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Circulating MicroRNA-21 as a Novel Noninvasive Biomarker for Hepatocellular Carcinoma Compared with Alpha Fetoprotein Gold Test

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Abstract

Hepatocellular carcinoma (HCC) is the greatest traditional kind of pre-eminent cancer worldwide, which happens mainly in chronic liver disease and cirrhotic patients. The available surveillance strategies for suspected HCC patients include serum alpha-fetoprotein (AFP) and liver imaging have been mainly recommended. However, the sensitivity and selectivity of these diagnostic strategies especially in the early stages of HCC have many obstacles. MicroRNAs (miRNAs) are non-coding RNAs that are 18–25 nucleotides in length. Plasma miRNAs may be a promising new biomarker for cancer detection and prognosis in the early stages. Assessment of Plasma MicroRNA-21 (miRNA-21) significance as a noninvasive Hepatocellular carcinoma marker compared with AFP gold standard test to improve HCC early diagnostic power. This is a prospective research project that included 90 patients in total, split into three classes., liver cirrhosis patients (LC) without any malignancies and (HCC) patients in addition to the healthy control group. Patients and controls were subjected to the clinical studies, routine investigations, imaging studies, and detection of plasma miRNA-21 & AFP. miRNA-21 showed a highly significant difference in the 3 studied groups. Control group with LC group, control group with HCC group, and LC group with HCC group P value (P 0.0001, P1 0.0001, P2 0.0001and P3 0.0001) respectively. Also, a highly significant difference was observed between pre-TACE and post-TACE miRNA-21 in the HCC group P value (0.0001).Circulating miRNA-21 may be used as a noninvasive co biomarker with AFP to increase HCC diagnostic accuracy in its early stages.

Keywords: Hepatocellular carcinoma, Early diagnosis, Alpha-fetoprotein, MicroRNA-21

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INTRODUCTION

The most common form of primary liver cancer is hepatocellular carcinoma (HCC), which occurs mainly in chronic liver disease and cirrhotic patients.[1]

It progresses with local expansion, intrahepatic spreading, and distant metastases.[2] HCC is the fifth most common tumor worldwide and the third leading cause of cancer-related deaths, with over half a million new cases diagnosed each year.[3] Mainly, HCC develops with a well-established chronic liver disease background. The majority of HCC cases occur in Eastern Asia and Sub-Saharan Africa, where hepatitis B virus (HBV), hepatitis C virus (HCV), and aflatoxin B1 exposure are the most common risk factors. On the other hand, in North America, Europe, and Japan, alcohol consumption and HCV are the main risk factors. However, in the USA, nonalcoholic fatty liver disease (NAFLD), or nonalcoholic steatohepatitis (NASH), and diabetes are the common risk factor for HCC.[4]

Inflammation, necrosis, fibrosis, and continuous regeneration characterize liver cirrhosis and take part in HCC development. HBV patients can develop HCC without any cirrhosis history; underlying fibrosis is usually present, with the suggestion of regeneration. By contrast, in HCV patients, HCC invariably presents, more or less, in the setting of cirrhosis.[5] Besides, many nodules are mainly found in the cirrhotic liver, including regenerative and dysplastic nodules. Prospective studies came to the fact that the occurrence of small-cell dysplastic nodules increases HCC risk, on the other hand, large-cell dysplastic nodules aren't related to HCC initiation. The relation of small-cell dysplastic nodules to HCC includes conserved proliferation markers presence in addition to the occurrence of nodule-in-nodule during pathological examination.[6]

Since surgical resection and liver transplantation, the two curative therapies for HCC, are only available to a small percentage of patients with early tumours, the HCC survival rate is very low. There is currently no successful systemic chemotherapy for HCC.[7] The available surveillance strategies for suspected patients include serum alpha-fetoprotein (AFP) and liver imaging that have been mainly recommended. However, the sensitivity and selectivity of these diagnostic strategies especially in the early stages of HCC have many obstacles. Many imaging and serological markers have been tested as early diagnostic tools for HCC in recent decades with different sensitivity and selectivity but till now imaging with AFP remains the gold standard test for diagnosis, staging, and follow-up of HCC, despite their failure, especially in the early stages.[8]

MicroRNAs (miRNAs) are non-coding RNAs that are 18–25 nucleotides in length. The regulatory regions in the 31 or 51 untranslated region (UTR) of target messenger RNAs are complementary to miRNAs[9] Specific miRNA binding inhibits the translation of target mRNAs or promotes mRNA degradation, resulting in a responsive and rapid gene-expression regulation cascade.[10] miRNA expression in liver tissues has proven that miRNA is associated with different liver disease stages also, by comparing plasma miRNAs before and after resection of a tumor it was found that circulating miRNAs like miRNA-15b, miRNA-21, miR-130b, and miRNA-183 are overexpressed with malignancies so circulating miRNAs may be a good indicator for early prediction of tumors.[11]

