

Decreased activity of glutathione peroxidase with oral ferrous iron administration: A cause of oxidative stress

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Abstract: Iron deficiency anemia (IDA) during pregnancy not only results in the disturbance of hematological parameters but has been found to be an additional cause of oxidative stress (OS). Moreover, oral iron for IDA during pregnancy has been found to worsen the condition. Present study aims to detect any alteration in glutathione peroxidase (GSH-Px) activity as an enzymatic anti-oxidant and its association with oral iron supplements. For study, 40 anemic pregnant women were prescribed with 200mg ferrous sulphate for 12 weeks (wks). A significant improvement in hemoglobin (Hb) and serum ferritin concentration (SFC) was seen after treatment ($p < 0.05$). In contrast to the values for hematological parameters, GSH-PX was found to be same for control and anemic groups before iron supplements ($p > 0.05$). A significant decrease in GSH-PX levels of anemic women was seen after iron therapy when compared with both the initial values and the control group ($p < 0.05$). We found a positive association between oral iron administration and OS. Our results showed a strong association between oral iron supplements and SFC which supports the iron overload theory. It is therefore concluded that OS is associated with oral iron supplements during pregnancy.

Keywords: Anemia, Iron-deficiency, oxidative stress, glutathione peroxidase, pregnant women.

INTRODUCTION

Nutritional iron deficiency is the most important cause of iron deficiency anemia (IDA) particularly during pregnancy (WHO, 2001). According to WHO, in developed countries about 23% of pregnant population suffers from IDA. While in developing countries including Pakistan this problem seems to be raised up to almost half of the pregnant population (WHO, 2008). Anemia during pregnancy frequently results in poor fetal and maternal outcome and even perinatal morbidity and mortality (Bothwell 2000).

As demand for iron increases during pregnancy which is mostly not fulfilled by diet alone, WHO recommends daily oral iron supplements during pregnancy and for postpartum period (Stoltzfus & Dreyfuss 1998). Disadvantages of oral iron supplements during pregnancy are mainly associated with the excess of free iron in intestine and iron overload resulting in OS, both locally and systemically (Milman, 2012).

An imbalance between the body's oxidant and anti-oxidant system results in OS. This imbalance could be due to excessive free radical production or due to deficiency of anti-oxidant defense systems naturally present in the cells. These changes are seen in many pathological and some physiological conditions. Pregnancy is a normal physiological condition but is associated with alterations in many metabolic and physiological functions (Dotsch *et al.*, 2001). Pregnancy

favors OS mostly because of placenta which is rich in mitochondria (Casanueva *et al.*, 2003). Moreover OS is found to be worsened with anemia (Yoo *et al.*, 2009) and particularly IDA during pregnancy (Chandra *et al.* 2011). Another factor that is mostly seen to cause OS during anemic pregnancy is the oral iron supplementation (Viteri *et al.* 2012). Iron, a transitional metal with the ability to generate free radicals, may encourage damage to the intestinal epithelial cells. In order to decrease the adverse effects associated with routine daily iron supplements intermittent iron supplements are prescribed as they have been found to be effective in maintaining hematological values (Khalid *et al.* 2012).

Studies have shown that even in normal pregnancy there are alterations in anti-oxidant levels of body (Adiga and Adiga, 2009). Patil *et al.* (2006) has reported that vitamins A, E and C, which serve as non-enzymatic anti-oxidants, are decreased in normal pregnancies. Although for non-pregnant population contrasting results for the antioxidants in IDA have been reported (Coghetto *et al.*, 2009).

Present study was designed to assess the activity of GSH-PX as an anti-oxidative status in local iron deficient pregnant population and any variation in GSH-PX levels following management of IDA with daily oral iron supplements.

MATERIAL AND METHODS

This 12 week, prospective observational study was carried out at a tertiary care center in Karachi involving pregnant,

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anemic ladies attending the antenatal clinics. Forty women with IDA and fulfilling the selection criteria were recruited for the study. Anemia was diagnosed according to the WHO (1992) criteria for pregnancy i.e. Hb less than 11g/dl during pregnancy. Selection criteria for women to be enrolled for the study were as follow; Singleton pregnancy with gestational age at least 12 wks, nonsmokers, Hb > 7g/dl., non-significant medical history, no history of any blood transfusion/iron supplements or multivitamin during current pregnancy.

Control group comprised of 20 non anemic pregnant women with normochromic and normocytic RBCs, non-addictive with no significant medical history. No placebo group was included for the study due to ethical reasons. Study was conducted after the approval from concerned department.

