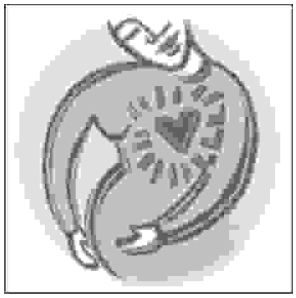
ORIGINAL PROF-1227

IRON STATUS IN PREECLAMPSIA



DR. TASNEEM ZAFAR
Professor
Department of Biochemistry,
Frontier Medical College, Abbottabad

DR. ZAFAR IQBAL
Associate Professor
Department of Biochemistry,
Women Medical College, Abbottabad

ABSTRACT... Objective: To evaluate iron status in pregnancy induced hypertension and role of iron in the etiology and pathogenesis of pre-eclampsia. **Design:** Coefficient correlation study. **Place and Duration.** At Department of Biochemistry, Frontier Medical College, Abbottabad with collaboration of Department of Obstetrics and Gynecology, Ayub Medical Complex, Abbottabad from March 2006-March 2007. **Material and Methods:** Study was performed on hundred pregnant women of age ranging between 15-35 years and having gestational age between28 to 34 weeks. Fifty obstetric patients were identified as having pre-eclampsia. Fifty healthy pregnant subjects were taken as controls, having uncomplicated pregnancies and were normotensive throughout gestation and without proteinuria. **Results:** Results depicts that mean age of pre-eclamptic group was significantly low ($P<0.001$) as compared to control. Both parameters, Hemoglobin and Haematocrit were significantly higher ($P<0.05$) in pre-eclamptic as compared to controls. Serum iron, serum ferritin and transferrin saturation were significantly higher ($P<0.001$) in pre-eclamptic in comparison with control group. Total iron binding capacity and unsaturated iron binding capacity were significantly lower ($P<0.001$) in pre-eclamptic group when compared to control group. Correlation coefficient between serum iron, total iron binding capacity (TIBC), serum ferritin, unsaturated iron binding capacity (UIBC) and systolic and diastolic blood pressure in pre-eclamptic group showed no significant positive correlation in any parameter. **Conclusion:** It is concluded that hemoglobin, haematocrit, serum iron, serum ferritin and transferrin saturation are significantly increased in pregnant women that later develops pre-eclampsia. Excess iron is postulated as casual factor in the oxidative stress ie; in its radical form, which may be involved in the pathogenesis of pre-eclampsia. Therefore, iron status of pregnant women should be assessed before giving iron supplements as these may cause more harm than benefit.

INTRODUCTION

Pre-eclampsia is a multisystem disorder of unknown etiology and is unique to pregnant women after twenty weeks of gestation. It is progressive disease with a variable mode of presentation and rate of progression[1]. Hypertension, proteinuria, excessive weight gain and edema are the classic clinical manifestation[2]. Other features include thrombocytopenia, hyperuricemia, abnormal liver function tests, and hemoconcentration [3,4]. Preeclampsia occurs in about 6% of the general population[5]. The incidence varies with geographic location. Predisposing factors are nulliparity black race, maternal age below 20 or over 35 years, low socio-economic status, multiple gestation, hydatidiform mole,

polyhydramnios, twins, obesity and underlying renal disease[6]. A number of reports indicate that blood levels of lipid peroxidation products are elevated in women with preeclampsia relative to normal pregnancy[7]. It has been suggested that lipid peroxidation may play a role in the etiology of the disease[8]. Iron and hematin proteins, play important roles as catalysts of lipid peroxidation in tissues. Iron promotes lipid peroxidation perhaps facilitated by the hyperlipidemia consequent to the tremendous mobilization of lipid that occur in the later half of human gestation[9,42].

This could further escalate the cycle by increasing circulating peroxide leveles[10,11]. Iron ions not safely sequestered in storage or transport proteins are hazardous because they can stimulate free radical reactions[12]. Biological examples of these are Fenton Chemistry leading to the formation of highly reactive species, such as the hydroxyl radical (OH) and the Ferryl ion (FeO_2+) and lipid peroxidation[13,14,15]. There is evidence that oxidative stress also occurs in pre-eclampsia[16].

