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Effects of Oral Iron Supplements in Pregnancy with IDA in Association with Oxidative Stress

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Abstract

Background: Iron deficiency anemia (IDA) is associated with adverse outcomes related to hemoglobin (Hb) and red cell indices and is now recognized as a major contributor to oxidative stress (OS). Maternal IDA during pregnancy is one of the most common nutritional deficiencies worldwide, exposing both the mother and developing fetus to the risk of OS. This study aims to identify the effects of IDA and oral iron supplements on oxidative stress during pregnancy.

Methodology: The IDA group comprised 90 pregnant women between 16 to 20 weeks of gestation. The study lasted 12 weeks with follow-up visits every 4 weeks. Two antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), were studied as OS parameters. Hemoglobin (Hb), serum ferritin concentration (SFC), and total iron binding capacity (TIBC) were also measured.

Results: The study found decreased levels of SOD, Hb, and SFC in the anemic group, which showed significant improvement after iron supplementation. GSH-Px and TIBC values were significantly lower compared to the control group. There was a direct relationship between SOD and Hb levels, with the highest SOD levels observed in conjunction with the highest Hb values.

Conclusion: Oral iron administration to treat IDA during pregnancy not only improves hematological parameters but also decreases oxidative stress by improving SOD levels. However, other micronutrient deficiencies might be responsible for the alterations in GSH-Px levels.

Keywords: IDA; oxidative stress; maternal anemia

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Introduction

High prevalence of Iron Deficiency Anemia (IDA) among women of reproductive age (WRA) is suggestive of its importance and negative effects on public health (1). Almost 41.9% of WRA are anemic in South-East Asia (2). Burden of anemia in developed countries, such as in Europe is 2–5%(3). However prevalence of IDA among WRA in Pakistan is 41.7% (4).

Maternal malnutrition has many worse effects on pregnancy outcome. Most common maternal deficiencies observed globally is IDA affecting health and wellbeing of mothers and offspring (5,6). Among the foremost contributing elements for pregnancy associated with IDA, low iron intake which is also associated with low bioavailability and an increased requirement have major role (7). Increase in iron demand during pregnancy is required for plasma dilution & expansion, fetal growth, placenta, and other fetal tissue development. Daily requirement of iron increases to 27mg/day during pregnancy (which is normally 8.7mg). This requirement cannot be fulfill by diet alone. World health organization (WHO) suggest iron supplementation program for treatment and prevention of IDA(8). The daily recommended dose for oral iron therapy is 100-200mg/day in divided doses(9).

Frequently IDA in pregnancy, worsen oxidative stress (OS) in maternal body because of imbalance between body's oxidant and anti oxidants defense system with excess production of reactive oxygen and nitrogen species (ROS/RNS)(10). IDA is also associated with decrease in anti-oxidants enzymes [Catalase, Superoxide dismutase(SOD)], & Glutathione Peroxidase (GSH-Px)(11).Outcomes of OS are particularly negative in the pre-natal & post-natal period . This is true because in fetal life rapidly multiplying cells and newly developing organ systems of fetus are quite sensitive to the harmful effects of ROS/RNS which are produced due to various environmental factors, metabolic conditions

or complications of pregnancy. OS in antenatal and neonatal period could be a possible cause for developing "oxygen radical disease in neonatology" resulting in increased risk for developing cardio, metabolic and immunological disorders in adult life(12).

OS is also a contributing factor for many chronic pathological processes in central nervous system (CNS), cardio vascular system (CVS) and inflammatory responses of body (13).Studies on animal models have also shown that prenatal IDA is adversely associated with CVS disorders in offspring(14). Risk in offspring associated with maternal OS are shown in Figure 1. OS can alter many essential reactions that disturb embryonic development. This occur through of gene expression modification, transcription factor signaling and alterations in cell cycle. It may affect baby's brain development or immune system(15). It can also be a reason behind sub fertility, miscarriages, maternal vascular disease and preterm labor.(16)

Considering importance of IDA in pregnancy, present study was aimed with primary objective of identifying effects of oral iron supplements in improving Hemoglobin and OS by measuring levels of two anti-oxidant enzymes (SOD & GSH-Px) . Secondary objectives were to study any relationship between the Hb concentration and studied antioxidant enzyme levels in IDA and normal pregnancy along with ferritin concentrations.

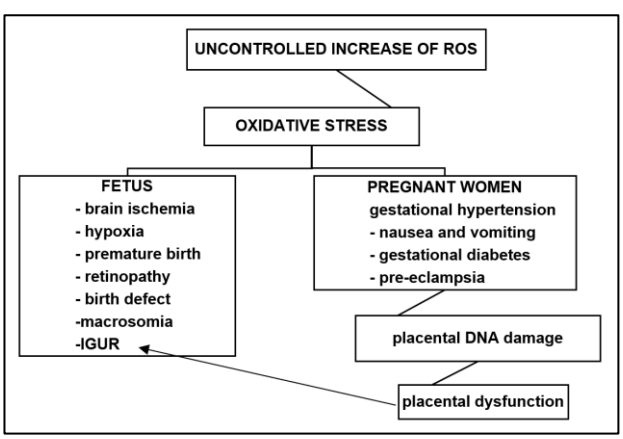

Figure 1. Fetal Risks associated with Maternal OS.

