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Serum fetuin-A levels in patients with type 2 diabetes mellitus

Fetuina-A u chorych z cukrzycą typu 2

INTRODUCTION

Human Fetuin-A (also known as Alpha 2-Heremans Schmid Glycoprotein, AHSG), is a multifunctional circulating plasma glycoprotein with a molecular weight of approximately 60 kDa and a half-life of several days. AHSG is synthesized abundantly during fetal development by multiple tissues, whereas in the adult it is secreted predominantly by the liver (>95%) reaching high serum concentrations (0.4–1.0 g/l) and representing a prominent part of the α2- band of serum electrophoresis. It is also a member of the cystatin superfamily of cysteine protease inhibitors [1, 5, 6, 9, 15].

More than 25 years ago, Lebreton et al. identified the relationship of low fetuin-A levels with acute inflammation and thus the regulation of this protein as a negative acute phase reactant [6]. Fetuin-A also displays additional biological activities as a soluble transforming growth factor-β (TGF-β). Furthermore, fetuin-A, a physiological inhibitor of insulin receptor tyrosine kinase, is associated with insulin resistance, metabolic syndrome and an increased risk for type 2 diabetes [5, 6, 15].

Among the roles of fetiun-A, inhibition of ectopic vascular calcification is its a prominent feature. Fetuin-A is a potent inhibitor of spontaneous hydroxyapatite formation from supersaturated calcium- and phosphate-containing solutions. It stabilizes colloidal complexes with Ca and P and prevents crystal growth by shielding mechanisms [6, 15]. Fetuin-A knockout mice develop severe soft-tissue and intravascular calcifications [10, 15].

Low fetuin-A levels in dialysis patients are associated with cardiovascular mortality, possibly via accelerating vascular calcification. The most serious complications of diabetes are atherosclerotic heart disease, cerebrovascular disease, and renal disease. The most common cause of death with diabetes mellitus is myocardial infarction [7, 12, 14]. The goal of this study was to determine serum fetuin-A concentration to elucidate its role in the development of cardiovascular complications in patients with type 2 diabetes.

MATERIAL AND METHODS

The study was conducted in 51 patients with type 2 diabetes (mean age 62.3±9.3 years). The average duration *of the disease from diagnosis was* 133.4±84.2 months. Macrovascular complications (coronary artery disease, peripheral arterial disease, stroke) were present in 30 and microvascular (nephropathy, neuropathy, retinopathy) in 19 diabetic patients. Among the enrolled subjects there were 26 women (51%) of the mean age 40.3±17.3 years and 25 men (49%) of the mean age 45.8±18.0 years. The patients were treated at the Endocrinology Clinic of the Independent Public Clinical Hospital No. 4 (SPSK 4) in Lublin.

The control group was composed of healthy subjects (n=30) with the mean age of 55.1±13.2 years, attending the periodic health checks at the Department of Laboratory Diagnostics of the Independent Public Clinical Hospital No. 1 in Lublin.

In all subjects determination of fetuin-A, fibrinogen, CRP, total cholesterol, triglicerydes, cholesterol HDL and LDL was performed in serum. The material for the study was the peripheral blood obtained from the ulnar vein. Blood samples were drawn after an 8–12 h overnight fast between 8:00 and 10:00 into clot tubes in volumes of 7 ml. Serum was separated from the collected blood samples by centrifugation for 10 min at 1000 rpm, aliquoted and stored frozen at -20˚C until analysis.

Serum fetuin-A concentration was determined with the use of Human Fetuin-A ELISA Kit (BioVendor Laboratory Medicine, Brno, Czech Republic) according to the manufacturer's protocol. The antibodies were specific for the human fetuin-A protein, with an assay sensitivity of 3.5 μg/mL. Serum total cholesterol (TCH), triglycerides (TG), HDL-cholesterol, fibrinogen and C-reactive protein (CRP) concentrations were measured using a biochemical analyser Konelab *bioMérieux* with dedicated reagents from the same company. LDL-cholesterol was calculated as TCH(TG/5)HDL-cholesterol.