Among the miRNAs that are dysregulated, miRNA-21 was identified as an oncogenic miRNA and its upregulation facilitates the proliferation, invasion, and metastasis of malignant cells.[11] It was found that miRNA-21 increases cell proliferation and suppresses cancer cell apoptosis in the xenograft model, further defining miRNA-21 as an oncogenic miRNA.[12]

According to sources, miRNA-21, miRNA-155, and miRNA-210 were raised in the plasma of B-cell lymphoma patients in comparison to healthy people, and miRNA-21 elevation was linked to the rate of relapse-free survival.[13]

So, Plasma miRNAs may be a promising new biomarker for cancer detection and prognosis in the early stages.[14]

Consequently, the aim of this project was the evaluation of circulating plasma miRNA-21 significance as a noninvasive marker for hepatocellular carcinoma compared with AFP.

MATERIALS AND METHOD

Design

This is a prospective research project. that was carried out at Sohag Centre of Cardiac

and Digestive System, Sohag, Egypt between May 2018 and January 2019.

Patients

The study included 90 patients (53 males and 37 females).; the mean age of the HCC group was 64.2±7.73 years with a range between 51 to 81 years while the LC group mean of was 63.1±8.12 years with a range between 48 and 78 years and in control healthy group, age's median was 41.73±13.59 years with range 18 years and 80 years. The studied participants were separated into three groups:

- Group, I included 30 healthy control.
- Group, II included 30 patients with liver cirrhosis (LC) without any malignancies.
- Group, III included Patients with Hepatocellular carcinoma (HCC).

Exclusion criteria

Patients with alcoholic liver cirrhosis, autoimmune liver cirrhosis, patients with any other malignancies, and HBsAg positive patients were not included in this report.

All patients underwent a comprehensive clinical review and a full history with particular focusing on the presence of jaundice, ascites, lower limbs edema, hepatomegaly, previous attacks of hepatic encephalopathy and splenomegaly. Complete blood count (CBC), liver function tests, specific HCV testing (anti-HCV antibodies by enzyme-linked immunosorbent assay (ELISA), and HCV RNA and viral load by real-time PCR were then done for all patients. Chronic HCV infection was defined by the persistence of HCV antibodies and HCV RNA in the patients' serum for at least 6 months. The abdominal sonographic examination was then done to detect the presence of hepatic fibrosis, HCC, splenomegaly, and to measure the portal vein diameter. The severity of liver cirrhosis was determined using the Child-Pugh score.[15]

According to the American Association for the Study of Liver Diseases (AASLD) guidelines, the diagnosis of HCC was confirmed by measuring of AFP in serum and triphasic computed tomography (CT) and/or magnetic resonance imaging.[16]

The Child-pugh score (CTP) was used to determine the seriousness of the disease, which takes into account five clinical (hepatic encephalopathy and ascites) and laboratory (albumin, prothrombin duration, and bilirubin values) parameters. According to the degree of abnormality, each variable is given a score of 1-3. Patients with compensated cirrhosis (class A) get a score of 5–6, those with mildly moderate decompensated cirrhosis (class B) get a score of 7–9, and those with extremely decompensated disease (class C) get a score of 10–15. All patients subjected to this study didn't receive any medications at the time of the study. The BCLC classification has been tested in various settings and provides treatment guidelines for hepatocellular carcinoma at all levels.[17]

TACE is the treatment of choice for intermediate-stage tumours (BCLC stage B). TACE uses a chemotherapeutic agent and embolization of occluding particles at the same time. The existence of a hyper vascularized tumor is a requirement for effective TACE. The combination of chemotherapeutic agent and occluding particles is administered selectively, resulting in a high local concentration of the chemotherapeutic agent in the tumor and low systemic distribution. The chemotherapeutic agent remains in the tumor region due to the occlusion of tumor vessels, and the resulting hypoxia enhances the chemotherapeutic agent's impact.[18]

Aside from a tumor size of less than 4 cm and improved vascularization, absorption of lipidol of at least 75% of the tumor volume is a favorable prognostic factor for good TACE.

Methodology

The following tests were performed on both patients and controls:

Clinical studies

including full medical history and complete clinical examination with focusing on the hepatobiliary system especially ascites, jaundice, previous attacks of hepatic encephalopathy, abdominal pain, or hematemesis

Laboratory studies

1. Routine investigations included kidney functions,[19] liver functions,[20] and complete blood count (CBC).[21]
2. Serological markers included HCVAb (22) and HBsAg.[23]
3. Molecular studies included detection of plasma miRNA-21 expression by Taqman Real time-PCR: where Isolation and quantification of total RNA were performed according to Mariner et al.[24] Briefly, EDTA blood samples were centrifuged for 15 minutes (1500 g) for

plasma separation and storage at - 80°C.

