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Association of Fibroblast Growth Factor 21 with Lipid Profile, MDA and AOPP in Patients with Type 2 Diabetes Mellitus

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes mellitus is rising all over the world due to population growth, aging, urbanisation, and the increase of obesity due to physical inactivity, characterized by persistent high blood glucose levels associated with aberrations in lipid, carbohydrate, and protein metabolisms leading to water and electrolyte imbalance. Cardiovascular diseases are the leading causes of mortality in diabetic patients. Mechanisms such as oxidative stress, lipid metabolism imbalance, as well as myocardial cell apoptosis are key factors to facilitate the progression of Diabetic cardiomyopathy.
Aim: The aim of this study was to assess FGF-21 levels and their association with lipid profile parameters and oxidative stress in patients with type 2 diabetes mellitus.
Methods: A patient based cross-sectional study was conducted among the subjects with history of type 2 DM for the past 10 years.

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Results: Variations in FBS, T.C, TG, LDL, HDL, VLDL, FGF-21, MDA and AOPP levels among cases and controls were depicted in Table 2. There was an increase in all these parameters in cases compared to controls whereas HDL showed a decrease among cases.
Conclusion: Our study concluded that there is a significant correlation between fibroblast growth factor 21 (FGF-21), oxidative stress, and abnormal lipid profile in type 2 diabetic patients. We would recommend further studies to explore the role of FGF21 as an important marker in predicting cardiovascular risk in diabetic patients.

Keywords: FGF21-Fibroblast growth factor21; Lipid Profile; T2DM-Type 2 Diabetes mellitus; MDA-Malondialdehyde; AOPP- Advanced oxidation protein products.

1. INTRODUCTION

Diabetes mellitus is a multifactorial disorder characterized by persistent high blood glucose levels associated with aberrations in lipid, carbohydrate, and protein metabolism leading to water and electrolyte imbalance [1]. The prevalence of diabetes is rising all over the world due to population growth, aging, urbanisation, and the increase of obesity due to physical inactivity [2]. Unlike the West, where the older are most affected, diabetes in Asian countries is comparatively high in young to middle-aged people [3]. Compared to Western population, Asians develop diabetes at a younger age with a lower body mass index and waist circumference as they have a strong ethnic and genetic predisposition for diabetes and have lower thresholds for the environmental risk factors [4]. All these complications have long-lasting adverse effects on a nation's health and economy, especially for developing countries [3]. The factor which contribute to the increased prevalence of Diabetes is longer survival rate of diabetic people, that is through early detection, better treatment strategies and therefore reduction in premature mortality. The International Diabetic Federation reported an estimate of prevalence rate of Diabetes in 2019 is 9.3% , that is 463 million people. It is predicted that 10.2 % (578 million people) of total population will have diabetes in 2030 and the number will increase by 51% (700 million) in 2045 [5].

Cardiovascular diseases are the leading causes of mortality in diabetic patients. Diabetic cardiomyopathy (DCM) is defined as a chronic myocardial disorder caused by DM .Hyperglycemia, insulin resistance, micro-vascular lesions and calcium overload in cardiomyocytes were reported to be involved in this disorder. Mechanisms such as oxidative stress, lipid metabolism imbalance, as well as myocardial cell apoptosis are key factors to facilitate the progression of DCM [6].

Dyslipidemia is described as high levels of triglycerides, small dense low-density lipoprotein (sdLDL) cholesterol particles, and low levels of high-density lipoprotein (HDL) cholesterol, which is more common in type 2 diabetes mellitus (T2DM). Various factors interplay in the development of dyslipidemia such as visceral fat, insulin resistance, and excessive fatty acids [7-8]. In T2DM, chronic hyperglycemia can lead to the generation of reactive oxygen species (ROS), and the ROS-hyperglycaemia interface is involved in the development of the microvascular and macrovascular complications of T2DM including diabetic retinopathy, nephropathy, and neuropathy (microvascular) and macrovascular complications such as ischemic heart disease, peripheral vascular disease, and cerebrovascular disease [9-10]. Free radical production is also stimulated by high glucose levels. Lower antioxidant enzymes due to chronic oxidative stress damage pancreaticβ-cells. Oxidative stress is caused by an imbalance between free radicals production and elimination thus producing alterations in cellular metabolism [11].

