دراسة سريرية لعامل نمو الخلايا الليفية21 في مرضى داء السكر نوع الثاني

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جامعة الموصل/ كلية العلوم/ قسم الكيمياء

Clinical study of Fibroblast growth Factor21 in diabetic type II

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Abstract :

The current study included determination the level of fibroblast growth factor21 (FGF21) hormone in (100)healthy subject their age are over 35 years old included both sexes, and comparing its level in (124)diabetic patients type II (DMT2).

The results showed increase FGF21 level in (DMT2) group as compare with its level with healthy control group, where the results showed the mean level of FGF21 in serum for healthy control was (75.4±3.2 pg/ml) and FGF21 in serum for (DMT2) group was (108.05±4.03 pg/ml).

Also the results show the effect of some factors such as body mass index (BMI) and age, where the level of hormone increases significantly according to the body mass index (BMI) and according to the age in the control group and the diabetes group (DMT2), While there was no effect of sex, fasting and smoking on the FGF21 level.

Also the results elucidated FGF21 level has positive correlation with Glucose, Total cholesterol, Triglycerides, Low-density lipoproteins (LDL-C), Very low-density lipoproteins (VLDL-C), Atherogenic index (AI), Aspartate aminotransferase (AST/GOT) and Alanine aminotransferase (ALT/GPT). While FGF21 level shows significantly negative correlation with High-density lipoprotein (HDL-C) concentration.

It was concluded this study show there was a relation between of FGF21 level and diabetic type II, and clinical parameter.

Key words:

Fibroblast growth Factor21, Diabetes typeII, BMI, Triglycerides, aminotransferase enzymes.

الملخص:

تضمنت هذه الدراسة تقدير مستوى هرمون عامل نمو الخلايا الليفية21 (FGF21) لدى [100] نموذج لأشخاص اصحاء اعمارهم اكبر من [35] سنة ولكلا الجنسين ومقارنة مستواه في [124] نموذج لأشخاص مصابين بداء السكر من النوع الثاني (DMT2).

حيث أظهرت النتائج زيادة مستوى الهرمون لدى مجموعة مرضى داء السكر مقارنة مع مستواه في مجموعة السيطرة حيث كان متوسط مستوى الهرمون في مصل دم مجموعة السيطرة هو (75.4±3.2 pg/ml) وفي مصل دم مجموعة مرضى داء السكر النوع الثاني (DMT2) كان متوسط مستواه (108.05±4.03 pg/ml) .

كما وتوضح النتائج تأثير بعض العوامل مثل مؤشر كتلة الجسم (BMI) والعمر، حيث اظهرت النتائج ان مستوى (FGF21) يزداد بشكل كبير وفقًا لمؤشر كتلة الجسم (BMI) ووفقًا للعمر في مجموعة السيطرة ومجموعة مرضى داء السكر (DMT2)، في حين لم يظهر تأثير للجنس والصيام والتدخين على مستوى (FGF21).

وبينت النتائج وجود ارتباط إيجابي لمستوى (FGF21) مع الكلوكوز والكوليسترول الكلي والدهون الثلاثية والبروتين الدهني الواطئ الكثافة (LDL–C) والبروتين الدهني الواطئ الكثافة جدا (VLDL–C) ومعامل مسبب التعاضدية (AI) وانزيم ناقل الامين الاسبارتيت (AST/GOT) وانزيم ناقلة الامين اللانين (ALT/GPT)، بينما وجد ارتباط سلبي لمستوى (FGF21) مع البروتين الدهني العالي الكثافة (HDL–C).

يستنتج من الدراسة وجود علاقة بين مستوى عامل نمو الخلايا الليفية21 وداء السكر والمتغيرات السريرية.

