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Original Article

Role of oxidative stress after routine Iron supplementation in normal and anemic women during pregnancy: Central Indian scenario

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ABSTRACT: Iron deficiency anemia (IDA) is one of the most common nutritional disorders worldwide, affecting people of all ages in developed and developing countries. causes dimunition of various antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase. The objective of the study was to detect impact of iron supplementation in anaemic & non- anaemic pregnant subjects on oxidative stress. Patients are divided into two groups, Control groups (60 non-anemic pregnant women) & Study groups (60 anemic pregnant women). The blood sample (5 ml) was collected from different groups of subjects. In controls groups, a fall in catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase were seen while lipid peroxidase was found to have increased significantly after iron therapy. In study group, and increment in all markers except lipid peroxidase was seen and the level of lipid peroxidase was decreased following iron supplementation which was statistically significant ($p<0.001$). it may be concluded that iron deficiency anaemia is associated with free radical generation and peroxidation of vital body molecules which implies increased risk for pregnant women.

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INTRODUCTION

Anemia is a commonest medical disorder, in which there is inadequate or defective formation of haemoglobin or defective maturation and formation of red blood cells and very frequent event among pregnant women [1]. During pregnancy, anemia can have deleterious effects on mother and as well as fetus in the form of maternal morbidity and mortality, intrauterine growth retardation, poor weight gain, premature labor, preterm delivery and perinatal morbidity and mortality. Iron deficiency anemia (IDA) is one of the most common nutritional disorders worldwide, affecting people of all ages in developed and developing countries. Among pregnant women at least half of all anemia cases have been attributed to iron deficiency. Surveys from different parts of India reveal that 87% of pregnant women suffer from anemia and about 10% have severe anemia (Hb < 7.0 g/dl) and > 90% of anemia cases are reported to be due to iron deficiency [2]. There are reports that iron deficiency can lead not only to anemia but it may also impair work performance, lead to an abnormal neurotransmitter function and result in immunological and inflammatory defenses [3]. Pregnancy is a stressful condition exhibiting increased susceptibility to oxidative stress, defined here as a disturbance in the prooxidant-antioxidant balance in favor of the former, leading to potential damage [4]. Dynamic changes in multiple body systems, resulting in increased oxygen consumption and in changes in energy substrate use by different organs including the fetoplacental unit. The human placenta is hemomonochorial, meaning that only one chorionic cell layer exists between maternal and fetal bloods, favoring exchange of gases, nutrients and metabolic products. Initially the placenta has a hypoxic environment.

As it matures and its vascularization develops, it changes to an oxygen-rich environment and its abundant mitochondrial mass favors the production of reactive oxygen species (ROS), which increases free iron liberated from iron-sulfur clusters [5]. Nitric oxide (NO) is also locally produced by the placenta and together with other reactive nitrogen species contributes to potential oxidative stress. To counter act with these free radical mediated damage our body possess a defense system, which comprises of numerous enzymes (catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase), proteins, vitamins and other small molecules [6].

Immune functi. Micronutrient deficiencies can reduce immunity even further enhancing these alterations [7]. Iron status and body iron can be monitored using serum ferritin, serum transferrin, serum transferrin receptor (sTfR) levels and sTfR/ferritin ratio. These are very sensitive biomarkers for iron deficiency in pregnant women. Serum ferritin yields information about the capacity of body iron reserves [8]. Oxidative stress causes dimunition of various antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione reductase (GR) [9]. The net effect of oxidative stress is DNA damage, impaired synthesis of protein, membrane lipids and carbohydrates and altered cell proliferation [10, 11].

Pregnancy is a condition exhibiting increased susceptibility to oxidative stress, leading to potential damage. Therefore, supplementation of iron without assessing iron level may make anemic women more vulnerable to oxidative stress during pregnancy. In view of the above considerations the present study was planned to evaluate the effect of oral iron supplementation on the marker of oxidative stress through plasma thiobarbituric acid reactive substances (TBARS) and the activities of anti-oxidative enzymes superoxide dismutase (SOD) and catalase (CAT) in pregnant anemic women.

MATERIALS AND METHODS

The study protocol was taken from the Institutional Ethical Committee of Uttar Pradesh University of Medical Science, Saifai, Etawah (U.P). The study population consisted of Pregnant women (n = 120) aged 20-30 years, was recruited from the outpatient Department of Obstetrics and Gynaecology, Uttar Pradesh University of Medical Science, Saifai, Etawah (U.P). The inclusion criteria of anemic subjects will be according to World Health Organization, which defines non-anemic women as Hb ≥ 11.0 g/dl and anemic women as Hb < 11.0 g/dl [12]. Women who had been using minerals and/or vitamin supplements were excluded from the study. We also excluded women having Hb less than 7.0 g/dl (severe anemic). Patients were divided into two groups, Control groups (60 non-anemic pregnant women) & Study groups (60 anemic pregnant women).

