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REVIEW ARTICLE

ROLE OF OXIDATIVE STRESS WHILE CONTROLLING IRON DEFICIENCY ANEMIA DURING PREGNANCY - INDIAN SCENARIO

Neeta Kumar, Nomita Chandhiok, Balwan S Dhillon and Pratik Kumar *

*Division of Reproductive Health and Nutrition, Indian Council of Medical Research, *Department of Medical Physics, IRCH, All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110029*

ABSTRACT

Iron Deficiency anemia ranks 9th among 26 diseases with highest burden. Asia bears 71% of this global burden. Adverse maternal and birth outcome associated with hemoglobin status renders the issue worth attention. Indian scenario has worsened over the period despite continuous international and national efforts. This indicates some lacunae in the approach and strategies applied. Various reports state that even with maximum effort to increase outreach and monitoring for adherence to Iron schedule, consumer's compliance remains abysmally low. Recent studies has pointed out biological basis of side effects (gastrointestinal complains and systemic events) as raised oxidative stress for which iron is the key catalyst. Up till now the only target of research has been to raise hemoglobin of pregnant women above 11gm/dl. With the reports of pregnancy specific morbidities i.e. hemorrhage and septicemia with low hemoglobin, eclampsia, small for gestation age, gestational diabetes with higher ranges of hemoglobin, alarm is raised to define optimum range. Use of oxidative stress as biochemical marker with different doses and schedules has been defined because India lack information for its own population upon oxidative stress status when iron is supplemented as per current guidelines. Studies done in India and abroad have defined that too much and too less, both may raise oxidative stress and studies of this sort may provide biochemical scale for optimization. This review therefore has evaluated currently available Indian research and reports to understand the need of future research area. Important findings from other countries have been incorporated for comparison.

KEY WORDS

Oxidative stress, Iron supplementation, Anemia during pregnancy.

Iron balance is critical to maintain normal erythropoiesis (1 ,2). Optimum balance is highly needed for growing children and pregnant women. Daily requirement for iron is 6 times greater for women in the last trimester of pregnancy than for non pregnant women. Currently 100mg elemental iron for 100 days is given during pregnancy in India (3). However the mode / strategies of supplementation have come under scrutiny. Investigations world wide have recently begun to measure the effect of ferrous iron (4) supplementation on indices of Oxidative Stress (OS) in pregnant women. A fine line between deficiency and overdoses in case of iron (or any micronutrient supplementation for that matter) is evident in literature and an utmost care is required while deciding doses and schedules (5 , 20). However during pregnancy these effects are not well studied. Efforts have begun to combat this situation without compromising beneficial effects of iron. Considering the age old problem of anemia in India and massive efforts going in vain this review applies extensive literature search by using key words Iron, pregnancy, oxidative stress, and anemia. Cross references and personal communications were collected through local and National Library to put forward a comprehensive Indian scenario in global perspective upon what happen to oxidative reactions when iron is deficient and consumed.

Address for Correspondence :

Dr. Neeta Kumar,
Scientist B, Div. of RHN,
Indian Council of Medical Research
Ansari Nagar, New Delhi-110029
E-mail: neeta@icmr.org.in, delhineeta@yahoo.co.in
Phone: 09313195247

Oxidative stress and its significance for maternal health:

Oxidative stress is defined as disturbance in the prooxidant-antioxidant balance in favour of the prooxidant, leading to potential damage producing oxidative stress. Free radical is any atom (e.g. oxygen, nitrogen) with at least one unpaired electron in the outermost shell, and is capable of independent existence. There are numerous types of free radicals that can be formed within the body, but here oxygen centered free radicals most commonly the superoxide anion (O^{2-}), the hydroxyl radical ($OH^{\cdot}$), singlet oxygen, and hydrogen peroxide (H_2O_2) are studied (6).

