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CORRELATIVE STUDY OF FERRIC REDUCING ABILITY OF PLASMA (FRAP) AN OXIDATIVE STRESS MARKER AND SERUM IRON LEVEL IN PREGNANT ANAEMIC WOMEN IN THREE TRIMESTERS OF PREGNANCY

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ABSTRACT

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 has been a source of great interest among researchers during the last decade and not without reason. Increased oxidative stress has been shown to be involved in the pathogenesis of a large number of disease processes directly or indirectly. Pregnancy favours a pro-oxidant environment mainly due to the mitochondria-rich placenta. Oxidative stress may not just be a threat to the mother but also to the growing fetus. Further anaemia is also a pro-oxidant condition and females in developing nations like India, suffer from mild to moderate anaemia which further worsens during pregnancy. In the rural female population, this problem is more acute keeping in mind that their diet is deficient in a wide array of nutrients including iron.

Material and Method: The objective of the study is to find a correlation of ferric reducing ability of plasma (FRAP) an oxidative stress marker and iron level in pregnant anaemic women in three trimesters of pregnancy. For this, a study group included a total of 150 subjects. All subjects were divided into control and study groups. Control group consisted of 25 non-anaemic pregnant women of the 1st trimester in 1st group, 25 non-anaemic pregnant women of the 2nd trimester in 2nd group and 25 non-anaemic pregnant women of the 3rd trimester in the 3rd group. While the study group consisted of 25 anaemic pregnant women of the 1st trimester in 1st group, 25 anaemic pregnant women of the 2nd trimester in 2nd group and 25 anaemic pregnant women of the 3rd trimester. Control and study group divided based on different trimester.

Results: Showed a direct positive correlation between Serum Iron levels and Ferric Reducing Ability of Plasma (FRAP) an Oxidative stress marker in the study as well as the control group.

Conclusion: Anemia in pregnancy shifts the oxidant-anti oxidant balance towards the oxidant spectrum resulting in a significant decrease in antioxidant activity in plasma of such females. This adds to the already existing pro-oxidant environment caused by the pregnancy. 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, Free radicals, Ferric reducing ability of plasma

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 defence mechanisms that neutralize free radicals. These antioxidant defence mechanisms can be categorized into 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 an 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 a threat to 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]

Anaemia is a pathologic condition marked by either a reduction in the red blood cell count (due to decreased production or increased destruction) or a decrease in the haemoglobin concentration in the red blood cells. Anemia also appears to be caused by the 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 anaemia (IDA) in approximately 500 to 600 million 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 anaemia. In India, the figure is even worse with 65-75 per cent pregnant females suffering from anemia.[9] Prevalence of anaemia in South Asian countries are among the highest in the world. WHO estimates that even among the South Asian countries, India has the highest prevalence of anaemia. What is even more important is the fact that about half of the global maternal deaths due to anaemia occur in South Asian Countries; India contributes to about 80 per cent of the maternal deaths due to anaemia in South Asia.[10] The most recent National family health Survey (NFHS-III, 2005-06) has reported a prevalence of anaemia at 57.9 per cent among pregnant women in India. Anaemia 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 of ferric reducing ability of plasma (FRAP) an oxidative stress marker and iron level in pregnant anaemic women in three trimesters of pregnancy

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, Shobha Nagar, Jaipur, Rajasthan. The institutional ethical clearance was obtained from the Ethical

Committee of the college.

2.2 Study population: The total number of subjects in the study were 150. All subjects were divided into control and study groups. Control group consisted of 25 non-anaemic pregnant women of the 1st trimester in 1st group, 25 non-anaemic pregnant women of the 2nd trimester in 2nd group and 25 non-anaemic pregnant women of the 3rd trimester in the 3rd group. While the study group consisted of 25 anaemic pregnant women of the 1st trimester in 1st group, 25 anaemic pregnant woman of the 2nd trimester in 2nd group and 25 anaemic pregnant women of the 3rd trimester. Control and study group divided based on different trimester. The subjects were selected from the Department of Obstetrics and Gynecology, NIMS Medical college, Shobha Nagar, Jaipur, Rajasthan. 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 haemoglobin 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 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(lll) reacts with chromazurol B (CAB) and cetyltrimethylammoniumbromide (CTMA) to form a coloured ternary complex with an absorbance maximum at 623 nm. The intensity of the 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 were 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 anaemic women and plasma level of FRAP. Statistical significance was defined as a p-value of <0.05.

3. Observations and results

Table-1: - Comparison of a haematological parameter of control and study group in three trimesters

	1st trimester		2nd trimester		3rd trimester	
Parameters	**Control group of 1st Trimester**	**A study group of 1st Trimester**	**Control group of 2nd Trimester**	**A study group of 2nd Trimester**	**Control group of 3rd Trimester**	**A study group of 3rd Trimester**
Hb(g/dl)	13.27 ± 0.70	10.24 ± 0.67a	13.73 ± 1.55	10.85 ± 0.82b	13.89 ± 0.92	10.96 ± 1.67c
TRBC (million/cumm)	3.94 ± 0.37	2.73 ± 0.53a	4.39 ± 0.52	3.41 ± 0.59b	4.94 ± 0.47	3.82 ± 0.89c
PCV (%)	35.97 ± 1.95	30.27 ± 2.95a	36.76 ± 3.96	34.12 ± 2.75b	40.28 ± 2.73	32.96 ± 4.97c
MCV (FL)	84.76 ± 5.20	67.80 ± 3.89a	88.48 ± 10.69	73.19 ± 3.90b	85.96 ± 8.89	76.89 ± 2.96c
MCH (pg)	28.43 ± 2.89	26.32 ± 2.32a	31.19 ± 2.94	26.63 ± 1.98b	32.19± 4.08	27.13 ± 3.89c
MCHC (gm%)	33.10 ± 1.29	29.19 ± 3.98a	34.98 ± 1.98	30. 42± 2.65b	32.19 ± 1.07	31.16 ± 1.52c

