

Association Between Oxidative Stress and Anaemia in Pregnancy

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ABSTRACT

Background: Oxidative stress is the topic of interest for most of the researches, as it is associated with the pathogenesis of a number of disorders. Pregnancy though a natural phenomenon is associated with increased susceptibility to oxidative stress. Also, pregnant women are more vulnerable to the nutritional deficiency disorders, most common being iron deficiency anemia. Both oxidative stress and anemia have become a potential threat to both mother and fetus. Therefore, the aim of our study was to determine the association of oxidative stress with anemia in pregnancy. **Methods:** This study was conducted in Jawahar Lal Nehru Medical College, Aligarh with inclusion of 25 healthy pregnant (Control) and 50 anemic pregnant women. Blood samples were collected and the parameters like Hemoglobin (Hb), malondialdehyde (MDA), Ferritin reducing ability of plasma (FRAP), Total peroxide (TP) and Oxidative stress index (OSI) were estimated. **Results:** We found that the levels of Hb and FRAP were significantly low and that of MDA, TP and OSI were significantly higher in the anemic pregnant women. Hb was positively correlated with FRAP but negatively with MDA, TP and OSI. The results were statistically significant ($p < 0.05$). **Conclusions:** Anemia when occurs in disturbs the oxidant-antioxidant balance which further adds to the pre-existing pro-oxidant cellular environment induced by pregnancy. Therefore, in order to prevent both maternal and fetal complications further evaluations are needed.


Key words: Oxidative stress, anemia, pregnancy

INTRODUCTION

Women during the period of pregnancy undergo a number of physiological and metabolic alterations in her body to sustain and foster the growth and development of her fetus.^[1] Normal pregnancy is associated with increased nutritional and oxygen demand that can result in oxidative stress and trigger the antioxidant defenses.^[2] Oxidative stress is a condition that occurs due to imbalance of free radical generation and the antioxidant defense system leading to potential damage.^[3] Of course, production of free radicals is a normal physiological process but production in excess amount leads to lipid peroxidation and promotes

maternal vascular malfunction since reactive oxygen species (ROS) such as superoxides promote endothelium activation. These free radicals are scavenged by the antioxidants present in the body thereby preventing the oxidative damage.^[4] The antioxidant systems present in the body may be categorized as free radicals scavengers (that include enzymatic antioxidants such as superoxide dismutase catalase, glutathione peroxides etc) or chain breaking antioxidants such as Vitamins C.^[5] In pregnancy placenta formed is rich in mitochondrial content thereby favoring the development of oxidative stress. Studies conducted in past have shown decreased superoxide dismutase activity in the 3rd trimester of pregnancy in comparison to non-pregnant women.^[6]

Anemia is regarded as the most common disorder in pregnancy leading to about 47% of maternal deaths in developing countries^[7] and about half of the anemia cases are due to iron deficiency.^[8] Anemia develops due to decrease in erythrocyte count or hemoglobin concentration.^[9] It promotes oxidative stress by causing inadequate supply of oxygen to the tissues that can lead to excess production of free radicals and decreased mobilization of free radical scavengers thereby promoting ROS mediated tissues damage.^[10] Further oxidative stress promotes pathogenesis of iron deficiency anemia by

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shifting the cellular microenvironment towards the favor of pro-oxidants.^[11] Several studies have shown increased oxidative stress in anemia.^[12,13] Deficiency of iron effects the synthesis of iron containing enzymes like catalase, peroxidase which are involving in free radical detoxification.^[14]

Therefore oxidative stress in association with anemia can causes adverse effect on both maternal and fetal health which can lead unhealthy pregnancy outcomes, thus we attempted this study to evaluate oxidative stress by measuring lipid per oxidation product (such as MDA), Total antioxidant capacity, total peroxide level and oxidative stress index in anemic pregnant females and assessed its association with anemia, so that our result may be fruitful in preventing pregnancy related complication, mediated by iron deficiency anemia and oxidative stress.

METHODS

This study was carried out in the department of Biochemistry in Jawahar Lal Nehru Medical College, Aligarh. 50 anemic pregnant women and 25 non-anemic pregnant women (Controls) were taken for the study.

Inclusion Criteria

Healthy non-anemic pregnant women with Hb > 11 gm% and anemic pregnant women with Hb ≤11 gm%

Exclusion criteria

Pregnant women with iron therapy, smoking and alcoholic history, diabetes, hypertension, infections, hepatic disorders, endocrine disorders and any other causes of anemia such as thalassemia, hemolytic disease etc were excluded from the study.

Sample collection

3-5 ml of blood sample was collected. 1-2 ml of sample transferred in EDTA vial and used for estimation of hemoglobin. 2-3 ml of sample was transferred in plain vial, allowed clot and serum was separated by centrifuging at 5000 rpm for 10 min. It was used for estimation of MDA, TP and FRAP level

Investigation

- Anemic status was checked by measuring hemoglobin by acid hematin method.
- MDA was estimated by kit based method.
- FRAP was determined by reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) which has an intensive blue colour that can be measured at 593 nm.^[15]
- Total peroxide level was measured by the method described by Aslan M, *et al.*^[16]
- OSI was calculated by the formula

$$OSI = \frac{TP}{FRAP}$$

Statistical analysis

The data obtained were recorded and analyzed by SPSS version 17. The level was significance was test by student's t-test. Association between various markers of oxidative

stress with anemia was determined by Pearson correlation coefficient. The p value of less than 0.05 was considered statically significant.

