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Original Research Article

A Study on Antioxidants and Iron nutritional status in Type 2 Diabetes Mellitus with and without Coronary Heart Disease

Veerabhadra Goud G. K[*1], Sudha Patil[2] and M.A. Rahman[3]

[1]*Department of Biochemistry, Akash Institute of Medical Sciences & Research Centre, Bangalore, India.*
[2]*Department of Obstetrics & Gynaecology, Akash Institute of Medical Sciences & Research Centre, Bangalore, India.*
[3]*Department of Biochemistry, SVS Medical College, Mahabubnagar, Telangana, India.*

*Correspondence Info:
Dr. Veerabhadra Goud G.K, MD. Biochemistry
Assistant Professor
Department of Biochemistry
Akash Institute of Medical Sciences & Research Centre, Bangalore, India.
E-mail: veerabhadrag2006@gmail.com

Abstract

Background: Diabetes mellitus is an endocrinal disorder. Patients with type 2 diabetes are at high risk for several cardiovascular disorders: coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy and congestive heart failure. Increased levels of MDA, Vitamin E and Ceruloplasmin are the markers for oxidative stress in type 2 diabetes mellitus. Studies suggest that magnesium, chromium, calcium and iron may have a role in Insulin Resistance or Diabetes Mellitus. The aim of the present study is to study the antioxidant and iron nutritional status in type 2 diabetes mellitus with and without coronary heart disease.

Materials & Methods: This, case control study was conducted on 60 subjects. Among them 20 were controls, 20 were known Type 2 Diabetes Mellitus patients and 20 were Type 2 Diabetes Mellitus patients with coronary heart disease. Patients with T2DM with other endocrinal disorders, patients on antioxidants, minerals and multivitamins as supplement form are excluded from the study. A laboratory investigations consisting of glucose, total cholesterol, triglycerides, HDLC, LDLC, Vitamin E, MDA, Ceruloplasmin, Ferritin, Iron, TIBC and Transferrin were carried out on fasting blood sample of subjects selected for this study.

Results: In the present study, FBS, PPBS, total cholesterol, LDL-Cholesterol, VLDL-Cholesterol and antioxidant parameters such as MDA, Ceruloplasmin and ferritin were significantly increased in T2DM patients and T2DM patients with coronary heart disease when compared to controls. Vitamin E and HDL cholesterol was significantly decreased. Iron, TIBC and Transferrin did not show significant difference. (p value <0.05).

Conclusion: The findings of this study is in accordance to earlier studies, that there is dyslipidemia, increased lipid peroxidation, inflammation and oxidative stress in diabetics compared to non diabetics; and the oxidative stress further increases as diabetes to cardiovascular diseases. These observations suggest that supportive therapy aimed at the reduction of dyslipidemia and oxidative stress may prevent the development and progression of vascular complications, responsible for the increased mortality and morbidity associated with type 2 diabetes mellitus. Future large prospective studies are recommended.

Keywords: Antioxidants, Coronary Heart Disease, Dyslipidemia, Ferritin, MDA, Type 2 Diabetes Mellitus

1. Introduction

India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken. [1] In 2012, an estimated 1.5 million deaths were directly caused by diabetes. More than 80% of diabetes related deaths occur in low- and middle-income countries. WHO projects that diabetes will be the 7th leading cause of death in 2030. [2]

Type 2 diabetes mellitus involves progressive development of insulin resistance and a relative deficiency in insulin secretion due to β-cell dysfunction, causing hyperglycaemia. Symptoms are often less marked delaying the diagnosis of the disease to several years after the disease onset. [2] Patients with type 2 diabetes are at high risk for several cardiovascular disorders: coronary heart disease, stroke, peripheral arterial disease, cardiomyopathy and congestive heart failure.

Type 2 diabetes mellitus causes deregulation of carbohydrate, protein and fat metabolism with excess production of free radicals. The main lipid abnormalities in type 2 diabetes are hyper-triglyceridemia, hyper-cholesterolemia and low HDL. Low plasma HDL level is a powerful risk factor of coronary heart disease. [2]

Dyslipidemia contributes to the rate of progression of atherosclerosis. Reactive oxygen species (ROS) are constantly formed in the human body under physiological as well as under pathological conditions. The action of these free radical is antagonized by the enzymatic and non-enzymatic antioxidant defense system [3].

