Original Research Paper

Volume - 13 | Issue - 04 | April - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Obstetrics & Gynaecology

EVALUATION OF OXIDATIVE STRESS MARKERS IN ANTENATAL WOMEN RECEIVING ROUTINE IRON

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ABSTRACT **Background:** Anaemia is low haemoglobin concentration affecting 47.74% of pregnant women globally.9 WHO recommends routine supplementation of iron to all antenatal mothers irrespective of the haemoglobin status. The aim of this study was to evaluate the levels of oxidative stress markers in antenatal women receiving routine iron supplementation and correlate their maternal and fetal outcomes. **Methods:** A prospective observational study of 100 pregnant women was conducted over 1.5 years at St. John's Medical College Hospital. On screening for inclusion criteria, at baseline, information on maternal socio-demography, anthropometry, dietary intake and clinical examination was collected. A venous blood sample at baseline and 3rd trimester of pregnancy and at delivery was collected for estimation of oxidative stress markers i.e., catalase and lipid peroxidase and results were correlated with pregnancy and birth outcomes. **Results:** The levels of oxidative stress markers were found to decrease from first to third trimester in a subset of 30 patients participating in the study. There was a greater incidence of preeclampsia in women with Hb > 13 g/dl receiving routine iron supplementation (37.5%). Also, there was increased risk of GDM, PROM and febrile morbidity although not statistically significant. **Conclusion:** Our study suggests that there is a trend towards increase in oxidative stress and incidence of pre-eclampsia when excess iron is supplemented to pregnant women with normal haemoglobin. Assessment of other oxidative stress markers in a larger study may be helpful in deciding the need for iron supplementation and its dosage.

KEYWORDS : iron supplementation, non-anaemic women, pregnancy outcome, oxidative stress

INTRODUCTION

Iron deficiency anaemia ranks 9th among 26 diseases of highest burden. Asia accounts for 70% of the global burden. Adverse maternal and birth outcomes associated with the haemoglobin status renders the issue worth attention.[1] According to the National Rural Health Mission, anaemia is defined as haemoglobin less than 11 g/dl[2]. Maternal anaemia when diagnosed before mid-pregnancy is associated with an increased risk of preterm birth. Hence, WHO recommends routine supplementation of iron to all antenatal mothers irrespective of the haemoglobin status.

On the other hand, it has been suggested that high levels of haemoglobin, haematocrit, and ferritin are also associated with an increased risk of foetal growth restriction, preterm delivery, and preeclampsia.[1] It has been reported that pregnancy specific morbidities i.e. haemorrhage and septicaemia with low haemoglobin; eclampsia, small for gestational age, gestational diabetes with high haemoglobin, alarm is raised to define the optimum range.[3] Oxidative stress is defined as a condition where there is disturbance in the pro-oxidant and antioxidant balance.[4] There is increased susceptibility to oxidative stress during pregnancy.

Fe^{2+} or Cu^{2+} reacting with unsaturated fatty acids in the presence of O_2 initiates the lipid peroxidation cascade in lipoproteins and biological membranes by the production of highly reactive $OH^{\cdot}$. Therefore other iron based or transition metal based radicals may be responsible for initiating the peroxidation of lipids.[5]

Having this information, and the fact that iron supplementation is done to all pregnant women in India, irrespective of the initial iron status, it appears worthwhile to investigate the effect of such supplementation on oxidative stress and birth outcomes, and to extend this information to possible long term effects such as susceptibility to metabolic syndrome in adulthood.

MATERIALS AND METHODS

A prospective observational study was conducted at St John's Research Institute and St John's Medical College Hospital (SJMCH), Bangalore, India from December 2014 to July 2016. The institutional ethical review board of SJMCH, Bangalore approved the study procedures and a written informed consent was obtained from each participant at enrolment.

All pregnant women aged 18 – 40 years and between 11 to 14 weeks of gestation, registered for antenatal screening at the Department of Obstetrics and Gynaecology of SJMCH, were eligible for this study. On the other hand, women with multiple fetuses; clinical diagnosis of chronic illness such as diabetes mellitus, hypertension, heart disease, and thyroid disease; positive for hepatitis B surface antigen, HIV, or syphilis infection (venereal disease); or who anticipated moving out of the city before delivery—were excluded. Socio-demographic details were collected at baseline (12.64 ± 0.78 weeks of gestation) along with information on maternal anthropometric measurements, dietary intake, clinical status, and routine antenatal blood biochemistry. These data were also collected in their second (21.67 ± 2.61 weeks) and third trimester (35.4 ± 2.34 weeks) of pregnancy.

