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Original Research Article

Serum Levels of Antioxidants Micronutrients in Pregnant Women & Their Neonates: A Prospective Observational Study

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Abstract:
Introduction: Pregnancy is associated with numerous biological & hormonal changes. An imbalance between the antioxidant agents and oxidative stresses may lead to pregnancy-associated complications.
Aims & Objectives: To evaluate the levels of oxidative stress markers and the levels of antioxidants in normal pregnant women & their neonates as compared to non-pregnant women.
Material & Methods: This prospective observational study recruited 26 pregnant women from the outdoor/indoor of Zanana Hospital and Mahila Chikitsalaya of SMS Hospital, Jaipur. Pregnant females > 18 yrs of age, with > 28 weeks of gestation, not suffering from any chronic disease affecting the dietary intake pattern were included in the study. Healthy symptomless non pregnant females in the reproductive age group were taken as the controls. Venous blood samples were collected at baseline & after parturition, within 24 hrs. from mothers & their neonates (cord blood) and sent for biochemical estimation. The Haemoglogin (Hb) %, proteins, albumin, globulin, A/G ratio, SOD, MDA, catalase, Zinc & copper, ascorbic acid, tocopherol, retinol & β carotene levels were assessed. On the basis of weight of the neonates delivered the women were categorised into three groups: Group A - Women who delivered neonates having weight < 1.5 kg. Group B- Women who delivered neonates having weight between 1.5 kg – 2.5 kg. Group C - Women who delivered neonates having weight >2.5 kg.
Results: The mean values of Hb % & total proteins were statistically significantly lower in Group A & Group B as compared to Group C. The mean A/G ratio was statistically significantly higher in the Group A as compared to Group B(p< 0.01) & in higher in Group B as compared to Group C(p< 0.05). The mean SOD & MDA levels were not found to be statistically significant between the three Groups. The mean catalase, Cu & Zn levels, ascorbic acid , α tocopherol & β carotene were statistically significantly lower in Group A & B as compared to Group C(p< 0.01).In neonates, weighing <1.5 kg wt the mean levels of Hb %, total proteins were found to be statistically significantly lower. The mean MDA levels were statistically significantly higher in neonates weighing <1.5. The mean levels of ascorbic acid, α tocopherol & β carotene were statistically significantly higher in neonates weighing >2.5 kg.
Conclusion: A gradual decline in the levels of the antioxidant vitamins have been observed along with increase in MDA levels, which correspond to increase in oxidative stresses both in pregnant women & their neonates. Thus, proper diet counselling & vitamin supplementation is essential for the prevention & management of pregnancy associated complications.
Keywords: Antioxidant, Micronutrient, Oxidative Stress, Pregnancy, Cord Blood.

Introduction

An increased demand for nutrients arises during the pregnancy, the fulfilment of which is essential for the mother and the fetus. Although physiologically normal, pregnancy period involves a lot of stress & the growth of the fetus is totally dependent on the nutritional status of the mother .Free radicals (ROS) are molecules having one or more unpaired electrons and are so are highly reactive. During oxidation, electrons or hydrogen ions are transferred from one molecule to another, leading to unstable products called free radicals which can trigger chain reactions at a cellular level and cause cell damage. [1]

Our body has developed protective systems to counteract the damage caused by ROS through the "antioxidant defence system". This defence system includes enzymatic antioxidants such as copper/zinc and manganese superoxide dismutase (Cu/Zn and Mn SOD), catalase, and selenium-dependent glutathione peroxidase [2], thioredoxin reductases, selenoprotein-P. These play an important role in protecting the placenta from the harmful effects of oxidative stresses in the mother & fetus. SOD acts as a good therapeutic agent against ROS mediated diseases. [3]

Oxidative stress develop when the balance between the production of free radicals and antioxidant defences is lost leading to failure of the neonatal antioxidant system during, before, and after delivery. [4] Malondialdehyde (MDA) is one such reactive metabolic product which is produced as a result of action of ROS on tissues & also from reactions occurring during lipid peroxidation. [5]The plasma MDA level is a sensitive indicator of lipid peroxidation and thus of oxidative stress during the perinatal period. [6]

Studies highlight the increase demand of certain vitamins during pregnancy such as ascorbic acid, tocopherol, and retinol .The deficiency of these vitamins increases steadily as the pregnancy progresses & it is significantly low in the third trimester, the sustainability of which may cause obstetric problems. [7] The lower levels of these vitamins could be attributed to increased, insufficient intake, changes in the blood volume or hormonal changes. These vitamins have shown to exhibit ROS-scavenging properties. Studies have associated low levels of ascorbic acid to premature / low birth, preeclampsia, anemia & premature rupture of foetal membrane.[8] Also, the fetus during the first stages of development is exposed to low levels of oxygen & after birth the environmental oxygen becomes fatal as reactive oxygen species(ROS) & free radicles are produced. [9] Thus, these three antioxidants ascorbic acid, α tocopherol, retinol play an important role in protecting from diseases, acting as ROS-scavengers, maintaining balance between ROS & antioxidant concentrations. In recent times, increasing oxidants & decreasing antioxidants have gained importance as they are a threat to the normal pregnancy. [7]

