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Microrna-122: A Prognostic Marker of the Pathological State of the Liver

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Abstract: This article provides information on the role of miRNAs in the progression of chronic diffuse diffuse liver diseases from the stage of chronic viral hepatitis to severe fibrosis. The study of new parameters for predicting the nature of the course, the rate of progression of liver fibrosis, the risk of developing HCC is of particular importance. In this direction, great prospects are opening up in the field of genomics. Among the total array of works, a significant share is occupied by studies of single nucleotide polymorphisms, freely circulating DNA, endosomal RNA and their significance in the pathogenesis of various diseases. The discovery in 2002 of microRNA-122 (miR-122) was an event in hepatology due to the functional features of this RNA cluster.

Purpose: to study the expression level of microRNA-122 as a prognostic parameter for the risk of complications in patients with chronic viral hepatitis.

Material and methods: to study the role of microRNA-122, 32 patients were selected from the total number of examined patients: 17 (53.1%) of them with a diagnosis of chronic viral hepatitis made up 1 subgroup and 15 (46.9%) with a diagnosis of compensated cirrhosis of the liver 2 subgroup. The expression level of microRNA-122 in blood plasma was measured using reverse transcription PCR in accordance with the TaqMan microRNA analysis protocol. The study corresponds to the priority areas for the development of science and technology in the Republic of Uzbekistan.

Results: Intervals of the miRNA-122 expression level range were determined, 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 liver fibrosis, being indicators of the functional capacity of the liver. It has been established that in patients with chronic viral hepatitis and cirrhosis of the liver, the range of miRNA-122 expression levels varies depending on the stages of fibrosis;

It has been proven that a decrease in the expression level of miRNA-122 is a prognostic marker in the development of complications of chronic viral hepatitis. The prognostic criteria of microRNA-122 were assessed in relation to the unfavorable prognosis of the risk of complications.

Conclusion: the significance of the results of the study lies in the need to determine the level of miRNA-122 expression as a prognostic marker for the development of complications in chronic viral hepatitis and to include molecular genetic research methods in the standard of patient examination, which prevents early disability and reduces mortality.

Keywords: chronic viral hepatitis, microRNA, liver fibrosis

Introduction

MicroRNAs, evolutionarily highly conserved, small (18–25 ribonucleotides) noncoding RNAs, are involved in the regulation of almost all cell functions [2, 10]. They are currently emerging as a new class of biomarkers. MiRNA dysregulation plays a key role in the pathogenesis of various diseases. A specific feature of microRNAs is their stability in the bloodstream [9, 10]. Many international studies have shown their importance as innovative markers in various pathological conditions of the body [1,4,7]. The advantages of microRNAs in this regard include versatility of detection, relative stability, and high sensitivity during sample storage. To date, more than 4000 miRNAs have been described [3,5,6]. Circulating miRNAs in serum are stable and protected from disappearance in body fluids, which makes them universal biomarkers in many diseases [9,10]. In the liver, miRNA-122 accounts for approximately 70% of all miRNAs and is important for the functional state of the hepatocyte, while other organs express a much smaller amount of this miRNA. MicroRNA -122 regulates many genes in the liver that control the cell cycle, differentiation, proliferation, and apoptosis [4,7].

Loss of miRNA-122 in the liver leads to dedifferentiation of the liver with a malignant phenotype. 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 LF seem to be a very important task for modern practical hepatology [8,11].

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 the pathological state of the liver.

Purpose of the study is to optimize the methods of non-invasive diagnosis of liver fibrosis in patients with chronic viral hepatitis.

To study the role of miRNA-122 in the study, 32 patients were selected from the total number of examined patients: 17 (53.1%) of them with a diagnosis of chronic viral hepatitis made up subgroup 1 and 15 (46.9%) with a diagnosis of compensated cirrhosis of the liver, subgroup 2 , from among those hospitalized for inpatient treatment in the department of hepatology of the RIEMIDRepublic of Uzbekistan. Genetic studies were carried out on the basis of the laboratory of molecular medicine and cellular technologies of the Republican Scientific and Practical Center for Hematology of the Republic of Uzbekistan.

We isolated 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

Material and methods: to study the role of microRNA-122, 32 patients were selected from the total number of examined patients: 17 (53.1%) of them with a diagnosis of chronic viral hepatitis made up 1 subgroup and 15 (46.9%) with a diagnosis of compensated cirrhosis of the liver 2 subgroup.

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 determining the load of HCV, the PCR method was used. HCV genotypes 1a, 1b and 3 were found in patients with CHC. RNA isolation and reverse transcription. RNA was extracted from blood plasma samples using a 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 anhydrous 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 evaluated spectrophotometrically: by the ratio of the optical density of the solution (A) at wavelengths of 260/280 nm using the ND2000 system (NanoDrop Technologies, USA). The acceptable degree of purification corresponded to the value A260/280 ≈ 1.8–2.0. To obtain cDNA, an 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 DNases nor RNases and stored at –80°C. The level of miRNA-122 expression in blood plasma was measured using reverse transcription PCR in accordance with the TaqMan miRNA analysis protocol. Total RNA was extracted with TRIZOL reagent (Invitrogen, CA, USA) according towith 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 studied using the miScript SYBR Green PCR Kit (QIAGEN, Germany) according to the manufacturer's instructions.

