*Orginal Article*

# Role of Maternal Iron Status in the Pathogenesis Of Gestational Diabetes Mellitus

M R Sarker[1], F Jebunnesa[2], T Khatun[3], R Helal[4], L Ali[5], A T M ARahim[6]

## Abstract

*Gestational diabetes mellitus (GDM) is one of the commonest complications of pregnancy; but its pathophysiology is still not fully understood. Recently attention has been focused on the relation between iron metabolism and glucose intolerance in the genesis of GDM. The present study was conducted to investigate the association of body iron store with various covariates of metabolic syndrome. A total 100 subjects were included in this study: 43 were healthy nondiabetic and nonanemic pregnant women (Control group) and 57 were pregnant women having Diabetes Mellitus (GDM group). Glucose level was measured by using glucose-oxidase method, fasting serum C-peptide by chemiluminescent enzyme immunoassay, Glycosylated hemoglobin (HbA1c) by using a modified high performance liquid chromatography (HPLC) method and insulin sensitivity (HOMA%S) and insulin secretory capacity (HOMA%B) were calculated by Homeostasis Model Assessment. Serum transferrin receptor (STfR) was measured by Enzyme-Linked Immunosorbent Assay and serum ferritin level was assessed by Microparticle Enzyme Immunoassay. Serum iron concentration was measured by IRN method.*

*The age of the study groups were found to be matched (p=0.522). Gestational weeks and parity of the study groups were significantly higher in GDM than Controls (p=0.004 and p=0.015 respectively). HbA1c level (%, M±SD) was significantly higher in GDM group (6.09±1.1) as compared to Control Among the marker of body iron status hemoglobin level showed no difference between GDM (11±1.25) and Control groups (10.6±0.8), but serum iron concentration [median (range)] was significantly lower in GDM group [6(2-19)] as compared to Control [12(2-36)].Serum Iron was strongly correlated with HOMA%B in univariate Spearman correlation analysis (r =0.347, P=0.008).On multivariate linear regression analysis also found Serum Iron associated (p=0.011) with HOMA% B in GDM group.*

*GDM in Bangladeshi subjects does not seem to be associated with iron deficiency or elevated body iron store. GDM subjects may show lower serum iron, but this is probably related to chronic inflammatory state of diabetes rather than iron deficiency.*

**Key words:** GDM, Iron overload, Ferritin, Soluble Transferrin Receptor, Anemia in pregnancy, Anemia in pregnancy

## Introduction

Pregnancy is a diabetogenic condition1 and abnormalities of carbohydrate metabolism occur frequently during pregnancy. Gestational diabetes mellitus (GDM) is defined as glucose intolerance that has its onset or first recognition during pregnancy.[1] Women with a history of GDM are at significantly increased risk of developing Type 2 diabetes in the future.[2] In majority of GDM patients, glucose regulation will return to normal after delivery.[3] Clinical and historical risk factors are associated with an increased risk for gestational diabetes and those include older maternal age, obesity, a history of diabetes in a first degree relative, a previous pregnancy with gestational diabetes, delivery of an infant with macrosomia and unexplained near term fetal death4. Recently attention also has been focused in the relation between iron metabolism and glucose intolerance in the genesis of GDM. There may be some association between iron metabolism and onset of GDM. It has been suggested recently that an elevated ferritin concentration is a part of picture of insulin resistance and that leads to GDM. Another marker of total body iron status is the transferrin receptor (sTfR). Transferrin receptor is a cellular transmembranous protein found especially on cells requir ing large amounts of iron such as hemoglobin synthesizing cells of reticuloendothelial system and placenta.[5]

