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The Importance of Microrna21 as Biomarker in Hepatocellular Carcinoma

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ABSTRACT

Objective: *Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer-related deaths worldwide.The diagnosis of HCC patients remains difficult, especially early in the development of the disease, and yet early and accurate diagnosis of HCC patients is vital in order to improve prognosis. Promising biomarkers for diagnosis of HCC have been successfully identifiedin several studies. MicroRNA-21 is one of the oncogenic miRNAs which may be a potential diagnostic biomarker for HCC.Our aim in the study was to investigate microRNAs as a biomarker in HCC and to compare the most unique MiR-21 in the literature as a biomarker and prognostic factor.*

Materials and Methods: *Ten patients diagnosed with HCC and ten healthy individuals of the same age and gender were selected as the control group. Six miRNAs (Let-7c, miR-1, miR-21, miR-29, miR-34, miR-335) selected and MiR 181 and miR 192 used as the endogenous control group.*

Results: *MiR21 was upregulated and statistically important compared with the endogenous control miR181(p=0.041). In the ROC analysis curve, AUC was significantly high for Delta181CT miR-21 (0.778). The sensitivity and specificity values of Delta181CT miR-21 with an optimal cut-off value of -3.508564949 were 77.8% and 90.0%. respectively. None of six miRNA statistically important compared with the endogenous control miR192.*

Conclusion: *MiR21 is important for determining as biomarkers at diagnosis of HCC.*

Keywords

Hepatocellular Carcinoma, Biomarker, MicroRNA miR21.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second leading cause of cancer-related deaths worldwide. Although the prognosis of patients with HCC is generally poor, however, early diagnosis of HCC is complicated by the coexistence of inflammation and cirrhosis [1].

The diagnosis of HCC without a pathological diagnosis can be achieved by assessing serum α-fetoprotein (AFP) levels and

diagnostic imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI) [2].

The diagnosis of HCC patients remains difficult, especially early in the development of the disease, and yet early and accurate diagnosis of HCC patients is vital in order to improve prognosis. Promising biomarkers for diagnosis of HCC have been successfully identified in several studies [3].

MicroRNAs (miRNAs) are endogenous, non-coding RNAs that regulate various molecular biological phenomena by suppressing the translation of target messenger RNAs (mRNAs). miRNAs, which often become dysregulated in malignancy, control cell proliferation, migration, invasion, and development in HCC by promoting or suppressing tumors It is important the molecular and functional roles of miRNAs in the pathogenesis of HCC [4]. MicroRNA-21 is one of the oncogenic miRNAs which may be a potential diagnostic biomarker for HCC [5].

Our aim in the study was to investigate microRNAs as a biomarker in HCC and to compare the most unique MiR-21 in the literature as a biomarker and prognostic factor.

Materials

Ten patients diagnosed with HCC and ten healthy individuals of the same age and gender were selected as the control group. After each individual included in the study was informed and signed a written consent form, 5cc EDTA blood samples were taken from the patients and the control group.

Methods

Approval was obtained from Faculty of Medicine Clinical Research Ethics Committee of Akdeniz University. Ethics committee approval number and date 471 and 17.08.2016. Ethics Committee.

Selected miRNAs

Six miRNAs (Let-7c, miR-1, miR-21, miR-29, miR-34,, miR-335) selected and MiR 181 and miR 192 used as the endogenous control group in line with their binding potentials and gene expression levels.

