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Circulating MicroRNA-143 and MicroRNA-122 as Prognostic Biomarkers in Egyptian Hepatocellular Carcinoma Patients

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Abstract: In this study, we investigate the expression profile of miRNA-122 and miRNA-143 in HCC patients and assess their clinical utility as novel HCC prognostic markers as early as feasible to enhance patient prognosis. This prospective case-control study involved 150 Egyptian participants, 50 of whom had HCC, 50 had liver cirrhosis (LC), and 50 were healthy. The microRNAs (miR-122 and miR-143) were quantified using reverse transcription real-time PCR. While miR-122 expression levels were downregulated compared to controls and LC groups; miR-143 expression levels were upregulated in HCC patients. MiRNAs (miR-122 and miR-143) had a strong diagnostic potential for HCC. With a sensitivity of 82% and specificity of 100%, combined measurement of miRNA 143+ miRNA 122 was statistically significant in differentiating between HCC and LC patients at AUC 0.89. By univariate analysis, miR-143 showed its value as a significant predictor of mortality (P<0.001). Studied microRNAs (miR-122 and miR-143) may be used as early, non-invasive indicators for both diagnosis and prognosis of HCC.

Keywords: Hepatocellular carcinoma; biomarkers; microRNA; prognosis.

1 Introduction

Hepatocellular carcinoma (HCC) is regarded as the second most prevalent cause of cancer-related mortality globally and the sixth most common cancer worldwide. Despite direct antiviral treatments and immunization campaigns, virus-related HCC is still high [1]. The transitioning countries, such as Egypt, have the highest prevalence rates [2, 3]. Viral hepatitis and alcohol-related liver disease (ALD) are the main sources of the burden of chronic liver disease globally [4]. HCC, which has an insidious onset, a poor prognosis, and a high mortality rate, accounts for around 75% to 85% of primary liver cancer [2].

Imaging examinations, such as CT, X-ray CT, MRI, and other imaging techniques, are widely used to diagnose liver cancer [5]. Less X-rays may pass through the high-density tissues, causing white shadows to appear on the pictures. In addition, white shadows are produced because X-rays cannot travel through thicker tissues as quickly. In another way, computed tomography (CT) uses X-rays to pierce tissues with varying densities and thicknesses. Subsequently, obtaining a diagnosis creates varying degrees of absorption [6]. Blood collection is not as hazardous to the body as other medical procedures, so finding liver cancer indicators in peripheral blood has significant potential. Alpha-fetoprotein (AFP), out of all the serum biomarkers examined, has been shown to increase diagnostic efficacy and help assess therapy response in patients with HCC [7]. According to Tsuchiya et al., [8] the specificity of this method may reach up to 72–90%, while the sensitivity is only 39–65%. The limited sensitivity and specificity of AFP in identifying early HCC, however, continues to cast doubt on its therapeutic use [9, 10].

One of the proteins that regulates the carcinogenesis process is microRNAs (miRNAs) [11]. It is generally known that miRNAs play a role in carcinogenesis and tumour progression since they can either suppress tumour growth or stimulate it, depending on the biological function of their targets [12]. By controlling several biological and

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physiological processes, miRNAs have a significant role in the initiation, growth, and metastasis of HCC [13]. MicroRNA-122 (miR-122) is a liver-specific miRNA, the excess of miR-122 in the liver facilitates HCV's genome translation and replication [14, 15]. A useful predictive tool for HCV-induced HCC may be serum miR-122 [15]. Additionally, it has been extensively observed that miR-143-3p functions as a tumour suppressor by regulating cell proliferation, apoptosis, invasion, and metastasis in various cancers, including HCC [16]. Serum miR-143 may serve as therapeutic targets for treating HCC and as diagnostic and prognostic indicators [17].

Therefore, the need for new, reliable, and non-invasive biomarkers for HCC enables a full understanding of the molecular pathways causing hepatic carcinogenesis. The current study's objective was to evaluate the correlation between miR-122 and miR-143 expressions as potential biomarkers for the early detection of hepatocellular carcinoma in Egyptian patients.

2 Methodologies

Study design and population

Each participant in the study gave their informed consent. The protocol for the study was approved by the National Liver Institute Committee and complied with both the Declaration of Helsinki's ethical criteria and Good Clinical Practice requirements. Between November 2019 and December 2020, 150 individuals were collected for this study and divided into three groups, which are as follows:

Group I: fifty individuals who also had liver cirrhosis and hepatocellular carcinoma. Group II: fifty cirrhosis patients who do not have HCC. Group III: As the control group, 50 healthy individuals who were age and sex matched and showed no signs of liver disease were included.

The diagnosis of cirrhosis was made using information from ultrasound, biochemistry, and clinical studies. Triphasic computed tomography criteria, ultrasonography, and serum AFP levels were used to diagnose HCC. Patients from groups I and II were recruited into the outpatient clinics of Menoufia University's Tropical Medicine Department, National Liver Institute's Hepatology and Gastroenterology Department, and both. Exclusion from the research was applied to patients with severe infections, autoimmune diseases, heart, lung, kidney, persistent alcoholism, and other cancers.

The following was applied to each patient. comprehensive history taking, clinical and general examination, ultrasonography, C.T. haematological parameters, including complete blood count (CBC), and other tests. Tests for liver function include AST, ALT, albumin, T. protein, total and direct bilirubin, prothrombin time, concentration, INR, alkaline phosphatase, and gamma glutamyl transferase (GGT). To identify the early stages of HCC, tumour markers such as alfafucosidase (AFU) and Alfa Fetoprotein (AFP) levels were measured for each sample. RNA isolation kit was used to extract RNA from the blood samples, then miR-122 and miR-143 expression was measured by qRT-PCR.

