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ORIGINAL ARTICLE

Study on Impact of Iron and Folic Acid on the Plasma Trace Minerals in Pregnant Anemic Women

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Abstract Iron deficiency anemia is one of the causes that lead to significant mortality and morbidity among pregnant women and fetus. The present study was undertaken to explore oral iron supplementation can modify the metal contents in pregnant anemic women. Iron and folic acid supplementations was given to 500 anemic women (mild = 200, moderate = 200, and severe = 100) and 100 age matched non-anemic controls daily for 100 days. Blood index values and plasma trace minerals were estimated as per standard protocols. Haemoglobin and ferritin levels were found significantly increased ($p < 0.001$) in anemic and control subjects after treatment. Moreover, the serum transferring receptor levels and total iron binding capacity were found significantly decreased in all treated groups. Iron (Fe), zinc (Zn) and copper (Cu) levels were found increased ($p < 0.01$) after oral iron supplementation groups. Moreover, selenium (Se) manganese (Mn) and were found to be decreased in all treated groups. Data provides the conclusion that iron and folic acid supplementation recovered the essential trace minerals, except manganese, which may lead to various complications including peroxidation of vital body molecules resulting in increased risk for pregnant women as well as fetus.

Keywords Iron deficiency anemia · Pregnancy · Iron supplementation · Trace minerals · Blood index · Nutrition

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Introduction

Iron deficiency anemia is a common public health problem in pregnant women [1]. It is responsible for maternal death in our country directly or indirectly from hemorrhage, cardiac failure, pre-eclamptia. It also increases perinatal mortality and morbidity consequence to preterm deliveries, growth retardation, cognitive and affective dysfunction in the infants. Women tend to have substantially lower iron stores, making them more vulnerable to iron deficiency when iron intake is lower or need increase. Women of reproductive age lose iron during menses and have a substantially higher need for iron during pregnancy, because of the increase in red cell volume of the mother and placental and fetal growth [2].

To overcome this problem during pregnancy iron supplementation is common used strategy to constitute the increased requirements. Pregnant women need perceptible amount of iron that cannot be acquired from diet alone, therefore in women with low risk for nutritional deficiency daily supplementation of iron at a dose of 60–120 mg/day to treat anemic women and a high dose is recommended for area where the prevalence of anemia is high [3]. Variation of each bivalent metal ion concentrations such as Fe, Cu, Se, Zn, Mn and Mg may change the concentration of other ion metals [4]. Almost all of the supplemental programs in developing countries mainly focus on iron supplementation [5]. However, these recommendations exceed the iron dietary allowance for women, which may eventually lead to oxidative damage, mainly mediated by iron, oral iron supplementation may also lead to various health problems due to increase bioavailability of free iron in the body i.e. abdominal cramps, nausea, vomiting, heart burn, diarrhea and constipation [6] and it also interact with intestinal

absorption of bivalent metals such as Cu, Zn, Se, Mg and Mn.

Bivalent minerals have important role as cofactors which play a vital roles in various metabolic pathways in the body. Fe, Se, Zn and Cu are main trace elements and the variations of their serum levels in pregnancy may be very important for both mother and fetus. Their ability to accept or donate electrons makes them central to the catalytic activity of many redox-active enzymes. Copper (Cu) is a central component of many enzymes involved in angiogenesis, oxygen transport mechanisms, lysine oxidase, cytochrome c oxidase, tyrosinase, ceruloplsamin, dopamine-β-hydroxylase, monooxygenase and copper-zinc superoxide dismutase (CuZn-SOD; SOD1), known as oxidant defense enzyme [7]. Cu deficiency may cause many disorders in the body. Previous reports showed that Cu and Hb levels are inversely correlated. This correlation is more significant in anemic pregnant women [8]. Iron (Fe) is an integral part of several classes of enzymes including cytochromes, enzymes involved in the synthesis of steroid hormones, detoxification of foreign particles in the liver, synthesis of neurotransmitters, DNA synthesis and breakdown [9]. Many benefits of selenium (Se) are related to its role as a cofactor in the production of selenoproteins, glutathione peroxidase, thyroid hormone deiodinase, thioredoxin reductase and methionin-r-sulfoxide reductase. The important role of selenoproteins for body as antioxidant enzyme is to help detoxification of the body and to protect the cell against oxidative damage by peroxidase produced from lipid metabolism. It is reported that there is increased requirement for Se during pregnancy presumably for fetal growth. There are reports that pregnant women had significantly decreased plasma Se levels with glutathione peroxidase in the several second and third trimesters and at delivery [10]. Zinc (Zn) has three functional classes: catalytic, structural and regulatory. These roles play a central role in cellular growth, differentiation, gene transcription system, hormone receptors, signal transduction pathways for human metabolism and reproduction [11]. There is report that 77% of pregnant women suffered from deficiency of vitamins, essential trace elements and minerals [12]. King and Cousins [13] reported that Zn deficiency causes growth retardation and multiple fetal abnormalities. Abnormal synthesis of nucleic acids, proteins, impaired cellular growth and morphogenesis, chromosomal defects, excessive cell death and excess lipid peroxidation of cellular membrane may all occur in severe Zn deficiency. Deficiency of this mineral may shorten the RBC lifespan because Zn is a cofactor for RBC-SOD contributing to protection from oxidative stress and to cell integrity [14, 15]. The aim of the present study was to investigate the hypothesis that oral iron supplementation can modify the metal contents in pregnant anemic women.

