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RESEARCH ARTICLE

Interplay between TGF-β1 and miRNA-122 biomarkers in hepatocellular carcinoma progression in patients with chronic hepatitis C

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ABSTRACT

Hepatocellular carcinoma (HCC) is a highly lethal malignancy and clinically validated medications have not yet been developed since there are no reliable diagnostic and prognostic biomarkers. Based on bioinformatics tools, TGF-β1 gene was the first target gene of miRNA-122, therefore this study was intended to assess the potential interconnection between TGF-β1 and miRNA-122 as a diagnostic and prognostic biomarker in the progression of HCC in patients with chronic hepatitis C (CHC) genotype (4). In this study, 100 people were included and split into two groups; group I: CHC patients without HCC that were classified into patients CHC without cirrhosis and CHC cirrhotic patients, group II: CHC patients with HCC, and healthy volunteers as control. The expression of miRNA-122 and TGF-β1 genes were analyzed using Real-Time PCR. An upregulation of miRNA-122 gene in cirrhotic and HCC patients compared to both chronic HCV non-cirrhotic, and cirrhotic patients, while, a decrease in expression of TGF-β1 was found in cirrhotic patients compared to HCV non-cirrhotic patients. Although significantly downregulated in HCC patients. Regression analysis indicated that the expression levels of miRNA-122 and TGF-β1 could be regarded as important indicators of the alterations in cirrhotic and HCC patients versus HCV non-cirrhotic patients, also with the chances of HCC versus cirrhosis patients. Our data indicated an interaction between miRNA-122 and TGF-β1, regulated gene expression and recommended the use of these parameters as noninvasive predictive biomarkers and therapeutic targets for HCV induced liver cirrhosis and HCC.

Keywords: Hepatocellular carcinoma; TGF-β1; MiRNA-122; liver cirrhosis; biomarkers.

INTRODUCTION

Infection with Hepatitis C virus (HCV), a hepatotropic RNA virus, is one of the leading causes of chronic liver disease including fibrosis, cirrhosis, and increased risk for development of Hepatocellular carcinoma (HCC). One of the main causes of chronic liver disease, including fibrosis, cirrhosis, and an increased risk of developing hepatocellular carcinoma, is infection with the hepatitis C virus (HCV), a hepatotropic RNA virus (HCC). The World Health Organization (WHO) estimates that 71 million people worldwide have chronic HCV infection (WHO, 2019), and that HCC causes more than 700,000 deaths annually (Jemal *et al*., 2011; Siegel *et al*., 2019). The prevalence of chronic hepatitis C is declining overall, but as the infected population ages and liver fibrosis, cirrhosis, and HCC proceed, the disease's effects are becoming more severe (Schanzer *et al*., 2014; El-Araby *et al*., 2020).

Hepatic stellate cells (HSCs) were assumed to be the main cause of pathologically increased extracellular matrix (ECM) in disease situations, therefore, it is thought that the activation of HSC and subsequent differentiation into myofibroblasts are the main causes of liver fibrosis (Xu *et al*., 2014).

HCC ranks is the fifth most prevalent cancer source and the second most common cause of cancer-related deaths worldwide (Jemal *et al*., 2011). It is a chemotherapy-resistant and inflammation-induced malignancy that is highly lethal, especially for the 40% of affected patients who have advanced-stage disease (Lohitesh *et al*., 2018). The development of clinically validated HCC agents has been hindered by a lack of knowledge of the diverse mechanisms underlying HCC tumorigenesis and progression, the intricate interactions between liver tumors and their immune microenvironment, and the absence of reliable diagnostic and prognostic biomarkers. (Mazzanti *et al*., 2016).

MicroRNAs (miRNAs) are small and non-coding RNAs that could be detected in serum or plasma and therefore can be used in the diagnosis or prognosis of numerous cancers, and considered as crucial controllers of the onset and progression of liver fibrosis (Lambrecht *et al*., 2015), cirrhosis, and HCC (El-Araby *et al*., 2020). miRNA-122 is a liver-specific miRNA and regarded as a key regulator

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in both liver development and liver disorders. In HCC patients' differences in miR-122 circulating level was observed when compared to healthy cases (Bandiera *et al*., 2015; Ors-Kumoglu *et al*., 2019; Parizadeh *et al*., 2019).

Transforming growth factor-β (TGF-β) is a potent cytokine that regulates cell proliferation, differentiation, and wound healing. It also plays a significant role in the onset and progression of liver fibrosis by encouraging the expression of genes relevant to fibrosis (Morikawa *et al*., 2016).

There are three distinct isoforms of TGF-β proteins: TGF-β1, TGF-β2, and TGF-β3. TGF-β1 is the most commonly and actively studied isoform in liver fibrogenesis (Dewidar *et al*., 2015).

TGF-β can facilitate the epigenetic changes in HSCs from epithelial to mesenchymal and matrix production through the regulation of expression levels of different miRNAs (Tu *et al*., 2019). Otherwise, epigenetic regulators control the expression and action of the components of the TGF-β signaling pathway (Tu *et al*., 2019). Numerous miRNAs were shown to be antifibrotic and connected to the TGF-β pathway (Gonzalez-Sanchez *et al*., 2021), of which TGF-β1 gene has been reported as the target of miRNA-122 (Xu *et al*., 2016). TGF-β pathway is a crucial immune modulator and biomarker for HCC (Chen *et al*., 2018b; Dituri *et al*., 2019; de Brito *et al*., 2020). Targeting the TGF-β pathway in hepatic immune cells may improve antitumor immunity in HCC patients since the TGF-β pathway has an immunosuppressive function in HCC (Chen *et al*., 2019).

Despite the rarity of new infections by HCV, nevertheless, the pathological effects are still present as fibrosis, cirrhosis, and HCC (El-Araby *et al*., 2020). Therefore, we are in desperate need of new non-invasive prognostic biomarkers. In the evolution of HCC in patients with chronic hepatitis C, the current study was created to assess the possible interaction between miRNA-122 and TGF-β1 as a diagnostic and predictive biomarker.

