A cross sectional study to assess the levels of serum FGF-23 in patients with type 2 diabetes mellitus with and without hypertension

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Abstract

Introduction: People with diabetes mellitus are known to have endothelial dysfunction and are at a higher risk of atherosclerotic cardiovascular disease (ASCVD)Fibroblast growth factor – 23 (FGF-23) is a hormone produced in bones and it primarily acts on kidneys to increase the urinary phosphate excretion

Aim: To assess the levels of serum fibroblast Growth factor-23 (FGF-23) in patients with type 2 diabetes mellitus with hypertension and without hypertension

Materials and Methods: This study was an institution based observational prospective case-control study. The subjects were divided into 2 groups such as patients with Type 2 DM with HTN & without HTN. After obtaining Institutional Ethics Clearance, the anthropometric measurements, BP, glycaemic parameters, lipid parameters & renal parameters were estimated along with serum FGF-23 levels among the study group. Statistical analysis was done with SPSS version 21.0. **Result & Conclusion:** Based on the findings of this observational prospective case-control study we can conclude that FGF-23 levels are significantly elevated in patients with type 2 diabetes mellitus with hypertension when compared to diabetes group alone. Assessment of FGF-23 can be used as a surrogate marker for early detection of atherosclerotic vascular disease and also for estimation of cardiovascular risk.

Keywords: Diabetes Mellitus, Hypertension, Fibroblast Growth Factor-23, Serum cholesterol, glucaemic index

Introduction:

Diabetes Mellitus refers to a group of common metabolic disorders that share hyperglycemia as the primary metabolic dysfunction. People with diabetes mellitus are known to have endothelial dysfunction and are at a higher risk of atherosclerotic cardiovascular disease (ASCVD)[1]. Hypertension is second only to diabetes in being the common cause of mortality due to non-communicable disease. Hypertension causes structural changes in the intima and medial layers of the large vessels and results in development of atherosclerotic changes in the arteries[2].

Fibroblast growth factor – 23 (FGF-23) is a hormone produced in bones and it primarily acts on kidneys to increase the urinary phosphate excretion and also inhibits the synthesis of biologically active form of vitamin D otherwise called calcitriol[3]. The action of FGF-23 in causing phosphaturia has adverse consequences on the myocardium and it mimics the FGF-2 in cardiac myocytes. Many research in the past have focused on the association between FGF- 23, chronic kidney disease and cardiovascular outcomes. There are not many studies and there is a paucity of data regarding the correlation between FGF-23

among diabetes patients with and without hypertension[4].

Aim & Objective of the study

- To assess the levels of serum fibroblast Growth factor-23 (FGF-23) in patients with type 2 diabetes mellitus with hypertension (DMHT group) and without hypertension (DM group) and to determine any correlation between FGF-23 among the study groups.

Place of study

This study was carried out in Department of Biochemistry and Department of Internal Medicine of Dhanalakshmi Srinivasan Medical College and Hospital in Perambalur, Tamilnadu.

Materials and Methods:

Study design:

The study was conducted from July 2018 till November 2020. This study was an institution based observational prospective case-control study with patients having both type 2 diabetes and essential hypertension included as cases and patients having only type 2 diabetes as controls.

Sample size calculation:

Considering the alpha α value of 5% with power of 90 % (ß=10%) as reported by Raju K et al[156] the minimum required sample size was 27 subjects in each group. with a total of 54 patients including both DMHT group and DM group. Therefore it was decided to recruit 40 participants in each group which is higher than the minimum sample size required.

Study participants:

The study sample included 30 patients with known type-2 diabetes mellitus and hypertension attending the Department of Internal Medicine, Dhanalakshmi Srinivasan Medical College and Hospital in Perambalur for treatment and 30 participants with only type 2 diabetes as controls.

Inclusion criteria:

- Patients with only type 2 diabetes mellitus for a duration of not more than5 years were included in DM group
- Patients with both type 2 diabetes mellitus and hypertension were included in DMHT group

Exclusion criteria:

- Patients with diabetes mellitus above 5 years duration
- Patients with history of kidney disease and any systemic medical illness
- Patients with history of hepatic disease
- Patients with history of CVD or any CVS complication.
- Patients with type 1 diabetes mellitus
- Patients with secondary hypertension

Data Collection:

After completion of history taking and physical examination, blood samples were collected from all 80 study participants. For all study participants: Blood samples were tested for urea, serum creatinine, serum uric acid, Total cholesterol, HDL cholesterol, Serum triglycerides, and serum FGF-23 levels.

