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Correlation between oxidative stress and Serum Iron level in pregnant anemic women in north Indian population

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ABSTRACT

The objective of the study is to find a correlation between anemia, oxidative stress and iron status in pregnant females in rural Rajasthan. A study group of 25 pregnant anemic women and a control group of 25 pregnant non-anemic women were chosen from Obstetrics and Gynecology OPD. Blood samples were taken and all relevant blood indices like, Hb%, PCV, MVC, MCH, MCHC were measured by automated cell counter. Serum iron level was measured by CAB method and FRAP easy was done on all the subjects and controls. The study showed a direct positive correlation (R value=0.46) between Serum Iron levels and Oxidative stress in study and control group. It also highlights a significant correlation ($p<0.05$) between Hb% level and a decreased ferric reducing ability of plasma. Anemia in pregnancy shifts oxidant-anti oxidant balance towards oxidant spectrum resulting in a significant decrease in antioxidant activity in plasma of such females. With oxidative stress already implicated in maternal and fetal complication in the outcome of pregnancy this increase is significant and calls for further evaluation.

KEYWORDS : oxidative stress, anti-oxidant, anemia

Introduction:

Oxidative stress (OS) results from an imbalance between reducing agents and enzymes involved in the removal of free radicals and/or reactive oxygen species. Oxidative stress affects a complex array of genes involved in inflammation, coagulation, fibrinolysis, the cell cycle, signal transduction and programmed cell death.[1]

Free radicals are generated during normal physiological processes but increased production of free radicals can cause alteration of biomolecules such as lipid peroxidation.[2] The cells have evolved a number of antioxidant defense mechanisms that neutralize free radicals. These antioxidant defense mechanisms can be categorized in to two types- free radical scavenging and chain breaking antioxidants.[3]The free radical scavenging mechanisms include enzymatic antioxidant like Superoxide dismutase (SOD), Glutathione peroxidase (GSH-Px), and catalase, which limit the cellular concentration of free radicals and prevent excessive oxidative damage.[3]

There exists a balance between the pro oxidant process/free radical generation and the antioxidant mechanisms of the cell. Under certain condition, this balance is disturbed there occurs a shift towards the oxidative processes resulting in increased level of oxidative stress.[4]

Pregnancy is a stressful condition in which many physiological and metabolic functions are altered to a considerable extent.[5] The mitochondria rich placenta becomes a major source of free radicals in the oxygen rich environment during pregnancy. In recent years the role of decreasing antioxidants and increasing superoxide is gaining importance as they are threat for the normal pregnancy. The studies found that there is reduced superoxide dismutase activity in the third trimester of normal pregnancy as compared to non-pregnant women.[6] Superoxide dismutase (SOD) is thought to play a central role in free radical scavenging because of its ability to scavenge superoxide anions, the primary ROS generated from molecular oxygen in cells.[7]

Anemia is a pathologic condition marked by either a reduction in the red blood cell count (due to decreased production or increased destruction) or decrease in the hemoglobin concentration in the red blood cells. Anemia also appears to be caused by shortened lifespan of erythrocytes. Increased ROS due to SOD1 deficiency makes their erythrocytes vulnerable to oxidative stress. In addition to SOD1 deficiency, GPx activity and protein levels of GPx1 were significantly lower in erythrocytes. Since GPx1 protein is prone to oxidative inactivation, oxidized GPx1 would be removed by the protease that degrades oxidized proteins in erythrocytes.[8]

Iron deficiency is the most common nutritional deficiency world over; resulting in iron deficiency anemia (IDA) in approximately 500 to 600million people.[9] Anemia is the commonest medical disorder of pregnancy. WHO has estimated that 14 per cent pregnant women in developed and 51 per cent pregnant women in developing countries suffer from anemia. In India the figure is even worse with 65-75 percent pregnant females suffering from anemia.[9] Prevalence of anemia in South Asian countries is among the highest in the world. WHO estimates that even among the South Asian countries, India has the highest prevalence of anemia. What is even more important is the fact that about half of the global maternal deaths due to anemia occur in South Asian Countries; India contributes to about 80 percent of the maternal deaths due to anemia in South Asia.[10] The most recent National family health Survey (NFHS-III, 2005-06) has reported a prevalence of anemia at 57.9 percent among pregnant women in India. Anemia is one of the important causes of maternal death either directly or indirectly. In 47% of maternal deaths in developing countries it is the cause of death.[11]

So, the present study was designed to find a correlation between the Iron level and Oxidative stress in pregnant anemic women.

