ORIGINAL RESEARCH

Serum Ferritin as a Risk Factor in Newly Diagnosed Type 2 Diabetes Mellitus: A Cross-sectional Study

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ABSTRACT

Background: The role of free Iron in Diabetes Mellitus is increasingly being recognized. It inhibits glucose production by the liver and decreases insulin metabolism causing peripheral hyperinsulinemia[1]. Iron released from ferritin undergoes a Fenton reaction to generate reactive oxygen species which damage the biological macromolecules.[2] **Material and Methods:** A cross-sectional study to compare the levels of fasting plasma glucose, sr. ferritin, HbA1C, and lipid profile in newly diagnosed 100 type 2 diabetes mellitus patients with 51 age and sex-matched healthy controls was carried out from November 2013 to June 2015 at MR Medical College, Kalaburagi after excluding patients with type 1 diabetes, Hemochromatosis, Thalassemia, and patients on Iron supplementation. The unpaired 't' test was used to compare the biochemical parameters between cases and control. ROC curves were plotted for sr. ferritin to find the diagnostic accuracy and area under the curve. Data was analyzed using SPSS 22 and P<0.05 was considered statistically significant. **Results:** Sr. ferritin in males was 457.9± 402.2 ng/ml and 184.46 ± 36.47ng/ml in females while in controls it was 84.6±36.8 ng/ml (P<0.001). The mean FBS was 181 in males and controls was 98 mg/dl. There was no significant association between Sr. Ferritin and Diabetic Nephropathy, Retinopathy and Neuropathy. **Conclusion:**The present studyshowed a significant increase in sr. Ferritin,Suggests oxidative stress as one of the major factors for pathogenesis in Type 2 diabetes mellitus patients.

INTRODUCTION

Diabetes Mellitus (DM) is defined as a heterogeneous group of diseases, characterized by a state of chronic hyperglycemia, resulting from a diversity of etiologies, environmental and genetic, acting jointly. Chronic hyperglycemia leads to several complications such as cardiovascular, renal, neurological, ocular, and recurrent infections.[1]

Recently, it has been found that increased body iron stores are associated with the development of glucose intolerance, gestational diabetes, type 2 DM, and insulin resistance syndrome. There is evidence that frequent blood donation leads to a decrease in the iron stores, which in turn leads to improvement in both beta cell secretion and peripheral insulin action in type 2 DM, followed by a drop in serum glucose, cholesterol, and triglycerides. It is also found that patients with uncontrolled diabetes have hyperferritinemia which is correlated with diabetic complications.[2]

Many studies have shown that abnormalities in the ferritin metabolism following glycation in a chronic hyperglycemic state might be a primary cause of hyperferritinemia in type 2 diabetes mellitus. Glycosylated ferritin has a longer serum half-life and glycemic control itself influences serum ferritin

concentration.[3] Elevated iron stores may induce diabetes and its complications through a variety of mechanisms including oxidative damage to the pancreatic ß cells, impairment of insulin extraction by the liver, and interference with insulin's ability to suppress hepatic glucose production.[4]

Free iron is toxic to cells and produces free radicals by undergoing a Fenton reaction. Iron is stored in ferritin, and only the ferrous form of iron is taken up by ferritin. Most of the ferritin is stored in the hepatocytes, spleen, bone marrow, heart, pancreas, and kidney and is affected by age and sex. Only a minute quantity of ferritin is present in the serum and this concentration is proportional to the body's iron stores.The levels of HbA1c in diabetes are used as a reliable index of glycemic control over the preceding 6 to 8 weeks.[5]The study aimed to evaluate iron overload status, and hyperglycemia by measuring serum ferritin, and HbA1c in cases of type 2 DM and compare it with healthy controls.

MATERIAL AND METHODS

A. Source of Data

A cross-sectional study comparing fasting plasma glucose, serum ferritin, HbA1c, and lipid profile levels in newly diagnosed type 2 diabetes mellitus patients with that of healthy controls was carried out from November 2013 to June 2015 at MR Medical College, Kalaburagi. Type 2 diabetes mellitus patients and healthy controls were selected from the general population. Written informed consent was obtained from each subject before starting the study. Patients and controls voluntarily participated in the study.

