Non-alcoholic fatty liver disease (NAFLD) remains the leading nosology among hepatic diseases around the world. Its complex pathogenesis is not still clearly understood. Besides metabolic changes one of potential mechanisms of NAFLD development is changes in hepatokines, fetuin-A in particular, secretion and metabolism.

**Objective** — to determine the role of fetuin-A in the development and progression of NAFLD.

**Materials and methods.** The study enrolled 78 NAFLD patients with metabolic disorders and 30 volunteers as a control group. Additionally to routine examination serum concentration of fetuin-A was determined. Statistical processing was carried out using SPSS 21.

**Results.** The results showed a statistically significant increase in fetuin-A concentrations in NAFLD patients with concomitant MS (p < 0.05). There was a direct correlation between fetuin-A levels and parameters of abdominal (WC and WHR, p < 0.05) and visceral obesity (V AT %, p < 0.05). Concentrations of fetuin-A were higher in polymorbid patients, especially in the combination of NAFLD with type 2 diabetes mellitus and obesity. Fetuin-A levels was associated with changes in carbohydrates and lipids parameters. Fetuin-A positively correlated with glycated hemoglobin (r = 0.32, p < 0.05) and index HOMA (r = 0.36, p < 0.05). There was a positive correlation between fetuin-A level and TG (r = 0.44, p < 0.05) and LDL cholesterol (r = 0.37, p < 0.05). Furthermore, an increase in fetuin-A was associated with an increase in activity of ALT (r = 0.39, p < 0.05) and elevation of the level of TNF-α (r = 0.33, p < 0.05). NAFLD patients with advanced stages of steatosis and fibrosis also demonstrated higher levels of fetuin-A (p < 0.05).

**Conclusions.** The obtained data indicate a significant pathogenetic role of fetuin-A in the development and progression of NAFLD.

**Keywords:** non-alcoholic fatty liver disease, fetuin-A, hepatokine.
autocrine, paracrine and endocrine signaling. Fetuin-A and B have the most significant influence on the regulation of metabolic processes. Fetuin-A is a multifunctional protein that is synthesized in the liver and secreted into the circulation. It is an endogenous inhibitor of insulin receptor tyrosine kinase in skeletal muscles and liver, leads to the development of insulin resistance. Fetuin-A acts as an endogenous ligand of Toll-4-like receptors, thus enhancing both insulin resistance and inflammation [12]. High levels of fetuin-A are associated with insulin resistance, atherogenic dyslipidemia, high levels of pro-inflammatory cytokines and reduced ones of adiponectin.

In humans, fetuin-A is considered a potentially important link between obesity and insulin resistance [11]. Fetuin-A concentrations correlate with fat accumulation in the liver and are increased in individuals with insulin resistance [7]. Higher serum levels of fetuin-A are associated with an increase in visceral adipose tissue, the main component of MS. On the other hand, a reduction in elevated fetuin-A levels was observed in individuals following exercise- and diet-induced weight loss [14]. The results of recent epidemiologic studies have proven the relationship between serum fetuin-A content and insulin resistance and diseases that develop on its background, namely MS and type 2 diabetes [4]. The direct relationship between fetuin-A levels and insulin resistance was confirmed in a large heterogeneous group of subjects using the HOMA-IR index to control insulin sensitivity. It is important to note the absence of a modulating effect of glucose level on the correlation of fetuin-A levels with insulin resistance [15]. The above suggests the possibility of other ways of implementing the relationship between fetuin-A and insulin resistance beyond glucose dysregulation.

Fetuin-A induces low-grade inflammation, inhibits adiponectin production in animals and humans, and may potentially affect visceral adipose tissue activity.

Objective — to determine the role of fetuin-A in the development and progression of NAFLD.

Materials and methods

The study included 78 patients with NAFLD and metabolic disorders and 30 volunteers as a control group who were examined on the basis of the department of gastroenterology and therapy and the outpatient clinic of the G1 «L. T. Mala National Institute of Therapy of the National Academy of Medical Sciences of Ukraine». Females predominated in both studied groups. The mean age of examined NAFLD patients was (54.6 ± 11.7) years.

Assessment of anthropometric parameters included height measurement and determination of body weight with calculation of body mass index (BMI). Adipose tissue distribution was assessed by measuring waist circumference (WC) and hip circumference (HC) with calculation of the waist-to-hip ratio (WHR). To carry out biochemical studies, blood was taken from patients in the morning from the ulnar vein after a 12-hour fast. All patients were assessed for the functional state of the liver, carbohydrate metabolism and lipid metabolism.

