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ARAŞTIRMA / RESEARCH

Importance of circulating microRNA-122 for hepatocellular carcinoma

Hepatoselüler karsinomda serum microRNA-122'nin önemi

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Abstract

Amaç: Bu çalışmanın amacı, hepatoselüler karsinomda dolaşımdaki mikroRNA-122'nin (miR-122) biyobelirteç olarak potansiyelini araştırmaktır.
Gereç ve Yöntem: Bu çalışmada, dolaşımdaki miR-122 seviyeleri, hepatoselüler karsinomun farklı klinik evreleri olan hastalarda gerçek zamanlı polimeraz zincir reaksiyonu ile ölçülmüştür. Ayrıca, bu veriler ile serum miR-122 seviyeleri arasındaki korelasyonu gözlemlemek için diğer laboratuvar ve klinik veriler de değerlendirilmiştir.
Bulgular: Hepatoselüler karsinom hastalarında serum miR-122 seviyeleri kontrol grubuna göre anlamlı olarak yüksek bulundu. (medyan miR-122 (min. - maks.); hastalar: 0,0104 (0-0,262); kontroller: 0,00187 (0-0,100), p <0,001). Serum miR-122 seviyesi 0.0038 cut-off değerinde eğri altında kalan alana göre hepatosellüler karsinom teşhisinde %67.4 sensitivite ve %74.4 spesifivite ortaya çıkardı. Bu cut off değerinde 1 yıllık, 3 yıllık ve 5 yıllık genel sağkalım oranları, bu değerin üstünde veya altında olan hastalar arasında istatistiksel olarak anlamlı bulunmadı.
Sonuç: Sonuçlarımız, dolaşımdaki miR-122'nin tek başına hepatoselüler karsinomun teşhisi, prognozu ve sürveyansı için uygun bir biyobelirteç olmayabileceğini göstermektedir. Bu nedenle, serum miR-122 ile birlikte diğer tanısal, klinik ve prognostik göstergelerin kullanılması ve bu konuda büyük ölçekli çalışmaların yapılması daha anlamlı olabilir.
Keywords:. Hepatoselüler karsinom, microRNA-122, serum microRNA-122.

Öz

Purpose: The main purpose of our study is to research the potential of circulating microRNA-122 (miR-122)as a marker for hepatocellular carcinoma.
Materials and Methods: Circulating miR-122 levels were measured by real-time polymerase chain reaction in patients with varied stages of hepatocellular carcinoma. In addition, various other laboratory and clinical data of the participants were evaluated in order to observe the correlation between these data and serum miR-122 levels.
Results: Serum miR-122 levels were found significantly higher in hepatocellular carcinoma patients compared to the control group. (median miR-122 (min. – max.); patients: 0.0104 (0-0.262); controls: 0.00187 (0-0.100), p <0.001). For the cut-off value of serum miR-122 levels (0.0038), the area under the receiver operating characteristic curve analysis revealed 67.4% sensitivity and 74.4% specificity in hepatocellular carcinoma diagnosis. At this cut-off value, 1-year, 3-year and 5-year overall survival rates were not statistically different between patients with above or below this value.
Conclusion: Our results show that only serum miR-122 is not a suitable biomarker for the hepatocellular carcinoma. Thus, using other diagnostic indicators together with serum miR-122 may be clinically meaningful for which large-scale studies are warranted.

Anahtar kelimeler: Hepatocellular carcinoma, microRNA-122, circulating microRNA-122.

INTRODUCTION

Globally, primary liver cancer is the sixth most diagnosed cancer and the fourth leading cause of cancer-related mortality[1]. Recent epidemiological studies revealed that hepatocellular carcinoma (HCC) is the most common histopathological type of liver cancer, accounting for more than 75% of liver

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carcinomas[2-4]. The highest risk among HCC development are chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, long-term alcohol use and aflatoxin-contaminated food intake[5].

