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Original Research Article

Association of fibroblast growth factor 21 with oxidative stress and lipid profile in type 2 diabetes

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ABSTRACT

Background: Cardiovascular disease is the most prevalent cause of morbidity and mortality in diabetic patients. Fibroblast growth factor 21 (FBG 21) is an endocrine factor that regulates glucose and lipid metabolism, insulin resistance, and obesity. Blood levels of FGF21 are elevated in patients with atherosclerosis, macrovascular, and microvascular complications of diabetes, possibly due to a compensatory up regulation. Studies reported that FGF21 is an important regulator of mitochondrial and oxidative stress. The role of FGF21 in chronic diseases and the diminished oxidative stress observed with anti-diabetic therapy has been the target of new studies. Current study aimed to evaluate serum FGF21 levels and its association with oxidative stress and lipid profile levels in type 2 diabetic patients.

Methods: 100 controls and 100 diabetic patients on oral hypoglycemic drugs between 35-55 years of age without any cardiac, renal, liver, and thyroid dysfunction were selected for this study. Oxidative stress (MDA), total antioxidant status (FRAP), and FGF21 were measured. FGF21 was analyzed by ELISA methods. Serum MDA was assessed by the method of Yagi serum total antioxidant status was measured by the method of Benzie et al.

Results: FGF21 level was increased in diabetic patients compared with controls. There was a significant positive correlation of FGF21 with MDA ($r=0.875$, $p<0.01$) and negative correlation with FRAP observed ($r= -0.867$ $p<0.01$). There was also positive correlation of FGF21 with total cholesterol ($r=0.499$, $p<0.01$), triglycerides ($r=0.648$, $p<0.01$), LDL-cholesterol ($r=0.337$, $p<0.01$) and negative correlation with HDL-cholesterol ($r= -0.172$, $p<0.05$) were observed.

Conclusions: Increased oxidative stress and decreased antioxidant status were observed in diabetics. This could be due to dyslipidemia and increased generation of free radicals. High levels of FGF21 observed in our study might represent its resistant state and the compensatory response to maintain metabolic homeostasis. Further studies are needed to explore the role of FGF21 as a novel marker in predicting cardiovascular risk in diabetic patients.

Keywords: Fibroblast growth factor, Malondialdehyde, Lipid profile, Type 2 diabetes mellitus

INTRODUCTION

Cardiovascular disease is a well known cause of mortality in diabetic patients. Several studies have indicated that diabetic cardiomyopathy (DCM) characterized by impaired cardiac structure and function is considered to be associated with oxidative stress and mitochondrial dysfunction.[1] Dyslipidemia is more common in type 2 diabetes mellitus (T2DM) and is typically characterized by high levels of triglycerides and small dense low-density lipoprotein (sdLDL) cholesterol particles, in combination with low levels of high-density lipoprotein

(HDL) cholesterol. Factors such as visceral adiposity, insulin resistance, and excess FFAs play a major role in the development of the lipid abnormalities.[2]

Oxidative stress is caused by an imbalance between free radicals production and elimination thus producing alterations in cellular metabolism.[3] Many chronic diseases developed from increased intracellular oxidative stress.[4] ROS can be formed during reactions by NADPH oxidases, xanthine oxidases, and lipoxygenases and mitochondrial oxidative phosphorylation.[5] High glucose levels can stimulate free radical production. Oxidative stress resulting from hyperglycemia initiate the progression to atherosclerosis and producing micro and macrovascular complications.[6] Chronic oxidative stress can destroy pancreatic β-cells due to lower amount of anti-oxidant enzymes.[7]

Fibroblast growth factor 21 (FGF21) produced in peripheral tissues have anti-inflammatory effect and also increases fatty acid oxidation and improving insulin sensitivity.[8] FGF21 may also lead to development of coronary heart disease.[9] Increased levels of FGF21 have been found in type 2 diabetes, metabolic syndrome.[10]

A study reported that cultured endothelial cells treated with oxidized low-density lipoproteins, resulted in an increased FGF21 mRNA expression and protein concentration.[4] FGF21 deficiency leads to impaired glucose tolerance, elevated blood insulin, and fatty liver development.[11] FGF21 deficiency enhanced the development of diabetic cardiomyopathy.[12] Fibroblast growth factor 21 exerts its cardiovascular protective activity through adiponectin, and sterol regulatory element binding protein 2 (Srebp-2). The amount and activity of brown adipocytes are inversely correlated with cardiovascular disease.[13] So the objective of the present study was to evaluate the serum FGF21 levels and its association with oxidative stress and lipid profile parameters in type 2 diabetes mellitus patients.

