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Evaluation of Plasma Manganese Levels in Pregnancy and Outcome Implications, Kinshasa, DR Congo

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ABSTRACT

Background: Although Manganese is one of the abundant elements in the earth's crust with essential roles involved in many metabolic functions in the human body, exposure to high levels of Mn has been linked to adverse outcomes in pregnancy and fetal development, including brain function and skeletal development. The aim of this work was to evaluate plasma Mn levels in pregnancy and their birth outcomes implications. **Methods:** Plasma-Mn levels were measured by atomic absorption (PG-Instruments combined Flame and Furnace -AA500FG- with graphite furnace, Germany) (18,20). Plasma samples (100 µL) were diluted quantitatively (1+10) with a matrix modifier solution containing 0.5% Triton X-100 (PA Sigma-Aldrich), 0.1% nitric acid (65% pure, Roth) and 0.1% magnesium (0.1 mg/mL Mg2+, Sigma-Aldrich). **Results:** Plasma-Mn levels were measured with a coefficient of variation of less than 10% in both samples and controls, and the detection limit (LOD) was 0.5 µg/L. Regarding differences between groups, levels of plasma Mn were observed in women with a family history of preeclampsia and diabetes mellitus (t-test, p=0.0271 and 0.0312, respectively). Plasma Mn levels were also significantly higher in 20-36 weeks of amenorrhea period as compared to other periods [means (±SD), 4.118 µg/L (± 3.911) in 20-36 weeks, 0.216 µg/L (± 0.873) in 10-19 weeks and 2.223 µg/L (± 2.091) at delivery (≥37 weeks), ANOVA, $p < 0.0001$] and newborns showed higher plasma Mn levels than their mothers [means (±SD), 5.151 µg/L (± 4.300) versus 2.467 µg/L (± 3.472), t-test, $p = 0.001$]. No significant associations were observed between maternal plasma Mn and birth weight, birth height, ponderal index or gestational age at birth.

Conclusions: Globally, no significant correlation between maternal plasma Mn and all of these outcomes (birth weight, birth height, ponderal index Apgar score, gestational age at birth, head circumference at delivery). However, women with a family history of preeclampsia and diabetes mellitus had significantly higher plasma Mn levels. Furthermore, the Mn levels above the 50th percentile (4.55 µg/L) found in fetal plasma samples constitute a major public health concern for newborns. Risk

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assessment of Mn exposure should take place at the earliest contact with pregnant and lactating women.

Keywords: Plasma manganese, birth outcomes, maternal outcomes, Prenatal exposure, Kinshasa

BACKGROUND

Manganese (Mn) is one of the abundant elements in the earth's crust with essential roles involved in many metabolic functions in the human body. Its main sources are dietary intake primarily and environment through water, soil, dust and air [1,2]. Exposure to high levels of Mn have been linked to neurotoxic effects and this poisoning remains a growing concern of public health, especially for pregnant women and children [3-11].

It is also well established that Mn crosses the placenta via active transport mechanisms [12], and cord blood Mn levels are significantly higher compared to concentrations in maternal blood at delivery [13]. There is growing concern about the transfer of Mn from pregnant women to the growing fetus [4] because the developing fetus is a prime target for the disrupting effects of Mn (1,14).

In DRC, Tuakuila, et al. [15] reported that high Mn levels measured in urine of the general population. Increased urinary excretion of Mn was also observed in preeclampsia by Elongi-Moyene, et al. [16] as compared to no-preeclampsia. In line with these results, the plasma Mn levels in pregnancy and their birth outcomes will be evaluated in Kinshasa. A conclusion will be given by providing recommendations to create a local Mn screening committee during pregnancy and lactating as suggested by committee opinion of the American College of Obstetricians and Gynecologists.