To study the body composition of patients (determining the total percentage of fat in the body, the percentage of visceral adipose tissue (VAT)) we used an electronic device — the Omron BF-511 body composition monitor scale. To determine the dysfunction of the VAT, the visceral adiposity index (VAI) was calculated according to the method of M. C. Amato [2].

Determination of serum C-reactive protein (CRP) levels was carried out by the enzyme-linked immunosorbent assay with the use of reagent kits hs-CRP Elisa kit — DRG International Inc. The serum level of TNF-α was determined using the Elisa-TNF-alpha reagent kit.

The serum concentration of fetuin-A was determined by the enzyme-linked immunosorbent assay method using the Fetuin-A Elisa reagent kit (manufactured by IBL, Germany).

The degree of steatosis (S1 — 1 steatosis degree, S2 — 2 steatosis degree, S3 — 3 steatosis degree) was assessed by determining the wave attenuation coefficient (WAC). Fibrosis stage (F0 — absence of fibrotic changes, F1 — 1 stage, F2 — 2 stage, F3 — 3 stage, F4 — cirrhosis) was assessed by shear wave elastometry (SWE), respectively.

Statistical processing was carried out using SPSS 21 for Windows XP using methods of primary descriptive statistics, Student’s t-test for dependent and independent samples, correlation analysis. The dependence of the indicator on the group was studied using Spearman correlation coefficient.

Results and discussion

NAFLD patients demonstrated statistically significant increase in serum levels of fetuin-A compared to the control group (0.86 ± 0.13 g/L vs 0.53 ± 0.05 g/L, p < 0.05). The obtained data may indicate the possible participation of this hepatokine in the pathogenesis of various stages of development and progression of NAFLD.

Therefore, we analyzed the serum levels of fetuin-A in patients with NAFLD on the background of MS, depending on the degree of abdominal obesity. The data are shown in Fig. 1.

The analysis of the results of the study showed that NAFLD patients showed a tendency to
elevation in fetuin-A concentration with an increase in body weight, but statistically significant changes in fetuin-A levels were found only for NAFLD patients with morbid obesity.

Subsequently, the relationship between the level of fetuin-A and anthropometric indices was evaluated (Table 1).

NAFLD patients showed a direct correlation between the level of fetuin-A and the parameters of abdominal (WC and WHR) and visceral obesity (VAT).

The data presented above suggest that fetuin-A takes part in the formation of an important component of MS, namely obesity, which can potentially contribute to the development of hepatic steatosis.

Our findings are supported by modern clinical studies, which show that a high-fat diet and overweight lead to an excess of free fatty acids and cause hepatocytes and adipocytes to secrete fetuin-A, which can enhance the infiltration of macrophages into adipose tissue [8].

Table 1. Fetuin-A correlations with anthropometric parameters in NAFLD patients

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Spearman’s coefficient</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>WC</td>
<td>0.41</td>
<td>0.0459</td>
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<tr>
<td>HC</td>
<td>0.18</td>
<td>0.0520</td>
</tr>
<tr>
<td>WHR</td>
<td>0.36</td>
<td>0.0343</td>
</tr>
<tr>
<td>VAT</td>
<td>0.44</td>
<td>0.0417</td>
</tr>
</tbody>
</table>

It is known that the activity of adipose tissue, and not its total mass, plays a significant pathogenetic role in the progression of NAFLD. Therefore, we analyzed the level of fetuin-A depending on VAI.

The obtained data are shown in Fig. 2.

According to the obtained data, the activation of VAT was accompanied by an increase in serum concentrations of the regulatory hepatokine fetuin-A, which confirms its trigger role in the development and progression of NAFLD. In the group of patients with high and moderate activity of VAT, a probable increase of this indicator was observed in comparison with the group of patients with a low level of VAI.

The general pathogenetic links of the development and progression of NAFLD have complex mechanisms and depend on the contribution of the constituent components of MS, in particular type 2 diabetes, obesity, hypertension, and others, which can combine and potentiate each other.

The concentration of fetuin-A in NAFLD patients was analyzed depending on the number of MS components (Fig. 3).