الكلمات المفتاحية : عامل نمو الخلايا الليفية 21 ، داء السكري من النوع الثاني ، مؤشر كتلة الجسم ، الدهون الثلاثية، إنزيمات ناقلة الأمين

1. Introduction:

Fibroblast growth factor 21 (FGF21) hormone is belong to the fibroblast growth factor family which contains 22members with abroad range of biological functions relevant to regulating cell growth & development, angiogenesis, wound healing and differentiation (Wan, *et al.*, 2014) and it consider as polypeptide with 210 amino acid residues (Baek, *et al.*, 2017), also it is a member of the FGF family of proteins and it is considered an endocrine hormone because it distribute through the blood system to regulate bodily functions (Cuevas-Ramos, 2016) numerous different metabolically active tissues express FGF21R but most of the hormone is produced by the liver and the levels of FGF21 are regulated by metabolic stressors such as obesity, metabolic diseases and lack of physical exercise such as type II diabetes (Gómez-Sámano, *et a*l., 2017), also FGF family member produced by liver and other tissues that plays an important role in the control of glucose metabolism and energy balance (Giralt, *et al.*, 2015).

Energy homeostasis in mammals is a regulated physiological process involving input of calories and expenditure of energy (Maratos-Flier, 2016), A mismatch of these two processes in favor of excess net calories results in obesity, which is now a major health problem such as type II diabetes, cardiovascular disease, nonalcoholic fatty liver disease (NAFLD) and increased risk of multiple cancers such as breast and colon cancer (Fisher, *et al.*, 2016), so FGF21 hormone is a key regulator of metabolism expressed in many tissues, including the liver (Zhu, *et al.*, 2014) and mediated weight loss appears to stimulates the oxidation of fatty acids and the production of ketone bodies in the liver with no change of food intake (Xiao-Long, *et al.*, 2017), Whether the insulin-sensitizing effects of FGF21 are dependent on reduced body fat, Interestingly these effects are attributable largely to enhanced insulin action in the liver, and yet recent evidence suggests that FGF21 regulates hepatic substrate metabolism by a direct effect on hepatocytes (David A, *et al.*, 2010), However some studies have shown that administrating FGF21 prevents diet-induced obesity and insulin resistance in humans and mice and there is a paradoxically positive correlation with elevated serum FGF21 levels and metabolic disorders like obesity, diabetes, mitochondrial diseases and aging. (Tezze, *et al.*, 2019)

In addition, when administered at pharmacologic doses FGF21 induces wide-ranging beneficial effects in animal models of obesity and diabetes Specifically in obese rodents, pharmacologic FGF21 treatment reduces body fat content and improves glucose tolerance, insulin sensitivity, and lipid parameters (both circulating and hepatic), Consequently FGF21 has emerged as a novel target for the treatment of obesity and associated metabolic dysfunction and has become a focus of metabolic disease research (Frayling, *et al.*, 2018; Wan, *et al.*, 2014).

2. Research problem:

Due to the prevalence of diabetes, this study was done to find the relationship between FGF21 and diabetes, because recent studies have highlighted for usage FGF21 as an indicator of diabetes, as well as its use as a Therapic agent for diabetes

3. Research hypothesis:

In this current study we will propose finding the relation between of FGF21 and some clinical parameter for diabetic type II as well as the effect of their age, sex, BMI, and also effect of fasting and smoking on FGF21 level

4. Location area of the research:

This study carry out in Chemistry Department of Science Collage in University of Mosul of with the help of al-waffa center for diabetic patients and Al-Salam hospital in Mosul city.

5. Aim and the importance of the research:

We propose to study this FGF21 hormone due to the few prior studies in Iraq, also due to the importance and association of this hormone with diabetic disease type II.

