STUDY OF IRON STATUS IN TYPE 2 DIABETES MELLITUS

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ABSTRACT:

Background: *Type 2 Diabetes Mellitus is a major growing health issue worldwide. Role of micronutrients is not well established. Studies have suggested that magnesium, chromium, calcium and iron have a possible role in Insulin Resistance or Diabetes.*

Objectives: *To study the levels of Serum Ferritin, Total Iron Binding Capacity, Iron and Hemoglobin in Type 2 diabetes patient and to observe for any statistically significant relationship exists between iron status and diabetes mellitus.*

Materials & Methods: *A total of 120 subjects were included in this study (60 with type 2 diabetes mellitus, and 60 normal subjects). Fasting plasma glucose was done on blood samples obtained after overnight fasting and 2-hour post-prandial plasma glucose was also done along with iron, total iron binding capacity (TIBC), Ferritin and hemoglobin.*

Results: *The level of serum Ferritin which is considered as sensitive marker of iron status is significantly higher in diabetic group. The other parameters like Hemoglobin, TIBC and Iron do not show any positive correlation.*

Conclusion: *We can conclude that serum Ferritin can be considered as sensitive marker of iron status in diabetic group. Serum Ferritin can be assessed in non-diabetic first-degree relatives of diabetic people for identifying the risk. Serum Ferritin can also be correlated with serum insulin and C – Peptide levels. There is no significant relationship between hemoglobin, TIBC, serum iron and diabetes mellitus. Excess tissue iron will increase the production of free radicals which in turn amplifies the steps involved in inflammatory lesion. Even though the level is not significant, serum iron and TIBC may be monitored at regular intervals in those with diabetes mellitus so that appropriate measures can be taken.*

Keywords: *Serum Ferritin, TIBC, Serum iron, type 2 diabetes mellitus, fasting and post prandial plasma glucose.*

INTRODUCTION

In India, in the year 2000, people with diabetes were 31.7 million and by 2030 it will be 79.4million. India is expected to have the highest number of people with diabetes by 2030. Type 2 Diabetes is diagnosed by elevation of Plasma Glucose greater than126 mg/dl in fasting state and greater than 200 mg/dl in 2hrs after 75gm of glucose load. Diabetic complications include retinopathy, neuropathy, IHD and stroke (1).

In the prevention of Type 2 Diabetes, diet and lifestyle plays a major role. The macronutrients like fat and carbohydrate have an impact on Type 2 Diabetes. Role of many micronutrients is not well established. Studies suggest that magnesium, chromium, calcium and iron may have a role in Insulin Resistance or Diabetes.

Iron, a potential catalyst involves in cellular reactions which produces Reactive Oxygen Species. These Reactive Oxygen Species induces oxidative stress and damage to tissues which alters the risk for Type 2 diabetes (2).

AIM

- To study the levels of Serum Iron, TIBC, Ferritin and Hemoglobin in Type 2 diabetes patient and normal healthy individuals
- To see whether there is any relationship between the iron status and diabetes

METHODS AND MATERIALS

The study was conducted over a period of four months. The study was done using oral glucose tolerance test for control, and fasting and post prandial plasma glucose for type 2 diabetes, among subjects attending SRMC & RI clinical lab as out patients or inpatients. The study includes 120 subjects of South Asian Urban Population. Out of which 30 were female and 30 were male with normal glucose tolerance; and 30 were female and 30 were male who were known diabetic. They were in the age group of 35 to 55 years. Each group was classified as Diabetic and Non Diabetic based on WHO criteria.

The data on family history and personal history of diabetes, smoking habits, alcohol consumption, dietary habits, recent blood donation, hypertension and treatment for any other diseases were collected through a standard questionnaire. Anthropometric measures like waist circumference measured at the level of umbilicus, height and weight, hip circumference etc were measured. Blood samples were collected after 12 hrs fasting in the vacutainers for estimation of glucose, iron, TIBC, Ferritin and hemoglobin. The samples were separated by centrifugation at 2400 rpm. Plain vacutainer is used for serum iron, TIBC, Ferritin and for plasma glucose sodium fluoride vacutainer were used. Glucose was analyzed in Konei lab 60 automated

systems using commercial kits. Ferritin was estimated by Automated Chemiluminescence's method using commercially available kit by **BAYER DIAGNOSTICS** (U.S.A) and Iron &TIBC were estimated by RXL Dimensions and commercial kits, reference material and standards from **DADE BEHRING** (Germany) were used.

