LIVER FIBROSIS AND METABOLIC CHANGES IN NON-ALCOHOLIC FATTY LIVER DISEASE: MODERN APPROACHES TO DIAGNOSTICS

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Abstract. *This study is devoted to the assessment of metabolic changes and the degree of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD), including steatosis of varying degrees. The study included 90 patients with NAFLD and 45 people in the control group.*

Research methods: clinical and laboratory methods, ultrasound elastometry (FibroScan), DEBQ questionnaire to identify eating disorders, molecular genetic analysis of the expression level of microRNA-221.

Key words: *Non-alcoholic fatty liver disease (NAFLD), liver fibrosis, FibroScan, microRNA-221, DEBQ questionnaire, liver enzymes, triglycerides, ALT/AST ratio.*

Introduction. Currently, non-alcoholic fatty liver disease (NAFLD) occupies a leading position among liver diseases and is considered as one of the most common chronic liver lesions globally [4]. The term NAFLD unites a wide range of liver pathologies, including various clinical and histological forms, such as fatty liver infiltration (steatosis), non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis. Liver steatosis most often has a favorable course and is not associated with an increased risk of mortality, but its progression, especially when turning into steatohepatitis, significantly increases the likelihood of developing cirrhosis and liver failure [7]. Population studies suggest that 60% to 80% of cases of cryptogenic liver cirrhosis are a consequence of non-alcoholic steatohepatitis (NASH)[3].

The main risk factors for NAFLD are obesity and overweight [8]. The incidence of hepatic steatosis in morbidly obese patients who have undergone bariatric treatment reaches 90%. A significant percentage of patients with type 2 diabetes mellitus (T2DM) suffer from NAFLD, the rate is about 69%. Half of the patients who have liver changes characteristic of fatty degeneration also have hyperlipidemia, mainly due to increased triglyceride levels. The development of NAFLD is influenced by demographic parameters such as age, gender, and ethnicity. Among the risk factors for disease progression, the most significant are: age over 45 years, obesity (BMI> 30), the presence of T2DM, hypertension, elevated triglycerides, and an ALT/AST ratio greater than one. Pathological obesity is almost always accompanied by hepatosis (in 95-100% of cases), and in 20-47% of cases by steatohepatitis.

Despite the high prevalence of NAFLD, more than 95% of cases (at any stage of the disease) remain undiagnosed, as shown by data from multicenter studies [5,2]. This is primarily due to the nonspecificity of the clinical picture of the disease. In many patients, the main manifestation is asthenovegetative syndrome [1,6]. The diagnostic process should include a comprehensive assessment of all aspects of metabolic syndrome. It is especially important to examine individuals with obesity, type 2 diabetes mellitus, and incidentally detected elevated liver enzymes in the presence of metabolic risk factors. During the diagnostic process at the stage of primary health care, it is important not to limit oneself to stating the fact that the patient has signs of liver steatosis and moderate hepatomegaly, but to continue the examination in order to exclude complications, select the necessary goal, and set priorities.

The aim of this study was to identify markers of liver fibrosis at different stages of NAFLD and to evaluate their relationship with clinical and biochemical parameters in patients with this disease.

Material and methods of research. In accordance with the objectives of the study, 90 patients diagnosed with non-alcoholic fatty liver disease were monitored during 2023-2024. All the data obtained were recorded in specially developed medical records. The study was conducted among patients undergoing inpatient treatment at the general therapy department of the multidisciplinary clinic of the Tashkent Medical Academy, based on the consent of the patients. Patients with non-alcoholic fatty liver disease were divided into 3 groups: out of 90 patients with non-alcoholic fatty liver disease, 26 patient had grade 1 steatosis according to ultrasound examination, 52 patient had grade 2 and 8 patient had grade 3 hepatic steatosis.The average age of patients in all 3 groups was approximately 57.6 years. The control group included 45 healthy people aged 57±10 years. There was no statistically significant difference in age between the groups. The following parameters were determined in the patients examined to assess the functional state of the liver and biliary tract: total bilirubin level, direct bilirubin, alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST), albumin, total protein, C-reactive protein (CRP), glucose content, on a Mindray BS-380 (Germany) automatic analyzer, lipid profile on a Mindray BS-88A (Germany) analyzer, complete blood count on a Mindray BS-5000 (Germany) analyzer.