Oxidative stress, secondary to persistent hyperglycaemia and dyslipidemia plays a key role in the pathogenesis of T2DM and its complications by excess ROS generation, auto-oxidation of glucose, non enzymatic protein glycosylation, lipid peroxides formation, impaired glutathione metabolism, impaired activities of antioxidant defence enzymes and decreased concentrations of low molecular weight antioxidants such as ceruloplasmin and uric acid. [4]

Malondialdehyde, as TBARS (ThioBarbituric Acid Reacting Substances), is frequently used to determine the pro-oxidant/antioxidant balance in type 2 diabetic patients as they are stable and easily measurable lipid peroxidation products. Ceruloplasmin acting as ferroxidase decreases the availability of the iron in free radical generating reactions [5]. Considering the pro-oxidant status of patients with T2DM, an increase in the level of Ceruloplasmin probably favours its protective action against free radical injury. Alternatively, an increase in serum Ceruloplasmin in type 2 diabetes could generate excess oxidized LDL, which causes atherosclerosis. It could also cause vascular injury by generating free radicals, such as hydrogen peroxide, in the course of oxidization of serum homocysteine. [6]

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. [7]

Vitamin E is one of the most important lipid-soluble antioxidants. Its function is to protect the integrity of membranes by inhibiting lipid peroxidation. Low plasma level of this anti-oxidant vitamin is associated with a 3.9-fold elevated risk of developing the disease. It has been suggested that vitamin E improves insulin sensitivity. The aim of the present study is to study the antioxidant and iron nutritional status in type 2 diabetes mellitus with and without coronary heart disease.

2. Materials & Methods

This study is a cross sectional case-control study, conducted in the Department of Biochemistry in association with Department of Medicine in Sri Venkata Sai Medical College, Yenugonda, Mahabubnagar, Telangana. The study was conducted on 60 subjects among them 20 were controls, 20 were known Type 2 Diabetes Mellitus patients and 20 were Type 2 Diabetes Mellitus with coronary heart disease. Patients with Type 2 Diabetes Mellitus with other endocrinal disorders, patients on antioxidants, minerals and multivitamins as supplement form are excluded from the study. A fasting (12 hours) venous blood sample (5ml) was drawn from the patients and controls into a sterile disposable syringe which was transferred into centrifuge tubes and was allowed to clot for 30 minutes. The sample was centrifuged at 3000 rpm for 10 minutes and the separated serum was used for the estimation of glucose (GOD-POD method), total cholesterol (CHOD-PAP method), TGL (GOP-PAP method), HDLC (CHOD-PAP method), LDLC (Enzymatic Colorimetric Assay), vitamin E (Baker's and Frank's method), MDA (Thiobarbituric acid), Ceruloplasmin (Copper oxide activity method), Ferritin (Immuno Enzymatic Assay) and Iron & TIBC (Ferrozine Method). Well designed proforma was prepared and patient's history was taken.

2.1 Statistical Analysis

Data were expressed as mean ±SD. P value <0.05 is considered as statistically significant. Statistical analysis was performed using Graph pad calculator software.

3. Results

In the present study, total of 60 subjects were included. 20 were Type 2 Diabetes Mellitus patients and 20 were Type 2 Diabetes Mellitus patients with Ischemic Heart Disease. Their ages ranging between 35 to 70 years. 20 age and sex matched healthy subjects were taken as controls. In this study, FBS, PPBS, total cholesterol, LDL-Cholesterol, VLDL-Cholesterol and antioxidant parameters such as MDA, Ceruloplasmin and ferritin were significantly increased in T2DM patients and T2DM patients with ischemic heart disease when compared to controls. Vitamin E and HDL cholesterol was significantly decreased. Iron, TIBC and Transferrin did not show significant difference, as illustrated in table 1 and 2.

Table 1: Control with Type 2 Diabetes Mellitus

Parameter	Controls (Mean±SD)	Cases (Mean±SD)	p-value
Fasting blood glucose (mg/dl)	88.6±6.51	220.60±92.69	0.0001*
Postprandial blood glucose (mg/dl)	126.75±11.19	284.70±104.06	<0.0001*
Total Cholesterol (mg/dl)	164.5±6.84	207.25±60.96	0.0035
Triglycerides (mg/dl)	134.80±12.22	153.20±74.24	0.2936
HDLC (mg/dl)	46.75±4.2	36.05±8.65	0.0001*
LDLC (mg/dl)	90.80±8.08	136.45±43.03	0.0071
VLDLC (mg/dl)	26.95±2.50	40.60±21.32	0.0071
MDA (nmol/l)	250.40±16.68	438.85±86.74	0.0001*
Vitamin E (mg/dl)	1.400±0.241	0.595±0.393	0.0001*
Ceruloplasmin (mg/dl)	31.05±4.33	37.35±7.86	0.0033
Ferritin (ngm/ml)	66.85±17.77	230.35±102.76	0.0001*
Iron (mcg/ml)	91.85±7.20	82.00±25.87	0.1092
TIBC (mcg/dl)	317.65±17.65	351±123.54	0.2394
Transferrin (mcg/dl)	222.30±12.30	253.30±90.58	0.1376

