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Expression of Circulating Microrna-199a and Microrna-155 in Chronic Viral Hepatitis B and Chronic Viral Hepatitis C

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Abstract: Viral hepatitis is one of the significant problems of hepatology in the world. Chronic viral hepatitis is known to have a high tendency to transform into liver cirrhosis and hepatocellular carcinoma. The high heterogeneity of the genomes of viral hepatitis and especially hepatitis C makes molecular characterization based not only on the determination of gene mutations and gene expression profiles, but also on new biological markers, fundamentally important. These new biomarkers are microRNAs (microRNA, miRNA, miR). Currently, a large amount of knowledge has been accumulated about changes in the expression of microRNAs in various pathologies, including chronic hepatitis, which makes it possible to create diagnostic and prognostic criteria based on microRNAs.

Key words: microRNA, gene expression, chronic viral hepatitis C, chronic viral hepatitis B.

Despite the successes achieved in the fight against many infectious diseases, in modern medicine the problem of chronic viral hepatitis (CVH) in Uzbekistan, as well as throughout the world, continues to remain relevant [1,3,7].

The World Health Organization (WHO) estimates that more than 325 million people worldwide are infected with hepatitis B or C and cause 1.4 million deaths each year. In terms of mortality, hepatitis B and C together rank second after tuberculosis, and the number of people infected with hepatitis is 9 times higher than the number of people infected with HIV. However, over 80% of people with hepatitis do not have access to prevention, testing and treatment [2,4].

The high heterogeneity of the genomes of viral hepatitis and especially hepatitis C makes molecular characterization based not only on the determination of gene mutations and gene expression profiles, but also on new biological markers, fundamentally important. These new biomarkers are microRNAs (microRNA, miRNA, miR). MicroRNAs are a class of small non-coding RNAs, 19 to 24 nucleotides in length, that regulate gene expression through various mechanisms that have not been fully studied to this day [8].

The results of numerous studies indicate the significant participation of hepatoviral infections in damage to the hepatobiliary system in humans. In recent years, numerous studies have been conducted to determine the participation and diagnostic value of small non-coding RNA molecules (microRNAs) in the formation of diseases [9]. MicroRNAs are molecules that regulate gene expression at the post-transcriptional level [10,11]. It has been proven that microRNAs potentially regulate every aspect of cellular activity, including differentiation, development, metabolism, proliferation and apoptosis of cells, including participation in the course of viral infections [12]. MicroRNAs circulating in serum are stable and protected from degradation in body fluids, making them universal biomarkers for many diseases. [13]. To date, about 2500 microRNAs have been discovered that can regulate the expression of more than 60% of all genes. Currently, a large amount of knowledge has been accumulated about changes in the expression of microRNAs in various pathologies, which makes it possible to create diagnostic panels based on microRNAs, however, on the way to the therapeutic use of microRNAs

there are a large number of unresolved issues [5]. The use of microRNAs as non-invasive biomarkers is of particular interest for liver diseases.

Aim: To study the expression level of microRNA-199a and microRNA-155 in CHB and CHCV.

Material and methods: The material for the study was blood plasma samples from 76 patients with chronic viral hepatitis: chronic viral hepatitis B (CVHB) - 36, chronic viral hepatitis C (CVHC) - 40, the control group consisted of 30 healthy individuals who had no markers of infection HBV and HCV infections.

Research methods: Total RNA was isolated from blood plasma using the MiRNeasy Serum/Plasma Kit according to the manufacturer's instructions (QIAGEN, Germany). To normalize the reaction and as an internal control, the "MiRNeasy Serum/Plasma Spike-In Control" kit containing C.elegans miR-39 miRNA mimic was used. Reverse transcription PCR (RT-PCR) was carried out using the MiScript II RT Kit (QIAGEN, Germany). RT-PCR reaction conditions: 37°C-60 min; 95°C-5 min. Real-time PCR was carried out using the MiScript SYBR® Green PCR Kit (QIAGEN, Germany). To detect internal control, a universal reverse primer, forward primer Ce_miR-39_1 miScript® Primer Assay and specific reverse primers (microRNA-199a and microRNA-155) (QIAGEN, Germany) were used. PCR conditions: 95°C - 15 min; 94°C-15 sec; 55°C-30 sec; 70°C-30 sec – 40 cycles. Interpretation of the results of quantitative assessment of microRNA expression was carried out using the following indicators: ΔCt indicator - the difference between the Ct values of the microRNA under study and the internal control; ΔΔCt indicator is the difference between the ΔCt values of the test sample and the control sample. The 2^-ΔΔCt method proposed by K.J.Livak [14] was used to analyze relative expression. Statistical processing of the obtained results was carried out using the Mann-Whitney U test.