RESULTS

The level of Hb was significantly lower in anemic pregnant women. Similarly, the concentration of MDA, TP and the value of OSI were significantly higher while FRAP levels were significantly low in anemic pregnant women in comparison to non-pregnant control.

Hb level was positively correlated with FRAP while there was negative correlation of Hb with MDA, TP and OSI. All the values were statistically significant (p<0.05).

Table 1: Comparison of study parameters among non-anemic (control) and anemic pregnant women (test)

Parameter	Control	Test	p
Hb	13.01±0.27	9.81±1.12	<0.05
MDA (µmol/l)	2.8 ±0.36	4.55 ± 0.27	<0.05
FRAP (mmol/l)	1.35 ±0.16	0.86 ± 0.124	<0.05
TP (µmol/l)	7.11 ± 0.15	10.31 ±0.33	<0.05
OSI	3.47 ± 1.3	5.75 ±0.25	<0.05

Table 2: Correlation of Hb with MDA, FRAP, TP and OSI

Parameter	r	p
MDA	-0.557	<0.05
FRAP	0.481	<0.05
TP	-0.536	<0.05
OSI	-0.602	<0.05

DISCUSSION

Pregnancy is normal physiological phenomenon accompanied by dynamic changes in women's body leading to increased nutrient demands and basal oxygen consumption making the women susceptible to the oxidative stress, a condition that occurs due to imbalance between pre-oxidant and anti-oxidant defense system.^[17] Oxidative stress increases production of reactive oxygen species thereby causing potential damage to mother and growing fetus. In this study, we attempted to study association of oxidative stress with anemia in pregnant women. Oxidative stress was assessed by estimation of MDA, FRAP, TP and OSI and its association with anemia was determined by calculating the correlation of oxidative stress parameters with hemoglobin. Anemia is one of the widest spread pathological condition associated with pregnancy. Anemia can further worsen the oxidative load by impairing the transport of oxygen^[18] and the potency of oxidative is related significantly to anemia.^[16]

In our study, the hemoglobin level was significant low in anemic pregnant women in comparison to control. MDA levels were also found to be significantly high in anemic pregnant women. Free radicals, being unstable are difficult to be measured directly. Hence their levels are measured indirectly by the estimation of lipid peroxidation products, most commonly (MDA). Studies have shown increase in levels of MDA in normal pregnancy too, but the condition is further deteriorated if anemia is associated with it. Anemia causes increased oxidative stress and lipid peroxidation.^[19] Study of Bhale DV *et al* showed higher

MDA concentration in pregnant women with iron deficiency anemia which was similar to that of Sujata M *et al.*^[20] In our study MDA levels in anemic and non-anemic pregnant women were respectively $2.8 \pm 0.36 \mu\text{mol/l}$ and $4.55 \pm 0.27 \mu\text{mol/l}$. Increased requirement of oxygen, reduced activity of anti-oxidant enzymes requiring iron for their activity may lead to increased ROS production which can further cause lipid peroxidation thereby increasing the levels of MDA.^[21]

We also estimated total peroxide levels in the study subjects and the values were significantly high in anemic pregnant women. Ishihara *et al* reported significant increase in lipid peroxide level in 2nd and 3rd trimester of pregnancy^[22] which was similar to that of Kodilowalmath *et al*^[23] while Bhale DV *et al* demonstrated them to be increased in all three trimesters.^[19] The arsenals that combat against free radicals are the various antioxidants present in the body.^[24] However, estimation of individual antioxidant levels may not provide the true antioxidants picture of the organism and therefore in this context estimation of total antioxidant capacity can provide the accurate antioxidant status.^[25] FRAP was assayed as a measure of total antioxidant capacity and it was found to be significantly low in the anemic pregnant group. According to Aslam MA *et al* total antioxidant capacity was significantly low and total peroxide level was significantly high in the pregnant patients with iron deficiency anemia.^[16]

We also found significantly high OSI in the test group in comparison to control which was similar to the findings of Aslam *et al.* When MDA, FRAP, TP and OSI was correlated with Hb (as marker of anemia), we found significant positive correlation of Hb with FRAP ($r=0.481$, $p<0.05$) while the correlation was significantly negative in case of MDA, TP, and OSI ($r=0.557$, 0.536 , 0.602 respectively, $p<0.05$), which was in accordance to previous study.^[16]

CONCLUSION

The findings of our study suggest that both oxidative stress and anemia are the common medical problem observed in pregnancy. The outcomes are further worsened when both these disorder are present in during pregnancy in association with each other. Iron deficiency anemia can lead to free radicals generation causing peroxidation of biomolecules thereby increasing the risk not only for pregnant women but also the growing fetus. Therefore, supplementation of antioxidants (in the form of vitamin) along with iron therapy can provide better response and early resolution of oxidative stress related symptoms in iron deficiency anemia. However further larger studies including larger sample size should be conducted for in depth analysis of association of oxidative stress with anemia in pregnancy.

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