Inclusion Criteria

Cases: 100 clinically newly diagnosed type 2 diabetes mellitus between the age group of 38-62 Years of either sex were included in the present study.

Controls: 51 Healthy Individuals of either sex with matching age groups were included as the control group.

Exclusion Criteria

Patients with Type 1 Diabetes Mellitus, Gestational Diabetes Mellitus,Hemochromatosis, Thalassemia, Hemosiderosis, and patients on iron supplementation, thiazide diuretics, antioxidant drugs, and steroids. Patients with chronic infection and inflammation, neoplasia, renal disease, liver disease, alcoholics, smokers, and critically ill patients admitted tothe intensive care unit.

B. Parameters measured

- Fasting plasma glucose and post-prandial plasma glucose by Glucose oxidase method.
- Glycated hemoglobin by turbidimetric immunoassay.
- Serum ferritin by chemiluminescence Immunoassay (Lilac Acculite CLIA). Serum ferritin < 10 ng/ml indicates iron deficiency anemia and levels > 250 ng/ml indicate hemochromatosis.

RESULTS

This study was conducted between November 2013 to June 2015for 20 months. A total of 100 newly diagnosed type 2 DM patients were enrolled. Along with this, a total of 51 healthy control subjects were selected who were age and sex-matched and were free from any systemic illness. The unpaired’t’ test was used to compare the biochemical parameters between cases and control. ROC curves were plotted for sr. ferritin to find the diagnostic accuracy and area under the curve. Data was analyzed using SPSS 22 and $P<0.05$ was considered statistically significant.

The Study includes 41 patients inthe age group 41-50, 53 patients in the age group of 51-60 yrs., and 3 patients in the age group of ≥ 60 Yrs. (Figure-1). Out of 100 patients, 32 were females (32%) and 68 were males (68%) (Figure 2).

In this study mean FBS in patients was 181.36±39.65 mg/dl in males and 184.46±36.47 in females, while in controls it was 98.06±7.28 mg/dl (Table 1). PPG levels were mean 300.33±46.75 mg/dl in males and 305.5±47.13 mg/dl in females, whereas in controls it was 121.60±13.72 mg/dl. Serum ferritin levels in males were 457.9+402.2 ng/ml in males and 451.8+399.76 ng/ml in females, while in controls it was 84.6± 36.8 ng/ml.In this study HbA1c levels were 10.40±2.02 in males and 10.5+1.97 in females,it was 5.46±0.72 in controls (Figure-3).

Total Cholesterol was 184.93±31.15 mg in males and 187. 43± 28.07 mg in females. Triglycerides level was 125.79+43.13 mg in males and 120.24+40.81 in females (P value 0.543).

HDL level was 40.7±5.5 mg in males and 41.9+4.5 mg in females. LDL level was 85.23+24.05 mg in males and 83.9±18.29 in females (Figure-4).

In this study, nephropathy was present in 7 patients (7%).The diagnostic accuracy of S. ferritin for nephropathy was 64.6%.There was no significant association between S. ferritin in the diagnosis of nephropathy.Out of 100 patients, 12 showed Neuropathy.The diagnostic accuracy of S. Ferritin for neuropathy was 58.2%. There was no significant association between S. Ferritin in the diagnosis of neuropathy.Retinopathy was present in 8 diabetic patients. The diagnostic accuracy of S. Ferritin for retinopathy accuracy was 57.7% and there was no significant association between S. Ferritin in the diagnosis of retinopathy (Figure-5,6,7,8).

Table 1: Levels (Mean ±SD) of FPG, PPG, Serum Ferritin, and HbA1C in newly diagnosed Type 2 diabetes mellitus and Healthy controls

Sl. No	Variables		Males		Females		P value
			Cases	Controls	Cases	Controls	
1	FBS mg/dl	mean± SD	181.36±39.65	98.06±7.28	184.46±36.47	97.2±6.95	**<0.001**
2	PPG mg/dl	mean± SD	300.33±46.75	121.60±13.72	305.5±47.13	121.60±13.72	**<0.002**
3	Sr. Ferritin	mean± SD	457.9±402.2	84.6±36.8	451.8±399.76	83.60±35.91	**<0.003**
4	HbA1C	mean± SD	10.40±2.02	5.46±0.72	10.5±1.97	5.36±0.68	**<0.004**
5	T. cholesterol	mean± SD	184.9±31.15	146.71±28.53	187.43±28.07	14.83±27.95	0.701
6	HDL	mean± SD	40.7±5.5	33.68±3.76	41.9±4.5	34.71±3.58	0.29
7	Triglycerides	mean± SD	125.79±43.13	99.74±18.64	120.24±40.81	99.84±17.81	0.543
8	LDL	mean± SD	85.23±24.05	77.84±6.57	83.9±18.29	76.93±7.43	0.781

