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Oxidative stress during early pregnancy and birth outcomes

Lisha Shastri[1], Rajkumar S Pammal[2], Indu Mani[3], Tinku Thomas[4] and Anura V Kurpad[3,5,*]
[1]St. John's Medical College, Bangalore, India: [2]Harvard College, Cambridge, MA, USA: [3]Division of Nutrition, St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore, India: [4]Division of Epidemiology and Biostatistics, St. John's Research Institute, St. John's National Academy of Health Sciences, Bangalore, India: [5]Department of Physiology, St. John's Medical College and Research Institute, Koramangala, Bangalore 560034, India

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Abstract

Objective: Routine high-dose Fe supplementation in non-anaemic pregnant women may induce oxidative stress and eventually affect birth outcomes. The aim of the present study was to measure oxidative stress markers in pregnant women with low/normal and high Hb values in trimester 1 (Hb_1) and to relate these to birth weight.
Design: A cross-sectional study where selected oxidative stress markers were analysed in both maternal (trimester 1; T1) and cord blood samples and correlated with birth weight.
Setting: A tertiary hospital in urban South India.
Subjects: One hundred women were chosen based on their Hb_1 values (forty women with low/normal Hb_1 (<110 g/l) and sixty women with high Hb_1 (≥120 g/l)).
Results: In T1, women with high Hb_1 values were found to have lower paraoxonase-1 (PON-1) activity (424·7 (SD 163·7) *v.* 532·9 (SD 144·7) pmol *p*-nitrophenol formed/min per ml plasma, $P=0{\cdot}002$) and higher lipid peroxides compared with women with low/normal Hb_1. Routine supplementation of Fe to these women resulted in persistent lower PON-1 activity in cord blood ($P=0{\cdot}02$) and directionally lower ($P=0{\cdot}142$) birth weights. Furthermore, women with high Hb_1 who delivered low-birth-weight babies were observed to have lowest PON-1 activity in T1. No changes were observed in other markers (myeloperoxidase activity and total antioxidant levels).
Conclusions: Routine Fe supplementation in pregnant women with high Hb_1 associated with increased oxidative stress, as reflected by low PON-1 activity in T1, could potentially lead to deleterious effects on birth weight.

Keywords
Oxidative stress
Hb
Trimester 1
Birth outcomes
Low birth weight

Fe deficiency is the most common nutritional disorder in the world and is often the cause of anaemia, a frequently encountered haematological problem in pregnancy care. Currently, the national policy in India is to provide universal supplementation equivalent to 100 mg elemental Fe/d to all pregnant women, regardless of their initial Fe/Hb status[1].

A number of recent studies suggest that Fe provided in excess may induce oxidative stress through the production of reactive oxygen species. Supplementation of 100 mg Fe/d to mildly anaemic women resulted in an increase in oxidative stress[2], while a dose of 50 mg/d given in the second trimester to pregnant women with high Hb (≥135 g/l) resulted in a higher number of babies born small for gestational age and a higher incidence of hypertensive disorders[3]. Intra-uterine oxidative stress is associated with adverse birth outcomes such as intra-uterine growth restriction, pre-eclampsia and an elevated risk of premature rupture of membranes[4], as well as an elevated risk of metabolic syndrome in later life[5].

An earlier Indian study showed that women with high Hb values in trimester 1 (T1), who consumed the recommended dose of supplemental Fe during pregnancy, had a higher risk of delivering a term low-birth-weight (LBW) baby[6].

The standard marker of oxidative stress is the level of lipid peroxides (LPO) formed from unsaturated lipids, mainly from oxidized LDL. One of the key enzymes that protects LDL from oxidation is paraoxonase-1 (PON-1). Studies have shown that decreased serum PON-1 and increased LPO may be indices of oxidative stress leading to early pathogenesis of atherosclerotic heart disease in pregnant women[7]. Another enzyme that is involved in the oxidative modification of lipoproteins, as well as in inflammation, is myeloperoxidase (MPO). Increased MPO activity has been reported in pregnant women with pre-eclampsia and intra-uterine growth restriction[8].

The assessment of oxidative stress in the current study was therefore based on measurement of the enzymatic

*Corresponding author: Email a.kurpad@sjri.res.in

activities of PON-1, LPO, MPO and total antioxidants (ToAx). The purpose of the current study was to assess if Hb status in T1 (Hb_1) was associated with oxidative stress and if this could be related to birth outcomes.