Despite great efforts in the last decades, still, there is no widely accepted reliable biomarker that may be used in the clinic to diagnose HCC at its early stages. Among biomarkers that are used in the clinic, the most commonly used is alpha-fetoprotein (AFP) which has been shown to have 41-65% sensitivity and 80-94% specificity[6]. Therefore, there is still an unmet clinical *need* to discover *biomarkers* for early diagnosis of *HCC* with higher specificity and sensitivity.

MicroRNAs (miRNA) are 20-22 nucleotide-long, single chain, non-coding RNA molecules that have extensive biological roles in cellular proliferation, differentiation and apoptosis. miRNAs negatively regulate genes by binding to and suppressing the mRNAs of target genes. They constitute one of the major groups of gene regulators and are estimated to account for %1-4 all of the genes[7].

miRNA expression profiles have been assessed for the development and progression stages of various cancer types[8]. These findings made miRNAs, levels of which can be measured from serum or plasma, a possible novel biomarker candidate for cancer diagnosis and prognosis[9]. Indeed, many scientific studies have investigated diagnostic and prognostic values of miRNAs in different cancers such as lung, prostate, colorectal, ovarian, breast and HCC[10,11].

It has been shown that HCC tissues present different miRNA expression patterns compared to adjacent normal tissues. As a result of these studies, in HCC tissues, the levels of miR-10b, miR-18, miR-20, miR-221, miR-222 and miR-224 were observed to be increased, whereas the levels of miR-199b, miR-145, miR-214, miR-199a, miR-200b, miR-150, miR-223 and miR-122 levels were observed to be decreased[12,13]. All these miRNAs have important roles in hepatocarcinogenesis. Among these, miR-122 is specific for liver and decrease of miR-122 has been shown to accompany the malignant phenotype of HCC[14].

On the other hand, studies based on the circulating miR-122 for HCC patients are less common but research on this topic has been gaining popularity. The objective of this study was to measure the levels of miR-122 in the serum of HCC patients compared to healthy controls with the hypothesis that HCC patients have higher levels of miR-122 and this marker might serve as a marker in the early diagnosis of HCC.

MATERIALS AND METHODS

The study was completed between 2011 and 2013 at the university of Cukurova, Adana, Turkey. Forty-three HCC patients and forty-three healthy controls attended in the study. The use of patient sample protocols was endorsed by the medical committee of ethics in Cukurova University (approval number: 13). The study was applied according to the Declaration of Helsinki Ethical principles for medical research involving human subjects. *Informed consent forms were obtained* from all participants before conducting the study. Healthy participants who were negative for viral hepatic markers, had normal transaminases and coagulation parameters with normal findings and liver echogenicity on ultrasonography were age- and sex-matched to the patients.

Procedure

We analyzed circulating miR-122 levels in serum samples of well-characterized patients with clinically or histologically confirmed different stages of HCC. In this study, data on laboratory parameters such as serum alanine transaminase (ALT), aspartate aminotransaminase (AST), *serum alpha-fetoprotein* (AFP), albumin, bilirubin, calcium ion, HBV DNA, hematocritis (HCT), international normalized ratio (INR), creatinine, platelet (PLT), prothrombin time (PTT), and miR-122 levels, condition of patients such as cirrhosis or type 2 diabetes mellitus comorbidity, and presence of ascites, clinical staging of the disease such as Barcelona Clinic Liver Cancer (BCLC - a scoring system which uses tumor size, extent, liver function, and performance status), Child-Pugh (a scoring system based on patient's total bilirubin, serum albumin, prothrombin time, ascites status and hepatic encephalopathy status) and Model for End-Stage Liver Disease (MELD - a scoring system based on patient's serum bilirubin and creatine and the INR values to predict survival) scores, tumor characteristics such as diameter, vascularity and nodularity, and lifestyle such as alcohol consumption have been collected and evaluated. The overall survival time was defined as the time between inclusion into our study and death or last documented contact to the patient.

The thresholds for alcohol consumption consideration were 60 grams/day for male participants and 20 grams/day for female participants. Cirrhosis diagnosis was performed through physical examination and triple-phase computerized tomography imaging. Tumor diameter and nodularity were determined by triple-phase computerized tomography imaging, whereas vascular invasion was determined by portal venous Doppler ultrasonography.