Transitional metals, especially iron, which are abundant in the placenta, are important in the production of free radicals[17,9]. In the presence of Fe^{++} and lipid peroxides, lipid peroxidation of membrane phospholipids is stimulated by alkoxyl radicals generated by the Fenton reaction[18]. Oxidative stress, hyperlipidemia and increase iron levels in the maternal compartment in pre-eclampsia could be responsible for causing oxidative stress in placenta[19].

Increased transferrin saturation and decreased unsaturated iron binding capacity in preeclampsia may occur consequent to oxidative stress and then further promote oxidative stress by decreasing serum antioxidant buffering against redox-active iron[20]. The approximate doubling of transferrin saturation in predelivery sera of women with preeclampsia relative to controls result from combined effect of increased serum iron and decreased total stransferrin concentration[21].

A study of serum iron and ferritin levels in Indian women with pregnancy induced hypertension and eclampsia composed with controls of similar gestational ages, revealed that mean serum iron was elevated slightly in pregnancy induced hypertension and significantly in eclampsia as compared to controls. Mean ferritin levels were significantly elevated in both pregnancy induced hypertension and eclampsia as compared to controls[22]. Maternal ferritin concentration is primarily a reflection of maternal, iron status, and a high level is associated with unfavorable outcome[12].

Estimates of gestational iron requirements and of the proportion of iron absorbed from different iron supplemental doses suggest that with present supplementation schemes the intestinal mucosal cells are constantly exposed to unabsorbed with an increased risk of fetal growth restriction, preterm delivery and preeclampsia[24]. Mean value of serum iron is significantly increased in the preclamptic women in comparison to controls whereas mean values of both total iron binding capacity and unsaturated iron binding capacity are significantly decreased in pre eclampstic women in contrast to controls[25]. Study was carried out to evaluate iron status in pregnancy induced hypertension and to explore the possible contributory role of iron to the etiology and pathogensis of pre eclampsia.

MATERIAL AND METHODS

Study was carried out in the department of biochemistry, Frontier Medical College Abbottabad with collaboration of department of obstetrics and gynaecology, Ayub Medical Complex Abbottabad. Study was performed on 100 pregnant women of age ranging between 15-35 years and having gestational age between 28 to 34 weeks. Fifty obstetric patients were identified as having pre eclampsia according to specific criteria. Gestational hypertension was defined as an increase of 30 mm Hg systolic or 15 mm Hg diastolic blood pressure compared with values obtained before 20 week's gestation or an absolute blood pressure >140/90 mm Hg after 20 weeks gestation if earlier blood pressure were not known. Proteinuria was defined as >500 per 24 hr urine collection or >2+ on a voided or >1+ on a catheterized random urine specimen. Fifty healthy pregnant subjects

were taken as controls, having uncomplicated pregnancies and were normotensive throughout gestation and without proteinuria. Neither pre eclamptic nor control women received iron supplements. Subjects having hemolytic anemia, liver disease, chronic renal disease, chronic hypertension, history of repeated blood transfusion, hematomas and those having chronic disease such as tuberculosis and rheumatoid arthritis were excluded.

The subjects were classified into two groups. Group X: Normal , healthy pregnant women as controls. Group Y: women with pregnancy induced hypertension. The clinical characteristics recorded were maternal age, gestational age at the time of blood sampling, systolic and diastolic blood pressure degree of proteinuria and presence and distribution of edema. Weight in kilograms (Kg) and height in centimeters(cm) were determined by standard methods. The blood pressure was measured and pulse rate was recorded. Ten ml of blood was collected from all the selected subjects of which two ml of blood was transferred to a bottle, containing EDTA and was used for hemoglobin and haematocrit estimation. From the remaining blood, serum was obtained and stored frozen at 20C in iron free microtubes until assayed number.