Pregnancy-associated complications include hypertension, gestational diabetes, preeclampsia, miscarriage, premature birth, stillbirth, and intrauterine growth restriction. These complications can cause acute and chronic health problems for both the mother and fetus. Oxidative stress could result in fetal loss or dysregulation of infant physiology. Placenta is susceptible to oxidative stress & destruction. [10,11,12]

Thus, the aim of the present study was to evaluate the levels of oxidative stress markers and the levels of antioxidants in normal pregnant women & their neonates as compared to non-pregnant women.

Material & Methods

This prospective observational study recruited 26 pregnant women from the outdoor/indoor of Zanana Hospital and Mahila Chikitsalaya of SMS Hospital, Jaipur. Pregnant females > 18 yrs of age, with > 28 weeks of gestation, not suffering from any chronic disease affecting the dietary intake pattern were included in the study. Healthy symptomless non pregnant females in the reproductive age group were taken as the controls. Objectives of the study were explained to the study participants & written informed consent taken. A prior institutional ethics committee approval was sought.

Detailed sociodemographic details, Clinical history, dietary habits, smoking, alcohol, familial history were recorded to evaluate the clinical implications of these factors on the antioxidant status and vitamin levels. Venous blood samples were collected and sent for biochemical estimation at the baseline. After parturition, within 24 hrs the venous blood samples were collected from the women and their neonates (cord blood) and sent for biochemical estimation. The Haemoglogin (Hb) %, proteins, albumin, globulin, A/G ratio, SOD, MDA, catalase, Zinc & copper, ascorbic acid, tocopherol, retinol & β carotene levels were assessed.

On the basis of weight of the neonates delivered the women were categorised into three groups:

Group A - Women who delivered neonates having weight < 1.5 kg.

Group B- Women who delivered neonates having weight between 1.5 kg – 2.5 kg.

Group C - Women who delivered neonates having weight >2.5 kg.

Statistical Analysis

The study data was tabulated & statistically analysed using SPSS version 22.0 for Windows (IBM Corp, India). Quantitative data are presented as mean ± SD. Intergroup comparisons were made using Student's paired t-test used to analyse intergroup comparisons. P-value 0.05 at 90% confidence interval was considered to be statistically significant.

Results

This prospective observational study recruited 26 pregnant women. The mean Hb % in the pregnant women was 9.8±0.69 which was lower than normal (Table 1).

Table 1 Physical & Biochemical Parameters at baseline & at 24 hrs after parturition

Parameters	Mothers (Mean ±SD)	Mother at 24 hrs after parturition (Mean ±SD)	Neonates (Mean ±SD)
Age (yrs)	25.23±2.76	-	-
Weight(kg)	64.69±4.21	52.47±7.51	2.22±0.66
Hb(%)	9.8±0.69	9.46±0.99	15.11±0.96
Proteins (g/dl)	6.09±0.38	6.24±0.4	4.91±0.33
Albumin (g/dl)	3.43±0.3	3.5±0.46	2.54±0.24
Globulin (g/dl)	2.66±0.43	2.69±0.48	2.38±0.34
A/G	2.34±0.33	1.38±0.38	2.10±0.21
SOD (U/ml)	2.03±0.22	2.15±0.46	1.75±0.36
MDA (nmol/ml)	1.62±0.31	1.2±0.93	1.22±0.2
Catalase (μmol/H2O2/min/mg)	6.07±0.36	5.97±0.54	5.64±0.49
Cu(μg/ml)	183.71±22.39	152.9±5.61	57.90±5.6
Zn (μg/ml)	68.99±11.71	52.27±4.4	91.79±11.20
Retinol (μg/ml)	24.62±3.05	19.99±3.46	21.70±2.82
Ascorbic Acid (mg/dl)	0.98±0.19	0.81±0.21	0.87±0.17
αTocopherol (mg/dl)	0.93±0.14	0.71±0.18	0.82±0.17
βCarotene (μg/dl)	111.57±10.26	109.54±11.88	105.58±0.79

The mean values of Hb % & total proteins were statistically significantly lower in Group A & Group B as compared to Group C.