The miRNA level is calculated by the formula (2 Ct*100), normalized to U6 snRNA and presented in arbitrary units. Amplification was carried out on Rotor Gene 6000 and Rotor Gene Q instruments (Qiagen, Germany). The obtained data are analyzed using 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). (The significance level is 5% for the critical value. The analysis of the correspondence of the distribution to the law of normal distribution of signs was performed using the Shapiro-Wilk test (in the case of miRNA ($p=0.0152$), miRNA $p<0.05$, the distribution of the sign differs from the normal one. Considering that W then the probability of differences in the samples is carried out by a non-parametric method.)

Isolation of total RNA from blood plasma containing a fraction of mature miRNAs was performed using the Ribo-prep-100 kit (Central Research Institute of Epidemiology, Rospotrebnadzor, Russia) in accordance with the manufacturer's protocol. The isolated RNA was quantified on a Qubit-4™ fluorimeter (Invitrogen, USA) using a Qubit microRNA Assay Kit-100 reagent kit for the quantitative determination of microRNAs (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 ranged 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) for certain miRNA-hsa-miR-122, U6snRNA (TaqMan microRNA Assays, Thermo Fisher Scientific, USA). The average value of the relative level of miR-122 was determined after the polymerase chain reaction in duplicate for each sample in real time on a Rotor-Gene Q instrument (Qiagen, Germany).

The results ΔCT = miR122 Ct - U6 Ct were normalized in relation to the group of healthy individuals. The results of the determination of miR-122 in groups were presented in relative units as the arithmetic mean value and the error of the arithmetic mean. The sample size was not previously calculated. The Mann-Whitney test (U) and chi-square test ($\chi 2$) were used to analyze differences between groups.

Results and discussion

In the course of the study, an analysis was made of the dependence of miRNA 122 expression on sex and age. The correlation coefficient between the expression level of microRNA-122 and the duration of the pathological process was calculated (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 the 2nd group was clearly different and averaged 6.8±1.8 years for men and 3.8±2.2 years for women. 4 ranges were distinguished: low 0.001 - 0.14, medium 0.15 -1.05, high 1.05 - 12.88 and the highest -> 12.89. Analysis of miR-122 expression level showed that patients with low miR-122 levels in blood serum had the longest period of the disease.

Differences in the frequency of occurrence of patients in the 1st and 2nd subgroups of patients in the miR-122 range of 0.001–0.14 was 5.9% versus 26.7%, respectively. The calculated chance of detection and risk of complications in this range is 4.5 (95% CI 0.57 - 36.22) and 5.8 (95% CI 0.57 - 59.31), respectively. However, despite the high rates of OR=5.8 and RR=4.5, such a difference turned out to be statistically insignificant ($\chi 2$=1.3, P>0.3) (Table 1).

Table 1.Statistical difference in the frequency of miR-122 indicators in the group of patients with CVH and cirrhosis (case-control model)

Indicators miR-122	1 group n=17		2 groupn=15		χ^2	P	RR	95% CI	OR	95% CI
	абс	%	абс	%						
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-	15,7	1,67-

								55,58		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 frequency of miR-122 occurrence in patients in subgroups 1 and 2 was 11.8% versus 66.7%, respectively. The calculated chance of detection and 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 scores OR=15 and RR=5.7, the difference was statistically significant ($\chi2$=8.0 , P<0.001), In this range, the risk of developing liver cirrhosis increases, and we clearly see this.

Differences in the incidence of patients in the 1st and 2nd subgroups of patients in the range of 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 OR=15.7 and RR=7.9, the difference was statistically significant ($\chi2$= 5.9, P<0.01), In the third range, RR increases by almost 8 times, the risk of detection, i.e., OR increases by 15.7 times, In this case, a significant association was found between the level of miRNA-122 expression and fibrosis 4 stages according to USE,

