Features of the connective tissue metabolism, the content of adipokines and cytokeratin-18 in patients with non-alcoholic steatohepatitis combined with osteoarthritis and obesity

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Aim. To determine the indices of connective tissue metabolism, adipokines and cytokeratin-18 in non-alcoholic steatohepatitis patients with osteoarthritis and obesity comorbidities.

Materials and methods. 90 patients were examined and divided into three groups: group 1 (n = 30) included patients suffering from OA grade II–III according to Kellgren and Lawrence classification with normal body mass (BMI = 21–25 kg/m²), group 2 (n = 30) – patients with NASH and OB without OA (BMI > 30 kg/m²), group 3 (n = 30) – patients with OA with NASH and OB (BMI more than 30 kg/m²). The control group consisted of 30 age-matched practically healthy persons (PHP). The average age of patients was (62.3 ± 5.7) years.

Results. In NASH patients with OB and OA, there is a significant increase in the synthesis of collagen and glycosaminoglycans which is accompanied by ineffective resorption of newly formed collagen due to inhibition of the collagenolytic activity of blood plasma in NASH arising from activation of protease inhibitors (α2-MG), a significant imbalance in the metabolic system of connective tissue, which, particularly in OA and OB comorbidities, leads to progressive fibrosis of the liver and its functions impairment. It has been established that blood adipokines level not only depends on body weight, but also reflects the risk for occurrence of nosologies associated with OB.

Conclusions. In patients with NASH and morbid OB, a significant increase in collagen and glycosaminoglycans synthesis was observed. Adipokine deficiency, found in the work, can play a significant pathogenetic role in the development and progression of NASH as well as OB and OA. Adipokines leptin and adiponectin and also cytokeratin-18 may serve as sensitive risk markers for comorbid diseases development and could be candidates for their measurements inclusion in the diagnostic algorithm for NASH, OA, OB and their combination.
Материалы и методы. Обследовали 90 пациентов, которые разделены на 3 группы: 1 группу (n = 30) составили больные, страдающие ОА коленных суставов II–III стадии по Kellgren и Lawrence с нормальной массой тела (ИМТ = 21–25 кг/м²), 2 группу (n = 30) – пациенты с НАСГ и ожирением без ОА (ИМТ >30 кг/м²), 3 группу (n = 30) – пациенты с ОА с НАСГ и ожирением (ИМТ более 30 кг/м²). Контрольную группу составили 30 здоровых лиц соответствующего возраста. Средний возраст больных – 62,3 ± 5,7 лет.

Результаты. У больных НАСГ на фоне ожирения и остеоартроза установлено существенное повышение синтеза коллагена и гликозаминогликанов, которое сопровождается неэффективной резорбцией новосинтезированного коллагена вследствие торможения коллагенолитической активности плазмы крови при НАСГ, возникшем вследствие активации ингибиторов протеаз (α2-МГ), дисбаланса в системе метаболизма соединительной ткани. Это, особенно в условиях сопутствующей ОА и ОЖ, приводит к прогрессирующему фиброзированию печени и нарушению ее функций. Установлено, что уровень адипокинов в крови зависит не только от массы тела, но и отражает риск возникновения нозологий, ассоциированных с тучностью.

Выводы. У больных неалкогольным стеатогепатитом, который возник на фоне ожирения, установлено существенное повышение синтеза коллагена и гликозаминогликанов. Дефицит адипонectина может играть важную патогенетическую роль в развитии и прогрессировании как НАСГ, так и ожирения и остеоартроза. Адипонектин и адипонектин, а также цитокерatin-18 могут быть чувствительными маркерами риска развития коморбидных заболеваний, а значит могут стать кандидатами на включение их определения в диагностический алгоритм при НАСГ, ОА и ОЖ и их сочетания.

Relevance

Modern achievements in hepatology have contributed to the recognition of the leading role of the connective tissue (CT) system in the pathogenesis of liver disease progression [7]. Liver fibrosis is a progressive pathological process that persists on the background of inflammation and leads to excessive matrix components accumulation in the extracellular space. If this process is accompanied by an ineffective resorption of the CT, as well as an excessive regeneration, it leads to distortion of the normal liver architecture, and as a consequence, to liver cirrhosis [5]. Scientists are currently attempting to find possible biochemical markers of liver fibrosis degree [11].

