Effect of Iron Supplementation on Plasma Lipid Levels in Pregnant Anemic Women

Running Title: Iron Supplementation and Lipid Profiles in Pregnant Women

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Abstract: <u>Background & Aims:</u> The present study deals with an investigation on the effect of iron deficiency anemia and iron supplementation on anemia markers and plasma lipid profiles in pregnant women. <u>Methods:</u> A total of 350 pregnant women were taken and divided into four groups on the basis of haemoglobin levels viz. control (100), mild (100), moderate (100) and severe (50) patients. All the groups were treated orally with 100 mg of iron and 500 µg of folic acid daily for hundred days. <u>Results:</u> Iron deficient pregnant women reflected a significant increase in plasma low density lipoprotein (LDL), very low density lipoprotein (VLDL), triglyceride (TG), lipid peroxide levels (LPx) and serum transferrin receptor levels (sTfR) while reduced concentration of plasma high density lipoprotein (HDL), iron (Fe), total lipid (TL), total cholesterol (TC) phospholipid (PL), serum ferritin and haemoglobin (Hb) and haematocrit (Hct) levels. Iron supplementation with folic acid indicated reverse changes in plasma Hb, Hct, Fe TL, TC, PL, TfR and ferritin except HDL levels. Moreover, iron treatment increases the rate of LPx, LDL, VLDL and TG levels. <u>Conclusion:</u> Besides, the results entails that iron supplementation returns to Hb levels but at the cost of lipoprotein oxidation especially in control and base line anemic women. Conclusively data of this study provide new insights into exploring a relationship between iron status and lipoprotein oxidation. However, further studies, including bigger sample size are mandatory to further irresolution of this problem.

Keywords: Pregnancy, iron deficiency, iron supplementation, lipid profiles, pregnancy outcome

1. Introduction

Iron deficiency is the most widespread micronutrient nutritional deficiency. Women tend to have substantially lower iron stores, making them more vulnerable to iron deficiency when iron intake is lowered or need increases. Iron supplementation is the most widely used approach to controlling the global problem of iron deficiency anemia. It is recognized in terms of excess body iron and "free iron" stimulating lipid peroxidation followed by leading to cell and tissue damage [1-2].

Iron deficiency led anemia is characterized by salient modified features of erythrocytes, including decreased cellular deformability due to anomalies in the structure and function of erythrocyte membrane, microcytosis and hypochromia. Increased susceptibility of these microcytic red blood cells to in vitro oxidative stress has also been demonstrated [3]. Indeed, alterations in proteins and lipids may often be more important in oxidative stress situations in vivo, since they provide the viscoelastic properties essential necessary for membrane deformability [4]. While the length and degree of unsaturation of phospholipid fatty acids particularly influence the lipid bilayer fluidity [5], the proteins and glycophorin play a crucial role in maintaining erythrocyte integrity and antegenecity.

Although studies have pronounced modifications in the erythrocyte membrane structure and function in iron deficiency anemia [6], few and rather conflicting data are available on the Indian population with IDA [7].

Lipid metabolism alters during pregnancy [8]. The anabolic phase of early pregnancy encourages lipogenesis and fat storage in preparation for rapid fetal growth in late pregnancy [9]. Normal pregnancy is associated with the formation of susceptible oxidizable particles (high LDL score) and an increase in oxidative damage. Iron deficiency is an independent risk factor for the development of coronary artery disease and is also associated with the development of an atherogenic lipid profile and increased oxidative stress [10]. Pregnant women with iron deficiency may further amplify these changes. Epidemiological studies have shown that low serum iron-binding capacity and high serum iron concentrations following abnormal levels of blood lipids are risk factors for coronary artery disease (CAD) and myocardial infarction [11-12]. Despite the great deal of information available on the hematological and biochemical changes in iron deficient anemic women, very limited studies have been carried out to investigate the lipid profiles in women with iron deficiency. A study conducted by Choi et al. [13] revealed lipid levels in patients with iron deficiency anemia to be directly related