Sampling and Laboratory investigations

Three sterile vacationer tubes collected ten millilitres of venous blood from the individuals. One tube included ethylene di amine tetra acetic acid (EDTA) for complete blood count (CBC) and RNA extraction; the second tube had sodium citrate for prothrombin time (PT) assay, and the third tube with no anticoagulant for routine liver function tests, alphafeto protein (AFP), and alpha fucosidase (AFU). The number of erythrocytes counts, haemoglobin concentration, hematocrit, blood indices, the total number of leucocytes, and platelets were estimated by automated cell blood counter Sysmex XP-300. The result confirmed by Medonic automated haematology analyzer, Prothrombin time (PT), concentration (CONC) and international normalized ratio (INR) were done using Thrombrel-S (human thromboplastin containing calcium). In contrast, AFP estimation was done by chemiluminescent sandwich principle using a complete automated analyzer Cobas e 411 and Alpha-l-fucosidase (AFU) by spectrophotometric Stop Rate Determination method.

Extraction and Reverse Transcription of RNA

The RNeasy Mini Kit with Qiazol Reagent (Qiagen, USA) was used to extract total RNA, including miRNAs, according to the manufacturer's instructions. Following the manufacturer's instructions, reverse transcription (RT) was performed using the miScript II RT Kit (Qiagen, USA).

Quantitative Real-Time PCR

The miR-122 and miR-143 expression levels in sera were assessed utilizing the Qiagen, USA SYBR Green PCR Kit. To perform real-time PCR on each miRNA, the forward primer sequences were used for miR-122: 5'-UGGAGUGUGACAAUGGUGUUUG-3' and 5'-UGAGAUGAAGCACUGUAGCUC -3' for miR-143. Real-Time PCR was performed as follows: After 15 minutes at 95°C, 40 cycles of 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s were

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carried out. For miRNA expression, the Ct value was disclosed. The cycle threshold (Ct) was obtained by deducting the target miRNAs' Ct (miR-122 and miR-143) values from those of the endogenous housekeeping gene miRNA-16. Relative gene expression= $(2)^{-[\Delta][\Delta]Ct}$ Where, Δ ΔCt = ΔCt sample –ΔCt reference.

Statistical Analysis

For the statistical analysis, IBM Inc., Chicago, IL, USA's SPSS version 25 was utilized. The following statistical tests were used: the distribution of quantitative data was examined using histograms and the Shapiro-Wilks normal test to ascertain whether parametric or nonparametric statistical testing should be used. The standard deviation (SD) and mean were used to express parametric variables. Within the same group, comparisons between two variables were compared using the paired T-test. The chi-square test and Kruskal–Wallis test to compare categorical variables; then, for each pair of groups, the Mann-Whitney (U) test was used. The receiver-operating characteristic (ROC) curve's area under the curve indicates a test's diagnostic performance. The best score for the test is in the range of around 100%, while more than 50% indicates an acceptable performance. A statistically significant value was defined as a two-tailed P value < 0.05. The Kaplan-Meier survival curve was utilized, and Cox regression analysis was conducted to determine the most influential factor and the statistically significant association between overall survival and progression-free survival.

3 Results

A. Participants baseline characteristics

A total of 150 subjects, the HCC patients were 37males and 13 Females with a mean age± SD of 59.52 ± 5.71 years; the LC patients were 36 males and 14 females with a mean age of 58.36 ± 4.17 years, and the healthy control group were 31 males and 19 females with a mean age of 59.74 ± 4.05. There was no significant difference in age and sex between the three groups (p =0.292, 0.378). The two patient groups, HCC and LC, showed no significant variation in weight loss and smoking status (P= 1.000, 0.052).

As regards results of peripheral blood picture parameters showed a significant decrease in the HCC group (P<0.001), while the mean level of serum transaminases, bilirubin serum, AFP, GGT, ALP, and AFU were significantly higher in the HCC patient group (P<0.001), in addition, albumin, total protein, and coagulation profile were significantly lower in HCC patients compared to LC and healthy control groups (P<0.001) (**Table 1**). Regarding miR-143, the expression level was significantly higher in the HCC group compared to the LC and healthy control group (P<0.001); on the other hand, miR-122 was significantly lower in the HCC group (P<0.001) (**Table 1**).

The clinicopathological characteristics of the study participants, such as jaundice, hepatic encephalopathy, and splenomegaly, were higher in the HCC patient group with no significant variation. At the same time, Bilharzia antibodies were significantly higher (P=0.009). HCC and LC patients had grade 1 ascites (42%, 48%), respectively, while 22% of HCC patients had grade 3 ascites with a significant association (P=0.005). HCC and LC patient groups had Child-Pugh class A, B, C, and B, which was more frequent in both groups, with a significant difference (P=0.040). The comorbidities of diseases such as diabetes and hypertension were higher in the LC patient group with no significant differences (**Table 1**). The vascular invasion and LN Metastasis were positive in (24% and 30%) respectively, in the HCC patient group.