All subjects were asked to fast for a minimum of 12 hours before blood sample collection. **Estimation of serum FGF-23 levels[5]**

The kit used was Ray Bio® FGF-23 ELISA kit which was a in vitro enzyme-linked immunosorbent assay for the measurement of FGF-23 in a quantitative way in serum, plasma and cell culture supernatants.

Estimation of lipid parameters

Serum HDL - cholesterol estimation was done by PEG-CHOD-PAP method[5], with Lipid Clearing Factor (LCF)[6] Estimation of serum total cholesterol was done by Enzymatic CHOD-PAP Method[6].

Serum triglycerides estimation was done by GPO-PAP method[7].Estimation of serum urea was done by urease method and estimation of serum creatinine was done by jaffe's method

Calculation of estimated glomerular filtration rate (MDRD formula):

$eGFR = 175 \times (S_{cr})^{-1.154} \times (Age)^{-0.203} \times 0.742$ (if female) $\times 1.212$

Where eGFR: Estimated GFR, S.cr:Serum creatinine value.

Estimation of serum uric acid was done by enzymatic photometric test using TBHBA

Statistical analysis

The data analysis was done using SPSS version 21.0. Descriptive statistics was calculated as frequency (n), percentage (%), mean and standard deviation, and represented using tables, graphs, diagrams etc. Student't' test being used to compare the means of continuous variables after ensuring that the variable followed normal distribution using Kolmogorov Smirnov Test for normality. Since the Kolmogorov Smirnov Test was statistically not significant (p=0.152), the data was assumed to follow normal distribution. Chi-square test was used to compare the various categorical variables. Pearson's correlation was used to study the linear relationship between continuous variables such as FGF-23, age, eGFR, Serum Triglycerides, Total Cholesterol, HDL cholesterol levels, blood pressure, glycated hemoglobin, and waist circumference. p value of <0.05 was used to reject the null hypothesis and considered as statistically significant.

Result:

Table 1: Comparison of anthropometric parameters betweengroups (n=80)

Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
eight(cm)	DM group	160.20	8.10	2.20	0.191
	DMHT group	162.40	6.75		
eight(kg)	DM group	69.28	10.24	3.475	0.162
	DMHT group	72.75	11.70		
BMI (kg/m^2)	DM group	26.90	2.63	0.622	0.379
	DMHT group	27.52	3.59		

The difference in mean height, weight and body massindex between the 2 groups was not statistically significant. Therefore the 2 groups are comparable.

Fig 1: Comparison of weight and BMI between groups (n=80)

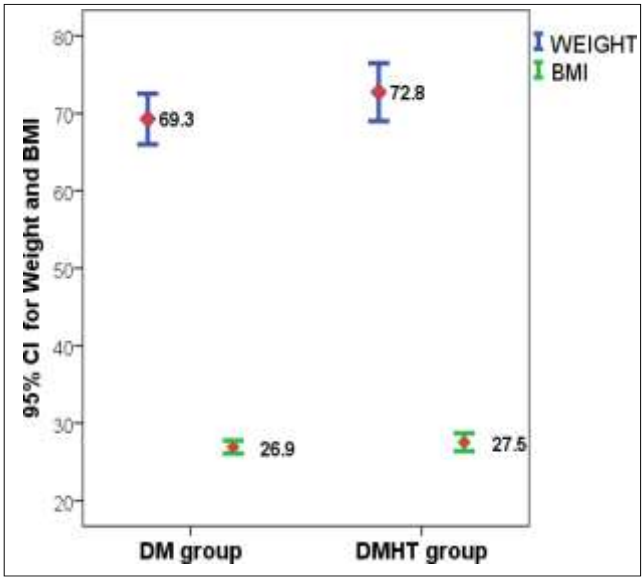


Table 2: Comparison of other anthropometric parametersbetween groups (n=80)

Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
Waist circumference(cm)	DM group	92.6	6.8	9.050	<***0.001***
	DMHT group	101.6	11.2		
Hip circumference(cm)	DM group	90.0	8.5	6.650	***0.005***
	DMHT group	96.7	11.8		
st Hipratio	DM group	1.03	0.09	0.024	0.267
	DMHT group	1.06	0.11		

The difference in mean waist circumference and hip circumference between the 2 groups was statistically significant with DMHT group having a higher mean waist circumference(abdominal/central obesity) than DM group.

