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Expression and diagnostic value of non-coding mir-142 in metastatic HCC patients

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ABSTRACT

Objective: the goal of this study was to see if miR-142 alone or with liver scores FIB-5 or AFP might be employed as an early sign in the diagnosis of hepatocellular carcinoma. *Methodology*: In this study, there were 25 patients with chronic hepatitis C (CHC), twenty-five patients with hepatitis C-induced hepatocellular carcinoma (HCC), and 25 healthy controls. A detailed medical history, a thorough physical examination, and laboratory testing such as a complete blood count, liver function tests, and abdominal ultrasound were all performed on all patients. RT-PCR was applied to define the expression pattern of miR-142. *Results*: The fold differences in miR-142 expression levels between the HCC and cirrhotic groups were statistically and significantly enhanced. The difference in AFP between the HCC and cirrhotic groups was not statistically significant. The AUC of the predicted HCC miR-142 was 0.89. Merging AFP with the anticipated miR-142 (AUC of 0.89), however, did not improve AFP. The AUC was 0.81 when the proposed FIB-5 cut-off of 1.40 was used to rule out advanced cirrhosis (P 0.051) combining the proposed HCC miR-142 with FIB-5 enhanced diagnostic effectiveness, with an AUC of 0.88. *Conclusion*: MiR-142, FIB-5, and AFP were found to be promising biomarkers for cirrhosis and HCC respectively.

Keywords: AFP, HCC, FIB-5, MiR-142, Promising biomarkers

1. INTRODUCTION

HCC is the sixth greatest frequent malignancy in the world, as well as the fourth-largest origin of cancer death (Herszenyi and Tulassay, 2010). Cirrhosis is a major risk factor for HCC (eg., HCV-infected cirrhotic patients and HBV-infected cirrhotic patients), which accounts for the vast majority of HCC cases (Singal et al., 2020). Despite this, the majority of HCC patients are discovered in future in the disease process, limiting therapeutic choices to a modest therapy with a one-year median survival rate (Galle et al., 2019). Individuals with chronic hepatitis B and C who do not have HCC have greater AFP levels than those with HCC (Zhou et al., 2011). However, blood AFP standard can be upraised in malignancies other than the liver, such as gastric cancer and cholangiocarcinoma (Ikeda et al., 2012). As a result, novel biomarkers with greater accuracy and the capacity to complement hepatic imaging might help

to enhance HCC diagnosis. As a result, finding new biomarkers to diagnose HCC is critical.

MiRs dysregulation is linked to the development of several cancers (Iorio and Croce, 2012). MicroRNA-142 is a tumor suppressor miR that targets some oncogenes and is significantly downregulated in a variety of cancers (Wang et al., 2018). The abnormal expression of miR-142 is not only diagnostic but also predicts cancer patients' prognosis (Wang et al., 2012). However, fewer studies in Egypt have been done to determine the importance of miR142 levels in HCC patients. As a result, the current study looked at blood intensities of miR-142 in HCC patients for seeing if they had any predictive significance.

2. MATERIALS AND METHODS

Ethical statement, Patients selection, and biochemical assays

Faculty of medicine, Umm Alqura University CASE #2021-2020-165 IRB approved this study; all work was carried out in compliance with the Ethical Values for Medical Research regarding the Helsinki Declaration in 1975. This study comprised Egyptian subjects with chronic hepatitis C (CHC; n=25); 25 Egyptian patients with hepatitis C-induced hepatocellular carcinoma (HCC), and 25 healthy controls. Patients with hepatic B virus (HBV) antigen or antibody, as well as human immunodeficiency virus (HIV) infection, were excluded from the research. Patients with HCC were also excluded if they had begun a chemotherapy regimen or had undergone surgery. Each participant had five milliliters of blood drawn. After that, their plasma samples were separated for biochemical analysis.

Furthermore, all HCV-mediated categories in this investigation were staged using triphasic CT and AFP evaluations. All of the patients in the research had a comprehensive medical history taken, a thorough physical examination, and laboratory testing such as a full blood picture, hepatic function tests, and an abdominal ultrasound. According to Shiha et al., (2017), the FIB-5 score was computed as follows: albumin (g/L) 0.3 + platelet count (10^9/L) 0.05+alkaline phosphatase (IU/L) 0.014+AST/ALT ratio×6+14.

