Original Article

Serum MicroRNA-122 as a Prognostic Biomarker in Patients with Liver Cirrhosis

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ABSTRACT

Background: Liver cirrhosis is associated with high morbidity and mortality. MicroRNAs (miRNAs), a class of endogenous small non-coding RNAs, are becoming increasingly recognized as crucial regulators in gene expression networks. In particular, a low serum miRNA-122 level was associated with hepatic decompensation so it can be used as a prognostic marker for liver decompensation.

Circulating miRNA-12 was examined aiming to clarify its prongnostic value in patients with liver cirrhosis and to discover its relation with patient's survival.

Methods: Gene expression level of *miRNA 122* was extracted and assessed in sera of 100 patients with liver cirrhosis, using quantitative reverse-transcription PCR (qRT-PCR) . MiRNA 122 expression levels were compared to liver function tests, MELD score, overall survival time and to different manifestation of decompensation.

Results: Serum samples from patients with hepatic decompensation showed significant down regulation of *miRNA122* compared to those from patients with compensated liver cirrhosis. Patients with ascites, and hepatorenal syndrome had significantly lower miRNA-122 levels than patients without these complications. A univariate Cox regression analysis revealed a significant association between miRNA-122 levels and overall survival Multivariate Cox regression analysis revealed that only miRNA-122 serum levels and platelet count were independent factor for survival. MiRNA-122 sensitivity and specificity for the predection of decompensation in cirrhotic patients were 100.0% and 90.1% respectively the best cut off point value of 0.892 (AUC= 0.9429)

Conclusions: Serum miRNA-122 is a useful new independent prognostic marker in patients with liver cirrhosis.

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Introduction

Cirrhosis can be a result of different mechanisms of liver injury that lead to necroinflammation and fibrogenesis. Chronic infection with HCV is the most common cause of chronic liver disease,it is complicated by cirrhosis in about 20 % of western world[1]. The morbidity increases rapidly when hepatic decompensation occurs,. After the first manifestation of ascites the one year mortality increases up to 40% [2]. With occurrence of spontaneous bacterial peritonitis or hepatorenalsyndrome ,the patients' prognosis further deteriorates [3]. If liver transplantation is indicated , the assessment of the risk of death in these patients will be of great interest and importance to optimize the time point for organ allocation [4].

Short term mortality was more precisely evaluated by the development of the Model for End Stage Liver Disease (MELD).MELD best predicts 3 month survival of cirrhotics, irrespective of etiology.Giving priority to patients who are most likely to die without livertransplanation such as those with hepatorenal failure,it is based on creatinine, bilirubin and INR, but lacks features of portal hypertension, such as ascites.[5] MicroRNAs (miRNAs), a class of endogenous small non-coding RNAs, are becoming increasingly recognized as pivotal regulators in gene expression networks . They can function as translational repressors by binding to the 3'untranslated regions (3' UTRs) of target messenger RNAs (mRNAs) and inforcing their degradation and/or leading posttranscriptional repression[6].

Dysregulated miRNAs play crucial roles in the pathogenesis of various diseases and characteristic miRNA patterns have been found in pathological tissues[7].Playing an important role in cell development, differentiation, and physiological function is not it's only value, but are also significant in the development of tumors, viral infections, and other closely related diseases.[8,9] MiRNA-122 family is the most abundant type of miRNAs in the liver [10]. And is important for the functional condition of the hepatocyte. It organizes many genes in the liver that adjust the cell cycle, differentiation, proliferation and apoptosis[11].Moreover, its Loss in the liver leads to hepatic undifferentiation witha malignant phenotype[12], a finding which is frequent in hepatocellular carcinoma (HCC), and was referred to correlate with migration, invasion and in vivo tumorigenesis[13]

Decreased level of miRNA-122 in fibrotic liver biopsies may be explained as the result of compromised normal hepatocytic activity or as the forsaken suppressive function of miRNA-122 that inhibits fibrogenesis.[14] The objectives of this research were studying miRNA-122 as apotential prognostic marker for liver cirrhosis, its correlation with other standard laboratory parameter,association with complications of liver cirrhosisand discovering its role as a predictor of survival in decompensated patients

Material and Methods

The current study was approved by the Local Ethics Committee of Faculty of Medicine, Benha University and all study participants gave a written informed consent prior to enrollment in the study.

The study was designed as a cohort prospective study. It copmrised100 patients with liver cirrhosis, 68 male and 32 female, selected from the departments of Hepatology, Gastroenterology & Infectious Diseases and Internal Medicine, Benha University Hospital between January 2014 and May 2015. The patient were categorized into

Group I: with22 patients diagnosed as compensated liver cirrhosis, and

Group II: with78 patient with decompensated liver cirrhosis.

Exclusion Criteria Included : age below 18 years, former liver transplantation and history of cancer in the last 5 years other than hepatocellular carcinoma.