METHODS

Study Population and Data Collection

Pregnant women were recruited at the maternity hospitals [Hôpital Général de Référence de Kinshasa (Gombe), Maternité Delvaux (Binza), Centre de Santé Saint-Christophe (Binza); Centre de Santé et Maternité Saint-Raymond (Matete), Maternité Esengo (Kisenso), Maternité Lisanga (Lemba); Centre de Santé et Maternité Bomoyi (Tshangu)]. Enrollment was implemented between June 2019 and June 2020 during the pregnancy visit. Eligible women (400) received a detailed explanation of study procedures before consenting to participate (living in Kinshasa ≥ 6 months, amenorrhea period ≥ 10 weeks, not planning to move out of the city before delivery, etc.). Positive responses were obtained from more than 95% (396 pregnant women) of those approached. The research protocol was approved by the Bio-ethics Committee of the School of Public Health at the University of Kinshasa.

Data Collection

During the pregnancy visit, women provided venous blood samples in 10 mL metal free tubes containing K2EDTA as described elsewhere [17-19]. At delivery, both maternal venous blood and umbilical cord blood samples were collected. All blood was immediately centrifuged (10 minutes, 3000 g) and the plasma fraction was transferred into 2.5 mL pre-cleaned glass vials (Supelco®) and stored at -80°C for the Mn analysis. The plasma samples were transported to the Analytical chemistry and Environmental toxicology laboratory of the University of Kinshasa. Pregnancy and delivery information collected in the questionnaires were clinics, socio-demographics, Anthropometrics, current and previous pregnancies, current and previous preeclampsia or diabetes mellitus, smoking during pregnancy, and lifestyle.

Analytical Methods

The samples were brought to room temperature and vortexed after thawing. Pb was measured by atomic absorption (PG-Instruments combined Flame and Furnace -AA500FG- with graphite furnace, Germany) [18,20]. Plasma samples (100 µL) were diluted quantitatively (1+10) with a matrix modifier solution containing 0.5% Triton X-100 (PA Sigma-Aldrich), 0.1% nitric acid (65% pure, Roth) and 0.1% magnesium (0.1 mg/mL Mg^{2+}, Sigma-Aldrich). Determinations were calibrated with Mn solutions prepared from Mn standard solution suitable for atomic spectrometry [1000 ppm Mn, 1 mg/mL Mn – Sigma-Aldric]. Because the Plasma Mn levels were low, triplicate samples were analyzed, repeated for each sample with a coefficient of variation less than 10%, and the detection limit (LOD) was 0.2 µg/L. Analytical validity was confirmed using commercial standard serum (Seronorm L1 and L2) at the beginning of the run and the end of each run of 20 samples, as previously described [21].

Statistical Analysis

Statistical data analysis was completed using Prism GraphPad 9.41 (GraphPad Soft - ware, San Diego, CA, USA). The normality of residuals was evaluated using Kolmogorov–Smirnov test for continuous variables. For the descriptive statistics, results are presented as percentage for categorical variables and as means (± standard deviation), percentiles (P25, P50, P75, P95), and minimum-maximum for continuous variables. Differences between groups were analyzed with analysis of variance (ANOVA), t-test, and trend test after log transformation of skewed variables. Differences in proportions were analyzed with chi-square test. Multiple linear regression was used to estimate the association between log-transformed continuous plasma Mn and other continuous or categorical variables. Two-sided $p < 0.05$ was considered statistically significant. Mn levels below the LOD were assigned a value of LOD/2 for statistical calculations [22,23].

RESULTS

Of the 396 women included in this study, 177 (45%) had 30 years of age or more, 306 (77%) had lower or middle school degree, 81 (20%) were unmarried, 186 (47%) earned less than 100$ USD monthly, 212 (54%) were multiparous,69 (17%) had diabetes mellitus, 142 (36%) had a history of preeclampsia and 243 (62%) were underweight, overweight or obese women. 40 (10%) consumed alcohol during pregnancy. None of them smoked during pregnancy (Table 1). Among the 56 births that occurred, 11 (20%) were pre-term or post-term, 35 (63%) were female, 1 (2%) was under 7 Apgar score in 5 minutes, and 7 (12%) were underweight or overweight newborns (Table 1).

