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Study of Umbilical Blood Lead Level and Its Relation with Pregnancy Outcome

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Abstract: Background: Lead exposure continues to be a major health problem that needs more attention in different health disciplines.

Objective: to explore the correlation between maternal exposure and cord blood lead levels and to identify potential predictors for umbilical cord blood lead levels and its effect on pregnancy outcome.

Methodology: This study is a cross sectional study, which included 100 newborns of healthy pregnant women with singleton pregnancy. The study took place from November 2017 to April 2018. All mothers complete a questionnaire about their demographic data, obstetric history. In addition, their pregnancy outcome was documented and an umbilical cord sample was drawn for determination of lead levels. Patients were divided into two groups according to umbilical cord blood lead levels (high group ≥10 µg/dl) and low group (<10 µg/dl).

Results: High umbilical cord blood lead levels were associated with living or working near highly trafficked streets or roads, living or working near painting workshops, living near waste burning, working near buildings being demolished, regularly using heavy traffic streets or roads, and drinking tea, coffee or green tea, while low lead concentration was associated with taking iron and calcium supplementation. Mothers in high group had significantly lower hemoglobin, and there was a significant decrease of birth weight, length and head circumference in such group. Umbilical blood lead level (UBLL) correlates negatively with mother's hemoglobin, neonatal birth weight, head circumference and length.

Conclusion: higher umbilical cord blood lead concentrations were associated with adverse maternal and neonatal outcome. Exposure usually occupational or environmental.

Keywords: umbilical cord; blood lead; pregnancy; neonates; outcome.

دراسة مستوى الرصاص في دم الحبل السري وعلاقته بنتائج الحمل

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الملخص: مازال التعرض للرصاص يمثل مشكلة صحية تحتاج للاهتمام من الانظمة الصحية المختلفة. تم تصميم الدراسة الحالية لاستكشاف وجود علاقة بين التعرض الأمومي للرصاص ومستويات الرصاص في دم الحبل السري وتحديد العوامل المسببة المحتملة التي قد تؤثر على مستويات الرصاص في الدم السُري العالية وتأثيرها على نتائج الحمل.

وقد شملت الدراسة مائة مولود حديث الولادة من أمهات حوامل أصحاء يحملن حملاً مفرداً. تم استخدام نموذج استبيان لجمع المعلومات من الأمهات المشاركات فيما يتعلق بالبيانات الديموجرافية وتاريخ الحمل وتم سحب عينة دم من الحبل السري لكل طفل حديث الولادة لتحديد مستوى الرصاص بها.

كشفت الدراسة أن مستويات الرصاص العالية في دم الحبل السري كانت مع الأمهات الحوامل اللاتي يسكن أو يعملن بالقرب من الشوارع أو الطرق المزدحمة وأيضا العيش بالقرب من ورش الطلاء وأماكن حرق النفايات وأيضا شرب الشاي أو القهوة أو الشاي الأخضر. وأيضا الأمهات الحوامل اللاتي كانت نسبة الهيموجلوبين لديهن منخفضة. بينما كانت مستويات الرصاص المنخفضة في دم الحبل السري مع الأمهات الحوامل اللاتي يتناولن مكملات الحديد والكالسيوم.

كما كشفت نتائج الدراسة أيضا أن هناك علاقة بين ارتفاع مستوى الرصاص في دم الحبل السري ونتائج الحمل متمثلة في انخفاض ملحوظ في القياسات البشرية (الوزن، الطول، ومحيط الرأس). أن وجود مستويات عالية من الرصاص في دم الحبل السري يعكس أضراراً جانبية على صحة الأم والجنين.

الكلمات المفتاحية: الحبل السري. الرصاص في الدم حمل؛ حديثي الولادة.

Introduction

Intrauterine exposure to heavy metals has been studied but there is a growing concern about the effects on pregnancy outcomes at levels lower than international guidelines[(1)].

Lead exposure remains a public health problem for subpopulations of women of childbearing age and for the developing fetus and nursing infant for several important reasons. Prenatal lead exposure has known to influence maternal health, infant birth and neurodevelopmental outcomes[(2)]. Research findings suggest that prenatal lead exposure can adversely affect maternal and child health across a wide range of maternal exposure levels. In addition, adverse effect of lead are being identified at lower levels of exposure than previously recognized in both child and adult populations[(3,4)].