PATIENTS AND METHODS

This study was authorized by the Theodor Bilharz Research Institute's Research Ethics Committee (TBRI-REC number PT: 523), and each subject provided written, informed consent before taking part in the investigation.

Study design

The study's participants were accepted into the Gastroenterology and Hepatology Department of the TBRI in Giza, Egypt. Patients with HCV genotype 4 who had been persistent for more than 6 months (HCV RNA Positive) and who had not received any particular HCV treatment in the previous 6 months met the inclusion criteria. Any concomitant cause of chronic liver disease was prohibited, including those with a history of schistosomiasis, chronic viral illnesses other than HCV, dual HBV and HCV infection, nonalcoholic steato-hepatitis (NASH), autoimmune hepatitis, biliary disorders, malignancies other than HCC, regular hepatotoxic drugs, alcohol abuse, diabetes, and HCV-infected patients receiving DAAS or immunomodulatory interferon-β therapy.

Hundred patients were included in this study based on the inclusion and exclusion criteria, and their distribution is shown in Table 1.

Table 1. Different groups included in the study

Healthy control		age- and sex- matched healthy adults (n = 25)	
Patients	**Group I**	**subgroup Ia**	CHC without cirrhosis (n = 25)
	CHC without HCC	**subgroup Ib**	CHC cirrhotic patients (n = 25)
	Group II	CHC with HCC (n = 25)	

Bioinformatics analyses

The following integrated online databases for miRNA target prediction and functional analysis served as the driving force behind the selection of miRNA-122 in the current study. Using a computational knowledge base called miRò http://microrna.osumc.edu, it was possible to establish miRNA-122's candidacy for the connection in this case study. GeneCards (The Human Gene Database), which is accessible at https://www.genecards.org, was used to get additional detailed information about miRNA-122. Using miRNet, a miRNA-centric network visual analytics platform (https://www.mirnet.ca), which predicts miRNA target genes based on support vector machines and open-access high-throughput experimental data, as well as many miRNAs list targeted the TGF- 1 genes, microRNA-122 target genes were identified.

Plasma sample preparation

Peripheral venous blood (7 ml) was collected from different individuals under strict sterile conditions. The blood samples were divided into 3 parts; Part 1: 2.5 ml fixed by EDTA for complete blood picture investigations. Part 2: 2.5 ml fixed by EDTA, centrifuged at 2000 rpm for 10 min, plasma was distributed in micro scaping tubes and stored in aliquots at –80°C until use for viral RNA extraction and HCV genotyping. Part 3: 2 ml blood were permitted to clot at room temperature, then centrifuged for 10 min at 3000 rpm, then serum was collected and stored in aliquots at –80°C until use for specific serological tests.

Laboratory analyses

Liver functions including albumin, total protein bilirubin, alanine aminotrasferase (ALT), aspartate aminotransferase (AST) was tested using the standard protocols. Alpha fetoprotein (AFP) tumor marker was analyzed using the autoanalyzer (Hitachi 736, Hitachim Japan).

Molecular analyses

RNA Extraction: The total viral RNA isolation was done from 200µl plasma using high pure viral RNA isolation kit (cat. No: 11858882001, roche) according to the manufacture procedure. **cDNA synthesis** was done according to Transcriptor First Strand cDNA Synthesis Kit (cat. No: 04379012001, roche) and HCV genotyping was employed using hot start reaction mix detection for PCR using HybProbe probes with the lightcycler carousel-based system (cat. No: 03003248001, roche). **Quantitative Real-time (qRT-PCR)** was performed using HERA SYBR Green RT-qPCR kit (version 08hp421191), easy to use reaction mix for single step qRT-PCR (Cat. No: WF10303001, willowfort, UK). The primer sequences were showed in Table 2.

Data analysis were applied depending on the SYBR green I filter combination (465–510), on lightcycler EvoScriptRNA SYBR green I master, comparative CT methods. B-actin (used as an endogenous control to normalize the amount of total mRNA in each sample) of GSTP1 between different samples.

Gene expression were calculated relative to the control samples (used as the calibrator sample) using the formula 2–ΔΔCT according to the method of Livak & Schmittgen (2001) and were expressed as fold-change https://bitesizebio.com.

Statistical analysis

Microsoft Excel 2016 and statistical package for social science 'IBM SPSS Statistics for Windows, version 26 (IBM Corp., Armonk, N.Y., USA) were used to analyze the data. A p value of 0.05 was used to determine statistical significance for continuous normally distributed data, which were given as mean ± SD with a 95% confidence interval and using frequencies and percentages for categorical variables. Non-normally distributed data were compared using Mann-Whitney tests, and categorical variables were distributed between groups using the Kruskal-Wallis H test in multiple groups, the ANOVA for normally distributed variables, and $\chi 2$ test or Fisher's exact test. Receiver operating characteristic (ROC) curves were used to evaluate

Table 2. Primers of genes included in the study

	Gene	Sequence	Tm	Reference
miRNA122-5p	Forward	5-TAGCAGAGCTGTGGAGTGTG-3	57.0°C	(http://www.mirbase.org)
	Reverse	5-GCCTAGCAGTAGCTATTTAGTGTG-3	57.0°C	
U6 RNA (Used as a specific internal control for miRNA)				[21]
	Forward	5'-CTCGCTTCGGCAGCACA-3'	57.0°C	
	Reverse	5'-AACGCTTCACGAATTTGCGT-3'	57.0°C	
TGF-β1	Forward	5-AAATGAAGGGAGGCGATCAGG-3	57.0°C	(https://www.ncbi.nlm.nih.gov)
	Reverse	5-AATTGGTGCCACATGGCTTG-3	57.0°C	
B-actin (Used as an endogenous control to normalize the amount of total mRNA in each sample)				
	Forward	5-AAATGAAGGGAGGCGATCAGG-3	57.0°C	(http://hgsv.washington.edu)
	Reverse	5-AATTGGTGCCACATGGCTTG-3	57.0°C	

GSTP1's diagnostic performance. As a measure of accuracy, the area under the ROC (AUC) was determined. As a measure of the prognostic performance of particular tests, the area under the ROC (AUC) was computed. The greatest combined sensitivity and specificity was used as the cutoff for a group of the study's diagnosis. To assess the co-expression, Spearman's rank correlation coefficient (r) was used. By stratifying the effects, the statistical interaction was analyzed, and the logistic regression model's major effect variables and their product terms were included.

