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STUDY OF MICRONUTRIENTS, FREE RADICAL AND ANTIOXIDANTS IN PREGNANT WOMEN

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ABSTRACT

Introduction: Nutritional anemia is more prevalent among women and contributes to maternal morbidity and mortality, as well as to low birth weight. The relationship between decreased iron availability in the nutrient media and the possible onset of oxidative stress is becoming more evident, because of the dual role played by iron in cell metabolism as either an antioxidant or a pro-oxidant factor.

Methods: The present study we comprised two groups, including low and high socioeconomic pregnant women (25-30 yrs). In these groups we studied complete blood cell count by sysmax cell counter; Serum Iron, TIBC and Zinc level by standard colorimetric methods; Plasma Vitamin C, Serum Malondialdehyde, Superoxide Dismutase was estimated by spectrophotometrically.

Observations & Results: We found statistically significant increased levels of lipid peroxidation and marked decrease in enzymatic and non-enzymatic antioxidants in anemic as compared to non-anemic pregnant women. We observed negative correlation between serum MDA and iron level ($r = -0.60$) in moderately anemic pregnant women. We also found negative correlation of serum MDA with vitamin C, SOD and Zinc ($r = -0.48$, $r = -0.44$, $r = -0.53$ resp.) and positive correlation between serum MDA and TIBC ($r = 0.35$) in moderately anemic pregnant women.

Conclusion: Over-all socio-economic development is recommended that all women of childbearing age should receive basic nutrition education. Thus there is an urgent need to promote healthy weaning practices and consumption of nutritionally sound, complementary foods to prevent anemia and decrease oxidative stress.

KEYWORDS : Micronutrients, Anemia, lipid peroxidation, antioxidants.

INTRODUCTION:-

Micronutrients encompass vitamins and minerals which are essential for normal human development and functioning and are needed in minute quantities(1). Pregnant women have been known to have high demand for nutrients during different stages of their reproductive cycles(2). The significance of adequate nutrition before and during pregnancy has long been known to optimize both mother and baby's health and well-being. Pregnancy is a time of enhanced metabolic demands with modifications in the physiology of a woman and increasing fetus requirements. Insufficient supply of vital micronutrients can result in a state of biological competition(3).

Although the fact that human adults requires only about 1 mg of absorbable iron per day, the problem of iron deficiency anemia is widespread, especially among women of reproductive age. While iron deficiency anemia (IDA), especially the more severe form has been shown to be associated with increased maternal mortality, even mild iron deficiency anemia may increase the rate of premature delivery and perinatal mortality(1). WHO's 2002 Global Burden of Disease Report identified iron deficiency as the 12th most important risk factor for all mortality globally, and the 9th most important risk factor for the global burden of disease.

Recent assessment by WHO about causes of maternal death has shown that hemorrhage contributes significantly to maternal death in developing countries(4).

In a separate analysis, IDA was an underlying risk factor for maternal and peri-natal mortality and morbidity, and was estimated to be associated with 115,000 of the 510,000 maternal deaths (22%) and 591,000 of the 2,464,000 peri-natal deaths (24%) occurring annually around the world. The consequences of anemia are serious, like reduces a woman's ability to survive due to bleeding during and after childbirth post-partum hemorrhage (PPH) and may result in premature and/or lower birth weight babies with a higher risk of death(4).

Pregnancy is a stressful condition associated with many physiological and metabolic alterations in functions to a considerable extent (5). Normal pregnancy is associated with high metabolic demand and higher requirement for tissue oxygen resulting in increased oxidative stress and antioxidant defences (6). There is a complex interaction of the pro-oxidants and antioxidants that results in the maintenance of intracellular homeostasis. Antioxidants play an essential role in the development and growth of the fetus, maintenance of a healthy pregnancy - and even before pregnancy, in fertility and conception (7). A state of oxidative stress is initiated due to the imbalance between the pro-oxidants and antioxidants (8). Oxidative stress (OS) has found to affect multiple physiological processes, from oocyte maturation to fertilization, embryo development and pregnancy. OS plays a role during pregnancy and normal parturition and in initiation of pre-term labour (9). Persistent formation of free radicals with the absence of proper antioxidant balance causes pathological changes in cells and tissue of female reproductive tract affecting functions like oocyte maturation, ovulation, implantation, and formation of amniotic fluid cavity, in pregnancy (10).

The generation of free radicals, is a normal physiological process, they act on lipids causing lipid peroxidation (11). There are two major types of free radical species— Reactive oxygen species (ROS) and reactive nitrogen species (NOS). In a healthy person, ROS and antioxidants remain in balance. Disruption in this balance, towards an overabundance of ROS causes OS. In most cases, OS appears to be a result of increased generation of ROS, rather than a depletion of antioxidants (10).