Timing of exposure has a determinant role on the consequences of the exposure (e.g., If exposure occurs during the time when organogenesis, permanent structural abnormalities might occur, while exposure after organogenesis is complete might lead to only functional abnormalities). Since bone lead stores persist for decades, women and their infants may be at risk for continued exposure long after exposure to external environmental sources has been terminated[(5)].

Lead reached the fetus by trans-placental transfer[(6)]. Placenta is a barrier that permits nutritional substances and oxygen to reach the fetus and prevent toxic compounds[(7)].

Pregnant women may be exposed to lead in their work in specific high-risk occupations[(8)]; and those practicing certain behaviors, such as pica[(9)], use of culturally-specific remedies and products[(10)], and renovating older homes[11]. Women living near hazardous wastes site or active smelters[12] and residents in countries still using leaded gasoline[13] may also be highly exposed.

Although lead exposure remains an important potential risk to the fetus, until now, little emphasis has been placed on developing guidelines for prenatal health care providers and women of childbearing age. There are currently no national recommendations or guidelines by any obstetric, family practice, pediatric, or nursing groups that cover lead risk assessment and management during pregnancy and lactation.

Methodology

This is a cross-sectional study, which included 100 newborns -of healthy pregnant women with singleton pregnancy from multiple general hospitals in Damietta Governorate. The study took place from November 2017 to April 2018. The study was carried out in the following hospitals through systematic random sample on a two days' work per week, in non-regular pattern according to the circumstances, with 20 newborns were included from each hospital: 1) Damietta general hospital; 2) Damietta specialized hospital; 3) Al-Azhar university hospital, new Damietta; 4) Kafr saad central hospital; and 5) Faraskour central hospital.

Full term healthy newborns of healthy pregnant women with a singleton pregnancy with maternal age ranges between 18——35 years were *included* in the study. On the other hand, newborns of mothers with one or more of the following were *excluded* from the study: high risk pregnancy as preeclampsia, renal or circulatory disease or any other chronic disease, Consumption of illegal drugs or alcoholic beverages, Any psychiatric disorder, On medication for neurological or psychiatric condition, and Smoker.

Official permissions were taken from the hospitals administrations that were involved in this study. A written agreement was taken from participants prior to the interview and blood sample collection.

Data was collected by a pre-designed questionnaire form. It included questions related to maternal age, parity, current and previous pregnancies, abortions, premature rupture of membranes, socio-demographic data, and lifestyle issues. In addition, it asks about living and working conditions as regard to occupation and environmental exposures; health habits as coffee and tea consumption during the third trimester of pregnancy, milk and milk products consumption, smoking behavior and cosmetics uses, consuming canned foods and drinks and the usage of pottery, aluminum utensils and newspapers in preparation of food. The last part of the form included questions concerning, history of chronic and acute diseases during current pregnancy & history of taking iron and calcium supplements. The questionnaire was partially based on the "prenatal-risk evaluation for lead exposure" questionnaire of Illinois department of public health and "lead exposure risk assessment questionnaire for children" of New York state department of health.

Then for each mother, the following were searched for: premature rupture of membranes, preterm delivery and mode of delivery and hemoglobin level.

For each neonate, the following data were collected: Anthropometric measurements at the moment of birth (weight, height and head circumference) and values of the APGAR scores at 1^{st} and 5^{th} minute. the Anthropometric measurements were then charted on Fenton premature growth chart 2013 to determine if the measurements are average, small or large for gestational age, with the tenth and ninetieth percentiles were the lower and higher limits respectively.

A venous blood sample of 5 mL was drawn from cord blood and collected in lead free ethylenediaminetetraacetic acid (EDTA) tubes immediately after the birth of the fetus and was immediately centrifuged to separate plasma from whole blood, thus avoiding transfer of lead from erythrocytes. Each plasma fraction was then pipetted into two ultra-cleaned Eppendorf tubes (2 mL) and levels of lead are determined immediately if possible or plasma samples were stored at -20°c, till the time of analysis. For each sample, lead concentration was measured using atomic absorption spectrophotometer at Mansoura University, faculty of science, chemistry department, and atomic absorption lab using "GBC Scientific Equipment atomic absorption spectrometer (SENSAA model)" According to Agency for Toxic Substances and Disease registry (ATSDR, 2007), the cutoff levels of lead ≥10 μg/dl was considered to be excessive for infants, children and women of child-bearing age. Thus, in the present study, cases were divided into two groups, the first included cases who had lead ≥10 μg/dl and the second who had lead concentration <10 μg/dl.