For adequate quality control both normal, abnormal reference control serum solutions and calibrators were run before each batch. Other factors influencing the quality, like proper functioning of instrument, temperature, glassware, cuvettes, distilled water were taken care.

RESULTS

A total number of 120 samples were selected to study the level of Hb, Iron, TIBC, Ferritin, FPG, and PPPG in Type 2 Diabetes patients and normal individuals. The subjects were divided into 2 groups. The 2 group include Normal Glucose Tolerance (NGT) and Type 2 Diabetes Mellitus (DM). 60 subjects were NGT and 60 subjects were DM.

Data evaluation was done using SPSS programme. The results were expressed as Mean (standard deviation). The P value was used to compare the different groups. The P value <0.05 was considered significant.

The mean and standard deviation of both clinical and biochemical characteristics of the two groups were calculated. The clinical parameters include the age and BMI. The biochemical parameter include Fasting Plasma Glucose (FPG), Post prandial Plasma Glucose (PPPG), Hemoglobin (Hb), Iron, TIBC and Ferritin

The results are depicted in tables 1 to 7 and in figure 1 to 6.

Table 1: Demographic table of 2 groups

Parameters	NGT (n-60)	Diabetes (n-60)
Age	43.5 (5.9)	48.7 (5.7)
Sex M/F	30 / 30	30 / 30
Family History %	36.7	53.3
Smoking %	26.7	30
HT %	35	60

Table 2: Comparison of age between Male and female NGT group and DM group.

Parameters	Sex	NGT	DM	P value
Age (yrs)	Male	49.03 (5.9)	44.8 (5.8)	0.008
	Female	48.4 (5.5)	42.2 (5.7)	0.000

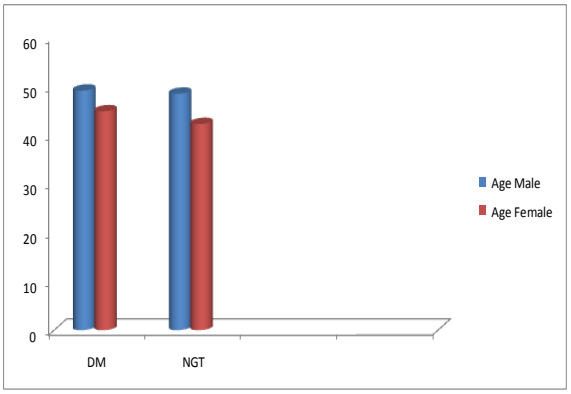

Fig 1: Age for Male and Female DM and NGT Group

Table 3: Comparison of BMI between Male and female NGT group and DM group.

Parameters	Sex	NGT	DM	P value
BMI	Male	20.9 (1.5)	24.7 (3.6)	0.000
	Female	19.8 (1.7)	23.9 (3.1)	0.000

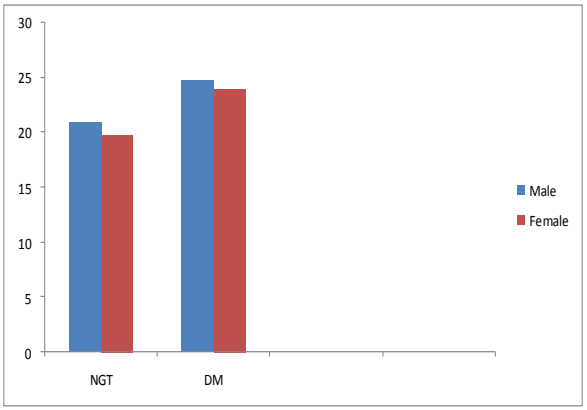

Fig 2: BMI for Male and Female DM and NGT Group

Table 4: Comparison of biochemical parameters for male NGT and DM group

Parameter	Male NGT (n-30)	Male DM (n-30)	P value
FPG (mg/dl)	95.7 (8.1)	156.8 (61.5)	0.000
PPBG (mg/dl)	109.1 (13.5)	234.2 (76.6)	0.000
Ferritin (ng/ml)	73.38 (31.5)	100.75(55.8)	0.002
Hb (gms %)	14.57 (1.5)	14.27 (1.8)	0.502
TIBC (µg/dl)	337.6 (52.2)	324.7 (71.7)	0.429
Iron (µg/dl)	111.3 (42.7)	91.3 (29.0)	0.039

