Original Research Article

A study on Serum Ferritin, HbA1c, Nitric oxide, Uric Acid levels in type 2 Diabetes Mellitus

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Received: 21-05-2021 / Revised: 19-06-2021 / Accepted: 20-07-2021

Abstract

Background: Diabetes mellitus (DM) is a commonest metabolic disorder affecting the people all over the world. There is growing evidence that increased iron stores in the body leads to developmentof glucose intolerance, type 2 DM and insulin resistance syndrome. Several studies have shown that development of endothelial dysfunction is the major cause for vascular complications in type 2 DM subjects and altered bioavailability of serum nitric oxide is found be the underlying cause for endothelial dysfunction in diabetic subjects. **Objectives:** To estimate fasting plasma glucose, serum ferritin, serum nitric oxide, $HbA_{1c}$and uric acid levels in type 2 DM subjects and compare the values with healthy controls and to do the correlation analysis between these biochemical parameters in type 2 DM subjects. **Materials and methods:** A case control study is conducted in a total of 56 diagnosed type 2 DM subjects and 31 healthy controls. Fasting plasma glucose, serum ferritin,serum nitric oxide, HbA_{1c}, and serum uric acid are estimated in all cases and control group. **Results:** Intergroup comparison of biochemical parameters was done by unpaired "t" testand correlation between parameters by Pearson coefficient analysis. In DM subjects, mean values of serum ferritin, serum nitric oxide, HbA_{1c}, and serum uric acid were found to be significantly increased ($p<0.001$) when compared to controls. Moreover, Serum ferritin has shown significant positive correlation with $HbA_{1c}$and serum nitric oxide in type 2 DM patients with „p" value of <0.05. **Conclusion:** 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 over load in type 2 DM subjects which may occur through many ways. Decreasing iron stores may reduce the oxidative stress, improve the vascularendothelial dysfunction andalso improves insulin sensitivity in type 2 DM subjects.

Keywords: HbA_{1c}, Serum Ferritin, Serum Nitric Oxide, Serum Uric acid andType 2 Diabetes Mellitus.

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, actingjointly. The underlying cause of diabetes is the defective production or action of insulin, a hormone that controls glucose, fat and amino acid metabolism.[1]Characteristically, diabetes is a long term 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 is found that increased body iron stores are associated with the development of glucose intolerance, gestational diabetes, and type 2 DM and insulin resistance syndrome. There is the evidence that frequent blood donation leads to 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 the drop in serum glucose, cholesterol and triglycerides. It is also found that patients with uncontrolled diabetes have hyperferritinemia which correlated 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].

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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. Iron is stored in the form of ferritin and only 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 minute quantity of ferritin is present in the serum and this concentration is proportional to the body iron stores.HbA1c is glycated hemoglobin formed by a post translational non enzymatic, substrate concentration dependent, irreversible process of combination of aldehyde group of glucose and other hexose with the amino terminal valine of the beta chain of hemoglobin. The levels of HbA_{1c}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, non-cholinergic 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].

Serum uric acid is produced by Xanthine Oxidase from xanthine and hypoxanthine, which in turn are produced from purine. It is a strong reducing agent and in human, over half of the antioxidant capacity of blood comes from serum uric acid[8].

Serum uric acid level is found to be increased with increasing HbA_{1c}levels up to the category 6-6.9% and there after decreases with further increase in HbA_{1c}levels (bell shaped relation)[9]. Hyperuricemia found to be associated with insulin resistance and components of metabolic syndrome. It is also a predictor of

cardiovascular disease in type 2 diabetes.

Objectives

1. To estimate the levels of serum ferritin, HbA_{1c}, serum nitric oxide, serum uricacid in Type 2 diabetic patients and compare it with control subjects.
2. To study the correlation between levels of serum ferritin as a marker of iron overload andHbA_{1c}in type 2 diabetic patients.
3. To study the correlation between serum nitric oxide levels as a marker of endothelial dysfunction and serum ferritin levels in Type 2 diabetic patients.
4. To study the serum levels of uric acid as an inflammatory marker, antioxidant incorrelation to $HbA_{1c}$and serum ferritin in Type 2 diabetic patients.

Methodology

Source of Data

A cross sectional study to compare the levels of fasting plasma glucose, serum ferritin, serum nitric oxide, $HbA_{1c}$and uric acid in type 2 diabetes mellitus patients with that of healthy controls was carried out from June 2019 to June 2020. Type 2 diabetes mellitus patients were selected from MGM General Hospital, Warangal (Hospitals was attached to Kakatiya . Medical College, Warangal) and healthy controls from the general population. Written informed consent was obtained from each subject before starting the study. Institutional Ethical Committee of Kakatiya Medical College, Warangal approval was taken before staring the study. Patients and controls voluntarily participated in the study.

Inclusion criteria

Cases-56 clinically diagnosed type 2 diabetesmellitus between the age group of 30-60yrs of either sex with 5years history of diabetes were included in the present study.