Lipids are reported as one of the primary targets of reactive oxygen species. Hydroperoxides have toxic effects on cells both directly and through degradation to highly toxic hydroxyl radicals. Free radicals cause cellular damage by reacting with phospholipid membrane and damages cellular transport process and also causes lysis of red blood cells. Therefore, membrane lipids are sensitive to lipid peroxidation. Malondialdehyde (MDA) is formed as byproduct of both enzymatic and non-enzymatic lipid peroxidation reactions [12]. MDA has been documented as a primary biomarker of free radical mediated lipid damage and oxidative stress [13]. Advanced oxidation protein products (AOPPs) are the recently investigated marker of protein oxidation during oxidative stress which represents the overall status of the protein in the cell/tissue [14,15]. In chronic oxidative stress,

AOPPs are formed by reactions between plasma proteins and chlorinated oxidants.

Fibroblast growth factor 21 protein consists of 210 aminoacids and synthesized mainly from liver. The gene for FGF 21 is located in chromosome number 19 [16]. Fibroblast growth factor 21 (FGF21) produced in peripheral tissues have anti-inflammatory effect and also increases fatty acid oxidation and improving insulin sensitivity. Increased levels of FGF21 have been found in type 2 diabetes and metabolic syndrome. The aim of this study was to assess FGF21 levels and their association with lipid profile parameters and oxidative stress in patients with type 2 diabetes mellitus.

2. METHODOLGY

Data were obtained from the subjects with history of type 2 DM for the past 10 years (including male and female) who were attending diabetic clinic at MES Medical College and hospital, Perinthalmanna, Malappuram district, Kerala. A patient based cross-sectional study was conducted from December 2020 to May 2021. Patients including both individuals who were willing to participate with an age limit of 30-55, without any serious illness such as liver diseases, kidney diseases, endocrine diseases and malignancy were included for this study. All the subjects were matched according to the age and sex. Healthy subjects with an age limit of 30-55 without any clinical evidence of major diseases based on the baseline investigations were selected as controls. Anthropometric measurements like age, sex, BMI, WHR, blood pressure was recorded.

BMI is calculated from measured height and weight and WHR from waist circumference and hip line measurement [15]. Fasting blood glucose (FBS), postprandial blood glucose (PPBS), lipid profile, MDA, AOPP and FGF21 were estimated.

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2.1 Sampling Procedure

To determine the required sample size

$$n = \frac{r+1}{r} \frac{SD^2 (Z\beta + Z\alpha/2)^2}{d^2}$$

SD – Taken from previous studies, d = Expected mean difference between case and control

r = ratio of case and control, Z (α/2) =1.96 (5% alpha error), Zβ = 0.84 (20% beta error)

SD – 0.65, d = 0.29, r = 2, Z (α/2) =1.96 (5% alpha error),Zβ = 0.84 (20% beta error

2.2 Sample Collection

Anthropometric measurements – Age, Sex, Body Mass Index (BMI), Waist Hip ratio (WHR) and blood pressure were registered. 5ml of venous blood with overnight fasting were collected in the next morning (before breakfast) for the study. Fasting blood sample for serum separation was collected in a clot activator tube under aseptic conditions and serum was separated. Fasting blood glucose and lipid profile was estimated using J &J Vitros.1FS autoanalyzer. Serum was separated and kept in a deep freezer at -20°C for a month and analyzed for malondialdehyde (MDA), AOPP and FGF21 [18]. Oxidative stress parameter malondialdehyde (MDA) and AOPP were estimated by ELISA. FGF21 was also analyzed by enzyme linked immunosorbent assay [19].