All Individuals in The Study were Subjected to: Full history taking including: age , sex, duration of disease and identification of the causes of liver cirrhosis as Hepatitis C, Hepatitis B, Primary sclerosing cholangitis, Autoimmune hepatitis, Non alcoholic steatohepatitis, Cardiac cirrhosis, Hemochromatosis, Primary biliary cirrhosis, Hepatocellular carcinoma and alcohol abuse .

Liver cirrhosis was assessed by:

- 1-Histopathological examination or pathognomic result abdominal ultrasound examination.
- 2-Coumpter tomography or magnetic resonance imaging.
- 3-Biopsies were performed only for patients with inexplicit stage of fibrosis or unclear cause of liver cirrhosis.

Assessment of Symptoms of Hepatic Decompensation: (variceal bleeding, sponteannous bacterial perotionitis, ascitis, hepatorenal syndromeandhepatic encephalopathy) was done by clinical, radiological, laparoscopic and laboratory investigation.

Diagnostic criteria for hepatorenal syndrome were:

1. A plasma creatinine concentration above 1.5 mg/dl that progresses over days to weeks.
2. The absence of any other apparent cause for the renal disease, including shock, ongoing bacterial infection,

current or recent treatment with nephrotoxic drugs, and the absence of ultrasonographic evidence of obstruction or parenchymal renal disease.

3. Urine red cell excretion of less than 50 cells per high power field (when no urinary catheter is in place) and protein excretion less than 500 mg/day.

4. Lack of improvement in renal function after volume expansion with intravenous albumin (1 g/kg of body weight per day up to 100 g/day) for at least two days and withdrawal of diuretics.[15]

The Patients were followed up until death (=overall survival), Liver Transplantation or Last Contact.

Blood Sampling: Peripheral blood samples were withdrawn from all patients under aseptic condition, then each sample was divided into 3 parts: (1.8) ml blood for each 0.2 ml Na citrate for determination of PT and international normalized ratio, 2 ml on EDTA for measurement of platelet count, and 4 ml sample collected in plain vacutainers for separation of serum . Serum was separated by centrifugation at 4ºc at 1500 x g for 10 mins. followed by an additional centrifugation at 4ºc at 2000 x g for 15 mins. The sera were aliquoted into 2 tubes: one was used for chemical analysis of liver function tests using Biosystem A15 autoanalyzer (Biosystem. Spain). The other serum aliquot was used for assessment of *miRNA 122* expression level.

Data were Used for Calculation of MELD Score.

Assessment of *miRNA 122* expression level by qPCR: MicroRNA was first extracted from fresh serum samples using the miRNeasy Mini Kit (Qiagen, Germany)

Next, the Reverse transcription step was performed using the miScript II RT Kit (Qiagen, Germany) and the cDNA products were kept at -20 till further processing.

Finally, qPCR of *miRNA122* was performed by StepOne™ Real-Time PCR System (Life Technologies, USA) using miScript SYBR Green PCR Kit supplied by (QIAGEN, Germany)the manufacturer instructions were followed throughout all steps. Briefly, PCR was initially activated at 95°C for 15 mins., then 40 cycles of denaturation at 94°C for 15sec., annealing at 55°C for 30sec.and a final extension step at 70°C for 30sec.The primers for *miRNA-122* and housekeeping gene (*RNU6B*) were supplied by Qiagen, Germany. After PCR*miRNA122*quantificationwas analysed using StepOne software (Life Technologies, USA) and expressed as relative mRNA level compared to *RNU6B* according tothe $2^{-\Delta\Delta Ct}$ method[16]

Statistics: The statistical analysis was conducted using STATA/SE 11.2 for Windows (STATA corporation, College Station, Texas). Tests used were (Chi-square test (χ2), Fisher's Exact Test (FET) , Student t-test (t) , Mann-Whitney test (z) , Kruskal Wallis test (χ2) and Spearman correlation coefficient (rho; ρ). Survival analysis was carried out using both univariate and multivariate Cox regression models to detect important predictors for patients' survival based on potential risk factors and liver functions.

Receiver Operator Curve (ROC) was used to evaluate the predictability of serum miRNA 122 levels for the presence of liver decompensation in cirrhotic patients. The Area Under the Curve (AUC), the best cut off point and the corresponding sensitivity and specificity were determined. The corresponding P-values were obtained. A P-value < 0.05 was considered statistically significant (S), a P-value < 0.001 was considered statistically highly significant (HS), while a P-value > 0.05 was considered statistically non-significant.

Results

The patients' characteristics are shown in (Table 1). The follow up period ranged between 200 and 500 days, with median of 343.4±83.44 days and. During the study time 22 out of 100 patients died and 6 patients were dropped out due to loss of contact with them. No patients underwent liver transplantation.