METHODS

Current study is a case control study. The experiments were conducted from August 2019 to January 2020 at Rajah Muthiah medical college and hospital, Annamalai University, Chidambaram, Tamil Nadu, India. Written informed consent was obtained from all subjects after clearly explaining the nature, and purpose of the study.

One hundred type 2 diabetic patients on oral hypoglycaemic drugs, in the age group 35-55 years without any other systemic diseases were selected for our study. Diabetes is confirmed based on fasting and postprandial blood glucose levels by WHO norms. ECG was done to rule out the cardiac problem. 100 control subjects in the same age group were selected for this study. Fasting blood samples were collected from study subjects. Blood samples were centrifuged at 3000 x g for 10 min. The routine investigations, glucose, lipid profile (total cholesterol, HDL, LDL, triglycerides) were carried out by ERBA EM-360 fully automated analyzer. Serum was separated and kept in a deep freezer at -20°C for a month and analysed for malondialdehyde (MDA), FRAP and FGF21. Oxidative stress parameter malondialdehyde (MDA) was estimated by Yagi et al method.[14] Total antioxidant status ferric reducing antioxidant power assay (FRAP) by Benzie et al method.[15] FGF21 was analyzed by enzyme linked immunosorbent assay (ELISA).

Statistical analysis

The data were expressed as mean±SD. Statistical analysis was carried out by SYSTAT. The comparison of parameters in the study groups was done by the student's'‘t’ test, while the correlation was determined by Pearson's correlation coefficients. $p<0.001$ and $p<0.05$ indicated statistical significance.

RESULTS

The baseline parameters in control and diabetic patients are shown in (Table 1). Baseline parameters like height, weight, BMI, waist-hip ratio, systolic BP, Diastolic BP were within the normal range in the control and diabetic group.

Table 1: Baseline parameters in control and type 2 diabetic patients.

Parameters	Control N=100, mean±SD	Type 2 diabetic patients, N=100 mean±SD	P value
Age (year)	45.53±5.33	46.2 ±5.24	0.365
Height (cm)	163.90±6.43	169.77±7.40	0.001
Weight (cm)	62.17±6.31	76.21±7.12	0.001
BMI	23.17±2.54	26.48±2.24	0.001
Waist hip ratio	0.895±0.02	0.916±0.02	0.001
Systolic BP (mmHg)	113.94±5.08	126.22±8.97	0.001
Diastolic BP (mmHg)	76.38±3.0	79.88±3.96	0.001

The FBS, PPBS, and HbA1C in controls and diabetes are shown in (Table 2). There was an increase in the level of FBS, PPBS, and HbA1C in diabetic patients compared to control statistically significant subjects ($p<0.001$).

The levels of serum cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol are shown in (Table 3). The level of serum cholesterol, triglycerides, and LDL cholesterol were increased in diabetics compared to controls and statistically significant ($p<0.01$). HDL

cholesterol was within the normal range in both control and diabetic patients.

There was a statistically significant increase in the level of MDA in diabetics compared to control ($p<0.001$) (Table 4). Serum levels of total antioxidant status (FRAP) were decreased in diabetics in comparison with controls ($p<0.001$). Serum FGF-21 level was statistically increased in diabetics compared to controls ($p<0.001$).

Table 2: FBS, PPBS and HbA1c in control and type 2 diabetic patients.

Parameter	Control N=100 mean±SD	Type 2 Diabetic patients N=100 mean±SD	P value
FBS (mg/dl)	94.64±9.14	155.14±33.93	0.001
PPBS (mg/dl)	115.29±10.74	252.14±44.20	0.001
HbA1C (%)	5.87±0.30	8.29±0.51	0.001

Table 3: Lipid profile parameters in control and type 2 diabetic patients.

Parameter	Control N=100 mean±SD	Type 2 Diabetic patients N=100 mean±SD	P value
Total cholesterol (mg/dl)	176.34±12. 97	202.38±26.97	0.001
Triglyceri- des (mg/dl)	99.31 ±15.42	155.44±30.19	0.001
HDL (mg/dl)	39.75±3.94	38.27±3.20	0.004
LDL (mg/dll)	106.20±16. 36	136.17±29.46	0.001

Table 4: Oxidative stress marker, total antioxidant status and FGF21 in control and type 2 diabetic patients.

