International Journal of Biochemistry Research & Review
8(3): 1-9, 2015, Article no.IJBcRR.19982
ISSN: 2231-086X

SCIENCEDOMAIN *international*
www.sciencedomain.org

Comparison of Plasma Glucose, Serum Ferritin, HbA1c and Serum Nitric Oxide Levels between Diabetic and Non Diabetic Individuals: An Indian Scenario

Mahalaxmi S. Petimani[1*], Prabhakar Adake[2] and P. Suresh Babu[3]

[1]Department of Biochemistry, Yenepoya Medical College, Yenepoya University, Mangalore-575018, Karnataka, India.
[2]Department of Pharmacology, Yenepoya Medical College, Yenepoya University, Mangalore-575018, Karnataka, India.
[3]Department of Biochemistry, J. J. M. Medical College, Davangere-577004, Karnataka, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author MSP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors PA and PSB managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2015/19982
Editor(s):
(1) Yi-Ren Hong, College of Medicine, Kaohsiung Medical University, Taiwan.
Reviewers:
(1) Jaspinder Kaur, Punjab Institute of Medical Sciences, Jalandhar, Punjab, India.
(2) Vivek Sharma, Himachal Pradesh Technical University, India.
(3) Anonymous, Mahidol University, Thailand.
Complete Peer review History: http://sciencedomain.org/review-history/10698

Original Research Article

Received 6th July 2015
Accepted 28th July 2015
Published 24th August 2015

ABSTRACT

Aims: To estimate fasting plasma glucose (FPG), serum ferritin, HbA1c and serum nitric oxide levels in type 2 diabetes mellitus (DM) subjects and compare the values with non diabetic individuals and also to assess the correlation analysis between the biochemical parameters in type 2 DM subjects.
Study Design: A case control study.
Place and Duration of Study: Study was carried out from June 2012 to June 2013 in Bapuji

**Corresponding author: E-mail: mahalaxmi.petimani@gmail.com;*

Hospital and Chigateri General Hospital, Davangere, Karnataka, India.
Methodology: A total of 87 subjects were included in the present study of which 56 type 2 Diabetes Mellitus patients and 31 control subjects. FPG, serum ferritin, HbA1c and serum nitric oxide were estimated in all subjects.
Results: Intergroup comparison of biochemical parameters was done by unpaired "t" test and correlation between the parameters by Pearson's coefficient analysis. The estimated mean levels (mean ± SEM) of FPG, serum ferritin, HbA_{1c} and serum nitric oxide in control group were 98.06±1.30, 84.6±6.61, 5.46±0.15 and 39.0±0.84 respectively. Similarly, in type 2 diabetic patients mean levels of 179.5±7.11, 457.9±53.7, 9.49±0.25, and 100.9±3.5 were obtained for respective parameters. Mean values of all parameters were found to be significantly increased In DM subjects (P=.001) when compared to control group. Moreover, Serum ferritin has shown significant positive correlation with HbA1c and serum nitric oxide in type 2 DM patients with 'P' value of .05.
Conclusion: The present study suggests that iron over load is one of the major factors in the pathogenesis of type 2 DM. Decreasing iron stores may reduce the oxidative stress, improve the vascular endothelial dysfunction and also improves insulin sensitivity in type 2 DM subjects.

Keywords: Endothelial dysfunction; iron overload; insulin sensitivity.

1. INTRODUCTION

Diabetes Mellitus (DM) is a heterogeneous group of diseases, characterized by a state of chronic hyperglycemia, resulting from a diversity of etiologies, environmental and genetic, acting jointly. The underlying cause of diabetes is the decreased production or action of insulin, a hormone that controls glucose, fat and amino acid metabolism. Diabetes is a chronic disease with variable clinical manifestations and progression. Chronic hyperglycemia leads to a number of 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. Frequent blood donation leads to decrease in the iron stores, which in turn leads to improvement in both beta cell function and peripheral insulin action followed by the drop in serum glucose, cholesterol and triglycerides in DM patients. It is also found that patients with uncontrolled diabetes have hyperferritinemia which correlates with diabetic complications [2].