to the level of iron. The total cholesterol level in the severely anemic group was observed to be significantly lower than that found in the other group. The triglyceride level was twofold higher in subjects with haemoglobin > 14.0 g/dl than in subjects with haemoglobin < 8.0 g/dl. The serum lipid concentrations in the females with severe anemia were significantly higher after iron supplementation. The study showed that blood haemoglobin levels correlated significantly with serum cholesterol concentrations. The results of the above study were contradicted by a study done accomplished by Tanzer et al. [14], according with higher serum total triglyceride, total cholesterol, and VLDL levels in iron deficient patients than in healthy controls. A study revealed that triglyceride, total HDL and LDL cholesterol levels having been higher during pregnancy except LDL-cholesterol that remained constant [15]. There is a study reported that serum triglyceride, total cholesterol LDL-c levels increased gradually as pregnancy proceeded except HDL-c level did not change significantly during pregnancy [16]. Therefore, the present study was undertaken to investigate the changes in iron status, oxidative stress and lipid levels in response to iron deficiency and iron supplementation in pregnant anemic women.

2. Materials and Methods

The study was approved by the Institutional Ethical Committee of King George"s Medical University, Lucknow, U.P., India and the objectives of the study were explained and a written concept was taken from each subject. The study was performed in the Department of Biochemistry, King George"s Medical University, Lucknow, U.P., India. The present study comprised of 250 pregnant anemic women [viz. mild (100), moderate (100) and severe (50)] aged between 20-40 years and pregnant non-anemic women as control (100), Hb as > 11 g/dl ranging in same age. The subjects were selected amongst those attending the Department of Obstetrics and Gynaecology, King George"s Medical University, Lucknow, U.P., India. Care was taken to ensure that all the subjects belonged to middle socioeconomic group (who were able to meet the basic necessities of life and have same requirements of confort). Selected subjects were all consumers of normal mixed Indian diet, not taking any drug for preceding one 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 [17]. Exclusion criteria were Hb less than 6.5 g/dl, no Hb rise by 1% after 3-week of iron-folic supplementation, alcoholic, smoker, metabolic diseases such as diabetes mellitus, malignancy, heart disease, infections such as tuberculosis, HIV, regularly using minerals/vitamins supplements and endocrine disorders.

Treatment

Each subject participated in the study for 100 days. At recruitment, all pregnant women (non-anemic and anemic) were first dewormed by giving them a single dose of Albendazole following Metronidazole (400 mg) three times daily for five days. Two days later i.e. after one week of recruitment, all pregnant women were given iron supplements (100 mg as ferrous sulphate and 500 mg folic acid) orally once a day, daily for 100 days. After three weeks of iron supplementation, we assessed Hb; if Hb did not raise then the subjects were excluded from the study and referred to other investigations. All blood collections were done in the Department of Obstetrics & Gynaecology, King George"s Medical University, Lucknow, U.P., India. The subjects were instructed not to change their dietary or activity habits during the study. After deworming, venous blood of all women (non-anemic and anemic) were taken for the estimation of biomarkers of blood index values, iron status, lipid profiles and oxidative stress parameter (pretreatment). All tests were repeated after 100 days of treatment (post-treatment).

Sample Collection

6 ml venous blood was taken from each of the subjects and divided into three aliquots. One ml of blood was transferred to an evacuated tube containing EDTA solution used to determine Hb and Hct. Three milliliters of whole blood was also transferred into EDTA containing tube and then centrifuged; plasma separated and used for the estimation of Fe, LPO, total lipid, total cholesterol, HDL, LDL, VLDL, triglyceride and phospholipid. Serum from remaining 2 ml blood after clotting separated out and collected in clean centrifuge tube and again centrifuged for five minutes at 3000 rpm. The serum thus obtained was used for the estimation of ferritin and transferring receptors levels.

Analytical Estimation

Blood haemoglobin was determined by using the cyanomethemoglobin method [18]. Haematocrit (Hct) was determined by using Sysmax A-380 automated cell counter. The concentration of iron in plasma was measured with flame atomic absorption spectrophotometer (Perkin Elmar AAS-700 Ueberlinger, Germany) [19]. Serum ferritin and serum transferrin receptor levels were determined by enzyme-linked immunosorbentassay kits (Spectro Ferritin, S-22 and TfR, TF-94, Ramco Laboratories Inc, Houston, Texas, USA). Serum total lipid, total cholesterol, high-density lipoprotein, triacylglycerol and phospholipids were determined using Randox Laboratory kit reagents and VLDL was calculated using the formula TG/2.2 mmol/L. Low density lipoprotein (LDL) cholesterol was determined by differential substraction of the sum of the cholesterol fractions from the total cholesterol.

