RESEARCH ARTICLE

Clinical Significance of MiR-130b and MiR-125b as Biomarkers in Hepatocellular Carcinoma

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Abstract

Objective: This study aimed to assess the role of miR-130b and miR-125b expression in Hepatocellular Carcinoma (HCC) progression. **Subjects and Methods:** This study was carried out on 150 subjects classified into three groups: Group I, 50 healthy controls; Group II, 50 patients with liver cirrhosis; Group III, 50 patients with HCV related HCC. The controls were frequency matched based on age and sex with the other groups. All individuals were subjected to testing for liver function, alpha-fetoprotein (AFP), and viral markers. miR-130b and miR-125b were detected in plasma using a quantitative real-time RT-PCR. **Results:** miR-130b was significantly upregulated, whereas miR-125b was significantly downregulated in HCC patients compared with cirrhotic patients and healthy controls. There was a significant correlation between miR-130b and AFP and tumor size. Receiver operating curve (ROC) analyses suggested that plasma miR-130b had a significant diagnostic value for HCC with a sensitivity of 92% and a specificity of 77.5%. A sensitivity of 85.5% and a specificity of 82.5% was observed for miR-125b for HCC. **Conclusion:** Plasma miR-130b and miR-125b may play a role in disease progression and development of HCC and may act as potential biomarkers for the diagnosis of HCC.

Keywords: miR-130- miR-125b- liver cirrhosis- hepatocellular carcinoma (HCC)

Introduction

Hepatocellular carcinoma (HCC) is the fifth world malignant tumor and the most prevalent type of liver cancer, with a high incidence and morbidity (Carr et al., 2014). Chronic hepatitis B (HBV) and C (HCV), hepatic cirrhosis, diabetes, nonalcoholic steatohepatitis (NASH), aflatoxin exposure, obesity, and nonalcoholic fatty liver disease (NAFLD) are all variables that influence the incidence of HCC (Fujiwara et al., 2018). In Egypt, HCC is a major health issue. HCV infection plays a significant role in the development of liver cancer (Giannini et al., 2015).

MicroRNAs are small, noncoding RNA molecules with 21–30 nucleotides in length that attach to the 3'-untranslated region (3'-UTR) of mRNAs to negatively regulate target genes. These complexes are posttranscriptional effectors involved in RNA-mediated interference and pathological conditions, including tumors, by affecting the expression of genes in numerous biological processes, which may have a role in the pathogenesis of HCC (Morishita et al., 2021; Elghazaly et al., 2018).

miRNAs are tissue-specific and remain stable in plasma or serum in a form protected from endogenous RNase activity even when exposed to unstable environments such as high temperatures or low pH, increasing their potential use as noninvasive biomarkers in disease detection (Li et al., 2011).

Various studies have investigated the function of miRNAs as diagnostic or prognostic biomarkers in human cancers, including HCC. miRNAs can serve as oncogenes or tumor suppressor genes depending on the cellular affection of their targets (Hung et al., 2016).

The miR-130 family contains mature miR-130a and miR-130b with nearly identical sequences but are coded by two loci. The level of miR-130b in numerous kinds of tumors has been higher in renal cell carcinoma, glioma, and gastric cancer while being lower in papillary thyroid carcinoma and endometrial carcinoma (Wang et al., 2014).

miR-125a is found at human chromosome 19q13 and plays a role in the occurrence and progression of ovarian cancer, breast cancer, lung cancer, and gastric cancer (Liu et al., 2012) by affecting the expression of multiple target genes that suppress and control cancer, including tumor protein P53, cyclin-dependent kinase inhibitor 1A, and Erb-B2 receptor tyrosine kinase 2 (Tang et al., 2015).

On the other hand, miR-125b has been found to

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decrease the proliferation of the metastasis of human liver cancer cells and may function as a tumor suppressor (Xiong et al., 2016).

