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Toxic and essential metals in placenta and its relation with lipid peroxides/glutathione status in pre-term and full-term deliveries

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ABSTRACT

Aims and Objectives: Pre-term birth is worldwide problem, little is known about oxidative stress in placental tissue due to metals exposure of women during pregnancy. To seek correlation between concentration of metals and redox status in placental tissue of women with pre-term delivery and compared with those of women with the full-term delivery. **Materials and Methods:** A hospital based case–control study was conducted, total 80 pregnant women were selected for this study i.e., 30 females who delivered pre-term babies (gestational age <37 week) serve as study group and 50 females who delivered full term babies (gestational age > 37 week) serve as control group. Concentrations of metals lead (Pb), cadmium (Cd), iron (Fe), zinc (Zn), and (Cu), malondialdehyde (MDA) end product of lipid peroxidation and glutathione (GSH) were measured in placenta of study group and control group. Data were analyzed using Students t- test, chi square and linear regression. **Results:** In preterm cases level of malondialdehyde (MDA) and metals (Cd and Pb) were significantly higher, while level of GSH and some metals (Fe, Zn and Cu) were significantly lower in pre-term cases when compared to full-term cases. There were significant positive correlations of placental metals (Pb and Cd) with MDA ($p<0.05$), suggests that metals might have influenced pre-term deliveries. **Conclusions:** The results showed that elevated level of placental Cd and Pb induced oxidative stress which might implicate in pre-term deliveries and higher concentration of Zn and Cu may be concerned with the defence against oxidative stress in placental tissue of full-term cases.

Key words: Pre-term birth, Placental tissue, Oxidative Stress, Metals

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INTRODUCTION

Gestational age of a neonatal which calculated from the last menstrual period is before 37 completed weeks is a pre-term. According to World Health Organization (WHO) report 2012, India has maximum number of pre-term delivery cases followed by China and top of the list in the world.[1] Pre-term delivery is responsible for 70% of mortality and 75% of morbidity in the neonatal period, especially in industrialized countries. In Agra region premature birth (33.6%) and LBW (low birth weight) are two major factors for high neonatal mortality.[2] We selected Agra city for this study because it has more than 13 larger and 7200 Small Scale Industrial Units (SSIU) in the district, these industries are responsible for highly polluted environment.[3] During the pregnancy women are exposed to a wide variety of foreign chemicals including metals through maternal medication, lifestyle factors (smoking, drug abuse, alcohol consumption), occupational and environmental sources and other risk factors such as socioeconomic status, race/ethnicity and domestic smoke (as from burning of biomass fuel for cooking).[4] These metals readily move across the placental barrier. Placenta serves as an intermediary and binds two genetically distinct individuals, the mother and the fetus.[5] In addition, accumulation of these metals in placenta, may affect the normal placental function, consequently upsetting the fetal development and lastly, at the terminal phase of placental life, i.e., delivery.[6] Many

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studies suggest association between toxic/essential metals in the placenta and premature birth, while some metals such as copper (Cu), zinc (Zn) and iron (Fe) are essential nutrients required for normal pregnancy and intrauterine growth but Pb and Cd are highly toxic at low concentration, could affect fetal growth and gestational age. Excess or deficiency of crucial metals during pregnancy is closely related to mortality and morbidity in the new born, pre-term and chronic metabolic disturbances.[7]

On the other hand, during pregnancy placenta is mostly mitochondria rich. This condition favours oxidative stress, which may also be enhanced by the addition to enzymatic activity and toxicity of metals capable of generate reactive free radicals. These free radicals interact with nuclear proteins, causing oxidative deterioration of lipid bilayer. As mentioned previously, oxidative stress has been associated with early deliveries as well as preeclampsia and trigger reduction of placental blood flow, which may even lead to fetal death.[8] Formation of Free radical increases during normal pregnancy, since increased cell turn-over or decreased antioxidants defence mechanisms; but source of reactive free radical in pre-term is not fully understood.[9] Excessive amount of ROS production during pregnancy, however, may overpower the antioxidant defence system. Deficiencies of specific antioxidant activities associated with the micronutrients copper and zinc. Results regarding decreased antioxidants defence activities implicated with poor pregnancy outcomes and the associated increased risk of diseases in adulthood, including cardiovascular disease and type 2-diabetes. This unwanted condition may be a consequence of an imbalance between toxic and essential metals in favour of the former during normal pregnancy. The effect of placental oxidative stress is controlled by the interception, modification and destruction of ROS through the action of endogenous antioxidants proteins including glutathione peroxidise enzymes and copper/zinc-dependent superoxide dismutase (Cu/Zn- SOD). Antioxidants require Zn and Cu as cofactors enzymes and protect the fetus. Although reduced activities of some antioxidants enzymes are associated with increased ROS, may be implicated with pre-term deliveries.[10] In view of these observations, we therefore, designed this present case-control study to evaluate the association between level of metals (Cd, Pb, Zn, Cu and Fe) exposure, with oxidative stress/antioxidant in the females who delivered pre-term or full-term babies, residing in Agra city area northern part of India.