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 Neuman-Keules 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).

3. Results

Effect of Iron and Folic Acid Supplementation on Anemic Markers in Pregnant Anemic Women

The pre and post-treatment bold index parameters of all pregnant healthy and anemic women are summarized in **Table 2**. The Haemoglobin (Hb) level in venous blood of healthy pregnant women was 12.19 ± 0.90 mg/dl. On the other hand, it was found decreased in „anemic condition‟ viz. mild (14%; p<0.001), moderate (34%; p<0.001) and severe (49%; p<0.001) subjects. After treatment with iron and folic acid (100 mg and 500 mg/d) for 100 days, the levels of Hb were reversed significantly in control (10%: p<0.001), mild (16%; p<0.001), moderate (33%; p<0.001) and severe (28%; p<0.001) pregnant anemic women. We also observed that haematocrit (Hct) in venous blood of control group was 35.84 ± 5.43%. However, this level was found significantly decreased in different groups of pregnant women, who were under anemic; such as mild (13%; p<0.001), moderate (20%; p<0.001) and severe (35%; p<0.001). Treatment with iron and folic acid increased the level of Hct in control (9%; p<0.01), mild (11%; p<0.001), moderate (13%; p<0.001) and severe (13%; p<0.001) men. Similarly, iron (Fe) level in blood plasma of mild (10%; p<0.01), moderate (43%; p<0.001) and severe (68%; p<0.001) anemic pregnant women was found decreased as compared with control group. Treatment with the drug restored the levels of Fe in control (44%; p<0.001), mild (40%; p<0.001), moderate (35%; p<0.001) and severe (32%; p<0.05) women.

Ferritin levels in blood serum of control group were 37.72 ± 12.96 μg/L. On the other hand, these levels were found significantly decreased in different groups of pregnant anemic women, i.e. mild (10%; p<0.01), moderate (46%; p<0.001) and severe (68%; p<0.001) when compared with control. After treatment with elemental iron and folic acid (100 mg and 500 mg/d) for 100 days showed a significant reversal in control, mild, moderate and severe by (44%; p<0.001), (40%; p<0.001), (35%; p<0.001) and (32%; p<0.05), respectively when compared with its respective pre-treated groups. We also observed sTfR level in blood serum of control group was 3.69 ± 1.64 mg/L. However, this level was found significantly increased in different groups of pregnant anemic women, such as mild (15%; p<0.01), moderate (35%; p<0.001) and severe (48%; p<0.001). Treatment suppressed the level of sTfR in control (41%; p<0.001), mild (35%; p<0.001) moderate (28%; p<0.001) and severe (20%; p<0.01) pregnant women when compared with its respective pre-treatment subjects.

Effect of Iron and Folic Acid Treatment on Lipid Profiles in Pregnant Anemic Women

We observed alterations in the lipid profiles (TL) following iron and folic acid (100 mg and 500 mg/d) for 100 days treatment (**Table 3**). In normal healthy pregnant women total lipid (TL) level was 9.77 ± 3.30 mg/dl, total cholesterol (TC) was 4.27 ± 1.94 mmole/L and phospholipid (PL) was 2.25 ± 0.31 mmole/L. It was found that in iron deficient pregnant anemic women viz. mild,

moderate and severe patients there was a decline in their TL level by 10% (p>0.05), 40% (p<0.001) and 61% (p<0.001); TL by 8% (p>0.05), 19% (p>0.05) and 29% (p<0.01) and PL by 2% (p>0.05), 7% (p<0.05) and 9% (p<0.01), respectively as compared with control. Treatment of above mentioned subjects with iron and folic acid showed reversal of above aforementioned lipid parameters. Our results showed that following treatment, the TL levels of control, mild, moderate and severe anemic women were significantly increased by 27% (p<0.001), 28% (p<0.001), 41% (p<0.001) and 34% (p<0.05), respectively when compared with their respective pre treatment parameters. Similarly, TC levels were also found significantly increased by 38% (p<0.001), 39% (p<0.001), 44% (p<0.001) and 41% (p<0.05). PL levels were also increased in post treated patients when compared with its pre treated groups. It was observed that the triglyceride (TG) level in blood plasma of control was 1.28 ± 0.20 mmole/L. However, these levels in pre treated mild, moderate and severe anemic pregnant women were increased by 7% (p<0.05), 18% (p<0.001) and 33% (p<0.001), respectively. Furthermore, after treatment the levels of TG were elevated in control (9%; p<0.01), mild (7%; p<0.05), moderate (4%; p>0.05) and severe (5%; p>0.05) pregnant women when compared with their respective pre treated levels. Table 3 shows that the pre-treatment mean levels of lipid peroxide in the blood plasma of pregnant non-anemic women (control) was 2.33 ± 0.31 nmole MDA/mg protein. The pre-treatment mean levels of these parameters were significantly (p<0.01 or p<0.001) increased in all groups of anemic women when compared with controls. Furthermore, after treatment the levels of LPx were also elevated in control (24%; p<0.001), mild (19%; p<0.001), moderate (11%; p<0.001) and severe (9%; p<0.01) women when compared with respective pretreated levels.

