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Effect of iron administration on oxidative stress in anemic and non anemic pregnant women

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Abstract

Background: Pregnancy is a condition exhibiting increased susceptibility to oxidative stress and iron plays a central role in generating harmful oxygen species. The objective of present study was to compare oxidative stress between anemic and non anemic pregnant women and to analyze the effect of oral iron supplementation on markers of oxidative stress and antioxidant enzymes in anemic and non anemic pregnant women.

Material and Methods: 35 pregnant women with iron deficiency anemia, supplemented with oral iron in therapeutic doses for 8 weeks were selected in group A and 35 pregnant women with hemoglobin >11 g/dl, supplemented with oral iron as prophylaxis for 8 weeks were enrolled in group B. Complete blood count, plasma lipid peroxides, enzymatic and non enzymatic antioxidants were measured and statistical analysis was done.

Result: The levels of Hb, serum iron, hematocrit, antioxidant enzymes and vitamin C were found significantly reduced and the levels of lipid peroxides were found significantly raised in iron deficient anemic women as compared with non anemic women. Following 8 weeks of iron supplementation there was a significant increase in antioxidant enzy mes and vitamin C levels and a significant decrease in lipid peroxide levels in anemic pregnant women, while there was a significant decrease in antioxidant enzymes and vitamin C levels and a significant increase in lipid peroxide levels in non anemic pregnant women, as compared with baseline levels before oral iron supplementation.

Conclusion: Oral iron treatment significantly improves hemoglobin level in iron deficiency anemia and recovers antioxidant defense system, while iron supplementation at usual recommended doses in non anemic pregnant women may contribute to increased oxidative stress.

Keywords: oxidative stress, iron supplementation, iron deficiency anemia

Introduction

Anemia among pregnant women is a serious global health concern. The World Health Organization estimates that globally 38% of pregnant women are anemic [1].

Iron deficiency is the most common cause of anemia during pregnancy. Other causes include parasitic diseases such as malaria, hookworm infections, and Schistosomiasis; micronutrient deficiencies including folic acid, vitamin A, and vitamin B 12; and genetically inherited haemoglobinopathies such as thalassemia [2].

The incidence of iron deficiency anemia in India is 60% in urban and 69% in rural population [3]. It increases the risk of adverse pregnancy and perinatal outcomes including maternal and neonatal deaths, particularly in severe anemia. In contrast, high levels of hemoglobin, haematocrit and ferritin are associated with an increased risk of preeclampsia, gestational diabetes mellitus, fetal growth restriction and preterm delivery [1].

Pregnant women are more prone to oxidative stress as a result of an imbalance between the pro-oxidant and antioxidant balance. Anemia leads to increased oxidative stress and increased lipid peroxidation [4].

The Centre for Disease Control and Prevention recommends universal iron supplementation with 60 mg of elemental iron once or twice daily from second trimester of pregnancy, to meet the iron requirements of pregnancy except in the presence of certain genetic disorders, such as hemochromatosis [3].

World Health Organization also recommends prophylactic supplementation of all pregnant women with 60 mg of elemental iron and 400 μg of folic acid daily. Ministry of Health, Government of India has recommended intake of 100 mg of iron and 500 μg of folic acid daily for 100 days in the second half of pregnancy [5].

Ferrous iron used for oral iron therapy in pregnancy is a potent pro-oxidant that propagates free radical reactions through Fenton chemistry, both locally and systemically. Consequently, although iron supplementation may improve pregnancy outcome when the mother is iron deficient, it may increase risk in the absence of iron deficiency. Many previous studies have reported worsening of oxidative stress following oral iron supplementation in iron deficient women, however some studies also reported improvement in oxidative stress following oral iron supplementation [6].

It is recognized that excess body iron and “free iron” stimulate lipid peroxidation that leads to cell and tissue damage. However, only a few studies have been conducted to investigate the effect of iron supplementation on oxidative stress in humans and results are inconclusive.

In the light of this background, the current study was conducted to compare oxidative stress between anemic and non anemic pregnant women and to analyze the effect of oral

iron supplementation on markers of oxidative stress and antioxidant enzymes in anemic and non anemic pregnant women.