RESULTS

According to the bioinformatics analyses, TGF-β1 gene was the first target gene of miRNA-122 target gene list after the exclusion, which depending on disease selections and the relation with the hepatic immune cells. In addition, at the same time many miRNAs targeted the TGF-β1 genes, like miRNA-122 and many other miRNAs showed in (Figure 1) https://www.mirnet.ca.

There were 100 individuals included in the present study. The descriptive data and laboratory analysis showed in Table 3 indicated that the healthy adults were mainly females (92%) and their mean age were ~45 years old. On the other hand, the patient group of CHC without cirrhosis their mean age was ~47 years old with 56.0% females and 44.0% males; while CHC cirrhotic patients their mean age was ~61 years old with 52.0% females and 48.0% males. However, patient group of CHC with HCC were ~58 years old with more males (76%) than females (24%). The AFP significantly increased ($P < 0.001$) in CHC cirrhotic patients and CHC with HCC.

The expression levels of both plasma TGF-β1 and plasma MiRNA-122 were examined in CHC cirrhotic patients and CHC patients with HCC compared to healthy controls, as well as when compared to patients of CHC without Cirrhosis. Besides that, actual fold-change of up and downregulated genes among subgroups (Ia), (Ib) and group II are illustrated in (Table 4).

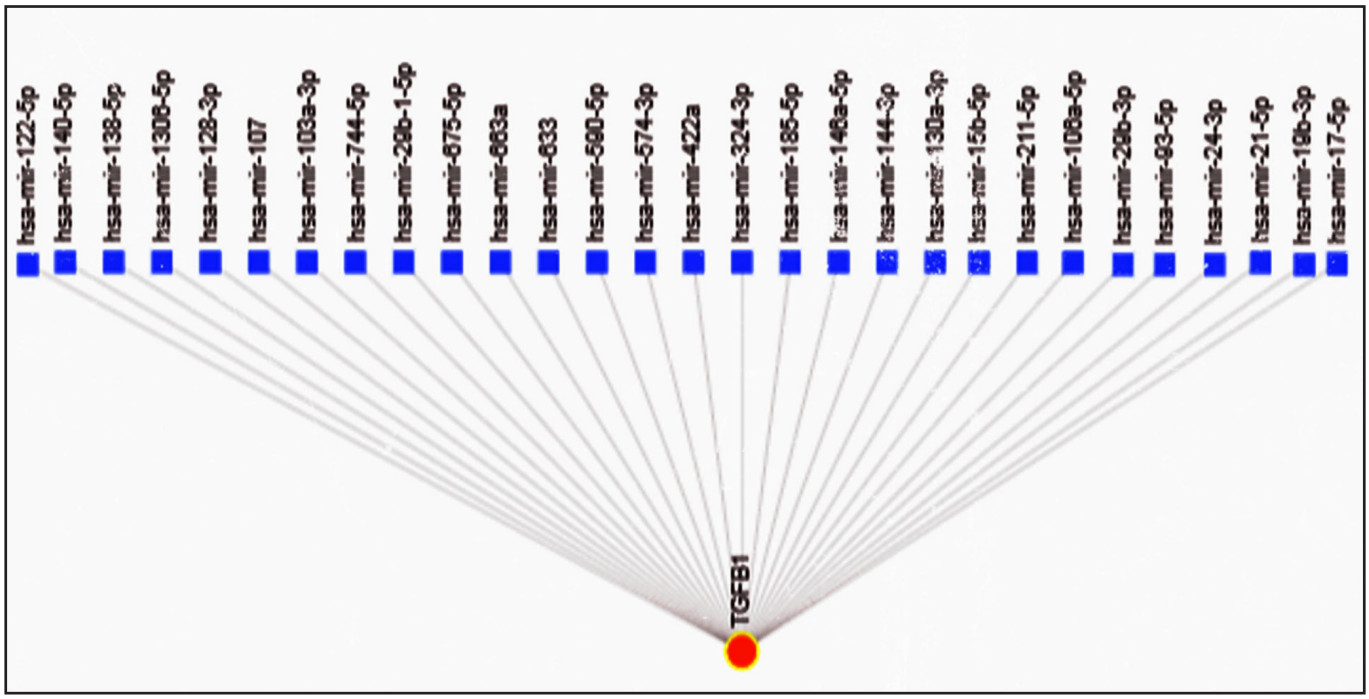

Figure 1. miRNetR: showing possible functional related miRNAs to TGFB1 in HCC cases.