Plasma samples were de-frozen in ice and centrifuged for 15 min at 5°C (16,000g). Total RNA was extracted from plasma samples using phenol, chloroform, and ethanol according to total RNA isolation protocol (ThermoFisher), centrifuged at 4°C for 30 minutes (16,000g) followed by washing and collection in preheated nuclease-free water. A Nanodrop spectrophotometer was used to determine RNA accuracy (NanoDrop Technologies, Wilmington). The absorbance ratio at 260nm has been used as a measure of RNA purity. 10 microliters of the extracted RNA were reverse transcribed into cDNA. The Step One TM Plus Real-Time PCR System (Applied Biosystems, USA) was used to conduct the real-time PCR reactions in triplicate.

The Syber-green PCR master mix (Applied Biosystems) was used to evaluate miRNA-21 expression by the following primers: miRNA-21 Forward primer: TAGCTTATCAGACTGATGTTGA and universal reverse: GCGAGCACAGAATTAATACGAC. U6 gene was used as internal control reference for miRNA-21 expression data normalization. Primers used for detection of the U6 gene were the U6-F: CGCTTCGGCAGCACATATAC and U6-R: TTCACGAATTTGCGTGTCAT.

4. Immunological investigations for detection of serum AFP.[25]

Imaging studies included abdominal ultrasound confirmation of liver cirrhosis[26] and Triphasic CT abdomen.[27]

Statistical analysis

- For statistical data analysis, IBM-SPSS (Statistical Package for Social Sciences), version 24 IBM-Chicago, USA (May 2016) was used.
- The mean, standard deviation (SD), number, and percentage are all used to express data. For quantitative data, the mean and standard deviation were used as descriptive values, while qualitative data was defined using numbers and percentages.
- The means of two groups were compared using the student t-test, and the means of more than two groups were compared using the one-way analysis of variance (ANOVA) test. In the case of non-parametric data, the Mann-Whitney test was substituted for the student t-test.
- The percentages of qualitative data were compared using Pearson Chi-square, and non-parametric data was compared using Fisher's Exact Test.
- To compared two quantitative variables, a Pearson correlation test was used. The following is how the value of (r) was calculated: r <0.2 negligible correlation, r 0.2-0.4 weak correlation, r 0.4-0.7 moderate correlation, r 0.7-1 strong correlation, r positive was considered as positive correlation and r negative was considered as a negative correlation.
- For all these tests, the level of significance (P-value) was explained as: No significance $P > 0.05$, Significance $P < 0.05$ and High significance $P < 0.001$.
- The best cut-off for AFP and MicroRNA 21 in HCC prediction was determined using ROC curve analysis. Also, sensitivity, accuracy, positive predicted value, and negative predictive value were measured.

Ethical Considerations

- The International Ethical Guidelines for Biomedical Research Involving Human Subjects (CIOMS/WHO, 1993) were used to conduct this analysis. Ethical approval by Research and Ethical Committee of Sohag Centre of Cardiac and Digestive System, Sohag, Egypt. All of the participants in this study gave their informed consent. Cases were assigned a serial number, and personal information was kept private.

RESULTS

Our study included 60 patients with chronic Hepatitis C virus (CHCV). We divided patients with CHCV into two groups: group HCV with liver cirrhosis (LC) and group HCV with hepatocellular carcinoma (HCC).

Age & Sex

A statistical significance in age difference between the studied groups. Control group with LC group, and control group with HCC group p-value ($p <0.0001$, $p1 <0.0001$ and $p2 <0.0001$) respectively but a non-significant difference in age when compared LC group with HCC group.