Fig 2: Comparison of waist and hip circumference betweengroups (n=80)

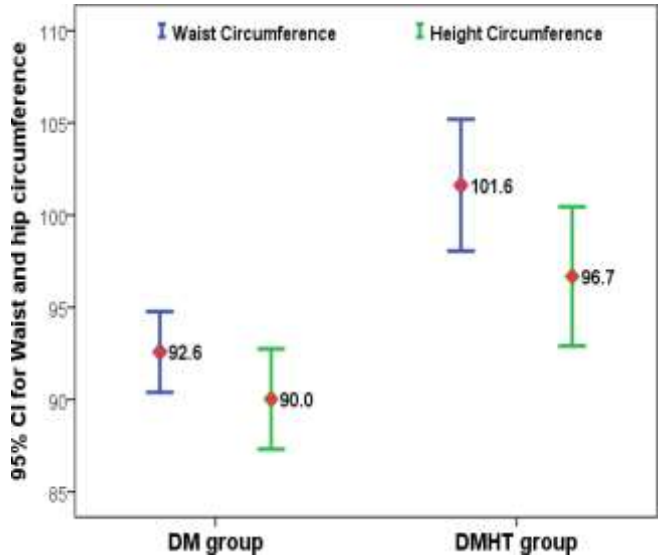


Table 3: Comparison of blood pressure between groups (n=80)

Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
Systolic blood pressure (mmHg)	DM group	111.10	6.66	29.35	***<0.001***
	DMHT group	140.45	8.44		
Diastolic blood pressure (mmHg)	DM group	75.60	4.71	13.85	***<0.001***
	DMHT group	89.45	3.79		

The difference in mean systolic and diastolic blood pressure between the 2 groups was statistically significant as expected with DMHT group having a higher mean systolic and diastolic blood pressure than DM group.

Fig 3: Comparison of blood pressure between groups (n=80)

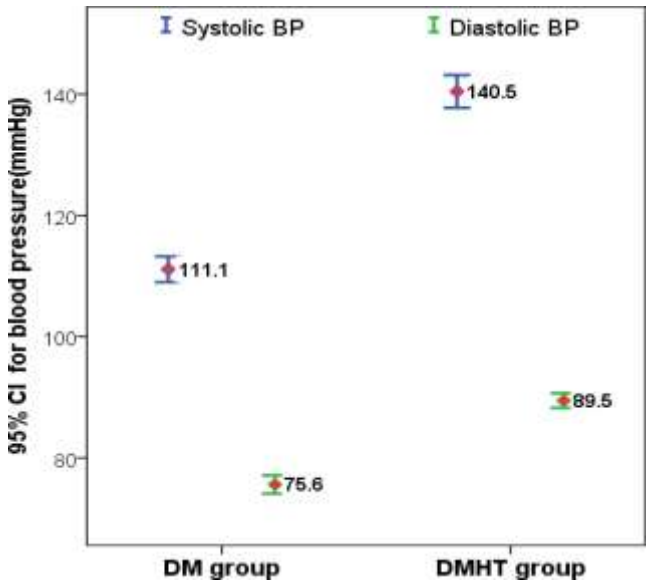


Table 4: Comparison of glycaemic parameters between groups(n=80)

Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
Fasting plasma glucose (mg/dl)	DM group	157.6	32.1	4.90	0.470
	DMHT group	162.5	28.1		
Post-prandial plasma glucose (mg/dl)	DM group	228.4	50.9	19.72	0.104
	DMHT group	248.1	56.3		
HbA_1C	DM group	7.42	0.71	0.272	0.093
	DMHT group	7.69	0.72		

There was no statistically significant difference in mean fasting, post-prandial plasma glucose and glycated hemoglobin values between the 2 groups. Hence the two groups are comparable with regards to glycaemic control.