Figure 1: Pie diagram showing age age-wise distribution of subjects

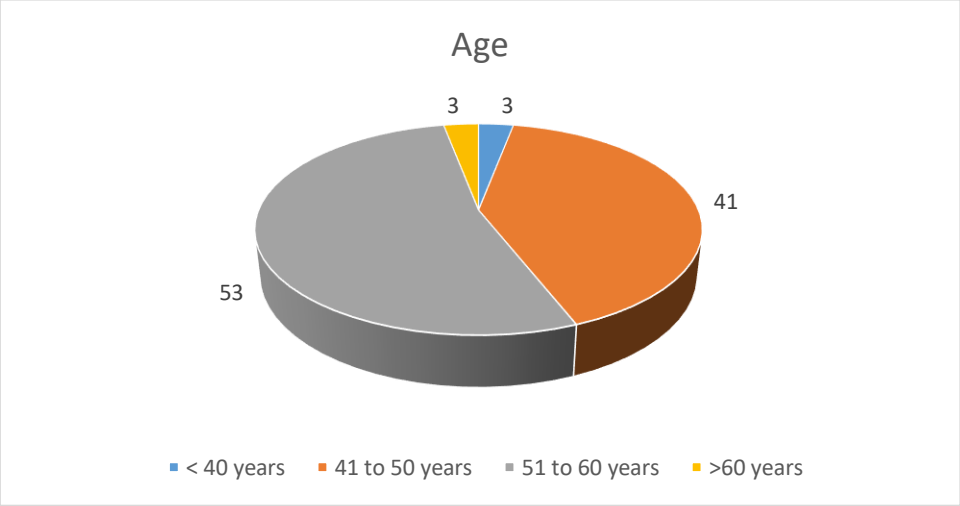


Figure 2: Pie diagram showing gender distribution

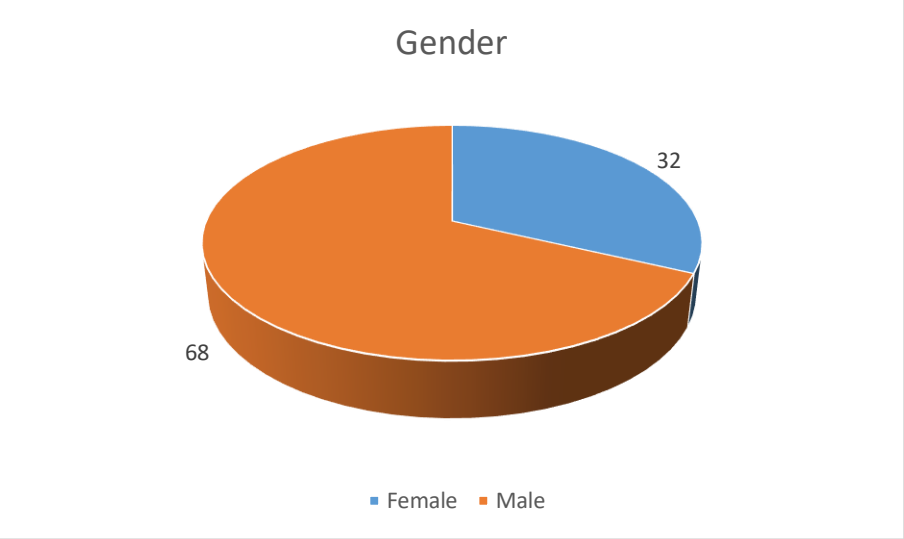


Figure 3: Bar diagram showing HbA1C levels

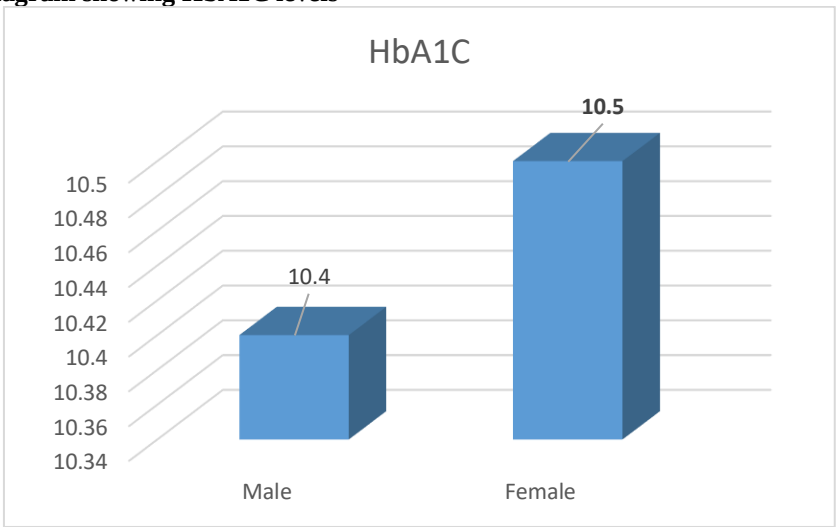


Figure 4: Bar diagram showing the lipid profile of subjects

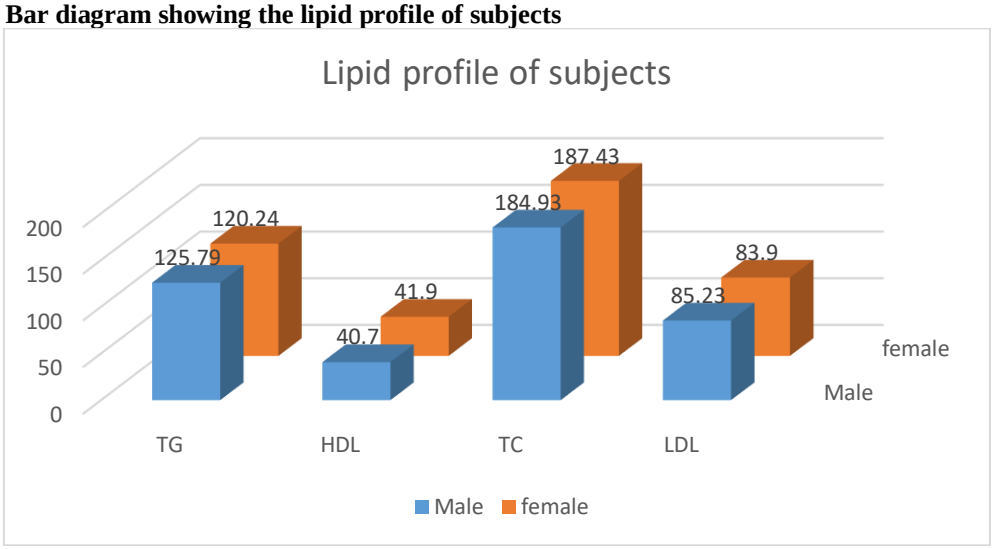


Figure 5: Bar diagram showing mean serum ferritin in complications of diabetes

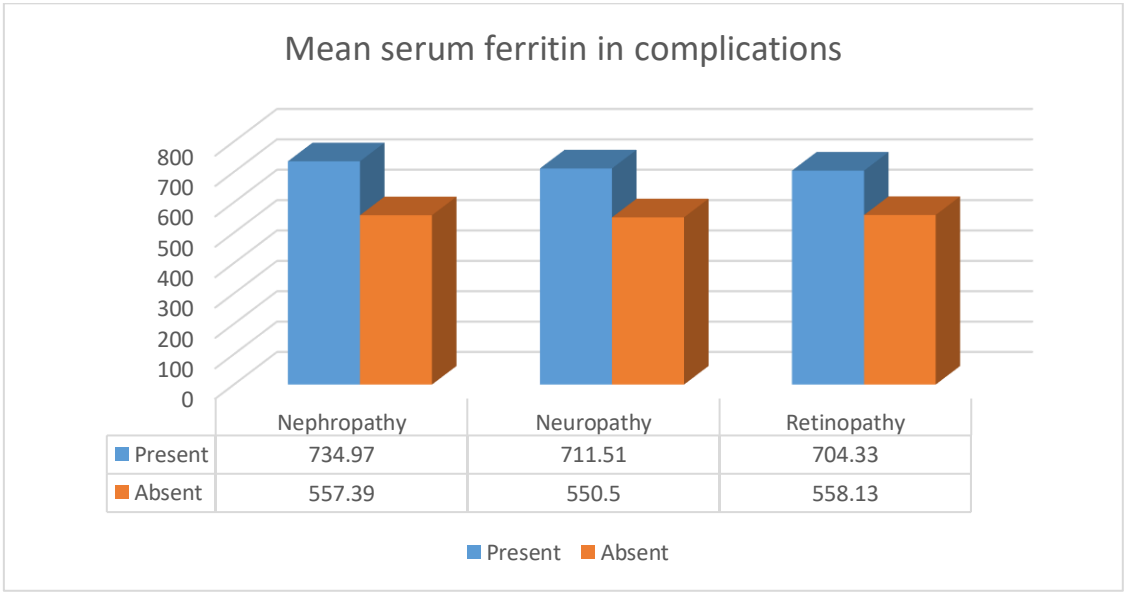


Figure 6: ROC curve showing sr. Ferritin in Diabetic patients for prediction of Nephropathy

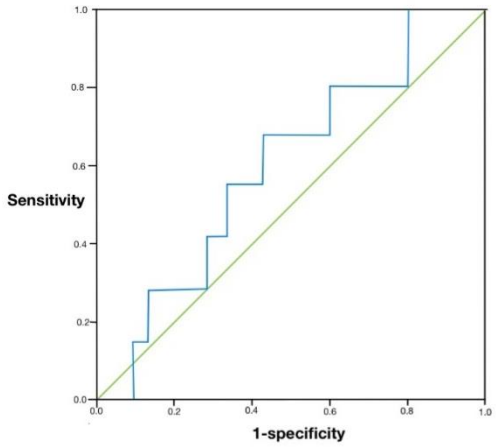


Figure 6: ROC curve showing sr. Ferritin in Diabetic patients for prediction of Neuropathy