Table 1: Clinic-pathologic characteristics of all studied groups

Variables	**HCC (n = 50)**	**LC (n = 50)**	**Control (n = 50)**	**p**
Hb (gm/dl)				
Mean ± SD.	9.93 ± 1.20	11.30 ± 1.29	13.49 ± 0.98	<0.001*
Platelets (x10^3/mm^3)				
Mean ± SD.	107.36 ± 48.01	131.72 ± 66.09	266.9 ± 66.28	<0.001*
WBCS (x10^3/mm^3)				
Mean ± SD.	3.41 ± 0.83	4.07 ± 1.27	6.56 ± 1.76	<0.001*
ALT (U/L)				
Mean ± SD.	56.62 ± 26.40	49.82 ± 22.74	25.46 ± 8.04	<0.001*
AST (U/L)				
Mean ± SD.	55.60 ± 22.42	50.24 ± 22.72	25.48 ± 7.55	<0.001*
Serum albumin (gm/dL)				
Mean ± SD.	3.17 ± 0.46	3.49 ± 0.43	4.39 ± 0.40	<0.001*

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T. protein (gm/dL)				
Mean ± SD.	5.70 ± 0.87	6.29 ± 0.69	7.11 ± 0.46	<0.001*
Total bilirubin (mg/dL)				
Mean ± SD.	3.43 ± 3.83	1.47 ± 1.06	0.58 ± 0.22	<0.001*
Direct bilirubin (mg/dL)				
Mean ± SD.	1.93 ± 2.76	0.71 ± 0.67	0.16 ± 0.07	<0.001*
GGT (U/L)				
Mean ± SD.	71.36 ± 21.52	50.72 ± 13.56	19.88 ± 6.52	<0.001*
ALP (U/L)				
Mean ± SD.	119.9 ± 35.39	83.92 ± 22.99	51.04 ± 14.41	<0.001*
PT (%)				
Mean ± SD.	72.79 ± 9.94	78.84 ± 8.86	98.06 ± 2.09	<0.001*
INR				
Mean ± SD.	1.33 ± 0.16	1.24 ± 0.13	1.02 ± 0.02	<0.001*
AFP (ng/mL)				
Mean ± SD.	377.35 ± 520.59	51.99 ± 78.40	5.12 ± 2.12	<0.001*
AFU (µmol/L)				
Mean ± SD.	142.28 ± 123.60	23.17 ± 18.53	4.80 ± 2.51	<0.001*
Micro RNA 143 RQ				
Mean ± SD.	14.46 ± 7.0	4.43 ± 1.41	1.94 ± 0.82	<0.001*
Micro RNA 122 RQ				
Mean ± SD.	0.43 ± 0.38	0.88 ± 0.18	1.34 ± 0.76	<0.001*
Jaundice	18 (36%)	11 (22%)	--	0.123
Bilharzia antibodies	34 (68%)	21 (42%)	--	0.009*
Hepatic encephalopathy	10 (20%)	4 (8%)	--	0.084
Splenomegaly	34 (68%)	32 (64%)	--	0.673
Ascites				
No	12 (24%)	19 (38%)	--	0.005*
Grade 1	21 (42%)	24 (48%)	--	
Grade 2	6 (12%)	7 (14%)	--	
Grade 3	11 (22%)	0 (0%)	--	
Child-Pugh class				
A	12 (24%)	19 (38%)	--	0.040*
B	25 (50%)	27 (54%)	--	
C	13 (26%)	4 (8%)	--	
Comorbidities				
DM	11 (22%)	20 (40%)	--	0.052
HTN	13 (26%)	16 (32%)	--	0.509
Heart diseases	0 (0%)	0 (0%)	--	--

Hb: Hemoglobin; WBC: White blood count; PLT: Platelets; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; ALB: Albumin; T.protein: Total protein; ALP: Alkaline phosphatase; GGT: Gamma glutamyl transferase; PT: prothrombin time; INR: international normalized ratio; AFP: alpha-fetoprotein; AFU: alpha fucosidase; DM: Diabetes mellitus; HTN: Hypertension; SD: Standard deviation; RQ: Relative quantification

B. Computed tomography and Survival analysis of HCC subjects

Based on nodule characteristics, multiple nodules were detected in 62%, and 44% had Large focal lesions (>5 cm) in

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diameter. The common site of HCC was the right lobe with 46%, followed by both lobes at 28% and 26% in the left lobe. TNM stage IIIa is more dominant (38%) than other staging (I, II, III b, and IV a). Similarly, BCLC Stage-C is more prevalent than BCLC-B (60% vs. 40%). Among the 50 HCC patients, 70% who received 1-year survival monitoring are still alive. Minimum and maximum survival times were six months and 12 months, respectively. The mean overall survival time (11.60 months) is shown in the Kaplan-Meier curve in **Figure 1**.

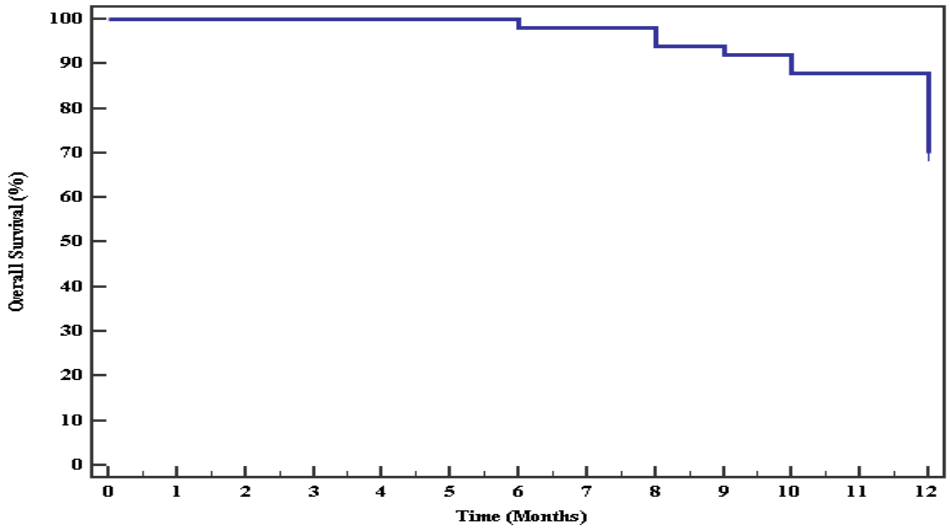

Fig. 1: Kaplan-Meier survival curve for Overall Survival

C. The correlation between biochemical, clinicopathological characteristics and miRNA in HCC subjects

Statistical analyses were conducted on subjects with complete baseline clinical data and the ΔCq level of miRNA. **Table 2** displays the relationship between miRNA expression levels and clinical laboratory investigations. There was a positive and significant correlation between mir-143 expression level and ALT, T.bilirubin, d.bilirubin, GGT, AFP, and AFU, while PLT, WBCs, and albumin were negatively correlated. On the other hand, mir-122 showed a positive significant correlation with PLT, WBCs, and albumin, while ALT, T.bilirubin, d.bilirubin, GGT, AFP, and AFU were significantly negatively correlated.