Previous studies have shown that abnormalities in the ferritin metabolism following glycation in 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 influence serum ferritin concentration [3]. Elevated iron stores may induce diabetes and its complications through a variety of mechanisms including oxidative damage to the pancreatic β cells, impairment of insulin extraction by liver, and interference with insulin's ability to suppress hepatic glucose production [4]. Free iron is toxic to cells and leads to production of free radicals by undergoing Fenton reaction.

HbA1c is a glycated hemoglobin formed by a post translational non enzymatic combination of aldehyde group of glucose and other hexose with the amino terminal valine in the beta chain of hemoglobin. The level of HbA1c in diabetes is used as a reliable index of glycemic control over the preceding 6 to 8 weeks [5].

Nitric oxide (NO) is a potent vasodilator and also an endothelial relaxing factor. NO is a short lived free radical, involved in variety of physiological functions like smooth muscle relaxation, inhibition of platelet aggregation and non-noradrenergic-noncholinergic neurotransmission [6]. Uncoupling of endothelial Nitric oxide synthase enzyme occurs in the blood vessels of diabetic subjects leading to endothelial dysfunction and excessive production of superoxide anion causing decreased NO bioavailability [7].

Previous have found that increased levels of FPG, HbA1c and serum nitric oxide levels are responsible for diabetic complications. However, role of hyperferritinemia in DM is still contradictory. So the present study is carried out to evaluate iron overload status, endothelial dysfunction; antioxidant and long term glycemic control status in Indian population.

2. MATERIALS AND METHODS

A total of 87 subjects were included in the present study of which 56 type 2 Diabetes

Mellitus patients and 31 non diabetic individuals. Study was carried out from June 2012 to June 2013 in Bapuji Hospital and Chigateri General Hospital, Davangere, Karnataka, India (Hospitals attached to J.J.M Medical College, Davangere). The study protocol was confirmed to the ethical guidelines of Declaration of Helsinki (Sixth revision, 2008). Institutional Ethical Committee approval was taken from J.J.M Medical College, Davangere Karnataka, India before starting the study. Written informed consent was obtained from each subject before starting the study. Patients and controls were voluntarily participated in the present study.

2.1 Inclusion Criteria: Cases

Clinically diagnosed type 2 diabetes mellitus between the age group of 30-60 years of either sex with five years history of diabetes were included in the present study.

2.1.1 Controls

Healthy individuals of either sex with matching age group were included as control group.

2.2 Exclusion Criteria

Patients with type 1 diabetes mellitus, gestational diabetes mellitus, hemochromatosis, Thalassemia, Hemosiderosis, patients on iron supplementation, thiazide diuretics, antioxidants drugs and steroids, patients with chronic infections and inflammation, neoplasia, renal disease, liver disease, alcoholics and smokers, critically ill patients admitted in intensive care unit and pregnant women, were excluded from the study.

2.3 Collection of Blood Sample

About 6 ml of venous blood was drawn from all the subjects (from large peripheral vein) under aseptic precautions, using a sterile disposable syringe. Out of 6ml, 3 ml of blood was transferred to plain vaccutainer and remaining 3 ml into EDTA containing vaccutainer. 3 ml of plain vaccutainer blood was subjected to centrifugation and the serum was separated which was used for estimation of serum ferritin and nitric oxide. Out of 3 ml of anticoagulated blood sample, 1 ml was used to estimate HbA1c and remaining 2ml was used to separate plasma to estimate fasting plasma glucose.

2.4 Parameters Measured

From the blood sample collected by the above method fasting plasma glucose, HbA1c, serum ferritin and serum nitric oxide levels were measured in both cases and controls. Fasting plasma glucose was measured by glucose oxidase method [8]. Kinetic Cadmium-Reduction method was used to measure serum nitric oxide levels. For HbA1c and serum ferritin estimation turbidimetric Immunoassay and Chemiluminescence Immunoassays were followed respectively [9,10].

