Oxidative stress associated with altered activity of glutathione peroxidase and superoxide dismutase enzymes with IDA during pregnancy

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Abstract: Iron deficiency anemia (IDA) during pregnancy, although associated with disturbances of hematological parameters, is now also considered as a source of oxidative stress (OS). Present study aims to detect any alteration in superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) enzymes activity in pregnant women with IDA. Levels of GSH-Px and SOD were measured in 156 anemic, pregnant women and compared with similar levels in 20 non anemic, pregnant women. Activity of SOD was found to be reduced in the anemic group when compared with the control group. We found a non- significant increase in GSH-Px activities in the anemic group. These findings could be explained in terms of OS under hypoxic condition which preserves the activity of GSH-Px with a decrease activity of SOD. A positive association was seen between IDA during pregnancy and OS with results suggesting that, apart from the deficiency of iron, some other factors are also associated for the increased OS seen during pregnancy.

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

INTRODUCTION

Iron deficiency anemia (IDA) is the most frequently observed medical condition during pregnancy which affects developing as well as developed countries (WHO, 1992). The national health survey of Pakistan reported that 43% to 47% rural and 35% to 40% urban women between 15-44 years of age are anemic (Mohyuddin, 1995). Ineffective erythropoiesis is found to be the most frequent outcome of iron deficiency (Khalid *et al*., 2011). In addition other iron-containing compound like cytochromes, myoglobin, peroxidases and catalases are also produced in decreased quantity (Rockey and Cello, 1993).

Pregnancy is a normal physiological condition in which there are various metabolic and physiologic adjustments (Khalid *et al.,* 2012). These changes are partly due to the requirements of the developing fetus and partly by the mitochondrial rich placenta (Casanueva and Viteri 2003).

High energy demand and increased oxygen requirement together with mitochondrial rich environment favors the imbalance between oxidative and anti-oxidant enzymes. The result of this imbalance, is oxidative stress (OS) which could be more marked in IDA, as mentioned in various studies possibly many iron containing compounds of body are involved in detoxification of free radicals. In addition to a limited anti-oxidant capacity, impairment of oxygen carrying capacity may further worsens the situation (Adiga and Adiga 2009; Kumar, 2015).

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OS results in a multi-faceted range of genes which are involved in inflammatory processes, fibrinolysis, coagulation, signal transduction, cell cycle, and apoptosis (Buonocore and Perrone, 2006). Although free radicals are produced during normal physiological processes but their increased generation can cause alteration of biomolecules like lipid peroxidation (Cheesman and Slater, 1996). In order to prevent the deleterious effects of reactive oxygen species (ROS), which include both free radicals and their non-radical intermediates , cells have a number of anti-oxidant defense mechanisms including enzymatic anti-oxidants like Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), catalase and some non-enzymatic defenses like ascorbate (vitamin C) and α-tocopherol (vitamin E) (Aslan *et al.,* 2006).

Anemia is associated with decrease in hemoglobin (Hb) concentration or decreased red blood cell (RBC) count either due to RBC destruction or their defective production. Studies have shown shortened lifespan of erythrocytes in anemic conditions. This is probably due to increased ROS due to SOD deficiency making them more vulnerable to OS. Besides SOD deficiency, significantly lower activities of GSH-Px and GSH-Px protein have been demonstrated in erythrocytes in anemia. As GSH-Px protein is susceptible to inactivation due to oxidation, oxidized GSH-Px would be removed by the protease that is responsible for degrading oxidized proteins in erythrocytes (Iuchi *et al.*, 2007).

The present study was designed to measure the activity of two anti-oxidant enzymes in IDA during pregnancy namely SOD and GSH-Px in local population.

MATERIALS AND METHODS

This observational study was carried out at an antenatal clinic of a public sector hospital of Karachi. Study approval was obtained with the concerned head of the department and ethical committee. A total of 200 pregnant women were screened for anemia. The cut off value for anemia was set according to the criteria of WHO (1992). WHO has defined anemia as Hb concentration of less than 11g/dl during pregnancy. Out of 200 screened pregnant women, 156 pregnant anemic women who fulfilled the other inclusion criteria were enrolled for the study. Inclusion criteria were: singleton pregnancy with gestational age between 8^{th} to 16^{th} weeks of pregnancy, not categorized as high risk pregnancy, no history of iron supplements during the current pregnancy and a non-significant medical history with no major surgical interventions. A detailed history was obtained for each woman. In addition, a detailed general physical and clinical examination was carried out which included the gestational age of fetus. Gestational age was investigated by both, the date of last menstrual period and ultrasound. Other information included height, weight and blood pressure. A detailed hematological work up including RBC count, Red cell Indices and reticulocyte count was done to rule out other types of anemia (results not mentioned). All the women were informed and written consent was obtained for participation in the study. For a control group 20 age matched non anemic pregnant women were selected.