Ultrasound elastometry (liver fibroscanning) was used as a non-invasive method to determine the degree of liver fibrosis using the FibroScan®502 Touch device. Patients were administered the DEBQ (Dutch Eating Behavior Questionnaire) to identify eating disorders.

Molecular genetic research methods include determination of the expression level of MicroRNA-221, extraction of microRNA using PCR and detection of changes in the expression of microRNA-221 using 0.75 ml of Trizol reagent. RNA isolation was performed using the “Ribo prep” kit (Russia). After the reverse transcription reaction, the levels of MicroRNAs were determined relative to U6 snRNA and presented in accepted units. Amplification was performed on the Rotor Gene Q (Qiagen, Germany) equipment.

Results and discussion. Patients with a mean age of 57.00±16.5 years with a diagnosis of NAFLD participated in the study. Men accounted for 45.6%, women for 54.4%. The mean BMI in the group of patients with non-alcoholic fatty liver disease was 28.1±5.8 kg/m^2 in 1 group, 28.9±5.9 kg/m^2 in 2 group, 29.0±2.0 kg/m^2 in 3group and 6 patients(6.6%) with normal body weight, 43 (47.7%) patients with excess body weight, 31 (34.4%) patients with obesity 1 degree, 9 (10%) patients with obesity 2 degree and 1 (1.1%) patient with obesity 3 degree.

When analyzing risk factors in patients with non-alcoholic fatty liver disease, smoking was identified in 2 (2.2%) patients from harmful habits.

During the study, the following diseases were identified among those associated with non-alcoholic fatty liver disease: hypercholesterolemia 25 (27.8%), type 2 diabetes mellitus 21 (23.3%), coronary heart disease 54 (60%), arterial hypertension 77 (85.6%), vegetative-vascular dystonia 17 (18.8%), chronic gastritis 21 (23.3%), gallstone disease 38 (42.2%), chronic pyelonephritis 79 (87.8%), chronic pancreatitis 13 (14.4) patients. **(Table 1).**

Table 1.

Frequency of co-occurrence of co-morbidities

Associated diseases	Category	Abs.	%	95% CI
Gender	Male	49	54,4	43,6 – 65,0
	Female	41	45,6	35,0 – 56,4
Smoking	Available	2	2,2	0,3 – 7,8
	Not available	88	97,8	92,2 – 99,7
Arterial hypertension	Available	77	85,6	76,6 – 92,1
	Not available	13	14,4	7,9 – 23,4
Coronary heart disease	Available	54	60,0	49,1 – 70,2
	Not available	36	40,0	29,8 – 50,9

Type 2 diabetes mellitus	Available	17	18,9	11,4 – 28,5
	Not available	73	81,1	71,5 – 88,6
Chronic pyelonephritis	Not available	11	12,2	6,3 – 20,8
	Available	79	87,8	79,2 – 93,7
Chronic gastritis	Not available	69	76,7	66,6 – 84,9
	Available	21	23,3	15,1 – 33,4
Gallstone disease	Available	38	42,2	31,9 – 53,1
	Not available	52	57,8	46,9 – 68,1
Chronic pancreatitis	Not available	77	85,6	76,6 – 92,1
	Available	13	14,4	7,9 – 23,4
Hypercholesterolemia	Available	25	27,8	18,9 – 38,2
	Not available	65	72,2	61,8 – 81,1

The results of clinical and biochemical parameters according to the stages of steatosis according to the ultrasound are presented. Total bilirubin (p = 0.001), urea (p = 0.033) and AST/ALT (p = 0.001) ratio were statistically significantly higher in the NAFLD groups compared to the control group. No significant differences were observed in other parameters, which indicates that some parameters of liver function change significantly depending on the stage in NAFLD. (Table 2)

Table 2.

Results of the main indicators of blood biochemical analysis in patients under investigation