2.5 Statistical Analysis

The values are expressed in mean ± standard error of mean(mean ± SEM). Unpaired 't' test was used to compare the biochemical parameters between cases and control and Pearson's correlation analysis was used to find the correlation between the biochemical parameters in cases.

3. RESULTS AND DISCUSSION

Among 56 DM patients, 24 were male and 32 were female. Similarly in control group 19 were male and 12 were females. Table 1 shows the demographic distribution of cases and controls included in the study. Mean age for type 2 diabetes mellitus patients was 50.09±8.2 years and 45.64±12.1 years in control group. Sex matched controls were selected and there was no significant difference between cases and controls with respect to age and sex of subject.

Table 2 Shows levels of various biochemical parameters in type 2 diabetic patients and healthy controls. The estimated mean levels of FPG, serum ferritin, HbA_{1c} and serum nitric oxide in control group were 98.06±1.30, 84.6±6.61, 5.46±0.15 and 39.0±0.84 respectively. Similarly in type 2 diabetic patients mean levels of 179.5±7.11, 457.9±53.7, 9.49±0.25, and 100.9±3.5 were obtained for respective parameters.

The statistical analysis by Unpaired 't'-test has shown that the levels of FPG, serum ferritin, HbA_{1c}, and serum nitric oxide are significantly increased in type 2 diabetic patients when compared to controls with P value of .001.

The Pearson's correlation analysis was done between the biochemical parameters of type 2 diabetes patients which showed statistically

Table 1. Age and sex-wise distribution of control and type 2 diabetic patients

	Cases	Controls	P value
No of subjects	56	31	
Age (years)Mean± SEM	50.09±1.0	45.64±2.1	.07*
Gender: Male	24 (42.9%)	19(61.3%)	.10*
Female	32(57.1%)	12(38.7%)	

**Not significant*

Table 2. Levels (mean±SEM) of FPG (fasting plasma glucose), serum ferritin, serum nitric oxide and HbA_{1c} in patients with type 2 diabetes mellitus and healthy controls

Variables		Cases	Controls	Mean difference	t value	P value
FPG	Mean±SEM	179.5±7.11	98.06±1.30	81.44	11.36	.001**
Mg/dl	Range	97-288	78-106			
Serum Ferritin	Mean±SEM	457.9±53.7	84.6±6.61	373.30	6.89	.001**
ng/ml	Range	34.4-174.5	12.3-162.2			
Serum Nitric	Mean±SEM	100.9±3.5	39±0.84	70.90	17.03	.001**
Oxide µmol/l	Range	38.4-146.0	30.1-45.8			
HbA1c	Mean±SEM	9.49±0.25	5.46±0.15	4.03	13.64	.001**
	Range	4.80-13.51	4.10-6.40			

*Unpaired 't' test, ** Highly significant*

Table 3. Showing the Pearson's correlation between biochemical parameters in type 2 diabetes mellitus patients

Correlation analysis		
Relationship between	**r value**	**P value**
Serum Ferritin and HbA1c	0.49	.001**
Serum Nitric oxide and HbA1c	0.35	.009*
Serum Nitric Oxide and Serum Ferritin	0.34	.01*

*r-Pearson's correlation coefficient *S-Significant; **HS-Highly significant*

significant positive correlation between serum ferritin and HbA1c with r value of +0.49 and P value .001. Serum nitric oxide and HbA1c also showed statistically significant positive correlation with r value of +0.35 and P=.05. Similarly, significant positive correlation was found between serum nitric oxide and serum ferritin with r value of +0.34 and P value of .05. The results of this study are in accordance with the previous studies, which also found that serum ferritin, HbA1c, FPG, serum nitric oxide levels are higher in DM and responsible for diabetic complications [11-13]. However, study by Nuria Freixenet et al. [14] disproves above concept and rules out hyperferritinemia in DM.