Osteoarthritis (OA) is one of the most common diseases of joints, which is accompanied by a decrease in the quality of life and disability. Recently, the data on certain OA-related phenotypes were obtained. The age-associated, estrogen-dependent, genetically determined, pain, post-traumatic, crystalline-induced and metabolic ones are the most common OA phenotypes, which differ in pathogenetic processes and clinical manifestations [3,10]. Metabolic variant of OA phenotype is one of the most frequent and associated with metabolic syndrome and low-grade inflammation. It has become an interest of scientists to find the relationship between the components of metabolic syndrome, proinflammatory cytokines activity and the metabolic state of CT components in patients with OA.

Non-alcoholic fatty liver disease (NAFLD) is a potential component of the metabolic syndrome including a range of clinical and morphological concepts: liver steatosis, nonalcoholic steatohepatitis (NASH) and may be complicated by liver fibrosis and cirrhosis development. The pathogenetic causes of NAFLD are mostly insulin resistance (IR) and an imbalance in adipocytokines and hormone-like substances regulating fat metabolism. Adiponectin and leptin are biologically active compounds produced by adipose tissue, which are not only essential for lipids and carbohydrate metabolism regulation, but also affect the vascular wall state, inflammatory processes and thrombus formation. The literature data provide evidence that low levels of adiponectin are associated with the development of OB and IR. Other authors also point to the key role of leptin in the regulation of fat and carbohydrate metabolism as a link to atherosclerosis and NAFLD [1]. On the other hand, adipocytokines can play a certain role in the development of arthritis and osteoarthritis [2].

Aim

To determine the indices of connective tissue metabolism, adipokines and cytokerin-18 in non-alcoholic steatohepatitis patients with osteoarthritis and obesity comorbidities.

Materials and methods

A total of 90 patients were examined and divided into three groups: group 1 (n = 30) included patients suffering from OA grade II–III according to Kellgren and Lawrence classification with normal body mass (BMI = 21–25 kg/m²), group 2 (n = 30) – patients with NASH and OB without OA (BMI >30 kg/m²), group 3 (n = 30) – patients with OA with NASH and OB (BMI more than 30 kg/m²). The control group consisted of 30 healthy age-matched individuals. The average age of patients was 62.3 ± 5.7 years.

NASH was diagnosed based on anamnestic, clinical, laboratory data, detection of the serum hepatitis B and C viruses markers, the results of USG according to the unified clinical protocol, approved by the Order of the Ministry of Health of Ukraine of 06.11.2014 No 826, in the presence of the criteria for exclusion of chronic diffuse liver disease of viral, hereditary, autoimmune or drug-induced genesis as causes of cholestatic or cytolytic syndromes, taking into account the 10th revision of ICE. OA was diagnosed based on the EULAR recommendations (2010) and the Order of the Ministry of Health of Ukraine of October 12, 2006, No 676 “Clinical Protocol for the Provision of Medical Aid to Patients with Osteoarthritis” under section 13 “Rheumatology” and the Protocol of the Ministry of Health of Ukraine of April 11, 2014, No 263 under section “Rheumatology”. The presence of abdominal OB in patients was assessed based on the Order of the Ministry of Health of Ukraine of January 14, 2013 No 16 “Methodical Recommendations for General Practitioners of Family Medicine on Counseling Patients regarding the Basic Principles of Healthy Eating”. Changes in the metabolism of the extracellular matrix components were determined by free oxyproline (FOP) content in the blood according to S. S. Telianets method (1985), protein-bound blood oxyproline (PBOP) by M. S. Osadchuk method (1979), hexosamines (HA) by O. G. Arhipova method (1988), sialic acids (SA), non-protein-bound fucose by "Danush Ltd" company sets (Lviv), ceruloplasmin (CP) with sets by Revin method (1976).
Table 1. Indicators of metabolic state of connective tissue in NASH patients with obesity and osteoarthritis comorbidities (M ± m)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Practically healthy persons (n = 30)</th>
<th>OA with normal body weight (n = 30) 1 group</th>
<th>NASH + OB (n = 30) 2 group</th>
<th>NASH + OB+ OA (n = 30) 3 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBOP, μmol/l</td>
<td>39.89 ± 0.30</td>
<td>56.96 ± 0.30*</td>
<td>64.08 ± 0.31**</td>
<td>84.64 ± 0.34**</td>
</tr>
<tr>
<td>FOP, μmol/l</td>
<td>11.98 ± 0.34</td>
<td>18.51 ± 0.32*</td>
<td>9.61 ± 0.43**</td>
<td>15.66 ± 0.42**</td>
</tr>
<tr>
<td>GA, mmol/l</td>
<td>5.77 ± 0.29</td>
<td>9.87 ± 0.27*</td>
<td>7.02 ± 0.28**</td>
<td>8.07 ± 0.25**</td>
</tr>
<tr>
<td>SA, mmol/l</td>
<td>2.24 ± 0.16</td>
<td>3.10 ± 0.13*</td>
<td>4.33 ± 0.13**</td>
<td>7.64 ± 0.15**</td>
</tr>
<tr>
<td>NPBF, μmol/l</td>
<td>37.87 ± 0.63</td>
<td>74.32 ± 0.52*</td>
<td>64.84 ± 0.53**</td>
<td>80.45 ± 0.44**</td>
</tr>
<tr>
<td>CP, mmol/l</td>
<td>12.16 ± 0.33</td>
<td>14.58 ± 0.38*</td>
<td>15.40 ± 0.41*</td>
<td>22.38 ± 0.34**</td>
</tr>
<tr>
<td>α2-MG, μmol/l</td>
<td>2.42 ± 0.09</td>
<td>3.70 ± 0.09*</td>
<td>3.18 ± 0.07**</td>
<td>4.21 ± 0.05**</td>
</tr>
</tbody>
</table>