Indicators	**Groups**				**p**
	NAFLD 1st.	NAFLD 2st	NAFLD 3st	Control group	
C-reactive protein (mg/l), Me [IQR]	18,5 [13,5; 22,8]	20,0 [13,5; 24,5]	19,0 [12,0; 26,0]	7,8 [6,4; 9,2]	0,290
Total protein (g/l), Me [IQR]	73,2 [68,9; 77,9]	74,2 [69,7; 81,0]	76,9 [75,2; 78,7]	76,0 [72,4; 80,4]	0,794
Total bilirubin (mkmol/l), Me [IQR]	16,6 [14,8; 19,7]	16,2 [13,8; 19,1]	19,1 [18,8; 19,3]	16,5 [14,6; 19,7]	$< 0,001^{*}p_{\text{control group – NAFLD1st.}} = 0,012 p_{\text{control group – NAFLD 2st.}} = 0,002$
Urea (mmol/l), Me [IQR]	5,8 [5,8; 5,9]	6,2 [5,3; 7,3]	6,6 [5,8; 7,6]	5,5 [5,3; 5,8]	$0,033^{*}p_{\text{control group}}$ – NAFLD 1st. = 0,020
Creatinine (mkmol/l), Me [IQR]	80,0 [74,8; 88,5]	82,6 [72,5; 90,8]	80,0 [77,6; 82,4]	82,9 [72,5; 91,2]	0,932
ALT (ed/l), Me [IQR]	24,0 [21,5; 26,8]	28,0 [24,5; 37,0]	26,0 [25,5; 26,5]	25,0 [21,0; 31,0]	0,237
AST (eed/l), Me [IQR]	14,0 [13,0; 19,0]	17,0 [14,5; 23,5]	14,5 [14,2; 14,8]	15,0 [11,0; 20,0]	0,259
AST/ALT (ed/l), Me [IQR]	0,6 [0,5; 0,7]	0,6 [0,5; 0,6]	0,6 [0,6; 0,6]	0,5 [0,1; 0,6]	$< 0,001^{*}$ $P_{\text{control group}}$ – NAFLD 1st.. = 0,002 $P_{\text{control group}}$ – NAFLD 2st.. = 0,004

** – differences between indicators are statistically significant (p < 0.05)*

The state of lipid metabolism is of particular importance for the pathogenesis of non-alcoholic fatty liver disease. It is known that the disease begins with the accumulation of lipids in organ tissues, and hyperlipidemia plays an important role in the development of the disease. In this study, metabolic parameters such as body mass index (BMI), glucose, and lipid profile were compared between patients with different stages of NAFLD and the control group. The parameters are presented as median and interquartile range (IQR). In patients with NAFLD, an increase in glucose and triglyceride levels was observed, especially in stages 2 and 3, which indicates the development of metabolic dysfunction and possible insulin resistance. However, no significant differences were observed in most cholesterol and lipoproteins. These results confirm that NAFLD is a disease that affects not only the liver, but also the general metabolic state.

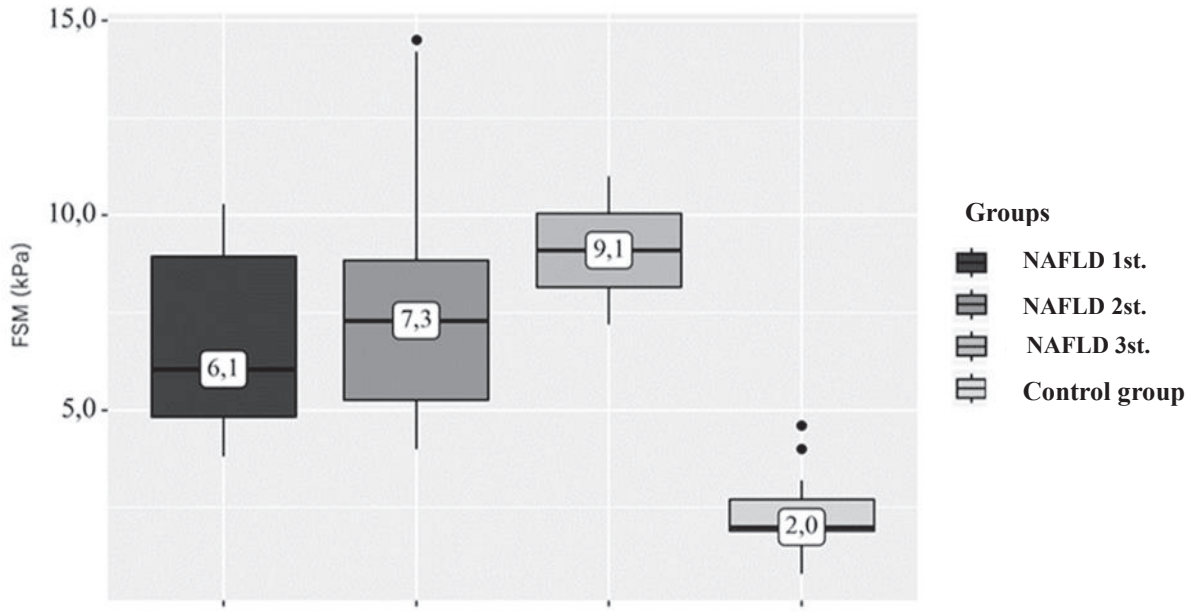
Table 3.