This study aims to assess the role of circulating miR-130b and miR-125b expression as a new miRNA may be involved in the development and pathogenesis of HCC

Materials and Methods

This study was conducted in the Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, and Internal Medicine Department of the National Liver Institute, Menoufia University, and this study included 150 subjects collected from April 2019 to December 2019. They were distributed into three groups: the control group included 50 age- and sex frequency matched healthy blood donors; the second group included 50 patients complaining of liver cirrhosis and diagnosed by their clinical examinations, radiological findings, and laboratory investigation; the third group included 50 HCV related HCC patients who were from the outpatient clinic at the National Liver Institute, Menoufia University. The diagnosis of HCC was determined by the presence of HCC by computed tomography (CT) ± histopathological examination if needed. Tumor staging was done using Barcelona Clinic Liver Cancer (BCLC) staging (Llovet et al., 1999). The Child–Pugh score was used to assess the severity of the liver disease (Pugh et al., 1973). Patients with acute hepatitis, chronic inflammatory disorders, and any tumor other than HCC were excluded from this study.

Methods

Under complete aseptic precautions, 5 ml of venous blood samples was taken from each subject. In tubes containing EDTA, 2 ml of blood was collected, then centrifuged at 5000 rpm for 10 minutes to separate plasma, and stored at −80°C for further processing of miRNA extraction. Moreover, 3 ml was collected in plain tubes and left to stand for 10 minutes. Then, the blood samples were centrifuged for 10 minutes at 5000 rpm. The supernatant serum was transferred into clean tubes for determination of liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, direct bilirubin, albumin, and AFP measurement using enzyme-linked immunosorbent assay (ELISA; DRG International Inc., USA.) Prothrombin time is determined using the STA-Stago Compact CT autoanalyzer (Colman et al., 1994). HCV RNA was detected by RT-PCR using HCV quantitative test COBAS TaqMan, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) (Ghany et al., 2009).

Detection of microRNA130b and microRNA 125b

MicroRNA was extracted from the plasma according to manufacturer instructions using kits supplied by Direct-zol™ RNA MiniPrep kit, Zymo Research. The quality and purity of RNA extract were determined using Nanophotometer N-60 (Implen, Germany). The extracted microRNA was reverse-transcribed (RT) using Reverse Transcription Kits (Applied Biosystems). For each RT

reaction, total volume was 15 μL: 5 μL miRNA extract was added to 7 μl RT master mix and 3 μL of RT primer, using a 2720 thermal cycler, Singapore; the product of RT was stored at -20°C.

miR-130b and miR-125b expressions were detected using real-time PCR. The PCR plate reaction was prepared using SNORD68 as an internal control. For each well, the master mix was prepared as follows: 12.5 μl SYBR Green master mix, 2.5 μl 10x miScript primer assay, 2.5 μl forward and reverse primers of selected genes, variable amounts of diluted cDNA (4 μl), and 3.5 μl RNase-free water were pipetted into each PCR tube. The miScript primer assay (Cat# 4427975) containing miRNA-specific forward primers was used to detect mature miRNA130 (CAGUGCAAUGAUGAAAGGGCAU), and for miRNA 125, miScript primer assay (Cat# 4427975) containing miRNA-specific forward primers was used (UCCCUGAGACCCUAACUUGUGA). The cycling conditions were as follows: 95°C for 15 min for the initial denaturation; 45 cycles at 94°C, 30 s for denaturation; 55°C, 1 minute for annealing and extension; 70°C, 7 minutes for final extension using the 7500 Real-time PCR system (Applied Biosystems, Foster City, CA, USA). Analysis of data was done using the comparative ΔΔCt method to detect the relative quantification (RQ), so the amount of the target (microRNA 130b and microRNA 125b) was normalized to an endogenous reference SNORD68 as a control. Figure 1a and Figure 1c show amplification plots of miRNA expression and Fig. 1b and Figure 1d show melting curve analysis

Statistical analysis of the data

Data were analyzed using IBM SPSS (Statistical Package for Social Science) software package, version 20.0. Comparisons between groups for categorical variables were assessed using the Chi-square test (Fisher or Monte Carlo). Student's t-test was used to compare two groups for normally distributed quantitative variables, while ANOVA was used for comparing the three studied groups and followed by post hoc (Tukey's) test for pairwise comparison. Kruskal quantitative variables and post hoc test (Dunn's for multiple comparisons test) were for pairwise comparison. The significance of the obtained results was detected at the 5% level.