Diabetes mellitus in all its heterogeneity has taken the center stage as one of the ultimate medical challenges. 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 [15].

Chronic hyperglycemia is a major initiator of diabetic complications. It induces various metabolic and hemodynamic derangements, including increased advanced glycation end (AGE) product formation, enhanced production of reactive oxygen species (ROS), activation of protein kinase C (PKC), stimulation of the polyol pathway and the renin angiotensin system (RAS), contributing to the characteristic histopathological changes observed in diabetic vascular complications [16].

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 are generated that appears to induce the development and progression of diabetic vascular complications directly or indirectly via activation of intracellular signaling pathways, generation of proinflammatory and prosclerotic cytokines [17].

In diabetes mellitus chronic hyperglycemic state is known to be responsible for increased oxidative stress resulting in complications. The four major pathways responsible for worsening of diabetic condition are polyol pathway, increased

formation of AGEs, deregulated protein kinase C pathway and increased hexosamine pathway flux [18].

Hyperglycemia in DM is caused by both overproduction and under utilization of glucose. There is also a relative excess of glucagon in DM. As a consequence, glucose is synthesized rather than consumed by liver and glucose uptake into muscle and adipose tissues are reduced drastically leading to hyperglycemia [19].

3.1 Serum Ferritin

It has been found that iron influences glucose metabolism, even in the absence of significant iron overload. Excess of tissue iron amplify the injury caused by free radicals as well as modulate various steps involved in the inflammatory lesion [20]. 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 [21].

In this study serum ferritin levels were found to be significantly increased in type 2 diabetes mellitus patients when compared to healthy controls. This study also showed statistically significant positive correlation between serum ferritin with serum nitric oxide and HbA1c. Increase in serum ferritin indicates an iron over load status in type 2 diabetes mellitus cases which has a role in the pathogenesis of diabetes. These findings are in accordance with previous studies conducted by Sumeet Smotra, Wei Bao and N.G. Fourohi [2,22,23].

Hyperglycemia causes glycation of proteins, especially hemoglobin, releasing iron in free form. This makes a vicious cycle of hyperglycemia, glycation of hemoglobin and increase in levels of free iron. Increased level of free iron pool enhances generation of oxygen free radicals, causing damage to biomolecules [24]. Glycation of transferrin in diabetes mellitus, decreases its ability to bind ferrous iron thereby increasing the pool of free iron and hence stimulates 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.

Furthermore, Iron influences glucose metabolism by inhibiting internalization of insulin and its actions, resulting in hyperinsulinemia and insulin resistance. Thus, the increased oxidative stress and insulin resistance results in endothelial and tissue damage.

3.2 Nitric Oxide (No)

Nitric oxide is a pleiotropic mediator of inflammation released from endothelial cells that causes vasodilatation by relaxing vascular smooth muscle and was therefore called endothelium–derived relaxing factor [10]. NO is a lipid soluble gas which is ideally suited as a potent inflammatory mediator because of its strong reactivity with oxygen, superoxide and iron-containing compounds. This inherent reactivity of NO translates into a relatively short lived radical [25]. NO is generated by the nitric oxide synthase (NOS) enzyme from molecular oxygen and the terminal guanidine nitrogen of the amino acid L-arginine, yielding L-citrulline as a co-product.

In our study, the serum NO level was significantly increased (P=.001) in type 2 diabetes mellitus patients when compared to healthy controls. And also, serum nitric oxide levels were positively correlated with serum ferritin and HbA1c levels. This finding is in accordance with previous studies of Dilshad Ahmed Khan and Katsuyuki Maejima [7,26].

The main cause for increased nitric oxide level in type 2 diabetic patients is due to chronic hyperglycemia leading to overflow of the polyol pathway products along with depletion in the nicotinamide adenine dinucleotide phosphate (NADPH). NADPH is an important cofactor for the enzymes in the metabolism of the reactive nitrogen species (RNS) and reactive oxygen species (ROS).