Table 3. Descriptive data and laboratory analysis for all studied groups

			Control N=25	Group I (CHC without HCC) N=50		Group II (CHC with HCC) N=25	P. value
				Subgroup Ia (CHC without Cirrhosis) N=25	Subgroup Ib (CHC with Cirrhosis) (N=25		
	Age		45.3±9.1	47.7±7.1	61.8±10.0	58.2±8.9	<0.001**
Demographic data	**Sex**	**Female**	2(8.0%)	14(56.0%)	13(52.0%)	6(24.0%)	<0.001**
		Male	23(92.0%)	11(44.0%)	12(48.0%)	19(76.0%)	
	HB		13.2±0.8	11.2±4.1	10.2±2.1	11.3±0.9	<0.001**
	WBCs		7.1±1.5	6.7±1.7	7.8±2.3	9.4±3.2	<0.001**
	Platelets X103		268.2±84.7	243.5±69.9	110.2±48.0	155.2±74.1	<0.001**
	PT		12.4(11.35-13.3)	11.2(11.10-12.4)	17.1(15.45-20.4)	15.5(13.0-18.4)	<0.001**
	PC		98.4(77.9-100.0)	99.0 (45.0-100.0)	60.0(44.0-71.55)	74.4(61.6-90.0)	<0.001**
	INR		1.01(1.0-1.16)	1.01 (1.0-1.04)	1.43(1.3-1.82)	1.24(1.1-1.5)	<0.001**
Laboratory Investigation	**UREA**		29.0(25.0-39.5)	38.0(34.0-42.0)	44.0(37.0-53.5)	84.0(45.0-140.5)	<0.001**
	CREAT		0.9(0.65-1.00)	1.0(0.75-1.1)	1.1(1.0-1.32)	1.87(1.01-3.2)	<0.001**
	Albumin		4.2±0.6	4.1±0.6	3.1±1.0	2.8±0.7	<0.001**
	T. Protein		7.3±0.6	6.4±0.9	5.9±1.1	5.3±1.1	<0.001**
	T. Bil		0.7(0.4-0.9)	0.9(0.79-1.35)	1.2(0.75-1.45)	1.9(1.0-3.4)	<0.001**
	D. Bil		0.2(0.1-0.2)	0.4(0.3-0.65)	0.6(0.3-0.8)	1.1(0.2-1.5)	<0.001**
	ALT		27.0(18.0-35.0)	42.0(41.0-56.0)	49.0(22.5-83.0)	60.0(43.5-89.0)	<0.001**
	AST		30.0(21.0-40.5)	38.0(26.0-45.0)	53.0(30.0-65.5)	82.0(36.5-106.5)	<0.001**
	AFP		5.4 (3.7-7.2)	7.8(6.65-9.0)	69.0(25.0-116.0)	310.0(84.8-535.0)	<0.001**
Clinical presentation	**APRI**		0.29 (0.21 - 0.39)	0.36(0.3 - 0.46)	1.09(0.53 - 2.22)	1.12(0.48 - 1.93)	<0.001**
	U.S Finding	**Cirrhosis**	0 (0.0%)	0 (0.0%)	25 (100.0%)	7 (28.0%)	
		Splenomegaly	0 (0.0%)	4 (16.0%)	16 (64.0%)	20 (80.0%)	<0.01*
		Ascites	0 (0.0%)	0 (0.0%)	19 (76.0%)	25 (100.0%)	

Age, Hemoglobin (HB), White Blood Cells (WBCs), Platelets, Albumin, and Total Protein are all given as mean ± SD; the ANOVA Test was used to evaluate the data. The data were analyzed using the Kruskal-Wallis test, with PT, PC, and International Normalized Ratio (INR), Urea, Crat. T. Bilirubin, D. Bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alpha fetoprotein (AFP), and APRI core represented as Median and interquartile range (25%–75%). However, X^2 Test was used to assess the data, and sex and U/S findings are shown as frequency and percent.
The AST to Platelet Ratio Index (APRI) score is derived as follows: [AST Level (IU/L)/AST (Upper Limit of Normal) (IU/L)]/Platelet Count (109/L) X100. (Normal 0.05, CHC 0.5–1.5 without cirrhosis, and Cirrhosis 1.5).
P values 0.05 and 0.001 are considered significant and extremely significant, respectively.

Table 4. Plasma MiRNA-122 and TGF-β1 gene expression in the studied groups

Biomarkers	Control N=25	Group I (CHC without HCC) N=50		Group II (CHC with HCC) N=25
		Subgroup Ia (CHC without Cirrhosis) N=25	Subgroup Ib (CHC with Cirrhosis) N=25	
miRNA-122	0	0.62(0.13- 1.19)	3.46(0.58- 10.05) [a]	10.27(2.32-27.76) [aa, b, **]
TGF-β1	0	45.0(23.15- 78.0)	3.18(0.47- 11.36) [aa]	0.13(0.09-0.18) [aa, bb, **]

The fold change low affects the fold change results: The normalized gene expression (ΔCT) in the test sample divided by the normalized gene expression (ΔCT) in the control sample is known as fold-change (2-ΔΔCT). (Fold-change values below one signifies a down- or negative-regulation.)
The data were analyzed using the Mann-Whitney U test, and all parameters are shown as the median with an interquartile range of (25%–75%) of the fold change of the study groups.
[a] *p* value is significantly different comparing with HCV group.
[b] *p* value differs significantly from the Cirrhotic group.
[*] *p* differs significantly from the CHC group (I).
[1] Initial p values below 0.05 is significant, [2] Initial p values below 0.01 is highly significant.

Expression of plasma miRNA-122 in all studied diseased groups upregulated significantly ($P < 0.01$ and < 0.001) respectively compared to patients suffering from CHC without Cirrhosis. On the other hand, comparing to CHC cirrhotic patients, the expression of miRNA-122 in patients of CHC with HCC showed significantly upregulation ($P < 0.01$). Also, significant upregulation in miRNA-122 expression regarding patients suffering CHC with HCC when compared to patients suffering CHC without HCC as general ($P < 0.001$) (Table 4, Figure 2).

In contrast, regarding TGF-β1 expression, a significant ($P < 0.001$) gradually decreased in all diseased groups when compared to the healthy control group and patients suffering CHC without Cirrhosis. While the expression of TGF-β1 in patients suffering CHC with HCC showed significant ($P < 0.001$) downregulation compared to CHC cirrhotic patients, also significant downregulation in TGF-β1 expression regarding patients suffering CHC with HCC when compared to patients suffering CHC without HCC as general ($P < 0.001$) (Table 4, Figure 3).

