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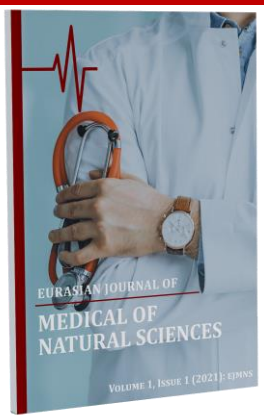

ROLE OF MICRORNAS IN CHRONIC VIRAL HEPATITIS: INSIGHTS INTO LIVER FIBROSIS AND PROGNOSTIC BIOMARKERS

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ABSTRACT

Chronic viral hepatitis is a significant global health concern with substantial economic consequences and increased risks of complications and mortality. Improved understanding of the disease and the development of effective management strategies are crucial. Researchers are studying new parameters, including microRNAs (miRNAs), to predict disease progression, liver fibrosis, and hepatocellular carcinoma (HCC) risk. Early diagnosis, identification of fibrosis markers, and accessible methods for fibrosis assessment are essential goals in modern hepatology.

Methods: This review summarizes recent advancements in the field of hepatology, focusing on genomics and miRNAs. Studies have explored the role of miRNAs as non-protein-coding RNA molecules in disrupting cellular processes involved in hepatic pathology, such as cell proliferation and programmed cell death. Understanding the molecular-genetic mechanisms underlying fibrosis and collagen regulation is essential in liver fibrosis research. Existing research has identified specific circulating miRNAs, including miRNA-122, miRNA-138, miRNA-143, and miRNA-185, as potential non-invasive biomarkers for stellate cell activation and liver fibrosis prognosis in patients with chronic viral hepatitis cirrhosis and HCC.

Results and Discussion: MiRNAs have demonstrated significant contributions to understanding intracellular regulation mechanisms and the pathogenesis of liver fibrosis. Notably, miRNA-122 has emerged as a key player due to its functional characteristics and diagnostic potential. By examining the prevalence of specific miRNAs, valuable insights into active fibrogenesis and fibrotic scar formation have been obtained. These advancements bring us closer to understanding the mechanisms underlying the complications of chronic viral hepatitis, notably liver cirrhosis.

Conclusion: The study of miRNAs holds promise for early detection of liver fibrosis and serves as a non-invasive tool for diagnosis and prognostic evaluation. Ongoing research on miRNAs and their role in liver fibrosis offers a better understanding of intracellular regulation mechanisms and potential therapeutic targets. By identifying specific miRNAs associated with

hepatic pathology, further advancements can be made to improve disease management and patient outcomes.

Introduction. Chronic hepatitis (CH) is in the center of attention of medical science and practical health care. The disease causes great damage to the economy of all countries. According to official data, 10-15 thousand US dollars are spent on treatment of a patient with chronic viral hepatitis. Risks of complications and cases of lethal outcomes from chronic diffuse liver diseases, despite the successes achieved in the diagnosis and treatment of many diseases, has a steady tendency to increase. In viral liver cirrhosis (LC) after 5 years of diagnosis, mortality reaches 70%. The vast majority of liver transplants are performed for the consequences of chronic viral hepatitis (CVH). The necessity of using expensive methods of liver transplantation for complications of CVH, such as LC and hepatocellular carcinoma (HCC) determines not only medical, but also socio-economic significance of the problem under study. It is worth noting that among all infectious diseases, viral hepatitis causes the greatest economic damage per case, second only to influenza and other acute respiratory diseases. This emphasizes the significant burden it places on healthcare systems and economies worldwide.

To improve our understanding of the disease and develop effective strategies for managing it, researchers are studying new parameters that can predict the course of the disease, the progression of liver fibrosis, and the risk of developing hepatocellular carcinoma (HCC). Early diagnosis, identification of laboratory and genetic markers of fibrosis, and the development of minimally invasive and accessible methods to assess the severity and progression of hepatic fibrosis are crucial goals in modern practical hepatology.

Liver fibrosis is characterized by the excessive accumulation of extracellular matrix, which occurs due to the activation of liver stellate cells. Recently, there have been promising developments in the field of genomics in this regard. Among the various research efforts, a significant focus has been placed on studying single-nucleotide polymorphisms, freely circulating DNA, endosomal RNA, and their significance in the pathogenesis of various diseases.