The value of metabolic indicators according to the stage of steatosis in non-alcoholic fatty liver disease

Indicators	Groups				p
	NAFLD 1st	NAFLD 2st	NAFLD 3st	Control group	
BMI (kg/m^2), Me [IQR]	30,5 [28,1; 33,9]	31,1 [28,9; 34,8]	31,3 [26,1; 33,5]	30,0 [29,0; 31,0]	0,792
Glucose(mmol/l), Me [IQR]	4,3 [4,0; 4,7]	5,1 [4,4; 8,6]	7,8 [6,2; 9,4]	4,8 [3,9; 5,5]	0,048*
Total cholesterol (mmol/l), Me [IQR]	4,9 [4,2; 5,5]	5,1 [4,2; 5,8]	5,4 [5,0; 5,8]	4,7 [4,2; 5,8]	0,748
LDLP (mmol/l), Me [IQR]	3,8 [3,2; 4,5]	3,5 [3,2; 4,8]	4,7 [4,2; 5,2]	3,8 [3,2; 4,4]	0,747
HDLP (mmol/l), Me [IQR]	1,5 [1,2; 1,7]	1,4 [0,8; 1,7]	0,9 [0,8; 1,1]	1,3 [0,8; 1,6]	0,318
TG (mmol/l), Me [IQR]	1,5 [0,9; 2,1]	2,2 [1,8; 2,4]	2,4 [2,2; 2,5]	1,6 [0,9; 1,9]	$< 0,001 * p_{\text{control group– NAFLD 1st}} = 0,018 p_{\text{control group– NAFLD 2st.}} = 0,002$
VLDLP (mmol/l), Me [IQR]	1,4 [0,9; 1,8]	1,7 [1,2; 2,0]	1,4 [0,9; 1,9]	1,4 [0,8; 1,8]	0,538

** – differences between indicators are statistically significant ($p < 0.05$)*

The method of determining the degree of liver fibrosis by FSM (FibroScan) is of great importance in assessing the condition of patients with NAFLD. In this analysis, FSM indicators were compared between different stages of NAFLD and the control group (Graph 1).



Graph 1. Analysis of the values of the "Fibroscan" ultrasound elastometry parameters by group of study participants

These results show that the increase in liver fibrosis in patients with NAFLD is statistically significant. Compared with the control group, FSM was significantly higher in NAFLD stages 1 and 2 ($p < 0.001$), and FSM was highest in NAFLD stage 3, with a significant difference of $p = 0.016$. This indicates that the degree of fibrosis increases with the development of NAFLD and that its early detection by FibroScan is important. (Graph 1).

The assessment of the type of eating behavior based on the results of the DEBQ (Dutch Eating Behavior Questionnaire) conducted to identify eating disorders in patients with stage 1 non-alcoholic fatty liver disease, identifying restrictive, emotional, and external types of eating disorders, is presented in **Graph 2.**

Statistically significant differences were found in the analysis of the restrictive domain of eating disorders ($p = 0.004$) (method used: Kruskal-Wallis test). Statistically significant differences were also found in the analysis of the emotional type domain ($p = 0.041$) (method used: Kruskal-Wallis test). Statistically significant differences were also found in the comparison of the externalizing type domain ($p < 0.001$) (method used: Kruskal-Wallis test).

The results of the study showed that in the group with grade 1 hepatic steatosis, we detected statistically significant changes ($p < 0.001$) (method used: Friedman criterion). The analysis showed that in patients with grade 2 non-alcoholic fatty liver disease, statistically significant changes were also detected ($p < 0.001$) (method used: Friedman criterion). In the group with grade 3 non-alcoholic fatty liver disease, no statistically significant changes were detected during the analysis ($p < 0.135$). (method used: Friedman criterion). During the study, statistically significant changes ($p<0.001$) were detected in the control group (method used: Friedman criterion).