The mean A/G ratio was statistically significantly higher in the Group A as compared to Group B ($p < 0.01$) & in higher in Group B as compared to Group C ($p < 0.05$). The mean SOD & MDA levels were not found to be statistically significant between the three Groups. The mean catalase , Cu & Zn levels were statistically significantly lower in Group A & B as compared to Group C($p < 0.01$).(Table 2) No difference was noted in the mean values of retinol among the three groups. The mean values of ascorbic acid, α tocopherol & β carotene were found to be statistically significantly lower in Group A & B as compared to Group C . (Table 2)

Table 2 Statistical evaluation between mothers of different weight neonates

Parameters	<1.5 kg v/s >1.5 to 2.5 kg	>1.5 to 2.5 kg v/s >2.5 kg
Age (yrs)	NS	NS -
Weight(kg)	NS	NS
Hb(%)	S< 0.001	S< 0.001
Proteins (g/dl)	S< 0.001	S< 0.001
Albumin (g/dl)	NS	NS
Globulin (g/dl)	NS	S< 0.01
A/G	S< 0.01	S< 0.05
SOD (U/ml)	NS	NS
MDA (nmol/ml)	NS	NS
Catalase (μmol/H2O2/min/mg)	S< 0.01	S< 0.01
Cu(μg/ml)	S< 0.05	S< 0.001
Zn (μg/ml)	S< 0.01	S< 0.01
Retinol (μg/ml)	NS	NS
Ascorbic Acid (mg/dl)	S< 0.01	S< 0.001
αTocopherol (mg/dl)	S< 0.001	S< 0.001
βCarotene (μg/dl)	NS	S< 0.001

In neonates, weighing <1.5 kg wt the mean levels of Hb %, total proteins were found to be statistically significantly lower than neonates weighing 1.5-2.5 kg. The mean MDA levels were statistically significantly higher in neonates weighing <1.5 kg than 1.5-2.5 kg weight & >2.5 kg weight neonates. The mean levels of ascorbic acid , α tocopherol & β carotene were statistically significantly higher in neonates weighing >2.5 kg as compared to 1.5-2.5 kg wt & higher in 1.5-2.5 kg wt as compared to <1.5 kg wt neonates. (Table 3)

Table 3 Statistical evaluation between different weight neonates

Parameters	<1.5 kg v/s >1.5 to 2.5 kg	>1.5 to 2.5 kg v/s >2.5 kg
Age (yrs)	-	-
Weight(kg)	S< 0.001	S< 0.001
Hb(%)	S< 0.05	S< 0.001
Proteins (g/dl)	S< 0.001	S< 0.05
Albumin (g/dl)	NS	NS
Globulin (g/dl)	S< 0.01	NS
A/G	NS	NS
SOD (U/ml)	NS	S<0.05
MDA (nmol/ml)	S< 0.01	NS
Catalase (µmol/H2O2/min/mg)	NS	NS
Cu(µg/ml)	S< 0.05	S< 0.001
Zn (µg/ml)	NS	S< 0.01
Retinol (µg/ml)	S< 0.01	NS
Ascorbic Acid (mg/dl)	S< 0.001	S< 0.001
αTocopherol (mg/dl)	S< 0.01	S< 0.001
βCarotene (µg/dl)	S< 0.01	S< 0.01

Discussion

The present study, highlighted the status of antioxidant micronutrient & peroxidative stress in pregnant women residing in Rajasthan. In Rajasthan, most population is vegetarian, due to which the women suffer from anaemia & low protein levels. At the time of pregnancy, it can lead to low birth weight neonates. The levels of α Tocopherol and ascorbic acid have also been found to be low. The human cells exhibit antioxidant mechanisms, to save cellular structures from oxidative stress and damage. [13] These are enzymes (Superoxide Dismutase (SOD) and Catalase (CAT)),proteins, as well as endogenous (lipoic acid, melatonin, bilirubin, glutathione, Coenzyme Q10) and exogenous (α Tocopherol and ascorbic acid, beta carotene, resveratrol, N acetylcysteine) low molecular weight anti-oxidant molecules. [14]

In the present study, mean Hb level was 9.8 ±0.69 mg/dl which was lower than normal. About 60% of women suffered from iron deficiency anaemia. The levels of Hb were statistically significantly lower in Group A as compared to Group B & C ($p<0.001$). Similar studies conducted by Magadum S et al [15] observed 47% of the pregnant females to be anaemic while in Anlaakuu P et al [16] it was 40.8%.

