CIDÉ-A GENE EXPRESSION IN PATIENTS WITH ABDOMINAL OBESITY AND LDL HYPERLIPOPROTEINEMIA QUALIFIED FOR SURGICAL REVASCULARIZATION IN CHRONIC LIMB ISCHEMIA

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According to the latest data, CIDÉ-A gene plays a key role in the regulation of body weight in both humans and mice, and therefore it is regarded a potential candidate gene for human obesity.

The aim of the study was to define the role of CIDÉA gene in patients with dyslipidemia and symptomatic limb ischemia.

Material and methods. The study group contained 28 patients, including 17 men and 11 women. Patients were enrolled in the study group, depending on the value of body mass index (BMI); there was BMI>30 for obese patients. The group included untreated patients (n=14) and patients (n=14) receiving atorvastatin 20 mg/day for at least three months prior to the initiation of the study. The control group (n=16) contained patients with no lipid disorders. A one-step isolation of RNA from lymphocytes and adipose tissue cells was carried out using the TRI method modified by Chomczyński and Sacchi. Next, gene expression was tested using real-time PCR.

Results. The highest mean relative expression of CIDÉ-A gene occurred in patients with normal body weight. The lowest mean relative expression of CIDÉ-A gene was observed in obese patients with lipid disorders. A high negative correlation (r=-0.7919) of CIDÉ-A gene expression, depending on BMI, was reported in the group of obese patients with lipid disorders.

Conclusions. Due to an important role of Cide-A protein demonstrated in the development of metabolic diseases such as obesity, metabolic syndrome, type 2 diabetes and their vascular complications, CIDÉ-A gene and protein are potential therapeutic targets in the case of these diseases.

Key words: CIDÉ-A gene, obesity, femoro-popliteal revascularisation, dyslipidemia

In the light of the current studies, obesity and type 2 diabetes are the main causes of secondary dyslipidemia (1, 2). The pathology accompanying these conditions, that results from lipotoxicity of ectopic lipids, carbohydrate metabolism disorders such as insulin resistance and impaired glucose tolerance as well as from impaired clotting and fibrinolysis, contributes to the development of chronic inflammation in the body and the accompanying atherogenic dyslipidemia. Persistent inflammation is associated with endothelial dysfunction, which initiates vascular pathology and the development of atherosclerotic complications (1, 3). The ability of adipocytes to capture free circulating fatty acids, their esterification
into triglycerides and accumulation in this form in adipose vacuoles of the fatty tissue cells are to a large extent controlled by the proteins of fatty droplets (4). The published results confirm a key role of CIDE-A gene in the regulation of body weight in both humans and animals. It is even suggested that it is a potential candidate gene for human obesity.

We therefore sought to define the role of CIDEA gene in patients with dyslipidemia and symptomatic limb ischemia.

MATERIAL AND METHODS

The study group contained 28 patients, including 17 men and 11 women. Patients were enrolled in the study group, depending on the value of body mass index (BMI); there was BMI>30 for obese patients. The group included untreated patients (n=14) and patients (n=14) receiving atorvastatin 20 mg/day for at least three months prior to the initiation of the study.

The patients with the second degree of lower limb ischemia according to Fontaine scale were qualified to the surgical femoropopliteal revascularisation treatment based on angiography and according to TASC protocol (6). The control group contained adipose tissue cells and whole venous blood cells obtained from the patients (n=16) with normal BMI and no lipid disorders – patients was qualified for mini-phlebectomy.

Adipose tissue cells and blood cells were always collected with the written consent of the patient. Venous blood samples were collected into a test-tube of 2 ml containing anticoagulant potassium versenate. Immediately after collection, adipose tissue samples were placed in a test-tube and frozen at -20°C. A one-step isolation of RNA from lymphocytes and adipose tissue cells was carried out using the TRI method modified by Chomczynski and Sacchi (7). The synthesis of cDNA was performed in 20 µl of the reaction mixture using the reagent kit High Capacity cDNA Reverse Transcription Kit produced by Applied Biosystems.

The gene expression test using real-time PCR

The cDNA preparations obtained after reverse transcription were amplified in real time using the technique of semi-quantitative expression analysis – Real Time PCR. The PCR procedure was done in the camera 7300 Real-Time System produced by Applied Biosystems using the SDS software. The reaction was carried out on the optical plate volume 25 µl.

The study used the following sets of FAM-NFQ-labelled TaqMan probes and primers:
- Hs.00154455_m1 gene CIDE-A,
- Hs.99999905_m1 gene GAPDH as endogenous control (Applied Biosystems). The reaction contained the following thermal cycles:
  - initial denaturation 95°C for 10 min,
  - 40 cycles: 95°C for 15 seconds, 60°C for 60 seconds.