Methodology

Present study was designed for 12 weeks (wks). Study was carried out in a public sector teaching hospital of Karachi. Study was randomized, longitudinal in nature. Ethical approval was obtained from IRB. A sample size of 74 per group was calculated based on 5% significance level (two tailed test), with power of 80% and difference in Hb Concentration of 5g/L and standard deviation of 10g/L. With a dropout rate of 20% and rounding of the figure 90 pregnant women in each group (control & IDA) were required. Pregnant women, attending the outpatient department with single fetus of gestational age of 16 weeks and above with Hb value between 7g/dl to 11g/dl with no obstetric complications or any co morbidity were eligible for study. Women with history of any drug intake or iron supplements in current pregnancy or intolerant to iron supplements in any previous pregnancy were not included in the study. Detailed history of every enrolled woman was obtained at the start of study along with general physical and clinical examination. Gestational age was investigated both by the date of last menstrual period and ultrasound. Written consent was obtained for participation and purpose of study was explained at the time of enrollment. Hb was determined by using the Cynamethemoglobin method as mentioned by INCAG, 1985. Serum ferritin was determined using a commercial kit (Enzymun-Test Ferritin; Roche Diagnostics GmbH, Mannheim, Germany). Follow up visits were planned at 4 wks interval. In each follow up all the physical and clinical examinations and biochemical measurements were repeated except for SOD and GSH-Px which were measured only of enrollment and at last follow up visit. These women were also interviewed regarding side effects (heart burn, nausea, vomiting, diarrhea and constipation) of iron supplements in each follow up visit. Statistical analysis was done by using SPSS –22 software. The values were given as mean ± SEM. Student's t-test was used to compare between the two groups.

Results

All the women completed the study period. Table 1 shows selected general physical exam parameters. Table 2 shows the Hb, serum iron, SFC, TIBC and GSH-Px values of control and anemic women before and after iron therapy. Hb, SFC and iron values showed significant improvement (P value <0.05) after iron therapy when compared to their pre supplemental values. No significant difference in GSH-Px values of control and anemic women was noted. GSH-Px and TIBC values decreased with iron therapy, (P value <0.05) when compared with pre supplemental values and with the control group. SOD levels were also improved (P value <0.05) when compared with pre supplemental values of anemic group. SOD of control group and post supplemental values showed non-significant difference. Table 3 shows SOD and GSH-Px values according to tertiles of Hb. It was noted that SOD has direct relationship with Hb levels, having highest value of SOD in the highest group of Hb. GSH-Px shows inverse relation to Hb with lowest value in highest group of Hb.

Table 1. Selected physical parameters of women in the study. (Maternal age , Blood Pressure and Body Mass Index(BMI) at the time of enrollment .) Values are expressed as mean (±SEM)

Groups	Age (Yrs)	Blood Pressure (mmHg) Systolic	Diastolic	BMI Kg/m²
Daily Group (n=42)	24.8 (±6.2)	111.5 (±2.18)	69.54 (±1.86)	21.49 (±0.47)
Once weekly Group (n=46)	24.7 (±4.8)	116.23 (±2.46)	70.16 (±1.54)	21.38 (±0.38)

Table 2. Mean values of Hb, SFC, Serum iron, TIBC, SOD and GSH-Px of control and anemic group (expressed as mean ±SD Range).

	Control group N= 20	IDA Pre N=156	IDA POST
Hb g/dl	11.3±0.5(11.1-13.2)	9.4 ±1.1*(7.3-10.7)	12.28±1.01* (11-13.1)
SFC ng/liter	55±30.4(32-65)	20.15±9.15 *(10.9-35)	38.46±19.4.4*(11-62)
Serum iron ng/dl	128±57.25(133-245)	81.05± 35.61*(35-149)	129.13±70.45* (51-290)
TIBC	317±46.21(267-371)	522.4±54.32 $^{\delta}$(427-695)	415.5±46.71$^{\delta\varepsilon}$ (284-591)
SOD u/gHb	3173 ± 120(2915-3324)	1161±109*(729-1284)	3203 ± 120*(2915-3324)
GSH-Px u/gHb	28.41± 5.2(24.16-32.3)	34.28±3.1(20.12-39.2)	24.17±3.1$^{\gamma}$ $^{\delta}$ (19.6-26.8)

* P value <0.05(significantly greater when compared with pre supplemental values)
$^{\gamma}$ P value <0.05(significantly low when compared with control group)
$^{\delta}$ P value <0.05(significantly low when compared with pre supplemental values)
$^{\varepsilon}$ P value <0.05(significantly greater when compared with control group)

Table 3. Mean values of SOD and GSH-Px according to tertiles of Hb (expressed as Mean ±SD Range) including control and anemic group.