2. Material and Method:

2.1 Ethical clearance: This study was carried out in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynecology, NIMS Medical College and Hospital, Jaipur, Rajasthan. The institutional ethical clearance was obtained from Ethical Committee of the college.

2.2 Study population: The total number of subjects in the study were 50, 25 non anemic pregnant women and 25 pregnant anemic women. The subjects were selected from the Department of Obstet-

rics and Gynecology, NIMS University Hospital, Jaipur.

The selected subjects were divided into two groups GROUP 1: Pregnant non anemic women as control. n = 2 5. GROUP 2: Pregnant Anemic women as study group n=25.

The personal and clinical history of the subjects was recorded with help of questionnaire at the time of examination.

Exclusion Criteria: Any patient with a known history of any chronic disorder such as Diabetes, Hypertension, infections such as Tuberculosis.

2.3 Estimation of hemoglobin and complete blood count: The CBC was observed using an ABX Penta 60 auto analyzer at the pathology lab of the central diagnostic division of NIMS Hospital.

2.4 Estimation of Oxidative stress: Ferric reducing ability of plasma: FRAP assay, which depends upon the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. Fe (II)-TPTZ has an intensive blue colour and can be monitored at 593 nm [12]. The automated method for measuring the FRAP or in other words the measurement of "antioxidant power" was modified by Varga et al. to a manual assay [13].

2.5 Estimation of Serum Iron level: CAB method, Iron(III) reacts with chromazurol B (CAB) and cetyltrimethylammoniumbromide (CTMA) to form a colored ternary complex with an absorbance maximum at 623 nm. The intensity of colour produced is directly proportional to the concentration of iron in the sample.

2.6 Statistical analysis: Statistical analysis was done, using SPSS 17 for Windows software Microsoft Excel 2007 and scientific calculator. The results were expressed as Mean ±Standard Deviation (SD). The difference in blood count & plasma level of FRAP between control and study group was analyzed using unpaired "t"-test. Difference between serum Iron level in control and study group was evaluated by unpaired "t"-test. Pearson's correlation was applied to determine the relationship between Serum Iron level in Pregnant anemic women and plasma level of FRAP. Statistical significance was defined at a p value of <0.05.

3. Observations and results. (Table:1) Comparison of Complete Blood Count in Pregnant Non anemic women and Pregnant anemic women.

Parameters	Control Group(n=25)	Study Group (n=25)	P value
Hb% [a]	12.37±0.38	9.70±1.16	P < 0.05
RBC [b]	4.26±0.26	3.67±0.36	P < 0.05
MCV [c]	90.24±3.18	86.12±5.93	P < 0.05
PCV [d]	38±2.51	30.99±3.26	P < 0.05
MCH [e]	28.29±2.40	26.64±2.63	NS
MCHC [f]	29.05±2.24	29.99±3.05	NS

Units a = gm/dl, b = Millions/Cu mm, c = Fl , d =% , e = Pg , f = Gm% , NS= Non significant Values are expressed as mean ± S.D. of 25 subjects compared to normal healthy control.

There was a statistically significant difference in the hematological indices between the study and control group. (p < 0.05.)

The mean value of FRAP in pregnant anemic women was 824±139.94 and Hb% was 9.704±1.16gm%. In control group the mean value of FRAP was 1005 ±203.23 and Hb% was 12.37±0.389gm%.

(Table:2) Comparison of Ferric Reducing Ability of Plasma (FRAP) in pregnant non anemic women and pregnant anemic women.

Parameters	Control Group(n=25)	Study Group(n=25)	P value
Ferric reducing ability of plasma(FRAP) [a]	1005±203.23	833±134.66	P<0.05

(Table No.3):- Comparison of Serum Iron level in Pregnant non anemic women and pregnant anemic women.

