	0.0005	0.02
	0.24	0.34
Third trimester dentine (n=74)	0.01 – 0.45	0.11 – 0.53
	0.04	0.003
	0.21	0.27
All postnatal dentine sampling points (n=76)	–0.02 – 0.42	0.04 – 0.47
	0.07	0.02