Likewise, the association between mir-143, mir-122, and clinicopathological variables in the HCC group is shown in **Table 3**, showing a significant association between tumour number, size, TNM staging, BCLC, and survival with both mir-143 and mir-122.

Table 2: Correlation between Micro RNA and biochemical parameters in the HCC group

Variables	Micro RNA 143		Micro RNA 122	
	r_s	p	r_s	p
Age (years)	-0.193	0.179	-0.100	0.488
Hb (gm/dl)	0.025	0.861	0.028	0.850
Platelets (x10^3/mm^3)	-0.294	0.038*	0.304	0.032*
WBCS (x10^3/mm^3)	-0.372	0.008*	0.322	0.023*
ALT (U/L)	0.352	0.012*	-0.362	0.010*
AST (U/L)	0.045	0.757	-0.151	0.295
Serum albumin (gm/dL)	-0.282	0.047*	0.317	0.025*
T. protein (gm/dl)	-0.029	0.842	-0.139	0.337
Total bilirubin (mg/dL)	0.352	0.012*	-0.375	0.007*
Direct bilirubin (mg/dL)	0.283	0.047*	-0.291	0.041*
GGT (U/L)	0.366	0.009*	-0.288	0.042*
ALP (U/L)	-0.035	0.809	0.163	0.258
PT (%)	0.007	0.959	-0.138	0.339

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INR	-0.018	0.903	0.149	0.303
AFP (ng/mL)	0.361	0.010*	-0.298	0.036*
AFU(µmol/L)	0.319	0.024*	-0.281	0.048*

rs: Spearman coefficient

Table 3: Relation between Micro RNA 143, Micro RNA 122, and clinicopathological variables in the HCC group

	N	Micro RNA 143		Micro RNA 122	
		Median (Min. – Max.)	**Mean ± SD.**	**Median (Min. – Max.)**	**Mean ± SD.**
Tumor number					
Single	**19**	11.19(2.24 – 24.56)	10.17 ± 6.37	0.68(0.01 – 1.46)	0.65 ± 0.45
Multiple	**31**	15.35(2.89 – 30.16)	17.09 ± 6.07	0.23(0.01 – 0.87)	0.30 ± 0.25
U(p)		**134.0*(0.001*)**		**157.0*(0.006*)**	
Tumor Size					
Small (<3 cm)	**9**	3.76(2.24 – 21.19)	8.02 ± 7.17	0.68(0.12 – 1.23)	0.75 ± 0.38
Medium (3 - 5 cm)	**19**	13.45(2.69 – 24.35)	13.82 ± 5.14	0.30(0.01 – 1.46)	0.37 ± 0.36
Large (>5 cm)	**22**	15.22(5.76 – 30.16)	17.66 ± 6.57	0.27(0.01 – 1.20)	0.35 ± 0.33
H(p)		**10.266*(0.006*)**		**7.333*(0.026*)**	
Tumour Site					
Rt Lobe	**23**	13.19(2.24 – 26.44)	13.92 ± 6.61	0.35(0.01 – 1.23)	0.45 ± 0.36
Lt Lobe	**13**	13.91(2.45 – 24.35)	13.57 ± 6.01	0.39(0.01 – 1.20)	0.48 ± 0.40
Both	**14**	15.32(2.69 – 30.16)	16.18 ± 8.55	0.27(0.01 – 1.46)	0.36 ± 0.40
H(p)		**0.857(0.652)**		**1.641(0.440)**	
TNM staging					
TNM-I	**7**	11.19(2.24 – 15.25)	8.82 ± 6.24	0.68(0.35 – 1.00)	0.72 ± 0.23
TNM-II	**3**	10.88(3.76 – 13.57)	9.40 ± 5.07	1.20(0.36 – 1.23)	0.93 ± 0.50
TNM-III a	**19**	13.19(2.69 – 21.35)	13.20 ± 5.12	0.34(0.04 – 1.20)	0.40 ± 0.31
TNM-III b	**9**	13.45(3.76 – 25.46)	15.20 ± 6.51	0.12(0.04 – 1.46)	0.40 ± 0.47
TNM-IV a	**12**	22.67(5.76 – 30.16)	20.47 ± 7.00	0.09(0.01 – 0.77)	0.21 ± 0.26
H(p)		**13.530*(0.009*)**		**15.240*(0.004*)**	
BCLC					
B	**30**	13.19(2.24 – 25.22)	12.65 ± 6.41	0.38(0.01 – 1.46)	0.49 ± 0.36
C	**20**	15.58(3.76 – 30.16)	17.19 ± 7.11	0.10(0.01 – 1.23)	0.34 ± 0.39
U(p)		**195.0*(0.038*)**		**199.0*(0.045*)**	
Fate					
Live	**35**	13.19(2.24 – 25.22)	11.93 ± 5.90	0.56(0.04 – 1.46)	0.58 ± 0.35
Dead	**15**	21.19(12.87 – 30.16)	20.37 ± 5.80	0.08(0.01 – 0.12)	0.07 ± 0.04
U(p)		**89.0*(<0.001*)**		**20.50*(<0.001*)**	