The concentration of sTfR is increased in the presence of iron deficiency anemia.[6] The main clinical use of sTfR measurement has been demonstrated in the assessment of erythropoietic activity[7,8] functional iron deficiency[9,10] and in distinguishing the iron deficiency of chronic diseases. It is still controversial whether sTfR can be used as an iron marker in an iron overload condition, because in some pathophisiological states which are related to iron overload its decrease parallel the ferritin concentration. Circulating concentration of sTfR is proportional to cellular expression of the membrane associated TfR.[11] Circulating sTfR are derived by proteolytic cleavage from sTfR expressed on the cell surface.[12] Intracellular iron influences the post-transcriptional regulation of expression of ferritin, the sTfR, and several other genes are important in mammalian iron metabolism.[13] Serum sTfR concentration is closely related to cellular iron demands and, as a consequence, the higher the ferritin levels the lower the sTfR concentration.[14,15] Iron supplementation during pregnancy increases maternal iron status during pregnancy including hemoglobin, serum iron, MCV, transferrin saturation, and serum ferritin. Iron overload and the associated oxidative stress contribute to the pathogenesis and increase risk of type 2 diabetes and other disorders. In iron overload, the accumulation interferes with the extraction, synthesis and secretion of insulin.[16] However, moderately elevated iron stores also increase the risk of type 2 diabetes.[17]

## Rationale

Poor health care infrastructure and widespread public ignorance of nutrition causes a large number of uncontrolled DM with anemia in Bangladesh. Iron is frequently prescribed without knowing its status in the pregnant, hence a probability of getting pregnancy with iron overload in

1. Manika Rani Sarker , Assistant Professor Dept: of Community Nutrition, BIHS, Dhaka.

2. Fatema Jebunnesa , Lecturer Dept of Biochemistry & Cell Biology, BIHS, Dhaka.

3. Taslima Khatun , Lecturer Dept: of Community Nutrition, BIHS, Dhaka.

4. Dr. Romena Helal , Dept: of Obstetrics & Gynecology, BIHS, Dhaka.

5. Dr. Liaquat Ali, Associate Professor& Director & Cell Biology, BIHS, Dhaka.

6. Dr. Abu Trab M A Rahim, Professor Institute of Nutrition & Food Science, University of Dhaka, Dhaka.

***Corresponding Author***

Manika Rani Sarker. e-mail: manika_rani128@yahoo.com

Bangladesh. In spite of the possibility of a link between iron status and diabetes only few studies (and mostly with insufficient number of subjects) have been conducted on this issue. Its manifestation also depends on racial variation and thus it should be studied in different populations. In western countries, due to better and early management of GDM, it is difficult to design a study to explore the interrelationship of glycemic status and maternal iron stores. Unfortunately, due to poor health care infra structure and ignorance in our country a large number of uncontrolled diabetes mellitus with anemia is available. On the other hand, there is a chance of getting patients with iron excess because during pregnancy iron is prescribed without knowing iron status. This provides a opportunity to study the correlation between the degree of hyperglycemia and body iron status in pregnant women. Availability of up to date markers of body iron store can add substantial value in such studies. Materials and methods: Study design It was a cross-sectional study.

**Subjects** A total 100 subjects were included in this study: Among them, 43 were healthy nondiabetic with nonanemic pregnant women (Control group) and 57 were pregnant diabetic (GDM group).

**Selection criteria**

Inclusion criteria

• Control group: Healthy nondiabetic with nonanemic pregnant women.

• GDM Group: Positive on the basis of a 2 - sample OGTT (fasting and 2 hour after 75 g Oral glucose) following the WHO criteria (1997) as adapted in BIRDEM. GDM Subject also with anemia and non-anemia.

**Exclusion criteria**

• Diabetic pregnant women with acute or chronic diabetic complications.

• Pregnant subjects with renal disease.

• Pregnancy with acute or chronic medical diseases, connective tissue diseases and chronic hypertension without proteinurea.

• Pregnancy with acute infections.

• Pregnancy with advanced grading of heart disease.

**Place and duration of the Study**

The study was carried out for a period of 1 year 6 month from June 2006 to December 2007. The subjects were collected from the Department of Gynecology and Obstetrics Out-patient Department of BIRDEM on the basis of availability.