CIDE-A gene expression was measured using relative qualification (RQ), in short called 2-ΔΔCT, where relative expression of the tested CIDE-A gene was defined by the following formula: 2^{ΔΔCT} (GAPDH) \times 2^{ΔCT} (CIDE-A). The calculated values of the relative CIDE-A gene expression were used in further research for statistical calculations. Differences between the two independent analyzed groups were determined using the parametric Student’s t-test or non-parametric tests: the Mann-Whitney U test and the Kolmogorov–Smirnov test. The evaluation of mutual relationships between the analyzed variables was done based on the Spearman’s rank correlation coefficient. The results were considered statistically significant if p < 0.05.

RESULTS

The study involved 28 patients, including 17 men and 11 women. The age of subjects
The obese patients (n=28, BMI>30), with LDL hyperlipidemia were subjected (n=14) and not subjected (n=14) to hypolipemic therapy. The mean LDL concentration in the group of untreated patients was 188 mg% (range 140-251 mg%) and was significantly higher then in control group (p< 0.05) In all patients from study group (n=28) the femoro-popliteal venous by-pass graft was done. In 16 patients from the control group, with normal BMI, and no lipid disorders, mean age 57.6 years (range from 53 to 72) the mini-phlebectomy was done (tab. 1).

Mean relative expression of the tested CIDE-A gene in adipose tissue was as follows:
- in the group of obese patients with dyslipidemia – 0.48598 ± 0.08
- in the control group – 1.47588 ± 0.43

The relative expression of CIDE-A gene was lower in study group in comparison to control with statistical significance p< 0.01 (tab. 2).

In all diagnostic groups, the mean CIDE-A gene expression in the cells of adipose tissue was higher in women than in men (no statistical significance, p = 0.0601). In the group of obese patients, a correlation of the level of CIDE-A gene expression was analyzed in the subcutaneous fatty cells depending on BMI. In the group of obese patients with lipid disorder, a high negative correlation (r=-0.7919) of CIDE-A gene expression was reported depending on patients’ BMI. The analyzed correlation revealed strong features of statistical significance (p=0.002) (fig. 1).

None of the study groups of patients showed the expression of the tested gene in peripheral blood lymphocytes.

**DISCUSSION**

In the presented studies we have analysed CIDE-A gene expression in human cells of the subcutaneous adipose tissue as well as in peripheral blood lymphocytes in selected human lipid disorders. We have studied the expression of CIDE-A gene and its correlation with the selected clinical, morphological and biochemical parameters in the peripheral blood of patients. The highest mean relative expression of CIDE-A gene occurred in patients with normal body weight. The lowest mean relative expression of CIDE-A gene was observed in obese patients with lipid disorders. Our study confirmed high CIDE-A gene expression in the human cells of white subcutaneous adipose tissue and visceral abdominal tissue. In contrast, no expression of the tested gene has been observed in peripheral blood lymphocytes in both groups of patients. However, currently more and more attention is paid not to the apoptotic function of Cide-A protein, but to its vital role in the body lipid metabolism. Resistance to obesity demon-

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<th>Table 1. The characteristics of obese patient’s group</th>
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<th>Table 2. CIDE-A gene expression in the subcutaneous adipose tissue in patients with lipid disorders based on patients’ BMI</th>
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<td>BMI &gt; 30 n=28</td>
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<td>Control BMI &lt;25 n=16</td>
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<td>BMI &gt;30 no hypolipidemic therapy n=14</td>
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<td>Athorvastatin 20 mg n=14</td>
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<td>Age mean/SD</td>
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<td>CIDE-A expression mean/SD</td>
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CIDE-A gene expression in obese patients before revascularization in limb ischemia

Viguerie et al. (2005) was a promising discovery. They analysed shifts in gene expression in more than 8,000 obese women who had lost weight using a low-fat diet (13). Nearly a twofold increase in the mRNA expression of CIDE-A gene was observed in the adipose tissue. A rise in CIDE-A gene expression, which plays a role in inhibiting basic lipolysis in the adipose tissue, was all the more surprising, as in patients with normal BMI we should expect an increase in lipolysis rather than its inhibition. This confirmed, however, the previous reports pointing the existence of a close relationship between the mRNA expression of CIDE-A gene in isolated cells and the adipose tissue and the content of fat in the human body. The level of CIDE-A gene mRNA was by 50% lower in obese patients than in non-obese. At the same time, the basic lipolytic activity in the subcutaneous adipose tissue, determined on the basis of glycerol released into the blood, in obese patients was twofold higher than in patients with normal BMI (13, 14, 15). CIDE-A gene expression in the cells of subcutaneous adipose tissue in patients subjected to hypolipidemic therapy was higher than in untreated patients. The obtained results confirm literature data indicating higher metabolism and lipolytic activity of the abdominal adipose tissue compared to the subcutaneous fatty tissue (16, 17, 18). Due to an important role of Cide-A protein demonstrated in the development of metabolic diseases such as obesity, metabolic syndrome, type 2 diabetes and their vascular complications, CIDE-A gene and protein are potential therapeutic targets in the case of these diseases.

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