Hemoglobin was estimated by Cyanmet Hemoglobin Method and Haematocrit values were estimated by microhaematocrit method on Microhaematocrit Machine. Serum iron and iron binding capacity was estimated by using kit method. Transferrin saturation was calculated by the following formula.

$$\text{Transferrin Saturation (\%)} = \frac{100 \times \text{Serum Iron}}{\text{TIBC}}$$

Ultrasound iron binding cpacity (UIBC) denotes the amount of transferrin unsaturated (unbound to) iron. It is about two third of the total iron binding capacity, as normally about one-third is saturated. The UIBC is calculated by the following formula.

UIBC (mg %) = TIBC - Serum Iron Concentration. Serum ferritin was estimated by Enzyme-immunoassay Kit method.

RESULTS

Hundred pregnant women of age ranging between 15-35 years and having gestational age between 28-34 weeks were enrolled. Fifty were uncomplicated normal pregnant subjects and fifty were pre eclamptic women. Table I shows the comparison of age, weight and height between control and pre eclamptic subjects. It shows that mean age of pre eclamptic group (Y) was significantly low ($p<0.001$) as compared to control group (X).

Table-I. Comparison of age, weight and height in control and pre eclamptic patients

Parameters	Group (X) (n=50)	Group (Y) (n=50)
Age year	24.88±0.60	19.98±0.43*
Weight Kg	58.60±0.65	57.40±0.87
Height (m)	1.54±0.01	1.53±0.87

**p<0.001 when compared to control, Group X= Control, Group Y=Pre eclamptic*

The comparison of haemoglobin and haematocrit between control and pre eclamptic was shown in table II. Both parameters were significantly higher ($p<0.05$) in pre eclamptic group as compared to control group.

Table-II. Comparison of haemoglobin and hematocrit between control and pre eclamptic patients

Parameters	Group (X) (n=50)	Group (Y) (n=50)
Haemoglobin (g/dl)	10.33±0.15	10.84±0.18
Haematocrit(%)	31.30±0.46	32.80±0.53

The mean values of serum iron, total iron binding , serum ferritin unsaturated iron binding capacity and percent saturation of transferring are shwon in table III. Serum iron, ferritin and transferring saturation were significantly higher ($P<0.001$) in pre eclamptic (Group Y) when compared to control (group X).

Table-III. Comparison of serum iron, ferritin, total iron binding capacity which unsaturated iron bidning capacity and % saturation of transferrin in normal and pre eclamptic patients.

Parameters	Group (X) (n=50)	Group (Y) (n=50)
Serum iron (mg/dl)	53.70±2.99	86.38±4.57*
Total iron binding capacity (mg/dl)	443.98±5.83	403.78±5.57*
Serum ferritin (ng/dl)	12.88±0.88	48.33±3.41*
Unsaturated iron binding capacity (mg/%)	390.28±8.20	317.35±9.61
Transferring saturation (%)	12.05±0.81	21.68±1.38

**=P<0.001 when compared to control)*

Table IV shows correlation coefficient between serum iron, total iron binding capacity (TIBC), serum ferritin, unsaturated iron binding capacity (UIBC) between systolic and diastolic blood pressure in pre eclamptic group. No significant positive correlation was observed in any parameter.

Table-IV. Correlation coefficient of serum iron, ferritin , total iron binding capacity and unsaturated binding capacity b/w systolic and diastolic blood pressure in pre eclamptic patients.

	Systolic BP (mm Hg)	DiastolicBP (mm Hg)
Serum iron (mg/dl)	r=0.04	r=0.16
Total iron binding capacity (mg/dl)	r=0.08	r=0.12
Serum ferritin (ng/dl)	r=0.05	r=0.16
Unsaturated iron binding capacity (mg/dl)	r=0.07	r=0.4

r=coefficient of correlation, statically non significant.