6. Method of the research:

The present study was carried out on (100) healthy group (46 female, 54 male), with age matching to the patients group as control. Also, (124) patients with diabetes mellitus type II ([64female, 60male)] from al-waffa center for diabetic patients in Mosul city.
The samples are divided into three groups according to age:
Group I: [less than 35-45] years old included both sexes.
Group II: [46-55] years old included both sexes.
Group III: more than 55 years old included both sexes.
After an overnight fast [12 hours] and non-Fasting, blood samples were obtained from the participants, the serum was isolated (Haque, *et al.*, 2019) and used to estimate the following clinical parameters:
-FGF21 :was measured by enzyme linked immunosorbent assay [ELISA technique] (João Paulo G., *et al.*, 2013) using SHANGHAI YEHUA Biological Technology Co., Ltd kit (China), This analysis was performed by using (BIO-TEK INSTRUMENTS, INC), in the immunity laboratory in Al-Salam hospital in Mosul city.
-Blood Glucose: was estimated by using the enzymatic colorimetric method, using **Randox kit** (United Kindom).
-Total Cholesterol: was measured by using enzymatic colorimetric method, using BIOLABO kit (France).
-Triglycerides: was determined by enzymatic colorimetric method, using **BIOLABO kit** (France).
-High density lipoprotein-cholesterol (HDL-C): was determined by precipitation method, using **BIOLABO kit** (France).
-Very low density lipoprotein-cholesterol (VLDL-C): was estimated by using the following equation: VLDL Conc. (mmol/L) = TG Conc./2.2. (Fischbach, 2000)
-Low density lipoprotein-cholesterol (LDL-C) :was estimated by using the following equation:
LDL Conc. (mmol/L) = Cholestrol Conc.-HDL Conc.-(TGconc./2.2). (Fischbach, 2000)
-Atherogenic Index (AI): was estimated by using the following equation:

Atherogenic Index, (AI) = Log (TG/HDL-C) (Nansseu, *et al.*, 2016).
-Body mass index (BMI): was calculated as weight in kilogram divided by the squared height in meters. (Liu, *et al.*, 2019)
-Uric acid: was estimated by using the enzymatic colorimetric method. (Walker, *et al.*, 1990)
-Aspartate aminotransferase (AST/GOT): was measured by Fuji dri-chem-slide by using FUJIFILM DRI-CHEM NX500i.
-Alanine aminotransferase (ALT/GPT): was measured by Fuji dri-chem-slide by using FUJIFILM DRI-CHEM NX500i.

6.1 Data Analysis :

Also the obtained data was analyzed by using statistical package for social sciences (IBM SPSS)

1. P-Value ≤ 0.05 was considered to be statistically significant.
2. Standard statistical methods were used to determine the mean and standard error.
3. One-way Anova (Duncan-test) to compare between more than two parameters.
4. T-test to compare between two parameters.
5. Linear regression analysis [Pearson correlation coefficient (r)] was performed to identify the relationship between different clinical parameters.

6.2 .1 Results and Discussion:

The data in (Table l) showed that the normal FGF21 level was (75.4±3.2 pg/ml) in healthy control group, this result was approximately close and compatible the results (68.3±18.3 pg/ml) in the literature (Samms, *et al.*, 2017) and near to the result obtained by other researcher (Magdas, *et al.*, 2019; Olszanecka-Glinianowicz, *et al.*, 2015; Esteghamati, *et al.*, 2016) in control group (64.2±10.4 pg/ml, 62.3±16.4 pg/ml, 99.0±12.68 pg/ml) respectively.

6.2.2 Level of FGF21 in DMT2 compared with control.

Result in (Table 1) elucidated that diabetic patients type II group have significantly higher FGF21 level (108.05±4.03 pg/ml) as compared with healthy control group, These results were accepted with those found by (Esteghamati, *et al.*, 2016; Hong, *et al.*, 2019; Panahi, *et al.*, 2016), many studies have reported that FGF21 level in DMT2 patients were higher than those in healthy control and it is assumed that this increase in the Level of FGF21 occurred because of FGF21 resistance (Mraz, 2009) also might be due to hyperglycemia in diabetic which make glucose induces human gene expression in liver through carbohydrate response element-binding protein (ChREBP) activation also in improve insulin sensitivity through inducing of hormone by the receptor peroxisome proliferator activated receptor-gamma (PPARγ) rise expression of glucose transporter GLUT-1 (Cuevas-Ramos, *et al.*, 2012)

6.2.3 Level of FGF21 according to age and sex in control group and DMT2 group.

In (Table 1) the results showed no significant differences in FGF21 level between males and females in control group and DMT2 patients group these results were agree with those found by (Chen, *et al.*, 2011).

