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THE ROLE OF MIRNA 122 IN LIVER DISEASES

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Abstract
The last decade has been accompanied by the emergence of a large number of studies on the role of small, non-coding RNA molecules (micro-RNAs). The study of miRNAs is important not only for a fundamental understanding of the mechanisms of intracellular regulation, but also has a high practical value as non-invasive biomarkers for early diagnosis of liver fibrosis. Current research provides new potential approaches to the therapy of liver fibrosis.

Key words: micro-RNA, chronic hepatitis, liver fibrosis.

Introduction

Chronic viral hepatitis (CVH) is the focus of medical science and practical health care. In about 80% of patients, the disease becomes chronic. Timely diagnosis of these conditions is the key to developing optimal patient management tactics and preventing complications. Liver fibrosis is reversible; it is a key pathological process in the development of all chronic liver diseases

The last decade has been accompanied by the emergence of a large number of studies on the role of small, non-coding RNA molecules (micro-RNAs). The key role of micro-RNA in the disturbance of the balance of proliferation, differentiation and programmed cell death in the development of various diseases, including liver pathology, was revealed. The micro-RNA profile often differs between cells of different tissues, which in turn can help determine, predict the response to therapy and determine the prognosis of the course of the disease. In view of the fact that the stage of liver fibrosis is a priority in the choice of treatment tactics, it remains relevant to

search for predictors of an unfavorable outcome. Molecular genetic mechanisms of the development of fibrosis regulate the synthesis and breakdown of collagen. The prevalence of one or another nomenclature of miRNAs can provide information about active fibrogenesis, therefore, can develop a rapid replacement of the hepatic tissue with fibrous tissue. Research in this area will make it possible to get closer to understanding the mechanisms of the formation of such complications of chronic viral hepatitis as cirrhosis of the liver and hepatocellular carcinoma.

Object

To study the expression level of miRNA-122 as a predictor of the risk of complications in patients with chronic viral hepatitis.

Material and Methods

To study the role of microRNA-122 in the study, 32 patients were isolated from the total number of examined patients: of them 17 (53.1%) with a diagnosis of chronic viral hepatitis made up 1 subgroup and 15 (46.9%) with a diagnosis of compensated liver cirrhosis 2 subgroup. The expression level of miRNA-122 in blood plasma was measured by reverse transcription PCR in accordance with the TaqMan miRNA analysis protocol.

Results

The intervals of the range of the expression level of miR-122 looked 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. The lowest levels of miRNA-122 were found in patients with severe hepatic fibrosis as indicators of the functional capacity of the liver.

In May 2016, at the World Health Assembly, 194 countries adopted a global viral hepatitis strategy with a goal of eliminating chronic viral hepatitis by 2030. In the absence of care for patients with CHB and CHC, 19 million deaths are expected from 2015 to 2030. Regions of Central and East Asia, North Africa, as well as the Middle East have the highest infection rate (> 3.5%) [1,2,8]. During monitoring of patients with CVH, the development of CP and HCC was established, characterized by a low 5-year survival rate (less than 5%). About 700 thousand people die annually from complications, which testifies to the extreme urgency of this disease, which causes social and economic damage to the state. It should be noted that among all infectious pathology, viral hepatitis causes the greatest economic damage per 1 case of the disease, and in terms of total damage it is second only to influenza and other acute respiratory infections [5,7,12, 13].

Early diagnosis, determination of laboratory and genetic markers of fibrosis, development and implementation of minimally invasive, effective and affordable methods for assessing the severity and rate of progression of AF are very important tasks for modern practical hepatology [4,3].

The last decade has been accompanied by the emergence of a large number of studies devoted to the role of small, non-coding RNA molecules (micro-RNA). The key role of micro-RNA in the disturbance of the balance of proliferation, differentiation and programmed cell death in the development of various diseases, including le liver pathology. Molecular genetic mechanisms of the development of fibrosis regulate the synthesis and breakdown of collagen. The prevalence of one or another nomenclature of miRNAs can provide information about active fibrogenesis, therefore, can develop a rapid replacement of the hepatic tissue with fibrous tissue. Research in this area will make it possible to get closer to understanding the mechanisms of formation of such complications of chronic viral hepatitis as cirrhosis of the liver.