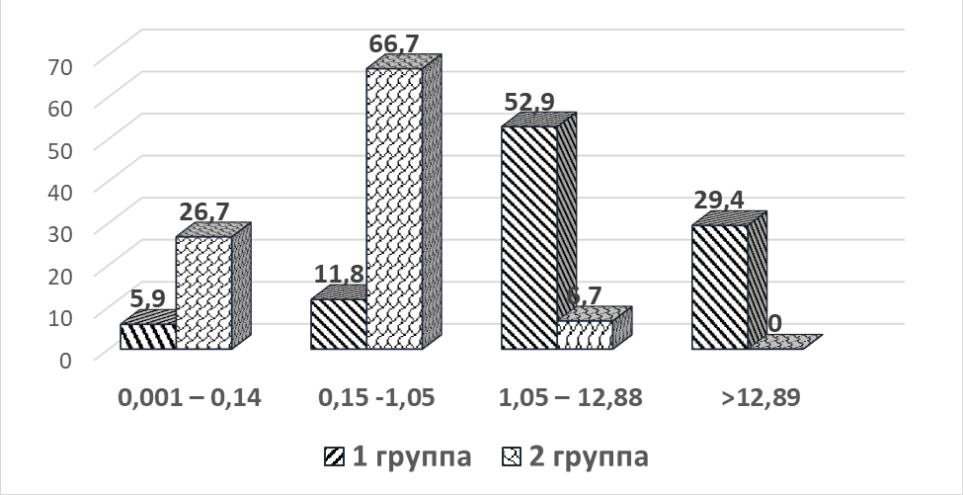

Figure 1. Expression level of microRNA-122

Differences in the frequency of occurrence of patients in groups 1 and 2 of patients in the range of 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% CI 1.13-55.58) and 15.7 (95% CI 1.67-148.1), respectively. However, despite the high rates of OR=15.7 and RR=7.9, the difference turned out to be statistically significant ($\chi2$=5.9, P<0.01). In the third range, RR increases almost 8 times, the risk of detection, t ,e OR increases by 15.7 times. In this case, a significant association was found between the level of miRNA-122 expression and stage 4 fibrosis according to ultrasound data.

Differences in the incidence of patients in groups 1 and 2 of patients in the miR-122>12.89 range was 29.4% versus 0%, respectively. The calculated chance of detection and 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 of OR=6.2 and RR=4.7, such a difference turned out to be statistically insignificant ($\chi2$=1.6, P<0.05), since no patients were observed in this range in group 2. Perhaps, with an increase in the number of patients, the differences would be more pronounced.

Conclusion

1. Consequently, a decrease in the level of miRNA-122 expression in the blood plasma is undoubtedly one of the pathophysiological links in the development of fibrosis and its complications in patients with CVH.

2. Modern medicine regards CKD as a multifactorial pathology, which, in addition to etiological factors, is based on complex violations of biochemical and molecular genetic processes.

3. The introduction of molecular genetic research methods, in particular, the determination of the miRNA-122 expression level in standard methods for the study of patients with CVH, taking into account the ranges, will help to identify risk groups for complications in advance, choose the right treatment tactics and, therefore, prevent disability caused by complications, and also extend the period to CP and reduce mortality rates.

References

1. Bala S., Circulating microRNAs in exosomes indicate hepatocyte injury and inflammation in alcoholic, drugeases / Bala S., Petrasek J., Mundkur S. [et al.] // Hepatology. — 2012. — Vol. 56. — P. 1946—1957.
2. Blancooa A Circulating microRNAs: molecular microsensors in gastrointestinal can cer / Blancooa A. [et al.] // Sensors (Basel). — 2012. — Vol. 12. — P. 9349—9362
3. Cermelli S. Circulating microRNAs in patients with chronic hepatitis C and non J. A. Marrero // PLoS One 6. — 2011. — e23937.
4. Elfimova N. Hepatocyte growth factor (HGF) inhibits collagen I and IV synthesis in hepatic stellate cells by miRNA /Elfimova N. et al. // PLoS One. — 2011. — Vol. 6. — e 24568.
5. Gao B.. Alcoholic liver disease: pathogenesis and new the rapeutic targets / B. Gao and Bataller R // Gastroenterology. — 2011. — Vol. 141. —1572—1585.
6. Gui J . Serum microRNA characterization identifies miR al marker for detecting liver pathologies / Gui J., Tian Y., Wen X. [et al.] // Clin. Sci (Lond). — 2011. — Vol. 120. — P. 183—193.
7. Ji J. MicroRNA expression, survival, and response to interferon in liver can cer / Ji J., Shi J., Budhu A. [et al.] // N. Engl. J. Med. — 2009. — Vol. 361. — P. 1437—1447.
8. Marquez R. T., Correlation between microRNA expression levels and clinical parame ters associated with chronic hepatitis C viral infection in humans /Marquez R. T., Bandyopadhyay S., Wendlandt E. B. [et al.] // Lab Invest. — 2010. — Vol. 90. — P. 1727—1736.
9. Roderburg C . Microofiling reveals a role for miRfibrosis / Roderburg C., Urban G. W., Bettermann K. [et al.]// Hepatolo gy. — 2011. — Vol. 53. — P. 209—218.
10. S.Ryder . Progression of hepatic fibrosis in patients with hepatitis C: a prospective repeat liver biopsy study / S.Ryder et al. // Gut. — 2004. — V. 53. — P. 451 —455.
11. Shu J. Genomewide microRNA downegulation as a negative feedback mechanism in the early phases of liver regeneration / Shu J., Kren B. T., Xia Z. [et al.] // Hepatology. — 2011. — Vol. 54. — P. 609—619.
12. Winter T. N Differential plasma MicroRNK Profiles in HBeAg Positive and HBeAg Nega tive Children with Cronic Hepatitis B / Winter T. N., Bang Heiberg I. L., [et al.] // PLOS ONE. — 2013. — Vol. 8, Issue 3. — P. 58236.
13. Zaret K. S. Regulatory phases of early liver development: paradigms of organogenesis / K. S. Zaret // Nat Rev Genet. — 2002. — Vol. 3. —499—512.