**Data Collection instruments**

Fasting and 2 hour after (75 gm glucose) blood sample were collected, who satisfied the selection criteria of the study subjects. Detailed sociodemographic data, family history and medical history were recorded on a data sheet appropriately. All interviews were conducted in the hospital. Physical examination was done and anthropometric measurements (height, weight) of each subject were taken and recorded in a pre-designed checklist form. Obstetric examination was performed and recorded for every patient. The data and specimen (blood) were collected on every morning at BMRG, BIRDEM. The subjects were biochemical investigated for glycemic status (fasting & 2hr ABF glucose and HbA1c), insulinemic status (fasting C-peptide) and body iron status (serum iron, ferritin & transferrin). Glucose level was measured by using glucose-oxidase method (Randox laboratories, UK), fasting serum C-peptide by chemiluminescent enzyme immunoassay (Immulite, EURO/DPC,UK), HbA1c by using a modified HPLC method (Bio-Rad,USA) and insulin sensitivity (HOMA%S) and insulin secretory capacity (HOMA%B) were calculated by Homeostasis Model Assessment. Serum transferrin receptor (STfR) was measured by Enzyme-Linked Immunosorbent Assay (ELISA), and serum ferritin level was assessed by Microparticle Enzyme Immunoassay (MEIA) technology (Immulite, EURO/DPC,UK). Serum iron concentration was measured by IRN method (Dade Behring,UK).

**Data Processing and analysis**

Data were expressed as M±SD for parametric values and median (range) for non-parametric values. Comparison between groups were done using Independent t-test to compare means and Mann-Whitney U test for skewed data. The relationship between serum Iron, hemoglobin, ferritin and serum transferrin receptor with other variables was examined using Pearson's parametric coefficient correlation (r) analysis. Multiple regression analysis was done to better assess the relationships within the variables and the influencing other variables. A p value of $<0.05$ was considered as significant. All the statistical analysis were performed with the SPSS data (SPSS Inc, Chicago, IL, USA).

**Ethical Aspect**

Before participation, informed and written consent was obtained from all subjects. The study was approved by the ethical committee of BIRDEM ACADEMI and was in accordance with the principles of the Declaration of BMRG, BIRDEM.

**Results**

The age of the study groups were found to be matched ($p=0.522$). Gestational weeks and parity of the study groups were significantly higher in GDM than Controls ($p=0.004$ and $p=0.015$ respectively). HbA1c level (%, M±SD) was significantly higher in GDM group (6.09±1.1) as compared to Control (5.8±0.7, $P=0.001$). Serum insulin level [ng/ml, Median (range)] as assessed by C-peptide, was significantly higher in GDM group [2.5(1-6.6)] compared to Control [1.5(1-3.3), $P<0.001$], but the insulin secretory capacity (HOMA% B) was not different between the two groups [median (range) 144(102-270) in GDM vs 165(100-281) in Control]. HOMA%S [Median (range)] was significantly lower in GDM group

[38(20-90)] as compared to Control [60(32-99), P<0.001]. (table:1) Among the marker of body iron status hemoglobin level showed no difference between GDM (11±1.25) and Control groups (10.6±0.8), but serum iron concentration [median (range)] was significantly lower in GDM group [6(2-19)] as compared to Control [12(2-36)]. (table:2) Serum Iron was strongly correlated with HOMA%B in univariate Spearman correlation analysis (r =0.347, P=0.008) (Fig:1). On multivariate linear regression analysis (table:3), also found Serum Iron associated (p=0.011) with HOMA%B in GDM group.