Results: As a rule, during pathology, the level of a particular microRNA changes depending on its function in regulating the pathological processes of the affected organ. Many authors point out the non-random nature of the identified associations of various microRNAs with the development of pathological processes [15]. The function of microRNA-199-a suggests, according to the literature, a protective function in the process of cell proliferation and the life of hepatocytes. According to some studies, liver damage by the hepatitis B virus is accompanied by an increase in the level of microRNA-199-a, which may not only reflect pronounced disturbances in immune regulation in the liver tissue during viral damage, but also indicates the decisive role of 199-a as a regulator of viral replication [16].

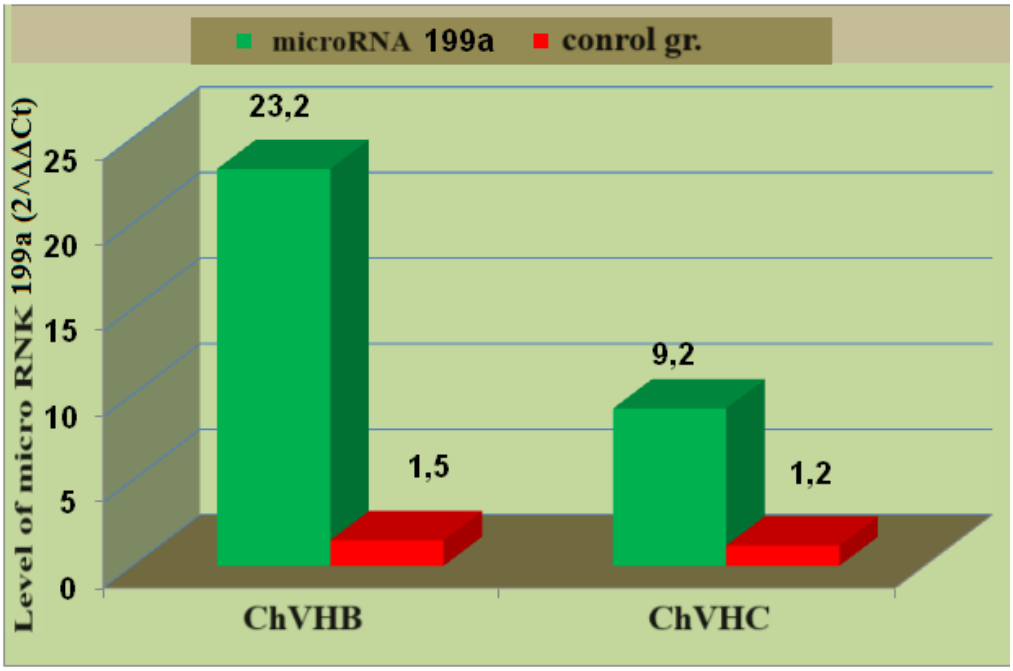

Figure 1. MicroRNA-199a expression level in chronic viral hepatitis.

The results we obtained from studying the level of expression of miR-199a in patients with chronic hepatitis B, C and in healthy individuals are presented in Figure 1. When examining patients with CHB and CHCV, we found (Figure 1) that the level of expression of miR-199a in patients with CHB and CHB CHCV significantly exceeds similar indicators in the control group ($p < 0.05$). The expression of microRNA-199a was much higher in CHBV compared to CHCV ($p < 0.05$).

No change in the expression level of miR-155 was detected in CHB (Fig. 2), and this may be due to the fact that during chronic HBV infection, the expression of miR-155 can be inhibited and its dysregulation is observed. In CHCV, the miR-15 indicator was increased and exceed similar indicators in the control group by 3.9 times. In a comparative analysis, the expression level of microRNA-155 was higher in the group of CHCV patients and was 5.4 times higher than the CHB levels.