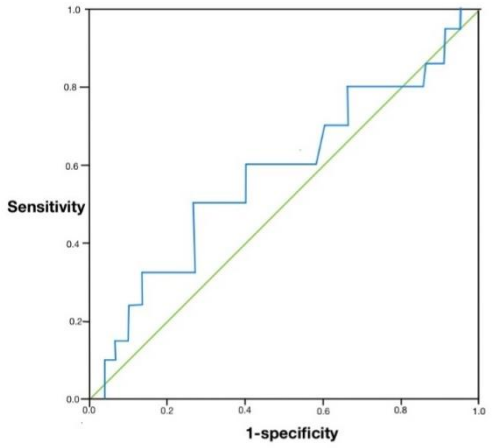
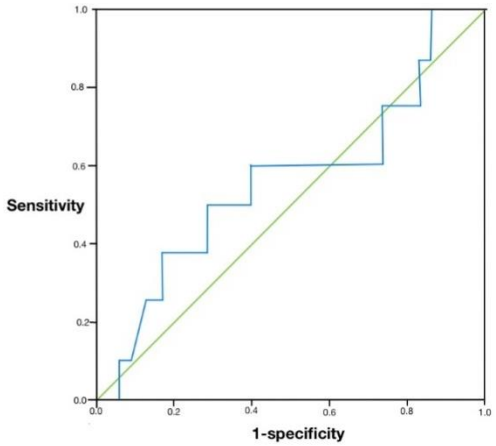


Figure 6: ROC curve showing sr. Ferritin in Diabetic patients for prediction of Retinopathy



DISCUSSION

Diabetes mellitus in all its heterogeneity has taken center stage as one of the ultimate medical challenges. The diabetic vascular complication is a leading cause of end-stage renal failure, acquired blindness, neuropathies, and accelerated atherosclerosis. These complications are the major cause of morbidity and mortality in patients with DM.[10]

Chronic hyperglycemia is a major initiator of diabetic complications. It induces various metabolic and hemodynamic derangements contributing to the characteristic histopathological changes observed in diabetic vascular complications.[11]

The biochemical process of advanced glycation appears to be enhanced in the diabetic milieu as a result of hyperglycemia, oxidative stress, and lipid peroxidation. A heterogeneous group of chemical

moieties is generated that appears to induce the development and progression of diabetic vascular complications bythe generation of proinflammatory and pro-sclerotic cytokines and various pathological processes.[12]

In the present study a total of 151 subjects, of which 100 type 2 diabetes mellitus patients and rest healthy controls were included. The fasting plasma glucose PPG, serum ferritin, and HbA1c levels were estimated in all these subjects.