Status of Blood Plasma Lipoproteins before and After Treatment with Iron and Folic Acid in Pregnant Women

Table 4 shows that the levels of lipoproteins like LDL and VLDL in the blood plasma of pregnant non-anemic women (control) were 2.23 ± 0.51 mmole/L, 0.287 ± 0.07 mmole/L, respectively. These parameters were significantly increased in all groups of anemic women when compared with controls. Treatment with iron and folic acid further increased the levels of LDL in control (p<0.05), mild, moderate and severe women. Furthermore, after treatment the levels of VLDL were also elevated in control, mild, moderate and severe anemic women when compared with pre-treated levels. We also observed that the level of HDL in the non-anemic women (control) was 1.45 ± 0.44 mmole/L. This parameter was decreased in all pre-treated groups of anemic women as compared with controls. After treatment these were further decreased in control, mild, moderate and severe anemic and non anemic women when compared with pre-treatment levels.

Pregnancy Outcomes after Treatment

Birth outcomes after medication with iron and folic acid are depicted in Table 5. Our results showed that the term

Paper ID: NOV152814

715

delivery was present in 88% with control, 84% of women with mild anemia, 85% with moderate and 83% in severe anemia. Preterm delivery was occurred in 12%, 16%, 15% and 17% of control, mild, moderate and severe subjects, respectively. Mode of delivery like normal vaginal was encountered in 71%, 77%, 82% and 87% of control, mild, moderate and severe post treated groups. Instrumental and cesarean delivery were occurred in 8%, 7%, 4% and 2% and 21%, 16%, 14% and 11% of control, mild, moderate and severe patients after treatment. Our results were also found that the baby birth weight of control was 2823.62 ± 367 g. This birth weight was non-significantly decreased in mild and moderate pregnant anemic patients except severe group (p<0.05) when compared with post treated control subjects.

4. Discussion

Pregnancy generally induces significant metabolic changes. The concentrations of lipids, lipoproteins and apolipoproteins in the plasma increase appreciably during pregnancy, principally affected by maternal hormonal changes such as rise in insulin, progesterone, 17-β estradiol and human placental lactogen. The data of the present study reported that treatment of iron deficient anemic women with oral iron (100 mg) and folic acid (500 μg) daily for 100 days significantly improved Hb and and Hct levels. Recovery of Hb after supplementation of iron with folic acid in patients may be due to the fact that iron is an essential constituent of heame and folic acid enhances the erythropoietic mechanism [20]. Plasma iron levels were observed to be significantly decreased in anemic women and recovered enormously in all supplemented groups. Women of reproductive age lose iron during menses, and having a substantially higher need for iron during pregnancy, because of the increase in red cell volume of the mother and placental and fetal growth [21]. Ministry of Health, Government of India has recommended intake of 100 mg of iron and 500 μg of folate per day for 100 days in the second half of pregnancy [22]. Tiwari et al. [23] and King et al.[24] had also reported that daily oral iron, in form of ferrous sulphate (100 mg) with folic acid (500 μg) daily for 100 days and 98 mg of ferrous sulphate alone for 56 days daily improved iron in iron deficient women. Present finding showed that ferritin levels were found decreased in different group of pregnant anemic women, however, serum transferrin receptor levels (sTfR) were registered to increase in these subjects. Treatment significantly revert the levels of above aforementioned parameters. Present results are similar to previous reports [25-26]. 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.