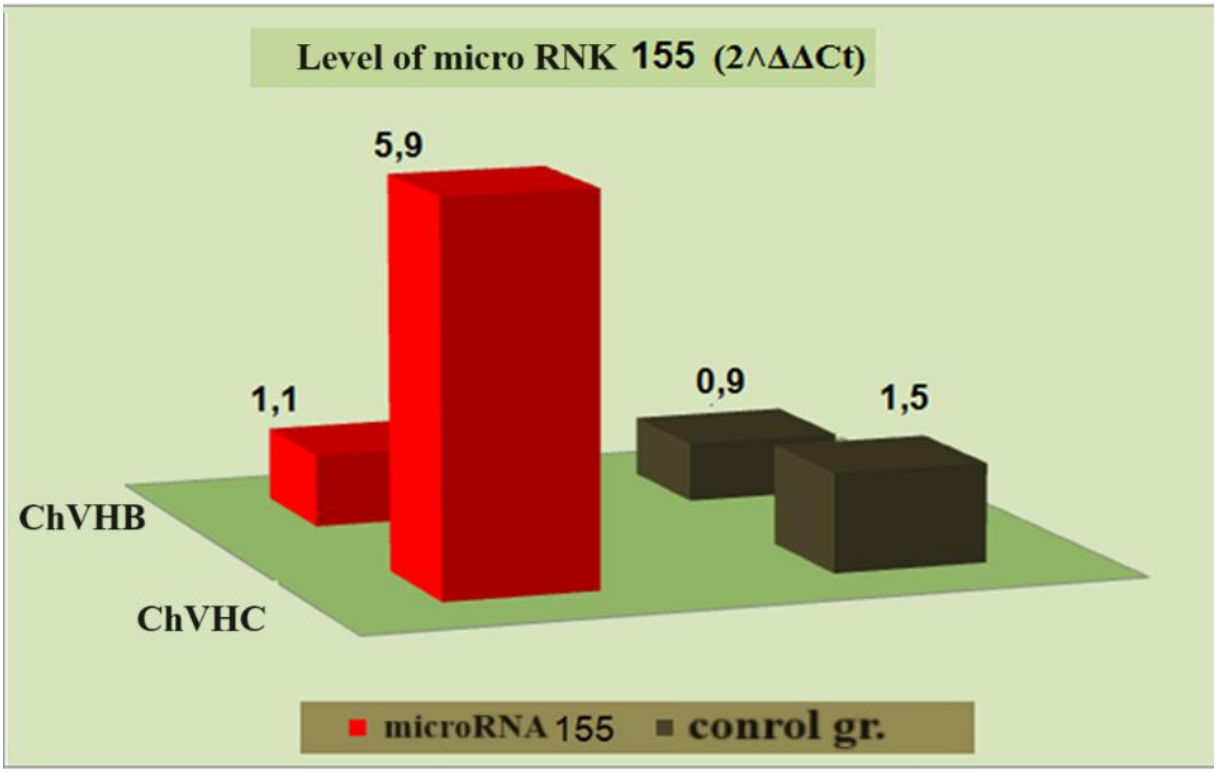

Figure 2. MicroRNA-155 expression level in chronic viral hepatitis

Similar studies were carried out by Chang C.C. et al. for HCV. In patients with HCV, the expression levels of miR-199a, miR-16, miR-193b, miR-222 and miR-324 were significantly higher compared to the healthy group. According to the authors, the expression levels of the above microRNAs may play a role as significant biomarkers of the risk of developing HCV infection [17]. Based on the results of the studies, the authors came to the conclusion that miR-122 can be used in laboratory monitoring of the management of patients with hepatitis C as an indicator of the severity of liver damage in acute hepatitis C and the rate of formation of liver fibrosis in chronic hepatitis C [6]. Other authors have shown that microRNA-199a is a potential biomarker that reflects the therapeutic effectiveness of treatment [18]. The work of Murakami Y. et al. showed that microRNA-199a directly regulates the replication of the hepatitis C virus and the possibility of its future use as a new antiviral therapy [19]. Ge J. et al. studied the expression and function of miRNA-155 in chronic HBV- infections where it was found that the expression of miR-155 in peripheral blood mononuclear cells was lower in patients with chronic HBV infection than in healthy individuals [20]. An increase in miR-155 in the blood serum was detected in patients with HCV infection compared to the control group, and the authors conclude that miR-155 is a positive regulator of inflammation, activated both in monocytes and in the blood serum of patients with chronic HCV -infection. The authors believe that a positive correlation between an increase in miR-155 and miR-122 in the blood serum of HCV-infected patients may be an indicator of inflammation caused by damage to hepatocytes [21]. According to Grek M., replication of

HCV RNA in peripheral blood mononuclear cells of patients with chronic HCV infection is associated with increased and coordinated expression of microRNA-155 and microRNA-196b [22].

Thus, the content of miR-199a and miR-155 in blood plasma can be considered another liver marker along with generally accepted specific markers, such as ALT and AST, for assessing liver damage in CHC. In the future, in correlation with biochemical blood parameters, viral load and instrumental methods for diagnosing liver fibrosis in chronic viral hepatitis, it may be of prognostic value for the formation of risk groups for the development of liver fibrosis, liver cirrhosis and HCC.

Conclusions:

1. Expression of microRNA-199a content is significantly higher in CHBV compared to CHCV.
2. The quantitative change in miR-155 in CHCV was significantly higher compared to CHB.
3. Further study of the expression of miR-199a and miR-155 as promising non-invasive markers will determine the potential of these miRNAs as early predictors of the development of liver fibrosis, liver cirrhosis and HCC.

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