The results in (Table 1) also elucidated that there was a significant difference in FGF21 level according to age group between first group & third group, also between second group & third group in the control, while there was no significant difference between the first group & second group in the control. on the other hand (Table 1) demonstrate there was a significant difference in FGF21 level according to age group between first group & third group also between second and third groups in DMT2 patients group, while there was no significant difference between the first group & second group groups in DMT2 patients group, These results were agree with those found by (Hanks, *et al.*, 2015), Increasing the FGF21 level with

age may be a compensatory pathophysiological processes related to energy metabolism and oxidative stress (Corton, *et al.*, 2000).

(Table 1) Level of FGF21 in serum for Control and DMT2 according to sex and age

Age (year)	Level of FGF21 hormone (pg/ml) mean ± S.E			
	Control group		DMT2 group	
	Male	Female	Male	Female
35-45	72.3±3.04	74.02±3.5	93.8±1.4	96.1±2.9
46-55	74.4±4.8	77.01±1.4	93.8±5.5	102.0±4.5
>55	86.7±2.4*	81.3±2.9*	124.9±3.7*	128.4±6.9*
Total mean ±S.E	75.4±3.2		*108.4±4.8	

*Significant difference at P≤0.05.

6.2.4 Level of FGF21 according to BMI in control group and DMT2 group.

The data in (Table 2) showed that there was a significant increase of FGF21 level in control group and also in DMT2 patients group with the rise of the value of body mass index (BMI), These results were accepted with those found by (Kralisch, *et al.*, 2013), the reason for the increase in the hormone level with the rise of the value of BMI in control group may be due to the contribution of high level of free fatty acid (FFA) which may elevated the regulation of FGF21 (Mai, *et al.,* 2010; Ting, *et al.*, 2017), whether the human is overweight or type II diabetes, metabolic disease aspect are present which may increase regulation of FGF21.

(Table 2) Level of FGF21 in serum for Control and DM T2 according to BMI

BMI kg/m^2	Level of FGF21 hormone (pg/ml) mean ± S.E	
	Control group	DMT2 group
<18.5	63.53± 2.2	96.27± 6.1
18.5-25	72.68±3.1*	97.91± 1.9
>25	80.16± 1.8*	113.3± 3.0*
Total mean ±S.E	75.4±3.2	108.4±4.8*

*Significant difference at P≤0.05.

6.2.5 Level of FGF21 according to fasting in control group and DMT2 group.

The results in (Table 3) demonstrates that there was no significant difference in FGF21 level in control group and DMT2 patients group between fasting (for 12 hour) and non-fasting, These results were agree with those found by (Gälman, *et al.*, 2008).

(Table 3) Level of FGF21 in serum for Control and DM T2 according to fasting

Variables	Level of FGF21 hormone (pg/ml) mean ± S.E	
	Control group	DMT2 group
Fasting	73.4±3.7	109.2±4.25
Non-Fasting	78.1±5.3	106.6±2.87
Total mean ±S.E	75.4±3.2	108.4±4.8

6.2.6 Level of FGF21 according to smoking in control group and DMT2 group.

Result in (Table 4) elucidated that there was no significant different in FGF21 level between smoking and non- smoking in control group and DMT2 patients group, These results were agree with those found by (Frayling, *et al.*, 2018).

(Table 4) Level of FGF21 in serum for Control and DM T2 according to smoking

Variables	Level of FGF21 hormone (pg/ml) mean ± S.E	
	Control group	DMT2 group
Smokers	74.7±5.0	105.3±6.2
Non- Smokers	76.1±3.8	110.9±2.8
Total mean ±S.E	75.4±3.2	108.4±4.8

6.3 The concentration of some clinical parameter in control group compare to DMT2 group.

The data in (table 5) shows that DMT2 group have significantly higher concentration of glucose (fasting or Random) as compared with control group, This results were accepted with those found by (American Diabetes Association, 2009; Allwsh, *et al.*, 2013), the increasing of glucose in patients with diabetic type II due to the insulin resistance at the cellular level where insulin resistance prevents the body from converting glucose into glycogen which make it difficult to remove excess glucose from blood (Holt, *et al.*, 2010).