In the present study, we also observed significantly increased levels of plasma lipid peroxide levels (LPx) in women with iron deficiency anemia and further elevated after treatment. Moreover, we found that in the control and mild group less iron is utilized instead of moderate and severe anemic patients, which leads to make available more free iron in the body that in term may eventually further enhance the oxidative stress levels in this group. There have been reports that lipid peroxidation was significantly accelerated in iron deficiency anemia [27-28]. 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. There are reports that the iron doses used for correcting iron deficiency anemia may further elevate the lipid peroxidation products, mainly due to increased bioavailability of elemental free iron in gastrointestinal mucosal cells of the subjects [20-23].

We observed alterations in the lipid profiles in iron deficient pregnant anemic women (**Table 3**). The concentration of total lipid, total cholesterol and phospholipid were found to be significantly decreased in pregnant anemic women. The reduction of lipids may be correlated with the iron deficiency anemia induced increased lipid peroxidation. These results are similar to those reported earlier in pre-eclampsic pregnant women [29]. It may be suggested that an excess of ROS in iron deficient patients caused the oxidative degradation of RBC lipids resulting in decreased level of lipids in these patients. The RBC is an important source of oxygen-related radicals in iron deficiency anemia (IDA). However, IDA, RBCs produces greater quantities of $O_2^{\cdot-}$, H_2O_2 and OH° than do normal RBC [30]. Besides, iron deficiency anemia, RBCs exhibit increased levels of thiobarbituric acid-reactive substances, suggesting that they are targets for oxidative stress [31]. During deoxygenation of haemoglobin, there is a transfer of electrons between iron atom and O_2 leading to the production of $O_2^{\cdot-}$. Physiological auto-oxidation of haemoglobin also leads to the production of free radicals. Within the RBC, one of the targets of oxidant damage is the plasma membrane. In the presence of $O_2^{\cdot-}$ generating system, iron (III) is reduced to iron (II) with subsequent formation of OH° and H_2O_2 [30-31]. The hydroxyl radical oxidizes unsaturated esterified membrane lipids, resulting in changes in fluidity of the bilayer. Cholesterol in RBC membrane normally comprises more than 99% of the neutral lipids in RBC membrane, has been found to exchange freely between both the inner and outer layers of the bilayer and is in equilibrium with non-esterified (free) cholesterol attached to the plasma lipoproteins [32]. However, Zailaie et al. [33] reflected that increased rate of exchange between plasma cholesterol and RBCs membrane cholesterol may be due to loss of RBC membrane phospholipids asymmetry following increase in oxidative stress, allowing more plasma cholesterol to be incorporated in RBC membrane possibly to retain some of the loss in RBC membrane integrity. After treatment with iron a significant enhancement in lipid levels of blood plasma profile was observed as compared with pre-treatment groups. These results are consistent with the reports of investigators [13-34], demonstrating that dietary iron supplementation

increases plasma lipids in iron deficient girls and in rats. Ohira et al. [35] found that total cholesterol concentrations were elevated following an increase in haemoglobin levels by transfusion and iron treatment. They also reported that the concentration of red blood cells might affect cholesterol synthesis or its mobilization from tissue to plasma.

Triglycerides were found to be elevated in iron deficient anemic women as compared with the controls and further increased after oral iron treatment. The increase in serum TG may be due to hypoactivity of lipoprotein lipase in blood vessels that breaks up TG. The concentration of HDL was found to be reduced following iron deficiency anemia and after iron supplementation (**Table 4**). Increased levels of LPO or VLDL and LDL in pre treated patients and even maximum increment of these lipoproteins in control and mild post treated subjects as compare to moderate and severe anemic patients are known to be cytotoxic to cells and tissues. Recently it has been emphasized that hyperlipemic β-lipoproteins are cytotoxic to cells and tissues presumably due to enhanced levels of associated LPO [36]. Lipids play significant role in various disorders like cardiovascular, hyperlipidemia fatty liver. It modifies the composition, structure, and stability of cellular membranes. Excess lipid in the blood is considered to accelerate the development of arteriosclerosis. An altered lipid metabolism can alter the cardiac function by changing the properties of cardiac cell membrane and these changes may contribute to the cell death. Recent reports indicate that cytotoxicity induced by LDL and VLDL can result from lipoprotein oxidation. Ultimately indicated by the relative amount of MDA equivalents or thiobarbituric acid-reacting substances (TBARS) residing on the lipoprotein [37].