The interaction of copper or iron and H_2O_2 also produce OH· as first observed by Fenton. Iron has the ability to gain and lose electrons i.e. ($Fe^{2+} \leftrightarrow Fe^{3+}$) very easily. This property makes iron and copper two common catalysts of oxidation reactions. The release of iron can be detrimental to cellular membranes because of the pro-oxidation effects it may have (7, 8). Raised OS have been implicated not only for chronic diseases like cardiovascular disease, cancer, diabetes, cirrhosis, atherosclerosis, Alzheimer's disease, and Parkinson's disease, it has been found altered with pregnancy, iron deficiency and overdoses (9, 26). Importance of attaining optimum range is desirable as low hemoglobin levels as well as high hemoglobin levels have been associated with adverse events (107). An elevated Hb level may be an indicator for possible pregnancy complications associated with poor plasma volume expansion, and should not be mistaken for good iron status (10). In addition to this, speculations have been made that an uncompensated OS experienced during pregnancy could predict or affect some pathological conditions of mother. Its effect on the developing fetus cannot be excluded either (11). Oxidative stress as biochemical indicator has been found proportionate to side effects in animal studies (64) and hence may be used to optimize iron status.

Burden due to low hemoglobin: An Indian review finds 19-22% of total maternal deaths due to anemia. Deaths due to septicemia and hemorrhage may indirectly be attributable to anemia (12). Indian Council of Medical Research (ICMR) reports prevalence of anemia among women 75% (31% moderate, 3% severe) (13). ICMR study on 1,66,996 live births across all over India found 11.5% direct maternal deaths due to anemia (14). Approximately 50% of the anemia is attributable to iron deficiency.

Average estimates for all anemia attributed mortality (both direct & indirect) are 7.26 for Asia which is high when compared with other developing region like Africa (6.37%) and Latin America (3.0%). Globally 841,000 deaths and 35,057,000 disability-adjusted life years lost go in to account of anemia. Asia bears 71% of this global mortality burden and 65% of the disability-adjusted life years lost as compared with North America which bears only 1.4% (15). Recently completed National Health and Family welfare Survey (NFHS-III 2006) reported 57.9% anemia (Hb <11gm/dl) among pregnant women. It is higher than 49.7%, reported in 1999 NFHS-II (16). Several factors identified for increasing burden i.e. low bioavailability of iron in Indian diet, high phytates, low content (17), high worm load & malaria prevalence(18), poor outreach and quality of iron supplementation, non- compliance and logistics difficulties (19 , 20).

Adverse impact of low hemoglobin (<11g/dl) on maternal status have been widely proved (21 ,22). Gomber et al reported rise in baby's birth weight from 2509 to 2803g when Hb rises from 7 to 11 g/dl (23). Sharma et al reported pregnant women (n=447) with 4-6 fold higher risk of prolonged labor at Hb<8.9 g%, 4.8-fold higher risk of delivery complications with Hb≤7.5 g% and maximum birth weight at Hb 9.6-10.5 g% (24). RR 1.35 (0.92-2.00) at Hb 40-80 g/L and 3.51 (2.05-6.00) at Hb<47 g/L is reported for maternal mortality (25).

An USA based retrospective analysis upon 173,031pregnant women showed odd ratio (OR) of 1.68 for preterm birth at Hb <7g/dl (Z score <-3.0) and 0.91 at Z score - 1.1-2.0 (26). Hematocrit (Hct) from 41% to 44% had lowest risk of premature birth and double at Hct<37% (27). Decreasing the Hct by single point had 24% rise in prematurity (OR 1.24), 5-point decrease tripled risk of prematurity (OR 2.98) (9). Scholl reported preterm delivery five fold high with iron deficiency anemia. Low birth weight and low pregnancy weight gain was three fold high in his famous Camden trial (28). Similar equations are yet to be derived for Indian population for all the ranges of Hb.

Burden due to high hemoglobin: Indian prevalence of high Hb is not known. Indirect evidence (ICMR study) finds 24% prevalence of pregnancy induced hypertension in a multicenter data. Approximately 10-24% maternal deaths attributed to high Hb, that comes equivalent to maternal deaths due to anemia (11.5%) and hemorrhage (23.6%) (14). Sirinivasan (Chandigarh, India) encountered 50 cases of severe cerebrovascular thrombosis due to hemoconcentration amongst 1000 deliveries performed (29). Iron overload, plasma volume nonexpanding due to protein nutritional deficiency are some factors identified behind high Hb at term (30). Pregnancy specific morbidities like PIH, eclampsia, septic

abortion and gestational diabetes are found to be associated with high Hb at term (31 ,32 ,33). Prevalence of such events in developing countries are 10 times more than in developed countries. Plasma volume study (n= 92 Indian, 191 Fijian women) showed lower red cell volumes, blood volumes & high incidence of toxemia, lower plasma volume for a given birth weight/ maternal size ratio in Indian women (34).