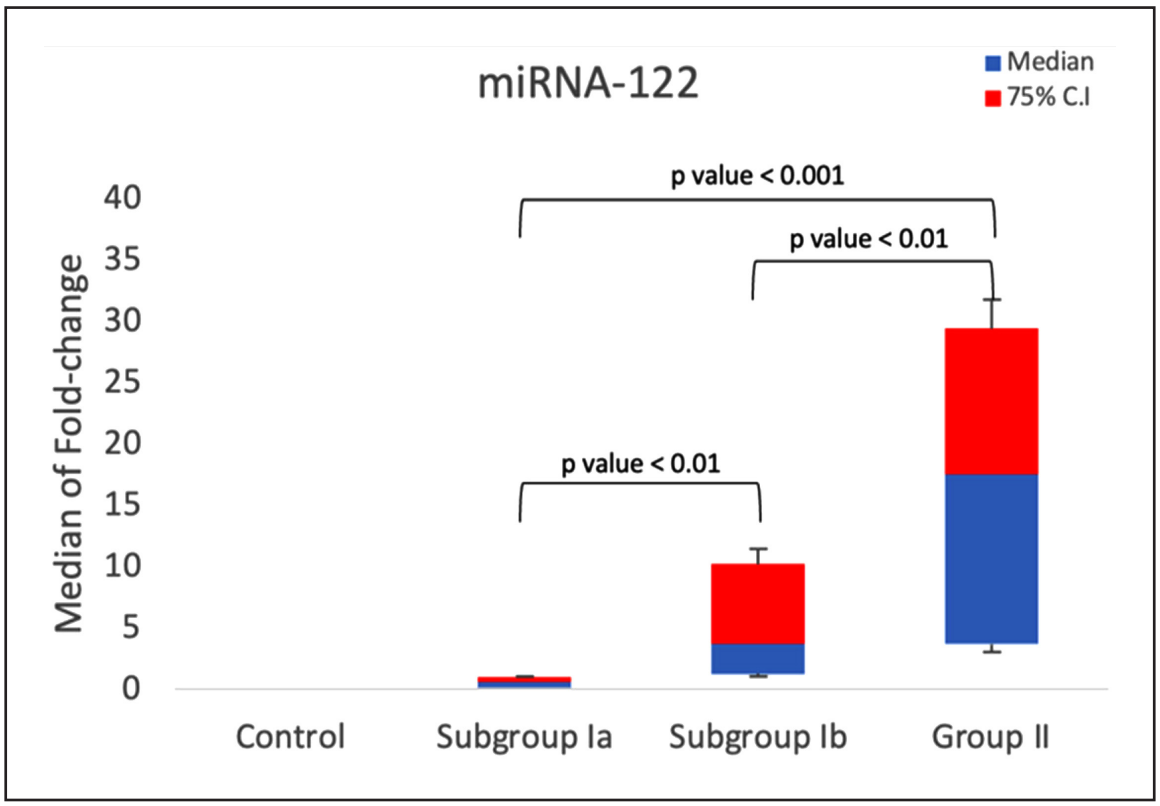

Figure 2. Box plot of miRNA-122 gene expression in the studied groups.

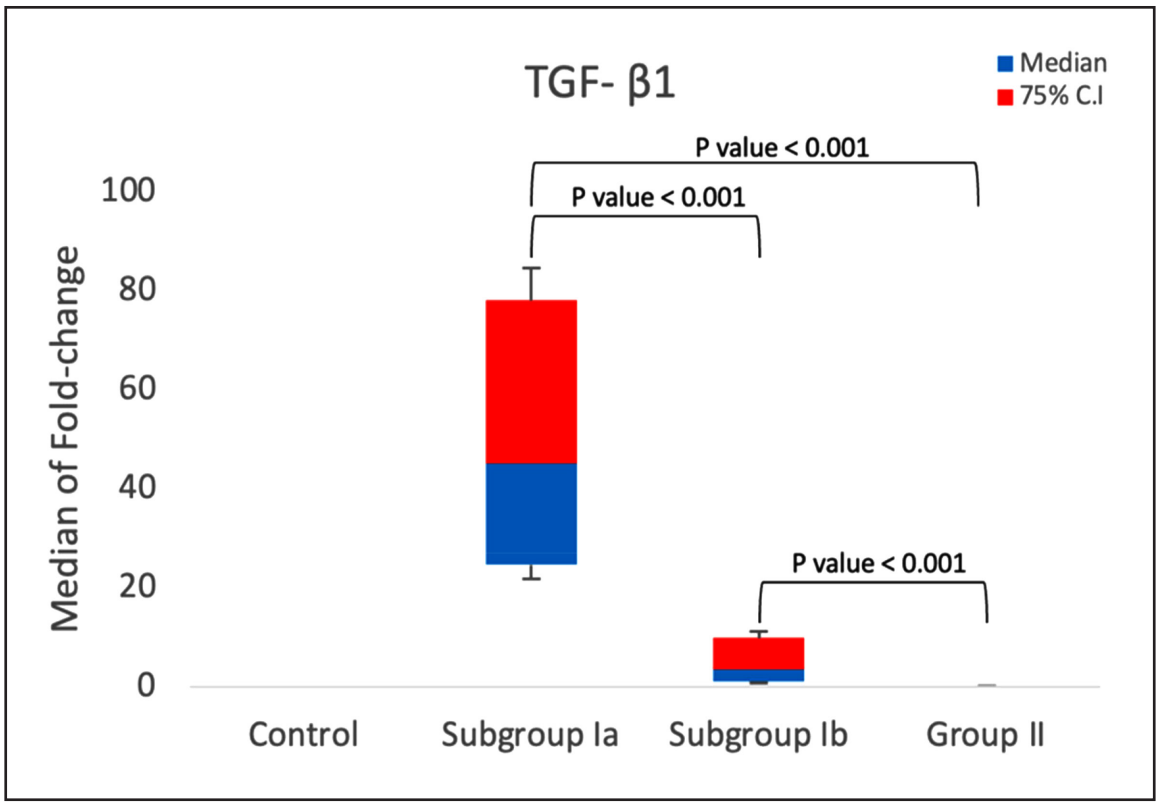

Figure 3. Box plot of TGF-β1 gene expression in the studied groups.

Using a ROC curve, diagnose the expression of several genes as indicators of CHC cirrhotic patients and CHC patients with HCC at various cutoff points. Calculated sensitivity, specificity, and diagnostic accuracy for the parameters under study to distinguish CHC cirrhotic patients from CHC patients without cirrhosis (Table 5).