Also, sex, there was a significant difference

Table 1. Demographic and Laboratory findings of the studied patients

Variables	Control N=30	LC N=30	HCC N=30	P	P1	P2	P3
Age/years							
Mean ± SD	41.73±13.59	63.1±8.12	64.2±7.73	<0.0001	<0.0001	<0.0001	1.00
Median (range)	40.5 (18:80)	63 (48:78)	65 (51:81)				
Gender							
Female	16 (53.33%)	15 (50.00%)	6 (20.00%)	0.02	0.80	0.01	0.02
Male	14 (46.67%)	15 (50.00%)	24 (80.00%)				
HCV Ab							
Negative	30 (100%)	0	0	<0.0001	<0.0001	<0.0001	1.00
Positive	0	30 (100%)	30 (100%)				
HBV Ag							
Negative	30 (100%)	30 (100%)	30 (100%)				
Positive	0	0	0				
TLC							
Mean ± SD	6.63±2.77	6.65±2.47	7.07±2.62	0.58	0.76	0.32	0.46
Median (range)	5.95 (3.5:14.8)	6.55 (2.7:12.3)	6.95 (2.7:14)				
HGB							
Mean ± SD	13.44±2.04	13.39±1.18	12.37±1.95	0.03	1.00	0.04	0.08
Median (range)	12.95 (10.5:18.9)	13 (11:16)	12 (9.6:18.7)				
PLT							
Mean ± SD	222.77±49.21	262.8±68.88	164.3±43.39	<0.0001	0.02	<0.0001	<0.0001
Median (range)	217 (155:338)	251 (152:421)	165.5 (102:289)				
Albumin							
Mean ± SD	4.4±0.49	4.15±0.55	3.70±0.64	<0.0001	0.33	<0.0001	0.007
Median (range)	4.3 (3.6:)	4.05 (3.3:5.3)	3.7 (2.6:5)				
Total bilirubin							
Mean ± SD	0.83±0.19	0.95±0.36	1.02±0.30	0.054	0.46	0.01	0.16
Median (range)	0.85 (0.5:1.1)	0.85 (0.6:2.1)	0.99 (0.5:1.5)				
Direct bilirubin							
Mean ± SD	0.23±0.06	0.61±0.23	0.41±0.24	0.0001	0.0001	0.01	0.01
Median (range)	0.2 (0.1:0.3)	0.6 (0.3:1.6)	0.4 (0.1:0.8)				
ALT							
Mean ± SD	40.10±16.16	98.7±24.51	47.6±25.22	0.0001	0.0001	0.33	0.0001
Median (range)	37 (7.5:82)	92 (65:147)	47 (8:108)				
AST							
Mean ± SD	35.35±11.42	72.83±17.21	48±21.93	0.0001	0.0001	0.008	0.0001
Median (range)	36.5 (13:59)	72 (39:108)	47.5 (9:99)				
PT							
Mean ± SD	12.82±0.76	13.14±1.85	14.60±1.80	<0.0001	1.00	<0.0001	0.001
Median (range)	12.9 (11.7:15.3)	12.2 (11:18.2)	14.25 (12:19)				
INR							
Mean ± SD	1.04±0.07	1.21±0.28	1.27±0.21	0.0001	0.004	<0.0001	0.88
Median (range)	1.0 (1.0:1.28)	1.0 (1.0:1.7)	1.26 (1.0:1.6)				
Creatinine							
Mean ± SD	0.67±0.22	0.89±0.33	1.01±0.22	0.0001	0.003	0.0001	0.01
Median (range)	0.6 (0.4:1.3)	0.8 (0.5:1.8) (0.7:1.6)	0.98				
Ascites	No [30 (100%)]	Yes [30 (100%)]	No [30(100%)]	<0.0001	<0.0001	1.00	<0.0001
Hepatomegaly							
No	100%	100%	26 (86.6%)	0.02	1.00	0.11	0.11
Yes	0%	0%	4 (13.3%)				
Splenomegaly							
No	30 (100%)	4 (13.3%)	7 (23.3%)	<0.0001	<0.0001	<0.0001	0.0001
Yes	0	26 (86.6%)	23 (76.6%)				

P compared the three group, p1 compared control & LC, p2 compared control & HCC, p3 compared LC & HCC. LC : Liver cirrhosis. HCC : Hepatocellular carcinoma. HCV Ab : hepatitis C virus antibody. HBV Ag : hepatitis B virus antigen. TLC : Total leucocytes count. HGB : Hemoglobin PLT : Platelets. Alt : alanine transaminase.Ast : aspartate transaminase. Pt :prothrombin time. INR : international normalized ratio.

between the studied groups, control with HCC group, and LC with HCC P value were (P1 0.02, P2 0.01, and P3 0.02) respectively (Table 1).

HCV Antibody Serology Test

By comparing between the 3 studied groups as regard HCV antibody (Ab), we found that there was a significant difference in HCV Ab, when compared control group with LC group and HCC group P value (p <0.0001, P1<0.0001, P2<0.0001) respectively but there was no significant difference between LC group and HCC group (Table 1).

Routine Laboratory Investigations

In terms of blood picture results of the studied groups included total Leukocytes Count (TLC), Hemoglobin (HGB), and platelets count (PLT). We found that there was no significant difference in TLC but there was a significant difference in HGB between the 3 studied groups, control with HCC group, LC group and HCC group P value (P 0.03, P2 0.04 and P3 0.08) respectively. PLT count showed a significant difference when compared the 3 studied groups, control with LC group & HCC group in addition to LC group and HCC group P value (P < 0.0001, P1 0.02, P2 <0.0001, and P3 <0.0001) respectively (table 1).

Preposition liver function levels in the 3 studied groups including Albumin, total bilirubin and direct bilirubin, ALT (Alanine Transaminase), AST (Aspartate Transaminase), PT (Prothrombin Time), and INR (International Normalized Ratio). Albumin results showed a significant difference between the studied group, when compared the control group and HCC and when compared LC group and HCC group P value (P < 0.0001, P2 <0.0001 and P3 0,007) respectively.

But total Bilirubin showed no significance when compared the control group and LC group and compared the LC group with the HCC group, otherwise, it showed a significant difference when compared the control group and HCC group P value (P 0.0001, P2 0.01) respectively.

As regards direct bilirubin, there was a significant difference between studied groups, control group with LC group, control group with HCC group, and LC group with HCC group P value (P 0.0001, P1 0.0001, P2 0.01and P3 0.01) respectively.