2.3 Statistical Analysis

Data were examined by using the Statistical Package of Social Sciences (SPSS-IBM) version 20. Descriptive statistics were computed for the variables. Mann whitney u test and Spearman

Rank correlation was used to establish the association between variables. 'P' value was less than 0.05 was used to indicate statistical significance.

3. RESULTS AND DISCUSSION

Demographic details of the cases and controls were shown in Table 1. Statistically significant difference with a higher value in diabetic group were obtained for the anthropometric measurements such as systolic, diastolic blood pressure, BMI and WHR (P <0.05). Variations in FBS, T.C, TG, LDL, HDL, VLDL, FGF-21, MDA and AOPP levels among cases and controls were depicted in Table 2. There was an increase in all these parameters in cases.

MDA shows significant positive correlation with FGF21 (R=0.261, p=0.013). AOPP also shows a positive correlation which was not found to be significant (R=0.116, p=0.277). FBS also shows mild positive correlation which was also not significant (R=0.025, p=0.815). Among the lipid profile, mild positive correlation was found among TC, TG, LDL and VLDL. HDL shows significant negative correlation (R=-0.404, P<0.0001) as depicted in Table 3 and Fig. 1.

Table 1. Demographic details of cases and controls

Variables	Cases	Controls	P value
Age	48.66±6.57	47.27±2.42	0.06
Males	45	55	0.183
Females	45	35	
Body Mass Index	24.99 ±4.54	23.81±2.675	<0.05*
Waste Hip Ratio	0.91±0.002	0.89±0.005	<0.05*
SBP	128± 16.5	113.11± 8.02	<0.05*
DBP	82.7± 8.8	73.66 ± 6.94	<0.05*

**Denotes statistical significance*

Table 2. Descriptive presentation of outcome parameters

Parameters	Diabetes Group		Control Group	
	Range	Mean±SD	Range	Mean±SD
FGF-21	7.62-355.14	55.08±65.25	7.99-79.30	24±14.57
MDA	0.79-1800	802.53±428.05	82.46-1793.51	768.9±423.97
AOPP	199.51-4800	782.5±980.14	257.27-2007.13	376.21±204.87
FBS	93-310	166.911±51.93	70-104	85.322±8.428
T.C	99-294	200.73±28.09	121-210	172.46±19.1
TG	69-407	141.9±47.832	55-155	116.34±34.34
LDL	43-182	129.64±27.862	71-150	109.6±19.6
HDL	21-55	36.98±4.51	36-44	39.34±2.91
VLDL	10.60-57	27.71±8.04	11-31	23.34±6.68

Table 3. Correlation of FGF 21 with FBS, oxidative stress parameters and lipid profile

Parameters	FGF21			
	Diabetes Group		Control Group	
	R Value	p Value	R Value	p Value
MDA	0.261	0.013*	-0.070	0.512
AOPP	0.116	0.277	0.042	0.693
FBS	0.025	0.815	-0.226	0.032*
T.C	0.064	0.550	-0.109	0.305
TG	0.192	0.070	0.081	0.449
LDL	0.042	0.695	-0.144	0.177
HDL	-0.404	<0.0001*	-0.021	0.843
VLDL	0.134	0.209	0.081	0.448