MATERIALS AND METHODS

Present study is based on the results from 80 pregnant women (30 pregnant women who had gestational age < 37 weeks and 50 pregnant women with > 37 weeks) whose placenta were analyzed for metal concentrations with oxidative stress in terms of lipid-peroxidation and glutathione. These pregnant women were selected from the Department of Gynaecology S.N. Medical College, Agra. Total 180 participants were enrolled in this study, out of them 80 women were excluded due to incomplete data, misplaced samples and questionnaires, 20 participants had changed hospital for delivery therefore could not collect samples. Placental tissues were taken from healthy pregnant women, after 28 weeks of gestational age after delivery. We took trophoblastic part of placenta tissue without sign of calcification, avoiding the decidua basalis and chorionic plate in accordance with recent study.[11] Placental tissues were collected during parturition. Approximately 25 gm of placental tissue taken from each subject were collected in wide-mouthed containers as coded samples; in an ice container for metals analysis and other biochemical assays. According to declaration of Helsinki all the pregnant women had given informed written consent and were personal interviewed after delivery as ascertain maternal age, weight and height, parity, residence area, source of drinking water, smoking habits, dietary, addiction habit, literacy, duration of lactation, used fuel for cooking, haemoglobin and reproductive history. In each case, data about maternal status and outcomes of birth were provided by gynaecology department of S. N Medical College Agra, India. The study sample population was rather homogeneous in terms of the socioeconomic status, age and nutrition habits.

A portion of placental tissues was used for preparation of homogenate (10%, w/v). Briefly, all placental tissues were washed carefully three times with ice cold physiological saline water, dried on filter paper, and homogenized in phosphate buffer saline (PBS) using homogenizer. An aliquot of homogenate used for determination of malondialdehyde (MDA) last product of lipid peroxidation by method of Ohkawa et al., (1979)[12] and spectrophotometrically evaluated at 412 nm. Remaining portion of homogenate was centrifuged for 30 min at 4000 rpm and supernatant (placental extract) was collected for determination of glutathione (GSH) by using Ellman (1959) method[13] and the absorbance finally measured at 530 nm. Remaining placentas were frozen at -4°C immediately until selective metals analysis. Special care taken in maintaining the ice-cold chain from the site of collection to the site of processing and all the biochemical parameters were assayed on the same day. For extraction of metals, 1.0 gm of placental tissue in a 50 ml Erlenmeyer flask was digested at 120ºC-150ºC with concentrated nitric acid till a clear solution was obtained. This solution was quantitatively transferred to a 10 ml volumetric flask and made up to volume with deionized water. A blank sample was also prepared with each set of samples in order to control possible metal contamination by external sources. Additionally, a quality check sample was always run with each set of samples for lead analysis to maintain accuracy.[14] Placental level of metals (Pb, Cd, Fe, Zn and Cu) were analysed by ICP-MS (Inductive Coupled Plasma- Mass Spectrophotometer) (Element XR, thermo Fisher Scientific, Germany).

Data were analysed statistically using IBM-SPSS (version-22) and the linear regression was used to determine the strength of relationship between placental metal concentrations with oxidative parameters. We used Chi square and Students- t-test for the association between categorical and continuous variables, respectively (Table 2). A p- value less than 0.05 was accepted as statistically significant. Results were reported as mean ± SD.

RESULTS

The characteristics (such as age, weight, BMI, drinking water supply and area of residence) of females with full-term or pre-term deliveries that were recorded in this study are shown in Table 1. No statistically significant differences were observed in maternal age, weights, height, number of children, duration of lactation and BMI (Body mass index) between two groups. However, there was significant difference in blood haemoglobin ($p < 0.001$). Concentrations of metals (Cd, Pb, Fe, Cu and Zn) in placental tissue with full-term and pre-term cases are shown in Table 2. The level of GSH ($p< 0.05$) was found significantly increased in full-term groups ($p< 0.05$) when compared to pre-term group, whereas, placental MDA level was significantly higher in pre-term group when compared with full-term group ($p< 0.001$) (Figure 1 a and b). Pb and Cd levels were higher in pre-term group while Fe, Zn and Cu levels were higher in the full-term group but difference were not found statistically significant in Fe, Zn and Cu, only Pb and Cd were found significantly higher in preterm cases. Figure 2, and 3 depicts the strength of relationship between MDA and placental Cd ($R^2 = 0.05$, $p<0.05$) and Pb ($R^2 = 0.05$, $p<0.05$). There was signsificantly negative correlation of placental Pb levels with GSH ($R^2 = 0.05$, $p<0.05$) (Figure 4).