A detailed history about dietary habits and any drug history was obtained at the time of enrollment. General physical and clinical examinations were also carried out, particularly for the estimation of gestational age of fetus and to exclude any sign and symptom of high risk pregnancy. Gestational age estimation was also done by the ultrasound scan and inquiring the date of last menstrual period. All the women were informed about the nature of study before their enrollment and written consent was obtained. They were prescribed with 200 mg Ferrous Sulphate daily. Follow up visits were planned after every 4 weeks till 12 weeks. At each follow up visit physical examination and bio-chemical tests were repeated & women were inquired about compliance or any side effect of iron therapy.

Hemoglobin was determined by using the cyanmethemoglobin method as mentioned by INCAG, 1985. Plasma iron was measured with a model 911 automatic analyzer (Hitachi Ltd, Mito, Japan). Plasma total iron binding capacity (TIBC) levels were measured by automatic analyzer (Kodak Ektachem 500) using the colorimetric method and standard kits. SFC was determined using a commercial kit (Enzymun-Test Ferritin; Roche Diagnostics GmbH, Mannheim, Germany). Measurement of GSH-PX activity was done with commercial kit according to Paglia and Valentine (1967). GSH-PX levels were expressed as U/g Hb.

The statistical analyses were performed with SPSS 12.5 for Windows. After iron therapy study participants were classified into tertile of Hb levels. Differences were considered significant at $P < 0.05$.

RESULTS

All the women completed the study period. Table 1 shows the Hb, serum iron, SFC, TIBC and GSH-PX values of control group 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.

Table 2 shows the mean values of iron, SFC, TIBC and GSH-PX according to tertiles of Hb of anemic women before & after iron supplements. SFC and iron showed a direct relation with Hb levels. Women with the highest tertiles of Hb after iron therapy showed the highest values of serum iron and SFC (P value < 0.05).

A highest value of TIBC lies in lowest tertile of Hb i.e. for the anemic women before the iron therapy. Highest tertile of Hb showed the lowest mean value of TIBC. The difference was significant ($P < 0.05$) when compared with the mean TIBC value of anemic women before the iron therapy. The upper tertile of Hb was inversely associated with GSH-PX level. Highest values of GSH-PX lies in lowest tertile of Hb while the highest tertile of Hb showed the lowest value of GSH-PX with significant difference ($P < 0.05$).

GSH-PX activity of women treated with iron was much lowered as compared to the control group. Although iron supplements improved the Hb levels up to the control values but the anti-oxidant enzyme was not improved.

DISCUSSION

Nutritional deficiency is one of the major causes of IDA (Camaschella, 2015). Iron supplementation programs to treat IDA have gained worldwide acceptance. Although providing iron to treat the problem seems a good choice but researches have shown the negative impact of iron on other parameters, except hematological.

The anti-oxidant enzyme system of erythrocytes is composed of highly active enzymes like super oxide dismutase (CuZn-SOD), GSH-PX and even non enzymatic systems which include antioxidant vitamins like vitamin C, vitamin E (Aslan *et al.*, 2006).

Studies have reported that iron deficiency (Chandra *et al.*, 2011) and iron supplements used to treat iron deficiency, both are associated with OS & lipid peroxidation (Khalid and Ahmad 2012, Amirkhizi *et al.*, 2008).

Various studies have supported the hypothesis that iron, in high doses (60 mg or more daily) may induce lipid peroxidation (Zhuang *et al.*, 2014, Lachili *et al.*, 2001).

We also found a negative effect of oral iron supplements on anti-oxidant defense mechanisms of body. In IDA it is

Table 1: Pre and post supplement data (expressed as mean \pm SD Range)

Blood Parameters	CONTROL	IDA PRE	IDA POST
Hb g/dl	13.6 \pm 1.6 (11.6-13.7)	9.5 \pm 1.5 ⁷ (8.9-10.8)	12.15 \pm 1.01*(11-13.1)
SFC ng/liter	50 \pm 32.4(35-61)	15.45 \pm 10.15 ⁷ (10.9-35)	33.46 \pm 19.4.4*(11-62)
IRON ng/dl	142 \pm 80.25(135-240)	91.05 \pm 45.89 ⁷ (33-148)	129.13 \pm 70.45*(51-290)
TIBC	327 \pm 49.21(278-362)	542.4 \pm 74.32(427-655)	410.5 \pm 46.71 ^{6c} (284-591)
GSH-PX(U/gHb)	28.48(33-37.1)	35.24 \pm 3.1(30.12-38.4)	20.17 \pm 3.1 ^{7d} (19.6-22.8)