The recorded data denotes a significant difference in ALT between groups, control group with LC group and LC group with HCC group P value (P 0.0001, P1 0.0001and P3 0.0001) respectively. Also, there was a significant difference in AST

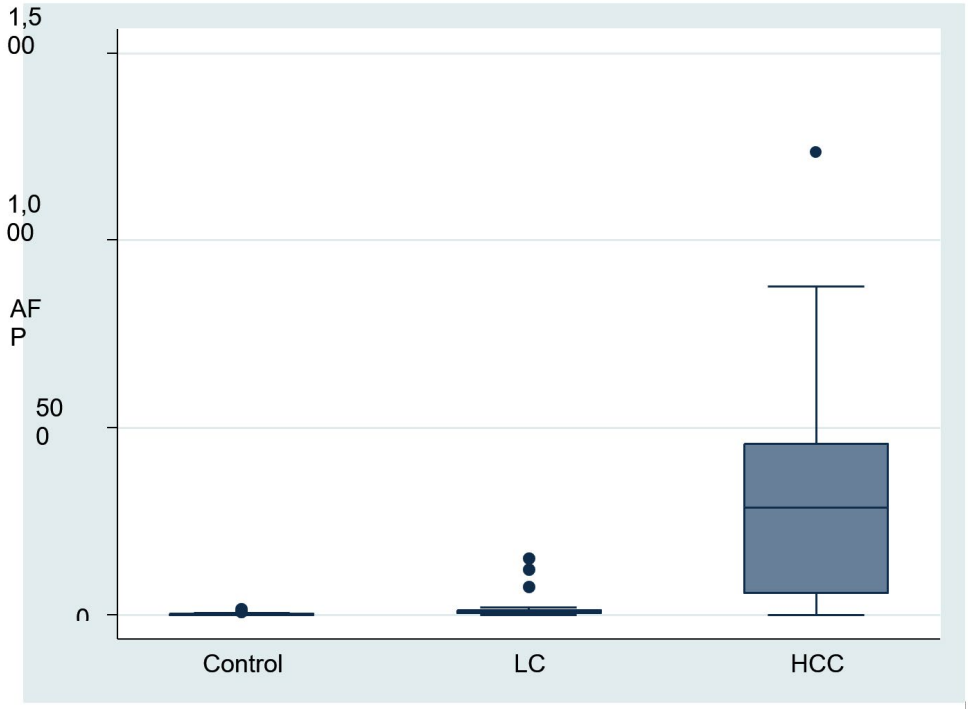

Fig. 1. Comparison Alpha fetoprotein level among 3 studied groups.

between groups, control group with LC group, control group with HCC group and LC group with HCC group P value (P 0.0001, P1 0.0001, P2 0.008and P3 0.0001) respectively. Data demonstrated a significant difference regarding Prothrombin Time (PT) &(INR) in this study, control group with LC group, control group with HCC group and when compared LC group with HCC group P value (P < 0.0001, P2 < 0.0001and P3 0.001) respectively (Table 1).

Regarding serum creatinine in the three studied groups, it was noticed that there was a highly significant difference between the studied groups, control group with LC group, control group with HCC group and LC group with HCC group P value (P 0.0001, P1 0.003, P2 0.0001and P3 0.01) respectively (Table 1).

It was shown that there was a highly significant difference in AFP&MicroRNA-21 levels between groups, control group with LC group, control group with HCC group and by compared LC group with HCC group P value (P 0.0001, P1 0.0001, P2 0.0001, and P3 0.0001) respectively (Table 2) (Fig. 1).

Imaging studies including abdominal ultrasound and Triphasic CT abdomen

With relation to the sonographic findings of the studied groups, as regards to the liver, we found that appearance of course and irregular in 100% of LC group and Hepatic Focal Lesion (HFL) in 90% of HCC group and no HFL in10% of HCC group while liver appears homogenous in 70% of the control group and mild bright in 30% of them. As regards spleen and presence of ascites, it was normal in the 3studied groups (Table 1).

Regarding the distribution of the number and size of lesions in CT findings of the HCC group. We found that 25 patients (83.33%) had one lesion, 2 patients (6.67%) had 2 lesions and 3 patients (10%) had 3 lesions, according to the size of lesions by CT Mean ± SD4.81±2.95 with Median (range) 4 (1.5:16) (Table 3) (Fig. 2).

Plasma miRNA-21

By comparing MicroRNA-21 in the 3 studied groups, it was found that there was a highly significant difference between the studied groups, control group with LC group, control group

Table 2. Comparison of Alpha fetoprotein level and Micro RNA-21 among 3 studied groups

Variables	Control N=30	LC N=30	HCC N=30	P	P1	P2	P3
AFP							
Mean ± SD	4.35±3.34	18.54±34.65	315.08±295.7	0.0001	0.0001	0.0001	0.0001
Median (range)	3.36 (0.4:14.1)	7.25 (0.9:150)	286.5 (1.49:1236)				
MicroRNA-21							
Mean ± SD	1.0±0	2.73±1.01	19.7±7.74	0.0001	0.0001	0.0001	0.0001
Median (range)	1.0 (1.0:1.0)	2.55 (1.2:4.8)	19.2 (3.1:33)				