Participants and methods

The current study was part of a prospective observational cohort of pregnant women conducted at St. John's Research Institute and St. John's Medical College Hospital in Bangalore, India. The details of the cohort have been described previously[6,9]. Briefly, the cohort consisted of 1838 pregnant women in the age group 17–40 years, who had no known morbidities and who delivered live babies at St. John's Medical College Hospital. All women of this cohort received routine Fe supplementation of 45 mg/d, although only 642 women could be considered anaemic in T1 ($Hb_1 < 110$ g/l). From this cohort, 100 participants were chosen based on their Hb_1 values: forty with $Hb_1 < 110$ g/l and sixty with $Hb_1 \geq 120$ g/l. All participants were supplemented with 45 mg elemental Fe/d in trimesters 2 and 3[6]. The study by Genc *et al.*[10] reported a difference of 40 U/ml in PON-1 between pre-eclampsia and normal pregnancy. To observe this difference between the low/normal and high Hb_1 groups, with 5% level of significance and 80% power, the sample size required was forty-five per group. We could get valid data in only forty low/normal Hb_1 women. To improve the power, we increased the number in the high Hb_1 group to sixty.

Blood sample collection and analysis

Venous whole blood samples were collected into EDTA-coated anticoagulant tubes (Becton Dickenson, NJ, USA) by trained laboratory assistants at T1 (11·9 (SD 2·3) weeks of gestation) and cord blood (CB) samples were collected at birth. Hb concentration was analysed using an automated cyanmethaemoglobin technique (ABX Pentra 60 C+ haematology analyser; Horiba ABX Diagnostics, Germany). The measuring range was 80–180 g/l with a within-run precision of >99%.

Analysis of oxidative stress parameters

The arylesterase activity of PON-1 was assayed using *p*-nitrophenyl acetate as the substrate as described by Burlina *et al.*[11]. Briefly, the rate of formation of *p*-nitrophenol was measured at 405 nm and arylesterase activity was expressed as pmol *p*-nitrophenol generated/min per ml plasma. MPO was measured as the rate of oxidation of *o*-dianisidine in the presence of H_2O_2 at 450 nm[12]. Enzyme activity was expressed as U/min per ml plasma (1 U = 1 OD change at 450 nm). LPO were measured as mmol thiobarbituric acid-reactive species formed on reaction of plasma with malondialdehyde. Briefly, plasma proteins were first precipitated with 15% (w/v) TCA and the supernatant was reacted with 20-mM thiobarbituric acid in the presence of 10% (w/v) SDS. The thiobarbituric acid-reactive species formed were measured in a fluorescence spectrophotometer with $\lambda_{ex} = 520$ nm and $\lambda_{em} = 570$ nm, and data were expressed as nmol malondialdehyde/ml plasma. ToAx were measured using the TEAC method (Trolox equivalent antioxidant capacity) based on the suppression of absorbance of the radical cation of 2,2′-azinobis(3-ethyl benzothiazoline sulfonate) by plasma antioxidants[13] and were expressed as nmol Trolox equivalent/ml plasma.

Delivery and birth information

Infants were weighed to the nearest 10 g on an electronic weighing scale (Salter Housewares 914 Electronic Baby and Toddler Scale, NY, USA) immediately after birth. LBW was defined as birth weight less than 2500 g irrespective of gestational age.

Statistical analysis

The comparison of the oxidative stress markers among the different groups of Hb_1 was performed using the independent-sample *t* test or the Mann–Whitney *U* test if not normally distributed. The data were also analysed based on dividing the participants into two groups based on birth outcome (LBW and others). Normally distributed data are presented as mean and standard deviation and the rest as median (quartile 1–quartile 3). For all analyses, two-sided *P* values <0·05 were considered statistically significant. The oxidative stress markers were compared between four combined groups of low/normal and high Hb_1 and low and normal birth weight using one-way ANOVA. All statistical analyses were performed using the statistical software package IBM SPSS Statistics for Windows, Version 18.0.

Results

The general characteristics and birth details of the women participating in the study (total, low/normal Hb_1 and high Hb_1) are summarized in Table 1. There were no differences in characteristics between the two groups except their Hb_1 values. Mean compliance to Fe supplementation was about 70% among all women. Among the women who had low/normal Hb_1, 25% of babies were born LBW, whereas 38% of women with high Hb_1 gave birth to LBW babies.