3.3 HbA1c

HbA1c is produced by covalent binding of glucose to hemoglobin. The red blood cell is completely permeable to glucose; the quantity of HbA1c formed is directly proportional to the average plasma glucose concentration that the red blood cell is exposed during its 120-day life span (6 to 8 weeks). Thus, in long term hyperglycemia, HbA1c constitutes a higher percentage of total hemoglobin than in normoglycemia. Transient elevations in plasma glucose level mildly affect HbA1c levels [27].

In this study, HbA1c levels were significantly increased in type 2 diabetes mellitus patients when compared to healthy controls. Statistically

significant positive correlation between HbA1c, serum ferritin, and serum nitric oxide levels were found in this study. These data are in accordance with previous studies done by Sumeet Smotra, Elizabeth Selvin and H.K. Choi [2,26,28].

Type 2 DM is a chronic disease with devastating consequences. Both macrovascular and microvascular complications such as retinopathy, nephropathy and neuropathy are associated with type 2 DM. An elevated HbA1c level has been shown to be a dominant predictor for the development and progression of microvascular complications in patients with type 2 DM. Patients with type 2 DM with HbA1c levels ≥7.5% have a 2.5-5 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 the macrovascular complications [29].

Acute glucose fluctuations above a mean value may trigger oxidative stress, which contributes to damage through oxidation of low-density lipoprotein, exacerbation of endothelial dysfunction, and other proatherogenic mechanisms leading to the development and progression of vasculopathies [29]. Diabetes control and complication trial (DCCT) has shown that 10% stable reduction in HbA1c results in 35% risk reduction for retinopathy, 25-44% risk reduction for nephropathy and 30% risk reduction for neuropathy [30].