Material and Methods

This prospective study was carried out on 70 pregnant women aged between 18-35 years, at Department of Obstetrics and Gynaecology, Kamla Raja Hospital, Gwalior (M.P.), during a period of 18 months from August 2015 to January 2017. Group A included 35 pregnant women with hemoglobin level ranging from 7 gm/dl to 10.9gm/dl (cases of mild and moderate anemia as per WHO classification of anemia in pregnancy) [7]. Diagnosis of iron deficiency anemia was based on Hb level below 11gm/dl and mean corpuscular volume less than 80 fl and / or serum ferritin less than 20 mcg/dl. Group B included 70 non anemic pregnant women with hemoglobin level more than 11 gm /dl (as per WHO definition of anemia) [7].

Women in group A were supplemented with one tablet of ferrous sulfate containing 60 mg elemental iron given three times daily for 8 weeks and women in group B were supplemented with one tablet of ferrous sulphate containing 100 mg elemental iron daily (as per recommendation of Ministry of Health, Government of India) [5] for 8 weeks.

The exclusion criteria were age >35 years, gestational age <14 weeks and > 28 weeks, multiple pregnancy, HIV positive status, women with malignancy, liver disease, kidney disease, chronic diseases e.g. tuberculosis, hypertension, diabetes mellitus, heart disease and obstetric complications such as preeclampsia and antepartum hemorrhage.

Detailed history was taken and a systematic general, systemic and obstetric examination was done. Routine investigations like ABORh, hemoglobin, serum iron, haematocrit, blood sugar screening, urine examination, VDRL, HIV, HBsAg were done. Specific investigations like Lipid peroxide levels, catalase, ascorbic acid, glutathione peroxidase, super oxide dismutase, glutathione reductase were done.

6 ml venous blood was taken from each pregnant woman and divided into three aliquots. Two ml blood was transferred to EDTA containing tube used to determine hemoglobin and haematocrit. 2 ml of whole blood was also transferred into heparin containing tube and then centrifuged; plasma separated and used for estimation of lipid peroxide levels (LPO). Remaining 2 ml of venous blood was also centrifuged at 3000 rpm for 15 minutes, serum separated and used for estimation of vitamin C. The RBC's were lysed by mixing chilled water and RBC lysate was used for the estimation of antioxidant enzymes namely catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR).

Each woman had a follow up visit after 8 weeks, when clinical examination and biochemical measurements were repeated.

The data collected was subjected to standard statistical analysis. Quantitative variables were presented as mean± SD. Qualitative variables were presented as median, range number and percentage.

Results

Table 1: Characteristics of pregnant women

Characteristics	Group A	Group B	p value
Age (years)	25.7±0.19	24.7±0.16	p = 0.962
Parity (%) Primigravida Multigravida	15(42.9%) 20(57.1%)	16 (45.7%) 19 (54.3%)	p =0.708
Gestational age (weeks)	16.09±1.58	16.11±2.04	p =0.141
Hemoglobin (gm/dl)	9.04 ± 0.84	11.64 ± 0.38	p<.001
Serum Iron (mg/dl)	27.04 ± 6.51	42.24 ± 4.51	p<.001
Hematocrit (%)	28.04 ± 3.51	36.08 ± 1.81	p<.001

Table No. 1 shows that there were no significant differences in age, gestational age and parity between the two groups. Before start of iron supplementation, group A had significantly lower mean values of hemoglobin, serum iron and haematocrit as compared with those of group B. (Table 1).

Table 2: Level of biochemical markers of oxidative stress at baseline

Biochemical marker	Group A			Group B			p value
	Range	Mean	SD	Range	Mean	SD	
CAT (U/mg protein)	28.50-48.19	37.99	5.65	45.84-62.01	54.89	4.32	<.001
SOD (U/mg protein)	0.55-1.20	0.86	0.17	0.94-1.35	1.21	0.11	<.001
GPx (U/mg protein)	24.19-34.19	29.01	3.17	30.14-42.73	37.18	2.96	<.001
GR (U/mg protein)	18.90-27.67	24.31	2.63	22.14-32.00	27.46	2.65	<.001
Vitamin C (mg/dl)	0.69-1.23	0.97	0.16	1.20-1,37	1.29	0.04	<.001
LOP (nmole MDA /mg protein)	2.80-4.17	3.42	0.43	2.11-2.45	2.25	0.10	<.001

CAT- Catalase, SOD- Superoxide Dismutase, Gpx- Glutathione Peroxidase, GR- Glutathione Reductase, LOP- Lipid Peroxide.