LC : Liver cirrhosis.HCC : Hepatocellular carcinoma. AFP : Alpha fetoprotein

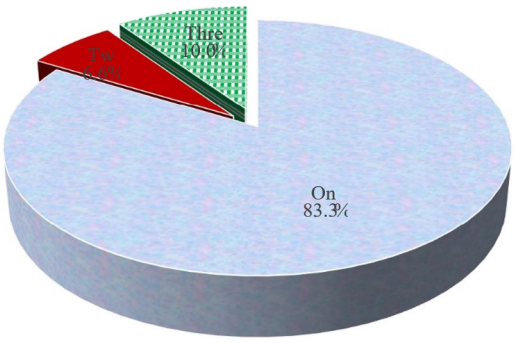

Fig. 2. Distribution of number of lesions in CT finding of HCC group.

Table 3. Distribution of number and size of lesions in CT finding of HCC group

CT finding	Number (%)
Number	
1	25 (83.33%)
2	2 (6.67%)
3	3 (10.00%)
Size	
Mean ± SD	4.81±2.95
Median (range)	4 (1.5:16)

Size is measured by millimeter

with HCC group and LC group with HCC group P value (P 0.0001, P1 0.0001, P2 0.0001and P3 0.0001) respectively (Table 4)

Number of lesions and MicroRNA-21 in HCC (Pre TACE and Post TACE)

In our study, by comparing the number of lesions and miRNA-21 in the HCC group, it was found that no statistical significance in PreTrans-Arterial Chemo Embolization TACE and post-TACE (Table 5).

Correlation between size of lesion and MicroRNA-21 (Pre TACE and Post TACE)

Correlation between the size of lesion and MicroRNA-21 in the HCC. It was found that the correlation coefficient was 0.01 in pre-TACE and 0.04 in post-TACE; there was no significant correlation between the size of lesion and microRNA-21 in the HCC group in pre-TACE and post TACE (Table 5).

Comparison between pre-TACE and post-TACE MicroRNA-21

By comparing pre-TACE and post TACE MicroRNA-21 in the HCC group, It was found that there is highly significant when comparedd between pre-TACE and post TACE Micro RNA-21

Table 4. Comparison between number of lesions and MicroRNA-21 in HCC group, PreTACE and PostTACE

Variables	One N=25	Two N=2	Three N=3	P	P1	P2	P3
MicroRNA-21pre TACE							
Mean ± SD	19.75±8.18	21±10.46	18.4±2.68	0.94	0.85	0.74	1.00
Median (range)	19.3 (3.1:33)	21 (13.6:28.4)	18.9 (15.5:20.8)				
MicroRNA-21post TACE							
Mean ± SD	2.26±0.93	3.55±0.35	3.03±1.07	0.10	0.08	0.17	0.56
Median (range)	1.9 (1.1:4.1)	3.55 (3.3:3.8)	2.8 (2.1:4.2)				

TACE: Trans Arterial Chemo Emobolization

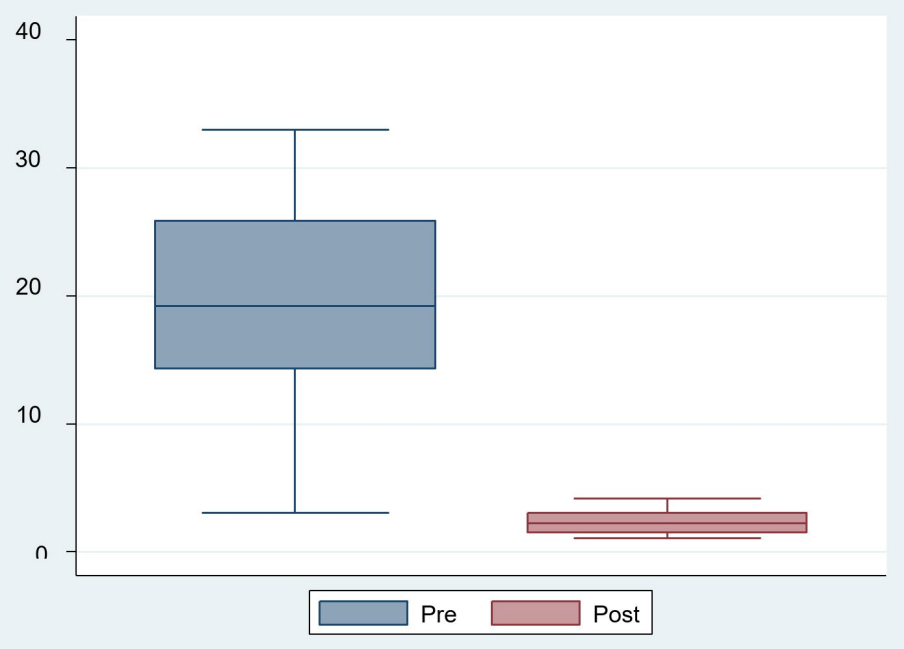

Fig. 3. Comparison between preTACE and post TACE MicroRNA-21 in HCC group.

in HCC group P value (0.0001) (Table 6) (Fig. 3).