*: the difference in indicators is significant (P < 0.05) as compared with the group of practically healthy individuals; **: the difference in indicators is significant (P < 0.05) between group 1 and groups 2 and 3; ***: the difference in indicators is significant (P < 0.05) between groups 2 and 3.

Table 2. Indicators of the serum content of leptin, adiponectin and cytokeratin-18 in NASH patients with obesity and osteoarthritis comorbidities (M ± m)

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Practically healthy persons (n = 30)</th>
<th>OA with normal body weight (n = 30) 1 group</th>
<th>NASH + OB (n = 30) 2 group</th>
<th>NASH + OB+ OA (n = 30) 3 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin, ng/ml</td>
<td>10.05 ± 0.54</td>
<td>12.80 ± 0.60*</td>
<td>43.62 ± 0.55**</td>
<td>50.47 ± 0.71**</td>
</tr>
<tr>
<td>Adiponectin, ng/ml</td>
<td>8.42 ± 0.55</td>
<td>6.73 ± 0.50*</td>
<td>4.03 ± 0.21**</td>
<td>5.72 ± 0.41**</td>
</tr>
<tr>
<td>Cytokeratin 18, U/l</td>
<td>60.00 ± 2.29</td>
<td>124.53 ± 1.53*</td>
<td>266.50 ± 9.48**</td>
<td>318.80 ± 15.44**</td>
</tr>
</tbody>
</table>

*: the difference in indicators is significant (P < 0.05) as compared with the group of practically healthy individuals; **: the difference in indicators is significant (P < 0.05) between group 1 and groups 2 and 3; ***: the difference in indicators is significant (P < 0.05) between groups 2 and 3.
in the control group; in patients with OA and normal BMI, this indicator was 1.27 times higher compared to the PHP (P < 0.05) with a significant intergroup difference.

The analysis of serum adiponectin revealed a decrease in their content in all study groups. In individuals with NASH and morbid OB, this indicator was the lowest and significantly 2.09 times (P < 0.05) lower then in the PHP group. In combined course of NASH with OA and OB, the level of serum adiponectin was 1.47 times lower in comparison with the PHP (P < 0.05). In the group of patients with OA and normal BMI, this indicator was 1.25 times lower than in the PHP (P < 0.05) with a significant intergroup difference.

Analyzing the level of cytokeratin-18 in the comparison groups, we found that it 2.08 times exceeded the control indicators in the patients of group 1; in the patients of group 2, it was 4.44 times higher in the control group, and 5.31 times higher in the patients of group 3. That is, we can say that in NAFLD patients at the stage of NASH, especially in the case of comorbidity, plasma levels of cytokeratin-18 were significantly increased.

As a result of the correlation analysis between the indices of the CT metabolism, adipokines and cytokeratin-18, the following data were obtained: adiponectin content was directly correlated with the content of FOP (r = 0.47, P < 0.05) and GA (r = 0.42, P < 0.05). The indicators of leptin were directly correlated with the content of PBOP (r = 0.65, P < 0.05), SA (r = 0.66, P < 0.05), NPBF (r = 0.55, P < 0.05) and CP (r = 0.63, P < 0.05). It should be noted that the indices of cytokeratin-18 were directly correlated with the indices of CT metabolism, in particular with the content of PBOP (r = 0.72, P < 0.05), GA content (r = 0.43, P < 0.05) and SA (r = 0.72, P < 0.05), with the content of NPBF (r = 0.65, P < 0.05) and CP (r = 0.74, P < 0.05).

