Correction of iron deficiency anemia in pregnancy and its effects on Superoxide dismutase

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Abstract: Iron deficiency anemia (IDA) affects not only the hematological parameters but also disturb the oxidative balance of body. In pregnancy, this is much more considerable as oxidative stress is considered to be one of the physiological changes during this period. This study aims to observe the effect of daily iron supplement on oxidative stress in pregnancy. In this study, 30 pregnant women with IDA were treated with daily oral iron supplements for 12 weeks. After 12 weeks hemoglobin (Hb), hematocrit, serum ferritin concentration (SFC) and superoxide dismutase (SOD) activity were measured and compared with pre-supplement levels. A significant increase in all the parameters was observed after treatment ($p<0.05$). When post supplemental values of SFC and SOD were compared with control group comprising of 10 non anemic pregnant women, they were significantly low ($p<0.05$). Our results indicate that oxidative stress in pregnancy associated with IDA decreases with daily iron supplements but fail to reach normal pregnant levels. This supports iron over load theory in daily iron supplements and suggests that oxidative stress increases if pregnancy is associated with iron deficiency.

Keywords: Iron deficiency, anemia, oxidative stress, pregnancy, superoxide dismutase.

INTRODUCTION

Iron deficiency is considered as frequently observed single dietary nutrient deficiency through out the world, predominantly during pregnancy (WHO, 2001). Ultimate result of iron deficiency is iron deficiency anemia (IDA). It comes out to be a serious issue with impact on health and well being of people living mostly in developing countries including Pakistan (UNICEF, 1995). According to estimates of WHO (2008) about 50% pregnant women in developing countries and 65.5% of South Asia suffer from IDA. This deficiency not only causes ineffective erythropoiesis but also results in decreased production of iron containing compound like cytochromes, myoglobin, peroxidases and catalases (Rockey and Cello, 1993). Many iron containing compounds of body are involved in detoxification of free radicals which are a potential source of oxidative stress in body.

Oxidative stress (OS) is a disturbance of balance between pro-oxidant-antioxidant systems. Results of such imbalances leads to increased pro-oxidant activity with a potential of tissue injury. Free radicals are those atoms, which in their outer most shell possess at least a single unpaired electron. These unpaired electrons have capability to exist independently. Human body is capable of forming various types free radicals like hydrogen peroxide (H_2O_2) superoxide anion (O_2^-), hydroxyl radical (OH^-) and singlet oxygen (Kim *et al.*, 2006). Electrons can be accepted or can be lost from iron without any difficulty i.e. ($Fe^{+2} \leftrightarrow Fe^{+3}$). Owing to this property, oxidation reactions frequently use iron as a catalyst. When iron is released in the tissue cells, its pro-oxidation properties could lead to injury of cell membranes and organelles (Walter *et al.,* 2002). Raised OS is a cause of disturbed function at cellular or molecular levels by damaging lipids, proteins and DNA (Kim *et al.*, 2006). This results in diseases like atherosclerosis, cardiovascular disease, Parkinson's disease, cancer, diabetes, Alzheimer's disease and cirrhosis (Somogyi *et al.*, 2007; Amirkhizi *et al.,* 2008).

Pregnancy has been labeled as a condition which exacerbates oxidative stress (Casanueva *et al.*, 2003). This is probably due to dynamic changes throughout the body systems. Placenta, highly vascular and rich in mitochondria greatly influences the maternal homeostasis. After its complete development, about 1% of basal metabolic rate is consumed by placenta. About 5% of electrons that are produced in mitochondria by electron transport chain are leaked out during cell respiration. These features can partly explain superoxide generation by placenta (Fridovich, 1979). In initial stages of pregnancy, placenta is exposed to hypoxic environment. As the pregnancy continues, it is then exposed to oxygen rich environment. These environmental changes in addition to the characteristics of placenta, further favours reactive oxygen species (ROS) production (Liochev and Friedovich, 1997). Not only this, but local production of Nitric oxide (NO) by placenta in presence of transition metals contributes to OS (Dotsch *et al.*, 2001). To make the situation further worse, macrophages are abundantly present in placenta. These macrophages are involved in local free radical production including reactive chlorine

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species (RCIS) which has free iron mixed up (Halliwell and Gutteridge, 1989).