Pregnancy, mostly because of the mitochondria-rich placenta, is a condition favouring oxidative stress (12). Many complications in pregnancy and birth defects have found to be related to the oxidative stress, free radical damage. Cells have developed a wide range of antioxidants systems in order to limit the production of ROS, inactivate them and to repair the cell damage. Thus the present study is aimed to determine the, complete blood cell count, serum iron, TIBC and zinc status in randomly selected pregnant women of the third trimester and to correlate them with oxidative stress (oxidants and antioxidants).

MATERIALS AND METHODS:-

The present study is comprised of 70 randomly selected pregnant women of 3rd trimester from low socio-economic status, which were divided into two groups on the basis of degree of anemia. Group 1st includes 20 non anemic and group 2nd includes 50 anemic pregnant women. Group 2nd further divided into two, group 2a includes 28 mild anemic and group 2b includes 22 moderately anemic pregnant women. This study was carried out at MGM Medical College, and Hospital, Navi Mumbai. A social demographic profile including education, family structure, diet consumed (vegetarian, non-vegetarian) was

noted. The study was carried out after obtaining free and informed verbal consent from all pregnant women. In this study subjects having severe anemia and metabolic and systemic disorder were excluded.

The anemia was diagnosed by estimating Complete Blood Cell Count on fully automated sysmax cell counter, serum Iron, TIBC was estimated by standard kit method. The antioxidant Vitamin C and serum Superoxide Dismutase (SOD) and marker of lipid peroxidation is serum Malondialdehyde level (MDA) was estimated by standard spectrophotometric methods. The statistical analysis was done by on SPSS version 20 with 95% confident interval.

RESULT AND DISCUSSION:

Table 1: Shows Distribution of Pregnant Women

Pregnant Women Age Group	Non Anemic Pregnant Women [Group-1]	Anemic Pregnant Women [Group-2] [n=50]	
		Mild Anemic [Group 2a]	Moderate Anemic [Group-2b]
20 to35 years	n=20 [28.5%]	n=28 [56%]	n=22 [44%]

Table 2 Hematological Parameters in Non-Anemic and Anemic Pregnant Women of third trimester.

Hematological Parameters	Non Anemic Pregnant Women [n=20]	Anemic Pregnant Women [n=50]	
		Mild Anemic [n=28]	Moderate Anemic[n=22]
Hb [gm %]	12.6±0.5	10.4±0.3*	9.2±0.6**
RBC[million/cu mm]	4.5±0.6	3.9±0.3*	3.3±0.4**
PCV [%]	37.5±1.6	31.8±1.3**	27.3±2.4**
MCV [fl]	83.0±9.2	80.8±6.1 NS	79.8±6.7 NS
MCH [pg]	28.1±4.1	27.4±2.7 NS	26.2±3.0 NS
MCHC [gm/dl]	33.8±1.4	33.6±1.6 NS	32.7±1.5 NS
RDW [%]	14.8±2.3	15.4±2.2 NS	15.6±2.5 NS
Serum Iron [µg/dl]	101.6±19.7	83.1±5.4**	73.1±5.8**
Serum TIBC [µg/dl]	277.7±26.6	305.8±15.6**	397.3±18.7**

Results are presented as mean ± S.D. NS p > *0.05*, S*p<*0.05*, HS**p <*0.001*

Table 3 Biochemical Parameters in Non-Anemic and Anemic Pregnant Women of third trimester

Biochemical Parameters	Non Anemic Pregnant Women [n=20]	Anemic Pregnant Women [n=50]	
		Mild Anemic [n=28]	Moderate Anemic [n=22]
Serum MDA [nmol/ml]	3.5± 0.6	3.7 ± 0.5 NS	4.4±0.76*
Plasma Vitamin C [mg/dl]	1.0± 0.2	0.79 ± 0.06*	0.66±0.12*
Serum SOD [Units/ml]	5.0 ± 0.4	4.7 ± 0.3 NS	4.15±0.6*
Serum Zinc [µg/dl]	90.5 ± 6.6	84.0 ± 8.0*	74.3±6.9**

Results are presented as mean ± S.D. NS *P >0.05,* S**P<0.05*, HS**P <0.001*

In the present study we determined complete blood cell count, levels of Serum Iron, TIBC, Zinc, MDA, Vitamin C and SOD activity in pregnant women of third trimester. We have studied prevalence of anemia and correlation of IDA with oxidative stress in control and study groups. In the study includes randomly selected 70 pregnant women of third trimester. Pregnancy is associated with increased demand for all the nutrients and deficiency of any of these nutrients could affects pregnancy, delivery and outcome of pregnancy.