Study from USA found 0.43% women with very high Hb (>14gm/l) (0.25% with very low Hb). Koller found 62% women having small for date baby at Hb >2SD (35). Lao, China reports high iron levels in the group with Gestational Diabetes Mellitus. Hb > 13 gm/dl has been taken as hemoconcentration in Cochrane meta-analysis of eight trials. Women on daily iron were three times more likely to have hemoconcentration (RR 3.01; 95% CI 1.46-6.19) (36). Steer found Hb above 105 g/l associtated to increased risk of low birth weight and preterm delivery (PTD) (37). This phenomenon is seen in all ethnic groups. Zhou et al reports RR 2.52 for PTD at Hb ≥ 130 g/l. Scanlon et al find OR: 1.79 with high Hb in small for gestational age (SGA) (10). Other studies too report similar findings (38 ,39 ,40).

Non compliance and side effects with Iron consumption: Various surveys from India found that even if logistics are taken care of, the compliance remains abysmally low. Its average is 22% and as low as 2% in some EAG states (Empowered Action Group states). This is despite increment of attendance at antenatal clinics (ANC) from 44% (NFHS-II, 1999) to 51 % in NFHS III 2006 (41 ,42). Recently launched NRHM (National Rural Health Mission-2005) has concerns that in spite of improved attendance at ANC clinics situation of iron consumption has not improved. Daily oral iron supplement has shown improvement of hematological indices but with frequent gastrointestinal (GI) symptoms. ICMR study evaluating dose effect of 60-120 mg iron supplementation in 115 participants faced 32 lost to follow-up (43). Despite constant monitoring to ensure compliance and manage the side effects 47.2% of the subjects consumed less than 90 tablets of 60 mg daily iron dose, 14% reported side effects (44). Prevalence of side effects doubled with 200 mg daily dose (44). Another study from New Delhi reported 40% compliance with daily 100 mg iron, while 85% in weekly. Majority (75%) of the non-compliance in oral iron group was due to GI side effects (45).

From outside India, RR1.13 is reported for nausea, 1.21 for stomach pain and 1.12 for hard stool at 20 mg iron daily (4). Cochrane analysis (6 trials) on women receiving daily oral iron reported more side-effects of any kind than women taking placebo or not taking any iron supplementation at all (26% versus 11.9%): (RR 1.90; 95% CI 1.09 to 1.33) (107). Vomiting in 15.7% iron users, 8.94% in nonusers, RR 1.69 (CI 95%1.15 to 2.47) has been reported. Upper tolerable dose of iron is 45mg according to CDC Atlanta.

Table 1 : Side effect profile at 60- 120 mg daily iron doses (currently in practice in Indian subcontinent)

Year	Journal/Country	Noncompliance / attrition (%)	Noncompliance due to side effects (%)	Causes for side effect and noncompliance -%
			Studies from Indian subcontinent	
1975	Q J Med (58)	30	6	Mostly due to non cooperation and refusal to draw blood
1989	GOI-Report (59)	No mention	No mention	GI side effects-1%
1991	ACC/SCN (57)	58	No mention	Lack of supply
1992	ICMR technical report (44)	47.2 (at 120 mg iron)	noncompliance due to communication skills in pursuing study subjects	GI side effects-14.2% nausea-8.8% gastritis-6.5%, Diarrhea-0.5%, Constipation- 0.8%, abdominal distention- 0.3%
1999	Natl Med J India (43)	38%		Marjory GI side effects
2000	Journal of Nutrition. (Pakistan) (60)	45%	Only one subject with minor side effect migration, change of center	No GI symptoms, withdrawal due to other personal reasons,
2002	J Health Popul Nutr.(Dhaka) (61)	18%	61.5	GI symptoms:(%): Heartburn 11.5, Nausea 14.4, Vomiting9.6, Diarrhoea 10.6, Constipation 59.6
2002	Indian Pediatr (23)	27%	No side effect reported	Not reported
2004	Am J Clin Nutr (21)	48%	21% had side effects	Dyspepsia10%,Constipation-5%, Diarrhea-3%, Vomiting-2%, Rash and itching-1%
2004	J Obstet Gynaecol Res (45)	60%	30% women with symptoms	75% of the non compliance had GI symptoms: nausea, vomiting, constipation
2007	Mescape general medicine (62)	Full compliance	78% subjects had side effects	GI intolerance 65.4%, Constipation 48%, Metallic taste 15.3%, Diarrhea 3.8%, Rashes 1.9%