Liver fibrosis is characterized by excessive accumulation of the extracellular matrix, which occurs due to the activation of stellate liver cells (synonyms: Ito cells, fat-storing cells, lipocytes) [6,8.9]. Circulating miRNA-122, miR-NK-138, miRNA-143, and miRNA-185 are potential non-invasive biomarkers of stellate cell activation and liver fibrosis for the purpose of prognosis in patients with viral hepatitis Cp and HCC. In this regard, the study of the expression level of miRNA-122, as a prognostic marker of the risk of complications, is an urgent problem of modern hepatology [10, 11, 12].

The study of miRNAs is important not only for a fundamental understanding of the mechanisms of intracellular regulation, but also has a high practical value as non-invasive biomarkers for early diagnosis of liver fibrosis. Current research provides potential new approaches to diagnosing liver fibrosis. MicroRNA-122 is an indicator of liver dysfunction and a new independent prognosis parameter for patients with CVH. According to the literature, with the development of liver diseases, in particular acute and chronic hepatitis, as well as their complications such as cirrhosis of the liver and HCC, the functioning of microRNA-122 is disrupted. Therefore, according to modern concepts, a change in the expression level of miRNA-122 can be a prognostic marker of a pathological state of the liver.

The aim of the study was to optimize methods for non-invasive diagnostics of liver fibrosis in patients with chronic viral hepatitis.

To study the role of microRNA-122 in the study, 32 patients were selected from the total number of examined patients: of them 17 (53.1%) with a diagnosis of chronic viral hepatitis made up 1 subgroup and 15 (46.9%) with a diagnosis of compensated cirrhosis of the second subgroup, from among those hospitalized for inpatient treatment in the department of Research Institute of Epidemiology, Microbiology and Infectious Diseases of the Republic of Uzbekistan

Genetic studies were carried out on the basis of 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-group 1 of 17 patients, the age ranged from 30 to 58 years (mean age 41.5 ± 6.8 years), and in group 2 of 15 patients whose age ranged from 31 to 60 years (mean age 31.5 ± 6.8 years).

Material and Research Methods

Peripheral blood samples were collected in tubes and citrate was added as an anticoagulant. Each sample was centrifuged at 3000 g for 7 min at 4 ° C to completely remove cellular components, and the supernatant was stored at –70 ° C. For genotyping and determination of HCV load, the PCR method was used. HCV genotypes 1a, 1b and 3 were found in patients with CHC. Isolation of RNA and reverse transcription. RNA was extracted from blood plasma samples using TRIZOL reagent (Invitrogen, United States). Briefly: 1 ml of TRIZOL reagent was added to 250 μl of blood plasma supernatant according to the manufacturer's recommendations. In order to improve the precipitation of small RNAs, the supernatant obtained from chloroform extraction was mixed with an equal volume of absolute ethanol (Merk) and left overnight at –80 ° C. The formed precipitate was separated by centrifugation at 12000 g for 30 min at 4 ° C and washed with 70% ethanol cooled to 0 ° C. After dissolution in RNase-free water, the purity of the isolated RNA was assessed spectrophotometrically: by the ratio of the optical density of the solution (A) at wavelengths 260/280 nm, using the ND2000 system (NanoDrop Technologies, USA). The value A260 / 280 ≈ 1.8-2.0 corresponded to an acceptable degree of purification. To obtain cDNA, the RNA sample was first treated with DNase, and then polyadenylation and reverse transcription were performed using poly (A) polymerase and specific primers, respectively. The resulting product was diluted 20 times with deionized water containing neither DNase nor RNase, and stored at –80 ° C. The expression level of miRNA-122 in blood plasma was measured by reverse transcription PCR in accordance with the TaqMan microRNA analysis protocol. Total RNA was extracted with TRIZOL reagent (Invitrogen, CA, USA) according to the manufacturer's instructions. The RNA concentration was measured using a NanoDrop ND2000 spectrophotometer (NanoDrop Technologies, USA). Reverse transcription was performed using the miScript Reverse Transcription Kit (QIAGEN, Germany). The expression level of mature miRNA-122 was investigated using the miScript SYBR Green PCR Kit (QIAGEN, Germany) according to the manufacturer's instructions. MiRN level A is calculated by the formula (2 Ct * 100), normalized to U6 snRNA and presented in arbitrary units. Amplification was performed on Rotor Gene 6000 and Rotor Gene Q instruments (Qiagen, Germany). The data obtained are analyzed using the software in the form of graphs. The obtained digital data are processed using the Microsoft Office 2007 and Statistica 6 software package (StatSoft Inc., USA). (For the critical value of the significance level, 5% is taken. The analysis of the correspondence of the distribution to the law of normal distribution of traits was performed using the Shapiro-Wilk test (in the case of miRNA ($p = 0.0152$), miRNA $p < 0.05$, the distribution of the trait differs from normal. that W then the probability of differences in samples is carried out by a nonparametric method.)