U: Mann Whitney test H: H for **Kruskal Wallis test**

p: p-value for comparison between the studied categories

*: Statistically significant at $p \leq 0.05$

D. Diagnostic Performance of HCC biomarkers and miRNAs (mRNA143 and mRNA 122)

The prognostic performance for HCC biomarkers (AFP, AFU), and (mRNA143 and mRNA 122) were presented in (**Figure 2, Table 4**). The ROC curve analysis showed the high sensitivity of mRNA143 and mRNA 122 (84% and 82%, respectively) and specificity (80% and 74%, respectively) in distinguishing HCC from LC patients. The combination of AFP+ AFU + miR-143 + miR-122 showed the highest sensitivity and specificity in distinguishing between all studied groups.

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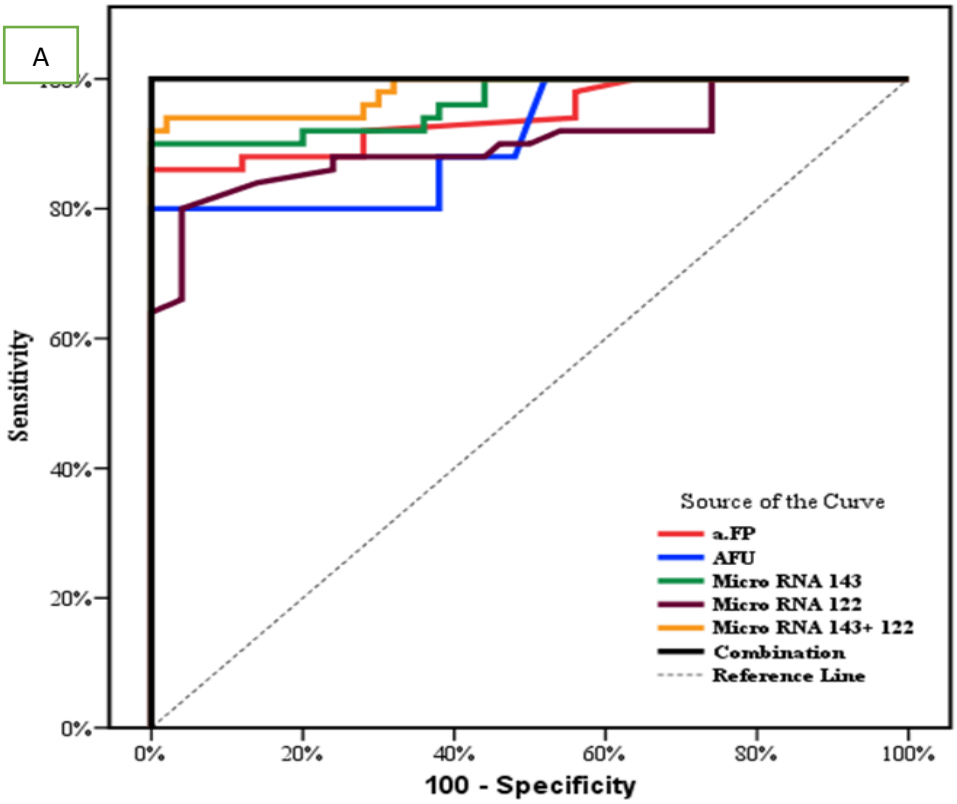
A



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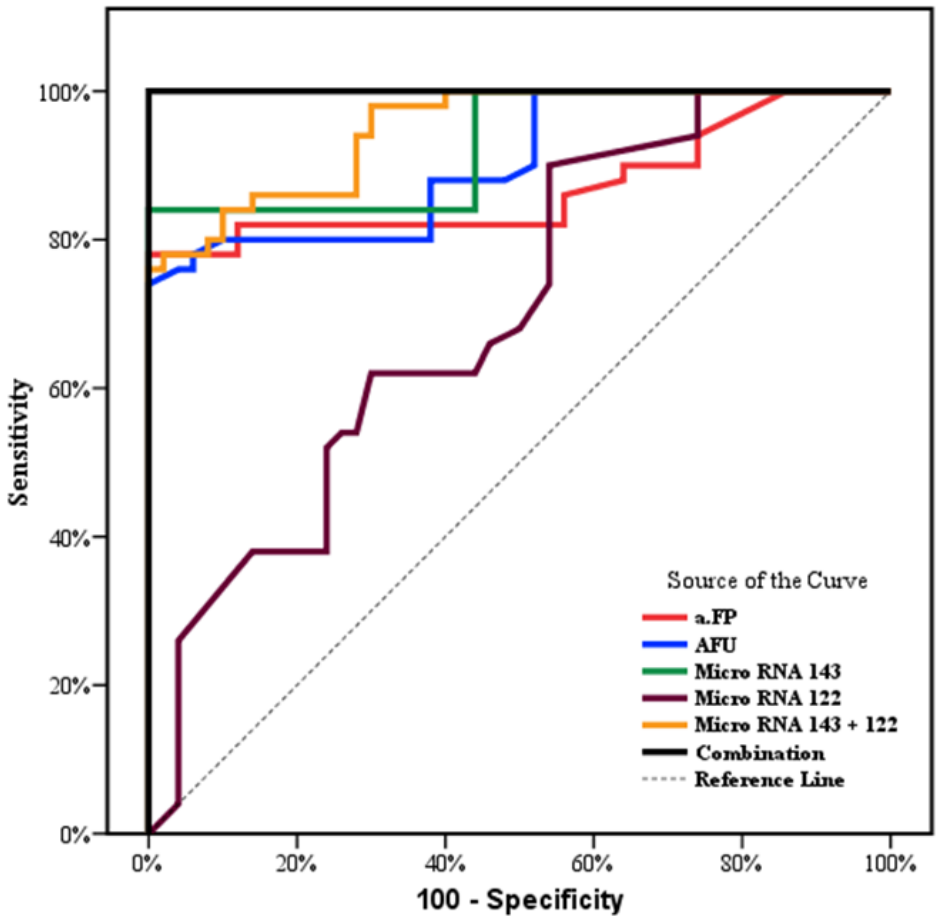