**Table 1: Clinical and Biochemical characteristics of the study subjects**

| Variables | Control (n=43) | GDM (n=57) | P value |
|---|---|---|---|
| Age (Years) | 25±5.7 | 25±5.5 | 0.522 |
| Gestational age in weeks | 30.5±4.5 | 27.2±6.5 | 0.004 |
| Parity | 2 (1-4) | 2 (1-4) | 0.015 |
| SBP (mm of hg) | 110±9.2 | 116±12.2 | 0.005 |
| DBP (mm of hg) | 70±8.2 | 75±7.7 | 0.002 |
| MBP (mm of hg) | 83.4±8.4 | 89.2±8.31 | 0.001 |
| TG (mg/dl) | 223.3±69.8 | 225.4±67.8 | 0.882 |
| Cholesterol (mg/dl) | 224±40.9 | 216.5±40.7 | 0.368 |
| HDL (mg/dl) | 38.9±9.87 | 41.1±9.1 | 0.256 |
| LDL (mg/l) | 142.2±39.1 | 128.3±41.9 | 0.095 |
| F Glucose (mmol/l) | 5.4±1.2 | 5.6±1.1 | 0.531 |
| ABF (mmol/l) | 6.5±0.80 | 10.1±2.1 | <0.001 |
| $HbA_{1c}$ (%) | 5.8±0.7 | 6.1±1.1 | 0.081 |
| C-peptide (ng/ml) | 1.5(1-3.3) | 2.5(1-6.6) | <0.001 |
| HOMA %B | 165(100-281) | 144(102-270) | 0.143 |
| HOMA %S | 60(32-99) | 38(20-90) | <0.001 |

Data are expressed as mean±SD; were compared using independent sample't' test and median (range) are presented skewed date and compared using man Whitney U test where as appropriate test; p<0.005 are considered as statistically significant; n=Number of subjects; SBP=Systolic Blood Pressure; DBP=Diastolic Blood Pressure; MBP=Mean Blood Pressure; TG=Tri Glyceride; Chol=Cholesterol; HDL=High Density Lipoprotein; LDL=Low Density Lipoprotein, F Glucose= Fasting Glucose; ABF=2 hr after Breakfast; HbA1c=Glycosylated Hemoglobin; HOMA%B=value of insulin secretory capacity and HOMA%S=value of Insulin sensitivity by Homeostasis Model assessment.

**Table 2: Iron status of the study subjects**

| Variables | Control (n=43) | GDM (n=57) | P value |
|---|---|---|---|
| Hemoglobin (%) | 10.6±0.8 | 11.0±1.2 | 0.085 |
| Serum Iron (µmol/L) | 12(2-36) | 6(2-19) | <0.001 |
| Ferritin (ng/ml) | 24(15-59) | 27(10-77) | 0.207 |
| STfR (nmol/l) | 16(10-50) | 16(10-45) | 0.574 |

Data are presented as mean±SD; mean were compared using independent sample 't' test and median (range) are presented skewed date and compared using man Whitney U test as a appropriate test, p<0.005 are considered as statistically significant; n=Number of subjects; STfR, Serum Transferrin Receptor.

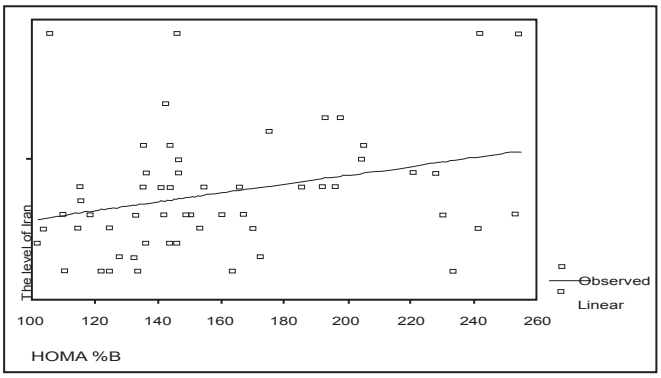


**Fig 1:The correlation between serum Iron and HOMA%B in the GDM subjectsy.**

**Table 3: Multiple linear regression analysis with dependent variable Serum Iron**

| Variable | β Value | P Value | 95% CI Lower Bound | 95% CI Upper Bound |
|---|---|---|---|---|
| ABF | 0.06 | 0.644 | -0.413 | 0.662 |
| $HbA^1C$ | 0.158 | 0.232 | -0.389 | 1.567 |
| HOMA %B | 0.352 | 0.011 | 0.009 | 0.065 |
| HOMA %S | 0.140 | 0.294 | -0.029 | 0.094 |