*P value <0.05(significantly greater when compared with pre supplemental values)

⁷P value <0.05(significantly low when compared with control group)

⁶P value <0.05(significantly low when compared with pre supplemental values)

^cP value <0.05(significantly greater when compared with control group)

Table 2: Mean values of iron, SFC, TIBC and GSH-PX according to tertiles of Hb (expressed as Mean \pm SD Range) anemic women before & after the iron supplements.

		Hb>7 <11 g/dl	Hb \geq 11 <12 g/dl	Hb \geq 12 g/dl
Hb g/dl	Mean \pm SD Range	9.5 \pm 1.5(8.9-10.8)	11.7 \pm 1.02(11-12.9)	12.6 \pm 1.51*(11.5-13.1)
SFC ng/liter	Mean \pm SD Range	15.45 \pm 13.15(10.9-35)	17.42 \pm 9.21(11-28)	49.52 \pm 45.04*(31-62)
IRON ng/dl	Mean \pm SD Range	91.05 \pm 45.89(33-148)	105.67 \pm 45.8(51-290)	152.6 \pm 49.01*(124-190)
TIBC	Mean \pm SD Range	542.4 \pm 94.32(427-655)	465 \pm 85.7(379-591)	356 \pm 62.4 ⁸ (284-548)
GSH-PX (U/gHb)	Mean \pm SD Range	35.24 \pm 3.1(30.12-38.4)	22.4 \pm 3.4(19.6-24.1)	19.15 \pm 2.8 ⁸ (17.7-22.8)

*P value <0.05(significantly greater when compared with values in lowest tertile of Hb)

⁸P value <0.05(significantly low when compared with values in lowest tertile of Hb)

not only iron that is deficient but some other important vitamins and trace elements like zinc, copper, and selenium are also significantly reduced (Traber & Kamal-Eldin 2007). Deficiency of antioxidant enzymes is also associated with the deficiency some of these trace metals (Gürögze *et al.*, 2006; Olivares *et al.*, 2006). Many studies have reported decreased activity of GSH-PX in IDA (Khanna, 2010; Tiwari, 2010). However Isler *et al* (2002) and Acharya *et al* (1991) reported similar activity of GSH-Px as for the non-anemic control group. In present study the anti-oxidant activity, measured in terms of GSH-Px level was also found to be same for both anemic and control groups. This is in accordance to the results of Isler *et al* (2002) & Acharya *et al* (1991) but contrary to others who reported a decreased level of anti-oxidant enzymes, particularly GSH-PX in IDA. Furthermore, we did not find any correlation between serum iron and GSH-PX levels as mentioned in earlier similar studies.

Moreover studies regarding vitamin and mineral supplements have shown to improve the anti-oxidant enzyme status in IDA (Kamp & Donangelo 2008; Madhikarmi & Murthy 2014). It is therefore postulated that it is not only the iron which is responsible for causing this imbalance but some other minerals and vitamins do have some role as well. This seems to be a reason of having almost similar values of GSH-PX in anemic and control groups of our study. Other possible explanation of this finding could be related to the increased production of NADPH by pentose phosphate pathway enzymes. These enzymes were found to be increased in IDA

(MacDougall, 1968). In view of the fact that GSH-Px activity is closely associated with NADPH levels, hence even in IDA, increased NADPH levels help to maintain the activity of GSH-Px as for the normal control values.

Iron, like any other transition metal, has the capability to enhance the production of free radicals both by RNAs and due to hyperglycemia by glycation. After administering iron orally, it bind with transferrin and enters into the circulation thus preventing the entrance of free iron. However, non-transferrin bound iron (NTBI) is found to be elevated in blood (Breuer *et al.*, 2000). This is possibly seen when a large amount of iron enters into the intestine causing a passive diffusion of iron. As a result, this NTBI may reach the liver and cause raised OS systemically (Kumar *et al.*, 2009). In order to decrease the possible burden of iron overload by daily iron supplements, studies have been conducted to find the effectiveness of oral intermittent iron administration during pregnancy (Khalid *et al.*, 2011).