MiRNA extraction and measurement from blood samples, the measurement methods of the four available miRNAs in patients and healthy individuals were performed as indicated in our previous study. Blood samples were centrifuged for 15 minutes and plasma was separated. MiRNA isolation (Invitrogen by Thermo Fisher Scientific-mirVana™ miRNA Isolation Kit) was performed from plasma. The obtained miRNA was measured in ng / µl on the QUBIT 3 FLUOROMETER device (Invitrogen by Thermo Fisher Scientific-Qubit ™ microRNA Assay Kit). The miRNA samples, whose concentration was found to be suitable, were obtained by using Thermal Cycler (Applied Biosystems by Life Technologies-TaqMan Advanced miRNA c DNA Synthesis Kit). cDNAs were kept at -20°C until the sufficient number was reached in two formats, 30 and 50. Gene expression levels of the component and cDNA prepared with a total volume of 20 µl in each well were measured with StepOne ™ Real-Time PCR (Catalog No: 4376357 Thermo Fisher) device. C_T values automatically taken from the system are reported in the excel file. The average CT values of the duplicated samples were compared with the control group miR 181 and miR 192, and the ΔC_T values were calculated. At the end of the study, the ΔC_T values of individuals with colon cancer were compared with the ΔC_Ts of the healthy control group like other our studies [6-8].

Statistical Method: The data were evaluated using the SPSS (Statistical Package for the Social Sciences) version 23.0 (SPSS Inc., Chicago, IL, USA) program.Descriptive findings are presented with number, percentage, mean ± standard deviation and median. Shapiro-Wilk test and skewness/kurtosis values were used to evaluate whether the data represented normal distribution. Independent samples t test was used if the data conformed to the normal distribution and Mann-Whitney U test was used if the data was not normally distributed. Comparisons were made between colon cancer group and healthy control group. A p value $p < 0.05$ was considered statistically significant. Receiver Operating Characteristic (ROC) curve analysis was performed to determine the sensitivity and specificity and diagnostic efficacy of miRNAs among the investigated groups [9].

Results

Ten patients with HCC cancer included in the study, seven male and three female of them, the mean age and range of them was 52.60 ± 16.2 and 55-67 years: In the control group, there was seven male and three female, their mean age 52.1 ± 16.6 and range 53-65 years.

MiR21 was upregulated and statistically important compared with the endogenous control miR181($p=0.041$).None of six miRNA statistically important compared with the endogenous control miR192 (Table 1 and 2).

The closer the value of area under the curve (AUC) was to 1.00, the more important was the miRNA that reflected the significant difference between hepatocellular carcinoma and healthy controls. In the ROC analysis curve, AUC was significantly high for Delta181CT miR-21 (0.778). The sensitivity and specificity values of Delta181CT miR-21 with an optimal cut-off value of -3.508564949 were 77.8% and 90.0%. respectively.

Discussion

The early detection of hepatocellular carcinoma (HCC) presents a challenge because of the lack of specific biomarkers. Plasme microRNAs (miRNAs) can discriminate HCC patients from controls. Wen et al. identified HCC-associated plasma miRNAs originating from the liver as early biomarkers for detecting HCC. In this multicenter three-phase study, they performed screening using both plasma and tissue samples. Then, they evaluated the diagnostic potential of the miRNAs in two case-control studies Finally,they used ten miRNAs, eight of which (miR-20a-5p, miR-25-3p, miR-30a-5p, miR-92a-3p, miR-132-3p, miR-185-5p, miR-320a and miR-324-3p) were significantly overexpressed in the

Table 1: Mirna Comparison of Patients with Hepatocellular Carcinoma and Healthy Subjects According To Endogen Control Delta181ct.

miRNA			
	Mean ± SD†	**Median**	**p**
Let7c			
Hepatocellular Carcinoma (n=9)	2.20986217922 ± 2.448346761650	1.97451019300	0.076*
Healthy Subject (n=9)	0.19106287122 ± 2.044921169629	0.20050430300	
mir29a			
Hepatocellular Carcinoma (n=9)	-1.06165440889 ± 3.432431513911	-1.48865127600	0.808*
Healthy Subject (n=7)	-0.72397636429 ± 1.144596999704	-0.70680999800	
Mir34a			
Hepatocellular Carcinoma (n=8)	0.25243401513 ± 1.615307785852	0.48969745600	0.939*
Healthy Subject (n=9)	0.34172433467 ± 2.853902802846	1.68600000000	
Mir1			
Hepatocellular Carcinoma (n=9)	-2.32150268567 ± 2.824469645406	-2.42631721500	0.186†
Healthy Subject (n=8)	-1.46233523000 ± 1.752284209587	-1.44645818350	
mir21			
Hepatocellular Carcinoma (n=9)	-2.32334009811 ± 3.357168549175	-2.27222251900	**0.041**†
Healthy Subject (n=10)	-4.17280411670 ± 1.073335738684	-3.99942012000	
Mir335			
Hepatocellular Carcinoma (n=8)	-2.85412955288 ± 4.315816779005	-1.87791061400	0.093*
Healthy Subject (n=7)	0.22938519500 ± 1.295162414177	0.11726379400	