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Table 1: Sociodemographic characteristics of the study subjects (2019 - 2020, Kinshasa, n = 396)

		n (%)
Maternal characteristics		396 (100)
Age (years)		
	<18	36 (9)
	18 - 29	163 (41)
	≥ 30	177 (45)
Education		
	Lower school or none	20 (5)
	Middle school	286 (72)
	High school or university degree	90 (23)
Marital status		
	Married or living as married	315 (80)
	Unmarried	81 (20)
Family income (month)		
	None	86 (22)
	<100 $ USD	100 (25)
	100$ - 500$	196 (49)
	≥ 500$	14 (4)
Smoking during pregnancy		
	Yes	0
	No	396 (100)
Alcohol use during pregnancy		
	Yes	40 (10)
	No	356 (90)
Parity		
	0 (primiparous)	184 (46)
	≥1 (multiparous)	212 (54)
BMI		
	≤18.5 (underweight)	5
	18.5 – 24 (normal)	149 (38)
	25 -29 (overweight)	141 (36)
	≥30 (Obese)	97 (26)

Diabetes mellitus		
	Yes	69 (17)
	No	327 (83)
Family history of preeclampsia		
	Yes	142 (36)
	No	254 (64)
Newborn characteristics		56(100)
Sex		
	Female	35(63)
	Male	21 (47)
Birth weight (g)		
	<2500 (underweight)	3 (5)
	2500 – 4000 (normal)	49 (88)
	> 4000 (overweight)	4 (7)
Ponderal Index (g/cm^3)		
	≤ 2.49 (Low ponderal index)	56 (100)
	2.50–3.16 (Normal Ponderal index)	0
	≥ 3.17 (high ponderal index)	0
Gestational age (weeks)		
	<37 (pre-term)	6 (11)
	37 – 41 (normal)	45 (80)
	>42 (post-term)	5 (9)
Delivery method (n)		
	Vaginal	56 (100)
	Caesarean section	0
Apgar score		
	5 min <7	1 (2)
	5 min ≥7	55 (98)

Table 2 lists the means (±SD), percentiles (P25, P50, P75, and P95), and minimum as well maximum of the continuous variables: maternal parameters including, plasma Mn (µg/L), age (years), weight (kg), height (m), amenorrhea period (weeks), BMI (kg/m2), SBP (mm Hg), DBP (mm Hg), and newborn parameters containing fetal plasma Mn, birth weight (g), birth height (cm), ponderal index (g/cm3), gestational age at birth (weeks), head circumference at birth (cm) and Apgar score. The plasma Mn means (±SD) were respectively 2.467 µg/L (± 3.472) in maternal and 5.151 µg/L (± 4.300) in newborns.

Table 2: Association between maternal-child parameters and plasma Mn levels.