Isolation of total RNA from blood plasma containing a fraction of mature miRNAs was carried out using the Ribot-prep-100 kit (Central Research Institute of Epidemiology,

Rospotrebnadzor, Russia) in accordance with the manufacturer's protocol. The quantitative determination of the isolated RNA was carried out on a Qubit-4 ™ fluorometer (Invitrogen, USA) using a Qubit MicroRNA Assay Kit-100 for the quantitative determination of microRNA (5-100 ng) (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. The RNA concentration in the sample was calculated automatically using a calibration curve. The yield of "pure" RNA was from 0.5 to 30 μg / ml. The reverse transcription reaction was carried out using the MMLV RT kit (Evrogen, Russia) for the synthesis of cDNA on an RNA template and stem-loop primers (20 μM) to certain microRNA-hsa-miR-122, U6snRNA (TaqMan microRNA Assays, Thermo Fisher Scientific, USA). The average value of the relative level of miR-122 was determined after carrying out the polymerase chain reaction in duplicate for each sample in real time on a Rotor-Gene Q device (Qiagen, Germany).

The results ΔCT = miR122 Ct - U6 Ct were normalized in relation to the group of healthy individuals. The results of determining miR-122 in groups were presented in relative units as the arithmetic mean and the arithmetic mean error. The sample size was not pre-calculated. Mann-Whitney (U) and chi-square (χ2) tests were used to analyze differences between groups.

Results and Discussion

In the course of the study, we analyzed the dependence of the expression of miRNA 122 on sex and age. The correlation coefficient was calculated between the expression level of miRNA-122 and the duration of the pathological process (r = -0.36). The duration of the disease in patients of group 1 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 for men and 3.8 ± 2.2 years for women. 4 ranges are highlighted: low 0.001 - 0.14, medium 0.15 -1.05, high 1.05 - 12.88 and the highest -> 12.89. Analysis of the expression level of miR-122 showed that patients with a low level of miR-122 in serum had the longest period of illness.

The differences in the incidence of patients in subgroups 1 and 2 of patients in the miR-122 range of 0.001–0.14 were 5.9% versus 26.7%, respectively. The calculated chance of detection and risk of complications in this range are 4.5 (95% CI 0.57 - 36.22) and 5.8 (95% CI 0.57 - 59.31), respectively. However, despite the high rates OR = 5.8 and RR = 4.5, this difference turned out to be statistically insignificant (χ2 = 1.3, P> 0.3) (Table 5.1).

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Table 5.1 Statistical difference in the frequency of miR-122 indicators in the group of patients with CVH and LC (case-control model)

Indicators miR-122	1st group n=17		2nd group n=15		χ^2	P	RR	95% CI	OR	95% CI
	abs	%	abs	%						
0,001 – 0,14	1	5,9	4	26,7	1,3	0,3	4,5	0,57-36,22	5,8	0,57-59,31
0,15 -1,05	2	11,8	10	66,7	8,0	0,005	5,7	1,46-21,86	15	2,4-93,0
1,05 – 12,88	9	52,9	1	6,7	5,9	0,01	7,9	1,13-55.58	15,7	1,67- 148,1
>12,89	5	29,4	0	-	1,6	0,2	4,7	0,614-36,03	6,2	0,64- 60,93

In the range of 0.15-1.05, the incidence of miR-122 in patients in subgroups 1 and 2 of patients was 11.8% versus 66.7%, respectively. The calculated chance of detection and the risk of complications in this range is 5.7 (95% CI 1.46-21.86) and 15 (95%, CI 2.4-93.0), respectively, High rates OR = 15 and RR = 5.7, the difference turned out to be statistically significant ($\chi 2 = 8.0$, $P < 0.001$), In this range, the risk of developing liver cirrhosis increases, and we can clearly see this.