In the present study, the neonates were divided in to two groups as regard to umbilical blood lead level regarding the cutoff point of 10μg/dl. The majority of study subjects were in the low umbilical blood lead level group (78 subjects; 54 had values < 5 μg/dl and 24 had values between 5 and 10μg/dl), while high umbilical blood lead level included (22 neonates).

The data were analyzed with Statistical Package for the Social Sciences (SPSS, Chicago, IL; USA) software v. 25. Descriptive results are presented as means± standard deviation (SD) or percentages. Quantitative variables were compared between two or more groups of subjects with Student's t-test. Qualitative variables were compared with Fisher's exact test between two groups. To determine the correlation between two quantitative variables, Pearson's correlation test was used. P values less than 0.05 were considered statistically significant.

Results

Both low and high umbilical blood lead level (UBLL) groups were comparable were comparable as regard to gestational age, newborn gender, mode of delivery, maternal age and mother's levels of education.

However, fathers with a higher (university) education were significantly increased in group with low UBLL (37.2% vs 18.2% respectively). In addition, high UBLL were significantly increased in urban populations (90.9%) when compared to rural inhabitants (33.3%). Females in both groups were comparable as regard to obstetric history.

In the present study, high umbilical cord blood lead levels was significantly associated with living or working near highly trafficked streets or roads, living or working near painting workshops, living near waste burning or recycling areas, working near buildings being demolished or rebuilt and regularly using heavy traffic streets or roads. In addition, high lead concentration was associated with drinking teach, coffee or green tea, while low lead concentration was associated with taking iron and calcium supplementation during pregnancy (table 1).

Table (1) Distribution of studied patients as regard to living conditions, working conditions or health habits in relation to umbilical cord blood lead levels

		Umbilical blood lead level			
		Low group N.(%)#	High group N.(%)#	test	p
Living conditions	Living near highly trafficked streets or roads	26(33.3%)	15(68.2%)	3.03	0.005*
	Living in house built before 1980	26(33.3%)	12(54.5%)	1.75	0.090
	Regularly visiting house built before 1980	22(28.2%)	11(50.0%)	1.80	0.08
	Living near building being demolished or rebuilt	12(15.4%)	4(18.2%)	0.299	0.767
	Living near painting workshop	14(17.9%)	10(45.5%)	2.348	0.026*
	Living near building being painted	17(21.8%)	7(31.8%)	0.89	0.37
	Using lead or cast-iron pipes	16(20.5%)	9(40.9%)	1.74	0.09
	Living near waste burning or recycling areas	12(15.4%)	10(45.5%)	2.58	0.015*
Working conditions	Working near highly trafficked streets or roads	25(32.1%)	13(59.1%)	2.25	0.031*
	Working in building built before 1980	15(19.2%)	4(18.2%)	0.11	0.91
	Working near building demolished or rebuilt	11(14.1%)	11(50.0%)	3.09	0.005*
	Working near painting workshop	13(16.7%)	11(50.0%)	2.84	0.008*
	Working near building being painted	11(14.1%)	8(36.4%)	1.98	0.06
	regularly using heavy traffic streets or roads	26(33.3%)	20(90.9%)	6.97	0.001*
Health habits	Drinking tea, coffee or green tea	21(26.9%)	13(59.1%)	2.71	0.011*
	Drinking milk and its products during pregnancy	33(42.3%)	10(45.5%)	0.25	0.79
	Taking iron and Cal. Suppl. during pregnancy	52(66.7%)	9(40.9%)	2.15	0.039*
	Pica	5(6.4%)	2(9.1%)	0.39	0.69
	Using Kohl regularly and other cosmetics	29(37.2%)	12(54.5%)	1.42	0.16
	Eating canned foods	37(47.4%)	11(50.0%)	0.20	0.83

		Umbilical blood lead level			
		Low group N.(%)#	High group N.(%)#	test	p
	Drinking caned drinking	17(21.8%)	3(13.6%)	0.922	0.362
	Using ceramic dishes pottery	4(5.1%)	1(4.5%)	0.112	0.911
	Using aluminum utensils or dishes	67(85.9%)	18(81.8%)	0.438	0.664
	Wrapping food in newspapers	30(38.5%)	7(31.8%)	0.574	0.570

(N) stands for number (frequency of observations), * indicates statistical significance

In the present research, there was statistically significant decrease of mother's hemoglobin in in high when compared to low group (8.21±0.49 vs 11.09±0.87 respectively). In addition, there was statistically significant decrease of neonatal birth weight, head circumference and length in high group when compared to low group. However, there was no significant difference between both groups as regard to AGPAR score at first or fifth minutes (table 2).