Fig 4: Comparison of glycaemic parameters between groups(n=80)

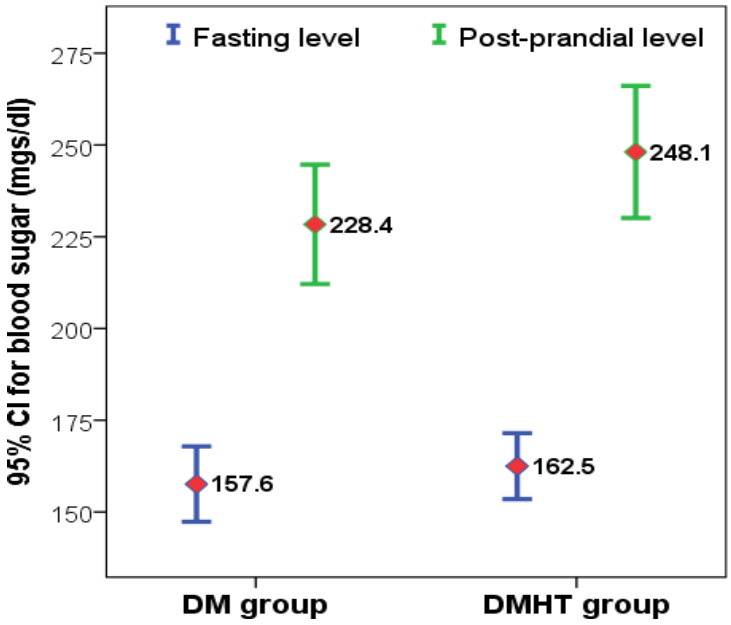


Table 5: Comparison of lipid parameters between groups (n=80)

Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
Total cholesterol(mg/dl)	DM group	181.3	19.8	7.375	0.265
	DMHT group	188.7	36.5		
lycerides (mg/dl)	DM group	169.1	42.5	22.72	***0.029***
	DMHT group	191.8	48.5		
HDL cholesterol(mg/dl)	DM group	40.0	3.7	2.475	***0.007***
	DMHT group	37.5	4.3		

The difference in mean triglyceride and HDL cholesterol levels between the 2 groups was statistically significant with DMHT group having higher mean triglyceride and HDL cholesterol than DM group.

Fig 5: Comparison of lipid parameters between groups (n=80)

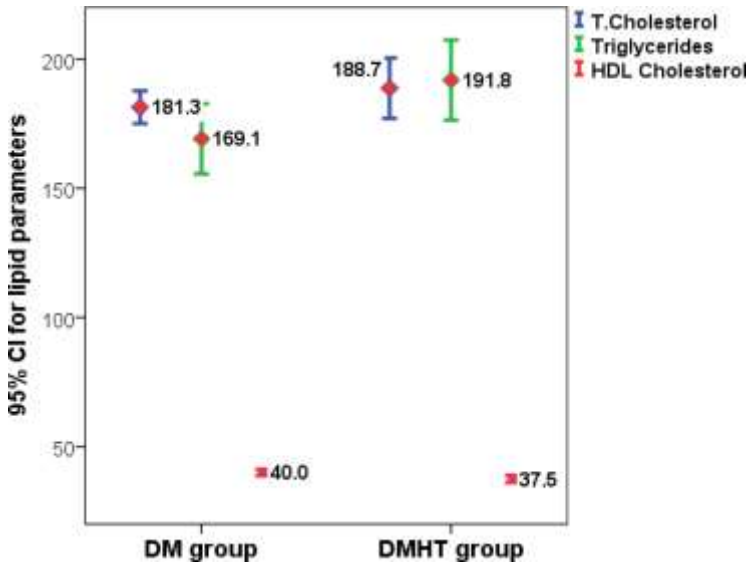


Table 6: Comparison of renal parameters between groups(n=80)

Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
ɔod urea(mg/dl)	DM group	20.25	3.410	1.525	0.074
	DMHT group	21.78	4.098		
reatinine(mg/dl)	DM group	0.755	0.1154	0.010	0.707
	DMHT group	0.745	0.1218		
uric acid(mg/dl)	DM group	3.823	.5673	0.037	0.773
	DMHT group	3.860	.5887		

There was no statistically significant difference in the distribution of renal function parameters between the 2 group

Fig 6: Comparison of renal parameters between groups (n=80)