Serum *miRNA-122*expression Levels in Patients with Liver Cirrhosis: Our results showed that serum miRNA-122 was down regulated in patients with hepatic decompensation with a high significance compared to patients with compensated liver disease (P <0.001). (Fig.1) On examining the relation of miRNA 122 with decompensating manifestation, There was significantly lower expression of serum miR-122 levels in patients with hepatorenal syndrome (p=0.03) and, ascitis (P = 0.038) than patients without the respective complications. (Fig.2). Furthermore, serum miRNA 122 expression levels showed no significant differences between patients with and without variceal bleeding (P = 0.81), hepatic encephalopathy (p=0.73), spontaneous bacterial peritonitis (P=0.94) and hepatocelluar carcinoma (p=0.94). (table 2)

Correlation of miRNA-122 Levels with Laboratory Parameters: There was a significant negative correlation between serum miRNA 122 expression levels and MELD score (p=0.003) (Fig. 3), serum creatinine (P=0.007), INR (p=0.048), total bilirubin (p=0.002) and GGT (p=0.045),

and it was highly significant with ALP (p<0.001). Meanwhile, there was significant positive correlation between serum expression of miRNA-122 levels and AST and ALT (p=0.03 and 0.047), respectively.

In contrast, there was no significant correlation between serum miRNA 122expression and neither total protein (p=0.79), albumin (p=0.58) nor platelet count (p=0.64)

The Sensitivity and Specificity for the Predection of Decompensation in Cirrhotic Patients: ROC curve showed that at the best cut off value for miRNA 122expression (0.892)the sensitivity and specificity for the prediction of decompensation in cirrhotic patients were 100.0% and 90.1% respectively with the area under the curve (AUC= 0.9429), while at the best cut off value for MELD score (15)the sensitivity and specificity for the prediction of decompensation in cirrhotic patients were

92.31%and 90.91%respectively with the area under the curve (AUC=0.9161).(Fig 4& 5)

The Serum miRNA-122 Level as a Predictor of Survival in Patients with Liver Cirrhosis: The study population were divided into tertiles according to the miRNA-122 levels at cutoff points 0.038 and 0.1805 .The third of patients with the highest miRNA-122 levels was compared to the two thirds of patients with lower miR-122 serum concentration. Aunivariate Cox regression analysis was performed. There was a significant association between miRNA-122 levels and overall survival p<0.001, hazard ratio 0.23, 95% confidence interval (0.10 – 0.52). (Fig. 6) Cox regression analysis using forward stepwise (likelihood ratio) revealed that miRNA-122 serum levels (P =0.02) and platelet count (P =0.02) were independently associated with patients' survival. (table3).

Table 1: Patient characteristics.

Variable No.		Compensated cirrhosis (No.=22)		Decompensated cirrhosis (No.=78)		Total (No.=100)		Test	P
		%	No.	%	No.	%			
Sex	Females	6	27.27	30	38.46	36	36.0	χ^2= 0.93	0.33
	Males	16	72.73	48	61.54	64	64.0		
Age (years)	Mean ±SD; (range)	52.82±5.29; (40-60)		55.05±6.27; (40-66)		54.56±6.11; (40-66)		t= 1.52	0.13

Table 2: Relation between miRNA-122 and decompensating manifestations

| Variable Mean		Micro RNA-122			Test	P
		± SD	Range			
Spontaneous bacterial peritonitis	No (No.=72)	0.120	0.183	0.0007-0.892	z= 0.07	0.94
	Yes (No.=6)	0.057	0.025	0.041-0.089		
Hepatorenal syndrome	No (No.=46)	0.177	0.13	0.0007-0.589	z= 2.11	0.03 (S)
	Yes (No.=32)	0.113	0.23	0.004-0.892		
Variceal bleeding	No (No.=46)	0.106	0.185	0.004-0.892	z= 0.24	0.81
	Yes (No.=32)	0.129	0.167	0.0007-0.589		
Hepatic encephalopathy	No (No.=64)	0.102	0.134	0.0007-0.589	z=0.34	0.73
	Yes (No.=14)	0.176	0.307	0.004-0.892		
Ascites	No (No.=22)	0.144	0.163	0.002-0.589	z= 2.07	0.038 (S)
	Yes (No.=56)	0.104	0.182	0.0007-0.892		
HCC	No (No.=72)	0.124	0.181	0.002-0.892	z= 0.07	0.94
	Yes (No.=6)	0.005	0.004	0.0007-0.009		

**Table 3: Univariate and multivariate analyses of factors associated with overall survival.**

Variable (No.=94)	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	p
Micro RNA-122 (High *vs.* low)	0.23	0.10 – 0.52	<0.001	0.36	0.15-0.88	0.02
Meld scores (>18)	5.46	1.89-15.79	0.002			
Sex (Male *vs.* female)	0.91	0.42-1.99	0.82			
Age (>65 years)	2.50	0.57-10.84	0.22			
Total protein (g/dl)	0.61	0.41 – 0.91	0.01			
Platelet count (x103/mm3)	0.98	0.96 – 0.99	0.001	0.98	0.97-1.00	0.02
Albumin (g/dl)	0.49	0.25 – 0.97	0.04			
Bilrubin (mg/dl)	1.10	1.04 – 1.17	<0.001			
GGT (Iu/l)	1.01	1.00 – 1.01	0.001			
ALP (Iu/l)	1.02	1.00 – 1.03	0.008			

