Measurement of novel adipokine visfatin in young patients with acute myocardial infarction. Clinical testing of a new ELISA

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Objectives. Adipose tissue produces a number of adipokines that have metabolic effect. Visfatin is a recently discovered adipokine whose concentration in plasma increases in obesity. It is also a proinflammatory mediator that promotes atherosclerosis and plays a role in plaque destabilization.
The aim of this study was to evaluate an assay for the determination of visfatin in human plasma and to investigate its clinical relevance as a marker of acute coronary syndrome (ACS) in a young population (Men under 45 y, Women under 55 y).
Design and Methods. We clinically tested a sandwich ELISA assay in young individuals with acute myocardial infarction (n=36) vs. a control group (n=21). The control sample was a healthy proband without inflammation, hepatic or renal injury and under 55 years of age.
Results. Visfatin in plasma was able to differentiate the control group from young patients with acute myocardial infarction (5 vs. 27 ng/L). Visfatin in the plasma of acute myocardial infarction (AMI) probands, correlated in individuals with acute coronary syndrome was related to plasma glucose (r=0.47; *P*=0.01), type 2 diabetes mellitus (r=0.65; *P*=0.01), plasma creatinine concentration (r=0.3, *P*=0.02), hsCRP (r=0.29; *P*=0.03), BMI values (r=0.18; *P*=0.04), triglycerides (r=0.5; *P*=0.01) and NT-proBNP (r=0.21; *P*=0.04).
In healthy subjects, these relations were not found. ROC analysis: visfatin cut-off concentration was 20 ng/L with a sensitivity of 84% and a specificity of 90%. The area under the curve (AUC) of cTNI was 0.96, the AUC of visfatin was 0.96. Thus, there was no difference.
Conclusion. We conclude that visfatin in serum may be a new independent potential marker of AMI.

Key words: visfatin, acute myocardial infarction, adipokines, ELISA

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INTRODUCTION

Visfatin is a protein present in several mammals and expressed highly in visceral fat. It is a peptide with many functions. It also has a role in the hypoglycaemic effect, causing insulin to decrease rapidly in the blood by lowering blood glucose and improving insulin sensitivity as an insulin receptor activator[1].

Adipose tissue is a large recently recognised endocrine organ secreting several adipokines, for example, adiponectin, leptin, FGF21, resistin, A-FABP and relatively newly presented vistatin. Adipokines play a role in obesity, insulin resistance, metabolic syndrome, beta-cell dysfunction, atherosclerosis, and endothelial dysfunction[2]. Visfatin in produced in reponse to inflammatory signals.The latter possesses anti-apoptotic effect on neutrophils in a clinical model of sepsis, it is also increased in acute pulmonary lesions, being useful as a marker of this condition. Visfatin is also diminished in patients with steatohepatitis compared to pure steatosis. However, increased visfatin levels correlate positively with portal inflammation. These observations could suggest an association of visfatin with inflammation. Negative correlation of visfatin with creatinine clearance and positive correlation with urinary albumin excretion has been demonstrated, suggesting that visfatin affects renal function[2].

The relationship of visfatin and inflammation could mean that visfatin levels could be used as a marker of acute coronary syndrome. Visfatin plays a role in the destabilization of unstable plaque.

METHODS

In this study, 30 men under 45 years of age and 27 women under 55 years of age were examined after approval by the Ethics Committee. The two groups did not differ significantly in BMI. Probands without signs of inflammation, atherosclerosis, or kidney damage (n=21) were selected as a control group.

For all 3 groups, a physical examination was carried out. This included medical history, ECG and echocardiog-

Table 1. Measured parameter values for monitored groups.

Group investigated	Parameter	X	Median	SD	Units
ACS men+women	Plasma visfatin	7	4	8	ng/L
Control group	Plasma visfatin	29	25	14	ng/L
ACS men+women	Plasma glucose	9	8	4	mmol/L
Control group	Plasma glucose	4	4,3	1.6*	mmol/L
ACS men+women	hs CRP	15	19	8	mg/L
Control group	hs CRP	2	3.5	4*	mg/L
ACS men+women	Triglycerides	3.2	2.8	1.9	mmol/L
Control group	Triglycerides	1.3	1.5	0.6*	mmol/L
ACS men+women	NT-proBNP	1100	1290	300	ng/L
Control group	NT-proBNP	520	705	400**	ng/L

*P<0.01 ** P<0.05

raphy. The following parameters were investigated in the blood plasma immediately after collection (Li-He): cTnI (Siemenes, Centaur), NT-proBNP (Siemenes, Cantaur), CRP (Siemens, Advia 1800), creatinine (Siemens, Advia 1800), ALT (Siemens, Advia 1800), AST (Siemens, Advia 1800), bilirubin (Siemens, Advia 1800), Na, K, Cl (Siemens, Advia 1800)), at the same time, aliquots of blood samples were frozen at - 80 °C. Visfatin was measured from these samples (BioVendor, ELISA, Czech Republic).

