ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 2, 18 SECTIO DDD 2010

[1]Chair and Department of Laboratory Diagnostics,
[2]Chair and Department of Endocrinology,
[3]Department of Biochemical Diagnostics, Medical University of Lublin

MONIKA LENART-LIPIŃSKA[1], BEATA MATUSZEK[1], DARIUSZ DUMA[1], MARCIN DZIEDZIC[1], IWONA KAZNOWSKA-BYSTRYK[1], BEATA WOJTYSIAK-DUMA[3], ANDRZEJ NOWAKOWSKI[2], JANUSZ SOLSKI[1]

Fibroblast growth factor - 21 as a novel marker up-regulated in type 2 diabetes?

Czynnik wzrostu fibroblastów -21 jako nowy marker w cukrzycy typu 2, regulowany w mechanizmie dodatniego sprzężenia zwrotnego?

INTRODUCTION

Fibroblast growth factor-21 (FGF-21), built of 181 amino acids, has recently been introduced as a novel adipocytokine which may be involved in the improvement of insulin sensitivity and pathogenesis of type 2 diabetes [11]. FGF-21 is believed to be a metabolic regulator, which in animal models has been shown to improve glucose metabolism and insulin sensitivity.

FGF-21 exerts its metabolic effect via FGFRs with the use of cofactor β Klotho [3]. Restricted expression of β Klotho limits FGF-21 action primarily in the liver, pancreas, and adipose tissue [5]. FGF-21 is mainly expressed in hepatocytes and in the pancreas, but also originates from adipose and muscle tissue, where it is regulated by the peroxisome proliferators-activated receptors, PPAR γ and PPAR α [10]. FGF-21 stimulates glucose uptake in adipocytes and this effect is insulin independent [6]. FGF-21 is believed to be a major metabolic regulator of glucose and lipid homeostasis and obesity. Kharitonenkov et al. demonstrated that in transgenic mice with overexpresion of FGF-21 a lean, insulin-sensitive phenotype was observed. FGF-21 transgenic mice have improved metabolic profiles: reduced glucose, insulin, cholesterol and triglyceride levels, insulin sensitivity and resistance to diet-induced and age-induced weight gain and fat accumulation [4]. Moreover, contrary to these observations, the lack of FGF-21 led to increased body weight, development of fatty liver disease, impaired glucose tolerance and increased insulin resistance. Numerous animal studies suggest that FGF-21 is a potent metabolic regulator with multiple beneficial effects on insulin resistance state. Interestingly, FGF-21 unlike classical FGFs, has not been reported to induce proliferation and to be a mitogenic factor. Nowadays, very little is known about changes in serum FGF-21 levels and its regulation in humans. It seems interesting to elucidate the role of this adipocytokine in patients with long- lasting type 2 diabetes.

The aim of the study was comparative assessment of FGF-21 concentrations in serum of patients with type 2 diabetes and evaluation of possible relationships between the studied adipocytokine and selected clinical and biochemical parameters.

MATERIAL AND METHODS

The study group comprised 64 randomly chosen adult patients with type 2 diabetes mellitus hospitalized at the Department of Endocrinology of the Medical University of Lublin, 34 women and 30 men aged 47–72 years, with the median duration of diabetes 10.9 ± 8.2 years. The study protocol was approved by the local Ethics Committee (KE-0254/135/2009). Written informed consent was obtained from every patient qualified to enter the study. All patients underwent clinical examination. The prevalence and degree of the severity of chronic vascular complications of the disease were evaluated clinically. In fasting serum samples, with the use of routine laboratory methods, we determined concentrations of glucose, insulin, CRP, fibrinogen, creatinine, triglicerides, total cholesterol, and HDL cholesterol (LDL cholesterol was calculated according to the Friedewald formula) at the time of admission to the Department of Endocrinology and after 2 years of follow-up. FGF-21 and adiponectin concentrations were also measured at this time using a solid phase enzyme-linked immunosorbentt assay, based on the principle of competitive binding (Human FGF-21 ELISA Kit, Human Adiponectin ELISA Kit; BioVendor, Modrice, Czech Republic), according to the manufacturer's instructions. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) formula, such as eGFR = 186 x Serum Creatinine $^{-1.154}$ x Age$^{-0.203}$ x (0.742 if Female). The control group comprised 20 healthy subjects matched for age to the study group, with no disturbances in carbohydrate metabolism, 14 women and 8 men, undergoing prophylactic examinations at the Department of Laboratory Diagnostics of Medical University of Lublin.