Table (2) Comparison between groups as regard to umbilical cord blood lead levels, mother's hemoglobin and neonatal outcome

	Umbilical blood lead level							
	Low group			High group			test	p
	Mean±SD	Median	Range	Mean±SD	Median	Range		
Blood lead levels	4.79±1.95	4.3	1.1 – 9.8	13.93±2.16	13.35	11 – 19.3		
Mother's hemoglobin	11.09±0.87	11.30	8.20 to 12.60	8.21±0.49	8.30	7.10 to 9.10	0.001	19.96
APGAR at 1 min	5.57±.79		4 to 7	5.63±.84		4 to 7	0.29	0.77
APGAR at 5 min	7.92±0.76		6 to 9	7.95±0.78		6 to 9	0.869	0.167
Birth weight	3.22±0.39	3.30	2.2 to 3.9	2.440±0.33	2.30	2.1 to 3.2	9.30	0.001*
Average for GA	65(83.3%)			9(40.9%)				
Small for GA	8(10.3%)			13(59.1%)				
Large for GA	5(6.4%(			0(0.0%)				
Head circumference	34.78±0.69	35.00	33 to 36	33.95±0.95	34.00	32 to 36	3.80	0.001*
Average for GA	71(91.0%)			22(100.0%)				
Below 10th percentile	0(0.0%0			0(0.0%)				
Above 90th percentile	7 (9.0%)			0(0.0%)				
Length	49.84±1.03	50	48 to 52	47.50±1.37	47.50	46 to 50	7.45	0.001*
Average for GA	74(94.9%)			19(86.4%)				

	Umbilical blood lead level						test	p
	Low group			High group				
	Mean±SD	Median	Range	Mean±SD	Median	Range		
Below 10th percentile	0(0.0%)			3(13.6%)				
Above 90th percentile	4(5.1%)			0(0.0%)				

SD: standard deviation, * indicates statistical significance; GA: gestational age

In the present study, there, proportional (positive), significant correlation between umbilical blood lead levels and premature rupture of membranes (PROM) of previous and current pregnancy. On the other hand, there was inverse (negative), significant correlation between umbilical cord blood lead levels and each of mother's hemoglobin levels, neonatal birth weight, head circumference and length (table 3).

Table (3) correlation between umbilical cord lead blood level and other variables

	Umbilical blood lead level	
	r	p
Number of pregnancies	0.014	0.88
Abortions	0.015	0.88
PROM	*0.31*	*0.001**
Previous premature deliveries	0.19	0.06
Mother's Hb level	*-0.835*	*0.001**
APGAR 1 minute	0.034	0.73
APGAR 5 minutes	0.081	0.42
Birth weight	*-0.61*	*0.001**
Head circumference	*-0.448*	*0.001**
Length	*-0.51*	*0.001**

(r): Pearson's correlation coefficient; * indicates statistical significance; PROM: premature rupture of membranes; Hb: hemoglobin.

Discussion

In the current study, the mean umbilical cord blood lead level for all study sample was 6.8 µg/dl (the mean in the low UBLL group was 4.8 µg/dl, while it was 13.9 µg/dl in the high UBLL group). When compared to other studies, Al-Jawadi *et al.* [14], in study in Mosul, Iraq, reported a mean of 2.3 µg/dl, which is much lower than the mean in the current study. On the other hand, Rahman *et al.*[15], reported a mean of 10.92 µg/dl in

Kuwait. The mean in our study was much higher than the mean of The National Health and Nutrition Examination Survey (NHANES) study from 1999 to 2002 (mean: 1.52 μg/dl; n=4525)[16]; shanghai (5.61 μg/dl; n=400)[17] and France (1.9 μg/dl; n=1017)[18].