Controls

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

Exclusion criteria

Patients with the following diseases were excluded from the study,

- Patients with Type 1 Diabetes Mellitus
- Patients with Gestational Diabetes Mellitus, Hemochromatosis, Thalassemia andHemosiderosis.
- Patients on iron supplementation, thiazide diuretics, antioxidants drugs andsteroids.
- Patients with chronic infections and inflammation, neoplasia, renal disease, liverdisease, alcoholics and smokers.
- Critically ill patients admitted in intensive care unit.

A. Collection of blood sample

About 6 ml of venous blood was drawn from all the subjects (from large peripheralvein) 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, nitric oxide and uric acid and out of 3 ml of anticoagulated whole bloodsample, 1 ml of whole blood was used to estimate HbA1c and 2ml was used to separate plasma to estimate fasting plasma glucose.

Parameters measured

From the blood sample collected by the above method, following parameters were measured in the present study.

1. Fasting plasma glucose
2. Glycated hemoglobin
3. Serum ferritin
4. Serum nitric oxide
5. Serum uric acid.

Estimation of fasting plasma glucose by Glucose oxidase (GOD-POD) method.

Estimation of serum ferritin by Method Chemiluminescence Immunoassay (Lilac Acculite CLIA). Estimation of serum nitric oxide (NO) by Kinetic Cadmium-Reduction method. Estimation of serum uric acid by uricase method. Estimation of glycated hemoglobin (HbA_{1c}) by turbidimetricimmunoassay[9-13]

Statistical analysis

Unpaired „t"test is used to compare the biochemical parameters between cases and controland Pearson"s correlation analysis is used to find the correlation between the biochemical parameters in cases. Statistical analysis is being done by SPSS version 16 and $p< 0.05$ is considered as statistically significant and $p< 0.001$ is taken as highly significant.

Results

A total of 87 subjects were included in the present study of which 56 type 2 Diabetes Mellitus patients and31 control subjects. Among 56 DM patients, 24 were male and 32 were female. Similarly in control group 19 were males and 12 were females.

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 ± SD	50.09±8.2	45.64±12.1	p=0.07 NS
Gender Male	24 (42.9%)	19(61.3%)	p=0.10 NS
Female	32 (57.1%)	12(38.7%)	

Table 2 shows the demographic distribution of cases and controls included in the study. Mean age of 50.09±8.2 years in type 2 diabetes mellitus patients and 45.64±12.1 years in controls were found. Sex matched controls were selected and there was no significant difference between cases and controls with respect to age and sex of subject(p value>0.05taken as not significant).

Table 2: Levels (mean ± SD) of FPG(fasting plasma glucose), Serum Ferritin, SerumNitric Oxide, HbA_{1c}, Uric Acid in patients with type 2 diabetes mellitus and healthy controls.

Variables		Cases	Controls	Control/Cases		
				MeanDiff	t value	p value
FPGmg/dl	mean±SD	179.5±53.2	98.06±7.28	83.44	11.36	<0.001**
	Range	97-288	78-106			
Serum Ferritinng/ml	mean±SD	457.9±402.2	84.6±36.8	373.90	6.89	<0.001**
	Range	34.4-1745	12.3-162.2			
Serum Nitric Oxide µmol/l	mean±SD	100.9±26.5	39±4.7	61.90	17.03	<0.001**
	Range	38.4-146.0	30.1-45.8			
HbA_{1c}	mean±SD	9.49±1.90	5.46±0.84	4.03	13.64	<0.001**

	Range	4.80-13.51	4.10-6.40			
Serum uric acid mg/dl	mean±SD	8.57±2.38	4.72±1.41	3.86	9.49	<0.001**
	Range	3.92-17.50	1.96-6.6			

Table 2 Shows levels (mean □ SD) of various biochemical parameters in type 2 diabetic patients and healthy controls.The estimated mean levels (mean □ SD) of FPG, Serum Ferritin, Serum Nitric Oxide, HbA_{1c},Uric Acidin control group were 96.06±7.28, 84.6±36.8, 39.0±4.7, 5.46±0.84 and 4.72±1.41 respectively.Similarly in type 2 diabetic patients mean levels of 179.5±52.2, 457.9±402.2, 100.9±26.5, 9.49±1.90, and 8.57±2.38 were obtained for respective parameters.

The statistical analysis by Unpaired „t"-test shows that the levels of Fasting plasma Glucose, Serum Ferritin, Serum Nitric Oxide, HbA_{1c}, Uric Acid are significantly increased in type 2 diabetic patients when compared to controls with p value of <0.001(statistically highly significant).

Discussion

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[14]. 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 changesobserved in diabetic vascular complications[15].

The biochemical process of advanced glycation appears to be enhanced in thediabetic milieu as a result of hyperglycemia, oxidative stress and lipid peroxidation. A heterogeneous group of chemical moietiesare generated that appears to induce the development and progression of diabeticvascular complications directly or indirectly via activation of intracellular signaling pathways, generation of proinflammatory and prosclerotic cytokines and various pathological processes[16].