Contrary the results of Salonen et al. [38], which would suggest that persons with iron deficiency should not be treated with supplements due to possible adverse events, the results of the present experimental study showed that measures of oxidative stress were affected by a therapeutic dose of iron in women with low iron status. This study clearly demonstrates that the levels of LPO, LDL and VLDL were increased maximum in control and mild than remaining groups because less iron is utilized in these groups due to different individual requirements facilitates the increasing oxidative stress. However, we can say that 100 mg of iron is excess for control and mild groups as moderate and severe patients. In our study, Iron status was improved as shown by an increase in ferritin, which is the most reliable marker of iron status, although the lipoproteins oxidation were affected by supplement intake in control and mild group instead of moderate and severe.

5. Conclusion

The results indicate that a therapeutic dose of iron affect measures of oxidative stress despite improved iron status in subjects receiving the supplement as considered by a significant increase in ferritin, and hemoglobin with alteration in lipid profiles and lipoproteins, although the support a probable relationship between iron status and lipoproteins oxidation is affected by aggressive supplementation with iron to improve iron status. Iron supplementation, at the dose we used, independently affects LDL oxidative susceptibility. Conclusively, as excess iron in fact plays a role in increasing risk of LDL oxidation during pregnancy.

Conflict Of Interest

None

Acknowledgement

References

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Paper ID: NOV152814   717

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Paper ID: NOV152814

Table 1: Characteristics of the subjects

Characteristics	Control (n=100)	Mild (n=100)	Moderate (n=100)	Severe (n=50)
Age (yrs)	26.12 ± 6.21	25.37 ± 7.71	25.88 ± 6.54	24.15 ± 5.17
Height (cm)	150.55 ± 4.52	150.16 ± 4.48	149.73 ± 3.34	150.02 ± 4.19
Literacy (%) illiterate				
< 10th grade	46 (46%)	51 (51%)	47 (47%)	23 (46%)
>10th grade	54 (54%)	49 (49%)	53 (53%)	27 (54%)
Parity (%)				
1st Pregnancy	16 (16%)	13 (13%)	11 (11%)	12 (24%)
≥ 2nd Pregnancy	86 (86%)	87 (87%)	89 (86%)	38 (76%)
History of miscarriages (%)				
Yes	10 (10%)	13 (13%)	15 (15%)	7 (14%)
No	90 (90%)	87 (87%)	85 (85%)	43 (86%)
Socioeconomic status (%)				
Low	15 (15%)	20 (20%)	21 (21%)	16 (32%)
Middle	69 (69%)	63 (63%)	65 (65%)	28 (56%)
High	16 (16%)	17 (17%)	14 (14%)	6 (12%)
Occupation (%)				
Housewife	97 (97%)	98 (98%)	97 (97%)	50 (100%)
Informal business	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Employee/students	3 (3%)	2 (2%)	3 (3%)	0 (0%)
Gestational age at recruitment (wk)	21.33 ± 2.56	19.72 ± 3.18	21.36 ± 2.32	22.61 ± 3.15

Table 2: Blood index values (mean ± SD) before and after iron and folic acid supplementation in subjects

Parameters	Control (N=100)		Mild (N=100)		Moderate (N=100)		Severe (N=50)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Hb	12.19 ± 0.90	13.46 ± 1.22***	10.49 ± 0.23[a]	12.18 ± 0.59***	8.03 ± 0.54[a]	10.70 ± 1.20***	6.73 ± 0.22[a]	8.65 ± 0.67***
Hct	35.84 ± 5.43	39.08 ± 5.47***	31.06 ± 2.77[a]	34.44 ± 2.93***	28.55 ± 3.86[a]	32.52 ± 3.46***	23.19 ± 2.25[a]	26.12 ± 2.77***
Fe	41.94 ± 7.56	58.87 ± 10.62***	38.86 ± 8.32[b]	51.30 ± 9.37***	23.97 ± 5.42[a]	30.47 ± 6.40***	19.64 ± 3.04[a]	24.12 ± 3.34**
Ferritin	37.72 ± 12.96	54.38 ± 11.92***	34.04 ± 8.96[b]	47.66 ± 10.12***	20.46 ± 8.44[a]	27.65 ± 10.18***	11.99 ± 3.06[a]	15.88 ± 3.15*
TfR	3.69 ± 1.64	2.19 ± 0.90***	4.26 ± 1.80[b]	2.78 ± 1.33***	4.99 ± 1.79[a]	3.59 ± 1.39***	5.46 ± 1.74[a]	4.38 ± 1.56**