also the results in (Table 5) show that DMT2 patients group have a significantly higher total cholesterol as compared with control group, These results were agree with those found by (Christodoulides, *et al.*, 2009), the reason of this may be due to the lowering in the effectiveness of lipase enzyme, which causes decrease in lipolysis proses (Frühbeck, *et al.*, 2014), as well as (Table 5) demonstrates that there are a significant increase of triglyceride concentration in DMT2 patients group compare with control, These results were agree with those done by (Picu, et al., 2017), this may be due to decrease the enzymatic activity of lipoprotein lipase in diabetic patients (Magacz, *et al.*, 2019), on the other hand (Table 5) shows that DMT2 patients group have low concentration of HDL-C as compared with control group, These results were agree with those done by (Haniye, et al., 2009), the reason may be due to defect in the activity of lipoprotein lipase which associated with diabetic diseases (Brites, *et al.*, 2017), also (Table 5) shows that DMT2 patients group have a significantly higher LDL-C concentration compared with control group. This may be because failing the enzyme activity of lipoprotein lipase in diabetic (Allwsh, *et al.*, 2019), also the results in the same table demonstrate that DMT2 patients group have a significantly increase in VLDL-C concentration as compared with control group because of the lowering in the activity of lipoprotein lipase in diabetes (Magacz, *et al.*, 2019), furthermore data in (Table 5) shows that DMT2 patients group have a significantly higher level of Atherogenic index (AI) as compared with control group because of high triglyceride concentration and low HDL-C concentration in patients with diabetic group compare with control group (Nansseu, *et al.*, 2016).

on the other hand results in (Table 5) elucidates that DMT2 patients group have high concentration of uric acid (U.A) as compare with control group the reason for the rise of U.A may be due to the inhibition of its re-absorption by the kidney tubules due to the high level of glucose (Liu, *et al.*, 2019), and this agree with our results (increasing glucose concentration), also the result in (Table 5) demonstrate that DMT2 patients group have high concentration level of Aspartate aminotransferase (AST/GOT), These results were agree with those done by (Najim, *et al.*, 2017), also the result in (Table 5) demonstrate that there were a significant increase in Alanine aminotransferase (ALT/GPT) level compare with healthy control group, this agree with those found by (Goyal, *et al.*, 2014).

Serum AST rises in Diabetes because AST usually associated with hepatitis and other hepatocellular diseases in an acute phase. (Bladh, *et al.*, 2017), also the serum ALT activity were good marker of liver disease and presence of cardiovascular disease and risk of having non-alcoholic fatty liver disease. (Kim, *et al.*, 2008), as known diabetes shows a close

correlation with hepatitis, liver cirrhosis and chronic liver disease (CLD) also there are association between Cardiovascular complications occur in most diabetic populations and diabetes mellitus (DM) (Kumar, 2018; Zhao, *et al.*, 2019; Allwsh, 2019).

(Table 5) Concentration of some clinical parameters in control and diabetic type II patients.

Clinical parameters	Control means ±S.E	DMT2 means ±S.E
Fasting Glucose (mg/dl)	82.88±2.8	200.9±5.6**
Random Glucose (mg/dl)	112.03±5.8	271.1±6.8**
Cho. (mg/ml)	125.6±6.6	197.5±4.7**
Tri (mg/ml)	85.37±3.3	160.44±7.1**
HDL-C(mg/ml)	160.4±1.4	38.8±1.4*
LDL-C	49.3± 7.3	120.6±5.11**
VLDL-C	17.0±1.8	32.09±0.2*
Atherogenic Index (AI)	2.15±0.13	5.1±0.2**
U.A(mg/dl)	4.8±0.2	8.86±0.29*
AST (U/L)	7.4±0.18	14.25±0.32**
ALT (U/L)	3.2±0.26	7.27±0. 54*

Significant difference at **P<0.01 , * P<0.05.