Table 1: Demographic characteristics of the females with pre-term and full-term deliveries

Variables	Pre-term (n=30)	Full-term (n=50)
Age of mother (years)	24.63±3.10	25.54±3.87
Height (cm)	154.32±3.66	155.11±5.30
Weight (kg)	56.95±3.01	58.78±7.14
BMI (kg/m²)	23.96±2.96	24.50±2.71
No. of children	1.76±0.95	1.44±0.75
Haemoglobin (g/dl)*	8.15±1.10	9.88±0.866
Duration of lactation (months)	15.1±8.76	11.5±8.96
Abode		
Urban	36.66% (11)	62% (31)
Rural	63.33% (19)	38%(19)
Addiction (Smoking habit)		
Yes	56.66% (17)	52% (26)
No	43.33% (13)	48% (24)
Dietary habit		
Vegetarian	73.33% (22)	62% (31)
Nonvegetarian	26.66% (8)	38% (19)
Source of drinking water		
Private	66.66% (20)	56% (28)
Government	33.33% (10)	44% (22)
Literacy		
Below 10th	60% (18)	60% (30)
Above 10th	40% (12)	40% (20)
Used fuel for cooking		
LPG	36.66% (11)	70% (35)
Biomass fuel	63.33% (19)	30% (15)

Values represent mean±SD, No. of subjects %, $P<0.05$*

Table 2: Concentration of metals in the Placenta of the females from pre-term and full-term deliveries

Metals concentration	Pre-term (n=30)	Full-term (n=50)
Pb*	3.86±1.08	2.00±2.05
Cd*	0.626±0.729	0.187±0.264
Fe	50.60±41.52	58.94±38.42
Cu	0.220±0.116	0.255±0.430
Zn	17.26±13.68	18.28±14.36

Results are in μg/dl, $P<0.05$*

DISCUSSION

Pre-term birth is complex and unresolved public health problem but cause of these problems is surely complex and unknown. There are number of factors responsible for preterm delivery, but it now appears that oxidative stress in trophoblastic placental tissue may play a crucial role. The generation of free radical in the human placenta can occur as in other tissues.[15] Previous studies have demonstrated an increase in MDA and decrease in GSH in placental tissue lead to preterm.[9] Our data shows, similar results in pre-term groups, MDA level was significantly higher ($p<0.05$) while GSH level was significantly lower ($p<0.05$) in placental tissue of women with the pre-term delivery compared to women with the full-term delivery (Figure 1 a). Increased MDA and decreased GSH level in pre-term than full-term cases indicate diminished ability to resist oxidative damage and associated with an imbalance between oxidative parameters;[16] Increased MDA is indirectly proportional to gestational age. Decreased level of GSH in IUGR (intra uterine growth retardation) and preterm placentas in comparison with the control group has been determined[17] similar to our study. Level of serum MDA was significantly higher in preterm deliveries as compare to full term deliveries in both maternal and cord

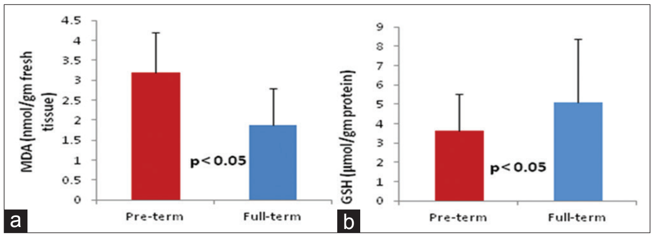

Figure 1: (a) Comparsion of levels of MDA in pre-term and full-term delivery cases. (b) Comparsion of levels of GSH in pre-term and full-term delivery cases