For discrimination of CHC cirrhotic patients from CHC patients without Cirrhosis, regarding miRNA-122, the AUC= 75.7% with (95% CI 61.0% – 90.4%, *P* = 0.001) and for TGF-β1, the AUC = 96.6% with (95% CI 92.9% – 100.0%, *P* < 0.001) (Figure 4a and 4b).

While in case of CHC patients with HCC versus CHC patients without Cirrhosis, the miRNA-122 showed AUC of 68.2% (95% CI 53.5% – 82.9%, p = 0.02) and for TGF-β1, the AUC was of 83.7% (95% CI 70.4% – 97.0%, p < 0.001) (Figure 4c and 4d).

We demonstrated that AUC for miRNA-122 = 78.8% (95% CI 67.6% – 90.0%, p = 0.001). Additionally, there was a noteworthy outcome, AUC = 91.8% for TGF-β1 (95% CI 84.9% – 98.8%, *P* < 0.001) (Figure 4e and 4f).

With respect to the univariate logistic regression analysis of cirrhosis, there was an increase in 1 degree of miRNA-122 increased the likelihood of having cirrhosis by a factor of 1.775 with (*P* = 0.02). For TGF-β1, a 1 degree rise in its expression level multiplied the likelihood of having cirrhosis by 1.289 (*P* = 0.002).

There was an increase in miRNA-122 at 1 degree, which increased the probabilities of having HCC by a factor of 1.056 (p = 0.04) with respect to the predictive value of TGF-β1 and HCC advancement. With respect to TGF-β1, a 1 degree rise in expression level raised the likelihood of having HCC by a factor of 16.099 (P = 0.001).

Remarkably, the group of CHC patients without HCC showed an increase in 1 degree of miRNA-122, which increased the odds of having HCC by a factor of 1.097 (P = 0.001); additionally, an increase in 1 degree of TGF-β1 expression level increased the odds of having HCC by a factor of 15.291 (P = 0.001) (Table 6).

Through all of the examined groups, correlation analysis found a significant negative association between miRNA-122 and TGF-β1 (r = -0.251 and P = 0.04). (Figure 5).

DISCUSSION

Over the past few years, Egypt made a remarkable progress in the elimination of HCV virus, through the Egyptian program (Abdel-Razek *et al*., 2019); however, cirrhosis and HCC remains prevalent particularly in patients with hepatitis infection. The biomarker for HCC that is currently most frequently utilized in clinics is serum AFP. Nevertheless, AFP is not secreted from all HCC cases (Terentiev & Moldogazieva, 2013). New noninvasive prognostic biomarkers are therefore required for the early diagnosis, mitigation, and therapy of liver cirrhosis and HCC.

Due to their great stability in plasma or serum, miRNAs are potentially useful as non-invasive biomarkers for HCC and are involved in the gene regulation of HCV infection and HCC progression (Ratnasari *et al*., 2022). Various miRNAs were identified as antifibrotic and implicated in innate and acquired immunity in hepatic inflammation, fibrosis, and activated HSC (Gonzalez-Sanchez *et al*., 2021). Several studies indicated that miRNA expression can be regulated by TGF-β signaling (Suzuki, 2018; Tu *et al*., 2019; Gonzalez-Sanchez *et al*., 2021).

Based on the miRNA gene networks analysis, numerous miRNAs targeted the TGF-β1 genes, like miRNA-122 and many other. However, based on HCC and its relationship to hepatic immune cells, the TGF-β1 gene was the top target gene on the list of miRNA-122 target genes. This study inspected the interplay between TGF-β1 and miRNA-122 biomarkers in the development of HCC in CHC patients.

This study found that plasma miRNA-122 gene expression levels were significantly higher in cirrhotic patients than in noncirrhotic patients, as well as in HCC patients than in both chronic HCV without cirrhosis and cirrhotic patients. This upregulation in miRNA-122 may be due to hepatocyte injury, primary source of miR-122, due to HCV infection led to higher release of miR-122 into the circulation increasing the plasma levels. Although the increased plasma levels of the liver-specific enzymes ALT and AST suggest that the liver is the main organ to experience acetaminophen-induced tissue damage, our investigation did not explicitly characterize the mechanisms connecting higher miR-122 blood levels to hepatocyte injury. In a previous investigation, Qi *et al*. (2011) revealed a marginally significant difference of raised blood miR-122 in HCC compared to without HCC, and the substantial diagnostic efficacy of serum miR-122 was proposed. A number of studies have also shown that HCC patients express higher miRNA-122 than healthy individuals (Ali *et al*., 2017; Dai *et al*., 2019). Additionally, Demerdash *et al*. (2017) reported that plasma miR-122 expression levels were significantly higher in CHC and HCC cases compared to healthy individuals. Contradictory results, however, claimed that miRNA-122 expression levels were down-regulated in HCC tissues and cell lines compared to normal tissues and cell lines, indicating that the miRNA-122 gene may act as a cancer suppressor (El-Ahwany *et al*., 2019; Yang *et al*., 2020).

TGF-β1 possesses de novo activity in human cancer cells, and it has recently been demonstrated that the signaling pathway plays a dual function in the development of hepatocarcinogenesis. Depending on the cellular context, it has been recognized for its function as both a tumor promoter and a tumor suppressor (Tu *et al*., 2019). TGF-β1 was substantially expressed in HSCs and fibroblasts, and act as primary sources of fibrogenic cells in liver tissues (de Brito *et al*., 2020).