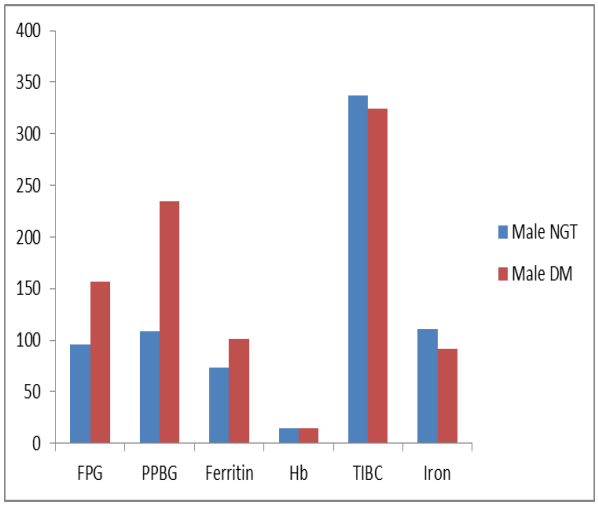

Fig 3: Biochemical Parameters of Male DM and NGT Group

Table 5: Comparison of biochemical parameters for female NGT and DM group

Parameter	Female NGT (n-30)	Female DM (n-30)	P value
FPG (mg/dl)	91.7 (15.6)	182.0 (84.2)	0.000
PPBG (mg/dl)	107.3 (17.0)	267.7 (121.9)	0.000
Ferritin (ng/ml)	35.14 (16.8)	58.02 (40.8)	0.006
Hb (gms %)	12.26 (1.3)	12.36 (1.1)	0.747
TIBC (µg/dl)	378.4 (72.4)	342.7 (105.0)	0.131
Iron (µg/dl)	76.9 (28.1)	66.2 (43.8)	0.266

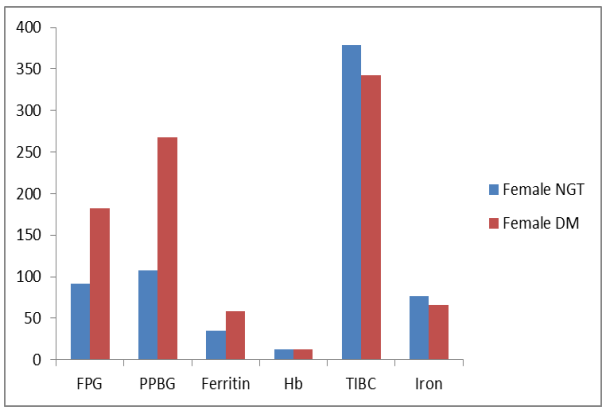

Fig 4: Biochemical Parameters of Female DM and NGT Group

Table 6: Comparison of biochemical parameters for male NGT and female NGT group

Parameter	Male NGT (n-30)	Female NGT (n-30)	P value
FPG (mg/dl)	95.7 (8.1)	91.7 (15.6)	0.217
PPBG (mg/dl)	109.1 (13.5)	107.3 (17.0)	0.553
Ferritin (ng/ml)	54.26 (31.82)	35.14 (16.8)	0.000
Hb (gms %)	14.57 (1.5)	12.26 (1.3)	0.000
TIBC (µg/dl)	337.6 (52.2)	378.4 (72.4)	0.015
Iron (µg/dl)	111.3 (42.7)	76.9 (28.1)	0.001

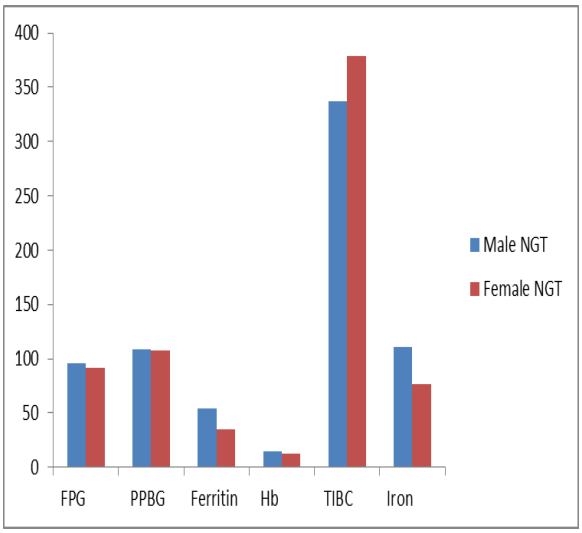

Fig 5: Biochemical Parameters of NGT Group

Table 7: Comparison of biochemical parameters for male DM group and female DM group