Haemoglobin (Hb) and iron (Fe) are expressed in mg/dl. The values of Haematocrit (Hct), ferritin and sTfR (serum transferring receptor levels) are expressed in %, µg/ L and mg/L, respectively.
Significance: [a]$P < 0.001$ and [b]$P < 0.01$ as compared with the pre control group.
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ as compared with the pre treatment group.
Pre = pre treatment; Post = post treatment

Table 3: Lipid profiles (mean ± SD) before and after iron and folic acid supplementation in subjects

Parameters	Control (N=100)		Mild (N=100)		Moderate (N=100)		Severe (N=50)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
TL	9.77 ± 3.30	12.44 ± 3.21***	8.75 ± 2.53	11.19 ± 2.74***	5.85 ± 2.49[a]	8.26 ± 2.84***	3.81 ± 1.82[a]	5.11 ± 1.81*
TC	4.27 ± 1.94	5.89 ± 2.41***	3.93 ± 1.88	5.48 ± 2.46***	3.45 ± 1.63	4.96 ± 1.96***	3.02 ± 1.13[b]	4.27 ± 1.69*
TG	1.28 ± 0.20	1.40 ± 0.18**	1.37 ± 0.22	1.47 ± 0.20**	1.51 ± 0.35[b]	1.57 ± 0.35*	1.70 ± 0.31[b]	1.78 ± 0.30
PL	2.25 ± 0.31	2.36 ± 0.35	2.20 ± 0.33	2.26 ± 0.34	2.10 ± 0.41[c]	2.19 ± 0.42	2.04 ± 0.29[b]	2.10 ± 0.28
LPx	2.33 ± 0.31	2.88 ± 0.39***	2.57 ± 0.43[b]	3.05 ± 0.49***	3.13 ± 0.75[a]	3.49 ± 0.85***	4.15 ± 0.73[a]	4.52 ± 0.78**

Total lipid (TL) is expressed in mg/dl. Total cholesterol (TC), triglyceride (TG) and phospholipids (PL) are expressed in mmole/L. Lipid peroxide levels is expressed in nmole MDA/mg protein.

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.

Pre = pre treatment; Post = post treatment

Table 4: Lipoprotein index profiles (mean ± SD) before and after iron and folic acid supplementation in subjects

Parameters	Control (N=100)		Mild (N=100)		Moderate (N=100)		Severe (N=50)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
LDL	2.23 ± 0.51	2.43 ± 0.55[*]	2.36 ± 0.56	2.58 ± 0.60	2.48 ± 0.61[c]	2.67 ± 0.59	2.62 ± 0.62[a]	2.78 ± 0.65
VLDL	0.287 ± 0.07	0.295 ± 0.07	0.302 ± 0.08	0.310 ± 0.08	0.345 ± 0.12[b]	0.351 ± 0.12	0.435 ± 0.17[a]	0.440 ± 0.17
HDL	1.45 ± 0.44	1.34 ± 0.42	1.40 ± 0.38	1.30 ± 0.37	1.34 ± 0.33	1.25 ± 0.33	1.25 ± 0.28	1.17 ± 0.29

The value of low density lipoprotein (LDL) is expressed in mg/dl. Very low density lipoprotein (VLDL), high density lipoprotein (HDL) and phospholipids (PL) are expressed in mmole/L.

Significance: [a]$P < 0.001$, [b]$P < 0.01$ and [c]$P < 0.05$ as compared with the pre control group.

[*]$P < 0.05$ as compared with the pre treatment group.

Pre = pre treatment; Post = post treatment

Table 5: Birth outcomes in the post-treated groups

Variable	Control (n=100)	Mild (n=100)	Moderate (n=100)	Severe (n=50)
Term delivery (%)	88	84	85	83
Preterm delivery (%)	12	16	15	17
Birth weight (g)	2823.62 ± 367	2781.7 ± 441	2727.3 ± 457	2575.7 ± 482
Mode of delivery (%)				
Normal vaginal delivery	71	77	82	87
Instrumental delivery	8	7	4	2
Cesarean section	21	16	14	11

There were no significant differences between the groups.