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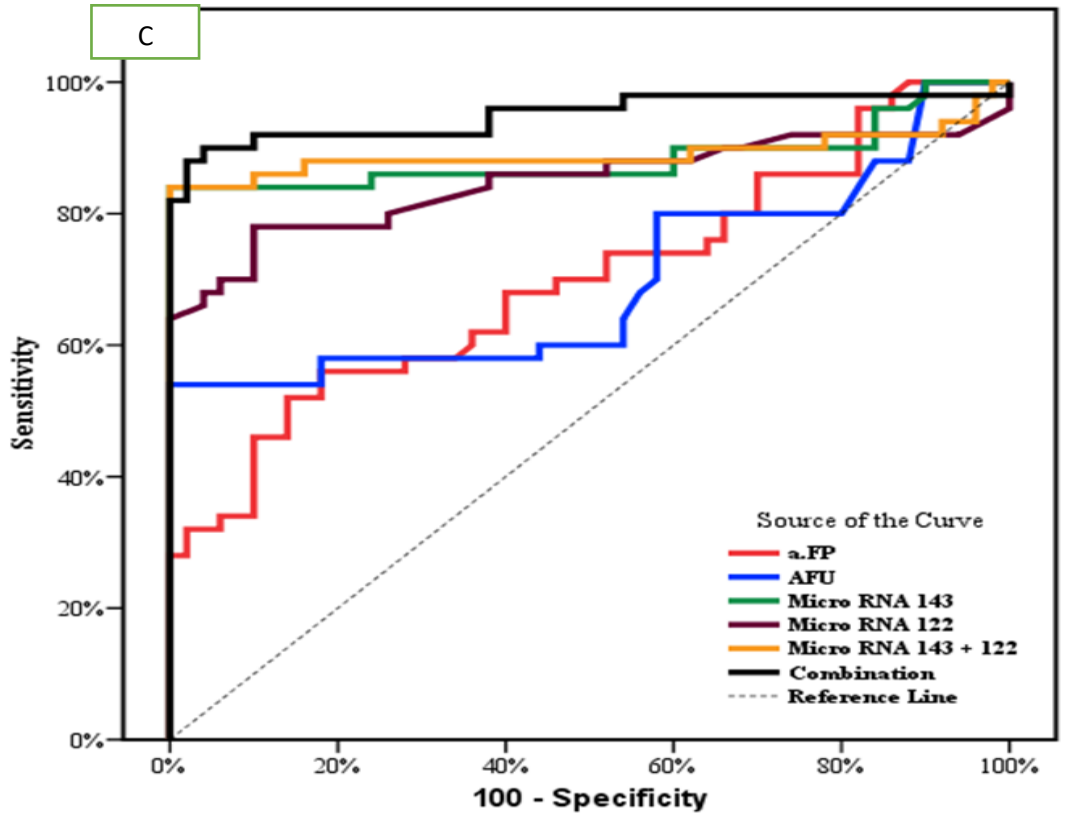

Fig. 2: ROC curve for tumour markers and miRNAs among studied groups **A**: HCC patients from control; **B**: LC patients from control; and **C**: HCC patients from LC

Table 4: Prognostic performance for tumour markers and miRNAs among studied groups

Groups	Parameters	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
	AFP (ng/mL)	0.944	<0.001*	0.898 – 0.989	>6.1	88.0	72.0	75.9	85.7
	AFU (µmol/L)	0.910	<0.001*	0.852 – 0.967	>7.1	80.0	70.0	72.7	77.8
	Micro RNA 143 RQ	0.964	<0.001*	0.930 – 0.998	>2.9	90.0	82.0	83.3	89.1
HCC vs. control	**Micro RNA 122 RQ**	0.903	<0.001*	0.838 – 0.966	≤0.765	82.0	96.0	95.3	84.2
	Micro RNA 143+ 122	0.982	<0.001*	0.960 – 1.0		90.0	100.0	100.0	90.91
	Combination #	1.000	<0.001*	1.0 – 1.0		100.0	100.0	100.0	100.0
	AFP (ng/mL)	0.870	<0.001*	0.793 – 0.948	>6	82.0	72.0	74.5	80.0
	AFU (µmol/L)	0.904	<0.001*	0.846 – 0.963	>7.2	80.0	72.0	74.1	78.3
LC vs control	**Micro RNA 143 RQ**	0.930	<0.001*	0.879 – 0.980	>2.77	84.0	80.0	80.8	83.3
	Micro RNA 122 RQ	0.703	<0.001*	0.601 – 0.804	≤0.95	62.0	60.0	60.8	61.2
	Micro RNA 143 + 122	0.949	<0.001*	0.911 – 0.986		86.0	86.0	86.0	86.0
	Combination #	1.000	<0.001*	1.0 – 1.0		100.0	100.0	100.0	100.0
	AFP (ng/mL)	0.699	<0.001*	0.507 – 0.727	>29	60.0	64.0	62.5	61.5
HCC vs LC	**AFU (µmol/L)**	0.699	0.001*	0.590 – 0.807	>31.1	58.0	68.0	64.4	61.8
	Micro RNA 143 RQ	0.885	<0.001*	0.807 – 0.963	>5.8	84.0	80.0	80.8	83.3
	Micro RNA 122 RQ	0.849	<0.001*	0.765 – 0.933	≤0.76	82.0	74.0	75.9	80.4
	Micro RNA 143 + 122	0.890	<0.001*	0.811 – 0.970		82.0	100.0	100.0	84.75
	Combination #	0.950	<0.001*	0.901 – 0.999		88.0	98.0	97.78	89.09