At the onset of study, lipid peroxide levels in group A were significantly higher as compared with group B. Mean values of antioxidant enzymes ant vitamin C were found to be significantly lower in group A as compared with group B, thereby showing that in cases with compromised hemoglobin status (group A), the oxidative stress was higher. (Table 2)

After 8 weeks supplementation with oral iron, the mean hemoglobin level in group A was 10.35 ± 0.84 (gm/dl) and in group B it was 11.74 ± 0.28 (gm/dl). In group B, no significant change in hemoglobin level was observed after 8weeks. However, in study group A, statistically significant increase in hemoglobin level was seen after 8 weeks of oral iron supplementation.

Table 3: Comparison of change in Oxidative stress biochemical markers in group A (Baseline and after 8 weeks)

Biochemical marker	Baseline		After 8 weeks		Change and Significance		
	Mean	SD	Mean	SD	Mean	SD	"p"
CAT (U/mg protein)	37.99	5.64	40.82	5.54	2.83	0.26	<.001
SOD (U/mg protein)	0.87	0.17	0.94	0.18	.07	0.01	<.001
GPx (U/mg protein)	29.01	3.16	30.41	3.07	1.41	0.17	<.001
GR (U/mg protein)	24.32	2.62	26.19	2.48	1.87	0.15	<.001
Vitamin C (mg/dl)	0.96	0.16	1.08	0.16	0.12	0.01	<.001
LOP (nmole MDA /mg protein)	3.43	0.43	3.18	0.46	-0.25	0.03	<.001

CAT- Catalase, SOD- Superoxide Dismutase, Gpx- Glutathione Peroxidase, GR- Glutathione Reductase, LOP- Lipid Peroxide.

Group A showed a statistically significant rise in levels of all antioxidant enzymes and vitamin C, while statistically significant fall in level of lipid peroxide was observed, after 8 weeks of oral iron therapy. (p<.001). (Table 3).

Table 4: Comparison of change in Oxidative stress biochemical markers in Group B (Baseline and after 8 weeks)

Biochemical marker	Baseline		After 8 weeks		Change and Significance		
	Mean	SD	Mean	SD	Mean	SD	"p"
CAT (U/mg protein)	54.90	4.35	52.01	4.79	-2.89	0.43	<.001
SOD (U/mg protein)	1.20	0.12	1.14	0.11	-0.06	0.005	<.001
GPx (U/mg protein)	37.16	2.96	34.43	2.48	-2.73	0.25	<.001
GR (U/mg protein)	27.45	2.67	25.07	3.15	-2.38	0.24	<.001
Vitamin C (mg/dl)	1.28	0.04	1.24	0.04	-0.04	0.003	<.001
LOP (nmole MDA /mg protein)	2.25	0.10	2.76	0.32	0.51	0.05	<.001

CAT- Catalase, SOD- Superoxide Dismutase, Gpx- Glutathione Peroxidase, GR- Glutathione Reductase, LOP- Lipid Peroxide.

After 8 weeks supplementation with iron, in group B we found a significant fall in levels of antioxidant enzymes namely catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and vitamin C, while lipid peroxide levels were increased significantly. (Table 4).

Discussion

Pregnant women are more prone to oxidative stress. Iron deficiency anemia not only affects hematological parameters but also disturbs body oxidative balance, which impairs pregnancy outcome. Iron supplementation is universally recommended during pregnancy to correct or prevent its deficiency. However, the pathological accumulation of the metal within the tissues aggravates the generation of reactive oxygen species and elicits toxic effects, which are mainly related to oxidative stress.