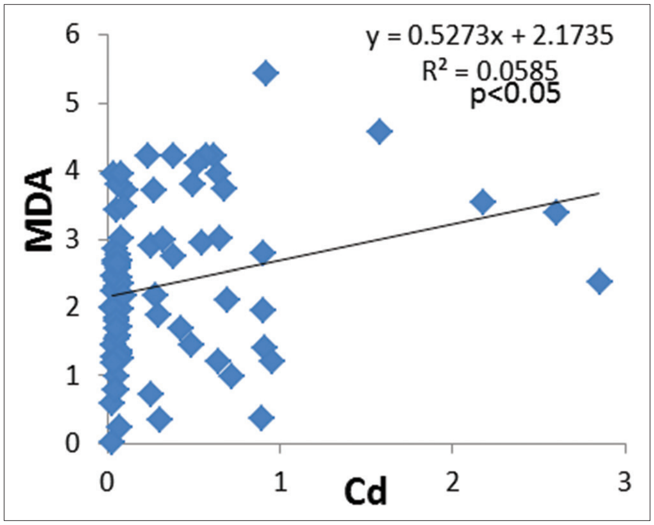

Figure 2: Placental MDA level plotted against placental Cd

blood was also reported in earlier study.[18]

Our findings are consistent with previous studies suggesting that MDA is an important factor in the pre-term delivery. The significant positive correlation in placental Cd and Pb with MDA (Figure 2 and 3) suggests that Pb and Cd may be associated with increased formation of ROS and thus produce oxidative stress in pregnant women. Cd and Pb are a strong inducer of oxidative stress in the placenta tissue, mainly through inhibition of antioxidant enzymes such as glutathione (GSH) has been reported by many researchers.[19, 20] In the present study, placental Cd and Pb levels were significantly higher in women with preterm deliveries as compared to the full-term delivery, which is supported by previous study, shown significantly higher placental Pb level in the cases of preterm delivery and premature rupture of membranes than those of the full-term.[21] Levels of oxidative stress parameters were significantly higher in women with preterm babies than full-term babies have been determined,[22] while role of Cd is not clear in oxidative stress. However, indirectly, Cd induces oxidative stress by a displacement of redox-active metals, inhibition of anti-oxidant enzymes and resulting in mitochondrial damage.[23] In our study, concentration of Cd found 1.5 times elevated in rural areas women when compared to urban areas women is agreement of previous study, has found higher level of Cd in placental tissue of rural areas women.[24] Level of placental Cd was found 3.4 times higher in women who used biomass fuel for cooking than women who used LPG (Liquid Petroleum Gas) for cooking purpose. Biomass fuel combustion are sources of metals such as Cd and Pd in indoor has been reported in many studies and especially women are exposed to these metals because they spend on the average 5-6 hours per day near stoves in the kitchen.[25]

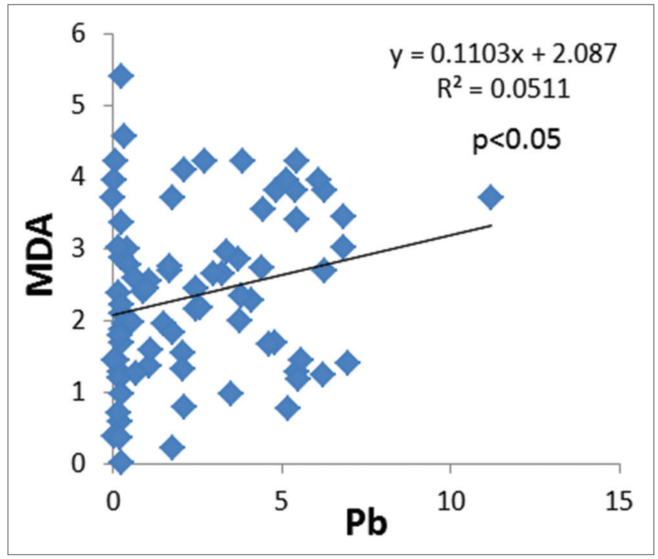

Figure 3: Placental MDA level plotted against placental Pb

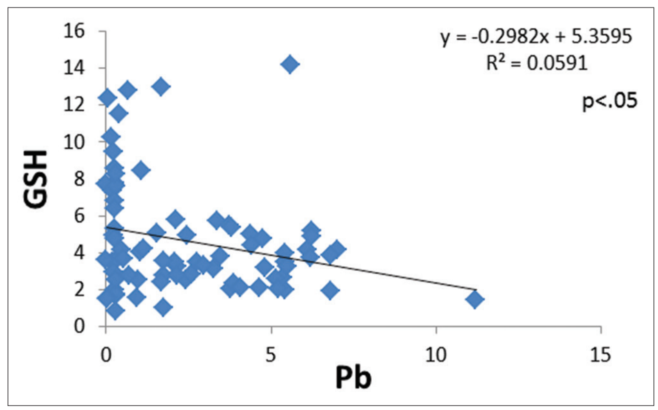

Figure 4: Placental GSH level plotted against placental Pb

Moreover, in the present investigation, placental Cd and Pb had significant positive correlations with MDA provoked oxidative stress and suggests increased oxidative stress leading to increased rate of lipid peroxidation in preterm deliveries which may be contributed in prematurity and its complications, whereas negative correlation between placental Pb and GSH (Figure 4) indicating lead-induced disruption of oxidant/antioxidant balance in the placenta of women having preterm deliveries. These data are similar to the only published Indian study which shows that Pb induces oxidative stress among pre-term cases, residing in around Lucknow.[26]