Parameters	Control N=100 mean±SD	Type 2 Diabetic patients N=100 mean±SD	P value
MDA (nmol/l)	1.89±0.19	9.20±0.46	0.001
FRAP (µmol/l)	514±15.22	336.28±14.21	0.001
FGF21 (pg/ml)	287.04±69.14	515.30±55.09	0.001

FGF-21 and MDA are positively correlated and FGF-21 and FRAP are negatively correlated as shown in (Table 5). Serum FGF-21 positively correlated with total cholesterol, triglycerides, serum LDL cholesterol, and negatively correlated with HDL cholesterol (Table 6).

Table 5: Correlation between FGF21, oxidative stress and total antioxidant status parameters.

Parameters	Pearson correlation coefficient (r)	P value
FGF21 versus MDA	0.875	0.01
FRAP	-0.867	0.01

Table 6: Correlation between FGF21 and lipid profile parameters.

Parameters	The Pearson correlation coefficient (r)	P value
FGF21 versus total cholesterol	0.499	0.01
Triglycerides	0.648	0.01
HDL	-0.172	0.05
LDL	0.337	0.01

DISCUSSION

Obesity increases the risk of many diseases including T2DM.[16] Current study showed a high BMI in diabetics compared to controls. Waist hip ratio was increased both in control and diabetic patients. BMI does not reflect body fat distribution, whereas the WHR as a measure of central adiposity is a major contributor to the development of diabetes, hypertension, insulin resistance, and dyslipidemia.[17] Serum cholesterol, triglycerides, and LDL levels were high normal in diabetic patients compared to the control group. Dyslipidemia in individuals with type 2 diabetes is very common, with a prevalence of 72-85%. This is associated with a significantly increased risk of coronary artery disease compared to individuals without diabetes.[18] Increased triacylglycerols and reduced HDL cholesterol are the main lipid abnormalities of diabetic dyslipidemia.[19]

FGF21 is a novel polypeptide ligand that has been shown to play a pivotal role in the regulation of glucose homeostasis and lipid metabolism. Increased FGF21 blood levels have been observed in mouse models of obesity associated diseases, such as non alcoholic fatty liver disease, chronic hyperglycaemia, and atherosclerosis, FGF21 levels are also increased in human population with many chronic diseases associated with atherogenic lipid profile.[20]

In our study, blood FGF21 levels are significantly increased in T2DM patients compared to controls. There was a positive correlation of FGF21 with total

cholesterol, LDL cholesterol, triglycerides, and negative correlation with HDL cholesterol. High serum FGF21 levels correlated positively with metabolic disorders like obesity, diabetes, mitochondrial diseases, and aging.[21] Several studies have proved that FGF21 stimulates the oxidation of fatty acids, the formation of ketone bodies, and the inhibition of lipogenesis.[22] FGF21 regulates glucose lipid metabolism thus proved to be a therapeutic target for the metabolic disease.[22]

Studies have found that rFGF21 treatment suppressed serum levels of low density lipoprotein (LDL), cholesterol, triglyceride, and free fatty acid (FFA) and enhanced the level of high-density lipoprotein (HDL) in addition to reduced body weight.[23] Previous studies have shown elevated blood FGF21 levels in impaired glucose tolerance and diabetes. It has also been observed in recent studies, that prevention of myocardial injury and apoptosis after ischemia-reperfusion occurs via FGFR1/β klotho/akt signalling cascade in cardiomyocytes.[24]

MDA is formed during lipid peroxidation after the rupture of the carbon chain of unsaturated fatty acids.[13] In our study MDA level was increased in diabetics compared to control and statistically significant ($p<0.001$). Total antioxidant status (FRAP) was decreased in diabetics in comparison with controls ($p<0.001$). There was a positive correlation of FGF21 with TBARS ($r=0.875$, $p<0.01$) and negative correlation of FGF21 with FRAP ($r= - 0.867$, $p<0.01$).

Oxidative stress is one of the most important pathogenesis of atherosclerosis. Oxidative stress leads to the generation of reactive oxygen species (ROS) and/or down regulation of the body's innate antioxidant defence systems.[25] Reports also suggests that FGF21 plays a vital role in the prevention of atherosclerosis.[24] The stimulation of antioxidative pathways by FGF21 reduced the oxidative stress in cardiomyocytes and prevented injury.[26]

CONCLUSION

Cardiovascular disease is the most prevalent cause of morbidity and mortality in diabetic patients. FGF21 can be a target to treat obesity and several metabolic disorders. High levels of FGF21 observed in our study could be compensatory responses to maintain metabolic homeostasis. Further studies are needed to explore the role of FGF21 as a novel marker in predicting cardiovascular risk in diabetic patients.

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