Model r2 = 0.141, CI=Confidence Interval, β= Standardized Coefficients

**Discussion**

GDM affects a considerable number of pregnancies and in spite of its well known clinical features the etiopathogenesis of the condition is still poorly understood. Recently the relation between iron metabolism and glucose tolerance has attracted attention and it has been implicated in the genesis of GDM[18]. It has been observed that secondary cause of iron overload such as excessive blood transferrin is associated with glucose intolerance in diabetes[19]. It has also been suggested that elevated ferritin concentration is a part of picture of insulin resistance which leads to GDM. Moreover, the relation between iron metabolism and

GDM in a possible iron deprived situation has also not been properly investigated.

Hemoglobin is a crude but commonly used marker for body iron status and it showed no significant difference in GDM subjects as compared Controls (p=0.085). The normal Hb in GDM was accompanied by a paradoxically low value of serum iron which was highly significant (p<0.001) when compared to control. The reference range for serum iron in Bangladeshi population has not yet been worked out, but in general, the reference values are 6 – 27 µmol/dl. Thus the GDM patients, with a median serum iron of 6 µmol/dl (range 2-19 µmol/dl), shows iron deficiency as measured by this parameter. Serum Iron in diabetic patients has been shown to be marginally lower, but so far it has not been investigated in GDM cases. It is interesting to see that the lower value of this rapidly available pool was not paralleled by any change in serum ferritin or STfR which are better and long-term markers of body iron stores. Ferritin (ng/ml) has found to be 24 (15-59) in Controls and 27 (10-77) in GDM cases. Serum ferritin has been claimed to be a reliable and sensitive parameter for the

assessment of iron stores in healthy subjects.[20,21,22] Quantitative phlebotomy has shown a close relationship between serum ferritin concentration and mobilizable iron stores, and it has been demonstrated that 1 ug/L of serum ferritin corresponds to 8-10 mg of storage iron.[23,24] In pregnant women, serum ferritin levels fall dramatically to below 20 ug/l during the second and third trimester, even in women taking supplements.[25] In a study it was found that women with mild GDM had raised serum iron and transferrin saturation, prior to diagnosis and treatment, and the post-partum Hb level was significantly higher. Furthermore, there were significantly positive correlations between the OGTT 2-h glucose value with not only ferritin concentration but also transferrin saturation. In a study Lao et al suggested a relationship Obetween increased maternal iron store and glucose intolerance in pregnancy.[26] Although this finding appears at variance with the reported features of iron deficiency in diabetic mothers and their offsprings[27,28,29,] it was compatible with the previous observation in the same population that the incidence of GDM was significantly lower in pregnancies complicated by iron deficiency anemia compared with pregnancies complicated by anemia as a result of thalassaemic traits[30]. In a study there was an extensive body of data suggesting that higher iron stores are associated with risk of type 2 diabetes in nonpregnant subjects.[31, 32, 33, 34] In pregnant women, Lao et al. found that higher Hb (>13 g/dl) was an independent risk for GDM[35] and that women with iron deficiency anemia had a reduced risk of GDM.[36] Lower STfR signify higher body iron store. The normal range for STfR is 3-9 mg/l. In a study by Flowers et al found to be 5.6 mg/l and for iron deficiency anemia was 18 mg/l.[37] Levels of three to four times normal have been reported for iron deficiency anemia[38], [39]. No significant difference between GDM and Controls was found regarding STfR. This may indicate that GDM may not be associated with change in total body iron store. In the present study a number of parameters which may potentially affect body iron stores were investigated in GDM cases. The GDM patient had significantly higher body weight, duration of gestation and also higher values of systolic blood pressure, diastolic blood pressure and mean blood pressure as compared to Control. GDM cases, however, didn't show any difference in lipid levels compared to Control. Thus, some clinical features of metabolic syndrome were present in GDM subjects which are known to affect iron metabolism. Serum Iron was strongly correlated with HOMA%B in univariate Spearman correlation analysis. On multivariate linear regression analysis, also found Serum Iron associated with HOMA% B in GDM group. GDM in Bangladeshi subject may be associated with iron deficiency or elevated body iron store. Lower serum iron in GDM is probably due to chronic inflammatory state rather than iron deficiency itself. The data also suggest that interpretation of body iron status in this condition, using the available markers (like serum iron, serum ferritin and STfR), needs to be made with caution due to the presence of concurrent factors like pregnancy and chronic inflammatory state.