The results of the conducted studies were statistically analysed using basic parameters of descriptive statistics (mean±SD or median and minimum, maximum). Partial Spearman correlation coefficient was used to establish the association between serum FGF-21 concentrations and other laboratory parameters. Statistical analyses were conducted using the Statistica version 8.0 programme. $P<0.05$ was considered significant.

RESULTS

Baseline characteristics of the study population are presented in Table 1. In the examined group of patients with type 2 diabetes significant differences concerning weight, BMI, waist circumference and parameters of blood pressure in comparison to the control group were observed. Biochemical characteristics of the study population are shown in Table 2 and Table 3. As a result of the conducted studies it was found that FGF-21 concentration in patients with type 2 diabetes was significantly elevated in comparison with the control group. Adiponectin concentrations were significantly lower than those observed in the control group. Glucose, HbA_1C, CRP, fibrinogen, TG and creatinine levels were significantly increased in the study group compared to the control group, whereas HDL cholesterol plasma concentration and eGFR values were significantly decreased. Univariate correlations of circulating FGF-21 concentrations are presented in Table 4. Serum FGF-21 concentrations were directly proportional to weight and waist circumference, fasting glucose and triglicerides. We demonstrated a significant negative correlation existing between FGF-21 and adiponectin, HDL cholesterol and eGFR values. Circulating FGF-21 levels were not correlated with age, sex, BMI, blood pressure, HbA_1C, CRP and fibrinogen. We did not observe any significant correlations between FGF-21 and insulin concentrations, HOMA-IR and the number and type of vascular diabetic complications. We failed to demonstrate any significant changes after 2 years of follow-up.

Table 1. Baseline characteristics of the study population

Number of cases	DM	Control group	p
	64	20	-
Duration of diabetes (years)	10.9 ± 8.2 (10.0)	-	-
Age (years)	61.5 ± 9.5	58.0 ± 13.0	NS
Weight (kg)	91.5 ± 16.9 (86.0)	69.0 ± 15.8 (64.0)	< 0.001
BMI (kg/m^2)	33.2 ± 4.4 (32.4)	24.7 ± 4.1 (24.1)	< 0.001
Waist circumference (cm)	115.0 ± 9.9 (114.0)	84.7 ± 16.9 (81.0)	< 0.001
Systolic blood pressure (mm Hg)	147.4 ± 13.2 (140.0)	121.8 ± 12.3 (130.0)	< 0.001
Diastolic blood pressure (mm Hg)	88.6 ± 9.5 (85.0)	73.6 ± 6.7 (70.0)	< 0.001
Pulse pressure (mm Hg)	60 ± 10	50 ± 10	< 0.01