Table 2 describes the results of oxidative stress parameters in women with low/normal *v.* high Hb_1. PON-1 showed a significantly lower activity in T1 in the high Hb_1 group (424·7 (SD 163·7) pmol *p*-nitrophenol formed/min per ml plasma) compared with the low/normal Hb_1 group (532·9 (SD 144·7) pmol *p*-nitrophenol formed/min per ml plasma, $P = 0{\cdot}002$). The same trend held for PON-1 in CB samples ($P = 0{\cdot}020$). The high Hb_1

Table 1 General characteristics of the study population (*n* 100): pregnant women attending a tertiary hospital in urban South India

	All		$Hb_1 < 110$ g/l		$Hb_1 \geq 120$ g/l		
	Mean	SD	Mean	SD	Mean	SD	*P* value*
Age (years)	23·9	3·2	23·3	2·9	24·4	3·3	0·076
Height (cm)	155·6	5·4	156·0	5·3	155·3	5·6	0·555
Weight (kg)	51·5	8·9	49·8	9·1	52·6	8·6	0·118
Final gestational age (weeks)	38·6	1·4	38·7	1·4	38·5	1·4	0·361
Birth weight (g)	2771	449	2836	482	2728	424	0·240
Hb_1 (g/l)	119	18	98	7	133	6	<0·001
Total supplemental Fe intake (mg)	7329	736	7392	674	7288	777	0·504
LBW (*n*)	33		10		23		0·165
%	33		25		38		

Hb_1, Hb in trimester 1; LBW, low birth weight.
*Calculated by the independent-sample *t* test for continuous data and by the χ^2 test for categorical data. $P < 0{\cdot}05$ was considered to be statistically significant.

Table 2 Comparison of oxidative stress markers within groups according to low/normal and high Hb in the first trimester, among pregnant women attending a tertiary hospital in urban South India

	$Hb_1 < 110$ g/l		$Hb_1 \geq 120$ g/l		
	Mean or median	SD or IQR	Mean or median	SD or IQR	*P* value*
PON-1					
T1	532·9	144·7	424·7	163·7	0·002
CB	361·5	138·2	302·0	101·6	0·020
MPO†					
T1	3·02	1·58–6·23	4·12	1·68–5·94	0·623
CB	4·12	0·98–7·80	2·35	0·74–5·36	0·525
LPO†					
T1	0·31	0·09–2·33	1·20	0·25–2·00	0·046
CB	0·42	0·07–0·79	0·37	0·23–0·65	0·981
ToAx†					
T1	1·53	1·30–2·44	1·47	1·16–2·44	0·918
CB	1·59	1·05–2·50	1·34	1·09–2·55	0·916

Hb_1, Hb in T1; IQR, interquartile range (quartile 1–quartile 3); PON-1, paraoxonase-1; T1, trimester 1; CB, cord blood; MPO, myeloperoxidase; LPO, lipid peroxides; ToAx, total antioxidants.
PON-1 expressed as pmol *p*-nitrophenol formed/min per ml plasma; MPO expressed as U/min per ml plasma; LPO expressed as nmol malondialdehyde/ml plasma; ToAx expressed as nmol Trolox equivalent/ml plasma.
**P* value calculated from the independent-samples *t* test. $P < 0{\cdot}05$ was considered to be statistically significant.
†Median and IQR with *P* value calculated from the Mann–Whitney *U* test. $P < 0{\cdot}05$ was considered to be statistically significant.

group also had a higher mean LPO level in T1 ($P = 0{\cdot}046$) but not in CB. Although not statistically significant, women with high Hb_1 had babies with a mean birth weight that was 108 g less than women with low/normal Hb_1 ($P = 0{\cdot}240$). No differences were seen in MPO activity or ToAx levels between the groups.

The participants were further sorted into two additional groups based on birth weight: Group 1, low/normal Hb_1 and normal birth weight (NBW); Group 2, low/normal Hb_1 and LBW; Group 3, high Hb_1 and NBW; and Group 4, high Hb_1 and LBW. Among these groups, both T1 and CB PON-1 were lower in Group 4 compared with the control Group 1 (Fig. 1), although only the T1 values were statistically significant.