**References**

1. Tracy L S, MD A J, Brown MD and Mark N, Feinglos MD. Gestational diabetes Mellitus. Clinical Diabetes 2005; 23(1): pp17-24.

2. Metzger BE, Cho NH, Roston SM, Radvany R: Pre pregnancy weight and antepartum insulin secretion predict glucose tolerance five years after gestational diabetes mellitus. Diabetes Care 1993; 16: 1598–1605.

3. Janzen C, Greenspoon J, Palmer SM, Diabetes Mellitus in Pregnancy. In: De Cherney AH, Nathan L. Current Obstetric and Gynecologic Diagnosis and Treatment. 9th edn. New york. Mc Graw Hill, 2003. pp 326-337.

4. Green JR, Pawson IG, Schumacher LB, Perry J, Kretchmer N. Glucose tolerance in pregnancy: ethnic variation and influence of body habits. Am J Obstet Gynecol 1990; 163: 86-92.

5. Sema G, Nilgun E, Mehmet A K: Soluble transferrin receptor and soluble transferrin receptor-ferritin index for evaluation of the iron status in elderly patients.Med:2004; 202:135-142.

6. Hernandez C, Lecube A, Carrera A, Simo R: Soluble transferrin receptors and ferritin in Type 2 diabetic patients. Diabet Med Jan 2005; 22(1): 97-101.

7. Kohgo Y, Niitso K, Hkato I, Tsoshima N, Sasaki K, Hirayama M, Numata T ,Nishisato T.& Urushizaki I: Serum transferrin receptor as a new index of erythropoiesis. Blood,1987; 70:1955-1958.

8. Huebers HA, Beguin Y, Pootrakul P, Einspahr D,

Finch CA: Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 1990; 75: 102–107.

9. Skikne BS, Flowers CH, Cook JD: Serum transferrin receptor a quantitative measure of tissue iron deficiency. Blood 1990; 75: 1870–1876.

10. Baynes RD, Cook JD: Current issues in iron deficiency. Curr Opin Hematol 1996; 3: 145–149.

11. Baynes RD, Cook JD: Current issues in iron deficiency. Curr Opin Hematol 1996; 3: 145–149.

12. Turkewitz AP, Amatruda JF, Borhani D, Harrison SC, Schwatz AL: A high yield purification of the human transferrin receptor and properties of its major extracellular fragment. J Biol Chem 1988; 263: 8318-8325.

13. Klausner RD, Rouault TA, Harford JB: Regulating the fate of mRNA: the control of cellular iron metabolism. Cell 1993; 72:19-28.

14. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA: Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 1990; 75: 102–107.

15. Beguin Y, Lipscei G, Thoumsin H, Fillet G. Blunted erythropoietin production and decreased erythropoiesis in early pregnancy. Blood 1991; 78:89–93.

16. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. 2002; 51:2348–54.

17. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently health women. JAMA 2004;291:711–7.

18. Frieinkel N, Metzger BE, Phelps RL et al. Gestational diabetes mellitus: Heterogencity of maternal age, weight, insulin secretion, HLA antigens and islet cell antibodies and the impact of maternal metabolism on pancreatic B-cell and somatic development in the offspring. Diabetes 1985; 34 (suppl 2); 1-7.