Results

Demographic data and liver function tests

There was no significant statistical difference between studied groups regarding age and gender. The levels of AST, ALT, total bilirubin, direct bilirubin, INR, and AFP were significantly increased in HCC patients compared with those in other groups. On the other hand, serum albumin levels significantly decreased in HCC groups compared to those in other groups (Table 1).

Regarding the tumor size, 54% of HCC patients had tumors ≤3 cm and 46% had tumors with >3 cm. According to the Child–Pugh classifications, most of the patients (80%) were classified as grade A. According to BCLC staging, 25 (50%) patients were in stage A, 13 (26%) patients in stage B, and 17 (34%) patients were in stage

Table 1. Comparison between the Three Studied Groups According to Different Parameters.

	Group I No (%)	Group II No (%)	Group III No (%)	P value
Sex				
Male	33 (66%)	35 (70%)	34 (68%)	0.864
Female	17 (34%)	15 (30%)	16 (32%)	
Age (years) Mean ± SD	46.7 ± 9.5	48 ± 6.5	47.3 ± 8.1	0.204
ALT (IU/L) Mean ± SD	20.4±7.3	45.0±10.4	69.5±19.6	<0.001*
AST (IU/L) Mean ± SD	22.6±6.4	55.4±13.5	65.7±14.8	<0.001*
Albumin (gm/dl) Mean ± SD	4.5±0.6	3.36±0.48	2.72±0.43	<0.001*
Total Bilirubin (mg/dl) Mean ± SD	0.45±0.19	2.12±0.53	3.5±0.95	<0.001*
Direct Bilirubin (mg/dl) Mean ± SD	0.19±0.07	0.35 ±0.24	0.41 ±0.36	<0.001*
INR Mean ± SD	0.92± 0.03	1.16± 0.04 a	1.13± 0.07 a	0.01
GGT (IU/L) Mean ± SD	34.5 ± 4.6	49.3 ± 4.55	86.4 ± 13.5	<0.001*
AFP (ng/ml) Mean ± SD	4.25±0.9	5.6±1.57	978±219	<0.001*

Group I, control group; Group II, liver cirrhosis; Group III, HCC; *, significant; ALT, alanine transaminase; AST, aspartate transaminase; AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

C, as demonstrated in Table 2.