Table 2: Controls and Type 2 Diabetes with IHD

Parameter	Controls (Mean±SD)	Cases (Mean±SD)	p-value
Fasting blood glucose (mg/dl)	88.6±6.51	163.0±24.42	0.0001*
Postprandial blood glucose (mg/dl)	126.75±11.19	223.65±50.17	<0.0001*
Total Cholesterol (mg/dl)	164.5±6.84	194.60±49.99	0.0112
Triglycerides (mg/dl)	134.80±12.22	178.9±59.55	0.0025
HDLC (mg/dl)	46.75±4.2	36.05±9.13	0.0001*
LDLC (mg/dl)	90.80±8.08	94.10±30.84	0.6460
VLDLC (mg/dl)	26.95±2.50	37.20±10.80	0.0002
MDA (nmol/l)	250.40±16.68	533.40±51.34	0.0001*
Vitamin E (mg/dl)	1.400±0.241	0.325±0.091	0.0001*
Ceruloplasmin (mg/dl)	31.05±4.33	44.10±3.61	0.0001*
Ferritin (ngm/ml)	66.85±17.77	229.70±33.18	0.0001*
Iron (mcg/ml)	91.85±7.20	89.00±18.89	0.9667
TIBC (mcg/dl)	317.65±17.65	366±103.23	0.0458
Transferrin (mcg/dl)	222.30±12.30	252.40±73.17	0.0775

4. Discussion

Diabetes mellitus is a complex and multifactorial disease, indulging severe insulin dysfunction in conjunction with gross abnormalities in glucose homeostasis, lipid and protein metabolism. It contributes for macrovascular and microvascular complications in diabetes. Of all cardiovascular cases are the leading causes of mortality and morbidity in diabetes mellitus.[8]

In the present study, MDA levels were significantly increased in cases of T2DM, as well as T2DM with CHD when compared to controls. MDA is marker for lipid peroxidation. Our results are in accordance with previous studies. [9-11]

In the present study, Ceruloplasmin levels were significantly increased in cases of T2DM as well as T2DM with ischemic heart disease when compared to controls. Increased levels of Ceruloplasmin indicating the presence of inflammation. Ceruloplasmin acts as a ferroxidase and decreases the availability of the iron in free radical generating reactions.[12] An increase in the level of Ceruloplasmin provides protective action against free radical injury. [13] Alternatively, an increase in serum Ceruloplasmin in type 2 diabetes could generate excess oxidized LDL, which causes atherosclerosis. [14] It could also cause vascular injury by generating free radicals, such as hydrogen peroxide, in the course of oxidation of serum homocysteine. [15]

In the present study, vitamin E levels were significantly decreased in T2DM as well as in T2DM patients with CHD when compared to controls. Vitamin E is one of the most important lipid-soluble antioxidants. Its function is to protect the integrity of membranes by inhibiting lipid peroxidation.

In the present study, no significant difference has been found in serum iron levels in T2DM as well as CHD as compared to controls. Our results are contrary to M. C. Thomas (2004), found increased iron indices in T2DM patients and evolved hypothesis that excess iron to have a role in development of DM and subsequently in glycemic control. But Ying-Hwa Chen (2008), found no significant differences in serum iron levels in T2DM as well as T2DM with CHD compared to controls. [16, 17]

In the present study, serum ferritin levels were significantly increased in T2DM as well T2DM patients with CHD compared to controls. This is in accordance with M.C. Thomas (2004), found elevated ferritin levels in T2DM. Excess circulatory free iron might be serving as a catalyst for

lipid and protein oxidation and the formation of reactive oxygen species which may be the cause of increase ferritin levels.

5. Conclusion

The findings of this study is in accordance to earlier studies, that there is dyslipidemia, increased lipid peroxidation, inflammation and oxidative stress in diabetics compared to non diabetics; and the oxidative stress further increases as diabetes to cardiovascular diseases. These observations suggest that supportive therapy aimed at the reduction of dyslipidemia and oxidative stress may prevent the development and progression of vascular complications, responsible for the increased mortality and morbidity associated with type 2 diabetes mellitus. Future large prospective studies are recommended. Limitations of the study are small sample size.

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