Materials and Methods

Subjects

The study was approved by the Institutional Ethical Committee of King George's Medical University, Lucknow, India. Before recruitment written informed consent was obtained from each subject. In total, four groups of subjects were selected for the study. Out of 600, 100 were pregnant non-anemic women as control and 500 were pregnant anemic (mild = 200, moderate = 200 and severe = 100) women as cases. Selected subjects were all consumers of normal mixed Indian diet, not taking any drugs for preceding 1 month. The inclusion criteria of anemic subjects were according to WHO, which defines mild anemia as Hb 10.0–10.9 g/dl, moderate as Hb 7.0–9.9 g/dl and severe as Hb < 7.0 g/dl [16]. Women who have been using mineral and/or vitamin supplements were also excluded. Patients, which did not show Hb rise by 1% after 3 weeks of supplementation, were also excluded from the study. Both groups were non-smokers, non-alcoholic and without any other symptoms known to influence the antioxidant status and minerals. Information on occupation and medical history, job description, socio-economic status and lifestyle of both groups were obtained through questionnaires. Subjects having a previous history of metabolic diseases such as hypertension, diabetes mellitus, malignancy, heart disease, infections such as tuberculosis, HIV and arthritis and endocrinal disorders were also excluded from the study.

Treatment

At recruitment, all women (non anemic and anemic) were first dewormed by giving them a single dose of Albendazole following Metronidazole (400 mg) three times daily for 5 days. Two days later i.e. after 1 week of recruitment, anemic women were given iron supplements (100 mg as ferrous sulphate and 500 μg folic acid) orally once a day, daily for 100 days.

Before deworming, venous blood of all women (non-anemic and anemic) was taken for the estimation of biomarkers of blood indices and trace minerals (pre treatment). All tests were repeated after 100 days of treatment (post treatment). After 3 weeks of iron and folic acid supplementation, Hb was assessed. Subjects, who's Hb was not improved, were excluded from the study and were referred for other investigations. All blood collections were done in the Department of Obstetrics and Gynaecology, Queen Mary's Hospital, King George's Medical University, Lucknow. The subjects were instructed not to change their dietary or daily activities during the study.

Sample Collection

Blood samples were collected in the Queen Mary's Hospital, King George's Medical University, Lucknow. At enrollment and after 100 days of iron and folic acid supplementation, 6 ml venous blood was taken from each of the subjects and divided into three aliquots. One milliliters of blood was transferred to an evacuated tube containing EDTA solution used to determine Hb, Hct, MCV, MCH and RBC counts. Three milliliters of whole blood was also transferred into EDTA containing tube and then centrifuged; plasma separated and used for the estimation of Fe, Se, Zn, Mn and Cu. Remaining 2 ml of venous blood was also centrifuged at 3000 rpm for 15 min, serum separated and used for the estimation of ferritin, transferring receptors and total iron binding capacity (TIBC).