All patients diagnosed with HCC at different stages by biopsy or triple-phase computerized tomography imaging were included in the study. No exclusion criteria were applied. The objective for sample size determination was to include all patients who applied to Cukurova University and Adana City Training and Research Hospital Gastroenterology and Oncology Department with the diagnosis of HCC between 2011 and 2013.

The control group consisted of healthy volunteers of the same age and gender, who applied to the hospital for screening/control and had normal physical examination, liver enzyme tests and laboratory values. They were negative for hepatitis markers, did not use alcohol or hepatotoxic agents, and had normal ultrasonographic imaging of the liver.

Collection of peripheral blood

Data collection forms were collected from all patients with HCC at different stages diagnosed by biopsy or triple-phase computerized tomography imaging at Cukurova University and Adana Numune Hospital between 2011 and 2013. Peripheral blood samples were collected at 8 a.m from patients and controls. upon fasting overnight the day after the collection of data collection forms. The collected peripheral blood samples were stored at -20°C in Paxgene® Blood RNA tubes until their use for RT-PCR.

Reverse transcription and RNA extraction

RNA was extracted from 150 µl blood sample using High Pure miRNA Isolation Kit following manufacturer instructions (one-column protocol). At the final step, total RNA was collected using 75 µl elution buffer. Lyophilized synthetic spike-in was diluted with 40 µl PCR grade water and was kept on ice for 15 min. Complementary DNA (cDNA) was obtained from total RNA using the Universal miRCURY cDNA synthesis kit following manufacturer instructions. Briefly, a total of 20 µl of the reaction mix (4 µl total RNA and 16 µl cDNA synthesis mix) was loaded onto a 96-well plate, and the reaction (60 minutes at 42°C followed by 5 minutes at 95°C) was run using LightCycler® 480 system. cDNA samples were stored at +4°C. Obtained cDNA samples were diluted with PCR grade water at a ratio of 1:80. Diluted SNORD48 and hsa-miR-122 PCR primer mixes were prepared according to the following manufacturer's recommendations. The reaction mix was prepared by using 4 µl diluted cDNA, 1 µl primer mix, and 5 µl 2x SYBR Green Master Mix. Prepared samples were loaded onto the 96 wells plates of LightCycler® 480 System. The following protocol was used: 95°C, 10 minutes; then 45 cycles of 95°C, 10 seconds and 60°C, 1 minute; then melting curve. Throughout the study, serum miR-122 levels are presented as normalized to each participant's own SNORD48 expression levels.

Statistical analysis

SPSS software was performed for statistical analysis (Version 25.0, SPSS Inc., Chicago, IL, USA). Statistical significance comparing groups were calculated using Student's t-test or one-way ANOVA for normally distributed data and using Kruskal-Wallis H or Mann Whitney U tests for the data that is not normally distributed. For serum miR-122 values, receiver operating characteristic curves (ROC curves) were constructed. Spearman's test was performed for assessing the correlation of serum miR-122 levels and variables. Statistically significance was considered under values of $p<0.05$. Wald test was used to analyze overall survival. $p<0.05$ was considered statistically significant in all tests.

RESULTS

As presented in Table 1, the participants in the patient and control groups were age- and sex-matched. At baseline, serum aspartate transaminase (AST, $p<0.001$), alanine transaminase (ALT, $p<0.001$), and bilirubin ($p<0.001$) were found statistically higher for patients compared to the controls. Moreover, it was observed that prothrombin time ($p<0.001$) and international normalized ratio (INR, $p<0.001$) were also statistically significantly higher in the patient group. In addition, serum platelet levels (PLT, $p< 0.001$) and calcium ion levels (Ca^{+2}, $p=0.005$) were statistically lower among patients.

Table 1. Baseline characteristics of patient and control groups.