* Independent samples t test, † Mann-Whitney U testi

Table 2: Mirna Comparison of Patients with Hepatocellular Carcinoma and Healthy Subjects According To Endogen Control Delta192ct.

miRNA			
	Mean ± SD†	**Median**	**p**
Let7c			
Hepatocellular Carcinoma (n=9)	4.57738473689 ± 3.794258003214	4.75871467600	0.337*
Healthy Subject (n=6)	2.13155862183 ± 5.761003809374	2.96516897600	
mir29a			
Hepatocellular Carcinoma (n=9)	1.30586814889 ± 1.649357800236	1.54111099200	0.266†
Healthy Subject (n=7)	2.48171235886 ± 4.230117449377	4.58884811400	
Mir34a			
Hepatocellular Carcinoma (n=8)	2.48308515538 ± 2.424055878041	2.92066955550	0.809*
Healthy Subject (n=7)	3.09366951429 ± 6.042838563720	4.11842727700	
Mir1			
Hepatocellular Carcinoma (n=8)	0.04601987200 ± 1.595538778849	0.62211608900	0.186†
Healthy Subject (n=7)	1.32640958529 ± 3.656892161918	2.04100000000	
mir21			
Hepatocellular Carcinoma (n=9)	0.04418245956 ± 5.060708096305	0.88399887100	0.627*
Healthy Subject (n=7)	-1.17338925600 ± 4.592451442893	0.40900000000	
Mir335			
Hepatocellular Carcinoma (n=8)	-0.63567471513 ± 3.704623094444	-0.76651859300	0.080*
Healthy Subject (n=4)	4.04852904125 ± 4.402973127986	5.03659085850	

* Independent samples t test, † Mann-Whitney U test

HBV-positive HCC patients.Furthermore, the reported miRNAs (miR-192-5p, miR-21-5p and miR-375) in two prospective cohorts potential for early HCC detection [10].

MicroRNA-21 (miR-21) is one of the oncogenic miRNAs which may be a potential diagnostic biomarker for hepatocellular carcinoma (HCC). Qu et al. systematically searched Medline, Embase, the Cochrane Library, ISI Web of Knowledge, Scopus and reference lists of identified primary studies. Two independent investigators extracted patient and study characteristics. The sensitivity and specificity of microRNA-21 for HCC detection and were analyzed with a random effect model. The area under summary receiver operating characteristic curve (AUC) was used to estimate overall test performance. A total of 515 HCC patients, and 338 healthy or chronic hepatitis controls from six published studies were enrolled in this meta-analysis. They published that MiR-21 is a helpful biomarker for early diagnosis of HCC [11].

Bharali et al. designed to find out whether the expression level of miR-21 and miR-122 was deregulated in HCC compared to controls without HCC. They used Real-time quantitative polymerase chain reaction was performed to find out the miRNA expression level using Ct value followed by statistical analysis where P value $\leq$ 0.05 was considered as significant like our study.They detected the

Overexpression of miR-21 and miR-122 in HCC, and published miR-21 and miR-122 could be considered as potential markers of HCC screening molecule in addition to other approved markers [12].