Parameters	Control Group(n=25)	Study Group(n=25)	P-Value
SERUM IRON a.	48.32 ± 4.06	21.29 ± 7.66	P <0.05

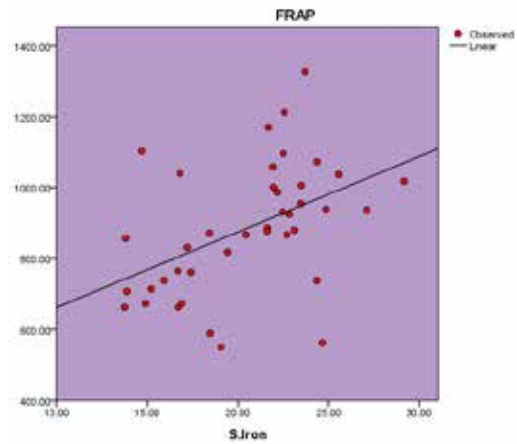
Units a = µg/dl .Values are expressed as mean ± S.D. of 25 subjects compared to normal healthy control.

Pearson's co relation was used to deduce a correlation between the FRAP of the subjects as well as the controls and their Iron level.

(Table No 4): Pearson's Correlation in pregnant anemic women between Serum Iron level and FRAP value.

Parameters	R –value
Ferric reducing ability of plasma	+0.46**

Units a = µ moles of FeSo4 equivalent/ L of plasma.** p<0.05



(Figure no 4)
Pearson's Correlation in pregnant anemic women between Iron levels and FRAP value.

4. Discussion:
In present study, the level of Hb%, TRBC, PCV, MCV among the study group showed significant difference when compared with control group (p<0.05).

The mean value of FRAP in pregnant anemic women was 833±134.66 and Serum Iron was 48.32 ± 4.06. In control group, the mean value of FRAP was 1005 ±203.23 and Serum Iron was 21.29 ± 7.66. It was found that the mean value of FRAP was significantly decreased in pregnant anemic women as compared to control (p<0.05).In pregnant women, the level of FRAP is directly proportional to the level of Serum Iron. i.e. as level of Serum Iron decreased level of FRAP decreased.

There was also a strong positive correlation (R-value= +0.46) between FRAP Values and Serum Iron in pregnant women. That meant that lower the Serum Iron level higher was the oxidative stress. So it showed that iron deficiency anemic pregnant women were exposed to increased oxidative stress in pregnancy compared to non anemic pregnant women.

Pregnancy is a physiological state characterized by a high-energy demand and an increased oxygen requirement. This is due to the increased demand for the mother as well as the fetus. To this end there occur various compensatory adaptive changes with advancing pregnancy, to meet the increasing requirements such as increased ventilation to account for enhanced oxygen demand. Such conditions may be responsible for raised oxidative stress observed during pregnancy. Anemia, due to impairment to the transport of oxygen, may further stress the body, worsening the oxidative load.

Aslam M et al. reported that serum total antioxidant capacity was significantly lower in patients with iron deficiency anemia than con-

trols ($p<0.05$), while serum total peroxide level and oxidative stress index were significantly higher (both $p<0.05$).[14] There was a significant correlation between hemoglobin level and serum total peroxide level, oxidative stress index and total antioxidant capacity ($r=-0.504$, $p<0.05$; $r=-0.503$, $p<0.05$; $r=0.417$, $p<0.05$, respectively).

Ishihara studied, lipid peroxide levels in non anemic pregnant and normal pregnant and reported remarkable increased levels of lipoperoxides in 2nd and 3rd trimesters of pregnancy in anemic pregnant as compared to normal pregnant women.[15] Similar observation was made by Kodliwadmath et al.[16]

Since RBC have no nucleus any increase in oxidative stress results in induction of antioxidant enzyme systems and this suggests of a role of superoxide dismutase in the protection of embryonic development against free radical damage, as observed by Carone et al [17].

Stephen Wisdom et al [18] and Davidge et al [19] reported of a reduction in superoxide dismutase activity in the third trimester of patients of pregnancy induced hypertension and pre eclampsia when compared to normal pregnant women hence suggesting a role of increases oxidative stress in the pathogenesis of these conditions.

5. Conclusion: The study concludes that serum iron level were directly proportional to FRAP values, an increase in serum iron levels results in a fall in the oxidative stress levels This is important keeping in mind that not only the pregnant female but the fetus is also exposed to this exaggerated oxidative stress.

Various studies have shown that exposure to certain factors in utero can have a lifelong effect on wellbeing of the individual[20]. It is important that studies are carried out to discern any long tern health issues faced by children born to anemic mothers battling higher oxidative stress.

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