The effects of reactive oxygen species (ROS) are maintained by antioxidants such as GSH, mechanisms against effects of oxidative stress are constituted by Cu/Zn-dependent enzymes, which protect the placenta from any undue harm. Serum or placental Zn concentrations have been reported to be low in early pregnancy women[27] and was similar in the present study. Maternal zinc deficiency can lead to an adverse pregnancy outcome, such as intrauterine growth retardation due to reduced estrogen and zinc binding protein levels.[28] According to earlier study placental metallothionein like GSH might play a protective role against cadmium toxicity by its binding to the metals.[23] The level of placental Zn and Cu were found higher in full-term groups than pre-term groups. Cu and Zn are main cofactor enzyme of GSH, thus increased level of GSH was found in full-term delivery compared to pre–term delivery (Figure 1 b) indicate oxidative stress inhibited by GSH, whereas the difference between Zn and Cu with GSH were not found statistically significant. Higher concentration of Zn and Cu in placental tissue of women with full-term deliveries may be shielding against oxidative stress in full- term, thus making the, the antioxidant defence system indirectly proportional to oxidative stress as observed in the present study.

About placental haemoglobin concentration, the level of haemoglobin was lower in pre-term groups than full-term groups. The difference between blood haemoglobin was found statistically significant ($p < 0.001$) in pre-term delivery. Its fact, Fe is an essential constituent of heme in blood, when Fe levels are low, as reported in our study, it may lead to decreased haemoglobin synthesis and statistically significant of blood haemoglobin may be implicated in early pregnancy. A possible explanation for the reduced content of Fe in the placenta of the women with pre-term delivery may be related to elevated levels of Cd and Pb found in the placenta of the women with pre-term in our study. This condition may the disruption of placenta Fe homeostasis and lead to premature birth.[29] A possible association between iron deficiency and excess with free radical mitochondrial damage, results oxidative stress is agreement with previous report.[30] An earlier study reported that the increased Pb level affects the essential metal level and its level increased with parity (number of children) whereas, there was a depletion of maternal stores of essential elements (Zn, Cu and Fe) with increasing parity.[14] Deficiency in level of Zn has been identified as a risk factor for maternal anemia has been determined.[31] In our study, level of Zn was found lower in preterm delivery may be concerned in anemia. According to a previous study, in maternal anemia, placental copper is not a forecaster, but significant copper deficiency has been observed in pregnant women with anemia. Deficiency of iron during pregnancy conducing to anemia and about 90% of anemia cases have reported in India that can have deleterious effects on mother and as well as fetus in the form of poor weight gain and preterm delivery.[32] Given that a low status of essential metals may raise the hazardous effect of lead and cadmium in placenta could be one of primary risk factor of preterm delivery.[33] In our study, diminished level of essential metals (Zn, Cu, Fe) and increased concentration of toxic metals (Pb, Cd) in study group may be associated with modulate the redox status in placental tissue. As expected, an interrelationship between increased levels of toxic metals, MDA and decreased essential metals, GSH levels in placenta, which may be coupled to preterm delivery.

CONCLUSION

The results of this study revealed that disturbance in levels of essential/toxic metals is related to unnecessary elevated level of oxidative stress and diminished the protective mechanisms such as GSH could be part of the pre-term deliveries. Significantly elevated levels of Pb and Cd were significantly correlated with MDA, suggests toxic metals-induced pre-term delivery in women of the present study through the altered the oxidant/antioxidants status in placental tissue. Future research should be directed at confirming the free radical mechanism of metals- induced pre-term delivery as observed in present study and needed to develop strategies to prevent the increased levels of toxic metals in pregnant women.

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Authors Contribution:
LS - Concept and design of the study, statistically analyzed and interpreted data, reviewed the literature, manuscript preparation and critical revision of the manuscript; **PA - C**ollected data and samples from hospital, helped in laboratory analysis of samples; **MA -** Conceptualized study, helped in preparing first draft of manuscript with statistically analysis and critical revision of the manuscript; **AT -** Concept of study, and critical revision of the manuscript.

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