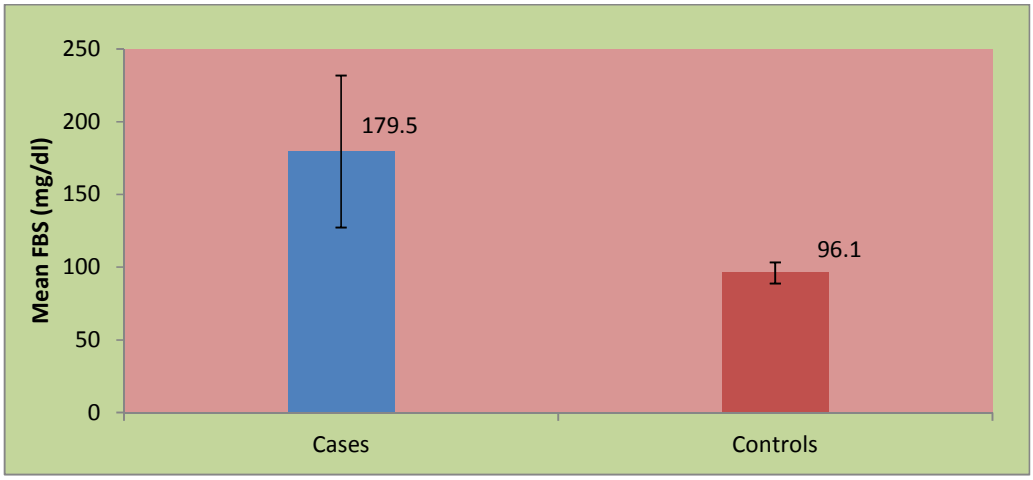

Fig. 1. Comparison of fasting blood sugar levels between cases and control

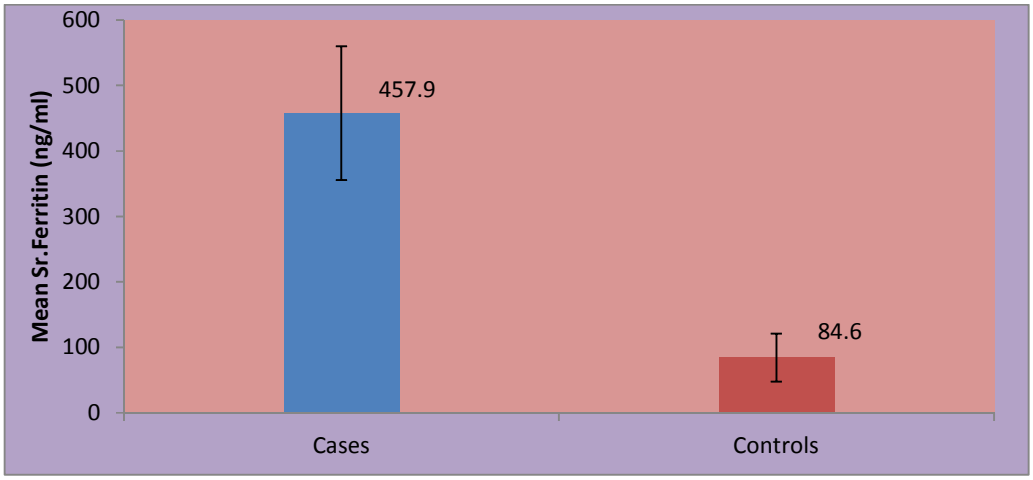

Fig. 2. Comparison of serum ferritin levels between cases of type 2 diabetes mellitus and healthy controls

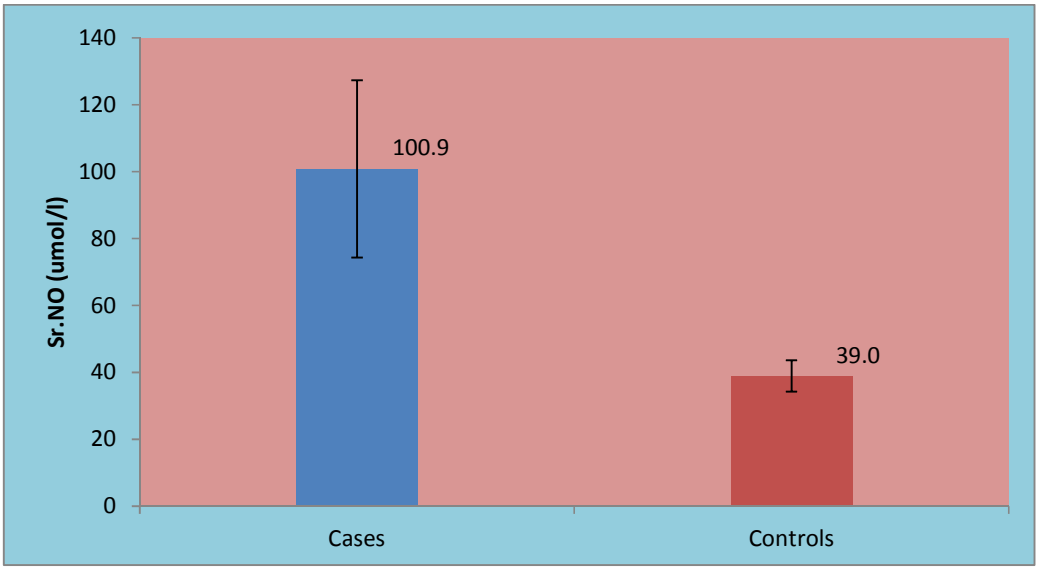

Fig. 3. Comparison of serum nitric oxide levels between type 2 diabetes mellitus patients and healthy controls