6.4 Correlation between the Level of FGF21 and some clinical parameters in control and diabetic patients

The data in (Table 6) showed that there was appositive correlation between serum FGF21 level and serum Glucose in patients with diabetic type II, this results were agree with those done by (Gao, *et al.*, 2019; Sung, et al., 2018) the cause might be due to that glucose induces human gene expression in liver through carbohydrate response element-binding protein (ChREBP) activation also in adipose tissue in addition improve insulin sensitivity through inducing of FGF21 by the receptor peroxisome proliferator activated receptor-gamma (PPARγ) and its agonist to increase expression of glucose transporter GLUT-1 (Cuevas-Ramos, *et al.*, 2012). Also the data in (Table 6) demonstrated that there was appositive correlation between FGF21 hormone level and cholesterol, triglyceride, LDL-C and VLDL-C concentrations, this results were accepted with those found by (Alexei, 2009; Gao, *et al.*, 2019), on the other hand a negative correlation between hormone level and HDL-C concentration, this results were accepted with those found by (Azam, *et al.*, 2015; Gao, *et al.*, 2019), the cause of the correlation of FGF21 and lipid may be resulted because FGF21 hormone is a nutritionally regulated hormone and it induced in a large part of peroxisome proliferator activated receptor which play a critical role in regulating metabolism through lipid oxidation in addition to impaired hepatic triglyceride clearance where PPAR target genes encoding rate-limiting enzyme and studies show the rule of plasma FGF21 in regulating lipid metabolism by multiple PPAR agonists (Christodoulides, *et al.*, 2009), Also the results show that there was appositive correlation between FGF21 and Atherogenic index (AI), the cause may be due to the positive correlation between FGF21 and triglyceride and a negative correlation with HDL-

C which made it may be associated with FGF21 (Alexei, 2009), on the other hand the data in (Table 6) demonstrates that there were a significant positive correlation between FGF21 level and uric acid, this results were agree with those found by (Esteghamati, *et al.*, 2016), the cause may be due to increase of uric acid (marker of kidney defect) where the hormone level increase when kidney failure appears to increase its level that correlate negatively with glomerular filtration rate (Cuevas-Ramos, *et al.*, 2012), Results in (Table 6) show positive correlation between FGF21 and Aspartate aminotransferase (AST/GOT) and Alanine aminotransferase (ALT/GPT) levels, the cause due to these enzymes may be consider as a good indicator of overall health and presence damage to the tissues and cells , also a sensitive marker of liver disease and cardiovascular disease risk score (Sahar, *et al.*, 2017; Kim, *et al.*, 2008), on the other hand also FGFs have been associated with established cardiovascular risk factors as well as with the severity and extent of coronary artery disease, in particular FGF21, may be useful as markers of cardiovascular risk (Domouzoglou, *et al.*, 2015).

(Table 6) Correlation between the level of FGF21 and clinical parameters in control and DMT2.

Clinical parameters	Control means	DMT2
	r-value	r-value
BMI	0.68**	0.72**
Fasting Glucose (mg/dl)	0.51*	0.76**
Random Glucose (mg/dl)	0.61**	0.49**
Cho. (mg/ml)	0.15*	0.31*
Tri (mg/ml)	0.49*	0.28*
HDL-C(mg/ml)	-0.27*	-0.68**
LDL-C	0.18*	0.24*
VLDL-C	0.15*	0.33*
Atherogenic index (AI)	0.20*	0.17*
U.A(mg/dl)	0.49**	0.54**
AST (U/L)	0.32*	0.24*
ALT (U/L)	0.22*	0.52*

**Correlation is significant at the 0.01 level.
**Correlation is significant at the 0.05 level.

7. Conclusion :

The studies has demonstrated that FGF21 level elevated in DMT2 the cause may be due to the compensatory responses or the FGF21 resistance and also showed rise of FGF21 level across age increase and BMI, In addition that study show there was a relation between of FGF21 and diabetic clinical parameter.

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