Common drawback with iron studies is that criteria for lost to follow up are found to be the major bias in final analysis of many hospital based randomized case control trials. For example a widely cited study evaluated effect of iron on birth outcome started with 513 pregnant women and only 144 women's status in IIIrd trimester could be obtained (46). Most of the studies did not analyze morbidities during pregnancy in different groups. Analysis to see the effect of supplementation in different trimester is also required. Majority Indian women are approaching for health care and supervised delivery either at the time of delivery or in third trimester. They are given extensive iron therapies (if detected anemic) in third trimester or just before delivery. Benefit to maternal health and birth outcome obtained by such last moment supplementation is not documented in India (38, 47). Many reports count lost to follow up as noncompliance (48). 18-60% noncompliance is reported in an Indian review. It is varying from 9-87% from other countries. (Table 1). Significant withdrawal due to side effects that was associated with hemo-concentration has been reported from Mexico and other countries outside India (36, 49-57).

OXIDATIVE STRESS ESTIMATION DURING IRON SUPPLEMENTATION

Animal studies:

Effects at the site of absorption: Studies from National Institute of Nutrition-ICMR reports oxidative status after iron consumption. Generation of apoptosis in GI tract in addition to reduction of microvillus height, mucosal erosion in rats was seen with different schedules of iron orally as observed by transmission electron microscope. EPR spectroscopy identified production of hydroxyl and methoxyl radicals in both the luminal and mucosal contents in the GI tract of iron supplemented rats (63). Surprisingly these changes became more pronounced when iron was given to anemic rats (64, 65).

Systemic effect: Elevated free radical levels from 25 to 40%, reduction of scavenging enzymes-catalase, SOD, glutathione peroxidase and alpha tocopherol in rat liver and kidney homogenates at 20 hours reported after iron administration at dose equal the dose of human consumption (100mg daily) (14). Continuous absorption of a fraction of supplemental iron at a rate beyond normal, perhaps by passive diffusion mass effect resulted in excessive liver iron levels, particularly in the previously deficient animals (66). Genotoxic and reproductive changes have been extensively highlighted in animal studies (Table 2). Organs have been found responding differently to different dose of iron (84).

Human studies: *In India* there is no randomized controlled study of oxidative status with iron during pregnancy, except currently ongoing trial at ICMR started as task force study at two Obstetrics and Gynae centers: AIIMS, New Delhi and PGIMER, Chandigarh. Preliminary findings (n=20) show TBARS level is decreased in nonanemic pregnant women in comparison to anemic group. Oxidized glutathione peroxidase is found raised during pregnancy ($p<0.05$) when compared with non pregnant age matched controls. Effect estimation of daily vs. weekly iron supplementation on oxidative stress is ongoing under this study.

An American pilot study (n=19) on healthy pregnant women with borderline anemia with oral ferrous iron in prophylactic doses (36 mg daily) found 2 fold elevation oxidized glutathione in Fe + group ($p=0.02$) (1). Lachili et al (2001) investigated the effect of a daily iron supplementation (100 mg/d as fumarate) and vitamin C (500 mg/d as ascorbate) for the third trimester of pregnancy on lipid peroxidation (plasma TBARS) and found them enhanced in the iron supplemented group($p < 0.05$) (23). Lund et al found fecal iron raised from 60 mmol/l at base line to 300 mmol/l after iron supplementation, which returned to base line within 2 wks after stopping iron (67). This resulted in 40% increased production of free radicals at colon, which could cause mucosal cell damage or increased production of carcinogens.

Iron found enhancing free radical production by RNAs and hyperglycemia through glycation. Elevated non-transferrin-bound iron (NTBI) was detected shortly after the ingestion of iron supplements in plasma and umbilical cord blood (68). Transferrin binds the iron leaving the cell and entering the circulation, thus avoiding the entrance of free iron. However, this mechanism appears to be overwhelmed by amounts of passively diffused iron when large iron boluses are presented to the intestine, and NTBI may reach the liver, causing systemically raised OS. Not only dose and schedule, effect of timing of supplementation has been highlighted in some reports (69). Important work done in India to seek answers of the questions rose by John in the year 2000 (108) is listed in Table 2**.** All human studies from India and seeing its limited number, some foreign studies reporting salient changes of OS status are included. Indian human data show increased OS during anemia and reversal with iron supplementation while findings from other countries are contrary to that (2, 4, 8, 10, 18, 80, 81, 82). Indian animal studies are also evaluated as their findings are contrary to what is found with human studies in India. However animal studies from foreign resonate the similar findings (70-71). Majority of them found raised OS with anemia and further rising with iron doses.