The clinical data and values of selected biochemical parameters in all subjects were expressed by using elements of descriptive statistics (mean X, standard deviation SD, median Me), results being shown in table. Distributions of the analysed variables were tested using the Shapiro-Wilk test. For a comparison of the obtained results of investigations in the case of normally distributed variables, the Student t-test was used. For variables that did not demonstrate compliance with the normal distribution, non-parametric test U Mann-Whitney was applied. Correlations between variables were investigated using Pearson's or Spearman's test. A p value ≤ 0.05 was considered as statistically significant in all analyses. For statistical analysis of the obtained results, Statistica 7.0 StatSoft was used.

RESULTS

Table 1 shows results of determinations of the selected biochemical parameters and fetuin-A concentrations in patients with type 2 diabetes and healthy participants. In the serum of diabetic patients the average fetuin-A concentration was 23.88±8.05 ng/l and was significantly lower ($p<0.05$) compared to the control group (26.66±6.38 ng/l). No differences in the fetuin-A levels were observed between males and females in the study group (25.77±9.95vs.21.91±4.89 ng/l) and between diabetic and healthy females (25.77±9.95vs.25.15±5.35 ng/l), whereas the males with

diabetes had a significantly lower ($p<0.01$) fetuin-A level than control males (21.91±4.89vs.30.82±7.48 ng/l).

Table 1. Serum levels of fetuin-A (ng/l) and selected biochemical parameters in the study and control group

Parameters	Study group		Control group		p level
	X±SD	Me	X±SD	Me	
Fetuin-A (ng/l)	23.88±8.05	22.44	26.66±6.38	26.17	< 0.05
Fibrinogen (g/l)	4.09±0.99	4.07	not performed		-
CRP (mg/l)	11.26±18.26	4.62	not performed		-
Total cholesterol (mg/dl)	194.1±53.5	193.0	207.2±38.5	196.5	NS
HDL-cholesterol (mg/dl)	40.9±10.2	40.0	60.3±17.4	56.5	<0.001
LDL-cholesterol (mg/dl)	119.9±46.4	117.0	124.6±30.5	121.0	NS
Triglycerides (mg/dl)	173.8±119.3	149.5	112.1±46.9	108.0	<0.01

p – level of statistical significance ($p\leq0.05$); NS – non-significance statistical

In the group of diabetic patients from among the selected r*isk factors* of *cardiovascular* diseases we observed a significantly lower HDL-cholesterol concentration ($p<0.001$) and a significantly higher triglycerides concentration ($p<0.01$) compared to the control group.

In thirty patients with macrovascular complications low fetuin-A concentration (below 22.44 ng/l), high concentrations of C-reactive protein (above 5 mg/l) and fibrinogen (above 4 g/l) were noted in 61%, 64% and 73% of cases, respectively. Furthermore, in the patients with low fetuin-A concentration (<22.44 ng/l), high level of C-reactive protein (>5 mg/l) were found in 56% and high concentration of fibrinogen (>4 g/l) in 75% of cases. In the group of patients with all of three risk factors frequency of macrovascular complications was 100%.

Based on statistical analysis, no significant correlations were observed between fetuin-A levels and both age and the values of selected biochemical parameters (fibrinogen, CRP, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) in diabetic patients.

DISCUSSION

Vascular calcification, which has recently received much attention because of its relationship with cardiovascular disease, is very common in dialysis patients. Several reports have shown that low fetuin-A levels were associated with high morbidity and mortality for dialysis patients, possibly through accelerated vascular calcification [1, 7, 12, 14].

Data from the literature have shown that vascular calcification is also often found in advanced atherosclerosis, especially in diabetic patients [1, 2, 6]. Mehrotra et al. [8] first reported a direct relationship between fetuin-A levels and coronary artery calcifications in non-dialysed patients with diabetic nephropathy.