Regarding miRNA 130b
    Plasma miR-130b levels were increased significantly in HCC patients in comparison with those in the cirrhotic patients (P < 0.0001) and healthy controls (P < 0.0001) and were elevated significantly in cirrhotic patients compared to healthy controls (P < 0.0001) (Table 3).
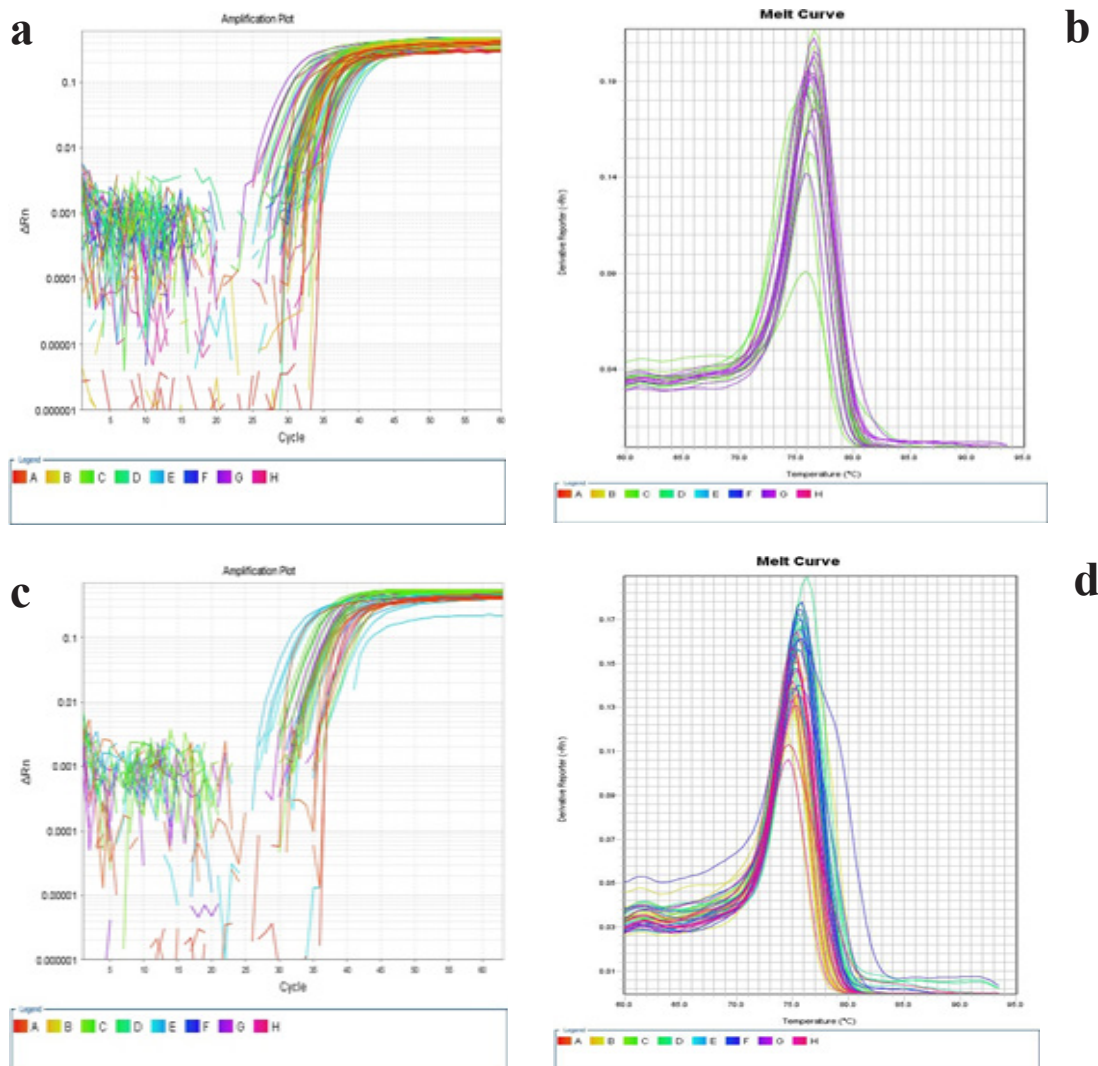

Figure 1. a, Amplification plot of miR-130; b, Melting curve analysis of miR-130; c, Amplification plot of miR-125; d, Melting curve analysis of miR-125
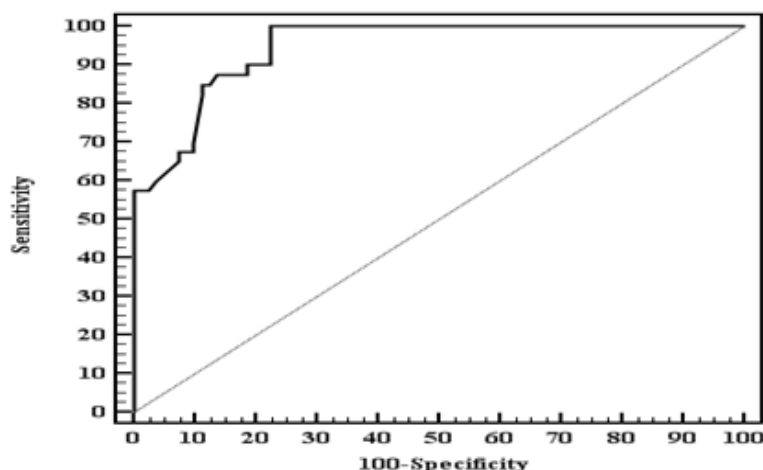
Figure 2. ROC Curve for RQ of miR130b to Predict HCC Cases vs. Chronic Cirrhosis and Control Group

Table 2. Distribution of the Studied Cases According to Different Parameters in the HCC Group (n= 50).