The optimal distribution points of the CK-18 biomarker for the diagnosis of liver steatosis in patients with OA and OB were determined using the ROC-analysis. The indicator had an area under the curve (AUC) of more than 0.8 (0.89), indicating the value of the selected biomarker (high quality model). Consequently, based on the performed ROC analysis, it has been proved that CK-18 is a sensitive marker associated with the presence of liver steatosis and can be used for early diagnosis of NASH in patients with OA and OB.

**Discussion**

According to the study results, an increase in the synthesis of collagen accompanied by a decrease in the processes of newly formed collagen resorption was found in NASH patients, while in OA, there was a catabolic activation of the extracellular matrix structural components induced by proteolytic enzymes overexpression. Thus, there was a degradation of collagen fibrils consisting mainly of type I collagen. In particular, these processes could be activated as a result of adipokines action, which are secreted by adipose tissue and affect the functions of cartilage and bone tissue.

High serum levels of GA and SA in NASH patients, especially in OA and OB comorbidities, could contribute to so-called collagen cementation in ECM reducing the probability of its resorption.

The obtained data indicate that there was a significant increase in the synthesis of collagen and glycosaminoglycans in NASH patients with OB and OA, which was accompanied by ineffective resorption of newly formed collagen due to inhibition of collagenolytic activity of blood plasma in NASH, arising from activation of proteinase inhibitors (α2-MG), a significant imbalance in the metabolic system of CT, which, particularly in OA and OB comorbidities, leads to progressive fibrosis of the liver and its functions impairment.

The combined course of NASH, OA and OB was characterized by adipokine imbalance, which manifested itself in the increase in leptin serum level and the decrease in adiponectin.

Cytokeratin-18 (CK-18) is one of the most promising markers for diagnosis of NAFLD. It is believed that apoptosis of hepatocytes plays a crucial role in NAFLD progression, liver fibrosis and cirrhosis formation. In the development of apoptosis of hepatocytes, caspases, which cleave cytokeratin-18, are generally activated [8]. Numerous studies have shown that these cleaved fragments can be detected using monoclonal antibodies.

CK-18 is the main cytoskeletal protein of hepatocytes and other epithelial cells. It has been previously reported as circulating mechanistic indicator of cell death [6]. Two forms of CK-18 can be observed in blood plasma: the full-length form (CK-18 M65) and the caspase-cleaved form (CK-18 M30). The CK-18 M65 is used to assess necrosis, while the CK-18 M30 is used specifically to evaluate the rate of apoptosis [4].

The accumulated data indicate that hepatocyte apoptosis plays an important role in chronic liver disease [13]. By a meta-analysis of eight case-controlled studies, the researchers found that plasma levels of CK-18 could be a significant risk factor for NASH, chronic hepatitis C (CHC) and chronic hepatitis B (CHB) [12]. Some reports also showed that plasma levels of the CK-18 M30 were associated with NASH progression in obese patients [9].

The above data suggest that adipokines and cytokeratin-18 are sensitive markers of hepatocyte apoptosis, CT imbalance, and, consequently, the progression of liver fibrotic changes.

**Conclusions**

In patients with NASH and morbid OB, a significant increase in collagen and glycosaminoglycans synthesis was observed. It was accompanied by an ineffective resorption of newly formed collagen due to collagenolytic activity of blood plasma inhibition in NASH resulted from activation of proteinase inhibitors (α2-MG), significant imbalance in the CT metabolism, which was worsened by accompanying OA leading to progressive liver fibrosis and dysfunction.

Taking into account that adiponectin was found to be positively correlated with the insulin sensitivity indices, active in body weight regulation, proinflammatory agent in joint diseases and involved in degradation of articular cartilage matrix, the adipokine deficiency, found in the work, may play a significant pathogenic role in the development and progression of NASH as well as OB and OA.

Thus, the blood level of adipokines and CK-18 not only depends on body weight, but also reflects the risk for occurrence of nosologies associated with OB. Besides, leptin and adiponectin may serve as sensitive risk markers for
comorbid diseases development and could be candidates for their measurements inclusion in the diagnostic algorithm for NASH, OA, OB and their combination.

**The prospect of further research.** The study of the CT metabolism, the content of adipokines and CK-18 under the influence of various hepatotropic drugs in NASH with OB and OA comorbidities.

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**Conflicts of interest:** authors have no conflict of interest to declare.

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Оригинальные исследования