It was shown that higher levels of fetuin-A were observed in polymorbid patients. As the number of components of MS increased, there was a tendency to an increase in the concentration of fetuin-A in comparison with NAFLD patients without metabolic disorders. However, there were no significant intergroup differences, which may be due not so much to the amount, but to the contribution of a specific component of MS to the imbalance of metabolic processes in the body of NAFLD patients. In particular, we analyzed the contribution of various components of MS to changes in the secretion of hepatokine A. According to the obtained data, the greatest contribution to the increase in the secretion of fetuin-A is made by diabetes mellitus 2, especially in obese patients. Higher levels of fetuin-A in patients with NAFLD in combination with diabetes, relative to isolated NAFLD, suggest the involvement of this hepatokine in the regulation of carbohydrate metabolism. In addition, clinical and
experimental studies have confirmed the hypothesis of a relationship between NAFLD and fetuin-A. In addition, it is well known that fetuin-A is significantly increased in hepatocellular injury and mediates insulin resistance as well as reduced glucose tolerance [13]. Therefore, we studied the relationship between the level of fetuin-A and the parameters of carbohydrate and lipid metabolism in patients with NAFLD on the background of MS. The results of the study are shown in Table 2.

The conducted studies did not reveal a relationship between fasting glycemia and insulin with the level of fetuin-A. However, a direct correlation between fetuin-A and the level of glycated hemoglobin and the HOMA-IR index was not statistically significant but had the character of a trend.

Disorders of lipid metabolism also play one of the key roles in the formation of metabolically associated diseases. Therefore, we analyzed the association of fetuin-A levels in blood plasma with indicators of lipid metabolism. The results are shown in Table 3.

The results of the correlation analysis demonstrated a weak direct relationship between the level of fetuin-A and indicators of lipid metabolism (TG, LDL). The obtained data suggest that fetuin-A has an effect on the development of dyslipidemia, which leads to the launch of a cascade of metabolic reactions with subsequent accumulation of lipids in the liver tissue. Similar data were obtained in the work of Verras et al., which demonstrated significant positive correlation of serum concentration of fetuin-A with TG, even after adjustment for gender and age, and this is consistent with the results of other studies [16].

Also, recent studies have shown some mechanisms of the relationship between fetuin-A and NAFLD. In particular, the study [9] showed that an increase in VAT leads to an excess release of fatty acids, which stimulate the secretion of fetuin-A by hepatocytes and adipocytes. Increased production of hepatokine A stimulates chemotaxis and penetration of macrophages into adipose tissue [8]. In this regard, active macrophages increase the secretion of inflammatory cytokines, such as TNF-α and IL-6, which can further worsen hepatocyte steatosis and the development of inflammation in liver tissue.

Therefore, we performed a correlational analysis of the relationship between fetuin-A and markers of hepatocellular damage and systemic inflammation. The results are shown in Table 4.

When comparing the levels of pro-inflammatory markers and cytolysis enzymes of hepatocytes and fetuin-A, a moderate direct correlation with the level of TNF-α and ALT was revealed. We did not observe the dependence of the level of fetuin-A on the activity of AST, GHT and CRP. The obtained data may indicate a possible predictive role of fetuin-A in the development of steatohepatitis in patients with NAFLD on the background of MS.

The increase in the levels of this hepatokine is the basis for further analysis of the relationship between fetuin-A and the main pathogenetic factors of the development and progression of NAFLD.
Fetuin-A levels were determined in the examined patients depending on the degree of steatosis, the data are shown in Fig. 4.

According to the obtained data, as the degree of liver steatosis progressed, an increase in fetuin-A levels was observed, which confirms our hypothesis about the involvement of this hepatokine in the processes of triggering a cascade of inflammatory reactions in liver tissue and metabolic disorders, which leads to increased accumulation of fat in hepatocytes. In patients with steatosis of the 2nd and the 3rd degree, statistically significant differences in the level of fetuin-A were observed comparing to patients with minimal signs of steatosis (p < 0.05).

We analyzed the role of hepatokine in the development and progression of liver fibrosis in patients with NAFLD in combination with MS (Fig. 5).

The formation of liver fibrosis in patients with NAFLD was accompanied by a significant increase in fetuin-A concentration, compared to the group without fibrotic changes. As the degree of fibrosis increased, a gradual increase in the level of this hepatokine was observed. Fetuin-A levels were highest in patients with severe stages of fibrosis. However, no significant differences between the groups were found.

**Conclusions**

The statistically significant increase in fetuin-A concentrations was observed in NAFLD patients on the background of MS compared to the control group (p < 0.05). A direct correlation between fetuin-A levels and parameters of abdominal (WC and WHR) and visceral obesity (VAT %) in the examined patients was revealed, which confirms its trigger role in the development and progression of NAFLD.