Guo et al. evaluated the diagnostic efficiency of serum miR-21 as novel biomarkers for patients with HCC and other controls. They found expression levels of miR-21 was significantly higher in primary HCC tissues than in adjacent noncancerous tissues (P<0.0001)and serum miR-21 diagnostic efficiency in AFP-negative HCC subgroups with AUC 0.831, sensitivity 81.2% and specificity 83.2%. In addition, the serum levels of miR-21 were significantly associated with clinical stage (P=0.006) and distant metastasis (P=0.000). Thus, their findings suggest that miR-21 together with AFP may help enhance the diagnosis of HCC, especially of AFP-negative HCC [13].

Tomimaru et al. evaluated the significance of plasma microRNA-21 level as a biochemical marker for HCC. They also compared miR21 in other groups of: 126 patients with hepatocellular carcinoma, 30 patients with chronic hepatitis, and 50 healthy volunteers. They found plasma microRNA-21 levels significantly diminished after surgery compared with the pre-operative values (p=0.0125). and in the 126 patients with hepatocellular carcinoma was significantly higher than in patients with chronic hepatitis and healthy volunteers (p<0.0001, p<0.0001, respectively). ROC analysis of plasma microRNA-21 yielded an AUC of 0.773 with 61.1% sensitivity and 83.3% specificity when differentiating hepatocellular carcinoma from chronic hepatitis, and an AUC of 0.953 with 87.3% sensitivity and 92.0% specificity [14].

Wang et al. published the expression of miR-21 in serum exosomes from patients with HCC or chronic hepatitis B (CHB) and enriched in serum exosomes which provides increased sensitivity of detection than whole serum. Exosomal miR-21 may serve as a potential biomarker for HCC diagnosis [15].

Liao et al. published the systematic evaluation of the performance of microRNA-21 as a diagnostic marker for HCC. Their findings suggested that circulating miR-21 can serve as a potential co-biomarker for early-stage HCC diagnosis. Thorough large-scale studies are needed to confirm the generalizability of our findings [16].

In our study, as in the literature, we investigated six microRNAs in plasma samples in the patient and control groups. Of the six microRNAs, only MiR21 was significantly upregulated in the patient group acording to the endogenous control MiR181. In the ROC analysis curve, AUC was significantly high for Delta181CT miR-21 (0.778). The sensitivity and specificity values of Delta181CT miR-21 with an optimal cut-off value of -3.508564949 were 77.8% and 90.0%. respectively.

MiR21 is also important in determining the prognosis of HCC. Yoon et al. investigated the clinical significance of miR-21 and miR-31 for HCC-specific prognostic effect in 93 patients with HCC. They published MiR-21 expression was significantly upregulated in HCC tissues relative to nontumor tissues. They published elevated miR-21 expression might represent a biomarker for HCC prognosis [17].

Wang et al. investigated the expression level of miR-21 in HCC tissues and its prognostic value among Asian population. They showed that, expression of miR-21 was evaluated by qRT- PCR in tumor and adjacent non-neoplastic tissues in 119 HCC patients. They published that miR-21 expression was significantly higher in HCC tissues compared with normal adjacent liver tissues (P<0.0001). and miR-21 expression level could be a novel potential biomarker for HCC prognosis [18].

Limitations

The small number of cases in our study was the most important limitation. It is planned to increase the number of patients and to evaluate the pathological diagnoses in detail in future validation studies.

As conlusion; MiR-21 may have an important role in the early diagnosis HCC. Further extensive studies are needed.

Main Points

The diagnosis of HCC patients remains difficult, especially early in the development of the disease, and yet early and accurate diagnosis of HCC patients MicroRNAs have attracted attention as promising biomarkers in other cancer.

In this study, our aim was to detect the most specific and sensitive microRNA by studying the microRNAs in the patient and control groups. MicroRNA-21 is one of the oncogenic miRNAs which may be a potential diagnostic biomarker for HCC. MiR-21 may have an important role in both the early diagnosis HCC. Further extensive studies are needed.

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