Fasting Plasma Glucose (FPG)

In the present study, the mean level of FPG in controls was 96.06±7.28, and in type 2 diabetic patients 181.36±39.65. Statistical analysis by unpaired student's t-test has shown that the level of FPG in type 2 diabetic patients was significantly increased as compared to controls ($p<0.001$).

There are 4 major pathways responsible for the worsening of diabetic condition polyol pathway, increased formation of AGES, deregulated protein kinase C pathway, and increased hexosamine pathway flux.[13]

Hyperglycemia in DM is caused by both overproduction and underutilization of glucose. There is also a relative excess of glucagon in DM. As a consequence, glucose is synthesized rather than consumed by the liver, and glucose uptake into muscle and adipose tissue is reduced drastically leading to hyperglycemia.[14]

Serum Ferritin

It is increasingly recognized that iron influences glucose metabolism, even in the absence of significant iron overload. Excess of tissue iron amplifies the injury caused by free radicals as well as modulates various steps involved in the inflammatory lesion.[6]Serum ferritin is a storage form of iron found in the liver cells, spleen, bone marrow, heart, pancreas, and kidney. Normally human serum contains a small quantity of ferritin.[7]

In our study serum ferritin levels were found to be significantly increased in type 2 diabetes mellitus patients when compared to healthy controls. We also found a statistically significant positive correlation between serum ferritin with HbA1C, This increase in serum ferritin indicates an iron overload status in type 2 diabetes mellitus cases which has a role in the pathogenesis of diabetes. These findings are similar to previous studies conducted by SumeetSmotraet al[2]and N.G. Fourohi et al[9].

Hyperglycemia in a poor glycemic controlled state causes glycation of proteins, especially hemoglobin, releasing iron in free form. This makes a vicious cycle of hyperglycemia, glycation of hemoglobin, and an increase in levels of free iron. Increased level of free iron pool enhances the generation of oxygen free radicals, causing damage to biomolecules[15]. Glycation transferrin in diabetes mellitus decreases its ability to bind ferrous iron thereby increasing the pool of free iron and hence stimulating ferritin synthesis. Glycated holotransferrin is also known to facilitate the production of free oxygen radicals, such as hydroperoxide, further amplifying the oxidative effects of iron[6].

Iron-induced oxidative stress can explain its close association with abnormalities in insulin sensitivity. Iron influences glucose metabolism. Iron causes inhibition of insulin internalization and its actions, resulting in hyperinsulinemia and insulin resistance. Free iron also exerts positive feedback on ferritin synthesis, while oxidative stress increases the release of iron from ferritin. The increased oxidative stress and insulin resistance result in endothelial and tissue damage[6].

Glycated Hemoglobin (HbA1c)

In long-term hyperglycemia, HbA1c constitutes a higher percentage of total hemoglobin than in normoglycemia. Transient elevations in plasma glucose levels only mildly affect HbA1c levels.[16]

In our study, HbA1c levels were significantly increased in type 2 diabetes mellitus patients with a mean value of 10.40±2.02 compared to healthy controls witha mean value of 5.46±0.84 ($p<0.001$). And correlation between HbA1c and serum ferritin, levels has shown a statistically significant positive correlation. These data correlate with previous studies done by SumeetSmotraet al[2] and Elizabeth Selvin et al[8].

Patients with type 2 DM with HbA1c levels ±7.5% have a 2.5 to 5-fold increased risk of developing microvascular complications. Retinopathy, nephropathy, and neuropathy have all been shown to correlate with the severity of hyperglycemia. Fortunately, achieving recommended blood glucose targets can substantially reduce the risk of microvascular complications, and possibly macrovascular complications[17].

Withthe present study, we did not find a significant correlation between diabetic retinopathy, neuropathy, and nephropathy. Probably this is because these are newly diagnosed cases of type 2 diabetes mellitus.