	Patients (n=43)	Controls (n=43)	p
Gender (m/f)	34/9	34/9	1.000
Age*	62.8 ± 10.2	62.9 ± 10.2	0.945
AST (u/l)†	62 (20-680)	20 (9-41)	0.0001
ALT (u/l)†	41 (18-488)	18 (7-39)	0.0001
Bilirubin (mg/dl)†	1.4 (0.3-18.9)	0.6 (0.1-2.0)	0.0001
PTT†	14.6 (10.7-24.2)	11.9 (10.6-52.4)	0.0001
INR*	1.30 ± 0.2	1.14 ± 0.6	0.0001
PLT (x1000)†	123 (51-985)	295 (142-556)	0.0001
Ca^{+2} (mg/dl)*	8.79 ± 0.9	9.09 ± 0.6	0.005
miR-122†,‡	0.0104 (0-0.262)	0.00187 (0-0.100)	0.0001
Albumin (g/dl)*	3.1 ± 0.6	3.2 ± 0.5	0.256
HCT*	35.2 ± 6.6	35.1 ± 5.6	0.983
Tumor Diameter (mm)†	45 (10-200)	-	n/a
MELD Score†	12 (7-34)	-	n/a
AFP (ng/ml)†	38.9 (1.03-48402)	-	n/a
HBV DNA (IU/ml)†	20 (0-29300000)	-	n/a

AST: aspartate aminotransferase, ALT: alanine aminotransferase, PTT: prothrombin time, INR: international normalized ratio, PLT: platelet, Ca+2: Calcium, miR-122: microRNA 122, HCT: hematocrit, MELD: Model for End-Stage Liver Disease, AFP: alpha-fetoprotein, HBV DNA: hepatitis B virus deoxyribonucleic acid, n/a: not applicable. *Mean ± standard deviation; †Median (Min. – Max.); ‡Normalized to SNORD48 expression.

Importantly, serum miR-122 levels were significantly higher in the patient group compared to control group (median miR-122 (min. – max.); patients: 0.0104 (0-0.262); controls: 0.00187 (0-0.100), $p < 0.001$). Statistical difference was not shown between groups regarding serum albumin and hematocrit percentage (HCT%) levels.

Patients in the study cohort had a mean 45 mm tumor size (between 10 mm and 200 mm) ± 46.9 mm, Model for End-Stage Liver Disease (MELD) score of 12 (between 7 and 34), ***serum alpha-fetoprotein*** (***AFP***) of 38.9 ng/ml (between 1.03 ng/ml and 48402 ng/ml) and ***HBV DNA level of*** 20 IU/ml (between 0 IU/ml and $29.3x10^6$ IU/ml). In this cohort, 25 patients had HBV, 4 patients had HCV, 1 patient had ASH, 3 patients had NASH and 10 patients had cryptogenic HCC.

In order to undertand the connection of serum miR-122 levels and different disease stages, Spearman's correlation test was used (Table 2). No statistically significant correlation levels were observed between serum miR-122 levels and Barcelona-Clinic Liver Cancer (BCLC) stages, MELD score, and Child-Pugh classes. However, statistically significant positive correlations were observed between BCLC, MELD score, and Child-Pugh classes (Spearman Correlation coefficient values -r- between 0.36 and 0.54, $p < 0.05$).

Table 2. Spearman correlation chart of various parameters in the patient group

	BCLC	MELD	Child-Pugh
Serum miR-122	-0.08	0.12	-0.08
BCLC	-	0.36*	0.54*
MELD	-	-	0.48*

miR-122: microRNA 122, BCLC: Barcelona Clinic Liver Cancer, MELD: Model for End-Stage Liver Disease; *p<0.05.