Spearman Rank Correlation, p<0.05 shows significance

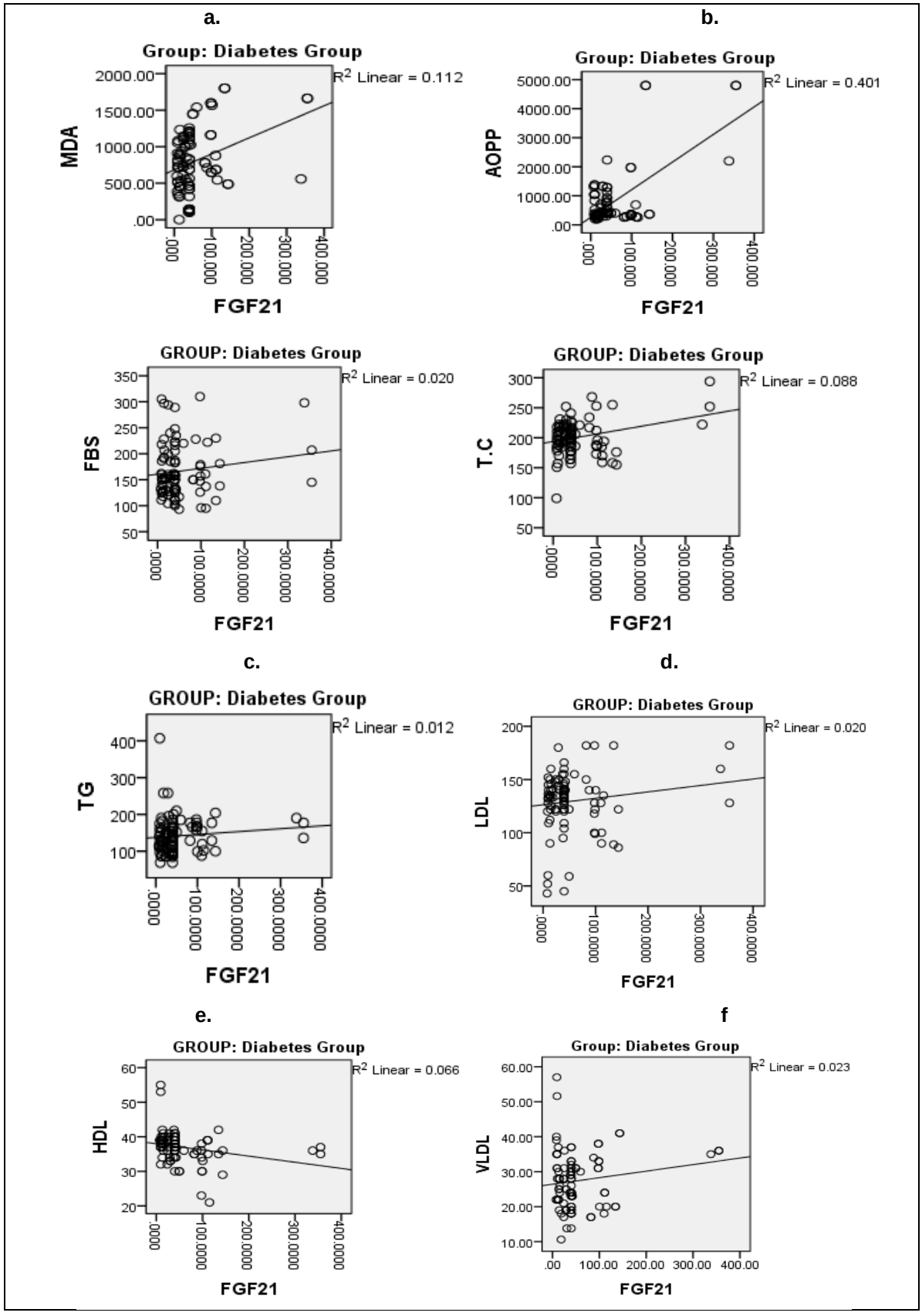

Fig. 1. Scatter plot for FGF 21 with FBS, oxidative stress parameters and lipid profile

Diabetes mellitus (DM) is a chronic metabolic syndrome which has reached epidemic proportions worldwide and represents a serious public health concern. The prevalence of DM, particularly T2DM, has rapidly increased in industrialized and many developing countries. Vascular complications are the main leading cause of morbidity and mortality in DM [20]. In our research, BMI, WHR, serum triglycerides, total cholesterol, LDL, MDA, AOPP levels were higher and a lower HDL were observed among cases as compared to the control group. Increased oxidative stress markers MDA and AOPP in Diabetic group suggest that both lipids and proteins are equally targeted by reactive oxygen species [2].