The limitation of the study was the small study population. A multicenter, large study will help to come to a definite conclusion.

CONCLUSION

The results of the present study support the concept that an increase in fasting plasma glucose, PPG glycated hemoglobin (HbA1C), and serum ferritin, play an important role in the pathogenesis of type 2 diabetes mellitus.

The present study suggests that oxidative stress is one of the major factors in the pathogenesis of type 2 DM. There is a need to prevent iron overload in type 2 DM subjects which may occur in many ways. Decreasing iron stores may reduceoxidative stress and improve insulin sensitivity in type 2DM subjects.

The present study also suggests that oxidative stress is one of the major factors for pathogenesis in type 2 DM. There is a significant increase in serum ferritin levels in type 2 diabetes mellitus patients. Serum ferritin a marker of iron overload, leads to oxidative stress through the Fenton reaction.

REFERENCES

1. Michael J. Fowler; Microvascular and Macrovascular Complications of Diabetes. *Clin Diabetes* 1 April 2008; 26 (2): 77–82.
2. Smotra S, Kudyar RP. Relationship between Serum Ferritin and Type 2 Diabetes Mellitus. JK Science, Oct-Dec 2008; 10 (4):170-174.
3. Sharifi F, Nasab NM and Zadeh HJ. Elevated serum ferritin concentrations in prediabetic subjects. DiabetVasc Dis Res 2008;5: 15-18.
4. Jehn ML, Gullar E, Clark JM, Couper David, Duncan B.B, Ballantyne CM.,et al. A Prospective study of plasma ferritin level and Incident Diabetes, The Atherosclerosis Risk in Communities (ARIC) Study. AmJEpidemiol 2007: 16:1047-1054.
5. American Diabetes Association standards of Medical care in Diabetes - 2011. Diabetes care 2011 Jan; 34 suppl 1:S11-61.
6. Fernandez- Real JM, Lopez- Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002;51:2348-54.
7. W, Bloodletting in high Ferritin Type 2 Diabetes, Diabetes care 2002;25:2249-2255.
8. Selvin E, Coresh J, Golden SH, Boland LL, Brancati FL, Steffes MW. Glycemic Control, Atherosclerosis, and Risk Factors for Cardiovascular Disease in Individuals with Diabetes. Diabetes Care 2005;28:1965-1973.
9. Rajpathak S, Ma J, Manson J, Willett WC, Hu Fb. Iron Intake and the risk of type 2 Diabetes in Women. Diabetes Care 2006;29:1370-1376.
10. Tietz NW. Textbook of clinical chemistry Philadelphia WB. Saunders Company 1999 pp. 794-795.
11. Srivatsan R. Das S, Gadde R, Manoj - Kumar K, Taduri S, Roa N, et al. Antioxidants and Lipid peroxidation status in diabetic patients with and without complications. Arch Iranian Med 2009, 12(2):121-27.
12. Yamagishi S, Nakamura K, Imaizumi T. Advanced glycation end products (AGES) and diabetic vascular complications. Curr Diabetes Rev 2005;1:93-106.
13. Goh SY, Cooper ME. The role of advanced glycation end products in progression and complications of diabetes. J ClinEndocrinolMetab 2008;93:1143-52.
14. Piconi L, Ceriello A. Oxidative stress, diabetes, and its complications. US Endocrine Disease 2007:36-38. NEX 67. Puri D. Integration of metabolism. In: Puri D. Textbook of medical Biochemistry, 3rd ed. New Delhi: Elsevier; 2011: 316-333.
15. Bao W, Rong Y, Rong S. and Liu L. Dietary Iron intake, body iron stores, and the risk of type 2 diabetes: A systematic review and meta-analysis. BMC Medicine 2012;10:119.
16. Shetty JK, Prakash M, Ibrahim MS. Relationship between free iron and glycated hemoglobin in uncontrolled Type 2 diabetes patients associated with complications, IJCB 2008;23(1):67-70.
17. Richard F. Dods. Diabetes Mellitus. In: Kaplan L, Pesce A. Clinical Chemistry, Theory Analysis and Correlation. Hickman P, Koerbin G. Sublett K (edts). Chapter 38.2010:5 ed. USA:Mosby.p.749.