	No. (%)
Ascites	25 (50%)
Metastasis	42 (84%)
Spleen enlargement	
Negative	26 (53%)
Positive	24 (48%)
Tumor size (cm)	
≤3	27 (54%)
>3	18 (46%)
Mean ± SD	3.088 ± 0.69
Child–Pugh score	
Stage A	40 (80%)
Stage B	10(20%)
BCLC	
Stage A	25 (50%)
Stage B	13 (26%)
Stage C	17 (34%)

Under receiving operating characteristic curve (ROC), at a cutoff point (>10.6) of the relative expression level of miR-130b between HCC patients and controls with an area under the curve of 0.945, the sensitivity of miR-130b relative expression level as a predictor of HCC was 92.5% and the specificity was 77.5% (Table 4 and Figure 2).

Expression of miR-130 showed a significant positive correlation with AFP and tumor size. However, it was not significantly correlated with other parameters (Table 5).

Regarding miRNA 125

Plasma miR-125 was decreased significantly in HCC patients when compared with that in the cirrhotic patients and healthy controls and was significantly downregulated in cirrhotic patients compared with healthy control (Table 3) with A sensitivity of 85.5 % and A specificity of 82.5 % (Table 4 and Figure 3).

Discussion

HCC is a primary liver cancer in adults; it is the fifth most common cancer in males and the eighth most
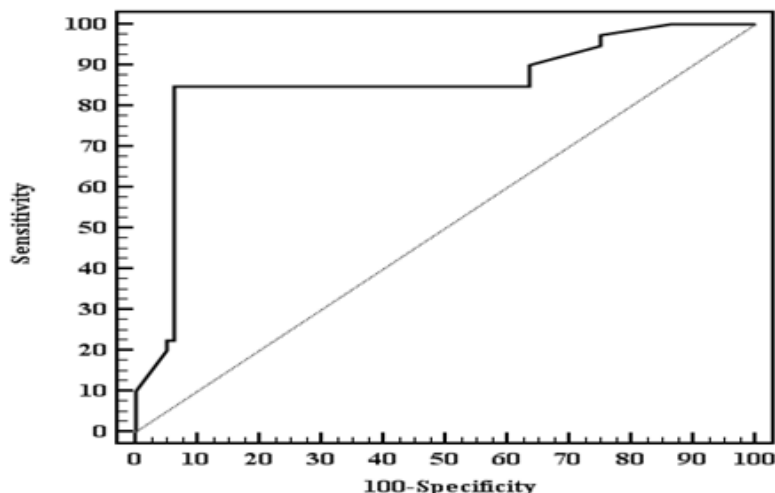

Figure 3. ROC Curve for RQ of miR125b to Predict HCC Cases vs. Chronic Cirrhosis and Control Group.

Table 3. Comparison between the Three Studied Groups According to miRNA Expression.

	Group I (n = 50)	Group II (n = 50)	Group III (n = 50)	P
RQ of miR-130b Mean ± SD	1.38 ± 0.49	9.7 ± 0.26	16.4 ± 1.96	<0.001*
Median (Min –Max.)	1.02 (0.93–4.2)	7.8 (3.4–14.5)	15 (9.5–21)	
RQ of miR-125b Mean ± SD	1.2 ± 0.2	0.7 ± 0.4	0.3 ± 0.4	<0.001*
Median (Min–Max.)	1.2 (0.8–1.8)	0.6 (0.1 – 1.7)	0.2 (0–1.3)	

Table 4. Agreement (Sensitivity, Specificity) for RQ 130 and 125 to Predict Hepatocellular Carcinoma Cases vs. Chronic Cirrhosis and Control Group.

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
RQ 130b	0.945*	<0.001*	0.888–0.979	>10.6	92.5	77.5	67.3	95.4
RQ 125b	0.852*	<0.001*	0.768–0.936	≤0.5	85	82.5	70.8	91.7

*, significant

common in females (Siegel et al., 2017).