		Hb >7<11 g/dl n =156	Hb ≥11 <12 g/dl n=14	Hb ≥12 g/dl n=6
Hb g/dl	Mean ± SD Range	9.4±1.1(7.3-10.7)	11.5±0.4(11.1-11.9)	12.3±0.2*(12-13.2)
SOD u/gHb	Mean ± SDRange	1161± 109 (729-1284)	3138 ± 101 (2915-3241)	3268± 114* (2991-3324)
GSH-Px u/gHb	Mean ± SDRange	34.28±3.1 (30.12-39.2)	30.14± 1.8 (26.41-32.1)	26.68± 1.6$^{\delta}$ (24.16-32.3)

*P value <0.05(significantly greater when compared with values in lowest tertile of Hb)
$^{\delta}$P value <0.05(significantly low when compared with values in lowest tertile of Hb)

Discussion

Iron Deficiency (ID) is a major contributing factor for the development of IDA (17). It is associated not only with ineffective erythropoiesis but also effects sufficient production of many iron containing compounds like myoglobin, cytochromes, catalases and peroxidases (18). Several iron containing compounds are involved in scavenging free radicals of body hence prevent OS. Although iron supplements have proved their efficacy in improving hematological parameters but have shown alteration in antioxidant enzymes of body. Erythrocytes have highly active antioxidant enzyme like CuZn-SOD, GSH-PX (19). It has been reported that daily iron supplements are an ultimate source of OS due to imbalance produces by them on enzymatic antioxidants. Another possible reason behind this OS caused by iron supplements is due to the fact that iron itself is a transition metal and has the ability to gain and accept electrons (13, 20, 21). Various studies have reported low levels of GSH-Px in IDA (22, 23). Though studies showing similar activity of GSH-Px as for the non-anemic control group are also available. (24, 25). Findings of our study also suggested a negative effect of oral iron supplements on GSH-Px. This is in accordance with the results of some other studies (24, 25). Studies involving minerals and vitamin supplements to both IDA and non-anemic groups have documented the improvement of antioxidant enzymes hence suggesting the involvement of different other minerals and vitamins for OS (26, 27). Yet another reason that could be associated with increased GSH-Px production, is increased NADPH production by pentose phosphate pathway enzymes (28). These enzymes are increased in IDA and ultimately GSH-Px activity even in IDA is maintained close to the normal control values. Iron given in form of oral supplements either in large amount or in daily dosing schedule also results in increase of non-transferrin bound iron (NTBI) in blood (29) causing OS systemically (30) or locally associated with inflammatory responses(31). OS due to daily iron supplements could be improved by intermittent iron supplements as suggested by various studies during pregnancy (32). Other findings of our study include decrease activity of SOD in IDA as suggested by other researchers (33,34). ROS mainly hydrogen peroxide is found to be enhanced and has free radical activity. This ultimately results in increase utilization of SOD (11). This is more marked in OS under hypoxic conditions particularly with different mineral deficiencies (24). Trace metals are found to have a very important role in antioxidant defense mechanism by direct contribution in the generation of antioxidant enzymes. Zinc (Zn), in conjunction with copper (Cu), serves as a cofactor for Cu/Zn-SOD, whose activity is compromised under Zn-deficient conditions (35). Both Fe deficiency and iron supplements during pregnancy are found as cause of OS with adverse outcomes for fetal development (35), with a higher risk of miscarriage, premature deliveries, low birth weights, and small for gestational age (SGA) (35).

Conclusion

Results of our study are suggestive for favorable outcome of oral iron supplements to treat IDA during pregnancy. Our study exhibited a positive relation of Hb level with serum iron and SFC. We also noted an imbalance of antioxidant enzymes namely GSH-Px and SOD in IDA as compared to non-anemic population. We found that oral iron supplements particularly the daily doses cannot restore the deficiency of antioxidant enzymes properly suggesting other mineral deficiencies are also responsible for OS. With these findings we may postulate that other trace metals like Zn, Cu etc should also be provided during IDA treatment. Limitations of our study included small sample size with only daily iron supplements. Hence more detailed studies with different dosing options are required to find out more close association of trace metal supplements during IDA to combat OS.

Ethical Approval:
This study was approved by Institutional Review Board Committee of the Jinnah Postgraduate Medical Centre, Karachi.
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Conflict of interest: None declared.

Authors' Contribution:
SK: Study design, research and drafting
SZ & AAH: Data collection.
SSA: Drafting, proofreading
HSIH: Statistical analysis

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