Our study showed a positive relation of Hb level and serum iron and SFC. We found a marked increase in SFC after iron supplements. Perhaps this could explain the resultant OS due to the reason that iron being a transition metal has the ability to generate free radicals. This free radical production is more evident as we have provided oral iron which could possibly result in intestinal mucosal cells filled with unabsorbed iron (Lund *et al.*, 1999). A continuous buildup of iron could serve as a source of OS with an inflammatory response locally or throughout the body (King *et al.*, 2008).

CONCLUSION

On the basis of the results of the present study, we may conclude that iron supplements used to treat IDA can no doubt correct the hematological values related to iron deficiency but could be associated with the production of free radicals and alterations in the oxidatative balance. However, more comprehensive studies are required due to our limitations in the present study as we conducted the study in a small sample of urban population. We also included only pregnant anemic women which could also be a reason of OS. We therefore suggest that further advanced studies are needed to assess the status of antioxidant minerals and molecules in IDA.

REFERENCES

- Acharya J, Punched NA, Taylor JA, Thompson RPH and Pearson TC (1991). Red cell lipid peroxidation and antioxidant enzymes in iron deficiency. *Eur. J. Haematol.*, **47**(4): 287-291.
- Adiga US and Adiga MNS (2009). Total antioxidant activity in normal pregnancy. *OJHAS*, **8**(2): 1-4.
- Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, and Chamari M (2008). Assessment of lipid peroxidation and activities of erythrocyte cytoprotective enzymes in women with iron deficiency anemia. *J. Res. Med. Sci.*, **13**(5): 248-254.
- Aslan M, Horoz M, Kocyigit A, Ozgonul S, Celik H, Celik M and Erel O (2006). Lymphocyte DNA damage and oxidative stress in patients with iron deficiency anemia. *Mutat. Res.*, **601**: 144-149.
- Bothwell, TH. (2000). Iron requirements in pregnancy and strategies to meet them. *Am. J. Clin. Nutr.*, **72**(1): 257s-264s.
- Breuer W, Ronson A, Slotki IN, Abramov A, Hershko C, and Cabantchik ZI (2000). The assessment of serum nontransferrin-bound iron in chelation therapy and iron supplementation. *Blood*, **95**(9): 2975-2982.
- Camaschella, C. (2015). Iron-Deficiency Anemia. *N. Engl. J. of Med.*, **372**(19): 1832-1843.
- Casanueva E, Fernando E and Viteri (2003). Iron and oxidative stress in pregnancy. *J. Nutr.*, **133**: 1700S-1708S.
- Chandra S, Mane S, Gupta U and Bhattacharjee J (2011). Effect of Prophylactic as well as Therapeutic Oral Iron Suppl ementation on Oxidative Stress in Normal Pregnancy and in Pregnancy with Iron Deficiency Anaemia. *Bombay Hosp J.*, **53**(2).
- Coghetto Baccin A, Lauerman Lazzaretti L, Duarte Martins Brandao V, Manfredini V, Peralba MCR and Silveira Benfato Mara (2009). Oxidative stress in older patients with iron deficiency anaemia. *J. Nutr. Health Aging.*, **13**(8): 666-670.
- Dotsch J, Hogen N, Nyul Z, Hanze J, Knerr I, Kirschbaum M and Rascher W (2001). Increase in endothelial nitric oxide synthase and endothelin-1 mRNA expression in human placenta during gestation. *Eur. J. Obstet. Gynecol. Reprod. Biol.*, **97**: 163-167.
- Gürgöze MK, Ölçücü A, A Denizmen AA, Taşkin E and Kiliç M (2006). Serum and hair levels of zinc, selenium, iron, and copper in children with iron-deficiency anemia. *Biol. Trace Elem. Res.*, **111**(1-3): 23-29.
- International Nutritional Anemia Consultative Group. (1985). Measurements of iron status, INCAG, Washington, DC.
- Isler M, Delibas N, Guclu M, Gultekin F, Suteu R, Aahceci M and Kosar A (2002). Superoxide dismutase and glutathione peroxidase in erythrocytes of patients with iron deficiency anemia: Effects of different treatment modalities. *Croat Med. J.*, **43**: 16-19.
- Kamp F and Donangelo CM (2008). Supplementing young women with both zinc and iron protects zinc-related antioxidant indicators previously impaired by iron supplementation. *J. Nutr.*, **138**(11): 2186-2189.
- Khalid S and Ahmad SI (2012). Correction of iron deficiency anemia in pregnancy and its effects on Superoxide dismutase. *Pak. J. Pharm. Sci.*, **25**(2): 423-427.
- Khalid S, Ahmad S and Zafar N (2012). Daily iron supplementation results in greater increase in red blood cell volume while intermittent iron results in greater increase in hemoglobin level in iron deficiency anemia of pregnancy. *Pak. J. Pharmacol.*, **29**(2): 15-21.
- Khalid S, Ahmad SI, Waqar S and Faisal M (2011). Effectiveness of weekly iron supplementation in anemia in pregnancy. *Pak. J. Pharmacol.*, **28**(1): 9-16.
- Khanna A, Dubey M and Sinha R (2010). Oxidative Stress and its Correlation with the Severity of Maternal Anemia. *Indian J. Prev. Soc. Med.*, **41**(3 & 4): 231-236.
- King SM, Donangelo CM, Knutson MD, Walter PB, Ames BN, Viteri FE and King, JC (2008). Daily supplementation with iron increases lipid peroxidation in young women with low iron stores. *Expl. Biol. Med.*, **233**(6): 701-707.
- Kumar N, Chandhiok N, Dhillon BS and Kumar P (2009). Role of oxidative stress while controlling iron deficiency anemia during pregnancy-Indian scenario. *Indian J. Clin. Biochem.*, **24**(1): 5-14.
- Lachili B, Hininger I, Faure H, Arnaud J, Richard MJ, Favier A and Roussel AM (2001). Increased lipid peroxidation in pregnant women after iron and vitamin C supplementation. *Biol. Trace Elem. Res.*, **83**(2): 103-110.
- Lund EK, Wharf SG, Fairweather-Tait SJ and Johnson IT (1999). Oral ferrous sulfate supplements increase the free radical generating capacity of feces from healthy volunteers. *Am. J. Clin. Nutr.*, **69**(2): 250-255.
- Macdougall LG (1968). Red cell metabolism in iron-deficiency anemia. *J. Pediatr.*, **72**(3): 303-318.
- Madhikarmi NL and Murthy KRS (2014). Antioxidant enzymes and oxidative stress in the erythrocytes of iron