Hb estimation was done by Cynamethemoglobin Hb method (Drabkin's method) as mentioned by Stoltzfus and Dreyfuss (1985). Serum ferritin concentration (SFC) was determined with Radioimmunoassay method by commercial kits. Plasma iron was determined by using automated analyzer. SOD and GSH-PX activity were measured using commercial kits as described by Paglia and Valentine (1967). SOD and GSH-PX levels were expressed as U/g Hb.

The statistical analyses were performed with appropriate statistical tests using SPSS 12.5 for Windows.

RESULTS

A total of 200 pregnant women were screened for anemia. Out of these, 156 women were found to be anemic according to WHO criteria, that is Hb level less than 11g/dl and recruited for the study, fulfilling the selection criteria. Thirty six women were having Hb levels less than 7g/dl. Eight women were having other medical issues or twin pregnancies. Pregnant women in control group had Hb levels greater than or equal to 11g/dl.

Table 1 shows the hematologic and anti-oxidant enzymes of control and anemic group. Significant difference (P value<0.05) has been found when Hb, SFC, serum iron, TIBC, SOD and GSH-Px values of control group were compared with the anemic group. With the exception of TIBC and GSH-Px rest of the parameters were found to be significantly increased (P value<0.05) in control group when compared with anemic group. It has been observed that mean value of TIBC for anemic group was significantly greater (P value<0.05) when compared with the mean TIBC value of control group. In contrast to the anti oxidant enzyme SOD that was found to be lower in anemic group (P value<0.05), GSH-Px was found to be statistically non-significantly increased in anemic group (P value>0.05).

Table 2 shows the mean values of SOD and GSH-PX according to tertiles of Hb of anemic and control groups when merged together. Although there was no significant association between SOD levels of non anemic women and Hb levels greater than 11g/dl still, women having the highest tertile of Hb were found to have the highest values of SOD. The upper tertile of GSH-Px was found to be inversely associated with the higher Hb levels with highest values seen in the lowest tertile of Hb. Again no significant association is found between the GSH-Px activity according to tertiles of Hb.

DISCUSSION

In this observational study, we studied the effect of IDA on two anti-oxidant enzymes namely SOD and GSH-P. It has been observed that one of the major causes of IDA, is nutritional deficiency (Camaschella 2015). This nutritional deficiency is also associated with the deficiency of different vitamins and minerals (Olivares *et al.,* 2006; Traber and Kamal-Eldin2007).

A simplest definition of OS is an imbalance between the pro-oxidant and anti-oxidant environment balance, favouring the pro-oxidant leading to cell injury. Raised OS has been implicated not only for chronic diseases like cardiovascular disease, cancer, diabetes, cirrhosis, atherosclerosis, Alzheimer's and Parkinson's disease (Somogyi *et al.*, 2007), it has been found altered also with pregnancy, iron deficiency (Chandra *et al*., 2011) as well as with iron supplements (Khalid and Ahmad, 2012).

Elements, like oxygen, have at least one unpaired electron in their outermost shell which can exist independently. These atoms are termed as free radicals. Superoxide anion (O_2^-), the hydroxyl radical (OH^-), singlet oxygen, and hydrogen peroxide (H_2O_2) are few of the studied free radicals which are generated within the body (Zwart *et al.*, 1999). Iron and copper are two common catalysts of oxidation reactions due to their ability to gain and loose electrons and can hence be injurious to the cell membranes due to their potential pro-oxidant nature. Furthermore we know that in pregnancy there is an

Table 1: 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	Anemic group N=156
Hb g/dl	11.9±0.5(11.1-13.2)	9.3 ±1.1*(7.3-10.7)
SFC ng/liter	58±30.4(32-65)	20.15±9.15 *(10.9-35)
Serum iron ng/dl	168±57.25(133-245)	81.05± 35.61*(35-149)
TIBC	317±46.21(267-371)	532.4±54.32 $^{\delta}$(427-695)
SOD u/gHb	3153 ± 120(2915-3324)	1061±109*(729-1284)
GSH-Px u/gHb	28.41± 5.2(24.16-32.3)	36.28±3.1(30.12-39.2)

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

Table 2: 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	9.3±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 ± SD Range	1061± 109 (729-1284)	3138 ± 101 (2915-3241)	3168± 114* (2991-3324)
GSH-Px u/gHb	Mean ± SD Range	36.28±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)

increase in oxygen and energy requirements. As pregnancy advances, various adaptative mechanisms come into play for compensatory adjustments (Gitto *et al.*, 2002). With these changes pregnancy itself becomes a situation favoring OS **(**Casanueva and Viteri 2003).