Values are shown as mean ± SD and median

Table 2. Biochemical characteristics of the study population

Parameter	DM	Control group	p
FGF-21	331.94 ± 248.20	158.11 ± 120.1	<0.01
Adiponectin	8.23 ± 3.10	10.86 ± 3.63	<0.05
Glucose (mg/dl)	166.0 ± 39.7	87.9 ± 9.7	< 0.001
HbA1c (%)	8.6 ± 1.9	5.8 ± 0.5	< 0.001
Total cholesterol (mg/dl)	190.3 ± 39.8	211.7 ± 47.5	NS
Triglicerides (mg/dl)	162.4 ± 68.5	120.2 ± 67.4	< 0.05
Cholesterol HDL (mg/dl)	47.0 ± 14.5	61.4 ± 14.5	< 0.01
Cholesterol LDL (mg/dl)	110.8 ± 29.3	116.3 ± 43.6	NS
CRP (mg/l)	4.35 ± 3.04	3.12 ± 1.02	< 0.05
Fibrinogen (g/l)	4.34 ± 1.17	2.62 ± 0.8	< 0.01
Creatinine (mg/dl)	0.95 ± 0.21	0.73 ± 0.17	< 0.05
eGFR (MDRD) (ml/min/1.73m^2)	77.9 ± 24.7	112.2 ± 20.3	< 0.001

Values are shown as mean ± SD

Table 3. Biochemical characteristics of the study population after 2 years of follow-up

Parameter	DM	DM after 2 years	p
FGF-21	331.94 ± 248.20	271.3 ± 325.3	NS
Adiponectin	8.23 ± 3.10	9.5 ± 2.75	<0.05
Glucose (mg/dl)	166.0 ± 39.7	166.1 ± 56.3	NS
HbA1c (%)	8.6 ± 1.9	7.4 ± 1.7	<0.05
Total cholesterol (mg/dl)	190.3 ± 39.8	187.5 ± 41.9	NS
Triglicerides (mg/dl)	162.4 ± 68.5	147.4 ± 75.2	<0.05
Cholesterol HDL (mg/dl)	47.0 ± 14.5	54.4 ± 15.1	NS
Cholesterol LDL (mg/dl)	110.8 ± 29.3	116.3 ± 43.6	NS
CRP (mg/l)	4.35 ± 3.04	5.48 ± 2.19	NS
Fibrinogen (g/l)	4.34 ± 1.17	4.08 ± 0.94 (3.60)	NS
Creatinine (mg/dl)	0.95 ± 0.21	0.90 ± 0.17	NS
eGFR (MDRD) (ml/min/1.73m^2)	77.9 ± 24.7	73.2 ± 23.5	NS

Values are shown as mean ± SD

Table 4. Univariate correlations of serum FGF-21 concentrations in the study population

Parameter	DM		DM after 2 years	
	r	p	r	p
Adiponectin (ng/ml)	-0.24	<0.05*	-0.41	<0.01*
Weight (kg)	0.27	<0.05*	0.25	NS
BMI (kg/m^2)	0.32	NS	0.22	NS
Waist circumference (cm)	0.36	<0.05*	0.33	<0.05*
Glucose (mg/dl)	0.27	<0.05*	0.39	<0.05*
HbA1c (%)	0.31	NS	0.32	NS
CRP (mg/l)	0.26	NS	0.26	NS
Fibrinogen (g/l)	0.18	NS	0.14	NS
Total cholesterol (mg/dl)	-0.13	NS	-0.13	NS
HDL cholesterol (mg/dl)	-0.26	<0.05*	-0.26	NS
LDL cholesterol (mg/dl)	0.08	NS	0.012	NS
Triglycerides (mg/dl)	0.27	<0.05*	0.41	<0.01*
eGFR (MDRD) (ml/min/1,73m^2)	-0.28	<0.05*	-0.40	<0.01*
Creatinine (mg/dl)	0.34	<0.05*	0.38	<0.05*