SAMPLE COLLECTION

The blood sample (5 ml) was collected from different groups of subjects. Thereafter, an aliquot was taken from blood sample to assess haemoglobin levels. A blood profile generated with the procedure described by WHO [13] which included haemoglobin level. The remaining sample utilized for measuring the activities of various enzymes and biochemical parameters. Blood sample was centrifuged at 1,000 g for15 min, and the plasma was removed. Then, erythrocytes were washed with 0.9% NaCl solution three times and kept at -800C until biochemical determination.

Biochemical parameters:

(1) Measurement of Lipid peroxide levels: The extent of per oxidation of lipid were evaluated on the basis of 2-thiobarbituric acid (TBA) with lipid peroxide, hydroperoxide and oxygen-labile double bond that gives rise to colour adducts with absorption maximum at 532 nm. A volume of 0.2 ml of plasma having about 3 mg protein was mixed with 0.5 ml of glacial acetic acid followed by the addition of 0.5 ml of 8% aqueous SDS (sodium dodecyl sulphate) and 1.5 ml of 0.8% TBA (thiobarbituric acid) solution. The reaction mixture was heated in a boiling water bath for 1 h. After cooling to room temperature, 3.0 ml of N-butanol was also be mixed and after thorough stirring for 5 min, the reaction mixture was centrifuged at 10,000 g for 15 min. A clear butanol fraction was obtained after centrifugation, absorbance of which were measured at 532 nm. Standard solution of malondialdehyde (10 nmol) was run simultaneously. Lipid peroxide content in the sample expressed as nmol of MDA/dl according to the method Ohkhawa and Ohishi [12].

(2) Assay of Catalase: The enzyme source is added to 2 ml of phosphate buffer and 1 ml of H2O2 into a cuvette and mixed thoroughly. The decrease in absorbance at 240 nm were recorded after every 30 s for 3 min by following the method of Aebi [14].

(3) Assay of Superoxide dismutase: Carried out in two setups, the set received 0.3 ml nitro blue tetrazolium, 0.2 ml phenazine methosulphate, 1 ml pyrophosphate buffer, 1 ml triple-distilled water and 0.2 ml enzyme source. The second setup tube received all the above reagents minus the enzyme source. The reaction was started simultaneously by adding 0.1 ml NADH. After an interval of 90 s, 1 ml glacial acetic acid was added to each tube for checking the reaction. The absorbance was read at 560 nm against blank [7].

RESULTS

The biochemical parameters of Non-anaemic pregnant women (healthy controls) and Anaemic pregnant women (Study groups) were determined before and after Daily prophylactic 100 mg Iron supplementation. All pregnant women either anaemic or non anaemic had given Iron supplementation for 3 months. Majority of subjects in both the groups were aged ≤ 24 years, there was no significant difference between the groups.

Serum Iron concentration and hemoglobin levels both were lower in anemic group as compared to controls (Table 1). The mean haemoglobin levels in control group were 11.5 ± 0.31 g/dL

and 8.68 ± 0.57 g/dL in study group. Statistically, study group had significantly lower mean value as compared to control.

The mean serum iron level in control group is 39.01 ± 3.66mg/dL and in study group it was 27.60 ± 4.0 mg/dL. Statistically, the difference between the groups was significant (p<0.001).

Table 1: Comparison of Haemoglobin levels (g/dL) & Serum Iron (mg/dL) in different groups at baseline (before iron therapy)

S. No.	Group	No.	Mean± SD (Hb) (g/dL)	Mean± SD (S. Iron) (mg/dL)
1.	Control Group (non-anaemic)	60	11.5±0.31	39.01±3.66
2.	Study Group (anaemic)	60	8.68±0.57	27.6±4.0

The oxidant parameters MDA was increased before supplementation (Table 2) in study group (Anaemic pregnant women) as compared with controls, but their levels were found to be lowered after iron supplementation (Table 3) in study group. Study group had statistically significance difference from Control group.

Levels of MDA in Study groups were significantly higher as compared to Control group before iron therapy. Mean value of all the other markers was found to be significantly lower in Study group as compared to Control group. The enzymatic antioxidant parameters SOD and CAT were increased before iron supplementation as compared to study group.