AUC: Area Under a Curve p-value: Probability value CI: Confidence Intervals

NPV: Negative predictive value PPV: Positive predictive value *: Statistically significant at $p \leq 0.05$
Combination: α FP+ AFU + Micro RNA 143 +Micro RNA 122

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E. The relationship between clinicopathological characteristics and mortality of HCC patients

Both univariate and multivariate Cox regression analyses were carried out to ascertain whether the calculated prognostic value of the study variables can behave as an independent risk factor. T. bilirubin, d. bilirubin, GGT, AFP, and BCLC were all the subjects of univariate analysis, demonstrating that prognostic value was a significant predictor of mortality (P<0.001). It was evident from miR-143's univariate analysis that continuous prognostic value was a strong predictor of mortality (P<0.001) with a hazard ratio of 1.170 (1.078 –1.270). Likewise, miR-122 in univariate analysis affects mortality with a hazard ratio of 0.0(0.0–0.005) and with multivariate analysis hazard ratio of 0.0 (0.0–0.121) with statically significant (P<0.05). Furthermore, multivariate analysis, including prognostic value and all demographic and clinicopathological features, showed a non-statically significant association with mortality (P>0.05) (**Table 5**).

Table 5: Univariate and multivariate COX regression analysis for the parameters affecting mortality

Variables	Univariate		#Multivariate	
	p	HR (LL – UL 95%C.I)	P	HR (LL – UL 95%C.I)
Sex (female)	0.877	1.060(0.509 – 2.207)		
Age (years)	0.820	1.007(0.949 – 1.069)		
Loss of weight	0.844	1.074(0.526 – 2.193)		
Smoking	0.798	0.913(0.454 – 1.835)		
Jaundice	0.815	0.916(0.440 – 1.908)		
Bilharzia	0.605	0.832(0.414 – 1.671)		
Abdominal pain	0.678	0.862(0.428 – 1.735)		
Hepatic encephalopathy	0.873	0.931(0.387 – 2.242)		
Splenomegaly	0.911	0.962(0.484 – 1.909)		
Ascites	0.625	0.752(0.239 – 2.362)		
Vascular Invasion	0.812	1.106(0.483 – 2.532)		
LN Metastasis	0.501	1.330(0.579 – 3.054)		
Child-Pugh class (C)	0.453	1.508(0.515 – 4.415)		
Comorbidities				
DM	0.833	1.131(0.360 – 3.555)		
HTN	0.052	2.741(0.993 – 7.567)		
Hb (gm/dl)	0.899	0.972(0.630 – 1.500)		
Platelets (x10^3/mm^3)	0.219	0.992(0.980 – 1.005)		
WBCS (x10^3/mm^3)	0.118	0.356(0.098 – 1.298)		
ALT (U/L)	0.057	1.015(1.0 – 1.031)		
AST (U/L)	0.438	1.008(0.988 – 1.029)		
Serum albumin (mg/dL)	0.294	0.555(0.184 – 1.669)		
T. protein (mg/dL)	0.349	1.333(0.730 – 2.433)		
Total bilirubin (mg/dL)	0.001*	1.192(1.072 – 1.326)	0.589	1.058(0.863 – 1.298)
Direct bilirubin (mg/dL)	<0.001*	1.329(1.147 – 1.540)	0.731	1.047(0.806 – 1.360)
GGT (U/L)	0.002*	1.028(1.010 – 1.046)	0.098	0.951(0.896 –1.009)
ALP (U/L)	0.603	1.004(0.990 – 1.017)		
PT (%)	0.603	1.013(0.964 – 1.065)		
INR	0.544	0.363(0.014 – 9.544)		
AFP (ng/ml)	0.006*	1.001(1.0 – 1.002)	0.275	1.001(0.999 – 1.002)
AFU (µmol/L)	0.074	1.004(1.0 – 1.009)		
Tumor number (Multiple)	0.297	1.840(0.586 – 5.779)		
Tumor Size(Large)	0.203	1.957(0.696 – 5.500)		
Tumour Site				
Rt Lobe	0.764	0.856(0.310 – 2.364)		
Lt Lobe	0.791	1.168(0.372 – 3.668)		
Both	0.933	1.051(0.334 – 3.304)		
TNM staging(III+IV)	0.189	30.688(0.186 – 5072.267)		
BCLC (C)	0.003*	5.556(1.759 – 17.550)	0.053	5.642(0.978–32.559)
Micro RNA 143	<0.001*	1.170(1.078 – 1.270	0.364	1.080(0.915–1.274)
Micro RNA 122	<0.001*	0.0(0.0 – 0.005)	0.026*	0.0(0.0 – 0.121)

HR: Hazard ratio C.I: Confidence interval LL: Lower limit UL: Upper Limit

#: All variables with p<0.05 were included in the multivariate *: Statistically significant at p ≤ 0.05

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4 Discussions

There is a limited sensitivity to diagnosing hepatocellular carcinoma early enough, postponing therapy until it is too late [18]. As a result of the characteristic dysregulation of miRNA expression in HCC, a distinctive expression profile that aids in early HCC identification might be observed [19]. HCC-related miRNAs are overexpressed, while suppressor miRNAs are under expressed [20]. A microRNA panel was shown in several trials to differentiate between HCC and liver cirrhosis [21, 22]. To enhance the patients' prognosis, we assessed the clinical relevance of circulating miR-122 and miR-143 as new predictive markers of HCC as early as feasible.