In this study we observed an important relationship between serum fetuin-A and development cardiovascular complications in diabetic patients. We demonstrated that in the patients with type 2 diabetes serum concentration of fetuin-A was significantly lower compared to healthy participants, which is consistent with reports of other authors [1, 2]. Clinical studies confirm that low serum fetuin-A levels are found in diabetic patients, and that a reduction of this liver-derived glycoprotein is associated with inhibition of calcification as well as inflammation but it also predicts cardiovascular morbidity and mortality in patients with type 2 diabetes [1, 2, 4, 5].

Eraso et al. [2] showed that low circulating fetuin-A is associated with peripheral arterial disease (PAD) in type 2 diabetes besides traditional cardiovascular risk factors. They suggest a potentially unique role for fetuin-A deficiency as a biomarker of PAD in patients with type 2 diabetes. Furthermore, Emoto et al. [1] demonstrate a negative correlation between serum fetuin-A and calcified atherosclerotic plaques in type 2 diabetic patients without kidney disease. They also support the hypothesis that fetuin-A in type 2 diabetic patients inhibits calcification of atherosclerotic plaques, but that cardiovascular disease and dialysis may modify or confound the relationship.

In contrast to our results and those of other authors, Fischer et al. [3] and Mehrotra et al. [8] have shown that high fetuin-A level is sensitive marker of coronary atherosclerosis in diabetic patients. One interpretation is that fetuin-A secretion may serve as a feedback defense mechanisms counteracting vascular calcification in early stages of diabetic and atherosclerotic disease, whereas dyslipidemia or hyperinsulinemia could potentially serve as triggers for hepatic fetuin-A release. Thus, reports in the literature suggest that high circulating fetuin-A, rather than low, is associated with incident type 2 diabetes and an increased risk of macrovascular complications [3, 4, 5, 11].

Moreover, data from the Heart and Soul study demonstrate that elevated fetuin-A levels are associated with impaired glucose and lipid metabolism [4, 5]. Recent studies by Ix et al. [4] and Stefan et al. [11] suggest that fetuin-A may be an independent risk factor for type 2 diabetes.

Of particular interest is the immune function of fetuin-A. It is classified as a negative acute-phase protein since its concentration in serum is down-regulated during episodes of acute and chronic inflammation. Recent reports suggest that inflammation may be one important cause of low circulating fetuin-A levels [1, 4, 13]. Serum fetuin-A concentations were inversely correlated with CRP levels and fetuin-A deficiency was identified as an inflammation-related predictor of all-cause and cardiovascular mortality in hemodialysis patients [14]. In our study we did not observe any significant correlation between fetuin-A levels and the values of selected markers of inflammation such as CRP and fibrinogen in diabetic patients. However, the patients with low fetuin-A concentration (<22.44 ng/L) had a high level of C-reactive protein (>5 mg/L) and fibrinogen (>4 g/L) in 56% and 75% of cases, respectively. In summary, our study demonstrates that the diabetic patients having macrovascular complications are associated with lower fetuin-A concentrations and higher levels of CRP and fibrinogen in serum. This corresponds to reports of other authors [1, 2, 13].

Further study is needed to explore a possible association between fetuin-A and vascular calcification, atherosclerosis, inflammation in diabetic patients.

CONCLUSIONS

Our data suggest that fetuin-A is an independent risk factor of development of macrovascular but not microvascular complications in type 2 diabetes mellitus patients. We concluded that determination of serum fetuin concentration together with high sensitivity CRP and fibrinogen levels may be a useful indicator of pending pathological process in cardiovascular system.