Table 2: Level of biochemical markers of Oxidative Stress in Control & Study groups (before iron therapy)

S. no.	Biochemical marker	Mean ± SD (Control group)	Mean± SD (Study group)	p value
1.	Catalase (U/mg protein)	50.16 ± 2.25	36.7 ± 3.48	<0.001
2.	Superoxide dismutase (U/mg protein)	1.11 ± 0.11	0.86 ± 0.15	<0.001
3.	Lipid peroxidase (nmole MDA/mg protein)	2.41 ± 0.09	3.09 ± 0.24	<0.001

Table 3: Level of biochemical markers of Oxidative Stress in Control & Study groups (after 3 months of iron therapy)

S. No.	Biochemical marker	Mean ± SD (Control group)	Mean ± SD (Study group)	p value	Mean % difference (control gp)	Mean % difference (study gp)
1.	Catalase (U/mg protein)	47.3 ± 4.57	38.51 ± 3.54	<0.001	-2.85	1.81
2.	Superoxide dismutase (U/mg protein)	1.09 ± 0.11	0.91 ± 0.15	<0.001	-0.02	0.05
3.	Lipid peroxidase (nmole MDA/mg protein)	2.43 ± 0.10	2.96 ± 0.23	<0.001	0.02	-0.13

In both the groups, significant changes were found in the level of antioxidant parameters after 3 months. In controls groups, significant decreases in Catalase and superoxide dismutase were seen while lipid per-oxidation level was found to be increased significantly (p<0.001). The independent t-test was carried out to find the significance between study and control groups which showed a highly significant (p<0.001) in the oxidant and enzymatic antioxidant parameters. The anemic study and non-anemic control groups were compared before and after iron supplementation.

DISCUSSION

Anaemia is common among the women in their reproductive years in particular if they are belonging to low socioeconomic status, in pregnancy, and members of an ethnic minority. Oxidative stress has also been reported to have an important role in the pathogenesis of Iron Deficiency Anaemia (IDA) [5, 9-11, 15]. Iron deficiency not only affects the production of haemoglobin but also of other iron containing proteins, viz. cytochromes, myoglobin, catalase (CAT) and peroxidase.

Since, in iron deficiency states enzymes of the antioxidant defense system are functionally defective, the balance tends to get tilted towards free radicals triggering oxidative damage [16]. However, data on oxidative and anti-oxidant defences in IDA is rather limited and often contradictory [6, 9, 10, 15, 17, 18].

In our study we observed increased level of plasma lipid peroxidation in Study group as compared with Control group, which may be contributed to over production of reactive oxygen species. Similarly Yang et al., 2006 also found increased lipid peroxidation (MDA level) and decreased Superoxide dismutase in pregnant women [19]. 24 concluded that DNA damage and oxidative stress were increased in iron deficiency anaemia [1]. Iron supplementation during pregnancy increases maternal iron status. Reactive iron species are products of oxygen and when brought into contact with a transition metal that is capable of changing valence, such as iron, ($Fe^{2+} \rightarrow Fe^{3+}$) a very reactive free radical, the hydroxyl radical is formed from oxygen via Fenton's reaction. These free radicals have the potential to damage cells, organs and tissues in the body.

Another experiment by Lachili et al., 2001, they examined the influence of an iron supplemental and vitamin C, an antioxidant that increases iron absorption, on oxidative stress during pregnancy [20]. They found that administration of 100 mg/day iron (as fumarate) supplementation with vitamin C 500 mg/day as ascorbate during the 3rd trimester of pregnancy increased measures of maternal iron status. An indicator of oxidative stress from lipid peroxidation, TBARS, was significantly increased in the supplemented women compared with controls. In our study, enzymatic levels of superoxide dismutase and catalase were increased in Study group as well as anaemia level was decreased when supplemented with iron. Kourtoglu Erdal et al., 2003 found catalase, SOD, and Glutathione peroxdase were significantly lower in anaemic patient [21]. After 6 weeks of iron supplementation these levels were increased. Isler et al., 2002, studied 28 patients with iron deficiency anaemia, they found decreased level of antioxidant enzymes [22].

After iron treatment antioxidant defense system was recovered by increasing SOD activity and maintaining Catalase level at near to control group. Our results provide strong evidence that anaemic pregnant women have increased oxidative stress as compared to non anaemic pregnant women. It is important for pregnant women to maintain healthy iron status for both themselves and their offspring. Oxidative stress improved with iron supplementation in anaemic pregnant women. Contrary to this, in our study oxidative stress was found increased in non anaemic pregnant women after iron supplementation. From our study, we concluded that there is a need of large, good quality trials assessing clinical outcomes of various iron supplementation strategies with respect to beneficial or adverse effects of oxidative stress status. We infer that optimum dose of iron should be decide at which oxidative stress would be nil or minimum. Therefore, multicenter study on large sample should be done to find out, what iron dosage and schedule will be most beneficial; what changes in oxidative stress pattern will be occurred, if normal as well as anaemic pregnant women supplemented with iron.

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