Analytical Estimation

Blood haemoglobin was determined by using the cyanomethemoglobin method [17]. Haematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell counts were determined by using Sysmax A-380 automated cell counter. Serum ferritin was determined by enzyme-linked immunosorbent assay kits (Spectro Ferritin, Ramco Laboratories Inc, Houston, TX, USA) [18]. Total iron binding capacity was estimated spectrophotometrically using ferrozine method [19], and validated by TIBC kit in semi-autoanalyzer, (Erba Chem 5 plus) [20]. The concentration of iron in plasma was measured with flame atomic absorption spectrophotometer (Perkin Elmar AAS-700 Ueberlinger, Germany) [21].

Se measurement was done in graphite furnace atomic absorption spectrophotometer (Perkin Elmer Analyst 800) using Zeeman background correction. Matrix modifiers were palladium (4 µg in 20 µl sample) and Mg sulfate (3 µg in 20 µl sample). Samples and calibration standards were diluted in 1:3 with 0.05% Triton X-100 to improve the sample viscosity and reproducibility of the results. Se levels in all groups were evaluated according to a standard curve. Se calibration standards were prepared from the commercial Se standard (1000 mg/l) by serial dilutions [22]. Plasma Cu levels were analyzed in flame photometer of atomic absorption spectrophotometer (Perkin Elmar AAS-700 Ueberlinger, Germany). Samples and calibration standards for Cu measurement were 1:2 dilutions with 10% glycerol. Commercial Cu calibrators were used as standards (1.000 mg/l) by serial dilutions and samples were evaluated according to a standard curve [23]. Plasma Zn levels were analyzed in flame photometer of atomic absorption spectrophotometer (Perkin Elmar AAS-700 Ueberlinger, Germany). Samples and calibration standards for Zn measurement were prepared in 1:4 dilutions with 5% glycerol. Commercial Zn standards (1.000 mg/l) were used by serial dilutions and samples were evaluated according to standard curve [24]. Besides, manganese (Mn) was estimated by using atomic absorption spectrophotometer. TIBC was determined by Ramsay's dipyridyl method [25].

Statistical Analysis

Healthy pregnant women (control) and pregnant anemic women (mild, moderate and severe) before and after treatment were compared together using one-way ANOVA analysis of variance by Newman–Keuls post hoc test between groups. A probability p value of <0.05 ($p < 0.05$) was considered statistically significant. The statistical analysis was performed on commercial software INSTAT 3.0, a demo version (Graph Pad Software, San Diego, CA).

Results

Blood Profile

The pre and post-treatment bold index parameters of all pregnant healthy and anemic women are summarized in Table 1. The Haemoglobin (Hb) level in venous blood of healthy pregnant women was 12.60 ± 1.08 mg/dl. On the other hand, it was observed decreasing significantly in all anemic conditions viz. mild ($p < 0.001$), moderate ($p < 0.001$) and severe ($p < 0.001$) subjects. Upon treatment with iron and folic acid (100 and 500 mg) per day for 100 days, the levels of Hb were reversed significantly in control ($p < 0.001$), mild ($p < 0.001$), moderate ($p < 0.001$) and severe ($p < 0.001$) pregnant anemic women. Similarly, we also observed that ferritin in control group was 37.53 ± 12.93 µg/l. However, this level was found significantly reduced in different groups of pregnant women, who were under anemic; such as mild ($p < 0.01$), moderate ($p < 0.001$) and severe ($p < 0.001$). Following treatment, there was enhancement in the level of ferritin in control ($p < 0.001$), mild ($p < 0.001$), moderate ($p < 0.001$) and severe ($p < 0.05$) women.

We also observed sTfR level in blood serum of control group to be 3.63 ± 1.62 mg/l. However, this level was observed significantly increased in different groups of pregnant anemic women, such as mild ($p < 0.01$), moderate ($p < 0.001$) and severe ($p < 0.001$). Treatment suppressed the level of sTfR in control ($p < 0.001$), mild ($p < 0.001$) moderate ($p < 0.001$) and severe ($p < 0.01$) pregnant women when compared with its respective pretreatment subjects.

