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 (FGF21) 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. 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 cholesterol.
(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 lipooxygenases 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.13 Total antioxidant status ferric reducing antioxidant power assay (FRAP) by Benzie et al method.15 FGF21 was analyzed by enzyme linked immunosorbent assay (ELISA).

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).

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 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 |
| Triglycerides (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/dl) | 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 |

**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...

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**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 |

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**References:**

1. Vineetha KR et al. Int J Res Med Sci. 2020 Dec;8(12):4343-4347
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. Studies have proved that FGF21 stimulates the oxidation of fatty acids, the formation of ketone bodies, and the inhibition of lipogenesis. FGF21 regulates glucose lipid metabolism thus proved to be a therapeutic target for the metabolic disease.

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. 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.

MDA is formed during lipid peroxidation after the rupture of the carbon chain of unsaturated fatty acids. 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. Reports also suggests that FGF21 plays a vital role in the prevention of atherosclerosis. The stimulation of antioxidant pathways by FGF21 reduced the oxidative stress in cardiomyocytes and prevented injury.

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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REFERENCES

1. Wu F, Wang B, Zhang S, Shi L, Wang Y, Xiong R, et al. FGF21 ameliorates diabetic cardiomyopathy by activating the AMPK-paraoxonase 1 signaling axis in mice. Clin Sci. 2017;131(15):1877-93.
2. Verhulst MJL, Loos BG, Gerdes VEA, Teeuw WJ. Evaluating all potential oral complications of diabetes mellitus. Front Endocrinol. 2019;10(56):1-49.
3. Raedschelders K, Ansley DM, Chen DDY. The cellular and molecular origin of reactive oxygen species generation during myocardial ischemia and reperfusion. Pharmacol Ther. 2012;133(2):230-55.
4. Gómez-Sámano MÁ, Grajales-Gómez M, Zuurth-Vázquez JM, Navarro-Flores MF, Martínez-Saaedra M. Fibroblast growth factor 21 and its novel association with oxidative stress. Redox Biol. 2017;11(2016):335-41.
5. Ren Y, Li Y, Yan J, Ma M, Zhou D, Xue Z, et al. Adiponectin modulates oxidative stress-induced mitophagy and protects C2C12 myoblasts against apoptosis. Sci Rep. 2017;7(1):1-12.
6. Tiwari BK, Pandey KB, Abidi AB, Rizvi SI. Markers of oxidative stress during diabetes mellitus. J Biomark. 2013;2013:378790.
7. Sharma R, Satyanarayana P, Pallavi A, Kumar S. Circulating serum adiponectin and oxidative stress biomarkers in prediabetes and type 2 diabetes mellitus patients. Asian J Pharm Clin Res. 2019;12(12):58-60.
8. Kim CS, Joe Y, Choi HS, Back SH, Park JW, Chung HT, et al. Deficiency of fibroblast growth factor 21 aggravates obesity-induced atrophic responses in skeletal muscle. J Inflamm. 2019;16:17.
9. Chen HF, Wu F, Lu N, Zheng WP, Lin KY. Decreased levels of serum fibroblast growth factor 21 in Chinese patients with coronary artery disease. Int J Clin Exp Pathol. 2016;9(9):9625-30.
10. Struik D, Dommerholt MB, Jonker JW. Fibroblast growth factors in control of lipid metabolism: from biological function to clinical application. Curr Opin Lipidol. 2019;30(3):235-43.
11. Chen H, Lu N, Zheng M. A high circulating FGF21 level as a prognostic marker in patients with acute myocardial infarction. Am J Transl Res. 2018;10(9):2958-66.
12. Tanajak P, Chattipakorn SC, Chattipakorn N. Effects of fibroblast growth factor 21 on the heart. J Endocrinol. 2015;227(2):R13-30.
13. Jin L, Lin Z, Xu A. Fibroblast growth factor 21 protects against atherosclerosis via fine-tuning the multigain crosstalk. Diab Metab J. 2016;40(1):22-31.
14. Yagi K. Lipid peroxides and human diseases. Chem Phys Lipids. 1987;45(2-4):337-51.
15. Benzi IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem. 1996;239:70-6.
16. Hauner H. Obesity and diabetes. In: Holt RIG, Cockram CS, Flyvbjerg A, eds. Textbook of diabetes. 4th ed. United States: Wiley-Blackwell; 2010:227.
17. Vasanthakumar J, Kambar S. Prevalence of obesity among type 2 diabetes mellitus patients in urban areas of Belagavi. Indian J Health Sci Biomed Res. 2020;13:21-7.
18. Turner RC, Millns H, Neil HA, et al. Risk factors for coronary artery disease in non-insulin dependent diabetes mellitus: United Kingdom prospective diabetes study. BMJ. 1998;316(7134):823-28.
19. Vergès B. New insight into the pathophysiology of lipid abnormalities in type 2 diabetes. Diabetes Metab. 2005;31(5):429-39.
20. Staiger H, Keuper M, Berti L, Hrabe de Angelis M, Häring HU. Fibroblast Growth Factor 21-Metabolic Role in Mice and Men. Endocr Rev. 2017;38(5):468-88.
21. Tezze C, Romanello V, Sandri M. FGF21 as Modulator of Metabolism in Health and Disease. Front Physiol. 2019;10:419.
22. Fisher FM, Maratos-Flier E. Understanding the Physiology of FGF21. Annu Rev Physiol. 2016;78:223-41.
23. Kohara M, Masuda T, Shiizaki K, Akimoto T, Watanabe Y, Homma S, et.al. Association between circulating fibroblast growth factor 21 and mortality in end-stage renal disease. PLoS ONE. 2017;12(6):1-14.
24. Sireesha K, Sailaja RP. Oxidative stress and diabetes: An overview. Asian J Pharm Clin Res. 2015;8(1):15-9.
25. Ying L, Li N, He Z, Zeng X, Nan Y, Chen J, et al. Fibroblast growth factor 21 Ameliorates diabetes-induced endothelial dysfunction in mouse aorta via activation of the CaMKK2/AMPKα signaling pathway. Cell Death Dis. 2019;10(9):665.
26. Planavila A, Redondo-Angulo I, Villarrota F. FGF21 and Cardiac Physiopathology. Front Endocrinol (Lausanne). 2015;6:133.

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