The results of study of 53 patients with NAFLD were presented in the article. It was revealed the association of SNPs in the gene PNPLA3 rs738409 with the development of NAFLD in patients of Uzbek nationality. It also provides reliable pathogenetic link the risk allele G with the progression of NAFLD. For patients with NAFLD and G allele is useful to add pentoxifylline on the background of the basic therapy.

**Keywords:** Non-alcoholic fatty liver disease, gene polymorphism, metabolites of nitrogen oxide

**UDC:** 616.36-002-036.12-085.2-092.4

**Introduction**

Non-alcoholic fatty liver disease (NAFLD) is a common cause of chronic liver disease and has emerged as an important global health problem (Adams, Angulo, and Lindor 2005). The reported prevalence of NAFLD in the general population is as high as 35% (Cheung and Sanyal 2010). NAFLD is a spectrum ranging from simple steatosis (fat in the liver) to non-alcoholic steatohepatitis (NASH - fat with inflammation and/or fibrosis) and cirrhosis. NAFLD represents the hepatic component of the metabolic syndrome and is strongly associated with obesity and insulin resistance (Vanni, Bugianesi, Kotronen, Minicis, Yki-Jarvinen, and Svegliati-Baroni, 2010). Genetic factors have been shown to play a significant role in the pathogenesis of NAFLD (Day, 2010). A familial aggregation study comparing overweight children with NAFLD and overweight children without NAFLD reported that fatty liver was more significant in siblings of children with NAFLD.

Adiponutrin encoded by *PNPLA3* has been reported to have both lipolytic and lipogenic properties; however, the exact function of adiponutrin is unknown. A common variant of *PNPLA3* gene (rs738409) has been reported to be associated with hepatic fat content in the Dallas Heart Study of different ancestries; Hispanics, European Americans and African-Americans. The Hispanics bear the highest propensity to develop NAFLD followed by the European Americans with African-Americans being the group with the lowest risk of NAFLD. Since then, several other studies in different populations have been conducted (Cox, Wing, Carr, Hightower, Smith, Xu et al., 2011; Hotta, Yoneda, Hyogo, Ochi, Mizusawa, Ueno et al., 2010; Kantartzis, Peter, Machicao, Machann, Wagner et al. 2009; Lin, Chang, Hu, Yang, Chang, and Ni, 2011; Romeo, Kozlitina, Xing, Pertsomilidis, Cox, Pennacchio et al., 2010).

The aim of study was to investigate association of *PNPLA3* rs738409 with the development and progression of NAFLD in patients of Uzbek nationality and assessment of the effectiveness of pharmacological correction.

**Material and methods**

53 patients in age 27-69 (mean 51.4 ±1, 27 years) with NAFLD were included in investigation. The study of the endothelial dysfunction (ED) was carried out measurements of the NO2 and NO3 in the blood serum. All patients were administered identification of single nucleotide polymorphisms (SNPs) in the gene PNPLA3 rs738409. Depending on the results of genotyping (C, G, C/G alleles) all the patients were divided into 3 groups. The control group amounted to 15 healthy volunteers by gender and age,
corresponding with the study. Patients administered ursodeoxycholic acid (UDCA) in doses of 15 mg/kg/day for 3 months. The results were evaluated before treatment, 1 and 3 months after treatment.

The results of genotyping identified the presence of SNPs in the gene PNPLA3 rs738409 allele of the C, G, C/G in patients with NAFLD. All of the examined were divided into three groups. I group consisted of 17 patients (8 men and 9 women) with NAFLD, with C allele, II group consisted of 16 patients (10 men and 6 women) with NAFLD, with G allele, III group-18 patients (8 men and 10 women) with identified C/G allele.