Of the 133 women contacted, only 120 qualified for participation. Among these 120 patients, 20 who intended to deliver at our hospital were lost to follow-up in the third trimester. Pregnancy outcomes all 100 women were included, whereas for birth outcomes women with birth data (n=81) were included. Owing to the sample size required to detect a difference in the primary outcome of the study, oxidative stress marker levels were measured in a subset of 30 patients (catalase and lipid peroxidase).

Antenatal care

Each patient was prescribed antenatal supplements of folic acid, iron, and calcium as per the antenatal schedule. At recruitment, patients were prescribed 5 mg folic acid supplement/d until the end of the first trimester. From the beginning of the second trimester until delivery, 100 mg ferrous ascorbate (equivalent to 100 mg elemental Fe) along with 0.5 mg folic acid/day was prescribed. In addition, during this period, the subjects were also prescribed 1000 mg calcium/day and 250 IU vitamin D3. No other multivitamin or multi-mineral preparations were prescribed. Supplement compliance with these prescriptions was recorded during the course of pregnancy in the form of a tablet count, to sum up the daily total iron intake.

Delivery And Birth Information

Post delivery, cord blood was collected and infants were weighed to the nearest 10 g on an electronic weighing scale (Salter Housewares 914 Electronic Baby and Toddler Scale, Oak Brook, IL, USA) immediately after birth. Infants born before 37 completed weeks of gestation were considered preterm. Infants weighing less than the 10th percentile for gestational age at delivery were defined as small for gestational age (SGA).

Blood Collection

Venous whole blood samples were collected into EDTA coated anticoagulant tubes (Becton Dickenson, NJ, USA) by trained laboratory assistants at T1 (trimester 1, weeks) weeks of gestation and

T3 (weeks). Haemoglobin (Hb) concentration was analysed using an automated cyanmethaemoglobin technique (ABX Pentra 60 C+ haematology analyser; Horiba ABX Diagnostics, Germany). For the analysis of oxidative stress markers, 1 ml of blood was stored in a plain vacutainer at -80ºC until analysis.

Oxidative Stress Marker Assays

Lipidperoxidase (LPO) enzyme activity was measured as mmol thiobarbituric acid-reactive species (TBARS) formed on reaction of plasma with malondialdehyde (MDA). Briefly, plasma proteins were first precipitated with 15 % (w/v) tri-chloro acetic acid and the supernatant was reacted with 20-mM TBA in the presence of 10 % (w/v) sodium dodecyl sulphate. The TBARS formed were measured in a fluorescence spectrophotometer with λ_{ex}=520 nm and λ_{em} =570 nm, and data were expressed as nmol MDA/ml plasma.

Catalase activity was assayed by incubating with excess substrate (hydrogen peroxide, H_2O_2) for 5 minutes on ice. The reaction was then quenched by the addition of sulphuric acid. The amount of H_2O_2 remaining in the reaction mixture (enzyme + substrate + cofactor + buffer) after 5 minutes of catalase action was determined spectrophotometrically at 450 nm using a multi plate reader system. Catalase activity was defined as the number of units/min/ml plasma of hydrogen peroxide decomposed in 5 minutes and was expressed as U catalase/min/ml plasma.

RESULTS

Out of the 100 patients who were followed up, delivery details of 81 patients was recorded and included in analysis. General characteristics and delivery details of study patients have been presented in table 1. The average age of patients was 25.0±3.8 years.

Table 1: General Characteristics And Delivery Details Of The Study Patients

Characteristics	n=81	Standard deviation
Age (years)	25.0±3.8	3.89
BMI	21.63	3.04
T1 Hb (g/dl)	11.99	0.61
T2 Hb (g/dl)	11.05	1.23
T3 Hb (g/dl)	11.62	1.15
Gestational Age at delivery (weeks)	38.3	1.6
Birth weight (kg)	2.9	0.45

BMI: body mass index; Hb: haemoglobin; TI: trimester 1; T2: trimester 2; T3: trimester 3

Analysis of oxidative stress markers was performed in a sub-set of 30 patients. The levels of LPO and catalase and LPO in the first and third trimester are represented in figure 2 and 3-. Activities of both these enzymes increased from the first to third trimester. It was seen that LPO activity was higher in patients with >12g/dL Hb (T1: 2.59 nmol/L, T3:0.89 nmol/L) than <12g/dL (T1: 2.27 nmol/L, T3: 0.99 nmol/L). Similarly, catalase activity was also higher in patients with >12g/dL Hb (T1: 37.58 U/ml/min, T3: 33.59 U/ml/min) compared to <12g/dL (34.31 U/ml/min, T3: 28.69 U/ml/min).