Table 2 Shows that hematological parameters Hb, RBC, PCV and serum Iron significantly decreased while serum TIBC level was significantly increased in mild and moderate anemic as compared to non-anemic pregnant women. We also found that prevalence of anemia is 71.4% in pregnant women, of this 56% were mild anemic and as 44% were moderately anemic, whereas surprisingly we could not found severe anemic pregnant women. Among the 71.4% anemic pregnant women, 55.4% were suffering from hypochromic microcytic anemia. This finding indicating that anemia in pregnancy is still a severe problem particularly in low socio-economic status. We observed positive correlation (r = 0.76) between hemoglobin and iron and negative correlation (r = -0.60) between serum MDA and serum iron level in moderately anemic pregnant women.

Our results are in par with earlier reports (1). Sheshadri S et al reported the high prevalence of micronutrient deficiency particularly of iron, zinc and folic acid in pregnant women in South East Asia. The study report indicates that iron deficiency anemia affects more than 50% of pregnant women, the prevalence of folic acid deficiency may be up to 30-50% and there is evidence to suggest that zinc deficiency is likely to be widespread but supportive data are scarce. Iron is an essential constituent of heme and when its levels is low, it affects synthesis of heamoglobin, which is required for maturation of RBCs. Defective heamoglobin synthesis impairs maturation of RBCs which results in hypochromic, microcytic anemia. It also may be due to plasma volume is expanding more rapidly than the red cell mass(1). In present study by the questionnaires indicates that anemic pregnant women are from lower income and less varied diets which is lack in essential nutrients. The nutritional requirement and caloric needs are difficult to meet due to extended families and large number of dependents.

Table 3 shows that MDA concentration was significantly increased and plasma vitamin C and serum zinc level significantly decreased in anemic as compared to non-anemic pregnant women. Serum SOD activity significantly decreased in moderate anemic pregnant women, but non significantly decreased in mild anemic pregnant women. We also correlated the level of MDA with Vitamin C, SOD, Zinc, and TIBC and the observations are negative correlation (r = -0.48, r= - 0.44, r = -0.53 resp.) of serum MDA with vitamin C, SOD and Zinc and positive correlation (r = 0.35) between serum MDA and TIBC in moderately anemic pregnant women.

Amit kumar mani tiwari et al showed that pregnant women with IDA have higher levels of lipidperoxidation (LPO) and lower levels of SOD and vitamin C levels than healthy control(13), Kurtoglu E reported same findings (14). Our results are in accordance with these findings, that there is significant decrease of SOD and vitamin C levels in anemic pregnant women. Ai-Guo Ma et al reported decrease in serum zinc level in last trimester of pregnancy, this may be because zinc is passively transferred across the placenta and there is also decrease zinc binding capacity of maternal blood during pregnancy, which facilitates efficient transfer of zinc mother to fetus(15). Idogun E Sylvester et al reported plasma ascorbic acid levels are reduced during pregnancy and socio demographic factors have mild effect on plasma vitamin C levels (16).

This decrease in ascorbic acid may be due to its extensive use as antioxidant to protect the gastrointestinal tract from the free radical damage during iron repletion and increased levels of lipid peroxidation products. The vitamin C acts as a reducing antioxidant and directly reacts with superoxides, hydroxyl radicals and various lipid hydroperoxides. In addition it can also restore the antioxidant properties of oxidized vitamin E(2).

The present study suggests that iron deficiency anemia is associated with over production of reactive oxygen species in terms of MDA or consequently decreased in antioxidant defense in terms of Vitamin C, SOD and Zinc. It also acts as a co-factor for many antioxidant enzymes; zinc is also constituent of SOD. Thus imbalance between oxidant and antioxidant causes peroxidation of vital body molecules which implies increased risk for pregnant women as well as for fetus. However, further in-depth studies are needed to assess the status of antioxidant minerals and molecules in pregnancy related anemia. In addition to this due to lack of rich sources of iron as well as vitamin C in the nutrition like green leafy vegetables, fruits, organ meat causes imbalance between oxidant and antioxidants. The questionnaire obtained from them clearly shows that there is lack of awareness, lack of self-wellbeing and negligence of health of pregnant women.

CONCLUSION:

Prevalence of anemia was significantly high with increased oxidative stress among pregnant women of third trimester belonging low to middle socio-economic status. Thus it is evident that over-all socio-economic development, adequate nutritional intake and combination of nutritional education, health awareness helps in reducing the prevalence of anemia in pregnant women and it can improve their health and wellbeing. The usual approach to improve micronutrient status during pregnancy i. e. for iron and vitamins supplementation, but in addition to this more attention should be focused on dietary approaches, including fortification of foods with micronutrients, which can be more useful and sustainable than supplementation during pregnancy.

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