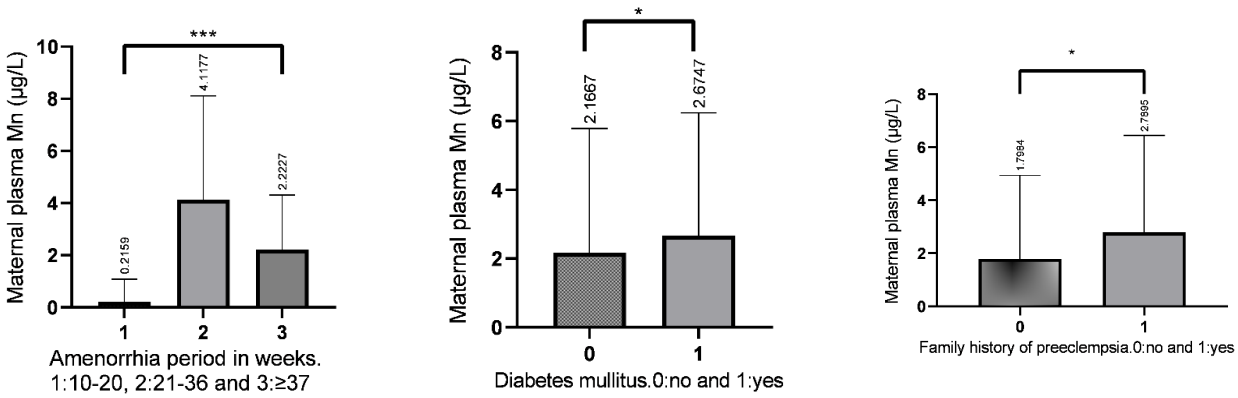
Variables	Mean ±SD	Percentiles				Min - Max
		P25	P50	P75	P95	
Mothers						
Mn (µg/L)	2.467 ± 3.472	0.564	1.127	3.789	11.72	0.100 – 17.160
Age (years)	26.57 ± 4.70	23.00	26.00	29.50	35.00	16.00- 45.00
Weight (kg)	68 ± 12	59	66	75	91	40 - 116
Height (m)	1.60 ± 0.07	1.60	1.60	1.70	1.7	1.00 - 1.80
Amenorrhea period	26.0 ± 8.5	19	26	32	39	10 - 42
BMI (kg/m²)	27 ± 5	23	26	30	35	16 - 45
Systolic blood pressure (mm Hg)	107 ± 15	100	110	110	113	69 - 221
Diastolic blood pressure (mm Hg)	66 ± 10	60	60	70	80	55 - 126
Newborns						
Mn (µg/L)	5.151 ± 4.300	1.364	4.500	7.239	13.970	0.100 – 18.310
Birth weight (g)	3190 ± 530	2820	3200	3543	4215	1500 - 4340
Birth height (cm)	49.0 ± 3.8	46	48	50	56	38 - 59
Ponderal Index (g /cm³)	1.393 ± 0.284	1.173	1.400	1.580	1.985	0.679 - 2.008
Gestational age at birth (weeks)	38.38 ± 1.53	33.00	38.50	39.00	40.45	33.00 - 42.00
Head circumference at birth (cm)	34 ± 2	33	34	36	37	29 - 38
Apgar Score	9 ± 1	9	10	10	10	5 - 10

Under limit of detection (LOD) rate (%)

Regarding differences between groups, levels of plasma Mn were observed in women with a family history of preeclampsia and diabetes mellitus (t-test, p=0.0271 and 0.0312, respectively). Plasma Mn levels were also significantly higher in in 20-36 weeks of amenorrhea period as compared to other periods [means (±SD), 4.118 µg/L (± 3.911) in 20-36 weeks, 0.216 µg/L (± 0.873) in 10-19 weeks and 2.223 µg/L (± 2.091) at delivery (≥37 weeks), ANOVA, $p < 0.0001$] and newborns showed higher plasma Mn levels than their mothers [means (±SD), 5.151 µg/L (± 4.300) versus 2.467 µg/L (± 3.472), t-test, $p = 0.001$]. No significant associations were observed between maternal plasma Mn and birth weight (g), birth height (cm), ponderal index (g/cm3), or gestational age at birth (weeks) (Figure 1,2).