In the present study, we found that GSH-Px activities in control group were lowered when compared to anemic group women. Although the difference when compared statistically appeared to be non-significant but still, as mentioned in various studies, we had the difference. On the other hand SOD activity was found to be reduced in anemic group when compared with control group. The reasons for increased oxidative stress and decreased anti-oxidant defense mechanisms in IDA have not been fully explained as studies have shown varying results. Some researchers have observed increased activity of SOD in anemic patients suggesting that this improvement was a compensatory response for increased OS (Acharya *et al.* 1991; Panchenko *et al.,* 1979). While many studies have reported decreased activities of SOD, GSH-Px, and catalases, in anemic patients (Kumerova *et al.,* 1998; Yoo *et al.*, 2009). In addition to these contrary results, studies have reported the improvement in OS with the treatment of IDA in anemic patients (Aycicek *et al.*, 2014). In normal pregnancy nonenzymatic anti-oxidant vitamins (A, E and C) are also produced in lower quantity as mentioned by Patil *et al.* (2006). Although some studies have showed contrary results for anti-oxidant levels in IDA but these results are for non-pregnant population (Baccin *et al.*, 2009).

Our findings could be explained in terms of OS under hypoxic condition. Increased oxidative stress results in decreased activity of SOD by ROS particularly by hydrogen peroxide (Isler *et al.*, 2002). Also SOD is a copper-dependent, anti-oxidant enzyme whose activity is closely associated with the serum copper levels (Mistry and Williams 2011). Although the correlation among iron deficiency, serum copper concentration and SOD activity have not been clearly understood, still low iron levels are considered as a source of OS (Amirkhizi *et al.,* 2010; Khoshfetrat *et al.,* 2008). Iron deficiency has been associated with altered serum levels of trace metals like selenium, zinc and copper (Nassef *et al.,* 2014; Angelova *et al.,* 2014). Studies have shown the improvement of Cu^{+2} with improvement of iron status and hence anti oxidant enzymes particularly SOD levels improved accordingly (Gropper *et al.,* 2002). Furthermore we did not find any correlation of SOD levels with the Hb values as in non anemic women there was a non significant difference of SOD levels when measured against tertiles of Hb. A highest mean value of SOD was found with the highest tertile of Hb suggesting some association with Hb status.

GSH-Px is a selenium-dependent enzyme .Interesting to note that selenium concentration has been found significantly lowered in patients with IDA (Yetgin *et al.*, 1992). Our findings are in accordance with Isler *et al.*, 2002 and Amirkhizi *et al.,* 2008 who reported similar GSH-Px activities in the anemic and control groups. Additionally some studies have shown the improvement in anti-oxidant enzymes with the use of vitamin and mineral supplements in anemic patients (Kamp and Donangelo 2008; Madhikarmi and Murthy 2014). With these findings, it could be speculated that in addition to iron deficiency, some trace elements like Cu, Zn, Mn, and Se, are also important for the activities of anti-oxidant enzymes. Furthermore, in IDA pentose phosphate

pathway enzymes activities are found to be increased. This enzyme has the major contribution in increased NADPH production, required for GSH-Px activity (Macdougall, 1968). This may perhaps explains the normal GSH-Px as compared to decreased SOD levels in IDA. Also as we found no significant association for GSH-Px activity when measured against different levels of Hb, this possibly suggest that although IDA is associated with altered activity of this enzyme, still some other factors are important in determining its production. Keeping in view all the possibilities which are mentioned earlier it can be postulated that even in IDA and with the deficiency of trace metals required for proper functioning of GSH-Px, human body tries to preserve its activity, possibly via pentose phosphate pathway enzymes. Still more investigations and research is needed to explain the changes in anti-oxidant enzymes during IDA.

On the basis of the results of the present study we can conclude that whether due to the free radical generation or due to the deficiency of minerals, IDA is associated with altered levels of two studied anti-oxidant enzymes, GSH-Px and SOD in local pregnant women. Though, more comprehensive studies are required in the field due to our limitations in the present study as we included only a small sample of anemic pregnant women of urban population. We therefore suggest that further advanced studies are needed to assess the status of anti-oxidant minerals and enzymes in IDA.

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