RNA extraction

Total RNA was secluded from plasma samples in line with the manufacturer's procedure using a miRNeasy extraction kit (Qiagen, Germany). Following that, the total RNA collected was reverse transcribed before being subjected to quantitative real-time PCR (RT-qPCR). In a nutshell, SYBER Green PCR master mix was supplemented with cDNA results from the RT process (Qiagen, Germany). The reactions were then amplified at 95°C for 10 minutes, at that point, for 40 cycles of 15 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 70°C.

RT-PCR assay

On a PCR technique, the relative gene expression of cDNA samples was measured (GeneXpert, California, USA). The proposed primer was then verified to assure perfect identification and little cross-reactivity, allowing for precise and repeatable quantification of the understudied miRs. Then, 20 liters of PCR reaction with 0.4 M primer, 10 liters of qPCR Master Mix, and 2 liters of 5 diluted cDNA were made. By subtracting the Ct-value of individual sample from the overall Ct value, Delta Ct (Ct) values approximating relative miR expression changes and equivalent to the spike in miR-142 controls of the same sample were determined. Fold change was then regarded as $2^{\wedge-\Delta\Delta Ct}$ (Livak and Schmittgen 2001).

Statistical analysis

GraphPad Prism v7 (GraphPad Software, San Diego, USA) was exploited to examine the records. Furthermore, specific data were debriefed as frequency and percentage; normally distributed data was presented as mean SD, and non-normally distributed data was presented as median and interquartile range (IQR). Furthermore, the chi-square test, ANOVA, or the Kruskal–Wallis tests were used to compare the three groups, whilst the Mann–Whitney U test was conditioned to match any two groups. All P-values were two-tailed, with $P<0.05$ indicating statistical significance. The diagnostic performance of miR-142, liver scores, and AFP in the individual groups was then assessed using the ROC curve.

3. RESULTS

Demographic analysis for all patients

Tables 1 and 2 describe the demographic and quantifiable data records. The allocation of gender through the 3 groups differed significantly (P=0.011). There were also significant gender disparities between the cirrhotic group and the HCC group. By comparing to the control group, cirrhotic and HCC patients did not have substantially higher ALT levels. Furthermore, as equated to the control, all groups had significantly higher AST levels (P<0.001). The CHC groups' total bilirubin levels were likewise

considerably higher than the control patients. In contrast, albumin levels in the sick groups were considerably lower than in the control group (P<0.001).There were significant changes in ALP levels across the groups (P<0.001), according to the findings. Furthermore, the diseased groups had highly unregulated level of AFP (P=0.032). However, when comparing CHC patients to HCC patients, higher AFP levels were not significant (P=0.065). Platelet counts were all recognised to be significantly different from the control group (P<0.001). FibroScan (kPa) was, on the other hand, substantially deregulated across the all groups. Patients with HCC had nonsignificant FibroScan findings when equated to the cirrhotic group (P=0.059).

Table 1 Demographic analysis for all studies patients.

Group / Variable	C N = 25	CHC N = 25	HCC N = 25	P-value			
				All	C vs. CHC	C vs. HCC	CHC vs. HCC
Age, yrs., mean ± SD	53.2 ± 5.6	55.9 ± 5.2	56.6 ± 3.2	0.023	0.031	0.020	0.541
Gender, male, n (%)	18 (72.0%)	14 (56.0%)	17 (68.0%)	0.011	0.411	0.041	0.004
Smoking, n (%)	7 (28.0%)	3 (12.0%)	6 (24.0%)	0.072	0.066	0.172	0.031
DM, n (%)	4 (16.0%)	10 (40.0%)	5 (20.0%)	0.054	0.145	0.431	0.023
Hypertension, n (%)	3 (12.0%)	8 (32.0%)	9 (36.0%)	0.551	0.278	0.298	0.789

(DM, diabetes mellitus)

Table 2 Comparison of laboratory data between the cirrhotic, control, and HCC groups