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SUMMARY

Fetuin-A is a multifunctional circulating glycoprotein that inhibits ectopic vascular calcification and induces insulin resistance in animals and humans. Low fetuin-A levels in dialysed patients are associated with cardiovascular mortality due to accelerating vascular calcification. The most serious complications of diabetes are atherosclerotic heart disease, cerebrovascular disease, and renal disease. The most common cause of death with diabetes mellitus is myocardial infarction. In this work we determined serum concentration of fetuin-A to elucidate its role in the development of cardiovascular complications in diabetic patients. We studied 51 patients with type 2 diabetes mellitus and 30 healthy humans. Macrovascular complications were present in 30 and microvascular in 19 diabetic patients. In these groups we determined serum concentration of fetuin-A, fibrinogen, CRP, total cholesterol, triglicerydes, cholesterol HDL and LDL. We found that serum levels of fetuin-A were decreased in the group of diabetic patients compared to control subjects. In patients with low fetuin-A concentration (below 22.44 ng/L) high levels of C-reactive protein (>5 mg/L) were found in 56 % and high concentration of fibrinogen (>4 g/L) in 75% of cases. In the group of patients with all of three risk factors the frequency of macrovascular complications was 100%. Our data suggest that fetuin-A is an independent risk factor of the development of macrovascular but not microvascular complications in type 2 diabetes mellitus patients. We concluded that determination of serum fetuin concentration together with high sensitivity CRP and fibrinogen levels may be a useful indicator of pending pathological process in cardiovascular system.

Key words: fetuin-A, C-reactive protein, fibrinogen, type 2 diabetes mellitus, macroangiopathies

STRESZCZENIE

Fetuina-A jest krążącą glikoproteiną, wykazującą działanie hamujące na procesy ektopowej kalcyfikacji naczyniowej oraz indukującą mechanizmy oporności na insulinę zarówno w badaniach na ludziach, jak i zwierzętach. Stwierdzono, że niskie stężenia fetuiny-A u pacjentów dializowanych są związane ze zwiększoną śmiertelnością z powodu chorób sercowo-naczyniowych w rezultacie nasilenia procesów kalcyfikacji śródnaczyniowej. Choroby sercowo-naczyniowe, w tym zawał mięśnia sercowego, również w przypadku cukrzycy typu 2 stanowią jedną z głównych przyczyn zgonów. Dlatego też postanowiono oznaczyć stężenia osoczowe fetuiny-A w celu wyjaśnienia jej roli w rozwoju powikłań sercowo-naczyniowych u pacjentów z cukrzycą. Badania przeprowadzono u 51 pacjentów z cukrzycą typu 2 oraz u 30 osób zdrowych. U 30 chorych z cukrzycą stwierdzono występowanie powikłań mikronaczyniowych, zaś u 19 pacjentów mikroangiopatie. U badanych osób oznaczano stężenia fetuiny-A, fibrynogenu, białka C-reaktywnego, cholesterolu całkowitego, triglicerydów, cholesterolu HDL oraz LDL. Stwierdzono, że stężenia fetuiny-A były obniżone w grupie pacjentów z cukrzycą w stosunku do grupy kontrolnej. U pacjentów z niskimi stężeniami fetuiny-A (poniżej 22,44 ng/L) wysokie stężenia białka C-reaktywnego (>5 mg/L) występowały w 56%, zaś wysokie stężenia fibrynogenu (>4 g/L) w 75% przypadków. W grupie pacjentów, u których stwierdzono obecność wszystkich trzech czynników ryzyka (niskie stęż. fetuiny-A oraz wysokie stęż. CRP i fibrynogenu), częstość występowania powikłań makronaczyniowych

wynosiła 100%. Nasze badania sugerują, że fetuina-A jest niezależnym czynnikiem ryzyka rozwoju powikłań makronaczyniowych u chorych z cukrzycą typu 2, zaś jej oznaczanie wraz ze stężeniami wysokoczułego CRP oraz fibrynogenu może być użytecznym narzędziem w wykrywaniu procesów patologicznych toczących się w obrębie dużych naczyń krwionośnych w tej grupie chorych.

Key words: fetuina-A, białko C-reaktywne, fibrynogen, cukrzyca typu 2, makroangiopatie