Lower levels of proteins were observed in Group A as compared to Group B & C. Higher level of A/ G ratio was observed in Group A as compared to Group B & C which was statistically significant ($p<0.05$). Thus a low iron status & low protein & albumin levels corresponded to a lower birth weight of the neonates. The primary etiology is nutritional deficiency & poor oral intake. Similar study conducted by Gohel MG et al. concluded low serum A/G ratio in second & third trimester of pregnancy compared to non-pregnant & first trimester. [17]

In the present study, the levels of SOD were significantly low in the test participants as compared to the control group. The MDA levels were within normal limits in the women. No statistically significant difference was found between the three Groups. In a study by Khan MM & Alam R observed statistically significantly higher mean levels of MDA & lower mean levels of SOD in pregnant women as compared to non-pregnant women. The MDA levels and SOD activity were found to be negatively correlated among pregnant women ($r= -0.65$, $P<0.01$). Thus, pregnant women had an elevated oxidative stress and reduced anti-oxidants, which could lead to pregnancy-associated complications. [18]

In the present study, Low MDA levels were observed in the cord blood of neonates of <1.5 kg & 1.5-2.5 kg weight neonates as compared to >2.5kg weight neonates. Similar findings noted by Suhail M 2009 that the MDA levels in pre-eclamptic women were significantly high as compared to that of control. Their level in pre-eclamptic cord blood was significantly low when compared to their pair-matched maternal blood. These findings may conclude that the cord blood has sufficient antioxidant capacity with adequate placental barrier which protects the fetus from the oxidative injury. [19] Gülbayzar S 2011 study, observed higher mean cord blood MDA levels in normal vaginal delivery and emergency caesarian section as compared to that of the elective caesarian group. [20]

In the present study, serum levels of catalase enzyme were found to be comparable between the test participants & the control group. Significantly lower levels were observed in Group A as compared to Group B & C women ($p <0.01$). The neonates of all the three groups did not show any difference in the catalase levels ($p>0.05$).

In the present study, the serum levels of Zn & Cu were highly statistically significantly lower in Group A as compared to Group B & C women. Copper is an essential cofactor required during various metabolic reactions, oxygen transport, protection against ROS [21] & embryonic development. [22] Increased serum Cu levels are observed during pregnancy which returns to normal levels after parturition. [23] Severe deficiency of Cu results in early embryonic death and gross structural defects, increased risk of cardiovascular events. [24]

In the present study, the levels of α Tocopherol and ascorbic acid were highly statistically significantly lower in Group A as compared to Group B & C women ($p<0.001$). The difference between serum levels of retinol were not significant In Group A & Group B & C. The serum levels of beta carotene did not show any significant difference between Group A & Group B ($p>0.05$) but the difference between Group A & Group C was statistically significant ($p<0.001$).

A literature review by Miazek K et al 2022 suggested significant potential of carotene, tocopherols and ascorbic acid to mitigate oxidative stress in various biological systems. [14]

Zinc is an essential component of over 200 metalloenzymes involved in carbohydrate and protein metabolism, nucleic acid synthesis, antioxidant functions. Its involvement in cellular division and differentiation makes it essential for embryogenesis. [25] During pregnancy, Zn facilitates brain development & helps during labour. [26]

In the present study, retinol levels were not found to be statistically different among the three groups. The ascorbic acid levels were significantly lower in Group A & B as compared to Group C. Similarly, Awoyelu CO et al 2004 study observed 10% of the pregnant females to be having marginally low ascorbic acid levels. Gupta et al 2020 concluded an increased susceptibility to premature preterm rupture of membranes in pregnant women with vitamin C deficiency. [27]

In the present study, α tocopherol levels were lower both in the Group A & B and their neonates as compared to Group C & their neonates. Similar findings shown by Kothari 1991. [28] Tazawa et al 1994, studied Vit E levels in infants, children & adolescents & noted 100% infants, 41% children & none of the adolescents suffered from Vit E deficiency.[29] Keikha, M et al 2021 study observed increased levels of ascorbic acid & α tocopherol in human milk during the first few days which support the antioxidant role of these two vitamins in counteracting the oxidative stresses of the neonates. In the present study, β carotene levels were not in the deficient range but Group A noted lower levels as compared to Group C.[30]

Pathak P et al 2004 concluded that pregnant women consumed a cereal-based (wheat) diet, which has been shown to have high phytate content. Also, 70% of the women were vegetarians, reflecting a low intake of animal foods (enhancers of zinc and iron absorption). This could be a possible explanation for high prevalence of zinc and iron deficiency.[31]

Thus, vitamin levels should be adequately monitored during pregnancy in order to replenish any deficiency by dietary supplementation & increased fruit intake as these function as antioxidants and counteract the oxidative stresses in the mother as well as neonates.

Conclusion

The present study observed high MDA levels & low serum levels of antioxidants vitamins & enzymes in the pregnant women. This imbalance between the oxidant and anti-oxidant ratio may result in complications during pregnancy. Oxidative stress can also have negative impact on the health of mother & fetus both. Thus preventive measures should be undertaken to replenish the micronutrients. The patients should be counselled about the proper diet during pregnancy. Management of the cases should be done while keeping these facts in mind.

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