Antioxidants are altered not only in IDA but also in normal pregnancy (Adiga and Adiga, 2009). Patil *et al.* (2006) has reported that nonenzymatic antioxidant vitamins (A, E and C) are produced in lower quantity in normal pregnancy. Some studies have showed opposite results for antioxidant values in IDA but these results are for non-pregnant population (Coghetto *et al.*, 2009).

The aim of this study was to evaluate the anti-oxidative status in terms of SOD level in local anemic pregnant population and alteration in enzyme activity after treatment with oral iron. Upto best of our knowledge no study has been done on local population regarding the anti-oxidant activity and effects of oral iron supplement on anti-oxidant levels.

SUBJECTS AND METHODS

Study was randomized, longitudinal in nature. This study was design for 12 weeks. Study was carried out on pregnant anemic women attending the antenatal clinic of a tertiary care hospital in Karachi. As in this study no extra invasive procedure or medicines were used, only approval of concerned head of department was obtained. Thirty pregnant anemic women who fulfill the inclusion criteria were recruited for the study. Informed written consent was obtained from each subject. Anemia cut off value was according to the criteria of WHO (1992). WHO has defined anemia as Hb concentration of less than 11 g/dl during pregnancy. Each woman was prescribed with 200 mg ferrous sulphate daily.

Inclusion criteria for the study were singleton pregnancy with a gestational age not less than 12 weeks with no history of iron or multivitamin supplement in current pregnancy. All the selected women were non-smokers and non-alcoholic. The women with Hb less then 7g/dl (severe anemia), or anemia due to any chronic illness or active co-morbidity like diseases of liver, cardiovascular system and kidney; tuberculosis, diabetes mellitus or gestational diabetes, multiple gestation; history of antepartum hemorrhage in current or any previous pregnancy and intolerance to oral iron in previous pregnancies were not included in the study. Due to ethical reasons, no placebo group was included in the study. 10 non-anemic pregnant women (Hb>11g/dl), age matched and having gestational age greater than 12 weeks were enrolled as control group. They had normocytic, normochromic RBCs with no history of any co-morbidity or any iron or nutrient supplement in current pregnancy. All were non-smokers and non-alcoholic. Dietary habits of these women included both heme and non-heme iron. They were informed and written consent was obtained.

Basic information of each patient included age, complete history of previous pregnancies, medical and surgical history. At booking visit, detailed general physical and clinical examination was done. This included maternal age, body weight, height, estimation of fetal age (both by examination and sonography) and Hb, hematocrit, serum ferritin and SOD values. Each women of IDA had a follow up visit after every 4 weeks. In each follow up all the physical and clinical examinations and biochemical measurements were repeated.

Venous blood samples (10ml) were obtained from median cubital vein with sterile syringe. Blood samples were collected in polystyrene standard tubes. Tubes contained an anti-coagulant namely ethylene diamine tetraacetic acid (EDTA). Half of the blood samples in standard EDTA-containing tubes were used for hematological assays and determination of the activities of SOD each. After collection, serum was separated by centrifuging for about 10 minutes at 3000 rpm. Plasma was then aspirated off and erythrocytes were washed 3 times with 0.9% normal saline solution and centrifuging at 3000rpm after each wash. The hemolyzate was prepared and stored at -2^{o}C for further analyses for SOD measurement.

Haemoglobin estimation was done by Cynamethemoglobin Hb method (Drabkin's method) as mentioned by INCAG (1985). In cyanmethaemoglobin (HbCN) method Hb converts into cyanmethaemoglobin on addition of KCN and ferricyanide. The absorbency is then measured at 540 nm in a spectrophotometer. Hematocrit concentrations were measured with the help of microhematocrit centrifuge. Serum ferritin concentration was determined with Radioimmunoassay method by commercial kits. Measurement of SOD activity was done with commercial kit. This method has been described by Paglia and Valentine (1967). SOD levels were expressed as U/g Hb.