Table 1 Blood index values (mean ± SD) before and after iron and folic acid supplementation in subjects

Subjects		Parameters			
		Hb	Ferritin	TfR	TIBC
Control N = 100	Pre	12.60 ± 1.08	37.53 ± 12.93	3.63 ± 1.62	329.72 ± 44.74
	Post	13.46 ± 1.15***	54.16 ± 11.94***	2.15 ± 0.84***	303.03 ± 46.57^{NS}
Mild N = 200	Pre	10.51 ± 0.22^{a}	34.14 ± 8.94^{b}	4.22 ± 1.78^{b}	363.35 ± 58.14^{c}
	Post	12.11 ± 0.57***	47.46 ± 10.13***	4.22 ± 1.78***	327.73 ± 52.23*
Moderate N = 200	Pre	8.05 ± 0.54^{a}	20.26 ± 8.24^{a}	4.95 ± 1.75^{a}	451.95 ± 90.47^{a}
	Post	10.81 ± 0.77***	27.25 ± 10.25***	3.55 ± 1.37***	379.56 ± 80.79***
Severe N = 100	Pre	6.74 ± 0.22^{a}	11.49 ± 3.16^{a}	5.41 ± 1.76^{a}	518.80 ± 98.04^{a}
	Post	8.65 ± 0.66***	15.48 ± 3.12*	4.32 ± 1.57**	474.10 ± 91.18**

Hemoglobin (Hb) and total iron binding capacity (TIBC) are expressed in mg/dl. The values of ferritin and sTfR (serum transferring receptor levels) are expressed in μg/l and mg/l, respectively. Ceruloplasmin is expressed in mg/l

Pre pre treatment, *Post* post treatment

Significance: ^{a} $p < 0.001$; ^{b} $p < 0.01$ and ^{c} $p < 0.05$ as compared with the pre control group

* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ as compared with the pre treatment group

The level of total iron binding capacity (TIBC) of pregnant non-anemic women (control) was 329.72 ± 44.74 mg/dl. This parameter was significantly increased in groups of anemic women by $p < 0.05$ in mild and $p < 0.001$ in moderate and severe as compared with its respective controls. Treatment with iron and folic acid reversed the levels of TIBC in control, mild, moderate and severe women at level of significance at $p > 0.05$, $p < 0.05$, $p < 0.001$ and $p < 0.01$, respectively.

Plasma Trace Minerals

The pre and post-treatment plasma trace minerals of all pregnant healthy and anemic women are summarized in Table 2. The values in control were as follows: iron (Fe) 43.75 ± 7.28 mg/dl, copper (Cu) 22.99 ± 4.75 μmol/l, zinc (Zn) 10.71 ± 1.68 μmol/l, selenium (Se) 1.74 ± 0.28 μmol/l and manganese (Mn) 0.106 ± 0.01 μmol/l. These parameters were found to be significantly decreased in all anemic subjects as compared with controls. Treatment of these anemic women with elemental iron and folic acid (100 mg and 500 μg/day) for 100 days showed a reversal of the above parameters. It was found that in iron deficient pregnant anemic women viz. mild, moderate and severe patients there was a decline in their Fe level by ($p < 0.001$), ($p < 0.001$) and ($p < 0.001$); Cu by ($p > 0.05$), ($p < 0.001$) and ($p < 0.001$), Zn by ($p > 0.05$), ($p > 0.05$), and ($p < 0.001$); Se by ($p < 0.01$), ($p < 0.001$), and ($p < 0.001$) and Mn by ($p < 0.05$), ($p < 0.001$) and

Table 2 Blood plasma trace minerals concentrations (mean ± SD) before and after treatment in subjects

Subjects		Parameters				
		Fe	Cu	Zn	Se	Mn
Control N = 100	Pre	43.75 ± 7.28	22.99 ± 4.75	10.71 ± 1.68	1.74 ± 0.28	0.106 ± 0.01
	Post	57.44 ± 9.66***	25.96 ± 5.06***	11.08 ± 1.47^{NS}	1.69 ± 0.28^{NS}	0.100 ± 0.01^{NS}
Mild N = 200	Pre	34.66 ± 5.97^{a}	21.10 ± 3.65^{NS}	10.22 ± 1.47^{NS}	1.59 ± 0.23^{b}	0.098 ± 0.01^{c}
	Post	44.40 ± 7.34***	24.34 ± 4.37***	10.59 ± 1.41^{NS}	1.52 ± 0.22^{NS}	0.092 ± 0.01^{NS}
Moderate N = 200	Pre	26.59 ± 6.56^{a}	24.34 ± 4.37^{a}	9.79 ± 1.90^{NS}	1.49 ± 0.19^{a}	0.092 ± 0.02^{a}
	Post	33.11 ± 6.94***	22.11 ± 3.52***	10.15 ± 1.88^{NS}	1.43 ± 0.17^{NS}	0.087 ± 0.02^{NS}
Severe N = 100	Pre	19.84 ± 3.53^{a}	16.89 ± 3.74^{a}	8.39 ± 1.80^{a}	1.33 ± 0.21^{a}	0.085 ± 0.01^{a}
	Post	23.95 ± 3.90**	19.67 ± 4.09**	8.63 ± 1.79^{NS}	1.25 ± 0.20^{NS}	0.078 ± 0.01^{NS}