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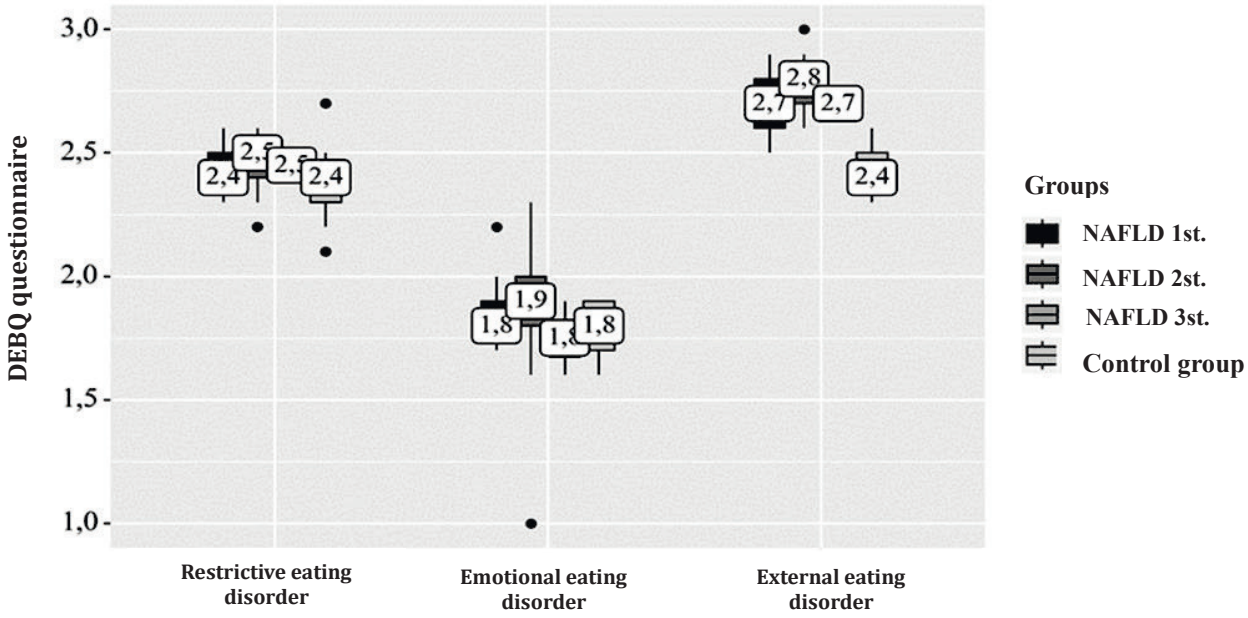
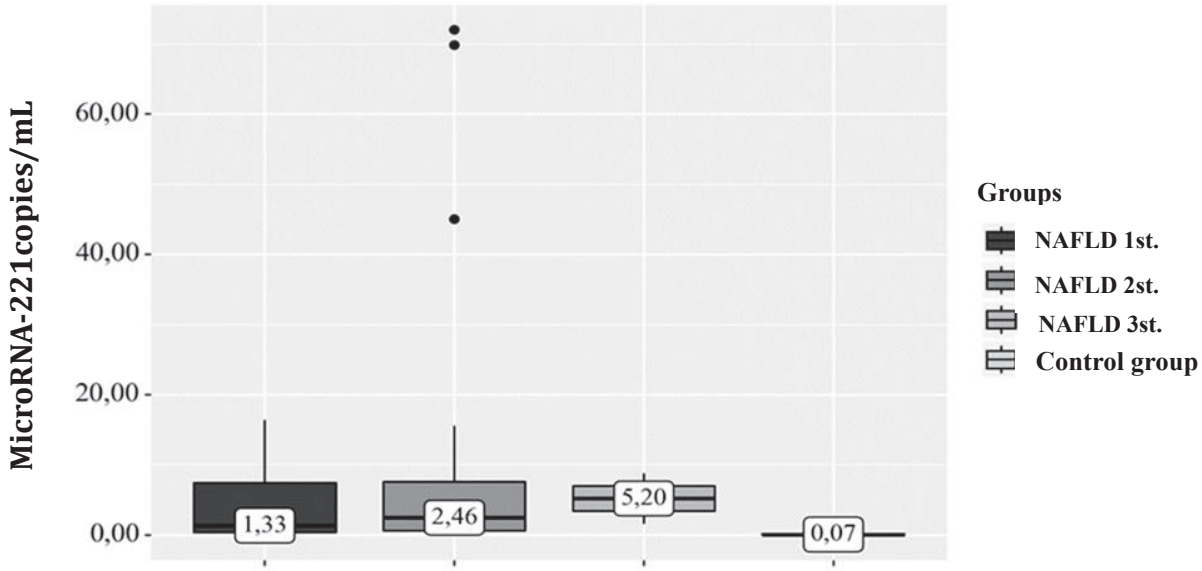


Graph 2. Results of the analysis of the DEBQ questionnaire by group of study participants

In patients with NAFLD, the DEBQ questionnaire identifies restrictive, emotional, and externalizing eating disorders. These changes may further exacerbate the progression of the disease. For example, emotional eating (e.g., stress) can lead to overeating or malnutrition, which in turn negatively affects the course of the disease.