STATISTICAL ANALYSIS

The data are expressed as means ±SD. The t-test was used for determining statistical significance of a difference between two groups. SPSS version 10 (Statistical Package for Social Sciences) software was used for all statistical calculations.

RESULT

Effects on physical parameters

All of the women successfully completed the study period. Physical parameters of IDA and control group including age, height, weight and gestational age, parity did not showed significant differences (table 1). In booking visit a detailed history about dietary habit was obtained for both IDA and control group. It clearly indicated that dietary intake of heme and non-heme iron

sources of control group was much greater than IDA group (data not shown).

Effects on hematological parameters

IDA group had significantly low Hb, hematocrit, serum ferritin concentration and SOD when compared to non-anemic pregnant women (table 2). IDA group was treated with daily oral iron supplements for 12 weeks. After 12 weeks of oral iron IDA group showed good recovery in all the parameters and then ranged within the normal limit. Mean Hb and hematocrit significantly increased after the treatment when compared to their pre-supplementation values ($p<0.05$) (table 2). When these parameters were compared with control group a non-significant difference was observed ($p>0.05$). Serum ferritin showed significant improvement ($p<0.05$) after the treatment (table 2). When post supplement serum ferritin levels compared with control group, they were significantly lower ($p<0.05$) (table 2). Same for SOD, as it increased significantly after iron treatment ($p<0.05$) (table 2) but when post supplemental values were compared with control group values, they were significantly low ($p<0.05$) (table 2).

DISCUSSION

The important findings of our study are: (1) SOD activity significantly decreased in pregnant anemic women when compared with non-anemic pregnant women. (2) When anemia was corrected after oral iron supplements for 12 weeks, SOD levels of those women were increased but still lower than non-anemic pregnant values.

The human body possess anti-oxidant systems which scavenge harmful free oxygen species. These could be enzymatic anti-oxidants namely superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalases, as well as non-enzymatic antioxidant mechanism like vitamin C and E (Aslan *et al.*, 2006). Not all the cell of body except for erythrocytes possesses highly effective anti-oxidants for instance SOD and GSH-Px. Studies have reported that in IDA, erythrocytes show a low level of anti-oxidant activity (Isler *et al.*, 2002; Yoo *et al.*, 2009). Results of these studies have concluded that anti-oxidant enzymes, for example SOD, GSH-Px and catalases were reduced in anemic conditions. Oxidative stress in IDA has also been demonstrated in murine model (Nagababu *et al.*, 2008). Findings of study by Amirkhizi *et al.* (2008) showed that lipid peroxidation increases in women with IDA. This increased lipid peroxidation could be a reason of excessive production of reactive oxygen species (ROS) or a decrease in anti-oxidant defense mechanisms. In our study, SOD activity of anemic pregnant women was found to be decreased as compared to non-anemic pregnant women. Our findings are opposite to the studies who reported an increased in anti-oxidant capacity as a compensatory response in iron deficiency (Coghetto *et al.*, 2009). In present study, SOD activity increased notably after treatment of patients of IDA with iron supplement. In IDA a decrease in anti-oxidant defense system and an increased lipid peroxidation is not the only reason of oxidative imbalance, here pentose phosphate pathway is also involve (Kumerova *et al.*, 1998). In iron deficiency, due to decreased activity of pentose phosphate pathway erythrocytes are more susceptible to oxidation. Although, they have a good capacity for recovery if anemia is corrected (Sundaram *et al.*, 2007).

In the present study, SOD activity in IDA group was lower than that of control group. This could be explained in terms of oxidative stress under hypoxic condition. In conditions of increased oxidative stress, activity of SOD is inhibited by ROS particularly by hydrogen peroxide (Isler *et al.*, 2002). Insufficient diet or diet with poor

Table 1: Selected physical parameters of women at enrollment in the study (expressed as mean ± SD).