Next, serum miR-122 levels were compared in patients sub-grouped by laboratory and clinical data, and clinical disease stages (Table 3). This analysis revealed that patients with cirrhosis had statistically increased levels of serum miR-122 levels in order to patients without cirrhosis ($p=0.019$). In addition, patients which has MELD score more than 20 had statistically higher levels of serum miR-122 levels compared to patients which has MELD score less than 20 (compared to patients with MELD score below 10, $p=0.011$; compared to patients with MELD score between 10 and 20, $p=0.008$). On the other hand, this analysis revealed that there were no

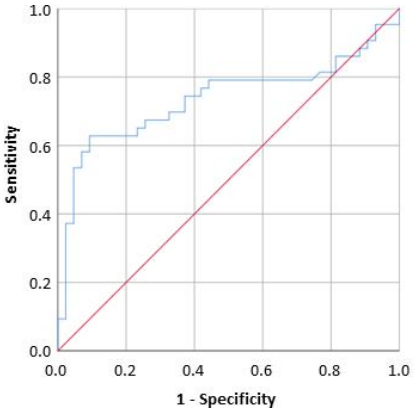
statistical significant differences when the patients were sub-grouped by their gender (p=0.414), alcohol consumption (p=0.241), type-2 diabetes status (p=0.434), Child-Pugh class (p=0.311), BCLC staging (p=0.700), ascites status (p=0.242), tumor size (p=0.891), nodularity (p=695), vascular invasion (p=0.437), bilirubin levels (p=0.419), AFP levels (p=0.557), ALT levels (p=0.866), creatine levels (p=0.299), HCT% (p=0.325), and AST levels (p=0.288).

Table 3. Serum miR-122 levels of patient subgroups based on clinical stages and laboratory and clinical data.

Variable	**miR-122**	**p**		
Gender				
Male (n:34)	0.0035 (0-0.1062)	0.414		
Female (n:9)	0.0041 (0-0.2620)			
Cirrhosis				
Yes (n:37)	0.0117 (0-0.2620)	0.019		
No (n:6)	0.0022 (0-0.078)			
Alcohol consumption				
Yes (n:10)	0.0105 (0-0.0445)	0.241		
No (n:33)	0.0104 (0-0.2620)			
Type 2 Diabetes Mellitus				
Yes (n:8)	0.0158 (0-0.445)	0.434		
No (n:35)	0.0094 (0-0.2620)			
Child-Pugh Criteria				
A (n:18)	0.0166 (0-0.2620)	0.311		
B (n:7)	0.0072 (0-0.0493)			
C (n:8)	0.0127 (0-0.2182)			
BCLC Staging				
A (n:11)	0.0207 (0-0.2182)	0.700		
B (n:12)	0.0062 (0-0.1062)			
C (n:13)	0.0072 (0-0.2620)			
D (n:7)	0.0138 (0-0.0451)			
MELD Score				
<10[a] (n:9)	0.0094 (0.0010-0.0831)	a-b	a-c	b-c
10-20[b] (n:30)	0.0068 (0-0.2620)	0.321	0.011	0.008
≥20[c] (n:4)	0.0728 (0.0445-0.2182)			
Ascites				
Yes (n:23)	0.0104 (0-0.2182)	0.242		
No (n:20)	0.0105 (0-0.2620)			
Tumour Diameter				
<5 cm (n:22)	0.0115 (0-0.2182)	0.891		
≥5 cm (n:21)	0.0086 (0-0.2620)			
Nodularity				
Single (n:21)	0.0094 (0-0.2620)	0.695		
Multiple (n:18)	0.0106 (0-0.1005)			
Diffuse (n:4)	0.0075 (0-0.0445)			
Vascular Invasion				
Invasive (n:14)	0.0056 (0-0.2620)	0.437		
Non-invasive (n:29)	0.0117 (0-0.2182)			
Bilirubin				
<1 mg/dl (n:10)	0.0086 (0-0.2182)	0.419		
≥1 mg/dl (n:33)	0.0108 (0-0.2620)			
AFP				
≤ 7 ng/ml (n:10)	0.0062 (0-0.1062)	0.557		
7-400 ng/ml (n:26)	0.0124 (0-0.2620)			
≥400 ng/ml (n:7)	0.0094 (0-0.4510)			
ALT				
≥45 u/l (n:17)	0.0078(0-0.262)	0.866		