The difference in the incidence of patients in subgroups 1 and 2 of patients in the range 1.05-12.88 was 52.9% versus 6.7%, respectively. The calculated chance of detection and risk of complications in this range is 7.9 (95% CI1, 13-55.58) and 15.7 (95% CI 1.67-148.1), respectively. However, despite the high rates OR = 15.7 and RR = 7.9, the difference turned out to be statistically significant ($\chi 2 = 5.9$, $P < 0.01$), In the third range, RR increases almost 8 times, the risk of detection, i.e. OR increases 15.7 times, In this case, a significant association was revealed between the expression level of miRNA-12 2 and stage 4 fibrosis according to USE,

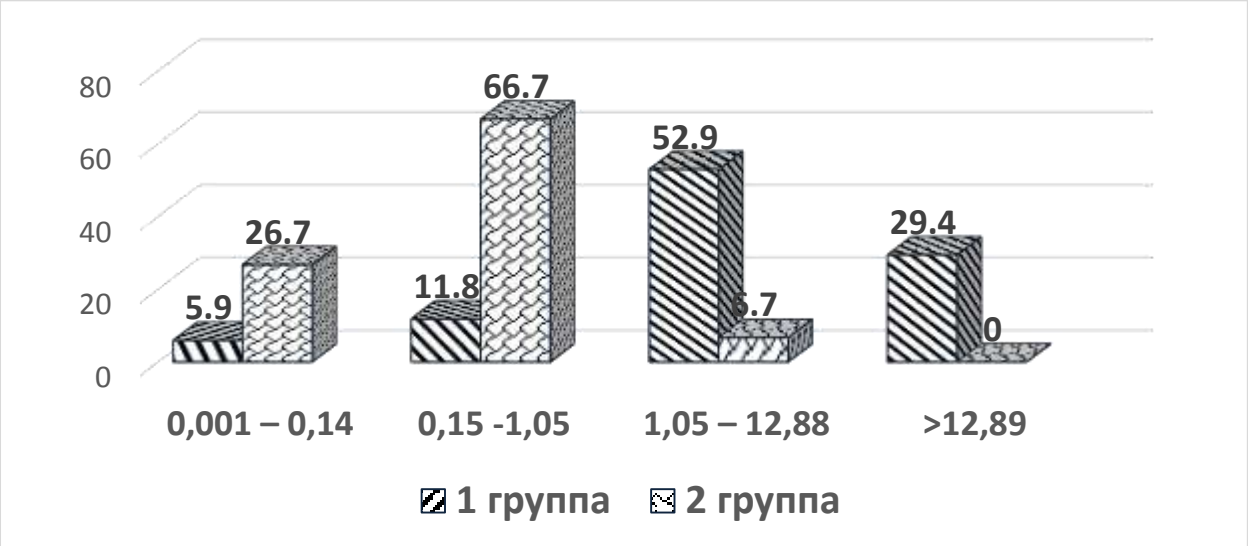

Figure. 5.1. Expression level of microRNA-122

Differences in the incidence of patients in groups 1 and 2 in the range of 1.05–12.88 were 52.9% versus 6.7%, respectively. The calculated chance of detection and risk of complications in this range are 7.9 (95% CI 1.13-55.58) and 15.7 (95% CI 1.67-148.1), respectively. However, despite the high indicators OR = 15.7 and RR = 7.9, the difference turned out to be statistically significant ($\chi 2$ = 5.9, P <0.01). In the third range, RR increases almost 8 times, the risk of detection, i.e., e OR increases by a factor of 15.7. In this case, a significant association was revealed between the expression level of miR-122 and fibrosis of stage 4 according to USE data.

The difference in the incidence of patients in groups 1 and 2 in the range of miR-122> 12.89 was 29.4% versus 0%, respectively. The calculated chance of detection and the risk of complications in this range is 4.7 (95% CI 0.614 - 36.03) and 6.2 (95% CI0.64 - 60.93), respectively. However, despite the high rates OR = 6.2 and RR = 4.7, such a difference turned out to be statistically insignificant ($\chi 2$ = 1.6, P <0.05), since no patients were observed in group 2 in this range, with an increase in the number of patients, the differences would be more pronounced.

Conclusions

1. Therefore, a decrease in the level of expression of miRNA-122 in blood plasma is undoubtedly one of the pathophysiological links in the development of fibrosis and its complications in CVH patients.
2. Modern medicine regards CKD as a multifactorial pathology, which, in addition to etiological factors, is based on complex disorders of biochemical and molecular genetic processes.
3. Serum microRNA-122 is a new potential parameter of liver dysfunction and a predictor of the risk of complications in patients with chronic viral hepatitis.

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