DISCUSSION

Pre eclampsia is still one of the leading cause of maternal and fetal morbidity and mortality. Despite active research for many decades, the etiology of this disorder remains exclusive to human pregnancy is an enigma. Recent evidence suggests there may be several underlying causes or predispositions leading to endothelial dysfunction and causing the signs of hypertension, pro-teinuria and edema-findings that allow to make the diagnosis of the syndrome of Pre eclampsia[26,27]. Many hypotheses have been offered and include prostacyclin thromboxane imbalance, endothelial dysfunction and immunogenetic and absolute or relative placental ischemia[28,29]. The current study is undertaken to evaluate iron status and its possible contributory role in oxidative stress in pre eclampsia[30,31]. There is a significant difference in maternal age between normal pregnant and pre eclamptic women. This was in disagreement to study conducted by[11], but no difference between weight and height was found in both groups. No significant difference was observed in hemoglobin concentration and haematocrit in pre eclamptic group[32,21], while in present study, it was observed that hemoconcentration occurs in pre eclampsia and altered hemodynamic may play a partial role in causing hyperferritinemia. Normal women has a decrease in serum iron and ferritin during the third trimester of pregnancy as their stores of iron are depleted because of fetoplacental demand and required expansion of red cell mass[33,34]. However, elevated level of serum iron is observed in pre eclamptic as compared to normal pregnant women, a study supported by[35,19,25]. Local iron excess and iron mediated oxidative stress have been demonstrated in the intestinal mucosa, liver spleen, bone marrow and placenta and the production of hydroxyl and methoxyl radicals in both the luminal and mucosal contents of the gastrointestinal tract verify the role of iron in free radical damage[36,9]. Total iron binding capacity (TIBC) is low in pre eclamptic group as compared to control[37]. Similarly, unsaturated iron binding capacity, a measure of the iron binding reserve of serum is also significantly lower in women with pre eclampsia relative to normal pregnancy[17]. Similar findings regarding the TIBC and UIBC were observed[25]. The results allude to the possible contribution of released iron free radicals from ischemic placenta in pre eclampsia to its etiology[38]. Serum ferritin is found elevated in pre eclamptic group, which is in agreement with study conducted by[35]. Serum

ferritin is a reliable indicator of total body iron status in non diseased individuals, with low concentration diagnostic of iron deficiency. However a high ferritin does not always signify iron excess[17].

Elevated serum ferritin occurs in a variety of clinical conditions with non utilization of iron and destruction of tissues such as in hemolytic anemia, hepatic damage or to suppression of erythropoiesis leading to accumulation of storage iron[2,39]. A prospective observational study was performed on 450 women by[12]. He observed that high ferritin was associated with increased risk for preterm delivery and neonatal asphyxia, while the lower ferritin level was associated with decreased risk of pre eclampsia, pre labour rupture of membranes[40]. Increased concentration of serum ferritin during third trimester may be part of an acute phase response, which suggests maternal infection and increased risk of poor pregnancy outcome[33]. Increased percent saturation of transferrin in pre eclamptic group is observed, which is in agreement with data collected by[17,37].

Serum transferrin concentration increases during the course of normal gestation, whereas serum iron concentration falls, resulting in a marked decrease in transferrin percent saturation. Iron bound to transferrin is known to be redox inert; it does not induce free radical oxidations[16]. On the other hand iron supplements and increased iron stores have recently been linked to maternal complications e.g. gestational diabetes and increased oxidative stress during pregnancy[5,27].

Consequently while iron supplementation may improve pregnancy otucome when the mother is iron deficient. It is also possible that prophylactic supplementation may increase risk when the mother does not have iron deficiency. Estimates of gestational iron requirements and of the proportion of iron absorbed from different iron supplemental doses suggest that with present supplementation schemes the intestinal mucosal cells are constantly exposed to unabsorbed iron excess and oxidative stress[23,41,42,43].

CONCLUSION

It is concluded that haemoglobin, haematocrit, serum iron, serum ferritin and transferrin saturation are significantly increased in pregnant women that later develops pre eclampsia. Excess iron is postulated as casual factor in the oxidative stress i.e in its radical form, which might be involved in pathogenesis of pre eclampsia. Therefore, iron status of pregnant women should be assessed before giving iron supplements as these may cause more harm than benefit.