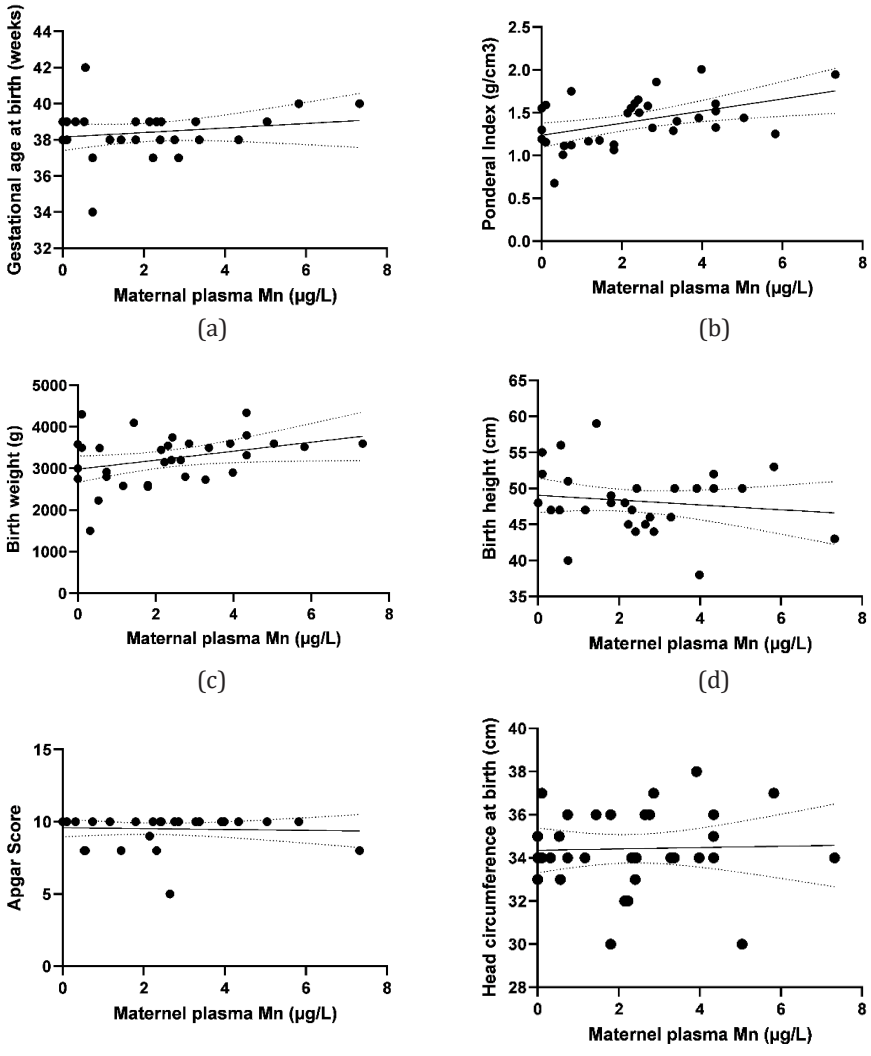
Table 3: Multiple regression analysis model

Parameter (dependent variable)	Partial R^2 (Independent variables)				Total R^2
	BMI	multiparous	Family history of preeclampsia	Amenorrhea period	
Log (maternal plasma Mn levels)	0.02018	0.02425	0.02019	0.02418	0.0888



(a) (b) (c)

Figure 1: Comparison between plasma Mn levels and maternal outcomes (t-test or ANOVA). Maternal plasma Mn levels (µg/L) against (a) Amenorrhea period in weeks, (b) Diabetes mellitus, and (c) Family history of preeclampsia.



(a) (b) (c) (d) (e) (f)

Figure 2: Scatter Plot of Maternal plasma Mn levels (µg/L) against birth outcomes. Maternal plasma Mn levels (µg/L) against (a) Gestational age at birth (weeks), (b) Ponderal Index (g/cm3), (c) Birth weight (d) Birth height (cm), (e) Apgar Score and (f) Head circumference at birth (cm).

DISCUSSION

The majority of Mn in whole blood is bound to red blood cells [24, 25]. And the remaining Mn in the plasma/serum which is bound with proteins such as albumin and globulin [26]. Mn crosses the placenta via active transport [27] and plays an important role in fetal development at a relatively high level, particularly in brain function and skeletal development [27, 28]. Indeed, several studies reported that higher blood Mn concentration during pregnancy may reflect increased physiological demands for fetal development [31]. However, under certain high-dose exposure conditions during fetal development, Mn can induce adverse birth outcomes [31-35].