- deficiency anemic patients supplemented with vitamins. *Iran Biomed. J.*, **18**(2): 82.
- Milman N (2012). Oral iron prophylaxis in pregnancy: not too little and not too much! *J. Pregnancy*, 2012. Available from URL <http://www.hindawi.com/journals/jp/2012/514345/>
- Olivares M, Araya M, Pizarro F and Letelier A (2006). Erythrocyte Cu Zn superoxide dismutase activity is decreased in iron-deficiency anemia. *Biol. Trace Elem. Res.*, **112**(3): 213-220.
- Paglia DE and Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*, **70**(1): 158-169.
- Patil SB, Kodliwadmath MV and Sheela K (2006). Lipid peroxidation and nonenzymatic antioxidants in normal pregnancy. *J. Obstet. Gynecol. India.*, **56**(6): 399-401.
- Stoltzfus RJ and Dreyfuss ML (1998). Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Ilsi Press, Washington, DC, pp.18-21.
- Tiwari AKM, Mahdi AA, Zahra F, Chandyan S, Srivastava VK and Negi MPS (2010). Evaluation of oxidative stress and antioxidant status in pregnant anemic women. *Ind. J. Clin. Biochem.*, **25**(4): 411-418.
- Traber MG and Kamal-Eldin A (2007). Oxidative stress and vitamin E in anemia. *Nutritional Anemia*, p.155.
- Viteri FE, Casanueva E, Tolentino MC, Díaz-Francés J and Erazo AB (2012). Antenatal iron supplements consumed daily produce oxidative stress in contrast to weekly supplementation in Mexican non-anemic women. *Reprod Toxicol.*, **34**(1): 125-132.
- World Health Organization (1992). The prevalence of anaemia in women: A tabulation of available information. 2nd ed. Geneva, p.6.
- World Health Organization (2001). Iron Deficiency: Assessment, prevention and control. Geneva, Switzerland, p.15.
- World Health Organization (2008). Worldwide prevalence of anemia 1993-2005. WHO Global database, p.10.
- Yoo J, Maeng H, Sun Y, Kim Y, Park D, Park T and Choi J (2009). Oxidative status in iron-deficiency anemia. *J. Clin. Lab. Anal.*, **23**: 319-323.
- Zhuang T, Han H and Yang Z (2014). Iron, oxidative stress and gestational diabetes. *Nutrients*, **6**(9): 3968-3980.