Parameter	Male DM (n-30)	Female DM (n-30)	P value
FPG (mg/dl)	156.8 (61.5)	182.0 (84.2)	0.191
PPBG (mg/dl)	234.2 (76.6)	267.7 (121.9)	0.207
Ferritin (ng/ml)	100.75 (55.8)	58.02 (40.8)	0.001
Hb (gms %)	14.27 (1.8)	12.36 (1.1)	0.000
TIBC (µg/dl)	324.7 (71.7)	342.7 (105.0)	0.442
Iron (µg/dl)	91.3 (29.0)	66.2 (43.8)	0.011

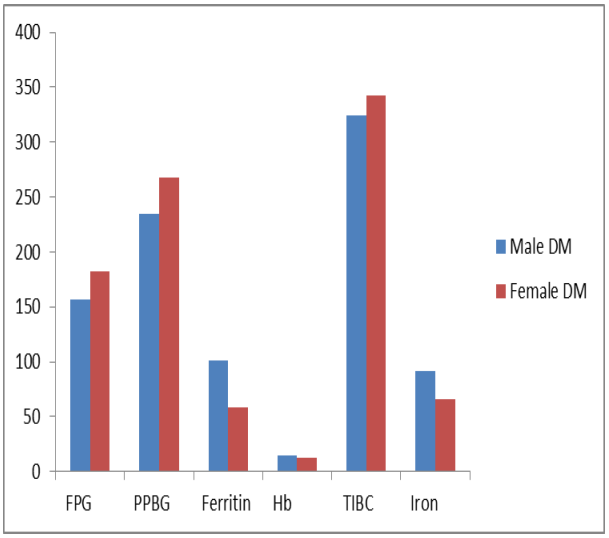

Fig 6: Biochemical Parameters of DM Group

DISCUSSION

Type II Diabetes results from the interaction of a genetic predisposition, diet and environmental risk factor. Diabetes Mellitus is a metabolic disorder with increased level of plasma glucose, relative deficiency of insulin secretion or with insulin resistance at the tissue level. Compared to people

with Type 2 DM who have substantial insulin resistance, people with Type 1 DM range from normal weight or under weight with predominant deficiency of insulin secretion (3).

Scientific evidence suggests, there are unsuspected influences between metabolism of iron status and type 2 diabetes. Since iron affects metabolism of glucose and glucose metabolism impinges on several iron metabolic pathways, the relationship between them is bidirectional. These relationships are influenced by oxidative stress and inflammatory cytokines which amplifies and potentiates the initiated events (4).

Recent studies shows increase in iron stores (ferritin) predicts the risk of developing type 2 diabetes, while decrease in iron level is protective. Damage caused by iron also triggers the events of chronic diabetes complication, in coronary artery responses and endothelial dysfunction (4). Tissue iron excess will increase the production of free radicals which in turn amplifies the steps involved in inflammatory lesion (5).

Absorption of non-heme iron by intestine is tightly regulated in keeping with the body requirements. When body iron stores are normal, iron absorption is minimal. Absorption of heme iron does not depend on body iron content. In the steady state, the circulating iron is bound to transferrin and is taken up by a high-affinity specific transferrin receptor from the blood. The transferrin-receptor complex is internalized through endocytosis and is released into a cellular compartment which is nonacidic. Here it can be used in the synthesis of essential cellular components (6). Insulin rapidly stimulates iron uptake by fat cells and redistributing transferrin receptors to the cell surface from an intracellular membrane compartment (7).

Iron influences insulin action reciprocally. Insulin action of inhibiting glucose production by the liver is interfered by Iron. As iron stores increases, insulin metabolism and hepatic extraction is reduced which leads to peripheral hyperinsulinemia (8). Liver insulin resistance is the initial and common abnormality which occurs in iron overload conditions. Studies also show that skeletal muscle, the main effector of insulin action is also affected by iron overload (9).

Iron is intimately linked to oxidative stress. Highly toxic free radicals, such as hydroxide and the super oxide anion, which induces lipid peroxidation, are produced by Iron through the Fenton reaction**.** Iron should be in free form to act as a pro-oxidant agent. From ferritin, Iron is released by the action of reducing agents that convert Fe^{3+} into Fe^{2+} (10). Synthesis of ferritin is stimulated by glycation of transferrin by reducing its ability to bind ferrous iron and by increasing the amount of free iron. Glycated holotransferrin also facilitates the production of free oxygen radicals, such as hydroxide, that accelerates the oxidative effects of iron (11). In diabetes mellitus there is an increase in reactive oxygen species (ROS) in tissues which leads to cascade of events that result in diabetic complications (12). Recent studies suggest pancreatic β-cells as a target of oxidative stress-mediated tissue damage (15). Physiological action of insulin causes increased uptake iron. Other factors such as aging, repeated infections, weight gain, periodontitis causing hyperinsulinemia also amplifies this process, resulting in increased deposition of iron, which further worsens insulin resistance.