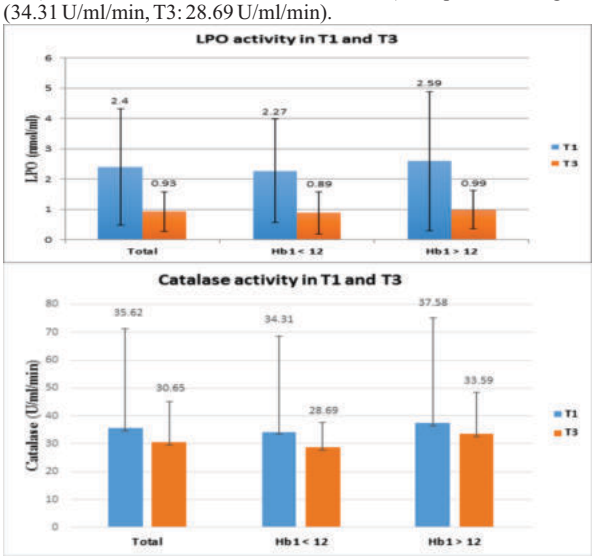

Figure 2: LPO levels in first and third trimester

Correlation analysis between oxidative stress markers and haemoglobin in three trimesters is represented Table 2. A significant, positive correlation was seen between Hb – T3 and V3 catalase activity (r= 0.328, P<0.05).

Table 2: Association Between Between Oxidative Stress Markers And HB

	T1 LPO	T1 CAT	T3 LPO	T3 CAT
Hb-T1	0.067	0.104	0.187	0.218
Hb-T2	0.193	-0.029	0.245	-0.079
Hb-T3	0.002	0.123	0.244	0.328*

*Spearman's correlation analysis. *P<0.05*

When the perinatal outcome of these patients was evaluated, it was found that there was an increased risk of preeclampsia in non-anaemic women on iron supplements. Also, there was increased risk of premature rupture of membranes (PROM), and gestational diabetes in these women although these were not statistically significant.

Table 3: Frequency Of Complications In Pregnancy:

COMPLICATION	n	%
Preeclampsia	23	28.4
GDM	6	7.41
Preterm	5	6.17
PROM	10	12.35
Febrile morbidity	6	7.41
Other morbidities	11	13.58
Uncomplicated	39	39

Furthermore, patients were grouped into tertiles based on their Hb status in the first trimester prior to iron supplementation. All three groups of women were supplemented with 100 mg elemental iron. With haemoglobin more than 13 g/dl, 37.5% of patients developed preeclampsia. When the haemoglobin was <12 g/dl, 22.95% of the patients developed preeclampsia. In this group an increased risk of PROM and gestational diabetes was also noted (Table 4).

Table 4: Table Depicting The Morbidities

Hb (g/dl)	Frequency (n)	Total morbidities	Preeclampsia	GDM	PROM
<12	48	30 (62.5%)	11 (22.92%)	5 (10.5%)	5 (10.5%)
12-13	44	25(56.81%)	11 (25%)	2 (4.54%)	5(11.36%)
>13	8	5 (62.5%)	3 (37.5%)	0	0

DISCUSSION

Iron deficiency anaemia is a common problem during pregnancy and can have adverse effects on the mother and the baby, manifesting as preeclampsia, low birth weight, PROM, GDM etc. Current recommendations prescribe iron to all pregnant women irrespective of their iron status. Therefore, the effect of prophylactic iron supplementation is likely to be highly variable, depending on the initial iron status, compliance to medication and the dosage received. This study was intended to evaluate the effects of excess iron intake by non-anaemic pregnant women.

In our analysis of non-anaemic women, in the subset of 30 patients, we found that there was an increase in LPO activity and catalase activity from first to third trimester. This is similar to a study done by Neeta Kumar et al[1].

We observed that the risk of preeclampsia has increased in non-anaemic women taking iron supplements although not statistically significant. This is comparable to the study done by Raman et al which suggests that pregnancy specific morbidities like preeclampsia, eclampsia are found to be associated with high Hb at term.[9] For example, in a study done by S Ziaei et al, [10] 727 pregnant women with Hb ≥ 13.2 g/dl were divided into two groups and 370 women received 50 mg of ferrous sulphate whereas 357 women received placebo and their pregnancy outcome was assessed. There was an increased incidence of hypertension disorder, small for gestational age birth rate in the case group compared to the placebo group.