The development and growth of HCC is a multistage process. Hepatitis C and B virus infections have been proven to play a significant role in the development of HCC. The progression involves the affection of important genes for different cellular processes such as apoptosis, cell cycle control, angiogenesis, invasion, and cell migration (Biselli-Chicote et al., 2011).

miRNAs may be good diagnostic and prognostic biomarkers and promising therapeutic targets for human tumors. miRNAs restrict gene expression by binding to 3'-UTR of target mRNA, causing mRNA destruction or blockage of protein translation (Rong et al., 2013).

miRNAs in carcinogenesis can play two different functions. They promote tumor development (known as oncomirs) through their effect on tumor suppressor genes or inhibit tumor growth by targeting oncogenes, so-called tumor suppressor microRNA (anti-oncomiRs), so miRNAs can serve two functions (Zhang et al., 2016).

Some miRNAs, such as miR-15b, miR-125b, miR-423-3p, miR-424, miR-494, miR-1225b, and miR-637, are involved in angiogenesis, which is one of the most important factors during HCC development (De Oliveira et al., 2020).

The present study aims to detect the role of circulating miR-130b and miR-125b expression in the development

Table 5. Correlation between RQ of miR130b and miR125b with Different Parameters in Group III (n= 50)

	RQ 130b		RQ 125b	
	rs	P	r	P
Age (years)	0.12	0.46	-0.05	0.77
ALT (IU/L)	0.14	0.45	0.02	0.90
AST (IU/L)	0.36	0.15	0.09	0.57
Serum albumin (g/dl)	-0.21	0.90	-0.12	0.47
Total bilirubin (mg/dl)	-0.50	0.90	0.00	0.99
Direct bilirubin (mg/dl)	0.11	0.47	0.05	0.82
BCLC	0.27	0.26	-0.05	0.84
GGT (IU/L)	0.15	0.45	0.07	0.51
AFP (ng/ml)	0.29	0.004*	0.23	0.15
Tumor size (cm)	0.67	<0.001*	-0.23	0.16

*, significant

and pathogenesis of HCC.

In this study, the liver biochemical profile is as follows: AST and ALT were significantly higher and serum albumin was significantly lower in HCC than that in the control group. These results agreed with Yakut et al., (2018), who said that this is due to the decreased synthetic ability of the liver for albumin.

In the present study, serum AFP level was significantly increased between cases and the control group. These results agreed with those of Carr et al., who reported that there was a significant elevation in HCC patients and higher AFP levels were associated with increased bilirubin levels, worse prognosis, and multifocality. Moreover, activation of the AFP gene in the malignant hepatocytes results in increased AFP production during the HCC development (Carr et al., 2014).

In this study, plasma miR-130b levels were increased significantly in HCC patients compared with those in the healthy controls and cirrhotic patients and were significantly increased in cirrhotic patients compared with healthy control with a sensitivity of 92.5 % and a specificity of 77.5 %.

That was in accordance with Liu et al., who identified miR-130b as an HCC biomarker with a high positive predictive value and that its expression increased when compared to noncancerous controls, with a sensitivity of 87.7 % and a specificity of 81.36 % (Liu et al., 2012).

Huang et al. also revealed that miR-130b was upregulated significantly in HCC patients tissues when compared with the noncancerous tissue (Huang et al., 2009).

The present study results show a positive correlation between miR-130 expression levels and serum AFP level and tumor size, and no significant correlations between miR- 130 and other parameters. Wang et al., (2014) [19] also found that the miR-130b expression level was higher in HCC tissues and correlated with poorer patient prognosis. The high level of miR-130b expression was correlated with tumor size and AFP serum level.

Xu et al., (2012) reported that miR 130b increases drug resistance and affects Wnt signaling in HCC treated by cisplatin. Furthermore, Lai et al., (2010) found that miR-130b regulates the expression of proapoptotic Bim, increasing cell viability and metastasis.

miR-130b in hepatocarcinogenesis acts as a cancer stem cell in HCC. It is highly expressed in tumor-initiating cells (CD133+) in HCC, thus supporting tumorigenesis and reducing resistance to chemotherapy (Ma et al., 2010).