In the past decade, there has been a surge in studies exploring the role of small non-protein-coding RNA molecules, known as microRNAs. These molecules play a key role in disrupting the balance of cell proliferation, differentiation, and programmed cell death, contributing to the development of various diseases, including hepatic pathology. Understanding the molecular-genetic mechanisms underlying fibrosis can help regulate the synthesis and breakdown of collagen, a critical factor in fibrosis development. By examining the prevalence of specific microRNAs, valuable information can be obtained regarding active fibrogenesis and the rapid replacement of healthy liver tissue with fibrotic scar tissue. Research in this area brings us closer to understanding the mechanisms behind the complications of chronic viral hepatitis, such as liver cirrhosis.

The study of microRNAs is not only essential for gaining fundamental insights into intracellular regulation mechanisms but also holds significant practical value as non-invasive biomarkers for early diagnosis of liver fibrosis. Modern studies have identified circulating microRNAs, such as miRNA-122, miRNA-138, miRNA-143, and miRNA-185, as potential non-invasive biomarkers for stellate cell activation and liver fibrosis prognosis in patients with

viral hepatitis cirrhosis and HCC. The discovery of miRNA-122 in 2002 was particularly noteworthy and has since contributed to advancements in the field of hepatology due to its functional characteristics.

Methods. To solve the set tasks we used clinical (general blood analysis, urine), biochemical (ALT, AST, alkaline phosphatase, total protein, bilirubin and its fractions, glucose content, total cholesterol, triglycerides, low-density lipoprotein (LDL) content, ultrasound (ultrasound), ultrasound elastometry (USE), molecular-genetic (expression level of microRNA-122) and statistical methods of research.

We examined the patient, collected anamnesis, assessed the severity of the condition and performed laboratory tests, general and biochemical blood analysis, determination of blood group and Rh factor, ultrasound, ECG, chest and abdominal radiography, consultations of allied specialists when indicated. Laboratory studies included hematologic studies of peripheral blood; determination of biochemical parameters: total protein and its fractions, bilirubin, transaminases, glucose; determination of the state of the blood coagulation system; general blood and urine analysis. All laboratory investigations were carried out according to standard methods.

Ultrasound of the abdominal cavity organs was performed using the device "MINDRAY DC-80, 21.5". During the ultrasound examination the size and acoustic structure of the stomach, liver, gallbladder were evaluated.

Ultrasound elastometry (liver fibroscanning) is a non-invasive method of diagnostics of liver fibrosis degree using FibroScan® 502 Touch device.

Molecular-genetic research. The expression level of microRNA -122 in plasma was measured by reverse transcription PCR according to the TaqMan microRNA analysis protocol. Total RNA was extracted with TRIZOL reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. RNA concentration was measured using a NanoDrop ND2000 spectrophotometer (NanoDrop Technologies, USA). Reverse transcription was performed using miScript Reverse Transcription Kit (QIAGEN, Germany). The expression level of mature microRNA -122 was examined using miScript SYBR Green PCR Kit (QIAGEN, Germany) according to the manufacturer's instructions.

The obtained numerical data were processed using Microsoft Office 2007 and Statistica 6 application program package (StatSoft Inc., USA).

Results and Discussion. The main complaints were comparatively evaluated during the study, and they were quite variable. Pain in the subcostal region was complained by 41 (29.9%) patients in group 1 and 5 (8.3%) ($P<0.001$ and RR=0.71).

In group 2, 82.7% more patients complained of decreased appetite than in group 1. Meteorism was noted in 13.3% of cases only in patients with cirrhosis, and signs of asthenia were predominant in the group of patients with CVH (68 (49.6%) and 28 (46.7%) $P>0.05$ and RR=2.07). Such symptoms as bleeding, heaviness in the left subcostal area and weight loss were characteristic of patients with liver cirrhosis (Table 1).

At objective examination of patients, liver thickening was reliably more frequently revealed in patients with CVH 43.8% of cases, jaundice - 19%. At the same time, in patients with LC in group 2, the most frequent signs were stellate telangiectasias - in 41.7% of cases,

liver thickening - 26.75%, palmar erythema - 13.3%, splenomegaly 10% and ascites -8.3%. (Figure 1).