Group / Variable	C N = 25	CHC N = 25	HCC N = 25	P-value			
				All	C vs. CHC	C vs. HCC	CHC vs. HCC
ALT, U/L, median IQR)	14.50 (35.0)	35.50 (25.0)	45.20 (39.0)	0.121	0.246	0.083	0.058
AST, U/L, median (IQR)	16.40 (11.0)	24.60 (15.5)	52.23 (58.0)	<0.001	0.005	<0.001	<0.001
Albumin, g/dL, median (IQR)	4.45 (0.35)	3.79 (0.66)	2.70 (0.99)	<0.001	<0.001	<0.001	<0.001
Bilirubin, mg/dL, median (IQR)	0.49 (0.31)	1.10 (0.21)	1.90 (1.63)	<0.001	<0.001	<0.001	<0.001
ALP (IU/L), median (IQR)	75.58 (19.98)	158 (22.98)	215 (55.62)	<0.001	<0.001	<0.001	<0.001
AFP, ng/mL, median (IQR)	2.65 (1.75)	7.45 (4.65)	15.45 (9.80)	0.032	0.006	0.001*	0.065
Hgb, g/dL, mean ± SD	13.82 ± 0.88	11.90 ± 2.04	11.21 ± 0.98	0.078	0.159	0.352	0.069
Plt, ×103/µL, mean ± SD	210.40 ± 14.56	89.50 ± 8.58	75.56 ± 11.25	<0.001	<0.001	<0.001	0.056
FibroScan, kPa, median (IQR)	2.98 (1.5)	25.23 (14.5)	36.56 (252)	<0.001	<0.001	<0.001	0.059
FIB-5, median (IQR)	0.5 (0.2)	3.2 (2.7)	NA	NA	0.042	NA	NA
APRI, median (IQR)	0.6 (0.2)	3.9 (1.9)	NA	NA	0.031	NA	NA

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; Hgb, haemoglobin; IQR, interquartile range; NA, not applicable; PLT, platelets.

Differential countenance of miR-142 in cirrhotic subjects and HCC patients

Between the HCC and cirrhotic groups, fold differences in miR-142 expression levels were statistically and substantially elevated (P <0.001). Nonetheless, the difference in AFP between the HCC and cirrhotic groups was not statistically significant (P = 0.065) (table 3).

Table 3 Comparison of patient groups regarding the miR-142 and AFP to differentiate between Cirrhotic subjects and HCC group

Group / Variable	CHC N = 25	HCC N = 25	P-value
Fold difference relative to control miR-142, median (IQR)	9.58 (5.32)	3.85 (2.17)	<0.001
AFP, median (IQR)	7.45 (4.65)	15.45 (9.80)	0.065

Receiver operating characteristics (ROC) comparison curves for identifying cirrhosis or HCC utilising AFP FIB-5 and FIB-5 alone or in conjunction with miR-142

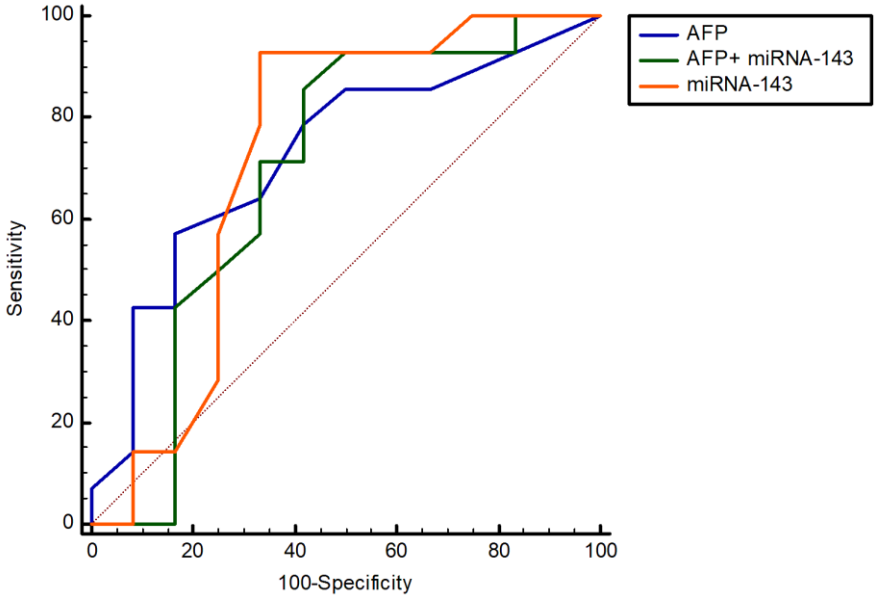

Figure 1A the ability of miR-142+AFP candidates to distinguish between cirrhosis and HCC was assessed using a ROC curve analysis.