In this study, plasma miR-125b was depressed significantly in HCC patients compared with that in the cirrhotic patients and healthy controls and was downregulated significantly in cirrhotic patients compared with healthy control with a sensitivity of 85.5% and a specificity of 82.5%.

Xu et al., (2018) found that levels of serum microRNA-125b could serve as potential mark¬ers for recognizing HCC from liver cirrhosis and healthy control. Sensitivity and specificity were >70% in dis-criminating HBV-HCC. The results suggest that serum microRNA-125b could act as a potential diagnostic marker for the detection of HBV related HCC.

Serum microRNA-125b could be used to predict microvascular invasion in patients with HCC, which might provide a powerful tool in making an accurate diagnosis of microvascular invasion and microRNA-125b, which was downregulated in HCC tissue and acted as a tumor suppressor (Jia et al., 2012).

Another study found that miR-125b was significantly reduced in HCC tissues and cell lines compared with that of adjacent noncancerous tissues, normal liver tissue, and cell lines. miRNAs play a critical biological role by causing pathophysiology changes in their target genes. Thus, miR-125b func¬tions by regulating the expression of SIRT7 (Zhao and Wang, 2015).

Chen et al., (2017) found that plasma miR-125b levels were significantly downregulated in HCC patients induced by HBV compared to those in HBV subjects without HCC and healthy controls, and a higher rate of metastasis was associated with the low miR-125b levels in HCC patients.

It can be concluded that miR-130b and miR-125b in plasma may be noninvasive biomarkers for detecting HCC, which will help diagnose HCC and predict prognostic factors in patients with HCC.

Author Contribution Statement

Sherin Sobhy: idea, methodology, and writing. Ebrahim Zid: revision. Fatma Reda: collection of data; Ali Nada: collection of samples. Eman Fouda: writing.

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Compliance with Ethical Standards
The ethics committee of the Faculty of Medicine, Menofia University, approved this study, and it has been performed according to the Declaration of Helsinki ethical standards. All participants included in the study provided informed consent before enrollment.

Conflict of Interest
The authors declare that they have no conflict of interest.

References

Biselli-Chicote PM, Oliveira ARCP, Pavarino EC, Goloni-Bertollo EM (2011). VEGF gene alternative splicing: Pro- and antiangiogenic isoforms in cancer. *J Cancer Res Clin Oncol*, **138**, 363–70.

Carr BI, Guerra V, Giannini EG, et al (2014). Association of Abnormal Plasma Bilirubin with Aggressive Hepatocellular Carcinoma Phenotype. *Semin Oncol*, **41**, 252–8.

Chen S, Chen H, Gao S, et al (2017). Differential expression of plasma microRNA-125b in hepatitis B virus-related liver diseases and diagnostic potential for hepatitis B virus-induced hepatocellular carcinoma. *Hepatol Res*, **47**, 312-20.

Colman RW, Hirsh J, Marder VJ, Salzman EW (1994). Hemostasis and thrombosis: basic principles and clinical practice, J.B. Lippincott, Philadelphia, pp 559-5.

De Oliveira AR, Castanhole-Nunes MM, Biselli-Chicote PM, et al (2020). Differential expression of angiogenesis-related miRNAs and VEGFA in cirrhosis and hepatocellular carcinoma. *Arch Med Sci*, **16**, 1150–7.

Elghazaly H, Gaballah A, Bahie Eldin N (2018). Clinic-pathological pattern of hepatocellular carcinoma (HCC) in Egypt. *Ann Oncol*, **29**, v5-v6.

Fujiwara N, Friedman SL, Goossens N, Hoshida Y (2018). Risk factors and prevention of hepatocellular carcinoma in the era of precision medicine. *J Hepatol*, **68**, 526–49.

Ghany MG, Strader DB, Thomas DL, Seeff LB (2009). Diagnosis, management, and treatment of hepatitis C: an update. *Hepatology*, **49**, 1335–74.