HR: hazard ratio

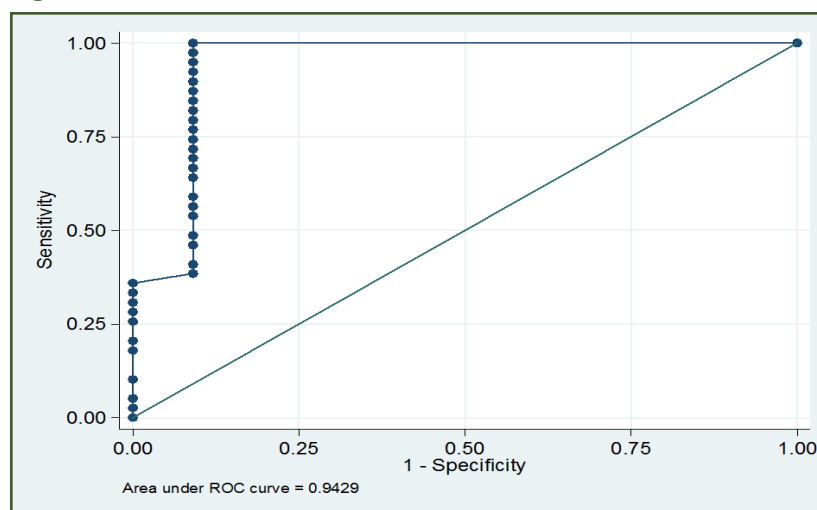HR<1 indicates reduced hazard risk and increased survival.