The aim of this study is to evaluate iron overload status,endothelial dysfunction, antioxidant and long term glycemic control status by measuring serum ferritin, serum nitric oxide levels, serum uric acid levels and HbA_{1c}in cases of type 2 DM and compare it with healthy controls.In the present study a total of 87 subjects, of which 56 type 2 diabetes mellitus patients and rest 31 healthy controls were included. The fasting plasma glucose, serum ferritin, serum nitric oxide , HbA_{1c}, serum uric acid 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 179.5±53.2. Statistical analysis by Unpairedstudent"s t-test has shown that the level of FPG in type 2 diabetic patients wassignificantly increased as compared to controls ($p < 0.001$).In diabetes mellitus chronic hyperglycemic state is known to be responsible for increased oxidative stress resulting in complications. The 4 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[17].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 liver, and glucose uptake into muscle and adipose tissue is reduced drasticallyleading to hyperglycemia[18].

Serum Ferritin

It is increasingly recognized that iron influences glucose metabolism, even in the absence of significant iron overload. Excess oftissue iron amplify the injury caused by free radicals as well as to modulate various steps involved in the inflammatory lesion.

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[19,20]

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 statistically significant positive correlation between serum ferritin with serum nitric oxide, HbA1c, and Uric acid.This 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 arein accordance with previous studies conducted by. Fourohi et al.[21]

Nitric oxide (NO)

In our study, the serum NO level was significantly increased ($p<0.001$) in type 2 diabetes mellitus patients as compared to healthy controls. The correlation analysis between serum nitric oxide with serum ferritin and $HbA_{1c}$was found to be positively correlated and is statistically significant.This finding is in accordance with studies of Dilshad Ahmed Khanet al[7]

Glycated Hemoglobin (HbA_{1c})

In our study HbA_{1c}levels were significantly increased in type 2 diabetes mellitus patients with mean value of 9.49±1.90 when compared to healthy controls having mean value of 5.46±0.84 ($p<0.001$). And correlation between HbA_{1c}, serum ferritin, and serum nitric oxide levels has shown a statistically significant positive correlation. However, correlation between $HbA_{1c}$and uric acid levels was found statistically non significant. These data are in accordance with previous studies done by Selvin et al[22].

Uric acid

In our study mean serum uric acid levels of 8.57 ±2.38 and 4.72 ±1.41 were noted in type 2 diabetes mellitus and healthy controls respectively.This difference in mean values were found to be statistically significant with p value (<0.05) and the Pearson"s correlation analysis between serum uric acid with $HbA_{1c}$and serum ferritin showed a positive correlation with r value +0.25 and +0.21 respectively. But correlation was not found statistically significant with $p >0.05$.These results are in accordance with studies done by Woo Yoo et al[23].

Conclusion

Diabetes mellitus is a complex and multifactorial disease characterized by absolute or relative deficiencies of insulin secretion and/or insulin action. DM is associated with chronic hyperglycemia and disturbances inthe carbohydrate, lipid and protein metabolism**.**

Chronic hyperglycemia leads to oxidative stress. Endothelial dysfunction is the major cause for the disease pathogenesis and progression in type 2 diabetes mellitus.

An excess iron store found to be the source for oxidative stress. Endothelial dysfunction in type 2 diabetes subjects is the major cause for development of vascular complications. Impaired Nitric oxide bioavailability, caused by an increased breakdown by reactive oxygen species lead to atherosclerotic changes in the blood vessels.

The results of the present study support the concept that increase in fasting plasma glucose, glycated hemoglobin (HbA_{1c}), serum ferritin, serum nitric oxide and serum uric acid plays an important role in the pathogenesis of type 2 diabetes mellitus.

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 when compared to healthy controls showing a state of iron overload in DM patients. Serum ferritin a marker of iron over load, leads to oxidative stress through Fenton reaction. Increase in the serum nitric oxide levels in DM patients can lead to endothelial dysfunction due to uncoupling of endothelium derived Nitric Oxide Synthase enzyme. This excess of nitric oxide is utilized for the free radical generation which may lead to atherosclerotic changes and complications in

diabetes. Increase in HbA_{1c}level is the main risk factor for the development and progression of disease in type 2 DM. Uric acid is a strong antioxidant and free radical scavenger. Increase in uric acid level in DM patients reflects a compensatory mechanism to counter increased oxidative stress.

There is a need to prevent iron over load in type 2 diabetes mellitus subjects which can occur through many ways like excessive iron supplementation, frequent blood transfusions as a measure to prevent and treat anemia. Decrease in dietary iron intake decreases 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 ensue adequate levels of antioxidant nutrients to the body to resist oxidative stress. Fiber rich green leafy vegetables are easily available and affordable also.

Limitations of the study

Limitation of our study is that we could not detect variations of these parameters in relation to the microvascular and macrovascular complications of DM.

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

Acknowledgment

The author is Thankful to Department of Biochemistry for providing all the facilities to carry out this work.

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Conflict of Interest: Nil

Source of support: Nil