ROC curve analysis of circulating miR-21and AFP for diagnosis of HCC

In this study, by ROC curve analysis of circulating miR-21 and AFP for diagnosis of HCC revealed that at a cut-off value of >4.8 in HCC with a sensitivity of 96.67%, the specificity of 100%; area under the curve (AUC) 0.99 (P<0.0001, 100% PPV and 98% NPV). However, the ROC curve analysis of AFP revealed that at a cut-off value of >21.4in HCC with a sensitivity of 80%, the specificity of 95%; AUC 0.91 (P< 0.0001, 88.9% PPV and 98% NPV) (Table 7) (Fig. 4).

DISCUSSION

HCC is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths.[28]

HCC has a high morbidity rate due to a lack of early warning markers and a poor prognosis. As a result, new frontiers in HCC diagnosis and therapeutics are still high- priority research areas.[29]

In Egypt, HCC is a public health concern. It accounts for 70.48 percent of all liver tumours in Egyptians, making it the second most common cancer after bladder cancer in men and breast cancer in women, as well as the second leading cause of death in men.[30]

The most commonly used tumor biomarker for HCC diagnosis is AFP. It has a low sensitivity and specificity, however. This emphasizes the need for other minimally invasive,

Table 5. Correlation between size of lesion and Micro RNA-21 in HCC group in preTACE and post TACE

Correlation between	Correlation coefficient	P value
Size & MicroRNA-21 pre– TACE	0.01	0.95
Size & MicroRNA-21 post–TACE	0.04	0.85

Table 6. Comparison between preTACE and post TACE Micro RNA-21 in HCC group

Variables	Summary statistics
MicroRNA-21 pre - TACE	
Mean ± SD	19.7±7.74
Median (range)	19.2 (3.1:33)
MicroRNA-21 post – TACE	
Mean ± SD	2.42±0.97
Median (range)	2.3 (1.1:4.2)
P value	<0.0001

HCC : Hepatocellular carcinoma. TACE: Trans Arterial Chemo Emobolization.

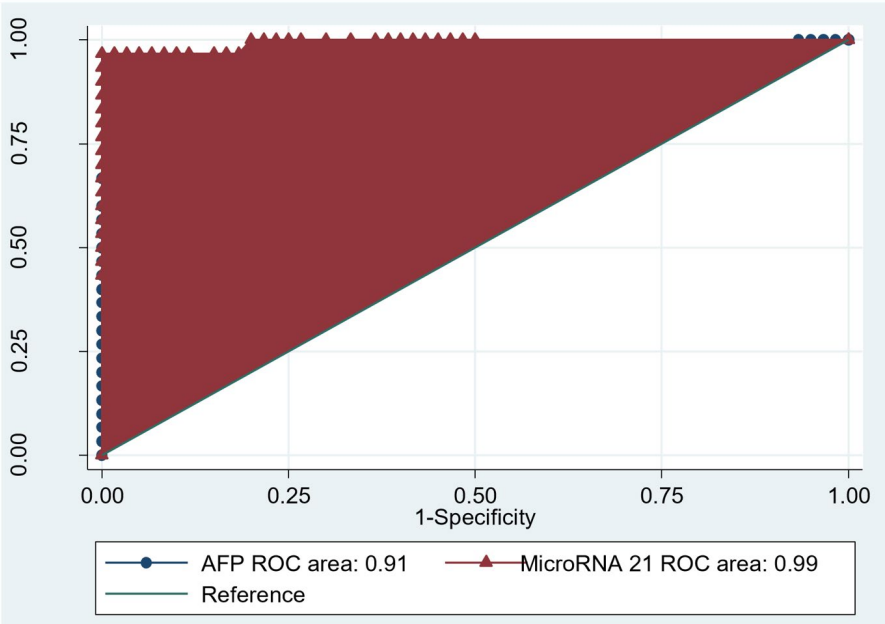

Fig. 4. ROC curve analysis of circulating miR-21 and AFP for diagnosis of HCC.

Table 7. Diagnostic performance of miR-21 and AFP for diagnosis of HCC

Cutoff	AUC	Sensitivity	Specificity	PPV	NPP	P value
AFP >21.4	0.91 (0.84-0.99)	80.0	95.0	88.9	90.5	<0.0001
Micro RNA21>4.8	0.99 (0.98–1.00)	96.67	100	100	98.0	<0.0001

P value comparing AFP & MicroRNA-21 =0.03

quick, and effective methods.[31]

miRNAs are a type of short non-coding RNA that plays a key role in post-transcriptional gene attenuation based on the sequence.[32]

MiRNAs play a role in a variety of cellular processes, including carcinogenesis. Furthermore, due to their resistance to RNase, extreme pH, and temperature, miRNAs are extremely stable in the plasma. As a result, they've been identified as potential biomarkers for detecting pre-symptomatic diseases in their early stages, such as HCC.[33,34]

The study's goal was to the evaluation of plasma miRNA -21 significance as a noninvasive Hepatocellular carcinoma marker and compared its diagnostic accuracy to AFP, to improve HCC diagnostic power at an early stage.