CONCLUSION

Oxidative stress worsene with anemia in most of the animal and human studies (43, 26-28, 33, 63, 64, 66). Status improved with iron in some animal and human studies; however majority animal as well as studies involving pregnant women found it increased (Table 2). Investigation to check whether OS increase is such that it can alter birth outcome are required. In Indian conditions where the ANC strategies are not much supervised, there is scarcity of information upon side effects and noncompliance too. In view of major studies and meta-analysis, it is concluded that there is a need of large, good

Table 2 : All Indian and prominent foreign studies done on human participants and animals to estimate oxidative stress with different iron doses at the site of absorption, systemic and at reproductive organs

Ref.	Iron dose	Sample/site	OS parameters					
			SOD	MDA/ TABRS	GSHPx	GSH Rd	Catalase	Other
			Human Studies : India					
Acharya et al (72)	Iron not supplemented	Human RBC cell lines, anemic subjects	↑		↓		↓	
Ramachandran et al (73)	-Do-	Human RBCs in iron deficiency						
Sundaram et al (74)	60mg iron daily for one month	n = 20 anemic adults, 16 control, serum tested		↑ pre Fe, ↓ ed after Fe		↑		↑ ed fructosamine pre Fe, Post therapy ↓ed
			Human Studies : from outside India					
Lachilim et al (8)	100mg/day as fumerate + 500mg vit. C in third trimester pregnancy	n = 27 Fe + Fe, 27 No supplementation Maternal blood used for OS parameters		↑ ed 3.62±0.36 in Fe + arm 3.01±0.37 in control				α-tocoferol ↓ in Fe+ group
Rehema et al (2)	36 mg ferrous iron supplemented Fe+ Group	n=19 pregnant women with borderline anemia, 13 Fe+, 6 Fe-ve			2 fold ↓ ed in Fe+ group			Other parameters no significant change
Scholl et al (28)	N=360, 60mg daily iron Camedon's ongoing Research	n=350 Urinary excretion of 8-hydroxyguanosine (8-OH-dG)						8-OH-dG ↑ ed (2.7 fold) post iron, AOR-3.3 at Serum transferring saturation>21.7%
Isler (75)	n=8 anemic received oral iron, 18 healthy control	Whole blood sample	↑		NS			
Ferreira et al (76)	effect of iron at concentrations (0, 1, 5, 10, 50 and 100 microM Fe3+)	Human normal RBC's antioxidant estimated, RBC incubated with colloidal iron hydroxide		Minimum at 5 & max. at 100 mcm	↓ with time at 10-100 mcm		NS	
Kurtoglu et al (9)	60 mg iron given	Adult RBCs studied for OS in 63 anemic before (b) and after (a)	↓ b ↑a	↑ b ↓ a	↓ b ↑ a		↓ b ↑ a	
Sevgi et al (77)	Supplemented iron	n=30 anemic & 25 normal children			↓ b ↑ a			
Olivares et al (78)	100 mg/d Fe, as ferric polymaltose	n=12 anemic, 12 control women RBC studied						↓ CuZn-SOD activity
Binkoski et al (79)	50 mg iron gr. A, 100 mg iron gr. B.	26 non anemic women		NS				
Kumerova et al (80)	No iron	n=56 anemic, 50 healthy control adults			↓	↓		↓ G6PD in anemic