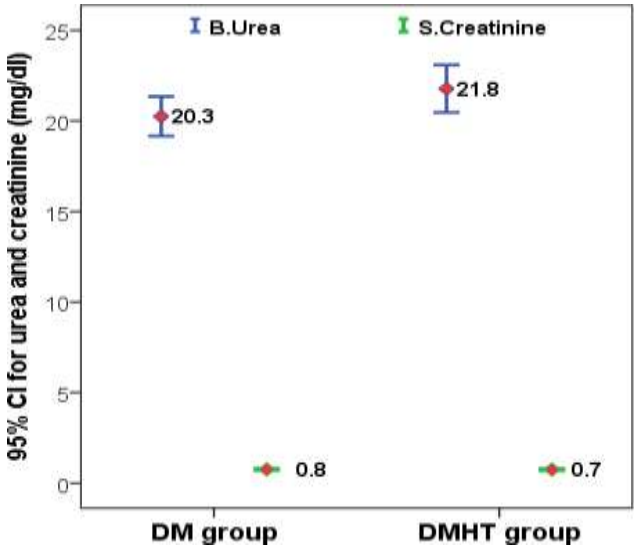


Table 7: Comparison of renal function based on estimatedglomerular filtration rate (eGFR) between groups (n=80)

Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
eGFR (mL/min/1.73m^2)	DM group	100.4	6.2	7.775	<***0.001***
	DMHT group	108.2	7.4		

The difference in mean eGFR levels between the 2 groups was statistically significant with DMHT group having a higher mean eGFR than DM group; however the eGFR values were in normal range in both the groups.

Fig 7: Comparison of renal function based on estimatedglomerular filtration rate (eGFR) between groups (n=80)

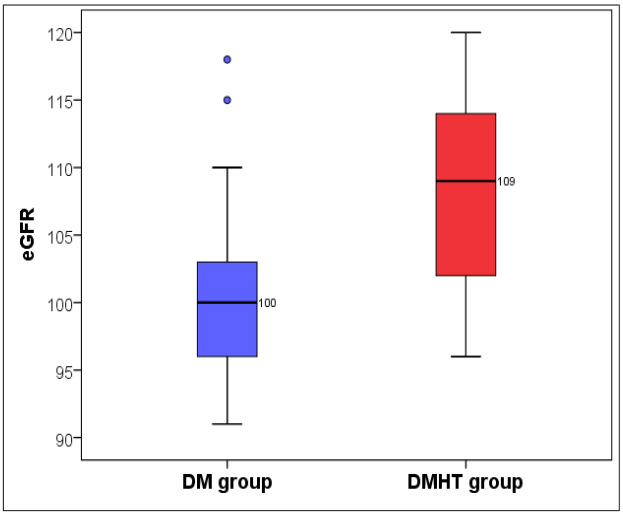


Table 8: Comparison of fibroblast Growth factor – 23 (FGF-23)levels between groups (n=80)

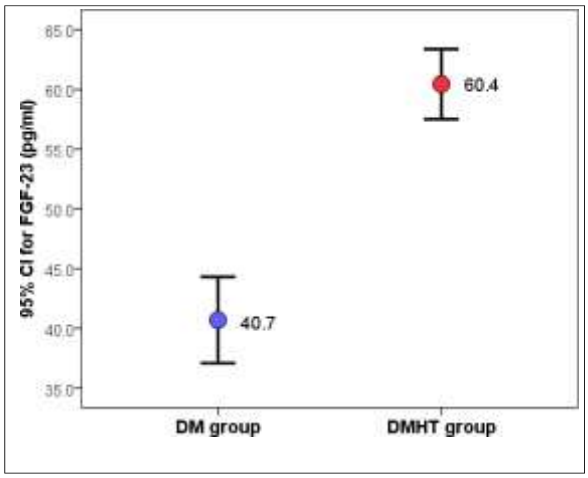
Variable	Group	Mean	S.D	Mean difference	Student 't'test p value
FGF-23 (pg/ml)	DM group	40.69	11.30	19.757	***<0.001***
	DMHT group	60.45	9.18		

The difference in mean FGF-23 levels between the 2 groups was statistically significant with DMHT group having a highermean FGF-23 than DM group.

Fig 8: Comparison of fibroblast Growth factor – 23 (FGF-23)levels between groups (n=80)

Discussion

Our finding indicates that both diabetes and hypertension are prevalent among the middle age group and affects the productive population besides predisposing these individuals for cardiovascular diseases in the future. This



finding reiterates the need for screening programmes targeting the middle age group for early diagnosis of both type 2 diabetes mellitus and hypertension. In the current study, type 2 diabetes mellitus and hypertension are prevalent in both males and females and was equally distributed in both genders. There was no difference in the gender distribution between the 2groups.

In terms of anthropometric measurements, the difference in mean height, weight and body mass index between the 2 groups was not statistically significant. And hence the 2 groups were comparable. The mean body mass index in DM group and DMHT group was 26.9 and 27.5 kg/m^2, respectively.

