CIDÉ-A GENE EXPRESSION IN PATIENTS WITH OBESITY QUALIFIED FOR ENDOVASCULAR TREATMENT OF ABDOMINAL AORTA ANEURYSM

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CIDÉ-A gene and the genes of LRP group play a key role in the regulation of the body weight and lipid metabolism in mammals. CIDÉ-A is defined as a potential human obesity gene and the LRP1 gene is associated with the development of abdominal aortic aneurysm (AAA).

The aim of the study was to define the role of CIDÉ-A gene in patients with dyslipidemia and asymptomatic AAA.

Material and methods. The study group consisted of 38 subjects, including 27 men and 11 women qualified for endovascular aneurysm repair (EVAR). The subjects with abdominal aortic aneurysm were enrolled in the study group, depending on the body mass index (BMI); in obese patients (BMI > 30). The control group (n = 16) included subjects without lipid disorders. One-step isolation of RNA from lymphocytes and adipose tissue cells was performed using the modified TRI method by Chomczynski and Sacchi, and then the gene expression was tested by real-time PCR.

Results. The highest mean relative of the gene expression for CIDÉ-A was reported in subjects with the normal body weight. The lowest mean relative of the gene expression for CIDÉ-A was observed in the group of obese patients with aortic aneurysm and lipid disorders. A high negative correlation (r = -0.7101) in the gene expression for CIDÉ-A was observed in the group of obese patients with aortic aneurysm, depending on the BMI.

Conclusions. Due to the important role of the CIDÉ-A gene and Cide-A protein in the development of metabolic syndrome, obesity and the accompanying vascular lesions such as abdominal aortic aneurysm, seen in this context, the tested gene and protein Cide-A represent a potential therapeutic target in these diseases.

Key words: CIDÉ-A gene, obesity, aneurysm, dyslipidemia

In the light of the current studies, obesity and metabolic syndrome are the main causes of secondary dyslipidemia, oxidative stress and vascular pathologies (1). 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 vascular pathologies including aneurysm formation (1, 2).
Persistent inflammation is associated with endothelial and smooth muscle cells dysfunction, which initiates vascular pathology and the development of atherosclerotic complications or aneurismal remodeling (1, 2, 3). The ability of adipocytes and 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, 5). The published results confirm a key role of CIDE-A gene in the regulation of body weight in both humans and animals (6).

Recent studies suggested that another regulator of lipid metabolism (Low density lipoprotein receptor related protein 1 (LRP1) gene could be a crucial agent in aneurysm formation (7). Authors showed an association between lipids metabolism genes, pro-inflammatory molecules (i.e. TNF-alpha, interleukine-6) and cardiovascular diseases (8, 9). LRP1 function is involved in several pathologies such as aneurysm formation, obesity and myocardial infarction (10). The metabolism of adipocytes, macrophages and monocytes is regulated by LRP1 so those cells show the highest level of its gene expression of this molecule (11).

We therefore sought to define the role of CIDE-A gene in patients with dyslipidemia and asymptomatic AAA.

MATERIAL AND METHODS

The study group contained 38 patients, including 27 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=21) and patients (n=17) receiving statin 20 mg/day for at least six months prior to the initiation of the study.

The patients with AAA were qualified to the EVAR procedure after angio-CT imaging according to AAA treatment consensus (12). The group of obese patients with diagnosed dyslipidemia without AAA (n=29) was included in the study. 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. Those patients was qualified for mini-phlebectomy.

Adipose tissue cells and blood cells were always collected during flebectomy procedure 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 -80°C. The whole venous blood samples were collected during routine blood cell count and biochemistry tests. The samples of subcutaneous fatty tissue were obtained during surgery performed due to AAA. 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 -80°C. The study protocol was approved by the Bioethics Committee at the Medical University of Lublin (Decision No. KE-0254/101/2008). A one-step isolation of RNA from lymphocytes and adipose tissue cells was carried out using the TRI method modified by Chomczyński and Sacchi (13). 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 as Hs.00154455_m1 gene CIDE-A and Hs.99999905_m1 gene GAPDH as endogenous control (Applied Biosystems). The reaction contained the thermal cycles of initial denaturation 95°C for 10 minutes and following by 40 cycles: 95°C for 15 seconds and 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^{CT_{(GAPDH)-C_{(CIDEA)}}}. 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 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 38 patients, including 27 men and 11 women. The age of subjects ranged from 50 to 73 years (mean 64.7). The obese AAA patients (n=38, BMI >30), with LDL hyperlipidemia were subjected (n=17) and not subjected (n=21) to hypolipidemic therapy. The mean LDL concentration in the group of untreated patients was 218 mg% (range 151-263 mg%) and was significantly higher then in control group (p< 0.02) In all patients from study group (n=38) the EVAR procedure was done. In 16 patients from the control group, with normal BMI, and no lipid disorders, mean age 52.1 years (range from 50 to 71) the minipheleectomy was done. In obese untreated patients without AAA (n=29) the mean LDL concentration was 198 mg% (range 121-289 mg%)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Women (%)</th>
<th>Men (%)</th>
<th>Altogether (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>11 (30%)</td>
<td>27 (70%)</td>
<td>38 (100%)</td>
</tr>
<tr>
<td>Age (mean) in years</td>
<td>61.31 (59-68)</td>
<td>64.3 (50-73)</td>
<td>64.7 (50-73)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.7 (30.1-34.1)</td>
<td>31.7 (30.4-35.2)</td>
<td>32.6 (30.1-35.2)</td>
</tr>
<tr>
<td>Subjected to hypolipidemic therapy</td>
<td>8 (73%)</td>
<td>9 (33%)</td>
<td>17 (45%)</td>
</tr>
<tr>
<td>Not subjected to hypolipidemic therapy</td>
<td>3 (27%)</td>
<td>18 (67%)</td>
<td>21 (55%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>0</td>
<td>10 (16.6%)</td>
<td>10 (27%)</td>
</tr>
</tbody>
</table>

Mean relative expression of the tested CIDE-A gene in adipose tissue

Mean relative expression of the tested CIDE-A gene was as follows:
- in the group of obese patients with dyslipidemia and AAA – 0.49598± 0.0798
- in the group of obese patients with dyslipidemia without AAA – 0.5972 ± 0.0102
- in the control group – 1.3867 ± 0.51.