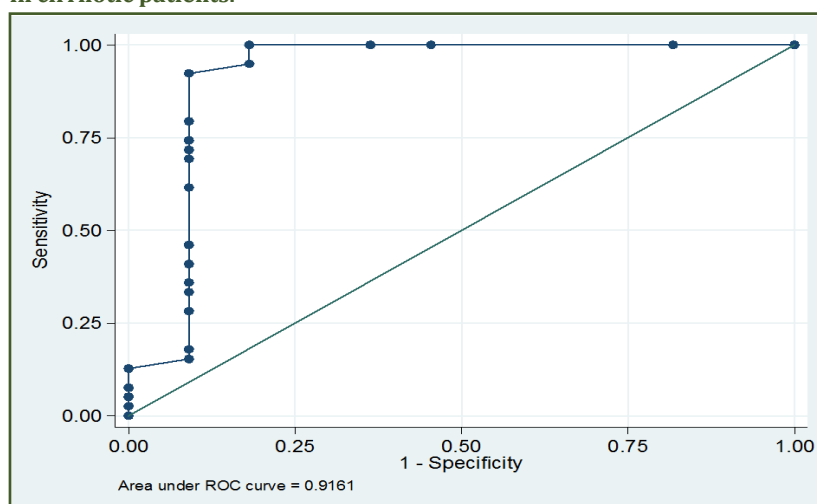HR>1 associated with increased hazard risk and reduced survival.
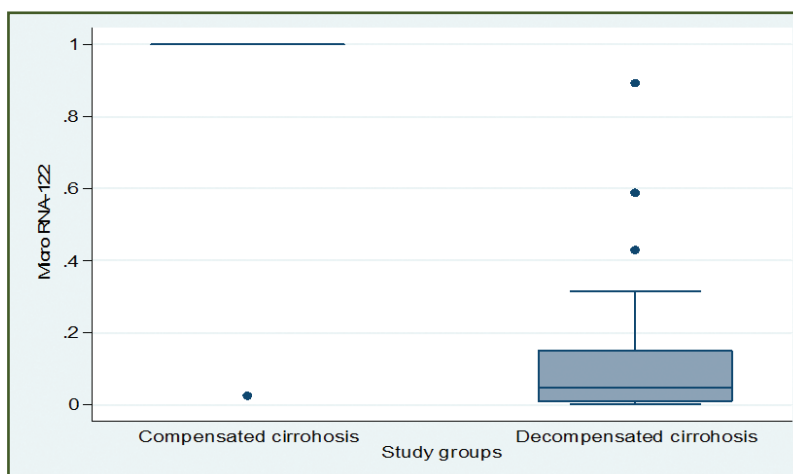
Fig. 1: Comparison between studied groups as regards microRNA-122.
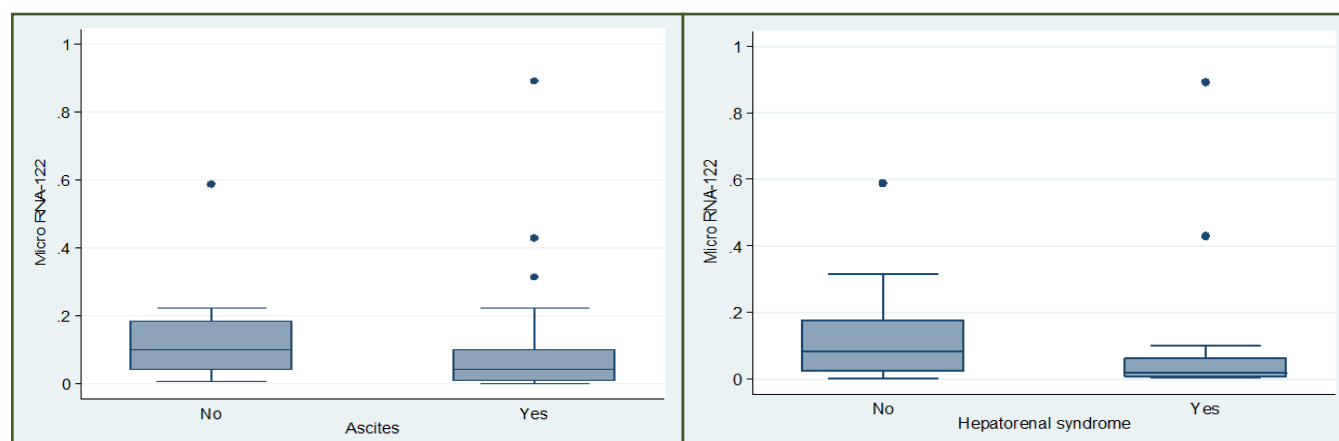
Fig. 2: Relation between miRNA-122 and ascitis and hepatorenal syndrome.
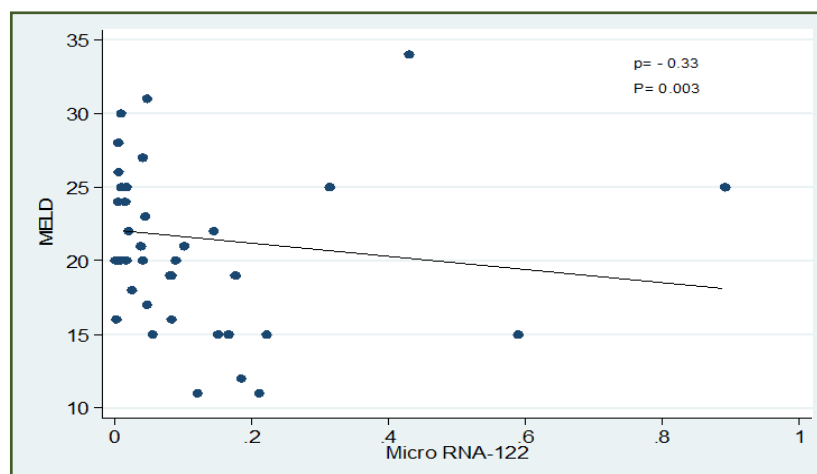
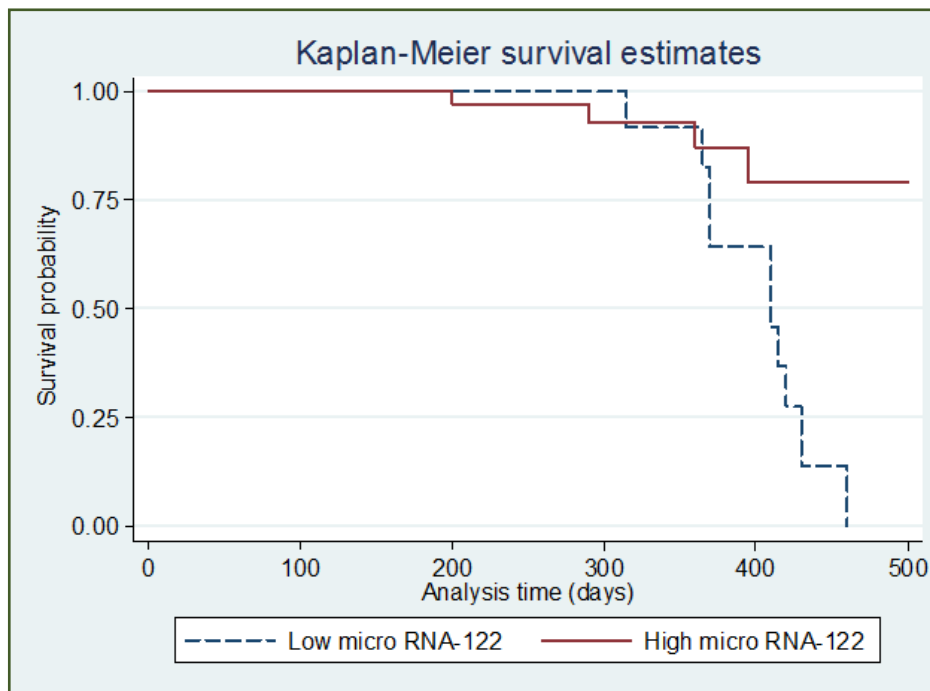**Fig. 3: Correlation between miRNA-122 levels and MELD score.**

Fig.4: ROC curve analysis of miRNA-122 for the prediction of decompensation in cirrhotic patients.