Iron (Fe) is expressed in mg/dl. The values of copper (Cu), zinc (Zn) selenium (Se) and Manganese (Mn) are expressed in μmol/l, respectively

NS non significant, *Pre* pre treatment, *Post* post treatment

Significance: ^{a} $p < 0.001$; ^{b} $p < 0.01$ and ^{c} $p < 0.05$ as compared with the pre control group

* $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ as compared with the pre treatment group

($p < 0.001$), respectively as compared with control. Our results showed that following the treatment, the Fe levels of control, mild, moderate and severe anemic women were significantly increased by ($p < 0.001$), ($p < 0.001$), ($p < 0.001$) and ($p < 0.01$), respectively when compared with their respective pre treatment parameters. Similarly, Cu levels were also found significantly increased by ($p < 0.001$), ($p < 0.001$), ($p < 0.001$) and ($p < 0.01$) after iron and folic acid supplementation. Treatment increased the level of Zn and Se in control ($p > 0.05$), mild ($p > 0.05$) moderate ($p > 0.05$) and severe ($p > 0.05$) pregnant women when compared with its respective pre-treatment subjects. Furthermore, after treatment with iron and folic acid the levels of Mn were also decreased in control, mild, moderate and severe women when compared with its respective pre-treated levels.

Discussion

The nutritional need for iron, copper, zinc, selenium and manganese in living organisms is derived from their role in the metabolism. Their ability to accept or donate electrons makes them central to the catalytic activity of many redox-active enzymes. They are components of many enzymes involved in metabolic reactions, including angiogenesis, oxygen transport, synthesis of steroid hormones, detoxification of foreign substances, synthesis of neurotransmitters, DNA synthesis and breakdown [7]. Owing to these vital roles in many of the body's biochemical processes, it is clear that deficiencies in copper, iron, zinc selenium, manganese and magnesium would lead to widespread problems, particularly if these deficiencies were to occur during times of rapid growth and development, such as pregnancy, infancy and puberty.

The results of the present study showed that plasma iron levels were decreased in anemic women and were recovered significantly in all supplemented groups. Women tend to have substantially lower iron stores, making them more vulnerable to iron deficiency when iron intake is lower or need increase. Iron supplementation is the commonly used strategy to meet the increased requirements of risk group, such as women with child-bearing age, especially during pregnancy. This is because high requirement of iron during pregnancy are not easily fulfilled by dietary intake alone [26]. It has been reported that daily oral iron supplementation improved the iron status in iron deficient women [6, 27]. Present finding showed that ferritin levels were found decreased in different group of pregnant anemic women, however, serum transferrin receptor levels (sTfR) and total iron binding capacity (TIBC) were registered to increase in these subjects. Treatment significantly revert the levels of above aforementioned parameters. Present results are similar to previous reports [28, 29]. Serum TfR is the most sensitive indicator of iron status when there is tissue iron deficiency and the serum ferritin concentration is the most sensitive indicator of iron status when there are residual iron stores. The sTfR concentration is usually increased owing to iron deficiency, leading to insufficient supplies of iron to the bone marrow. The consistent decrease in sTfR in the iron supplemented group was probably due to an increase in iron supply by the iron capsules, or to a decrease in iron demand while the iron stores were being replenished.