In polymorbid patients, especially in the combination of NAFLD with type 2 diabetes mellitus and obesity, a higher secretion of fetuin-A was observed compared to isolated NAFLD. The influence of fetuin-A on the development of dyslipidemia and insulin resistance, which are key links in the pathogenesis of NAFLD, was revealed.

A direct correlation between fetuin-A and the activity of ALT and the level of TNF-α was revealed, which may indicate the possible involvement of this hepatokine in the formation of inflammatory processes in liver tissue and the progression of NAFLD.

In patients with NAFLD, higher levels of fetuin-A were observed as the degree of liver steatosis increased. In patients with steatosis of the 2nd and the 3rd degrees, statistically significant changes in the level of fetuin-A were observed, compared to patients with minimal signs of steatosis (p < 0.05).

The formation of liver fibrosis in patients with NAFLD was accompanied by a significant increase in fetuin-A levels, compared to the group without fibrotic changes.

The obtained data indicate a significant pathogenetic role of fetuin-A in the development and progression of NAFLD.

**Table 4. Fetuin-A correlation with cytolytic enzymes and pro-inflammatory cytokines in NAFLD patients with MS**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Spearman’s coefficient</th>
<th>p</th>
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<tbody>
<tr>
<td>CRP</td>
<td>0.23</td>
<td>0.0611</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.39</td>
<td>0.0420</td>
</tr>
<tr>
<td>ALT</td>
<td>0.33</td>
<td>0.0380</td>
</tr>
<tr>
<td>AST</td>
<td>0.26</td>
<td>0.0650</td>
</tr>
<tr>
<td>HGT</td>
<td>0.17</td>
<td>0.0731</td>
</tr>
</tbody>
</table>

*The difference from the group S1 is statistically significant (p < 0.05).*

**Figure 4. Fetuin-A concentrations in NAFLD patients depending on the degree of liver steatosis**

**Figure 5. Indicators of fetuin-A in patients with NAFLD depending on the degree of liver fibrosis**

**Conflicts of interest: none.**

The authors have contributed equally to conception and design, acquisition and interpretation of data, drafting the article.
Патогенетична роль фетуїну-А у формуванні метаболічних порушень та системного запалення у хворих на неалкогольну жирову хворобу печінки

Неалкогольна жирова хвороба печінки (НАЖХП) є провідною нозологією серед захворювань печінки у світі. Її складний патогенез остаточно не вивчено. Окрім метаболічних змін, одним з потенційних механізмів розвитку НАЖХП є зміни секреції та метаболізму гепатокінів, зокрема фетуїну-А.

Мета — визначити роль фетуїну-А у розвитку та прогресуванні неалкогольної жирової хвороби печінки.

Матеріал та методи. У дослідження було залучено 78 хворих на НАЖХП з метаболічними порушеннями та 30 добровольців як контрольну групу. Додатково до планового обстеження визначали сироватку концентрацію фетуїну-А. Статистичну обробку проводили за допомогою SPSS 21.

Результати. Проаналізовано 215 даних у хворих на НАЖХП з метаболічними порушеннями та 65 у добровольців. Визначено, що концентрація фетуїну-А була вищою у поліморбідних пацієнтах, особливо при поєднанні НАЖХП з цукровим діабетом 2 типу та ожирінням. Рівні фетуїну-А були асоційовані зі змінами параметрів вуглеводного і ліпідного обміну.

ОРИГІНАЛЬНІ ДОСЛІДЖЕННЯ

References

індексу НОМА \((r=0,36; p<0,05)\). Визначена позитивна кореляція між рівнем фетуїну-А та ТГ \((r=0,44; p<0,05)\) і холестерином ЛПНЩ \((r=0,37; p<0,05)\). Крім того, підвищення фетуїну-А асоціювалося зі збільшенням активності АЛТ \((r=0,39; p<0,05)\) і підвищенням рівня TNF-\(\alpha\) \((r=0,33; p<0,05)\). Пацієнти з НАЖХП із пізніми стадіями стеатозу та фіброзу також продемонстрували вищі рівні фетуїну-А \((p<0,05)\).

**Висновки.** Отримані дані свідчать про значну патогенетичну роль фетуїну-А у розвитку та прогресуванні НАЖХП.

**Ключові слова:** неалкогольна жирова хвороба печінки, фетуїн-А, гепатокін.

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