This study included 60 chronic HCV patients attending the Tropical Department at Centre of Cardiac and Digestive System, Sohag, Egypt, and 30 individuals as a control group during the period from May 2018 to January 2019. We divided patients with HCV into two groups, HCV Group with LC and HCV Group with HCC.

Our recorded data denoted a significant difference in routine laboratory investigations when compared to the 3 studied groups. Our results were similar to the results of Tarek et al.[35] as they found that in terms of necro-inflammatory markers (AST & ALT), liver synthetic markers INR, albumin, bilirubin, and alkaline phosphatase, there was a highly significant difference between the three classes. These results were in line with those of other studies.[36,37]

In the current study, there was a highly significant difference when comparing miRNA -21 levels in the 3 studied groups. The study of Tarek et al[35] revealed that plasma miRNA -21 values were significantly different in the HCC group compared to both chronic liver disease and healthy control groups (p <0.01). Another study by Fang et al.[38] analyzed the expression of miRNA -7 using qRT-PCR in 10 pairs of clinical HCC tissue samples and reported repression of miRNA -7 expression in 7 of 10 HCC cases compared to paired normal tissues.

So, miRNA -21 was found to be down-regulated in HCC tissues and inhibit proliferation and metastasis in HCC cells in vitro and in vivo, according to our findings, which were consistent with other studies. MiRNA -21 was found to be down-regulated in HCC tissues and inhibit proliferation and metastasis in HCC cells in vitro and in vivo, through different target genes and signalling pathways.[39,40]

According to our results, Correlation between numbers of lesions in CT & Sonographic findings with plasma miRNA -21 level it is clear that miRNA -21 is associated with HCC degree that was confirmed by the reduction of plasma miRNA -21 after TACE as the size of the tumor was reduced. Furthermore, Guo et al.[41] and Huang et al.[42] compared the expression of miRNA -21 in HCC and noncancerous samples and found that HCC had higher miRNA -21 expression, while normal samples had lower miRNA -21 plasma levels than HCC. Taken together, these results indicated that miRNA -21 could be used to diagnose HCC as compared to healthy people.

Diagnostic performance of miRNA -21 and AFP for diagnosis of HCC showed that miRNA-21 has high sensitivity and specificity compared with AFP and this was in agreement with Liao et al.[43] who suggested that miRNA -21 had a combined sensitivity and specificity of 62.4 percent and 84.4 percent for distinguishing HCC from chronic hepatitis volunteers, respectively. Also, the presence of an AFP level of 400 ng/ml is thought to be an indication of HCC, but it is not seen in the early stages of the disease. Approximately one-third of all HCC cases with small lesions (less

than 3cm) cannot be detected early.[44]

Finally, according to our results, miRNA -21 is a potential noninvasive early diagnostic marker for HCC, circulating miRNA -21 has many unique advantages. First, plasma miRNA -21 possesses minimal invasion compared with histopathological examinations. Second, plasma microRNAs are stable and reproducible.[45] Third, plasma miRNA -21 cannot be influenced by cirrhosis or viral status. Fourth, high significant overexpression of plasma miRNA-21 was noticed even in early-stage HCC and after treatment.[46,47]

So, circulating miRNA -21 may be used as a new co-biomarker with AFP to increase the accuracy of early-stage HCC diagnosis.

CONCLUSION

From this study we conclude that circulating miRNA -21 may be used as a noninvasive co biomarker with AFP to increase the precision of early-stage hepatocellular carcinoma diagnosis especially the sensitivity and selectivity of the current HCC early diagnostic strategies have many limitations where miRNA -21 possesses many unique properties including, minimal invasion compared with histopathological examinations, its stability, and reproducibility doesn't influenced by cirrhosis or viral status, and has a high significant overexpression even in early-stage HCC and after treatment. For sure this study is a single centre study that has some limitations including the small sample size and need to be validated using a multicentre study.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

LY, KH and AN designed the research. AO, and LY performed the research. AO, LY and AN analyzed data. All authors designed the figures and wrote the text. All authors read and edited the manuscript.

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None.

ETHICS STATEMENT

This study was approved by the ethical committee of Center of Cardiac and Digestive System, Sohag, Egypt. (No.SCCDS/HU102/Pros18/TCU/2/21,2018). The study was carried out according to Internatinal Ethical Guidelines for Biomedical research involving human subjects (CIOMS/WHO, 1993), and as per the Helsinki Declaration of 1975, as revised in 2008 . (http://www.wma.net/en/20activities/10ethics/10helsinki/).

INFORMED CONSENT

Written informed consent was obtained from all the participants involved in the study.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

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