Recent epidemiological studies have shown that increased iron stores predicted the development of diabetes. Ferritin is the storage form of iron. Iron converts reactive free radical into highly reactive ones. As the serum Ferritin level increases it affects the insulin synthesis and secretion in pancreas and interferes with the insulin extracting capacity of liver. Deposition in muscles leads to muscle damage and decreases glucose uptake.

The complex process of advanced glycation end product formation produces reactive oxygen species by metal – catalyzed reactions. Advanced glycation end products themselves bind transition metals (14) potentiating their toxic effects, including insulin resistance. Decreasing iron stores would ameliorate insulin resistance by reducing this cascade of events. Therefore reactive oxygen species interfere with insulin signaling at various levels, impairing insulin uptake through a direct effect on insulin receptor function and inhibiting the translocation of GLUT 4 in the plasma membrane. Perhaps this could be one of the reasons for the increased incidence of type 2 diabetes in persons with more ferritin.

The initial and the most common defect in patients with an earlier stage of damage induced by iron overload is liver – mediated insulin resistance. Hepatic iron overload is characterized by hyperferritinemia, normal transferrin saturation, and increased prevalence of glucose tolerance and diabetes. Transition metals play an important role in protein glycation induced by hyperglycemia. Plasma glucose levels are strongly associated with serum ferritin levels even in healthy subjects (15).

In study done by Salonen et al, serum ferritin had significant positive correlation with plasma glucose, serum triglyceride and serum apolipoprotein B concentration and inversely correlated to serum HDL2 cholesterol levels, all of which are components of insulin resistant syndrome(16).

In a study by Nan Hee Kim et al, the serum ferritin had a positive correlation with fasting plasma glucose, BMI, and fasting C Peptide level, an indicator of Hyperinsulinemia (17).

Hemoglobin, Iron, TIBC when compared NGT group against DM group in both male and

female patients does not show any statistical significance. This results in agreement with those in the literature.

Ferritin values are found to be positively correlated in the male and female subjects when NGT group is compared against DM group. Serum Ferritin is a marker of insulin resistance. It is an independent determinant of poor metabolic control in diabetic patients. Diabetic microangiopathy is associated with abnormal increased ferritin level in serum. Men with moderately higher ferritin levels had a significantly worse coronary risk profile than men with lower levels. Mean serum ferritin levels are higher in men than in premenopausal women (18).

Unhealthy diets in affluent countries contribute to diabetes risk not only through excess fat intake but also through excess iron supply (for example, in meat or in iron supplemented food). This potentially explains the reduced risks for diabetes in premenopausal women and vegetarians. Thus lowering body iron stores may become a tool in preventing type 2 diabetes in selected groups.

Ferritin concentration in male NGT is higher than female NGT implies that hyperferritinemia occurs before the elevation of plasma glucose. NGT first degree relatives in the type 2 diabetic pedigrees have higher ferritin concentration than normal control subjects (19).

The low levels of serum iron and higher levels of TIBC in both female groups than the male group may be due to the reason that they are mostly anemic due to physiological process like menstruation and pregnancy leading to iron deficient state.

Increase in Serum Iron level contribute to macro vascular disease as iron has an adverse effect on endothelium and accelerates the development of atherosclerosis (20). During the course of atherosclerotic plaque formation, ferritin gene expression increases (21). In our study we observed there was no increase in serum iron among those with diabetes mellitus. Even though there is no increase in serum iron in diabetes, iron participates in the formation of free radicals which are highly toxic and capable of inducing lipid peroxidation. Invariably in iron overload, insulin resistance is reported. Hence periodic monitoring of serum iron may be needed among those with diabetes mellitus. Further long term prospective studies including all the parameters of iron metabolism may throw more information in this field.