Considering the center for disease control and prevention (CDC) updates, this study revealed that 24% of the study subjects had umbilical blood lead level between 5 and 10μg/dl and study subjects with UBLL higher than 5 μg/dl represented 46% of all study subjects. Comparing the results with other studies, the percentage of study subjects with UBLL ≥10μg/dl was much higher than reported by Al-Jawadi *et al.* [14], in Mosul, Iraq, where it was 5.7%, and percentage of study subjects with umbilical blood lead level between 5-10μg/dl was 7.7% (n= 370). It was also much higher than reported by Zhang *et al.*[19], with only 2.3% of cases had UBLL higher than 5 μg/dl. On the other hand, Rahman *et al.* [15] reported a percentage of 58%.

In our current work, there was a marked significant difference between the two groups as regard to residency with 90.9% of high UBLL lives in urban areas compared to 33.3% of the low UBLL group. These results are comparable to the results of Manzoor[20], who reported that, women living in the urban areas showed higher lead level in blood than women living in rural areas. On the other hand, Zhang *et al.* [19] reported that there was no relation between living in urban trafficked areas and elevated umbilical cord lead blood levels.

In this current study, there was marked significant difference between the two groups as regard the history of PROM, including current pregnancy. PROM was significantly associated with elevated umbilical blood lead level. These results are comparable with Vigeh *et al.*[21] who found that, blood lead concentrations were higher in PROM deliveries that in non-PROM deliveries. Also early rupture of the fetal membrane has been shown in subjects at higher blood lead concentration (>13 μg/dl).

In this present study, there was also a significant difference when comparing the two groups as regard to history of previous premature deliveries (p=0.034). With premature deliveries being significantly associated with the high umbilical lead level group. These results were comparable to the results of Taylor *et al.* [22], who reported that there is increased risk of preterm deliveries with maternal blood lead level >5 μg/dl. Furthermore, Patel and Prabhu [23], reported that there was a significant relationship between gestational age and umbilical cord blood lead level. In this way, increase of mean umbilical cord blood lead level to 1 μg/dl, gestational age was reduced by one week.

In the current study, high UBLL was associated with living, working near, using highly trafficked streets or roads. These results were comparable to the results of Manzoor[20], who reported that the mean blood lead levels (BLL) of the mothers living in heavy trafficked areas was higher than of those living in less trafficked areas. Rahbar *et al.*[24], in a study on blood lead concentration of children in Jamaica, reported that children living near a high traffic road had a higher mean of BLL than those living in low traffic road (3.43 μg/dl vs 2.52 μg/dl respectively).

In contrast, Zhang *et al.* [19] reported no association between living in traffic areas and elevated UBLL. Kirel *et al.* [25], also reported that the lead levels did not differ regarding the traffic load of the residency.

A worldwide initiative to phase-out lead in gasoline has already stimulated important reductions in ambient air lead levels and population blood lead levels in some countries[26]. A complete phase-out of leaded gasoline was completed throughout the Latin American and Caribbean region by 2005[27].

In this current work, high UBLL also associated with exposure to leaded-paint, living or working near painting workshop. Jacobs *et al.*[11] reported that, lead-based paint was commonly used in homes built before 1950, and was not banned from sale for residential use in the United States until 1978. Recent studies estimate that more than 38 million U.S. homes still contain some lead-based paint, with two-thirds of the houses built before 1960 containing lead-based paint hazards. In addition, a study by Patil *et al.*[28] found significant raised blood levels in spray painters.

In our current work, as regard to living near waste burning or recycling areas, there was significant difference between the two groups (p=0.015), with 45.5% of the high UBLL group and 15.4% the low UBLL group living near waste burning or recycling areas. These results were comparable to Huo *et al.* [29] who aimed to determine Blood Lead Levels of Children in Guiyu, an Electronic Waste Recycling Town in China, found that Statistical analysis showed that children living in Guiyu had significantly higher BLLs compared with those living in Chendian (non-Electronic Waste Recycling Town) (p < 0.01). Also, in a study by Amankwaa *et al.*[30] to determine the impact of electronic waste recycling on blood lead levels in Ghana, the mean BLL (3.54 μg/dL) of non-e-waste workers was slightly higher than that of e-waste workers (3.49 μg/dL), although higher BLLs ranges were found among e-waste workers (0.50–18.80 μg/dL) than non-e-waste workers (0.30–8.20 μg/dL). Workers who engaged in e-waste burning tended to have the highest BLLs.