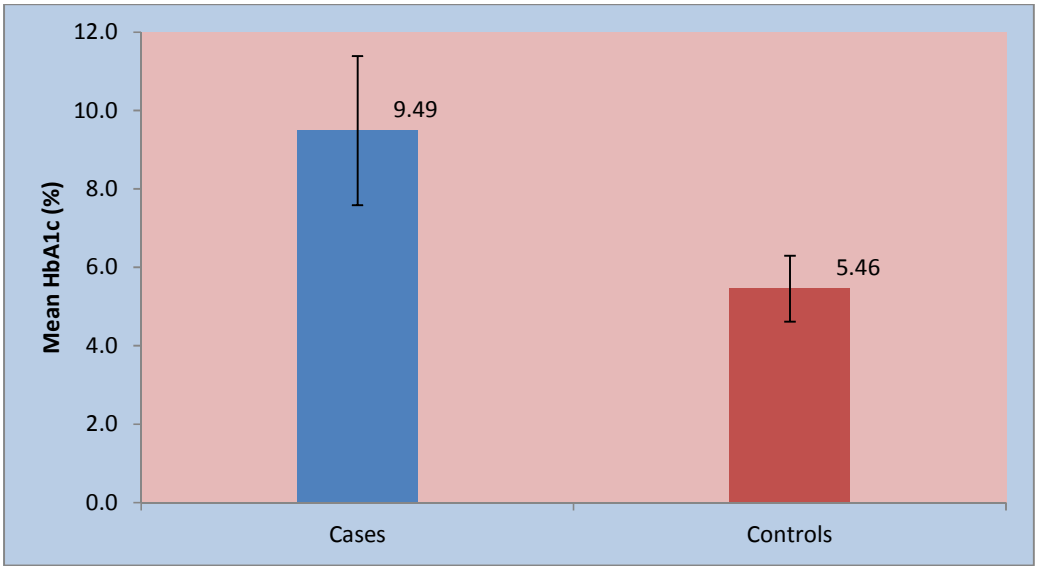

Fig. 4. Comparison of HbA1c levels between type 2 diabetes mellitus patients and healthy controls

4. CONCLUSION

The results of our present study support the concept that increase in fasting blood glucose, HbA1c, serum ferritin, and serum nitric oxide play an important role in the pathogenesis of type 2 diabetes mellitus.

Reduction in dietary iron intake by avoiding intake of meat and avoiding excessive iron medications might decrease the oxidative stress and improves insulin sensitivity in type 2 diabetes mellitus patients. Good glycemic control and consuming diet rich in green leafy vegetables will ensure adequate levels of antioxidant nutrients in the tissue and help the body to resist disease related oxidative stress.

Limitation of our study is that we could not detect variations in these parameters in relation to

diabetes induced microvascular and macrovascular complications. In future, further studies are required to know the relationship of these biochemical parameters in type 2 diabetes patients with complications in order to effectively control and prevent the disease progression.

CONSENT

Written informed consent was obtained from each subject before starting the study. Patients and controls voluntarily participated in the study.

ETHICAL APPROVAL

Institutional ethical committee approval was taken from J.J.M Medical College, Davangere, Karnataka, India before staring the study.