Giannini EG, Farinati F, Ciccarese F, et al (2015). Prognosis of untreated hepatocellular carcinoma. *Hepatology*, **61**, 184-90.

Huang X, Wang Q, Chen J, et al (2009). Bead-based microarray analysis of microRNA expression in hepatocellular carcinoma: miR-338 is downregulated. *Hepatol Res*, **39**, 786–94.

Hung C, Hu T, Lu S, et al (2016). Circulating microRNAs as biomarkers for diagnosis of early hepatocellular carcinoma associated with hepatitis B virus. *Int J Cancer*, **138**, 714–20.

Jia HY, Wang YX, Yan WT, et al (2012). microRNA-125b functions as a tumor suppressor in hepatocellular carcino¬ma cells. *Int J Mol Sci*, **13**, 8762-74.

Lai KW, Koh KX, Loh M, et al (2010). MicroRNA-130b regulates the tumour suppressor RUNX3 in gastric cancer. *Eur J Cancer*, **46**, 1456-63.

Li Y, Jiang Z, Xu L, et al (2011). Stability analysis of liver cancer-related microRNAs. *Acta Biochim Biophys Sin*, **43**, 69-78.

Liu AM, Yao TJ, Wang W, et al (2012). Circulating miR-15b and miR-130b in serum as potential markers for detecting hepatocellular carcinoma: a retrospective. Cohort study. *BMJ*, **2**, 160-6.

Ma S, Tang KH, Chan YP, et al (2010). miR-130b Promotes CD133+ liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1. *Cell Stem Cell*, **7**, 694–707.

Morishita A , Oura K , Tadokoro T, et al (2021). MicroRNAs in the Pathogenesis of Hepatocellular Carcinoma: A Review. *Cancers*, **13**, 1-29.

Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R (1973). Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg*, **1973**, 60646–649.

Rong M, Chen G, Dang Y (2013): Increased MiR-221 expression in hepatocellular carcinoma tissues and its role in enhancing cell growth and inhibiting apoptosis in vitro. *BMC Cancer*,

13, 1-14.

Siegel RL, Miller KD, Jemal A (2017). Cancer statistics. *CA Cancer J Clin*, **67**, 7-30.

Tang H, Li RP, Liang P, Zhou YL, Wang GW (2015). miR-125a inhibits the migration and invasion of liver cancer cells via suppression of the PI3K/AKT/mTOR signaling pathway. *Oncol Lett*, **10**, 681–6.

Wang W, Zhang H, Wang L, et al (2014). High expression of microRNA-130b correlates with poor prognosis of patients with hepatocellular carcinoma. *Diagn Pathol*, **9**, 1-6.

Xiong F, Ma Hong, Qu Y, Wen F (2016): Profiles of serum miR-99a, let-7c and miR-125b in hepatitis B virus (HBV)-associated chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. *Int J Clin Exp Pathol*, **9**, 7087-95.

Xu L, Wei B, Hui H, Liu Y (2018). Association of serum microRNA-125b and HBV-related hepatocellular carcinoma in Chinese Han patients. *Int J Clin Exp Med*, **11**, 3699-703.

Xu N, Shen C, Luo Y, et al (2012). Upregulated miR 130a increases drug resistance by regulating RUNX3 and Wnt signaling in cisplatin treated HCC cell. *Biochem Biophys Res Commun*, **425**, 468-72.

Yakut M, Özkan H, Karakaya MF, Erdal H (2018). Diagnostic and Prognostic Role of Serum Interleukin-6 in Malignant Transformation of Liver Cirrhosis. *Euroasian J Hepato-Gastroenterol*, **8**, 23-30.

Zhang D, Zhou P, Wang W, et al (2016). MicroRNA-616 promotes the migration, invasion and epithelial-mesenchymal transition of HCC by targeting PTEN. *Oncol Rep*, **35**, 366-74.

Zhao L, Wang W (2015). miR-125b suppresses the proliferation of hepatocellular carcinoma cells by targeting Sirtuin7. *Int J Clin Exp Med*, **8**, 18469-75