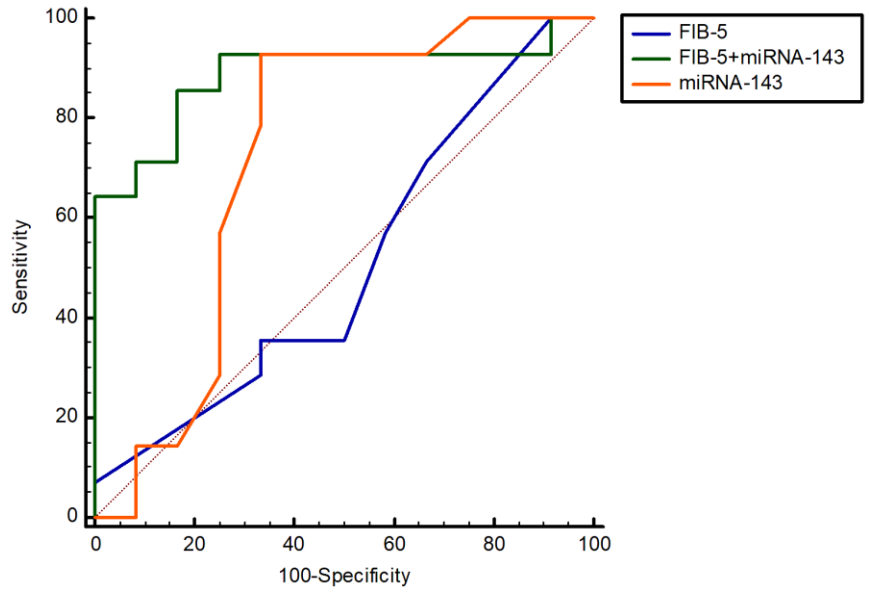

Figure 1B the ability of miR-142+FIB-5 candidates to discriminate among cirrhosis and HCC was assessed using a ROC curve analysis.

The commonly approved tumor marker AFP had an AUC of just 0.73 (P = 0.065) in this group, making it a weak discriminator of HCC. When an AFP cut-off of >15 ng/ml was applied to detect HCC, it yielded a sensitivity of 35%, specificity of 85%, PPV of 70.3%, NPV of 44.8 %, and overall accuracy of 66.8%. AFP was not improved by combining it with the suggested miR-142 (Figure 1A). In this cohort, the biochemical marker FIB-5 was tested for its power to discriminate cirrhosis from HCC. In this cohort, by the suggested FIB-5 limit of 1.40 to rule out progressive cirrhosis yielded an AUC of 0.81, with a sensitivity of 85%, specificity of 55%, PPV of 72.2 %, NPV of 90.3 %, and overall accuracy of 79.9 %. With an AUC of 0.88 (Figure 1B), combining suggested HCC miR-142 with FIB-5 increased diagnostic efficacy.

4. DISCUSSION

HCV contagion is a life-threatening disease that moves over 185 million people worldwide (Thomas, 2013). Chronic HCV infection that is not treated progresses to more serious stages like cirrhosis or HCC (Zaltron et al., 2012). Men's cancer is the sixth most prevalent cause of mortality (Rahib et al., 2014). However, a major difficulty is the absence of accurate biomarkers for initial detection and prognosis of HCC, necessitating the development of novel sensitive and specific diagnostic methods. As a result, the present study looked examined miR-142 levels in HCC patients' blood to determine if they had any prognostic value.

The patients with HCC in this study were on average 56.6±3.2 years old. HCC patients varied in age from 42 to 70 years old (mean 58+5.76 years), according to Castroagudin et al., (2005). This result can be explained by the considerable period between the onset of the hepatic inflammatory process and the occurrence of HCC. Parenteral anti-Schistosomal treatment and other iatrogenic exposures are responsible for the great majority of infections among Egyptians aged 30 and older (Mohamoud et al., 2013). Across practically all etiologies of liver disease, men have a 2:4 higher chance of HCC than women (El-Serag, 2004). The HCC group consisted of 17 (68.0%) males and 8 (32.0%) females, according to our findings. This is in line with the findings of Alavian et al, who looked at 1328 HCC patients and discovered that HCC was much greater in males (77.7%) than females (23.3%) (Alavian and Haghbin, 2016).