ACKNOWLEDGEMENT

Authors are thankful to all the individuals who have participated in the study without them the research would be incomplete. Authors extend sincere thanks to all the staff members who were the part of study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Epidemiology of chronic non communicable diseases and condition. In: Park K, editor. Park's text book of Preventive and Social Medicine. 19th ed. Jabalpur: Banarsidas Bhanot Publishers. 2007;327-32.
2. Smotra S, Kudyar RP. Relationship between serum ferritin and type 2 diabetes mellitus. JK Science. 2008;10(4):170-174.
3. Sharifi F, Nasab NM, Zadeh HJ. Elevated serum ferritin concentrations in prediabetic subjects. Diabet Vasc Dis Res. 2008;5: 15-18.
4. Jehn ML, Gullar E, Clark JM, Couper David, Duncan BB, Ballantyne CM, et al. A prospective study of plasma ferritin level and incident diabetes, The Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol. 2007;16:1047-1054.
5. American Diabetes Association. Standards of Medical care in Diabetes-2011. Diabetes Care. 2011;34(1):S11-61.
6. Kimura H, Esumi H. Reciprocal regulation between nitric oxide and vascular endothelial growth factor in angiogenesis. Acta Biochimica Polonica. 2003;50(1): 49-59.
7. Khan DA, Qayyum S. Evaluation of cardiac risk by oxidative stress and inflammatory markers in diabetic patients. Pak J Med Sci. 2009;25(5):776-781.
8. Principles of Immunochemical Techniques. In: Kricka L editor. Teitz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. New Delhi: Elsevier Publishers. 2006;219-244.
9. Tietz NW. Text book of Clinical chemistry. 3rd ed. Philadelphia: W.B Saunders Company Publishers. 1999;794-795.
10. Principles of Immunochemical Techniques. In: Kricka L editor. Tietz Textbook of Fundamentals of Clinical Chemistry. 6th ed. Philadelphia: W.B Saunders Publishers. 2008;167-168.
11. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Solonen R. High iron levels are associated with excess risk of myocardial infarction in eastern finnish men. Circulation. American Heart Association. 1992;86:803-811.
12. Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation. 1997;96: 25-28.
13. Maejima K, Nakano Shigeru, Himeno M, Tsuda S, Makiishi H, Ito T, et al. Increased basal levels of plasma nitric oxide in type 2 diabetic subjects. Journal of Diabetes and its Complications. 2001; 15(3):135-143.
14. Nuria Freixenet, Carme Vilardell, Gemma Llauradó, Olga Giménez-Palop, Eugenio Berlanga, Cristina Gutierrez, et al. Men with hyperferritinemia and diabetes in the Mediterranean area do not have a higher iron overload than those without diabetes. Diabetic Research and Clinical Practice. 2011;91(2):e33-e36.
15. Srivatsan R, Das S, Gadde R, Manoj-Kumar K, Taduri S, Rao N, et al. Antioxidants and lipid peroxidation status in diabetic patients with and without complications. Arch Iranian Med. 2009; 12(2):121-27.
16. Yamagishi S, Nakamura K, Imaizumi T. Advanced glycation end products (AGEs)

and diabetic vascular complications. Curr Diabetes Rev. 2005;1:93-106.
17. Goh SY, Cooper ME. The role of advanced glycation end products in progression and complications of diabetes. J Clin Endocrinol Metab. 2008;93:1143-52.
18. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;414:813–820,
19. Integration of metabolism. In: Puri D editor. Textbook of medical biochemistry. 3rd ed. New Delhi: Elsevier Publishers. 2011; 316-333.
20. Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes. 2002; 51:2348-54.
21. Fernandez-Real JM, Penarroja G, Castro A, Garcia-Bragado F, Lopez-Bermejo A, Ricart W. Bloodletting in high ferritin type 2 diabetes. Diabetes care. 2002;25: 2249-2255.
22. Bao W, Rong Y, Rong S, 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.
23. 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.
24. 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.
25. Weinberg JB, Lang T, Wilkinson WE, Pisetsky DS, St Clair WE. Serum, urinary and salivary nitric oxide in rheumatoid arthritis: Complexities of interpreting nitric oxide measures. Arthritis Research and Therapy. 2006;8:1-9.
26. Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation. 1997;96: 25-28.
27. 5th ed. USA: Mosby. 2010;749.
28. Selvin E, Coresh J, Golden SH, Boland LL, Brancati FL, Steffes MW. Gycemic control, atherosclerosis and risk factors for cardiovascular disease in individuals with diabetes. Diabetes Care. 2005;28: 1965-1973.
29. Fowler GC, Vasudevan DA. Type 2 diabetes mellitus: Managing hemoglobin A1cand beyond. Southern Med J. 2010; 103(9):911-16.
30. Calisti L, Tognetti S. Measure of glycosylated hemoglobin. Acta Biomed. 2005;76(3):59-62.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/10698