Ref.	Iron dose	Sample/site	OS parameters					
			SOD	MDA/ TABRS	GSHPx	GSH Rd	Catalase	Other
Rehman et al (81)	14 mg/day Gr A, 60mg/day-Gr B with 260mg vit C iron	n=38 healthy adults, DNA extracted from white blood cells, tested before (b) & after (a) 6 weeks of Fe consumption	↓ b ↑a	↑ b ↓ a	↓ b ↑ a		↓ b ↑ a ↓ a	↑ b Oxidative DNA damage
Yang et al (82)	14mg (Gr A), 60mg (Gr B) iron with Vit. C RDA	n=20 adults each their own control, platelet function & low density lipoprotein oxidation tested			NS with added Vit. C			↑ mean lag phase oxidation of low density lipoprotein (LDL) after iron
Troost et al (83)	Single 100mg dose	N=6, GI Lumen wash collected from double lumen perfusion tube		↑ (0.07 – to 3.35) on iron perfusion			↑ ed NPAC	Expression of 89 genes & six biological processes altered with iron
Devrim et al (84)	N=15 on 100 mg iron, 13-no iron	N=27 pregnant women maternal plasma & placenta tested		↑				
Yip et al (85)	No iron given	RBCs of iron deficient adults		↑				
Petukhov et al (86)	-do-	RBC of polycythemia subjects		↑				↓ ed selenium dependent GSHPx
Vives et al (87)	-do-	Micorcytic RBC of patients	↓	↑	↓			
Avissar et al (88)	-do-	RBCs of iron deficient adults	↓	↑	↓			
Riazantsev et al (89)	-do-	RBCs of anemic pregnant women			↑	↓		
Panchenko et al (90)	-do-	RBCs of anemic children	↓					
Cellerino et al (91)	-do-	n=9 normal, 15 anemic adults	↓	↑	↓			
Mehmet et al (92)	-do-	22 anemic and 22 healthy females	↓	↑	↓	↓		↑ lymphocyte DNA damage
Golovin and Konvai (93)	-do-	N=55 anemic adults	NS	NS	NS	NS	NS	
Krause et al (94)	-do-	n=29, Blood of anemic adults			↑			
		Animal Studies : India						
Jain et al (95)	rats fed Fe-deficient (2 ppm Fe) Fe diets	RBC membrane lipids and proteins tested		↑ ed in anemic			↑ ed NPAC in anemic	↓ ed RBC T½ in iron deficient group
Rao and Jagdeesan (96)	Fischer rats iron sufficient (C) or Fe deficient (d) diets	Hepatic enzymes	↓ by 28% Gr. D.	↑ by 50% Gr. D	by 50% Gr. D			
Srigiridhar and Nair (97)	effects of excess free iron, was tested in rats fed with iron	site of iron absorption-duodenum	↑ a		↓ a		↑ a	Ratios of SOD/Gpx, Cat/Gpx↑ ed in the Fe fed group
Srigiridhar and Nair (64)	WKY female rate 8 mg iron for 15 days	Effect after supplement	NS		NS			protein carbony1 formation
Srigiridhar and Nair (63)	Fe fed rats Given Vit E and C	Site-rat mucosa Before vit E & C (b), after (a)	↓ b ↑ a	↑ b ↑ a	↓ b ↑ a		↓ b ↑ a	Vit E & C acted antioxidant
Kaur and Mehmood (98)	Wistar rats fed iron 29 mg/kg body weight (or 6.58 mg/kg Fe) daily	Site-GI brush border						↓ Alp, sucrase maltase, lactase rehalase, NS- leucine amino peptidase & γ GT

quality trials assessing clinical outcomes of various iron supplementation strategies with respect to beneficial / adverse effects and OS status (99). Major thrust of public health policy is to increase demand and compliance to control anemia, but without addressing compliance, optimizing oxidative stress and minimizing side effects it may again be a series of failed strategies. Other factors responsible for altered OS i.e. pregnancy itself, age, parity, chronic diseases, diet, environment, season, emotional quotient/ stress, protein intake, intestinal parasites and malaria etc are unique in India. Therefore study should be a multi-center, on large sample to provide concrete answers pertaining to situations in India.

Questions asked by John, years ago still remain unanswered. He put queries (100) i.e. what approach will provide the most efficacious outcome? If iron supplementation is provided, what dosage and schedule are the most beneficial? Is there a real health risk of too much iron? Can iron supplementation prevent as well as induce oxidative stress? What changes in oxidative stress patterns occur, if normal as well as anemic women are supplemented? Upcoming information from Camedon study and ICMR task force study would be providing some of these answers.

Abbreviation: Malondialdehyde: MDA, Oxidized low density lipoprotein: OX-LDL, Superoxide dismutase: SOD, Glutathione peroxidase: GSH-Px, Glutathione reductase: GSHRd, OS: Oxidative stress, Thiobarbituric acid reacting substances (TBARs), ↑ raised, ↓ decreased, NS- No significant change, Iron = elemental iron doses, Non-protein antioxidant capacity-NPAC, Alp-alkaline phosphatase, b= before, a=after

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