* Significant correlations as assessed by Spearman correlation method

DISCUSSION

In the current study, FGF-21 plasma concentrations were determined for the first time in patients with long-lasting type 2 diabetes mellitus with micro- and macrovascular complications and the median disease duration of more than 10 years. We demonstrated that FGF-21 plasma levels are significantly elevated in patients with type 2 diabetes compared to the control group. As mentioned above, numerous animal studies suggest that FGF-21 is a potent metabolic regulator with multiple beneficial effects on insulin resistance state, so that our results in patients with type 2 diabetes seem to be controversial. However, our data are consistent with a recent study by Zhang and coworkers in which serum FGF-21 concentrations were determined in 232 subjects from the community-based Hong Kong Cardiovascular Risk Factor Prevalence Study [12]. Results of the current studies conducted in humans revealed that FGF-21 is increased in subjects with diabetes, obesity and lipids disorders [1]. These results of the first studies conducted in people were really surprising, since blood serum FGF-21 concentrations in obese people, especially with central obesity in the course of the metabolic syndrome, were found to be significantly increased. What is interesting, it could be observed that along with an increase in structural components of the metabolic syndrome, FGF-21 concentration increased progressively [12]. It was even suggested that FGF-21 could be a potential biomarker of an increasing risk of the metabolic syndrome. In our study we confirmed that circulating FGF-21 is significantly and positively associated with weight and waist circumference, fasting glucose and TG, whereas a negative correlation exists with HDL cholesterol. We failed to observe significant associations between markers of insulin resistance such as fasting insulin and HOMA-IR, which was proved in research of others authors [1, 12]. These studies support the notion that FGF-21 might be part of a physiological feedback mechanism improving insulin signalling in insulin resistance-associated conditions including type 2 diabetes. What is interesting to note, in Kharitonenkov's research the metabolic parameters were significantly influenced in diabetic rhesus monkeys by FGF-21 treatment [7]. Thus, FGF-21 induced a significant decrease in fasting insulin and TG, whereas HDL cholesterol and adiponectin were increased. On the other hand, the insulin resistance and/or dyslipidemia might cause resistance to FGF-21, leading to compensatory up-regulation of this antidiabetic adipocytokine.

The physiological relevance of increased FGF-21 levels in patients with type 2 diabetes remains to be elucidated. On the basis of the results obtained this may suggest the compensatory mechanism of the observed changes or tissue resistance to FGF-21. The mechanisms leading to increased levels of FGF-21 in patients with type 2 diabetes and insulin resistance are still unclear at present. It should be noted that FGF-21 is produced mostly but not only in the liver, also in a wide range of other tissues, such as the adipose and muscle tissue, and modulation of FGF-21 concentration might be tissue specific.

In our study no correlation with FGF-21 concentration was observed in the studied group of patients with diabetes complicated with micro- and macroangiopathy, regardless of the severity or number of complications. In our project we noticed a significant negative relationship between FGF-21 and the renal function expressed as eGFR calculated with the use of the MDRD formula. Our findings are in close accordance with Stein's data who demonstrated a statistically significant relation

of this adipocytokine to renal function [8]. They found that circulating levels of FGF-21 were > 15-fold higher in patients with chronic kidney disease maintained on hemodialyses in comparison to the control group. On the basis of the results obtained from the studies, we can suppose that this adipocytokine is eliminated via the renal route. A negative correlation of FGF-21 concentrations with eGFR values may also indicate accumulation of the examined compound in the body as an effect of progressing renal insufficiency. It can be also concluded that serum creatinine or other markers of renal function should always be included in studies concerning FGF-21 physiology. What is interesting, similarly to our data, a negative association between FGF-21 and circulating serum adiponectin has also been found in subjects with the metabolic syndrome [12]. The role of adiponectin as an independent metabolic marker of increased cardiovascular risk was previously well established [9]. In Dostalova's study, adiponectin remains an independent predictor of circulating FGF-21 [2].

Taken these observations together, FGF-21 serum concentrations showed a significant association with metabolic and vascular risk factors including decreased adiponectin and atherogenic lipid profile (increased TG, decreased HDL cholesterol) in univariate analysis. These results may suggest that FGF-21 might be a novel marker up-regulated in type 2 diabetic patients. This paradoxical up-regulation of FGF-21 might be a compensatory mechanism to improve glucose metabolism when insulin resistance and an atherogenic lipid profile are present. Prospective studies are needed to better elucidate the role of FGF-21 in metabolic and cardiovascular disease.