<45 u/l (n:26)	0.0111(0-0.218)	
Creatinine		
≥1 mg/dl (n:17)	0.0134(0-0.218)	0.299
<1 mg/dl (n:26)	0.0086(0-0.262)	
HCT		
≥%36 (n:22)	0.0158(0-0.218)	0.325
<%36 (n:21)	0.0078(0-0.262)	
AST		
≥45 u/l (n:28)	0.0086(0-0.262)	0.288
<45 u/l (n:15)	0.0182(0-0.218)	

AST: aspartate aminotransferase, ALT: alanine aminotransferase, PTT: prothrombin time, INR: international normalized ratio, PLT: platelet, Ca+2: Calcium, miR-122: microRNA 122, HCT: hematocrit, MELD: Model for End-Stage Liver Disease, AFP: alpha-fetoprotein, HBV DNA: hepatitis B virus deoxyribonucleic acid, BCLC: Barcelona Clinic Liver Cancer.



Serum miR-122	Sensitivity (%)	Specificity (%)	AUC (95% CI)
0.0027	76.7	58.1	0.74 (0.62-0.85)
0.0029	74.4	62.8	
0.0038	67.4	74.4	

Figure 1. Cut-off value determination for serum miR-122.

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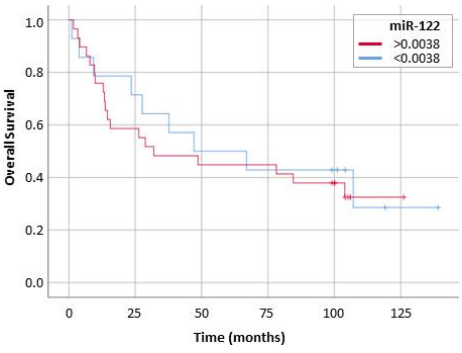

Serum miR-122 Level	Survival Mean (months)	Std. Error	95% Confidence Interval	Survival %			p
				1 year	3 years	5 years	
<0.0038	70.6	15.5	42.0 - 99.1	78.6	57.1	42.9	0.673
>0.0038	60.8	9.7	41.8 - 79.9	75.9	48.3	41.4	

Figure 2. Overall survival curve of patients separated by their serum miR-122 levels.

In order to determine the optimum serum miR-122 cut-off value, ROC curve was generated based on serum miR-122 levels obtained from both groups. The optimum cut-off value to be used as diagnostic criteria in HCC in these cohorts was calculated as 0.0038 with 67.4% sensitivity and 74.4% specificity (Figure 1. Table on the right shows sensitivity and specificity values for different cut-off values). At this cut-off value, area under the ROC curve (AUC) was calculated as 0.74 (95% CI: 0.62-0.85, p=0.0001).

Finally, the overall survival curve of patients was built based on the serum miR-122 levels of patients. As it can be seen in Figure 2, there was no statistical difference observed for the survival rates of the patient groups. The mean survival estimate was 70.6 months and 60.8 months for patients with serum levels below and above the cut-off (0.0038), respectively

DISCUSSION

Many studies in the literature have investigated the tissue miR-122 levels in HCC, and have demonstrated that tissue miR-122 was significantly decreased compared to the adjacent normal liver tissue[14-16]. In this present study, however, miR-122 was measured in serum of HCC patients and age- and sex-related controls.

The patients which have both chronic hepatitis and HCC, miR-223 serum miR-21 and miR-122 levels were detected higher compared to controls[17]. In addition, in a controlled prospective study with 120 participants (48 HBV patients with HCC, 48 HBV patients without HCC, and 24 control patients), serum miR-122 was found significantly higher in both patient groups compared to healthy controls[18]. In line with these findings, in this present study, significantly higher serum miR-122 levels were observed in HCC patients compared to controls (median miR-122 (min. – max.); patients: 0.0104 (0-0.262); controls: 0.00187 (0-0.100), p <0.001). miR-122 are presented as normalized to SNORD48 since SNORD48 has been shown to be one of the best small non-coding RNA references for relative quantification in miRNA expression studies[19,20].