Age and male sex are separate risk factors for HCC [23, 24]. In the current study, there was a minor but statistically significant increase in the risk of HCC among men. According to this study, liver functions (ALT, AST, bilirubin, ALP, GGT, AFP, AFU), in comparison to the control group, the HCC group exhibited significantly higher levels of miR-143 and liver cirrhosis, whereas the LC group showed significantly lower levels of both markers ($P<0.001$). This finding is in line with those reported by Shaker *et al*., [25] and Liu *et al.,* [26], who found that HCC patients had higher serum levels of ALT, AST, bilirubin, GGT, and AFP than those people with chronic liver disease. AFU was also shown to be useful in the early diagnosis of LC and HCC [27]. Regarding miR-143, Mamdouh *et al.,* [17] and El-Gohary *et al.,* [28] revealed that patients with chronic hepatitis and HCC had significantly higher serum levels of miR-143. In contrast to our findings, Liu *et al.,* [29] found that HCC patients had low levels of miR-143. The haematological parameters (HB, WBC, and PLT), albumin, total protein, prothrombin time, and miR-122 were noticeably decreased in the HCC group and LC group when compared to the control group, and the HCC group was noticeably higher than the LC group ($P<0.001$). These outcomes matched those of Shaker *et al.,* [25], who noted a decline in Hb concentration, platelet count, and albumin. Furthermore, HCC patients had significantly lower levels of miR-122 than the LC group [30]. This study's finding that bilharzia and HCC are significantly correlated follows Calvisi, [31] finding that *Schistosoma mansoni* infection and HCC are related. Ascites also appeared more frequently in HCC patients in this study than in LC patients, which shows that ascites may predict LC progression to HCC. Ascites are frequently observed in HCC patients, and as reported, it is linked to both tumour and cirrhosis variables and a lower long-term survival rate [32].

In this study, there was a significant positive association between mir-143 expression level and ALT, T.bilirubin, d.bilirubin, GGT, AFP, and AFU, but a negative correlation between mir-143 expression level with albumin, WBCs, and PLT. While ALT, T.bilirubin, d.bilirubin, GGT, AFP, and AFU were significantly adversely correlated with mir-122, PLT, WBCs, and albumin exhibited a positive significant association with mir-122. Like miR-143, miR-122, and clinicopathological data, miR-143 and miR-122 revealed a substantial correlation between tumour number, size, TNM staging, BCLC, and survival. After validation, we discovered that in HCC patients, miR-143 was increased, whereas miR-122 was downregulated. We also do ROC analysis to identify the possible use of miR-143 and miR-122 in the diagnosis and prognosis of HCC. Moreover, The ROC curve analysis of miR-143 and miR-122 showed good sensitivity and specificity in differentiating HCC from LC patients. In this work, miR-143 showed 84% sensitivity and 80% at a cutoff level of >5.8, and the AUC was 0.8, indicating good discriminative power with ($P\leq0.001$). This is consistent with a prior study that found that individuals with HCC had considerably higher serum levels of miR-143, which may be utilized as potential biomarkers for diagnosing HCC [28]. Furthermore, at a cutoff level >0.76 with ($P\leq0.001$), the serum miR-122 expression level demonstrated sensitivity (82%) and specificity (74%) for differentiating cirrhotic individuals with HCC from LC patients, this result in the line of Zhao *et al.,* [33]. The combined measurement of mRNA 143 and mRNA 122 had a highly significant difference in discriminating between HCC and liver LC patients with AUC 0.89 with a sensitivity of 82% and specificity of 100%. also, the combination of AFP+ AFU + Micro RNA 143 + Micro RNA 122. This result opens the window to the importance of the diagnostic panel, which has become a more powerful tool in diagnosing HCC. From a prognostic view and studying HCC behavior and invasiveness, we found in our study that miR-143 is much higher in more invasive HCC, including large focal lesions, TNM staging, and BCLC, where it's more in BCLC C than BCLC B and lower levels of miRNA 122 had the same behavior. After adjusting for other clinical variables, uni and multivariate Cox analyses showed that biochemical parameters (T.bilirubin, d.bilirubin, GGT, AFP, and BCLC), miR-143 and miR-122 indicated that prognostic value was a significant indicator for mortality ($P<0.001$). Multivariate miR-122 showed a hazard ratio of 0.0 (0.0–0.121) with statically significant ($P<0.05$). These results suggest that the miR-143 and miR-122-based scoring systems can be a significant independent predictor.

In evaluating the findings, the limitations of the study must be taken into account. The small sample size is the first. The second constraint pertains to the combination of miRNAs with additional biomarkers, and the accurate identification of their source may facilitate additional investigations. A more accurate technique may be anticipated to offer promising detection and enhance treatment outcomes with more precise regulatory advice.

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5 Conclusions

This study showed that miR-122 may function as a tumour suppressor and that miR-143 may function as a potential biomarker as an onco-miR in patients with HCC. Our research revealed that miR-143 and miR-122 had a higher degree of specificity and sensitivity than AFP, even though AFP is normally the marker utilized in clinical practice to predict HCC. AFP+ AFU + miR-143 + miR-122 showed the highest sensitivity and specificity for identifying HCC patients.

6 Recommendations

The research recommends the combination of miRNAs with different biomarkers, and the accurate identification of their source may facilitate additional investigations. A more accurate technique should be used to offer promising detection and enhance treatment outcomes with more precise regulatory advice.

Conflicts of Interest Statement

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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