MicroRNA-221 (miRNA-221) is an important biomarker involved in intracellular metabolic processes and inflammatory mechanisms. This analysis was aimed at comparing the expression levels of microRNA-221 between patients with different stages of NAFLD and the control group.

Based on the results of the study, significant differences were found in the assessment of microRNA-221 values depending on the group of study participants ($p < 0.001$) (method used: Kruskal-Wallis test). **(Graph 3)**



Graph 3. Analysis of microRNA-221 values according to the group of study participants

The control group had very low levels of microRNA-221, with a median of 0.07 copies/mL (IQR: 0.05–0.10). MicroRNA-221 levels increased consistently in stages 1–3 of NAFLD, with a median of 1.33 copies/mL in 1 stage, 2.46 copies/mL in 2 stage, and 5.20 copies/mL in 3 stage. The difference compared to the control group was statistically significant in all NAFLD groups ($p < 0.05$): NAFLD 1 and 2 stages: $p < 0.001$. NAFLD 3 stage: $p = 0.033$ (also not statistically significant, but still significant). These results suggest that microRNA-221 may be involved in the pathogenesis of NAFLD and its potential use as a biomarker.

We conducted a correlation analysis of the relationship between microRNA-221 values and Fibroscan ultrasound elastometry parameters (Table 4).

Table 4.

Results of correlation analysis of the relationship between microRNA-221 values and "Fibroscan" ultrasound elastography parameters

Indicator	Characteristics of correlation dependence		
	ρ	Correlation on the Cheddoka scale	P
MicroRNA-221 – FSM	0,819	High	< 0,001*

** – differences between indicators are statistically significant ($p < 0.05$)*

A high level of direct correlation was revealed when assessing the relationship between Fibroscan ultrasound elastometry parameters and microRNA-221 values.

Thus, the results of molecular genetic analysis in patients with non-alcoholic fatty liver disease show that the expression level of microRNA-221 is statistically significantly increased in NAFLD, which allows us to recommend it as a promising biomarker for early detection of NAFLD and assessment of disease progression. As the NAFLD stage increases, the increase in the amount of microRNA-221 reflects the progression of the disease.

Conclusions:

1. In patients with NAFLD, comorbidities were in the following order: arterial hypertension (85.6%), chronic pyelonephritis (87.8%), coronary heart disease (60%), hypercholesterolemia (27.8%), etc. Overweight and obesity were found in 92.2% of cases.

2. According to clinical and laboratory studies, the levels of bilirubin, urea and the AST / ALT ratio were statistically higher in the groups with NAFLD compared to the control. With the progression of NAFLD, an increase in glucose and triglycerides was observed, especially at stages 2 and 3 of steatosis. According to elastometry, liver stiffness indicators increased as steatosis progressed, indicating the progression of fibrosis. The differences between the stages were statistically significant ($p < 0.05$)

3. According to the results of the study, it was found that the DEBQ index indicators for all types of eating behaviors in patients with NAFLD were higher than in the control group. In particular: Restricted eating was approximately 4% higher in the NAFLD grade 2 group compared to the control group ($p = 0.005$). Emotional eating was 5–6% higher in this group ($p = 0.033$). External eating was approximately 12–17% higher in the NAFLD grade 1 and 2 groups compared to the control group, and the differences were statistically significant ($p < 0.001$). These disorders could contribute to the deterioration of metabolic state and progression of the disease.

4. It was found that the expression of microRNA-221 (miR-221) increases in line with the severity of liver fibrosis, indicating its potential use as a promising biomarker for assessing the progression of NAFLD.

The results obtained serve to justify an effective approach to improving the diagnosis and monitoring of NAFLD by using a comprehensive approach - assessing clinical, laboratory, instrumental and molecular indicators.

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