Subsequently, these parameters were also analysed based on a birth weight grouping (Table 3). Women who delivered LBW babies had a significantly lower T1 PON-1 activity compared with those who delivered babies with NBW. The LBW group also had a higher mean LPO level in T1, although this was not significant. No differences were seen in any of the parameters in the CB samples.

Discussion

Fe-deficiency anaemia is among the commonest problems encountered during pregnancy, especially in developing countries such as India. A routine supplementation of Fe and folic acid during the second and third trimesters of pregnancy is believed to be essential for the benefit of both the fetus and the mother. However, an increasing number of studies especially from developing countries suggest that high levels of Fe can cause undesirable secondary effects, including gestational diabetes mellitus and pre-eclampsia[5]. Epidemiological studies also show negative associations between birth outcomes and both low and high Hb levels during pregnancy[14,15]. However, in India, the current national guideline prescribes

a universal dosage of 100 mg elemental Fe/d during pregnancy[1], despite the possibility of high Fe supplementation leading to adverse outcomes[16,17], especially in women who start out the pregnancy with high Hb[6].

In the current study, all women were supplemented with 45 mg elemental Fe/d during their second and third trimesters of pregnancy, irrespective of their initial Hb status[6]. We have earlier reported that even this dose of Fe supplementation in pregnant women with a high Hb_1 has an adverse impact on birth outcome[6]. Similar results have been reported with 50 mg elemental Fe provided daily for 8 weeks during pregnancy[18]. Supplementing Fe to healthy, non-anaemic pregnant women has been reported to increase oxidative stress, resulting in deleterious effects on lipid peroxidation[19,20] as well as protein oxidation[18]. Some of these effects have been shown to be ameliorated by the concomitant consumption of antioxidants such as vitamin C[19]. In the current study, effects on both lipid peroxidation as well as protein oxidation (using PON-1 activity as a surrogate marker[21]) were investigated. It was seen that women who started their pregnancy with a high Hb_1 actually had a higher level of oxidative stress as evidenced by higher levels of LPO and lower PON-1 activity in T1 (Table 2).

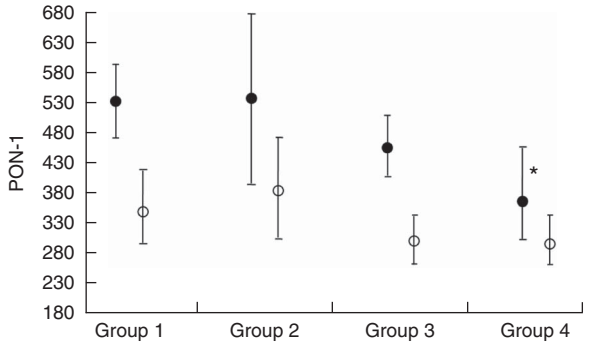

Fig. 1 Mean paraoxonase-1 (PON-1) in the trimester 1 (T1) blood of mothers (●) and the cord blood of babies (○) expressed as pmol *p*-nitrophenol formed/min per ml plasma. Values are means with their 95 % confidence limits represented by vertical lines. *$P = 0{\cdot}004$ when Group 4 of T1 PON-1 is compared with Group 1. All groups were given the routine iron supplementation (Group 1, low/normal Hb_1 and normal birth weight; Group 2, low/normal Hb_1 and low birth weight; Group 3, high Hb_1 and normal birth weight; Group 4, high Hb_1 and low birth weight; Hb_1, Hb in T1)

Furthermore, Fig. 1 suggests that women with high Hb_1, who eventually delivered babies that were LBW, had further lower PON-1 activity in T1 compared with those who had low/normal Hb_1. Considering the fact that all women had similar Fe supplementation during their pregnancy, irrespective of Hb_1 values, these data suggest that women with high Hb_1 associated with low PON-1 activity may be more susceptible to the possible deleterious effects of this routine Fe supplement. On the other hand, it is important to emphasize that anaemic women appear to have benefited by the Fe supplementation, as evidenced by the higher PON-1 activity in CB as well as NBW.

PON-1 is an important anti-oxidative enzyme associated with HDL which prevents the oxidation of LDL and reduces the formation of atheromatous plaques in the blood vessels. It therefore has a protective role against cellular damage from oxidative stress[22] and low serum PON-1 activity is associated with increased risk of atherosclerosis[7,23] as well as adverse birth outcomes[10,21].