One of the explanations why serum miR-122 levels increase but tissue miR-122 levels decrease in HCC patients may be due to the leakage of miRNA-122 from damaged hepatocytes to circulation as it has been suggested in other studies[15]. This might be due to the fact that many HCC patients have comorbid chronic viral hepatitis[21]. In support of this possible explanation, in the present study, 67.4% of the patients had comorbid chronic viral hepatitis (out of 43 patients, 25 patients had HBV, and 4 patients had HCV).

In this study, in order to evaluate whether there is a similar situation between HCC and miR-122 or not, the adherence between circulating miR-122 and various clinical and biochemical parameters together with disease stages were investigated. Among evaluated parameters (Table 2), only patients with cirrhosis and patients which has MELD score more than 20 had statistically higher levels of serum miR-122 levels compared to patients without cirrhosis and patients which has MELD score less than or equal to 20, respectively ($p<0.05$ in both cases). This is in contrast with what has been published previously by Köberle et al. which showed a strong positive correlation between ALT and AST levels and serum miR-122, and a negative correlation between miR-122 and MELD score in HCC patients[22].

Serum miR-122 levels were not statistically different between patients grouped for other parameters. Although miRNA-122 has been shown to increase AFP expression[23], in this study, no statistically significant correlation between serum miR-122 levels and serum AFP level were observed (p=0.557). Another example would be vascular invasion which is known as a poor prognosis in HCC and miR-122 has been shown to regulate intrahepatic metastasis in liver tissue[24]. In this study, serum miR-122 levels between patients with and without vascular invasion were not statistically significant (p=0.437). Yang et. al has shown that long noncoding RNA SNHG7 (small nucleolar RNA host gene 7) accelerates the proliferation, migration and invasion of HCC cells via regulating miR-122-5p[25].

In accordance with earlier studies[17,18], ROC-AUC calculations indicated that at serum levels of miR-122 value of 0.0038, HCC will be suspected with 67.4% sensitivity and 74.4% specificity. In line with our findings, in a recent research, it has been found that miR-122 can predict the development of HCC at a cut-off value < 0.67[26].

One of the limitations of our study can be listed as the low number of patients. Another limitation may be related to the non-classification of patients per

their prior HCC treatments, or to the fact that some patients were recently diagnosed while others have been treated. And finally, not including viral hepatitis patients within the control group can be a limitation to our study.

As a summary, in this study, circulating serum miR-122 levels and other diagnostic, prognostic and clinical parameters in HCC patients were studied in comparison to that of control participants. Our findings show that only circulating miR-122 is not an appropriate biomarker for HCC. As suggested for cardiovascular diseases[27], combining miR-122s with other diagnostic, clinical, prognostic indicators can be the meaningful approach to improve the diagnosis accuracy of HCC. More studies are required to figure out the role of serum miR-122 as an HCC biomarker.

Yazar Katkıları: Çalışma konsepti/Tasarımı: EO, HA; Veri toplama: EO, ABÖ; Veri analizi ve yorumlama: EO, OÜ; Yazı taslağı: EO; İçeriğin eleştirel incelenmesi: MÜS, ABÖ; Son onay ve sorumluluk: EO, HA, MÜS, OÜ, ABÖ; Teknik ve malzeme desteği: HA, MÜS; Süpervizyon: MÜS, OÜ; Fon sağlama (mevcut ise): yok.
Etik Onay: Bu çalışma için Çukurova Üniversitesi Tıp Fakültesi Girişimsel Olmayan Klinik Araştırmalar Etik Kurulundan 05.01.2012 tarih ve 13/4 sayılı kararı ile etik onay alınmıştır.
Hakem Değerlendirmesi: Dış bağımsız.
Çıkar Çatışması: Yazarlar çıkar çatışması beyan etmemişlerdir.
Finansal Destek: Yazarlar finansal destek beyan etmemişlerdir.
Yazarın Notu: Bu çalışma, 6-9 Haziran 2013 tarihlerinde Singapur'da düzenlenen 23. Asya Pasifik Derneği Karaciğer Çalışmaları Konferansı'nda poster olarak sunulmuştur.
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