The relative expression of CIDE-A gene was lower in study AAA group in comparison to control with statistical significance p< 0.01. The relative expression of CIDE-A gene was lower in study AAA group in comparison to obese patient with dyslipidemia without AAA (p< 0.05).

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 AAA, a high negative correlation (r=-0.7101) of CIDE-A gene expression was reported depending on patients' BMI. The analyzed correlation revealed strong features of statistical significance (p=0.001).

A very high negative correlation (r = -0.8074) in the expression of CIDE-A gene was found in the group of obese patients with AAA and lipid disorders, depending on the LDL/HDL ratio. The analyzed correlation exhibited the characteristics of statistical significance (p = 0.001) (fig. 1).

Table 1. The characteristics of obese patient’s group with AAA

<table>
<thead>
<tr>
<th>BMI &gt;30</th>
<th>BMI &gt;30</th>
<th>BMI &gt;30</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=38</td>
<td>n=21</td>
<td>n=17</td>
<td>n=16</td>
</tr>
<tr>
<td>Age mean/SD</td>
<td>64.7/3.9301</td>
<td>62.5/0.2984</td>
<td>63.9/0.4934</td>
</tr>
<tr>
<td>CIDE-A expression mean/SD</td>
<td>0.49598/0.0798*</td>
<td>0.611135/0.0689*</td>
<td>0.4811/0.0901</td>
</tr>
</tbody>
</table>

Table 2. CIDE-A gene expression in the subcutaneous adipose tissue in patients with AAA based on patients’ BMI>30 (* p< 0.01)
A high negative correlation \((r = -0.6525)\) in the expression of the \(CIDE-A\) gene was observed in the group of obese patients with AAA and lipid disorders, depending on the TCH/HDL ratio. The analyzed correlation exhibited the characteristics of statistical significance \((p = 0.021)\) (fig. 2).

None of the study groups of patients showed the expression of the tested \(CIDE-A\) 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 in patients with AAA. Our present study confirmed high \(CIDE-A\) gene expression in the human cells of white subcutaneous adipose tissue. In contrast, no expression of the tested gene has been observed in peripheral blood lymphocytes in both groups of patients as in our previous study (3). 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 AAA patients with lipid disorders.

However, currently the attention is paid not only to the vital role in the body lipid metabolism, but to its apoptotic function of Cide-A protein (14). The altered metabolism of lipids in obesity is associated with chronic inflammation of the adipose tissue, which normally protects the body from the ectopic deposition of the fat (15). The overload of adipocytes with lipids in obesity together with their insensitivity to insulin inhibit proliferation of preadipocytes and stimulate apoptosis of already mature adipocytes which are responsible for the regulation of the transcription factors: C/EBP and cSREBP-1, an increase in the secretion of chemotactic factors for macrophages and monocytes (MCP-1) as well as the recruitment of new macrophages infiltrating the inefficient adipose tissue. In obesity, these cells may constitute as many as about 50% of the adipose tissue cells (16). The secreted cytokines stimulate macrophages through the production of large amounts of TNF-\(\alpha\) by IKK–NF-B (inhibitor of nuclear factor (NF)-B (IB) kinase—NF-B) and the activation of the signalling pathway JNK–AP1 (Jun N-terminal kinase activation protein-1) along with MAP4K4 so in close relation to \(LPR1\) activity pathway (9, 16, 17, 18). TNF-\(\alpha\) negatively regulates the activity of PPAR\(\gamma\) in chronic inflammation which occurs in obesity. This influences the weakening of protein expression in the fat droplets, including protein Cide-A. The reduced level of these proteins impairs their ability to shield the fat droplets against the active lipases, enhances lipolysis of triglycerides in the adipose tissue and increases the number of circulating free fatty acids in the blood serum.

The presence of any kind of lipid metabolism chronic disorders leads to systemic pathologies, secondary to the toxicity of the ectopically stored lipids, and the development of insulin resistance (19, 20). According to the modern views, human protein Cide-A may play
a key role in the regulation of the lipid metabolism and the ability of the adipose tissue to store triglycerides by inhibiting primary lipolysis.

Numerous works have confirmed that CIDE-A protein coexists with perilipin and Cide-C/Fsp27 proteins, which are considered to be regulators of lipolysis, located around the fat droplets in the adipose tissue (21, 22, 23). The interactions have also been confirmed between TNF-α, PPARγ and Cide-A protein in the impaired regulation of the lipid metabolism in obesity (17, 18). PPARγ increases the expression of proteins within the fat droplets in the normal adipocytes. These proteins include Fsp27, Cide-A, perilipin, ADRP and S3-12 and their presence on the fat droplets inhibits primary lipolysis and the release of free fatty acids that stimulate the accumulation of triglycerides in adipocytes (16, 23). 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 (17, 24, 25). 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 (26, 27). Due to a role of Cide-A protein demonstrated in the development of metabolic diseases such as obesity, metabolic syndrome, and vascular complications, CIDE-A gene and protein are potential therapeutic targets in the case of these diseases.

REFERENCES