REFERENCES:

1. Sarah Wild, Gojka Roglic, Anders Green, Richard Sicree, and Hilary King. Global Prevalence of Diabetes Estimates for the year 2000 and projections for 2030.Diabetes Care1997; 27:1047 – 1053
2. Tuomainen T-P, Nyysonen K, Salonen R, Tervahuta A, Korpela H. Body iron stores are are associated with serum insulin and blood glucose concentrations. Diabetes Care 1997; 20: 426 – 428.
3. Jukka T Salonen, Tomi-Pekka Tuomainen, Kristiina Nyyssönen, Hanna-Maaria Lakka, and Kari Punnonen. Relation between iron stores and non-insulin dependent diabetes in men, a case-control study. British Medical Journal1998; 17:727 – 730.
4. Nan Hee Kim et al, Jung Heon Oh, Kyung Mook Choi, Young Hyen Kim and Sei Hyu Baik. Serum Ferritin in healthy subjects and type 2 Diabetes patients. Younsei Medical Journal 2000; 41: 387 – 392.
5. Jonathan E Shaw and Donald J Chisholm. Epidemiology and prevention of type 2 diabetes and the metabolic syndrome. Journal of Clinical Endocrinology and Metabolism 2003; 179(7): 379 - 383.
6. James B Meigs, Peter Wilson, Caroline Fox, David M Nathan and Lisa M Sullivan. Body mass index, metabolic syndrome, and risk of Type 2 Diabetes or Cardiovascular Disease. Journal of Clinical Endocrinology and Metabolism 2006; 91: 2906 – 2912.
7. Lo Ohlson, B larrhson, Svardsudd, Welin H Erikson, Bjorn torp and G Tiblin. The influence of body fat distribution on the incidence of diabetes mellitus. 13 years of follow up of the participants in the study of the men born in 1930. Diabetes Care 1985; 34: 1055 – 1058.
8. Sp Helmrich, Ragland, RW Leung, and Paffenberger. Physical activity and reduced occurance of non insulin dependent diabetes mellitus. NEJM 1991; 325: 147 – 152.
9. Duffy SJ, Biegelsen ES, Holbrook M, Russell JD and Gokce JK Jr. Iron chelation improves endothelial functions in patients with coronary artery disease. Circulation 2001; 103:2799 – 2804.
10. José Manuel Fernández-Real, Abel López-Bermejo, and Wifredo Ricart. Cross-Talk between Iron Metabolism and Diabetes. Diabetes 2002; 51: 2348 – 2354.
11. Medalie JH, PapieR CM, Goldbourt U, Herman JB. Major factors in the development of diabetes mellitus in 10,000 men. Arch Intern Med 1975; 135: 811 – 817.
12. Wilson PW, McGee DL and Kannel WB. Very low density lipoproteins and glucose intolerance over fourteen years, the Framingham Study. American Journal of Epidemiology and Obesity, 1981; 114:697 – 704.
13. Facchini FS, J.M Fernandar, Zinzhong Liang, Arroyo, Cabriro. Real effect of phlebotomy on plasma glucose and insulin concentrations. Diabetes Care 1998; 21:2190 - 2196.
14. Ascherio A, Rimm EB, Giovannucci E, Willett WC, Stampfer MJ. Blood donations and risk of coronary heart disease in men. Circulation 2001;103: 52 – 57.
15. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and ß-cell function. Diabetes Care 2000; 51:1000 – 1004.

16. J.M Fernandar Real, Wiferdo Ricart, Arroyo, Cabriro. Serum ferritin as a component of insulin resistance syndrome. Diabetes care 1998; 21(1): 62 – 68.
17. Nan Hee Kim, Jung eon ho, Kyung Mook Choi, Sei Hyun Baik, Sang Jin Kim. Serum ferritin in healthy subjects and Type 2 diabetes patients. Yonsei Medical Journal 2000; 41(3): 387 – 392.
18. Megan Jehn, Jeanne M Clarke, Eliseo Gullar. Serum ferritin and risk of metabolic syndrome in US adults. Diabetes Care 2004; 27(10): 2422 – 2428.
19. Yan Ren, Haoming Tian, Xiujun Lee, Zinzhong Liang. Elevated serum ferritin concentrations in a Glucose impaired population and in normal glucose tolerant first degree relatives in familial type 2 diabetic pedigrees. Diabetes Care 2004; 27(2): 622 – 623.
20. Gillum RF, Willett WC, Russell JD, G Tiblin. Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men: the Third National Health and Nutrition Examination Survey. International Journal of Obesity Related Metabolic Care 2001; 25:639 – 645.
21. Pang J, Jiang MJ, Chen YL, Wang FW, Wang DL, Chu SH. Increased ferritin gene expression in atherosclerotic lesions. Journal of Clinical Investigation 1996; 97:2204 –2212.