Fig. 5: ROC curve analysis of MELD scores for the prediction of decompensation in cirrhotic patients.

Fig. 6: Serum miR-122 levels are associated with survival in patients with liver cirrhosis Distribution of serum miRNA-122 levels throughout the patients. Survival curves for patients with high or low serum miRNA-122 levels. The analysis was performed with the Cox regression model.

Discussion

Liver cirrhosis (LC) is a late stage of progressive hepatic fibrosis characterized by distortion of the architecture and formation of regenerative nodules and different degrees of liver function impairment; these patients are prone to a variety of complications reducing life expectancy markedly[17]. In this prospective cross section study we evaluated if the serum levels of miR-122 might be a suitable prognostic parameter in patients with liver cirrhosis. Data obtained from the present study revealed a statistically significant decrease of miRNA 122 in patients with decompensated cirrhosis compared with those with compensated cirrhosis.

Trebicka and colleagues[18]explained the low level of miRNA-122 in decompensate liver cirrhosis by noting that miRNA 122 is present abundantly in hepatocytes with much lower levels in circulation in healthy subjects. With hepatocyte injury miRNA-122 is released in circulation more readily and serum level rise. With eventual loss of hepatocytes and development of fibrosis with proliferation of mylofibroblasts and accumulation of extracellular matrix, the circulating miR-122 levels drop again·

This indicates that in patients with liver cirrhosis, the miRNA-122 serum level might be a marker for hepatic functional capacity, whereas at earlier stages of liver disease, the serum miRNA-122 level is mainly an indicator of necroinflammatory activity and cell death in the liver. As release from damaged hepatocytes might be the major source of hepatocyte-derived miRNAs[19] Decompensation of liver reflected the stage of liver fibrosis, in a recent study where reduced level of miRNA-122 in stage F4 fibrosis as compared with stage F0was noted, miRNA-122 showed a negative correlation with fibrosis stage in fibrotic liver samples and, intriguingly, also with liver stiffness (LS values)[20].

That was most probably due to the fact that miRNA-122 positively regulates the accumulation of cholesterol and triglycerides and the metabolism of fatty acids . Thus, a decreased level of miRNA-122 in fibrotic liver biopsies may be interpreted as the result of compromised normal hepatocytic activity or as the eliminated suppressive function of miRNA-122 that hinders fibrogenesis·[21] Namely, miRNA-122 has been found to suppress the proliferation of hepatic stellate cells(HSCs), resulting in decreased maturation of collagen by down regulating the expression of P4HA1, a key enzyme in collagen maturation [14].

The present study further revealed a significant negative correlation between the serum miRNA-122 concentration and MELD score. This was approved by **Waidmannet al** .[22]and **Ko¨ berle et al.** [23] who found the same results.

That correlation could be explained by the suggestion that miRNA-122 serum concentration also reflects residual functional liver tissue in patients with end stage liver disease. In addition, we observed an even stronger positive correlation between miRNA-122 levels and AST and ALT. These findings go on line with another study performed by *Kholeif et al.*[24] who found a positive significant correlation between serum miRNA-122 and serum levels of ALT and AST in compensated cirrhosis indicating that miRNA-122 represents at least in part ongoing liver damage andcell death.

Finding of the inverse correlation between serum concentrations of miRNA-122 and creatinine in this study was agreed by *Waidmannet al.*[22] who stated that patients with hepatorenal syndrome had highly significant lower miRNA-122 serum levels than patients without this complication. In another publication no correlation between serum miRNA-122 and creatinine levels was found in patients with toxic liver damage[25]. Regarding the decompensated cirrhosis group and according to the results of this study correlation tests have revealed a significant negative correlation between miRNA 122 and both bilirubin, GGT and it was highly significant regarding ALP. While no significant correlation was found with albumin, total protein, INR and platelet. *Kholeif et al.*[24] suggested that serum miRNA-122 level did not reflect overall liver function finding no significant correlation between serum miRNA-122 level and serum albumin, serum bilirubin, platelet count in their study

In the present study patients with ascites and hepatorenal syndrome had significantly lower serum levels of miRNA-122 than patients without the respective complication. In contrast, no significant differences were observed between patients with and without variceal bleeding, hepatic encephalopathy, hepatocellular carcinoma or spontaneous bacterial peritonitis, higher volume distribution in patients with ascites could be an explanation[19]. This goes on line with a study done by *Waidmann et al.*[22] who reported that patients with ascites or hepatorenal syndrome had significantly lower serum levels of miRNA-122 in that study.