The activity of PON-1 in T1 was significantly lower among women who delivered LBW babies (Table 3).

Table 3 Comparison of oxidative stress markers within groups according to birth weight, among pregnant women attending a tertiary hospital in urban South India

	NBW		LBW		
	Mean or median	SD or IQR	Mean or median	SD or IQR	*P* value*
Hb_1 (g/l)	118	18	121	18	0·373
Birth weight (g)	3018	309	2269	197	<0·001
PON-1					
T1	487·5	157·9	406·3	169·5	0·031
CB	323·4	123·7	327·2	113·5	0·884
MPO†					
T1	4·05	1·55–5·94	3·43	1·84–6·78	0·640
CB	2·51	0·77–6·87	2·62	0·64–5·07	0·400
LPO†					
T1	0·51	0·12–1·99	1·53	0·73–2·53	0·095
CB	0·39	0·19–0·60	0·45	0·20–0·88	0·442
ToAx†					
T1	1·46	1·21–2·43	2·05	1·16–2·48	0·887
CB	1·36	1·09–2·53	1·97	1·08–2·57	0·847

NBW, normal birth weight; LBW, low birth weight; IQR, interquartile range (quartile 1–quartile 3); Hb_1, Hb in T1; PON-1, paraoxonase-1; T1, trimester 1; CB, cord blood; MPO, myeloperoxidase; LPO, lipid peroxides; ToAx, total antioxidants.
PON-1 expressed as pmol *p*-nitrophenol formed/min per ml plasma; MPO expressed as U/min per ml plasma; LPO expressed as nmol malondialdehyde/ml plasma; ToAx expressed as nmol Trolox equivalent/ml plasma.
**P* value calculated from the independent-sample *t* test. $P < 0{\cdot}05$ was considered to be statistically significant.
†Median and IQR with *P* value calculated from the Mann–Whitney *U* test. $P < 0{\cdot}05$ was considered to be statistically significant.

One explanation for this is that PON-1 is itself very susceptible to oxidative stress and can be inactivated by free-radical-induced oxidation[24]. PON-1 activity has been reported to decrease as pregnancy progresses, as it is consumed when stabilizing the oxidative stress levels in the blood[24]. In addition to the effects of haemodilution, it has been postulated that the lower levels could be a result of decreased protein synthesis due to cellular injury, which in turn decreases the enzymatic turnover, and thus contributes to the decrease in serum levels of the enzyme[23]. Recent studies have shown that LBW babies have low PON-1 levels in CB[23]; however, we did not observe such an effect. This might be due to the fact that the levels of LPO, which are the key influencers of PON-1 activity, were unaffected in the CB of the LBW group.

Studies have shown that increased protein oxidation markers and lowered PON-1 activity in early pregnancy could predict clinical complications like pre-eclampsia and gestational diabetes mellitus[10,21]. In combination with the current finding of higher oxidative stress and lower PON-1 activity in T1 also being associated with high Hb_1 as well as LBW, the potential of PON-1 being used as an early marker of gestational morbidities is worth investigating.

MPO is an enzyme that is produced and released by activated neutrophils in response to an inflammatory stress[8] and plays a key role in the pathogenesis of CVD. Many of the morbidities of pregnancy such as pre-eclampsia and intra-uterine growth restriction exhibit increased systemic inflammatory responses, and it has been hypothesized that MPO plays an important role here as well[25]. Hung *et al.*[8] recently reported that higher MPO levels in maternal blood before delivery were associated with pre-eclampsia and intra-uterine growth restriction. However, in the current study no differences were seen in MPO activity. This is not surprising since the maternal blood sampling in the current study was in the first trimester, where there probably was no inflammatory stress, even though there appears to be oxidative stress.

The limitations of the present study are mainly the small sample sizes, the focus on only T1 and CB rather than all trimesters, and the lack of heterogeneity of the population studied. Although many more such studies on larger populations are required, the present study reprises the question regarding the prudency of a universal high-dose Fe supplementation in pregnant women with high Hb_1. In conclusion, the study suggests that low PON-1 activity in the first trimester of pregnancy, particularly in women with high Hb_1, could be an early indicator of oxidative stress, which could potentially have adverse effects on the fetus.

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