Physical parameters	Non anemic pregnant women	Anemic pregnant women
Age (years)	24.5 (± 3.4)	24.8 (± 3.2)
Parity (n)	3.4 (± 2.2)	3.6 (± 2.5)
Height (cm)	147 (± 4.1)	149 ± (4.2)
Gestational age (wks)	13.5 (± 2.1)	13.8 (± 2.2)
Weight (kg)	55.5 (± 5.2)	58.1 (± 5.7)

Table 2: Pre and post supplementation data (Expressed as mean ±SD)

Blood parameters	Control group	IDA group (pre supplement)	IDA group (post supplement)
Hemoglobin g/dl	13.7 ± 1.5	9.4 ± 2.5*	13.21 ± 1.3†
Haematocrit %	44 ± 3	31.72 ± 3*	39.84 ± 4†
Ferritin g/L	39 ± 14	11 ± 5*	27 ± 6*†
SOD u/gHb	3168 ± 141	1050 ±114*	2580 ±125*†

*p value <0.05 (Significantly low values when compared with control group).
† p value <0.05 (Significantly greater values when compared with pre supplement values).

nutritional value could be an important reason of developing oxidative imbalances. Mineral deficiencies can decrease activity of certain enzymatic and non enzymatic anti-oxidants. This factor seems to be more important in our study patient. Most of the anemic women in our study were not consuming a balanced diet. With detailed interview, it was concluded that their diet was deficient in iron and other minerals. Hence it can be postulated that most of anemic pregnant women included in the study were not consuming diet which is required to maintain the increased nutritional requirements of pregnancy. Important to note that even when anemia was corrected, SOD increased but not upto the level of non-anemic group. It indicated that anti-oxidant activity was not corrected upto the full by treating anemia. One possible reason for this could be deficiency of minerals like zinc (Zn), copper (Cu), cadmium, selenium (Se) etc. Zn is considered to be an essential component of SOD structure (Stefanidou *et al.*, 2006). Studies have reported that activities of SOD and other anti-oxidant enzymes are decreased in deficiency of minerals like Cu, Zn and Se (Isler *et al.*, 2002; Gürgöze *et al.*, 2006; Olivares *et al*., 2006). Findings of our present study indicates that iron supplementation used was not sufficient to maintain the activity of SOD upto the control level possibly due to deficiency of other minerals. Other possible explanation of this could be that in this study we have only used daily iron regimen in order to correct anemia. In recent years, studies have shown that daily iron regimens are causing oxidative stress. Bhatla *et al.* (2009) found a considerable greater augmentation of lipid peroxidation with daily ferrous sulphate administration in comparison to weekly administration. Data by Yoo *et al.* (2009) has indicated a decrease in blood ROS in IDA. He found an increase in anti-oxidant and catalase activity after treating IDA patients when compared to their pre-treatment values. Yoo's research has supported the hypothesis of OS in IDA. This potential "oxidative stress" can be explained by studies on laboratory animals (Viteri *et al.*, 1995). They found that if oral iron is administrated daily, it could lead to impairment of iron absorption from consequent iron dosages and is a source of potential iron overload. It is well-known that ROS and in particular hydrogen peroxide causes inhibition of SOD activities (Hodgson and Fridovich, 1975). Besides, free radical production is also contributed by a low SOD activity. These are in accordance to the finding as we have observed in SFC. A statistically significant increase in SFC after treatment when compared to pre-supplement level suggests an effective treatment in IDA. At the same time when post treatment values were compared with the control group they were significantly low. Studies have shown that daily iron administration does not improve SFC much when compared with intermittent iron supplements (Zamani *et al.*, 2008). This might supports mucosal block theory.

Conclusion of this study is that daily iron supplement was able to correct the Hb, hematocrit, SFC and SOD levels upto normal values. Post-treatment values of SFC SOD when compared with the non-anemic pregnant levels, were significantly low. Our findings support the theory that daily iron supplements are a source of oxidative stress when given in IDA.

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