19. Lao TT, Chan PL, Tam KF. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess. Diabet Med 2001;18:218–23.

20. Jacob RA, Sandstead HH, Klevay LM: Utility of serum ferritin as a measure of iron deficiency in normal males undergoing repetitive phlebotomy. Blood 1980; 56: 786-791..

21. Walters GO, Miller FM, Warwood M: Serum ferritin concentration and iron stores in normal subjects. J Clin Pathol 1973; 26: 770–772.

22. Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. Am J Clin Nutr 1974;27:681–7.

Cross-talk between iron metabolism and diabetes. Diabetes

23. Walters GO, Miller FM, Warwood M: Serum ferritin concentration and iron stores in normal subjects. J Clin Pathol 1973; 26: 770–772.

24. Jacob RA, Sandstead HH, Klevay LM: Utility of serum ferritin as a measure of iron deficiency in normal males undergoing repetitive phlebotomy. Blood 1980; 56: 786-791..

25.Cook JD: Adaptation in iron metabolism. Am J Clin Nutr 51:301–308, 1990

26. Lao TT, Chan PL, Tam KF. Gestational diabetes mellitus in the last trimester—a feature of maternal iron excess. Diabet Med 2001;18:218–23.

27. ChockalingamUM, Murphy E, Ophoven JC, Weisdorf SA, Georgieff MK. Cord transferrin and ferritin values in newborn infants at risk for prenatal uteroplacental insuf®ciency and chronic hypoxia. J Pediatr 1987; 111: 283-286.

28. Petry CD, Eaton MA, Wobken JD, Mills MM, Johnson DE, Georgieff MK. Iron deficiency of liver, heart and brain in newborn infants of diabetic mothers. J Pediatr 1992; 121: 109-114.

29. Petry CD, Wobken JD, McKay H, Eaton MA, Seybold VS, Johnson, DE et al. Fetal transferrin receptor in diabetic pregnancies with increased fetal demand. Am J Physiol 1994; 207: E507-E514.

30. Lao TT, Pun TC. Anaemia in pregnancy is the current definition meaningful. Eur J Obstet Gynecol Reprod Biol 1996; 68: 53-58.

30. Lao TT, Pun TC. Anaemia in pregnancy is the current definition meaningful. Eur J Obstet Gynecol Reprod Biol 1996; 68: 53-58.

31. Tuomainen TP, Nyyssonen K, Salonen R, Tervahauta A, Korpela H, Lakka T et al: Body iron stores are associated with serum insulin and blood glucose concentrations: population study in 1,013 Eastern Finnish men. Diabetes Care, 1997; 20: 426-8.

32. Ford ES & Cogswell ME: Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 1999; 22: 1978-1983.

33. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently health women. JAMA 2004;291:711–7.

34. Salonen JT, Tuomainen TP, Nyyssonen K, Lakka HM, Punnonen K: Relation between pregnancy outcome. Am J Clin Nutr 59 (Suppl. 2):492S–500S, 1994.

35. Lao TT, Chan LY, Tam KF, Ho LF. Maternal hemoglobin and risk of gestational diabetes mellitus in Chinese women, Obstet Gynecol 2002 May; 99:807-12.

36. Lao TT,Ho LF, Impact of iron deficiency anemia on prevalence of gestational diabetes mellitus, Diabetes Care, 2004 Mar;27(3):650-6.

37. Flowers CH, Skikne BS, Covell AM, Cook JD. The clinical measurement of serum transferrin receptor. J Lab Clin Med 1989; 114:368–77.

38. Kohgo Y, Niitso K, Hkato I, Tsoshima N, Sasaki K, Hirayama M, Numata T ,Nishisato T.& Urushizaki I: Serum transferrin receptor as a new index of erythro

poiesis. Blood,1987; 70:1955-1958.

39. Huebers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA: Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 1990; 75: 102–107.