REFERENCES

1. Madaio MP el al. **The diagnosis of glomerular diseases, acute glomerulonephritis and the nephrotic syndrome.** Arch Intern Med 2001;161:25.-(PMID:11146695).

2. Luft FC: **Hypertensive nephrosclerosis: update.** Curr Ooin Nephrol Hypertension:2004; 13:147. (PMID:15202608).

3. Persu A et al. **Recent insights in the development of organ damage caused by hypertension.** Acta Cardiol 2004;59:369. (PMID 15368798).

4. Mushambi MC, Halligan AW. Williamson K. **Recent developments in the pathophysiology and management of pre eclampsia.** Br J Anaesthesia 1996;76:133-148.

5. Sibai BM. **Diagnosis, prevention and management of eclampsia.** Obstect Gynecol 2005;105:402.(PMID: 15684172).

6. Reynolds SC, Mabie WC, Sibai BM. **Hypertensive states of pregnancy In: Current Obstetric and Gynecologic diagnosis and treatment.** 9Y ed.De Chemey AH. Nathan L (eds) McGraw Hill Company, USA 2003;pp338.

7. Benzie IFF. **Lipid peroxidation. A review of causes, consequences, measurement and dietary.** Int J Food Sci Nutri. 1996;47:233.

8. Morris 1M, Gopaul NK, Endresen MIR, Knight M, Linton EA, Dhir S, Redman CWG. **Circulating markers of oxidative stress are raised in normal pregnancy and pre eclampsia.** B J Obstet Gynecol 1998;105: 1195-1199.

9. Casanueva E and Viteri FE. **Iron and oxidative stress in pregnancy.** J Nutri 2003;133:1700S-1708S.

10. Hubel CA. **Dyslipidemia, Iron and oxidative stress in pre eclampsia; Assessment of maternal and feto placental interactions.** Semin Reprod Endocrinol 1998;16(1):75-92.

11. Hubel CA, Roberts 1M, Taylor RN, Musci T1, Rogers GM, McLaughlin MK. **Lipid peroxidation in pre eclampsia. New perspectives on pre eclampsia.** Am J Obstet Gynecol 1989;161:1025-34.

12. Lao TT, Tam KF. Chan LY. **Third trimester iron status and pregnancy outcome in non anaemic women. Pregnancy unfavorably affected by maternal iron excess.** Human Re-production 2000; 151843-1848.

13. Hung TH, Skepper IN, Burton GJ. **In vitro ischemia-Reperfusion injury in term Human placenta as a model for oxidative stress in Pathological Pregnancies.** Am J pathol 2001; 1591031-1043.

14. Gutteerodge IMC. **Iron and oxygen. A biologically damaging mixture.** Acta Paediatr Scan Suppl 1989;361:1031-85.

15. Halliwell B and Chirico S. **Lipid peroxidation. Its mechanism, measurement and significance.** Am J Clin Nutr 1993;57(suppl): 715S-725S.

16. Takagi Y, Nikaido T, Toki T, Kita N, Kanai M, Ashida T, Ohira S, Konishi I. **Levels of oxidative stress and redox-related molecules in the placenta in pre eclampsia and fetal growth restriction.** Virchows Arch 2004;444(1): 49-55.

17. Hubel CA Bodnar LM, Many A, Harger G, Ness RB, Roberts IM. **Nonglycosylated ferritin predominantes in the circulation of women with pre eclampsia but not intra utrine growth restriction.** Clin Chern 204;50: 948-951.

18. Dowhan W, Bodanov H. **Functional Roles of Lipids in membrane in biochemistry of lipids.** Lipoproteins and membranes 4th ed Vancer ed Vance JE (editor) Elservier 2002.

19. Vaughan 1£ and Walsh Sw. **Oxidative stress reproduce placental abnormalities of pre eclampsia.** Hypertens pregnancy 20002;21(3): 205-23.

20. Raman L, Pawashe AB, Yasodhara P,. **Hyperferritinemia in pregnancy induced hypertension and eclampsia.** JPGM. 1992;38:2, 65-7.