About 70% and 55% were overweight in DM group and DMHT group, respectively. In addition, obesity was present in 7.5% and 25% in DM group and DMHT group. This finding points out that there is high prevalence of overweight and obesity among the study groups predisposing them for type 2 diabetes mellitus, hypertension and atherosclerotic cardiovascular disease (ASCVD) as well[8,9]. This high prevalence of obesity also points towards the slowly rising modern epidemic of obesity and diabetes[10].

Patients in DMHT group had a higher mean waist circumference and hip circumference than patients in DM group and this difference was statistically significant. Studies have shown that he higher prevalence of central obesity in DMHT group indicates the role of abdominal obesity in pathogenesis and development of hypertension in subjects with type 2 diabetes mellitus[11,12,13].

Blood pressure: The difference in mean systolic and diastolic blood pressure between the 2 groups was statistically significant as expected with DMHT group. As all the subjects with both diabetes and hypertension were on treatment, the mean systolic and diastolic blood pressure was around 140mm Hg which is the target blood pressure in many patients.

Glycaemic parameters: The mean fasting, post-prandial plasma glucose and glycated hemoglobin levels in DMHT group were higher than in DM group but there was no statistically significant difference in mean fasting, post-prandial plasma glucose and glycated hemoglobin values between the 2 groups.

Lipid parameters: The difference in mean triglyceride and HDL cholesterol levels between the 2 groups was statistically significant with DMHT group. In parallel with the higher prevalence of abdominal obesity in DMHT group, the mean levels of triglyceride were higher and HDL levels were lower in DMHT group.

Renal parameters: There was no statistically significant difference in the distribution of renal function parameters such as urea, creatinine and uric acid levels between the 2 groups making them comparable with each other. The difference in mean eGFR levels between the 2 groups was statistically significant with DMHT group having a higher mean eGFR than DM group; however the eGFR values were in normal range (above 90 mL/min/1.73m^2) in both the groups. It is to be noted that patients with renal damage were excluded in this study.

Fibroblast Growth factor – 23 (FGF-23) levels: The difference in mean FGF-23 levels between the 2 groups was statistically significant with DMHT group having a higher mean FGF-23 than DM group alone. In parallel to the findings of this study, He X et al [14] reported that FGF-23 levels are much higher in patients with diabetes and lower extremity peripheral atherosclerotic disease. They reported that even after adjusting for age, gender and eGFR, there was a statistically significant association between FGF-23 levels and femoral intima-media thickness (FIMT) among diabetespatients.

However there was no statistically significant correlation between mean FGF-23 levels and glycated hemoglobin levels in both the groups. This may be because that all the patients were on regular treatment with anti-diabetic drugs and had adequate glycaemic control with relatively lower levels of glycated hemoglobin. A multi-variate linear regression model indicated that even after adjusting/controlling for other independent variables such as age, blood pressure, waist circumference, the significant predictors were serum FGF-23 levels and HbA1C levels.

Balci et al[15] also reported in similar lines that there was a significant increase in FGF-23 levels especially among subjects undergoing hemodialysis and they suggested that FGF-23 levels can be used as an independent marker for aassessment of renal disease in patients with diabetes.

On the contrary, Kestenbaum et al [16] reported that for every rise of 20 pg/ml in FGF-23, there is a 19% higher risk of developing heart failure and 14% higher risk of coronary heart disease but FGF-23 levels did not correlate with carotid intima media thickness (CIMT) and they concluded that FGF-23 is an independent novel risk factor for cardiovascular disease in the general population.

Therefore, with the above mentioned findings suggest FGF-23 can be used as a marker for cardiovascular risk among diabetic patients especially with concomitant hypertension.

Conclusion:

Based on the findings of this observational prospective case-control study we can conclude that FGF-23 levels are significantly elevated in patients with type 2 diabetes mellitus with hypertension when compared to the DM group alone. Assessment of FGF-23 can be used as a surrogate marker for early detection of atherosclerotic vascular disease and also for estimation of cardiovascular risk.

Limitations:

- Relatively small sample size
- Long term follow-up of patients with elevated FGF-23 development of cardiovascular events would have been ideal but could not be done in this study.
- The study findings cannot be generalizable to all diabetic patients as only patients with less than 5 years duration and without kidney damage were included in this study.

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