Statistcal analysis was cared out using the Medcalc program (Belgium). In addition to descriptive statisticsan ROC analysis was performed.

RESULTS

The probands with ACS had higher BMI values (P=0.02). Women and men with AKS did not differ significantly in BMI values (BMI: 36 for probands with ACS vs. 21 for the control group).

Plasma visfatin concentration was significantly lower in the control group than in young ACS subjects (7 vs. 29 ng/L). The ALT, AST, and bilirubin were not significantly different. Further values are given in Table 1.

The diagnosis of ACS was related to plasma glucose (r=0.47; P=0.01), type 2 diabetes mellitus (r=0.65; P=0.01), plasma creatinine concentration (r=0.3, P=0.02), hsCRP (r=0.29; P=0.03), BMI values (r=0.18;P=0.04), triglycerides (r=0.5; P=0.01), NT-proBNP (r=0.21; P=0.04).

No similar correlations were found in the control group. Since the data were not normally distributed, the Spearmann correlation coefficient was used.

The cut-off visfatin concentration for the diagnosis of ACS was 20 ng/L with a sensitivity of 84% and a specificity of 90%. The AUC of cTnI was 0.94; The AUC of visfatin was 0.96 and did not differ.

DISCUSSION

In a recent review, the authors discussed cytokines as potential markers of patients with DM type 2 associated with high ACS risk[3]. Values of serum visfatin, intestine and peritoneum were increased not only in mesenteric ischemia but also in acute pancreatitis. These diseases were associated with plasma concentrations of visfatin[4].

The authors not only discussed the favorable adipokines, but also unfavorable ones like resisitin and visfatin, with the aim of finding potential biomarkers recommended for the clinical use in the diagnosis, prognosis and follow up of patients with T2D at high risk of developing cardiovascular diseases as well as leading to new therapeutic approaches[3]. Serum, intestinal and peritoneal visfatin levels were increased not only in the case of mesenteric ischemia, but also in acute pancreatitis. In these two clinical pathologies, the visfatin levels of the intestinal and peritoneal increased parallel to the serum visfatin levels[4].

In another publication on ACS, resistin and visfatin were studied and these were significantly higher (P=0.01), and adiponectin and apelin were significantly lower in ACS probands compared to stable angina[5].

Another group demonstrated during a 10-year follow-up, that the metabolic status of the study subjects worsened, with a significant increase in body mass index (BMI) (P<0.0001), waist-to-hip ratio (P<0.0001), triglycerides (TG) (P<0.0001), low-density lipoproteins (P=0.0305), Homeostasis Model assessment (P<0.0001), and presence of diabetes (P<0.0001). This change was accompanied by an increase in serum visfatin level, which showed a weak correlation with BMI (P<0.0001, r=0.27586) and presence of diabetes (P<0.0043, r=0.14188) (ref.[6]).

Other authors found an increased concentration of visfatin in blood of patients with AMI. They hypothesised that pro-inflammatory cytokines such as visfatin play a role in the development of atherosclerosis and rupture of atherosclerotic plaque[7].

Another team of authors found a link between biomarkers and visfatin and hypothesised that plasma visfatin could be used to estimate myocardial damage[8].

Since our AUC ROC for cTnI and visfatin did not differ significantly, visfatin may be a complementary and independent biomarker of ACS. Further studies will be necessary to determine the dynamics of visfatin in relation to the cTnI and the patient's history.

CONCLUSION

Given the recent clinical trials and our own results, we believe that visfatin could be a new independent, biomarker of ACS.

Limitation

Information on visfatin in the literature is disparate. On the one hand, it has been found to reduce blood glucose in experiments, improve insulin sensitivity and has an anti-apoptotic effect. On the other hand clinical studies have shown that its plasma concentration is increased in T2DM, ACS, dyslipidaemia and other metabolic disorders. This paradox must be verified in larger multicenter studies[1,3,6].

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