In the present study, Mn levels (2.467 µg/L and 5.151 µg/L in maternal and fetal plasma samples, respectively) were similar or slightly higher than those reported in previous studies [18,36- 39,40]. Mean Mn levels in fetal plasma were about twice as high as in maternal plasma reflecting active transport of Mn across the placenta [11,13,39,41-44]. Nonetheless, as the level of fetal Mn that may be considered ''safe'' has not yet been established, the results from Andersen et al. [4] showed a growing concern about the transfer of Mn from pregnant women to the growing fetus. Similar to this finding, previous studies reported that the disrupting effects of fetus have been first observed in the stage of developing fetus [14], serum Mn levels in the umbilical cord greater than 5.0 µg/L were associated with poorer performance on neurobehavioral tests [18] and a high level of umbilical cord serum Mn (≥ 9.1 µg/L) increased the risk of low neonatal behavioral neurological assessment scores in neonates [45]. In this work, the Mn levels above the 50th percentile (4.55 µg/L) found in fetal plasma samples constitute a major public health concern.

As reported in this work, systolic and diastolic blood pressures were normal in pregnancy. However, women with a family history of preeclampsia and diabetes mellitus had significantly higher Mn levels as compared to other ones (t-test, p=0.005 and 0.031, respectively). This may be because of increased excretion of Mn was observed in preeclampsia [16] and family history of preeclampsia and diabetes mellitus outcomes are considering among the principal risk factors for preeclampsia (46-8).

Like most studies, we found higher Mn plasma levels in 20-36 weeks of amenorrhea period compared with the period before (10-20 weeks) or at delivery (≥37 weeks) likely reflecting an increased need period for Mn in the fetus [27,28,31,49]. However, similarly to other studies, no relevant differences or associations in Mn levels were found according to age [50,51], BMI [40,52], birth weight (18,43), birth height, ponderal index at birth [18], or Apgar score [18,43].

A major limitation should be considered in evaluating present results. With regard to study population, data collection and analytical methods, the relatively small number of birth cohort studied. The sample collection methods used here were not robust but by chance, which were practically inevitable under present survey conditions and susceptible to errors associated with sample collection. Analytical problems at the low levels of Mn found in plasma are major reasons that plasma Mn should be measured routinely with much lower detection limits and with better accuracy by ICP-MS without advanced clean room facilities [18-20]. Moreover, potential contamination by analysis of plasma Fe, free hemoglobin, dietary Mn intake and environmental risk factors was not assessed [19].

CONCLUSIONS AND RECOMMENDATIONS

Although no significant linear negative correlation between maternal plasma Mn and all of these outcomes (birth weight, birth height, Apgar score, head circumference at delivery) and Mn levels in both maternal and fetal samples were similar or slightly higher than those reported in previous studies has been found in this study, possibly due to small number of birth cohort studied and scarce relevant data on associations between plasma-Mn and adverse outcomes, women with a family history of preeclampsia and diabetes mellitus had significantly higher plasma Mn levels. Furthermore, the Mn levels above the 50th percentile (4.55 µg/L) found in fetal plasma samples constitute a major public health concern for newborns. Risk assessment of Mn exposure should take place at the earliest contact with pregnant and lactating women.

DECLARATIONS

Ethical Approval

The research protocol was approved by the Bio-ethics Committee of the School of Public Health at the University of Kinshasa. Kinshasa, DRC.

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' contributions

The first draft of this manuscript has been written by the first author Y. M. T (1). The co-author 2 H.N. and 3 M.M. prepared Tables and Figures, respectively. The co-authors 4 D.K., 5 C.K., 6 JP.M. and 7 A.M. reviewed equally the manuscript. The co-author 8 contributed to supervise all the work and to correspond with the Journal.

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Availability of data and materials

Not applicable. However, the study results will report to individual sample donors with proper explanations.

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