When compared to healthy individuals, ALT and total bilirubin levels are considerably higher in the two diseased categories: cirrhosis, and HCC, according to our demographic data analysis. Albumin levels, on the other hand, were considerably lower in all diseased groups as compared to healthy controls. These results might be because HCC frequently occurs alongside liver cirrhosis, and these measures are used to stage CHC. Furthermore, CHC, which is existent in 80 % to 90 % of patients, was found to be the most crucial risk element for the advancement of HCC (Huo et al., 2008; Lim and Kim, 2008). There was no statistically significant difference in AFP levels across the three diseased groups, corroborating Di Bisceglie et al., (2005) results that AFP are raised in progressive CHC and that AFP are usually raised up, even when HCC is absent. This lends credence to the idea that AFP is too lacking specificity (Wei et al., 2018). The examination of fold differences in miR-142 expression level in our study revealed a substantial folding drop in countenance level in the HCC group 3.85 (2.17) compared to the CHC group 9.58 (5.32) (P<0.001). MiR-142 is a tumor-specific marker that contributes to carcinogenesis and circulates in the bloodstream, making it a potential new diagnostic marker. This is in agreement with a study held by Tsang FH et al., (2015) who discovered that miR-142 and miR-142-5p were often (>70%) down-regulated in HCC samples and that their expression levels decreased progressively to early HCC and then progressed HCC. As a result, miR142 and miR-142-5p show a character in the multistep development of HCC. In another study, the expression of miR-142 was considerably decreased in primary HCCs to venous metastases in a prior miR profiling research (Wong et al., 2012) which these data imply that miR-142 affects cell motility-related activities when taken together.

In our study, MiR-142 is down-regulated in HCC samples, according to previous studies, and functional studies have shown that miR-142 can reduce relocation, propagation *invitro* by targeting Family Small GTPase 1(RAC1) and TGF-β (Yu et al., 2017). Although methodological issues might account for disparities, one research found elevated stages of circulating and tissue miR-142 in HCC (Ghosh et al., 2015). The potential of miR-142 to differentiate CHC with HCC from cirrhosis was established utilizing ROC curve analysis using the suggested HCC miR-142. This result was much superior to AFP's diagnostic performance in detecting HCC (AUC = 0.73), showing the suggested miR-142-significant 3p's clinical advantages over the presently employed HCC tumor biomarker. Using AFP and the miR panel combined did not improve diagnostic performance. Obviously, although greater validation of a miRs panel incorporating other miRs with miR-142 in a large research population is required in the future, the panel's diagnostic potential is undeniably exciting.

However, there are certain limitations to this are a research that should be considered while assessing the data. These results need to be confirmed in a broader patient group, including those who have reached SVR, before they can be applied to the general HCV population. Only individuals with HCV genotype-4 were included in this investigation. The diagnostic value of a miR panel across all genotypes needs additional exploration, especially as DAAs therapy possibilities grow additional effectual and less

needful on genotyping. The present study established that only miR-142 separated CHC patients from HCC patients. Prospective validation of the suggested cirrhosis-specific miR would be beneficial. At all phases, the panel should be tested in a study cohort with biopsy-confirmed fibrosis to ensure that it can distinguish between mild and severe disease (F0-2).

5. CONCLUSION

To conclude, our study revealed that miR-142 expression in the individuals with CHC varies depending on their disease severity. We found miR-142, FIB-5, and AFP to be potential biomarkers of CHC and HCC development. Diagnostic panels that might be utilized to stratify CHC and identify early HCC have been developed by combining the levels of expression of these biomarkers. Although the miR panels need to be validated further before they can be utilised in clinical practise, these promising early results imply that miRs could be employed to help identify individuals with cirrhosis and HCC.

Author Contributions

Conceptualization, A.E.; validation, A.E, and A.O.B; formal analysis, A.O.B; investigation, A.E; data curation, A.O.B; writing—original draft preparation, A.E.; writing—review and editing, A.E, and A.O.B. All authors have read and agreed to the published version of the manuscript

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Institutional Review Board Statement

The study was approved by the Ethics Committee of College of medicine, Umm Alqura University (CASE #2021-2020-165 IRB)

Informed Consent Statement

All patients who took part in this research gave their informed consent.

Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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