* Shows P<0.05 of Hb, TRBC, PCV, MCV, MCH and MCHC between Control and study group of First Trimester.
** Shows P<0.05 of Hb, TRBC, PCV, MCV, MCH and MCHC between Control and study group of Second Trimester.
*** Shows P<0.05of Hb, TRBC, PCV, MCV, MCH and MCHC between Control and study group of Third Trimester

Table-2 Comparison of FRAP and Serum Iron of control and study groups in a different trimester.

Parameters	CONTROL GROUP(N=25)			STUDY GROUP(N=25)		
	1st Trimester	2nd Trimester	3rd Trimester	1st Trimester	2nd Trimester	3rd Trimester
FRAP [FeSO4 Equivalent of 1L of Plasma (µM)]	944.28 ± 85.26	1012.56 ± 128.64	1116.68 ± 164.64	760.75 ± 121.71*	774 ± 155.43**	797.15 ± 134.92***
SERUM IRON (µmol/L)	21.07 ± 2.40	22.17 ± 2.25	25.46 ± 2.30	18.17 ± 2.97a	18.94 ± 3.86b	19.67 ± 4.26c

a Shows P<0.05 of FRAP and S. Iron between Control and Study group of First Trimester.
b Shows P<0.05 of FRAP and S. Iron between Control and Study group of Second Trimester.
c Shows P<0.05 of FRAP and S. Iron between Control and Study group of Third Trimester

Fig-1: - Comparison of FRAP between control and study group in three trimesters

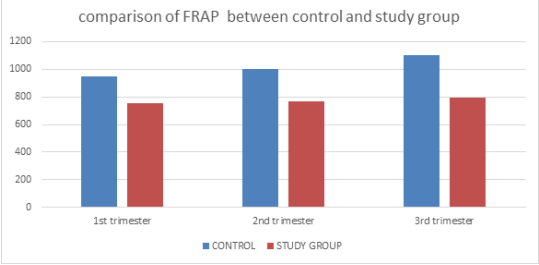
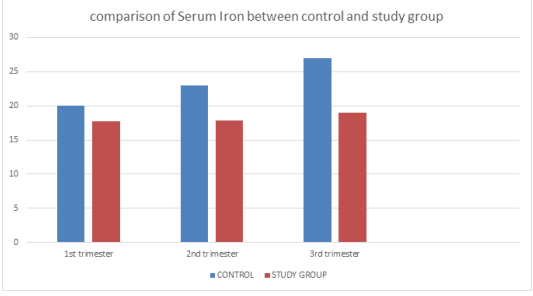
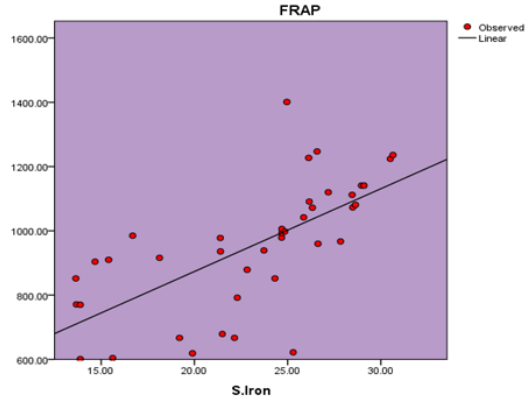


Fig-2:- Comparison of serum iron between the control and study group in three trimester



Correlation between FRAP and Serum iron in the 3rd trimester



Pearson's Correlation in pregnant anaemic women between Iron levels and FRAP value.

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

(µmol/l) and Hb in a pregnant woman with iron deficiency anaemia were significantly decreased ($p<0.05$) as compared to the controls in all three trimesters.

In the present study, it was found that the level of FRAP [FeSO4 Equivalent of 1L of Plasma (µM)] was significantly decreased in study groups as compared to control groups in all three trimesters. So, oxidative stress was significantly increased in anaemic pregnant women in every trimester as compared to their respective controls ($p< 0.05$). There was also a strong positive correlation between FRAP Values and Serum Iron in pregnant women. That meant that lower the Serum Iron level higher was the oxidative stress.

So it was shown that iron deficiency anaemic pregnant women were exposed to increased oxidative stress in pregnancy compared to non-anaemic 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. Anaemia, 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 anaemia than controls ($p<0.05$), 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-anaemic pregnant and normal pregnant and reported remarkably increased levels of lipoperoxides in 2nd and 3rd trimesters of pregnancy in anaemic pregnant as compared to normal pregnant women.[15] A similar observation was made by Kodliwadmath et al.[16]

Since RBC have no nucleus any increase in oxidative stress results in the 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 increased oxidative stress in the pathogenesis of these conditions.

5. Conclusion:

The study concludes that serum iron level was 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 the wellbeing of the individual[20]. It is important that studies are carried out to discern any long- term health issues faced by children born to anaemic mothers battling higher oxidative stress.

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