CONCLUSIONS

1. On the basis of the conducted research, it can be concluded that the higher FGF-21 concentrations observed in the study group of patients with type 2 diabetes may result from a compensatory reaction to metabolic disturbances or tissue resistance to this adipocytokine.

2. A negative correlation of FGF-21 concentrations with eGFR values may suggest renal elimination of the studied compound and indicate its accumulation in the body as an effect of progressing renal insufficiency.

3. Changes in parameters of metabolic control of diabetes are not connected with changes of FGF-21 concentrations.

4. Further investigations are required to determine the clinical consequences of the observed changes and answer the question whether FGF-21 might be a novel marker up-regulated in type 2 diabetic patients.

REFERENCES

1. Chen W.W., Li L., Yang G.Y. et al.: Circulating FGF-21 levels in normal subjects and in newly diagnosed patients with type 2 diabetes mellitus. Exp. Clin. Endocrinol. Diabetes, 116, 65, 2008.
2. Dostalova I., Kavalkova P., Haluzikova D. et al.: Plasma concentrations of fibroblast growth factors 19 and 21 in patients with anorexia nervosa. J. Clin. Endocrinol. Metab., 93, 3627, 2008.

3. Goetz R., Beenken A., Ibrahimi O.A. et al.: Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members. Mol. Cell Biol., 27, 3417, 2007.
4. Inagaki T., Dutchak P., Zhao G. et al.: Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab., 5, 415, 2007.
5. Kharitonenkov A., Shanafelt A.B.: FGF21: a novel prospect for the treatment of metabolic diseases. Curr. Opin. Investig. Drugs., 10, 359, Apr 2009.
6. Kharitonenkov A., Shiyanova T.L., Koester A. et al.: FGF-21 as a novel metabolic regulator. J. Clin. Invest., 115, 1627, 2005.
7. Kharitonenkov A., Wroblewski V.J., Koester A. et al.: The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology, 148, 774, 2007.
8. Stein S., Bachmann A., Lössner U. et al.: Serum levels of the adipokine FGF21 depend on renal function. Diabetes Care, 32, 126, 2009.
9. Takemura Y., Walsh K., Ouchi N.: Adiponectin and cardiovascular inflammatory responses. Curr Atheroscler. Rep., 9, 238, 2007.
10. Wang H., Qiang L., Farmer S.R.: Identification of a domain within peroxisome proliferator-activated receptor gamma regulating expression of a group of genes containing fibroblast growth factor 21 that are selectively repressed by SIRT1 in adipocytes. Mol. Cell Biol., 28, 188, 2008.
11. Wente W., Efanov A.M., Brenner M. et al.: Fibroblast growth factor-21 improves pancreatic beta-cell function and survival by activation of extracellular signal-regulated kinase 1/2 and Akt signaling pathways. Diabetes, 55, 2470, 2006.
12. Zhang X., Yeung D.C., Karpisek M. et al.: Serum FGF21 levels are increased in obesity and are independently associated with the metabolic syndrome in humans. Diabetes, 57, 1246, 2008.

SUMMARY

Numerous animal studies suggest that FGF-21, a newly discovered adipocytokine, is a potent metabolic regulator with multiple beneficial effects on insulin resistance state. The aim of the study was assessment of FGF-21 concentrations in the serum of patients with type 2 diabetes and evaluation of possible relationships between the studied adipocytokine and selected clinical and biochemical parameters. The study was conducted in 64 patients with type 2 diabetes, 34 women and 30 men aged 47–72 with the mean duration of diabetes 10,9 ± 8,2 years. In fasting serum samples concentrations of glucose, insulin, lipids profile parameters, creatinine, CRP, fibrinogen, HbA_1C, adiponectin and FGF-21 were determined at the time of admission to the Department of Endocrinology and after 2 years of follow-up. The control group comprised 20 healthy persons matched for age to the study group, with no disturbances in carbohydrate metabolism, 14 women and 8 men. Results: In patients with diabetes the mean FGF-21 concentration was 331,94 pg/ml and was significantly greater in comparison to the control group: 158,11 pg/ml, $p<0,01$. After 2 years of follow-up the mean FGF-21 concentration was 271,3 pg/ml. Significant correlations between FGF-21 concentrations and adiponectin ($r=-0,24$, $p<0,05$), weight ($r=0,27$, $p<0,05$), glucose ($r=0,27$, $p<0.05$), HDL ($r=-0,26$, $p<0.05$), TG ($r=0,27$, $p<005$) and eGFR ($r=- 0,28$, $p<0,05$) were observed. Conclusions: On the basis of the conducted studies it can be concluded that greater FGF-21 concentration observed in the