Objective examination data showed that palmar erythema as a stigma of liver damage was detected in patients with LC significantly more often than in group 1. Splenomegaly, ascites were indicative signs of portal hypertension. At objective examination of patients liver thickening was reliably more often revealed in patients with CVH 43,8% of cases, jaundice - 19%. A characteristic feature of the cholestatic syndrome was a high frequency of subicteric sclerae and skin in patients with CVH than in patients of group 2, which could be associated with the phenomena of intrahepatic cholestasis.

At the same time, in patients with LC in group 2, the most frequent signs were stellate telangiectasias in 41.7% of cases, liver thickening in 26.75%, palmar erythema in 13.3%, splenomegaly in 10% and ascites in 8.3%. We calculated the relative risk of liver infection for each indicator of the condition of the patients studied. A situation in which the relative risk is greater (RR>1) indicates that the risk of developing with the studied factor is greater than without it. When the relative risk is equal to one (RR=1), there is no association between the factor and the disease.

Analysis of epidemiologic anamnesis data showed that the cause of infection is the route of transmission: 5 (3.6%) patients were detected in group 1 due to surgical interventions, and 2 (3.3%) in group 2 (P>0.05, RR=1.12). The maximum risk was observed during dental manipulations in group 1 there were 10 (7.3%) patients, in group 2 there were 10 (16.7%) (P>0.05, RR=2.89), and also during gynecological interventions in group 1 there were 4 (2.9%) patients, in group 2 there were 9 (15%) (P>0.05, RR=2.11). The source of infection was not established in 111 patients (81%) in group 1, in group 2 in 36 (60%) (P>0.05, RR=1.61), most often these were patients with HCV.

Ultrasound elastometry (USE) was used to assess the processes of fibrosis. Fibroscan 502 device (Echosens, France) was used as a reference method. The results of sampling depending on the stage of fibrosis were as follows. In the first group the maximum number of patients was observed at fibrosis stage F0 (3,5-6,0), the number amounted to 52, in the 2nd group with these parameters no patients were observed. In the second group the maximum number of patients was observed at fibrosis stage F4 (20-24), their number amounted to 36 (Table 2).

Clinical symptomatology generally increased with increasing fibrosis stage, but often varied. The high frequency of asthenic symptoms in patients with advanced fibrosis probably reflects the severity of both hepatoprivate and psychosomatic mechanisms (Table 3).

In patients with LC almost all biochemical tests were significantly altered. We see the cholestasis syndrome due to hyperbilirubinemia and increased activity of alkaline phosphate. Decreased protein-synthetic function of the liver is evidenced by decreased levels of total protein, albumin and increased thymol assay, indicating dysproteinemia and confirming the syndrome of mesenchymal inflammation. Low values of platelet count confirm the syndrome of hepatic cellular insufficiency in LC.

In group 1 patients BMI had no significant differences from the control group. Metabolic parameters in patients with CVH were mainly within the reference values (Table 8). But, statistically significantly increased levels of TG were observed in 18% of patients. The same

picture is observed when analyzing the content of LDL, the indices of which in 35% of persons were much higher than these parameters of the control group. Proceeding from the above-stated, it is possible to assume, that on the average in one third of patients with CVH there were determined the violations of blood lipid spectrum parameters in the form of increase of TG, LDL, that clearly shows the presence of atherogenic risk in this group of patients. Progression of fibrosis in CVH leads to an increase in the severity of metabolic disorders.

In the majority of patients in comparison with the control group was diagnosed a decrease in glucose levels and violations of blood lipid spectrum parameters in the form of decreased levels of cholesterol and HDL, increased concentration of TG. The analysis of indicators indicates the interdependence of metabolic tests with biochemical parameters and liver density according to USE. Metabolic disorders, mainly in the form of triglyceride elevation, were observed in 28% of CVH patients. In group 2 metabolic disorders were manifested in the form of hypoglycemia, hypocholesterolemia, hypertriglyceridemia, which is the result of decreased synthetic function of the liver.

Fibrosis progression rate (FPR) was calculated in all patients with a certain duration of the disease and stage of fibrosis severity. This parameter was determined according to the results of liver ultrasound as a ratio of fibrosis stage (in points) to disease duration (in years), which allowed us to distinguish fast (up to 10 years) and slow (more than 10 years) rates of fibrosis progression in CVH, accordingly dividing them into 2 subgroups.