**SAMPLES OF RESTRICTION**

<table>
<thead>
<tr>
<th>K</th>
<th>C</th>
<th>G</th>
<th>C</th>
<th>C/G</th>
<th>C/G</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image.jpg" alt="Image of gel electrophoresis" /></td>
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</tr>
</tbody>
</table>

Note: K-control, C - allele, G - allele, C/G allele

Diagnostics of ED has revealed in patients with NAFLD significant increase of the nitrogen oxide metabolites in blood serum (Table 1), which was correlated with the alleles of the gene. So, in the presence of the C allele's amount of nitrogen oxide metabolites (NO2 and NO3) compared with those of healthy control was increased by 32.3% and 62.5% (p<0.05). In patients with the presence of C/G allele increase in NO2 totaled 74.2%, and NO3 has been increased more than 2 times (p<0.001). It has also been established that in the presence of G allele the number of NO2 was at 93.5% (p<0.05) more, and NO3 more than in 3 times in comparison with the indicators of healthy control. The analysis of the content of nitric oxide metabolites in blood serum of patients with NAFLD, depending on the duration of the disease, gender and the age of the patients, statistically significant differences are not revealed.

Thus, the carried out researches have shown, that in patients with NAFLD in blood serum, there was an increase in end-nitrogen oxide metabolites. The amount of nitrogen oxide metabolites were higher in patients with G allele, compared with the other groups.

**Table 1. The content of nitrogen oxide metabolites (NO2 and NO3, µmol/L) in the blood serum of patients with NAFLD before treatment**

<table>
<thead>
<tr>
<th></th>
<th>Healthy control</th>
<th>I group (CC)</th>
<th>II group (GG)</th>
<th>III group (C/G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO2</td>
<td>0.31± 0.02</td>
<td>0.41± 0.02*</td>
<td>0.60± 0.03*</td>
<td>0.54± 0.03*</td>
</tr>
<tr>
<td>NO3</td>
<td>0.24± 0.02</td>
<td>0.39± 0.03*</td>
<td>0.64± 0.04*</td>
<td>0.53± 0.03*</td>
</tr>
</tbody>
</table>

Note: *- difference significant against figure of healthy control (p<0.05)
After the monthly UDCA therapy we revealed the reduction of nitrogen oxide metabolites, however, in the 2 group of significant changes have not been noted, in this connection the patients with the presence of the G allele additionally was administered pentoxifylline 800 mg/day for 2 months.

As has been seen from the obtained results of the research, conducted by the treatment of patients with NAFLD contributed to the reduction of the content of final nitrogen oxide metabolites in blood serum (Table 2). So, in the first group the number of nitrogen oxide metabolites was reduced by 24% and 34.4% as compared to before treatment. In group II, who in addition took pentoxifylline we found a reduction by NO\textsubscript{2} 76.5% and NO\textsubscript{3} in 2.4 times. In group III decrease of NO\textsubscript{2} was by 45.2%, and NO\textsubscript{3} - in 1.7 times. Thus, the treatment contributed to the significant reduction of nitrogen oxide metabolites.

<table>
<thead>
<tr>
<th></th>
<th>I group</th>
<th>II group</th>
<th>III group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO\textsubscript{2} (µ mol/L)</td>
<td>0.41± 0.02</td>
<td>0.60± 0.03</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.33± 0.03*</td>
<td>0.34± 0.03*</td>
</tr>
<tr>
<td></td>
<td>NO\textsubscript{3} (µ mol/L)</td>
<td>0.39± 0.03</td>
<td>0.64± 0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.29± 0.03*</td>
<td>0.27± 0.03*</td>
</tr>
</tbody>
</table>

Note: *- difference significant against figure before treatment (p<0.05)

Discussion

On the basis of the received data, ED, perhaps, is a predictor of progression of the pathology of the liver and the intensification of vascular disorders. Taking into account the need to recognize the independent existence of such a trigger mechanism of the formation of NAFLD, as endothelial dysfunction (along with IR), the basic and pathogenetically grounded approach in treatment of this disease can be considered use of the preparation of bile acids - UDCA. The medication acts in the role of endothelial corrector as at the expense of direct and indirect actions on the endothelium.

Conclusion

Thus, as a result of research it was revealed the association of SNPs in the gene PNPLA3 rs738409 with the development of NAFLD in patients of Uzbek nationality. It also provides reliable pathogenetic link the risk allele G with the progression of NAFLD. For patients with NAFLD and G allele is usefully to addition pentoxifylline on the background of the basic therapy. Further study of the ED and ways of its correction with the consideration of the genetic polymorphism are promising for the development of an integrated speed multi-component therapy programs NAFLD.

References