21. Hubel CA, Kozov AV, Kagan EV, Evans RW, Davidge ST, McLaughin MK et al. **Decreased transferrin and increased transferrin saturation in sera of women with pre eclampsia.** Implications for oxidative stress. Am J Obstet Gynecol 1996; 175: 692-700.

22. Prieto I, Barry M, Sherlock S. **Serum ferritin in patients with iron overload and acute and chronic liver diseases.** Gastroentrol 1975;68:525-533.

23. Esther C. Fermando EY. **Iron and oxidative stress in pregnancy.** J Nutr. 133:1700S-1708, May 2003.

24. Theresa O, S. **Iron status during pregnancy. Setting the stage for motheer and infant.** Am J of Clin. Nutr. , May 2005;81(5): 1218S-1222.

25. Basher K, Deb K. **Alteration in iron status in pre eclampsia.** Mymensingh Med J. 2006 Jan; 15(1): 22-420.

26. Schlembach D. **Pre eclampsia. Still a disease of theories.** Fukushima J Med Sci 2003;49(2): 69-115.

27. Lain KY et al. **Compensatory concepts of the pathogenesis and management of pre eclampsia.** JAMA 2002; 287: 3183. (PMID 12076198).

28. Piering WF , Garancis IG, Beckeer CG., Beres IA, Lemann I. **Pre eclampsia related to a functioning extra uterine placenta.** Report of a case and 25 years follow up, Am J Kidney Disease 1993; 21: 310-313.

29. Johnson Ca et al. **Clinical practice guideline for chronic kidney diseases in adults. Part II Glomeerular filtration rate, Proteinuria and other markers.** Am Fam Physician 2004; 70: 1091, (PMID 15456118).

30. Adamson JW. **Iron deficiency and hypoproliferative anemia, Chapter 90, p 586.** In Kasper DL et al (editors). Harrison's Principles of internal Medicine, 16th ed McGraw Hill 2005.

31. Gurr MI. Harwood JL, Frayn K: **Lipid Biochemistry.** Blackwell Publishing 2002.

32. Roggensack AM, Zhang Y, Davidge ST. **Evidence for per oxynitrite formation in the vasculature of women with pre eclampsia.** Hypertension 1999;33: 83-89.

33. Theresa OS. **High third trimester ferritin concentration. Association with very preterm deliver, infection and maternal nutritional status.** Obstet, Gynaecol 1998;92:161-6.

34. Kaneshige E. **Serum ferritin as an assessment of iron**

stores and other hematologic parameters during pregnancy. Obstet Gynaecol 1981;57:238.

35. Raman L, Pawashe AB, Yasodhara P. **Hyperferritinemia in pregnancy induced hypertension and eclampsia.** J Postgard Med 1992;38:65-7.

36. Fogo AB. **Minimal change disease and focal segmental glomerulosclerosis.** Nepherol, Dial Transplant 2001;16(Suppl16);74, (PMID 11568250).

37. Rayman MP, Barlis I, Evans RW. Redman CWG, King IJ. **Abnormal iron parameters in the pregnancy syndrome pre eclampsia.** Am J Obstet Gynaecol 2002; 187: 412-8.

38. Migneco A et al. **Hypertensive crises. Diagnosis and management in the emergency room.** Eur Rev Med pharmacol Sc 2004; 8:143 (PMID 1563400).

39. Chun MJ e al. **Focal segment glomeruloscleerosis in nephrotic adults, presentation, prognosis and response to therapy of the histologic variants.** J Am Soc Nephrol 2004;15:2169 (PMID 15284302).

40. Theresa OS. **Iron status during pregnancy, setting the stage for mother and infant.** American Journal of Clinical Nutritin Vol 81(5): 1218S-1222S May 2005.

41. Christie W. **Lipid Analysis 3rd ed.** The Oily press; 2003.

42. Geisslere C, Power HJ Human Nutrition 11th ed, Elsevier 2005.

43. Warnock DG. **Towards a definition and classification of acute kidney injury.** J Am Soc Nephrol 2005;16: 3149. (PMID 16207828).