High rate of fibrosis progression is associated with male gender. Various liver function tests are altered during the progression of LF, which explains the inclusion of direct and indirect markers of fibrosis in fibropanels. Chronicization of the process and progression of fibrosis in adults is the higher the older the age of infection of the individual. It has been shown that the accrual of liver fibrosis is faster in individuals infected after 40 years of age.

Accumulation of fibrosis and formation of cirrhosis occurs with greater frequency in persons with increased activity of the pathologic process and a high degree of necrotic-inflammatory changes in the liver. In accordance with this, normal serum ALT level is associated with absence of liver fibrosis progression, whereas ALT level at least 1.5 times higher than normal is associated with rapid progression of liver fibrosis. In addition, it was also found that the duration of chronic viral hepatitis is correlated with the severity of liver fibrosis.

The data obtained when analyzing metabolic parameters depending on the rate of fibrosis progression revealed that differences were found only for glucose concentration, in the group with high rate of fibrosis development, this parameter had lower values.

In addition, the change of microRNA-122 expression level was investigated. To study the role of microRNA-122, 32 patients were selected from the total number of examined patients: 17 (53.1%) with the diagnosis of chronic viral hepatitis constituted the 1st subgroup and 15 (46.9%) with the diagnosis of compensated cirrhosis constituted the 2nd subgroup, from among those hospitalized for inpatient treatment in the hepatology department of RSSPMC of epidemiology, microbiology, infectious and parasitic diseases. Genetic studies were carried out at the Laboratory of Molecular Medicine and Cell Technologies of the Republican Scientific and Practical Center of Hematology of the Republic of Uzbekistan.

We selected 10 healthy volunteers from the control group. In the 1st subgroup, the age of patients ranged from 30 to 58 years (mean age 41,5±6,8 years), and in the 2nd subgroup, the age of patients ranged from 31 to 60 years (mean age 31,5±6,8 years). The range interval of microRNA -122 expression level was as follows, the division was four-level from the minimum values of 0.001 - 0.14; 0.15 -1.05; 1.05 - 12.88 and >12.89 to the maximum level.

The correlation of microRNA 122 expression with sex and age was analyzed during the study. No significant correlation was found. Correlation between the level of microRNA-122 expression was found with the duration of the pathologic process, particularly in group 2 patients with LC. The duration of the disease in patients of the 1st subgroup averaged 5.7±3.2 years in men and 3.7±3.3 years in women. The duration of the disease in patients of group 2 was clearly different and averaged 6.8±1.8 years in men and 3.8±2.2 years in women. The division of patients into groups with low, medium and high miRNA -122 concentrations showed that patients with low serum miR-122 levels had longer disease duration than patients with higher miR-122 levels. (Table 6).

The difference in the incidence of patients in subgroups 1 and 2 of patients in the miR-122 0.001-0.14 range was 5.9% vs. 26.7%, respectively. The calculated odds of detection and risk of complications in this range were 4.5 (95% CI0.57 to 36.22) and 5.8 (95% CI0.57 to 59.31), respectively. However, despite the high OR=5.8 and RR=4.5, such a difference was statistically insignificant ($\chi 2=1.3$, p=0.3). In the range of 0.15-1.05, the frequency of miR-122 occurrence in patients in patient subgroups 1 and 2, was 11.8% vs. 66.7%, respectively. The calculated odds of detection and risk of complications at this range are 5.7 (95% CI1.46-21.86) and 15 (95%, CI2.4-93.0), respectively. High OR=15 and RR=5.7, the difference was statistically significant ($\chi 2=8.0$, P<0.001). In this range, the risk of cirrhosis formation increases, and we clearly see it. The difference in the incidence of patients in 1 and 2 subgroups of patients in the range of 1.05-12.88 was 52.9% vs. 6.7%, respectively. The calculated odds of detection and risk of complications at this range were 7.9 (95% CI1.13-55.58) and 15.7 (95% CI 1.67- 148.1), respectively. However, despite the high OR=15.7 and RR=7.9, the difference was statistically significant ($\chi 2=5.9$, P<0.01). In the third range, RR increased almost 8-fold and the risk of detection, i.e., OR increased 15.7-fold. In this case, a significant association was found between microRNA-122 expression level and stage 4 fibrosis by USE.