In the current study, comparing the low and high UBLL groups regarding health habits that might influence UBLL, there was significant difference between the two groups concerning regularly drinking tea, coffee or green tea during pregnancy (p=0.011) with 59.1% of the high UBLL group admitting regularly drinking tea, coffee or green tea compared to 26.9% of the low UBLL group. These results were comparable to the results of Al-Jawadi *et al.* [14] who reported that increased coffee and tea consumption emerged as a significant risk predictor for UBLL>5 μg/dl (p = 0.034). Furthermore, Taylor *et al.*[22] also reported that caffeinated coffee and tea were significantly strong predictors of elevated BLL (p<0.001).

In the current study, as regard to taking iron and calcium supplementation during pregnancy, there was significant difference between the two groups (p=0.039) with 40.9% of the high UBLL group admitted regularly taking the supplementations compared to 66.7% of the low UBLL group. Thus, that may indicate that iron and calcium supplementation having protective effect. These results were comparable to the results of Al-Jawadi *et al.* [14] who reported that receiving iron and calcium supplementations during pregnancy had a significant protective effect against the development of high UBLL (p = 0.045). Taylor *et al.* [22] also reported that Dietary calcium and iron supplements were protective against high lead levels (p = 0.001 and p = 0.046, respectively).

In the current study, the mean maternal hemoglobin level was 10.5 g/dl, with the mean Hemoglobin (Hb) was much higher in the lower UBLL group than that of the high UBLL group (11 g/dl and 8.2 g/dl respectively). Also, there was also a marked negative correlation between maternal hemoglobin level and umbilical cord blood level (Pearson correlation coefficient=-0.835 & r=0.001). These results were comparable to the study of Al-Jawadi *et al.* [14], which suggests that hemoglobin level <11 g/dl was a significant predictor for UBLL ≥5 μg/dl (p=0.007).

In the current study, as regard to the birth weight, high UBLL was associated with low birth weight and there was negative, significant correlation between birth weight and UBLL. These results were comparable to those reported by Taylor *et al.* [22], who reported that increase in the blood lead level was significantly associated with reduction in birth weight. Zhu *et al.* [31], also reported that in a study on mothers with low lead level below 10 μg/dl, umbilical cord blood lead levels of between 5 and 10 μg/dl had a relationship with reduction of neonate weight by 61-87gm with the decrease in birth weight for a 1-μg/dL increase in PbB ranged from an estimated means value of 4 g (from 9 to 10 μg/dL) to 27 g (from 0 to 1 μg/ dL).

In contrast, Neda *et al.*[32] found no significant correlation between the umbilical cord lead blood level and birth weight (r=0.092, r=0.141). Rahman *et al.* [15] also reported that there was no significant correlation between umbilical cord blood lead level and birth weight.

As regard to the length of newborns, there was significant decrease in higher UBLL when compared to low UBLL group, and there was inverse significant correlation between UBLL and neonatal length. These results were comparable to those reported by Taylor *et al.* [22], who reported that increase in the blood lead level was significantly associated with reduction in crown-heel length. These results were also comparable to those reported by Neda *et al.* [32], who reported that there was reverse statically correlation between umbilical cord blood lead level and length newborns (r=-0.22, p=0.008).

On the other hand, Rahman *et al.* [15] reported that there was no significant correlation between umbilical cord blood lead level and either birth weight, length of newborns.

As regard to the head circumference, there was a significant decrease in High when compared to low UBLL and there was an inverse significant correlation between head circumference and UBLL. These results were comparable to those reported by Taylor *et al.* [22] who reported that increase in the blood lead level was significantly associated with reduction in head circumference. In addition, Neda *et al.* [32] reported that there was reverse statically correlation between umbilical cord blood lead level and head circumference of newborns (r= -0.20, p=0.01). On the other hand, Rahman *et al.* [15] reported that there was no significant correlation between umbilical cord blood lead level and head circumference of the newborns.

In conclusion, results of the present work revealed that, high lead concentration is associated with adverse pregnancy outcome. Possible risk factors include: living or working near highly trafficked streets or roads, living near painting workshops, regular use of heavy trafficked roads, drinking tea, coffee or green tea; while taking iron and calcium supplementation was associated with protective effects.

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