Table 5. Diagnostic performances of miR-122 and TGF-β1 in discrimination the disease progression

		Cutoff	Sn.	Sp.	PPV	NPV	Accuracy	AUC	95% C.I		p-value
									Lower	Upper	
CHC without Cirrhosis Vs CHC with Cirrhosis	miRNA-122	2.04	60.0%	92.0%	88.2%	69.7%	76.0%	75.7%	61.0%	90.4%	0.001**
	TGF-β1	18.50	84.0%	96.0%	95.5%	85.7%	90.0%	96.6%	92.9%	100.0%	0.001**
CHC with Cirrhosis Vs CHC with HCC	miRNA-122	24.08	32.0%	100.0%	100.0%	59.5%	66.0%	68.2%	53.5%	82.9%	0.02*
	TGF-β1	0.93	100.0%	76.0%	80.6%	100.0%	88.0%	83.7%	70.4%	97.0%	0.001**
CHC without HCC Vs CHC with HCC	miRNA-122	2.55	76.0%	72.0%	57.6%	85.7%	73.3%	78.8%	67.6%	90.0%	0.001**
	TGF-β1	0.93	100.0%	88.0%	80.6%	100.0%	92.0%	91.8%	84.9%	98.8%	0.001**

Sn: Sensitivity, Sp: Specificity, PPV: Positive predictive value, NPV: Negative predictive value, AUC Area under curve and C.I: 95% Confidence Interval.
Significant results are indicated by p values * <0.05 is significant, ** <0.01 is highly significant.

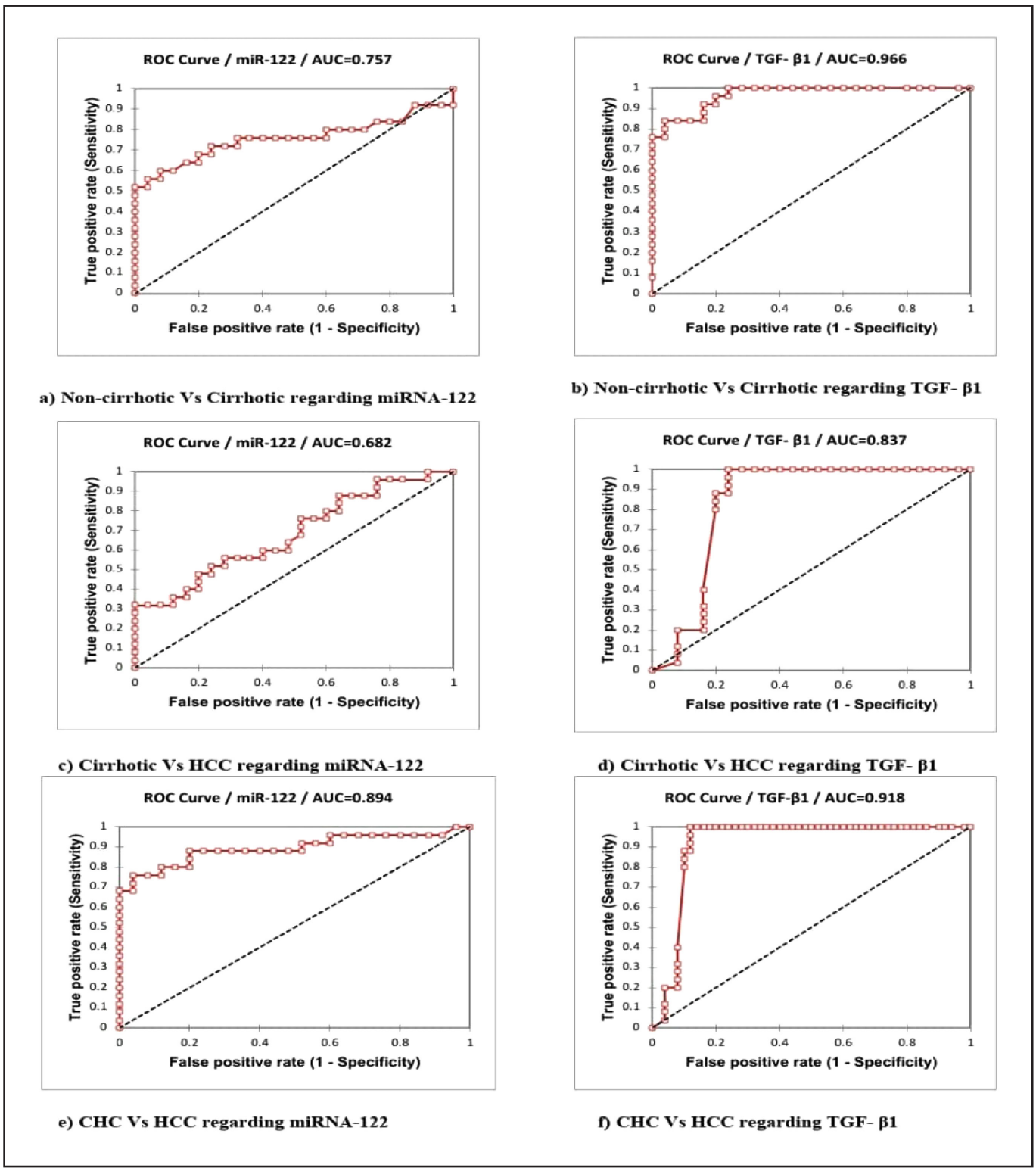

Figure 4. ROC Curve for the miRNA-122 and TGF-β1 in the studied groups.

Table 6. Univariate analysis in the studied groups

	Biomarker	OR	95% C.I	p value
CHC without Cirrhosis Vs CHC with Cirrhosis	miRNA-122	1.775	1.081 - 2.914	0.02*
	TGF-β1	1.289	1.094 - 1.518	0.002**
CHC with Cirrhosis Vs CHC with HCC	miRNA-122	1.056	1.002 - 1.112	0.04*
	TGF-β1	16.099	1.447 - 179.102	0.001**
CHC without HCC Vs CHC with HCC	miRNA-122	1.097	1.036 - 1.161	0.001**
	TGF-β1	15.291	1.546 - 156.151	0.001**

OR; Odd Ratio, C.I; Confidence Interval, *p* value calculated depend on logistic regression analysis.
* *p* value <0.05 is significant, ** *p* value <0.01 is highly significant.