The difference in the incidence of patients in subgroups 1 and 2 of patients in the miR-122 >12.89 range was 29.4% vs. 0%, respectively. The calculated odds of detection and risk of complications at this range were 4.7 (95% CI 0.614-36.03) and 6.2 (95% CI0.64-60.93), respectively. However, despite the high OR=6.2 and RR=4.7, such a difference was statistically insignificant ($\chi 2=1.6$, P<0.05. Possibly, the differences would have been more pronounced with a larger number of patients. We also studied the relationship of the range of microRNA-122 expression level with examination data, complaints, clinical and biochemical data, and elastometry parameters. At 1-2 range of microRNA-122 expression level is 0.14±0.04, at these indicators in the group with CVH the number of patients is 1. In this case, the results are considered insignificant.

In the group with LC, the number of patients was 4. All of them are male, age from 50-57 years, duration of disease more than 5 years. The category of patients included in this range of

miR-122 expression level had elevated levels of ALT (80.1±7.1), AST (71.2±5.8), alkaline phosphatase (61.3±1.3), albumin (40.4±0.08), platelet count (113±4.97). No change in bilirubin level was found. Fibroelastometry indices in this category of patients were F4 (20,0-24,0).

At range 2 the level of microRNA-122 expression is 0.15 -1.05, at these indicators in the group with CVH the number of patients is 2. In the group with LC, the number of patients was 10. The category of patients with this parameter in the range of microRNA-122 level had increased levels of ALT (80.1±7.1), AST (71.2±5.8), alkaline phosphatase (71.2±5.8), albumin (32.4±0.08), platelet count (113±4.97). No changes in bilirubin level were found. Fibroelastometry indices in this category of patients were F3 (9.0-20.0).

In the 3 range the expression level of microRNA-122 is 1.05 - 12.88, with these indicators in the group with CVH the number of patients is 9. In the group with LC the number of patients was 1. The category of patients with this range of microRNA-122 level had increased levels of ALT (80.1±7.1), AST (71.2±5.8), alkaline phosphatase (71.2±5.8), albumin (32.4±0.08), platelet count (113±4.97). No change in bilirubin level was found. Fibroelastometry indices in this category of patients were F2 (6.5). Thus, the level of microRNA -122 in may be a useful prognostic parameter in patients with cirrhosis, when assessing the risk of complications, particularly HCC. We also found a correlation between serum levels of microRNA -122 and albumin, total protein or bilirubin. One explanation for this may be that serum protein levels are altered in patients with severe liver dysfunction.

In addition, bilirubin levels also varied with cholestasis. The relationship of microRNA-122 expression level with the rate of fibrosis progression was also evaluated. Analysis of microRNA -122 level in relation to elastometry data in CVH patients showed that as liver fibrosis progresses, the level of microRNA -122 expression decreases. This is caused by replacement of the mass of functioning hepatocytes by connective tissue that does not contain microRNA -122. According to the level of microRNA -122, studied in dynamics, we can judge about the rate of liver fibrosis progression. The development of LC is accompanied by even more pronounced suppression of microRNA -122 expression, which makes this molecule promising as a prognostic marker. Determination of the relative amount of microRNA-122 in the blood determines the activity of the inflammatory process in the liver, therefore, it is a parameter of the severity of the course of hepatitis. Thus, according to the results of the conducted researches it is possible to assert that microRNA -122 can be used in laboratory monitoring of management of patients with CVH as an indicator of severity of liver damage and speed of liver fibrosis formation.

CONCLUSIONS

1. Comparative analysis of the results of clinical and laboratory, instrumental and molecular genetic methods of examination of patients with chronic viral hepatitis determined the association of the risk of liver fibrosis development with genetic factors.
2. Correlation analysis in patients with chronic viral hepatitis revealed numerous reliable correlations of metabolic indices with biochemical parameters and liver density according to USE.In particular BMI correlated ALT, alkaline phosphate and liver density, inverse correlation of markers of cytolysis, cholestasis with HDL.

3. In CVH patients, evaluation of microRNA-122 expression level as a potential biomarker will allow, early diagnosis of liver fibrosis with subsequent use for stage stratification during treatment.
4. Implementation of molecular genetic methods of research, in particular, determination of microRNA-122 expression level in standard methods of investigation of patients with CVH with regard to ranges will help to identify in advance the risk groups of complications development, to choose the correct tactics of treatment and, therefore, to prevent disability caused by complications, as well as to prolong the period to LC and reduce mortality rates.

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