In present study, plasma levels of lipid peroxides were found significantly increased in iron deficient pregnant women in group A when compared with group B. This is in agreement with the findings of Bhale *et al* and Sanaa *et al* [8, 9]. This may be attributed to over production of reactive oxygen species or a deficiency of antioxidant defense.

Antioxidant enzymes are the major defense system of cells in normal aerobic reactions. In present study, before iron supplementation mean values of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and vitamin C were significantly lower in group A as compared with group B. Kortoglu *et al* also reported significantly lower levels of catalase, superoxide dismutase, glutathione peroxidase in iron deficient anemic subjects [10]. Catalase is an iron dependent enzyme and is not unexpected to be decreased in iron deficiency. Decreased superoxide mutase level found in anemic women in present study may be due to increased oxidative stress, because it is well known that reactive oxygen species, especially hydrogen peroxide, inhibit SOD activity.

In present study, before iron supplementation mean values of vitamin C were significantly lower in group A as compared with group B. The decrease in endogenous ascorbic acid found in anemic women in present study may be due to its extensive use as an antioxidant to protect the gastrointestinal tract from the free radical damage during iron repletion and increased levels of lipid peroxidation products.

In present study, after 8 weeks supplementation with oral iron, group A showed a significant rise in levels of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and vitamin C. Similar findings were reported in other studies. In study by Kurtoglu *et al*, levels of catalase, superoxide dismutase and glutathione peroxidase were found increased after 8 weeks of iron supplementation in anemic subjects [10]. Khalid S *et al*, reported significant increase in superoxide dismutase activity after daily oral iron supplementation in anemic women [11]. In study by Isler *et al*, before treatment, superoxide dismutase activity was significantly lower in anemic patients than in the control group. After the treatment, SOD activity significantly increased in all three patient groups [12]. Olivares M *et al* also reported increased erythrocyte SOD activity following treatment with iron in adult women with iron deficiency anemia [13].

In present study, after 8 weeks supplementation with oral iron, in group A levels of lipid peroxides were significantly lower, while mean values of antioxidant enzymes and vitamin C were found significantly increased as compared with baseline values before start of treatment with oral iron. Our results indicate that oxidative stress in pregnancy associated with iron deficiency anemia decreased with daily iron supplements but fail to reach normal pregnant levels.

So after 2 months supplementation with ferrous sulfate in group A, there was a significant improvement in Hb, haematocrit and serum iron levels and levels of antioxidant

parameters were significantly increased and levels of oxidative parameters were significantly decreased.

After 8 weeks supplementation with iron, in group B we found a significant fall in levels of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and vitamin C, while lipid peroxide levels were increased significantly. This finding is in agreement with the finding of King SM *et al*, who reported more than 40% increase in plasma malondialdehyde (a marker of lipid peroxidation) level after 6 weeks of iron supplementation in non anemic iron depleted women [14].

Our findings are similar to the results reported by Chandra S *et al*, who reported a statistically significant rise in pro-oxidants NO and MDA and a significant fall in the levels of antioxidants SOD, GPx and GSH after iron supplementation in women without iron deficiency anemia [15].

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 non-transferring bound iron may reach the liver, causing systemically raised oxidative stress. This could explain the reason of increased oxidative stress found in non anemic patients following iron supplementation in present study. Also iron overload following treatment with iron in non anemic women could promote generation of free radicals, leading to an increase in oxidative stress and may result in cellular damage.

Thus iron is a double edged sword: although crucial for many central metabolic pathways and immune mechanisms, its increased accumulation may cause formation of toxic molecules and progressive tissue damage [16].

Conclusion

Prenatal oral iron treatment corrects the hematological parameters in iron deficiency anemia and recovers antioxidant defense system by increasing activity of antioxidant enzymes. However, further studies are needed to assess the oxidative stress in pregnancy related anemia.

Increased lipid peroxide levels found in the maternal plasma in the iron supplemented non anemic women suggests that iron supplementation at usual recommended doses in non anemic pregnant women may contribute to increased oxidative stress. However, further studies are needed to assess these effects on pregnancy outcome.

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