In a study done by *Abd Elmouttaleb1 et al.* (26)the highest serum miRNA–122 expression level was in hepatocellular carcinoma (HCC) group, followed by chronic HCV group. The lowest serum level of miRNA–122 was in cirrhosis group. The level of miRNA-122 in human serum is also being explored as a potential biomarker to detect hepatocellular carcinoma development and progression[27]. MiRNA-122 is hypothesized to regulate terminal hepatocyte differentiation, and loss of its expression (with subsequent upregulation of stem cell-associated proteins such as Pkm2) is a potential step towards hepatocellular carcinoma development.[28]

Previous studies suggested that the serum level of miRNA-122 reflects hepatic inflammation and cell death in patients with HBV- or HCV-induced chronic hepatitis, and depending on the context and stage of liver disease[29,30]. Supported by a previously reported study, comparison between the diagnostic performance of serum miRNA 122 and MELD score in prediction of decompensation in cirrhotic patients using ROC curve pointed to the high value of serum miRNA 122 level determination as an indicator for impaired liver function and disease severity[31].

A univariate Cox regression analysis revealed a significant association between miRNA-122 levels and overall survival and performing Cox regression analysis using forward stepwise (likelihood ratio)to evaluate the predictability of each parameter as independent factor of survival, it revealed that miRNA-122 serum levels and platelet count were independently associated with survival in liver cirrhosis. A previous study on patient with HCC identified serum miRNA-122 as an independent prognostic parameter for overall survival in patients suffering from HCC[23]. In that study the miRNA-122 serum level, however, was notan independent factor for overall survival, but correlated with the MELD score and clinical chemistry parameters of hepatic necroinflammation. Few studies shed light on platelet count prognostic value. In a study done by *Bureau et al.*[32] a platelet count above 75×10^9/L were predictive of survival inpatients with refractory ascites treated by Transjugular intrahepatic portosystemic shunt (TIPS).

Multiple factors can contribute to the development of thrombocytopenia in liver cirrhosis, including splenic platelet sequestration, bone marrow suppression by chronic hepatitis C infection, and antiviral treatment with interferon-based therapy. Reductions in the level or activity of the hematopoietic growth factor thrombopoietin (TPO) may also play a role[33].

Conclusion

The serum level of the hepatocyte specific miRNA-122 is decreased in patients with hepatic decompensation, and that decrease inmiRNA-122 levels are associated with ascites and hepatorenal syndrome.Suggesting that serummiRNA-122 is an indicator for hepatic functional capacity. Lower miRNA 122 concentration were associated with overall survival of patients with liver cirrhosis . Only low platelet count and low miRNA 122 were independently associated with mortality. Thus, serum miRNA122 level is a useful prognostic parameter in patients with liver cirrhosis.

References

1. Schuppan D and Afdhal NH:Liver cirrhosis. Lancet.2008,371: 838–851.

2. Ca´rdenas A and Gine`s P: Management of patients with cirrhosis awaiting liver transplantation. Gut.2011, 60: 412–421.

3. Garcia-Tsao G and Bosch J: Management of varices and varicealhemorrhagein cirrhosis. N Engl J Med.2010, 362: 823–832.

4. Wiesner RH: Patient selection in an era of donor liver shortage: CurrentUS policy. Nat ClinPractGastroenterolHepatol, 2005; 2: 24–30.

5. Chen H, Bai M, Qi X, Liu L, Affiliation: Department of Liver Disease and Digestive Interventional Radiology, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an, China He C, Yin Z, et al.: Child-Na Score: A Predictive Model for Survival in Cirrhotic Patients with Symptomatic Portal Hypertension Treated with TIPS. PLoS ONE. 2013, 8(11): e79637.

6. Etheridge A, Lee I, Hood L, Galas D, Wang K.: Extracellular microRNA: A new source of biomarkers: Mutation Research.2011,717(1): 85-90.

7. Esquela-Kerscher A and Slack FJ :Oncomirs – microRNAs with a role incancer. Nat Rev Cancer.2006, 6: 259–269.

8. Bartel DP: MicroRNAs: genomics, biogenesis, mechanism, and function. Cell, 2004; 116: 281-297.

9. Dykxhoorn DM and Lieberman J :The silent revolution: RNA interference as basic biology, research tool, and therapeutic. Annu. Rev. Med. 2005, 56: 401-423

10. Lewis AP and JoplingCL :Regulation and biological function of the liver-specific miR-122. Biochem. Soc. Trans. 2010, 38: 1553-1557.

11. Boutz DR, Collins PJ, Suresh U,Lu M, Ramírez CM, Fernández-Hernando C, et al., : Two-tieredapproach identifies a network of cancer and liver disease-related genes regulatedby miR-122. J BiolChem. 2011, 286: 18066–18078.

12. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS.: Loss of miR-122 expression in liver cancer correlates with suppression of thehepatic phenotype and gain of metastatic properties. Oncogene.2009, 28: 3526–3536.

13. Scisciani C, Vossio S, GuerrieriF,Find all citations by this author (default). Or filter your current search Schinzari V , Find all citations by this author (default). Or filter your current search De Iaco R , Find all citations by this author (default). Or filter your current search D'Onorio de Meo P, et al.,: Transcriptional regulation of miR-224 upregulated in human HCCs by NFκB inflammatory pathways. J Hepatol.2012, 56: 855-861.

14. Li J, Ghazwani M, Zhang Y, Lu J, Li J, Fan J, et al., : MiR-122 regulates collagen production via targeting hepaticstellate cells and suppressing P4HA1 expression. J Hepatol.2013,58: 522-528.

15. Arroyo V, Ginès P, Gerbes AL, et al. : Definition and diagnostic criteria of refractory ascites and hepatorenal syndrome in cirrhosis. International Ascites Club. Hepatology. 1996,23 (1): 164–176.

16. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-DeltaDelta C(T))Method. Methods. 2001, 25, 402.

17. Sørensen HT, Thulstrup AM, Mellemkjar L, et al., Longterm survival and cause-specific mortality in patients with cirrhosis of the liver: a nationwide cohort study in Denmark. Journal of Clinical Epidemiology. 2003, 56(1): 88–93.

18. Trebicka J, Anadol E, Elfimova N, Strack I, Roggendorf M, Viazov S, et al. :Hepatic and serum levels of miR-122 after chronic HCV-induced fibrosis. J. Hepatol. 2013, 58:234-239.

19. Wang K, Zhang S, Weber J, Baxter D, Galas DJ.: Export of microRNAs and microRNA-protective protein by mammalian cells. Nucl Acids Res. 2010, 38: 7248–7259.

20. Halász T, Horváth G, Pár G, et al., MiR-122 negatively correlates with liver fibrosis as detected by histology and FibroScan. World J Gastroenterol, 2015; 21(25): 7814-7823.

21. Hu J, Xu Y, Hao J, Wang S, Li C, Meng S.: MiR-122 in hepatic function and liver diseases. Protein Cell. 2012, 3: 364-371

22. Waidmann O, Kberle V, Brunner F,Zeuzem S, Piiper A, Kronenberger B. : Serum MicroRNA-122 Predicts Survival in Patients with Liver Cirrhosis PLoS ONE, 2012, 7(9): e45652.

23. Ko¨berle V, Kronenberger B, Pleli T, et al.,: Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. European Journal of Cancer. 2013, 49(16): 3442-3449.

24. Kholeif H, MohamedAboulEla A, Badawy D : Circulating Micro RNA-122 in Hepatitis C Virus Infected in Egyptian Group of Patients Clinical Medicine and Diagnostics. 2015, 5(6): 114-121.

25. Starkey PJ, Dear J, Platt V, et al., :Circulating microRNAs as potential markers of human drug induced liver injury. Hepatology.2011, 5: 1767–1776.

26. AbdElmouttaleb AT, Abd-Elatif DM, Soliman GM, Taher MS, Abonar AA. : Serum Micro RNA-122 as a Biomarker for Hepatocellular Carcinoma in Chronic Hepatitis C Virus Patients. Research In Cancer and Tumor. 2015, 4(2): 25-33.

27. Ding X, Ding J, Ning J,Yi F, Chen J, Zhao D, et al., : Circulating microRNA-122 as a potential biomarker for liver injury. Mol Med Rep. 2012, 5(6):1428-1432.

28. Jung CJ, Iyengar S, Blahnik KR, et al., : Epigenetic modulation of miR-122 facilitates human embryonic stem cell selfrenewal and hepatocellular carcinoma proliferation. PLoS One. 2011,6(11):e27740.

29. Bihrer V, Friedrich-Rust M, Kronenberger B, et al., : Serum miR- 122 as a biomarker of necroinflammation in patients with chronic hepatitis C virus infection. Am J Gastroenterol, 2011, 106: 1663–1669.

30. Cermelli S, Ruggieri A, Marrero JA, Ioannou G, Beretta L.: Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. PLoS One. 2011, 6:e23937.

31. Botta F, Giannini E, Romagnoli P, et al., MELD scoring system is useful for predicting prognosis in patients with liver cirrhosis and is correlated with residual liver function: a European study. Gut. 2003, 52(1): 134–139.

32. Bureau C, Métivier S, D'Amico M, et al., Serum bilirubin and platelet count: A simple predictive model for survival in patients with refractory ascites treated by TIPS. Journal of Hepatology.2011,54: 901–907.

33. Afdhal N, McHutchison J, Brown R, et al., :Thrombocytopenia associated with chronic liver disease. Journal of Hepatology. 2008, 48: 1000–1007.