examined group of patients with type 2 diabetes may result from compensatory reaction to metabolic disturbances or tissue resistance to this adipocytokine. Changes in parameters of metabolic control of diabetes are not connected with changes of FGF-21 concentrations.

Key words: FGF-21**,** type 2 diabetes, insulin resistance, adiponectin, adipocytokines

STRESZCZENIE

FGF-21 jest stosunkowo niedawno odkrytą adipokiną, mogącą odgrywać istotną rolę w poprawie insulinowrażliwości i patogenezie cukrzycy t. 2. Celem pracy była ocena stężenia FGF-21 w surowicy krwi pacjentów z cukrzycą t. 2 w porównaniu do grupy kontrolnej oraz znalezienie zależności pomiędzy tą cytokiną a innymi parametrami klinicznymi i biochemicznymi. Badania wykonano u 64 pacjentów z cukrzycą t.2, w tym 34 kobiet i 30 mężczyzn w wieku od 47 do 72 lat ze średnim czasem trwania choroby 10.9 ± 8.2 lat. W próbkach krwi pobranej na czczo wykonywano oznaczenia stężeń glukozy, insuliny, CRP, fibrynogenu, lipidogramu, kreatyniny, HbA_1C, adiponektyny i FGF-21 w momencie przyjęcia do Kliniki Endokrynologii oraz po dwu latach obserwacji. Grupę kontrolną stanowiło 20 osób (14 kobiet i 8 mężczyzn) dobranych pod względem wieku i płci do grupy badanej, bez zaburzeń gospodarki węglowodanowej. U pacjentów z cukrzycą średnie stężenie FGF-21 wynosiło 331.94 pg/ml i było istotnie wyższe w porównaniu z grupą kontrolną: 158.11 pg/ml, $p<0.01$. Po dwu latach obserwacji średnie stężenie FGF-21 wynosiło 271.3 pg/ml. Zaobserwowano istotne zależności pomiędzy stężeniami FGF-21 a adiponektyną ($r=-0.24$, $p<0.05$), masą ciała ($r=0.27$, $p<0.05$), glukozą ($r=0.27$, $p<0.05$), HDL cholesterolem ($r=-0.26$, $p<0.05$), TG ($r=0.27$, $p<005$) oraz wartościami eGFR ($r=-0.28$, $p<0.05$). Nie znaleziono istotnych zależności pomiędzy stężeniem FGF-21 a parametrami wyrównania metabolicznego, wykładnikami stanu zapalnego i insulinooporności, jak również obecnością powikłań naczyniowych choroby. Na podstawie przeprowadzonych badań można przypuszczać, iż obserwowane wyższe stężenia FGF-21 w badanej grupie pacjentów z cukrzycą t. 2 mogą wynikać z kompensacyjnej reakcji na zaburzenia metaboliczne bądź też są wynikiem tkankowej oporności na tę cytokinę. Zmiany parametrów wyrównania metabolicznego nie przebiegają ze zmianami stężenia FGF-21.

Słowa kluczowe: FGF-21**,** cukrzyca t. 2, insulinooporność, adiponektyna, adipocytokiny