Dyslipidemia in individuals with T2DM is very common and is associated with increased risk of coronary artery disease compared to individuals without diabetes. Increased triacylglycerols and reduced HDL cholesterol are the main lipid abnormalities of diabetic dyslipidemia [21,22]. Insulin resistance in Diabetes mellitus increases the release of free fatty acids from adipose tissue, which are taken up by the liver. Increased uptake of free fatty acids by liver leads to the synthesis of triglycerides. Increased triglycerides synthesis subsequently stimulates hepatic production of triglyceride-rich very low density lipoprotein cholesterol (VLDL) and increased secretion of ApoB. These triglyceride-rich LDL molecules are then hydrolyzed by lipoprotein lipase leading to the production of small dense LDL [23].

Fibroblast growth factor FGF21 is an endocrine hormone that has, besides its primary function of maintaining the energy homeostasis, beneficial effects on glucose homeostasis, including weight loss. FGF21plays a key role in regulating glucose homeostasis and lipid metabolism. FGF21 levels were also higher in the human population with several chronic disorders linked to atherogenic lipid profiles [24]. In this study, FGF21 levels in the blood were found substantially higher in T2DM patients relative to controls. FGF21 had a mild positive correlation with triglycerides, total cholesterol, and LDL cholesterol but had a negative correlation with HDL cholesterol. FGF21 controls glucose and lipid metabolism and has thus been identified as a potential therapeutic target for metabolic disease [25]. In our study mild positive correlation was observed for FGF21 with FBS._Recent studies have reported that rFGF21 therapy depresses the serum amounts of cholesterol, LDL, triglyceride, and free fatty acid (FFA) thus increasing high-density lipoprotein (HDL) and lowering body weight [26]. FGF21 levels in the blood are higher in people with reduced glucose tolerance and diabetes, as reported in previous literature. Several studies have pointed out that the role of FGF21 in lipid metabolism is favouring fatty acid oxidation, ketogenesis and inhibiting lipogenesis [27]. FGF21 act as an endocrine regulator in glucose and lipid metabolism since it is positively correlated with fasting blood glucose and adverse lipid profile parameters.

MDA is produced when the carbon chain of unsaturated fatty acids is ruptured during lipid peroxidation. In our study, diabetics had higher MDA levels than controls. FGF21 shown to have a significant positive correlation with MDA ($p<0.01$). AOPPs known as proinflammatory and prooxidative compounds that accumulate in aging patients with diabetes may play a major role in increasing prevalence of endothelial dysfunction and subsequent cardiovascular diseases. In our study higher AOPP levels were found in cases and showed a positive correlation with FGF21 which was not statistically significant. Several studies have pointed out that AOPPs and other oxidative stress markers increase in adult subjects with type 2 diabetes with and without micro or macrovascular complications [2].

One of the most significant pathogenesis of atherosclerosis is oxidative stress. Oxidative stress generates ROS and downregulates the innate antioxidant protection mechanisms of the body. FGF21 decreases oxidative stress in cardiomyocytes and prevents injury by stimulating antioxidative pathways. According to studies, FGF-21 has a role in the prevention of atherosclerosis [28]. Recently, a growing body of evidence demonstrates that FGF21 may be an effective drug for the treatment of DCM, especially in the aspects of reducing oxidative stress [6].

4. CONCLUSION

This study aimed to assess the association of fibroblast growth factor 21 with lipid profile and oxidative stress in patients with type 2 diabetes mellitus. Our study concluded that there is a significant correlation between fibroblast growth factor 21 (FGF-21), oxidative stress, and abnormal lipid profile in type 2 diabetic patients. We would recommend further studies to explore the role of FGF21 as an important marker in predicting cardiovascular risk in diabetic patients.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL AND CONSENT

The protocol was approved by Ethical Review Committee of MES Medical College on 10 October 2019 with IEC No. IEC/MES/09/2019. Written informed consent were obtained from each subject. Research participation, confidentiality, and consent were followed as per Helsinki declaration, with local adaptation to allow both verbal and written instructions.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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