But, when comparing the oxidative stress markers in the subset of 30 patients and the risk of preeclampsia, our studies shows that there was no significant increase in the preeclampsia state as seen in a study done by Morris et al.[74] They divided the study population into 3 groups: those with preeclampsia (n=19), control pregnant women (n=19), matched for gestation, age and parity and a group of non-pregnant

individuals (n=7). They found that there was no difference in lipid peroxide or Malondialdehyde levels between the groups of women with preeclampsia and the control group. However, the levels were significantly higher than in non-pregnant women.[11]

It is in contrast to a study by Casanueva[5] et al, which has demonstrated the negative effects associated with high Hb levels early in pregnancy, as well as in situations where Hb levels fail to show the decline secondary to physiological dilution after gestational week 20. The incidence of gestational hypertension, preeclampsia, eclampsia, low birth weight increase rapidly when Hb levels surpass 13 g/dl.[12,13]

In our study, when the study population was divided into 3 subgroups with Hb <12, 12.1-13 and > 13 g/dl, it was noted that the subgroup with Hb > 13 g/dl, had a high incidence of preeclampsia i.e., 37.5%. At the same time, when Hb < 12 g/dl, about 22.5% patients developed preeclampsia. There is a 10% increase in risk of gestational diabetes in the study group.

CONCLUSION

In our study of iron supplemented women, in the majority of women with Hb between 11-13 g/dl (92%), there was no statistically significant increase in the risk of preeclampsia. However since the sample size was small, further studies are warranted in various parts of the country to validate the same. Further policies looking at maternal iron supplementation should consider these evidences and recommend the same to pregnant women who are diagnosed of iron deficient anaemia to begin with.

REFERENCES

1. Neeta Kumar, Nomita Chandhiok, Balwan S Dhillon and Pratik Kumar. Role of oxidative stress while controlling iron deficiency anaemia during pregnancy – Indian scenario. Indian Journal of Clinical Biochemistry; 2009: 24 (1) 5-14.
2. Guidelines for control of Iron Deficiency Anaemia, National rural health mission, NRHM, Ministry of health, India, 2013: 9-25.
3. Saara Parkkali, Abascassamo F, Nwaru B I, et al. Comparison of routine iron prophylaxis and screening and treatment for anaemia: pregnancy results and preliminary birth results from a pragmatic randomised controlled trial (PROFEG) in Maputo, Mozambique. BMJ Open 2013; 3: e001948.
4. Cheeseman KH, Salter TF. An introduction to free radical biochemistry Br Medical. Bulletin; 1993: 49(3):481-493.
5. Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. J Nutr 2003; May 133 (5 Suppl 2): 1700S-1708S.
6. Sri Giridhar K, Nair KM. Supplementation with alphatocopherol or a combination of alpha-tocopherol and ascorbic acid protects the gastrointestinal tract of iron-deficient rats against iron-induced oxidative damage during iron repletion. Br J Nutr 2000; 84: 165–73.
7. Bedi N, Kambo I, Dhillon BS, Saxena BN, Singh P. Maternal deaths in India - Preventable tragedies (An ICMR Task force Study). J Obst Gyn of Ind 2001; 51(2): 86-92.
8. Gomber S, Agarwal KN, Mahajan C, Agarwal N. Impact of daily versus weekly hematinic supplementation on anemia in pregnant women. Ind Pediatr 2002; 39(4): 339-46.
9. Raman L, Pawashe AB, Yasodhara P. Hyperferritinemia in pregnancy induced hypertension and eclampsia. J Postgrad Med 1992; 38(2): 65-7.
10. Ziaei S, Norrozi M, Faghihzadeh S, Jafarbegloo E. A randomized placebo-controlled trial to determine the effect of iron supplementation on pregnancy outcome in pregnant women with haemoglobin ≥ 13.2 g/dl. BJOG. 2007; 114:684–688. [PubMed: 17516958].
11. Jonathan M. Morris, Nitin K. Gopaul, Narit J.R. Endersen, e al. Circulating markers of oxidative stress are raised in normal pregnancy and preeclampsia. British Journal of Obstetrics and Gynaecology. November 1998; 105: 1195-1199.
12. Lund, C. J. & Donovan, J. C. (1967) Blood volume during pregnancy. Am. J. Obstet. Gynecol. 98: 393–397.
13. Lao, T. T., Chan, P. L. & Tam, K. F. (2001) Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess? Diabet. Med. 18: 218–223.