We have also observed that plasma Cu concentration was decreased in all patients with iron deficiency anemia when compared with controls. However, treatment increased the Cu levels in all anemic subjects. Copper is present largely in the form of organic complexes, which are needed as co-factor for several enzymes, have been shown to be diminished with iron deficiency anemia [30–32]. We suggested that Cu is sequestered in tissue with iron deficiency. Shukla et al. [33] suggested that reduction of iron allows for enhanced transport of nonferrous minerals into tissues and corresponding reduction in plasma. Decreased plasma Cu levels in iron deficient subjects may be due to a deficient absorption and/or increased excretion of this metal in these patients. The significant increase observed in plasma Cu levels following 100 mg iron supplementation in the present study is similar to the finding of earlier reports, which found that 50 mg iron supplementation significantly increased the Cu concentrations in iron deficient females [34, 35].

The role of Zn is defined in three functional classes: such as catalytic, structural and regulatory. These roles explain why zinc plays a central role in cellular growth, differentiation, and metabolism. It is important for rapid growth, both pre- and post-natally for tissues such as those of the immune system and the gastrointestinal tract that undergo continuous cell renewal. Its absorption adapts to physiological needs. Stressful conditions such as infectious diseases may alter Zn absorption efficiency. Intestinal excretion and urinary losses can also be affected zinc status [13]. Zinc alters erythropoiesis. Alternatively, deficiency of this mineral may shorten the RBC lifespan since zinc is a cofactor for RBC-SOD contributing to protection from oxidative stress and to cell integrity [14, 15].

The plasma zinc (Zn) levels in iron deficient groups in our study were lower than that of the controls but this decline was not statistically significant in all groups. However, treatment with oral iron and folic acid recovered Zn levels in iron deficient women. There is an antagonism between Zn and Fe absorption from the gastrointestinal tract, as an increase iron concentration in the intestinal lumen may antagonize the uptake of Zn [36]. Our results are similar with previous reports, which found that iron and

vitamin C supplementation increases the plasma Zn levels in pregnant women [37].

The present investigation showed decrease in selenium level in pre-treated anemic women. Our result is similar with previous reports showing low Se levels in children with iron deficiency anemia and in anemia among older adults [38, 39]. Glutathione peroxidase, a selenium dependent enzyme, metabolizes the peroxides and protects cell membrane from peroxidative damage. It is well established that in iron deficiency anemia exhibit oxidative stress overload. In the present study, Se levels could have decreased significantly due to involvement of it in the synthesis of GPx enzyme, to counter act with free radical's neutralization. After treatment Se concentration further decreased in post-treatment groups. There have been reports that iron supplementation adversely affect the body selenium status in young women [40]. Arnaud et al. [41] supplemented 100 mg of iron during pregnancy from 6-months of gestation to delivery and found that Se concentration was significantly decreased in iron supplemented group.

Interestingly we observed that the manganese level was decreased significantly in pre-treated groups and it was further reduced after treatment with iron and folic acid. Taken together present data with earlier reports [6, 42, 43], it is well reflected that during pregnancy manganese is required for the proper functioning of enzymes such as superoxide dismutase that is important for the scavenging of free radicals. Less magnesium level during pregnancy may result in impairment of antioxidant potential of cells by reducing the superoxide dismutase activity, as well as increased lipid peroxidation [6, 44]. Indeed, the present report is propagated in continuation to our earlier finding [6] concerning with justified correlation between SOD activity and Mn levels in pre- and post-treated subjects. Besides, lipid peroxidation of RBC has been observed to be significantly accelerated in iron deficiency anemia [45, 46]. It is well known that lipid peroxidation is a free-radical-mediated phenomenon and that the lipids in RBCs are susceptible to peroxidation in the pathophysiology of iron deficiency anemia. Previous studies have reported that low manganese levels may cause accumulation of superoxides that could consequently cause oxidative stress and associated complications [46]. Correlating the present finding with our previous relevant study [6] the iron doses applied for correcting iron deficiency anemia may further elevate the lipid peroxidation products, principally due to increased bioavailability of elemental free iron in gastrointestinal mucosal cells of patients, leading to probability of decreased level of Mn. Also, significant depletion of plasma level of manganese ($p < 0.001$) may trigger the oxidative stress in iron deficiency anemia during pregnancy. Further work along this significant objective and notion is in progress.

Conclusively, on the basis of our results, iron deficiency anemia leads to decrease the essential trace minerals and iron and folic acid supplementation recovered these metals, except manganese that may probably lead to various complications including peroxidation of vital biomolecules resulting in increased risk for pregnant women as well as fetus.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

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