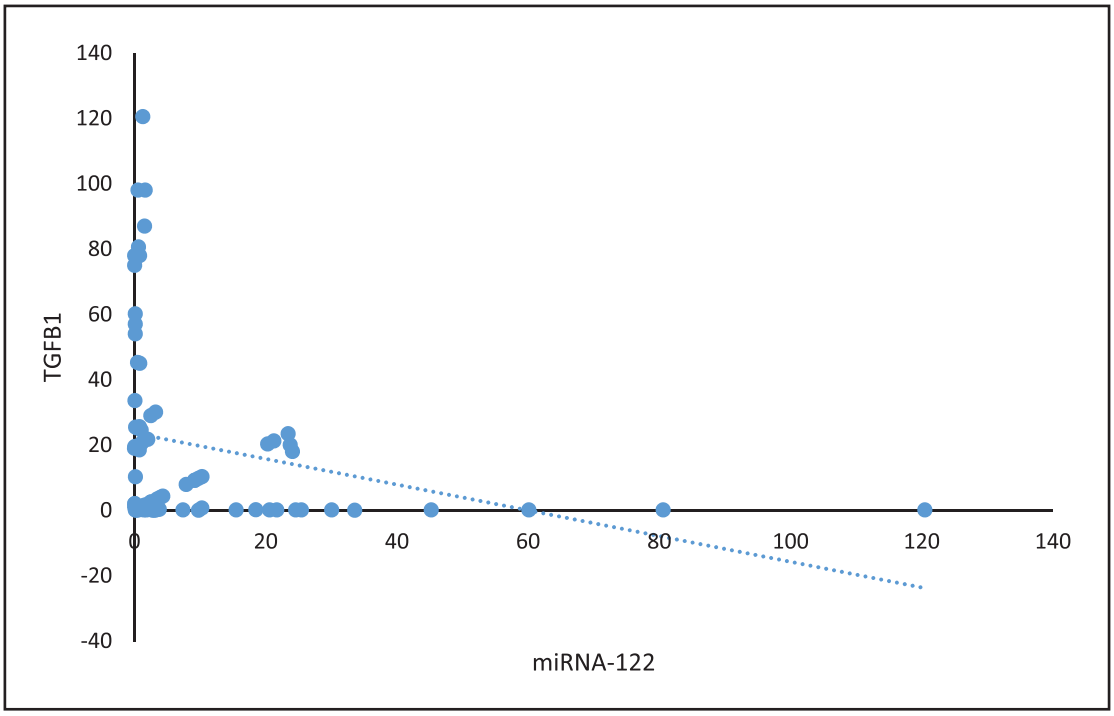

Figure 5. Correlation between TGF-β1 and miRNA-122 in the studied group.

According to this study, TGF-β1 expression was lower in CHC cirrhotic patients than it was in CHC patients without cirrhosis. When compared to CHC patients without cirrhosis and CHC patients without HCC, there was a substantial downregulation in CHC patients with HCC. Our results confirm the findings of Farid *et al*. (2014) that liver cancer patients exhibited lower TGF-β1 gene expression.

Using a ROC curve, the miRNA-122 gene expression as a biomarker in cirrhotic patients demonstrated diagnostic accuracy of 90.0% at various cutoff points. Group II had an accuracy rate of 83.7%. Although there is 91.8% accuracy when comparing CHC patients as a whole to CHC patients with HCC. The accuracy for the TGF-β1 gene in patients with cirrhosis was 76.0%. The accuracy for patients with HCC was 66.0%. While the accuracy rate for HCC patients compared to CHC patients was 73.3%.

As a biomarker for illness progression, the prognostic values derived from the ROC curve were stratified, and it was discovered that miRNA-122 gene expression increased with disease progression in a unique manner. On the other hand, TGF-β1 gene expression gradually decreased as the condition developed. This falls under the categories of HCC and CHC with cirrhosis.

Recent research has shown the possibility of using tumor-specific miRNAs as prognostic or diagnostic indicators for cancer in bodily fluids (Lan *et al*., 2015). This result is in line with the findings of Franck and colleagues (Franck *et al*., 2020), who suggested that miRNA-122 may have potential as a biomarker of liver damage and likely prognosis.

To identify miRNA-122 and TGF-β1 as a predictor and/or prognostic parameter for cirrhosis progression, logistic regression analysis was used. Regression analysis revealed that the expression levels of TGF-β1 and miRNA-122 were significant predictors of changes in CHC cirrhotic patients and HCC patients compared to non-cirrhotic CHC patients. Evidently, when chosen as relevant factors linked to the likelihood of diagnosing HCC vs cirrhosis patients, the expression levels of miRNA-122 and TGF-β1 raised the probability of having cirrhosis.

This mechanistic study's findings, which included an inverse correlation between the expressions of miRNA-122 and TGF-β1, suggest that miRNA-122 upregulation causes TGF-β1 to be downregulated, which in turn causes TGF-β1 to be switched off at both the mRNA and protein levels in the patients under study. We may underline the miRNA-122 targets the regulatory region (3-UTR) of TGF-β1 mentioned earlier. The link described above is mainly consistent with the research conducted by Chen and colleagues (Chen *et al*., 2018a).

CONCLUSION

These findings suggested that miRNA-122 and TGF-β1 might be utilized to distinguish between CHC patients with HCC and CHC patients without HCC as a diagnostic parameter with a substantial result. Additionally, the findings pointed to a connection between miRNA-122 and TGF-β1 in the regulation of gene expression, which strongly encourages us to employ these two variables as noninvasive prognostic biomarkers and new therapeutic targets for HCV-induced liver cirrhosis and HCC in HCV Genotype 4-infected patients. However, more extensive studies are required to confirm their veracity.

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Conflicts of interest

The Authors declares that there is no conflict of interest.

REFERENCES

Abdel-Razek, W., Hassany, M., El-Sayed, M.H., El-Serafy, M., Doss, W., Esmat, G. & Waked, I. (2019). Hepatitis C virus in Egypt: Interim report from the world's largest national program. *Clinical Liver Disease* **14**: 203-206. https://doi.org/10.1002/cld.868

Ali, H.E.A., Hameed, R.A., Effat, H., Ahmed, E.K., Atef, A.A., Sharawi, S.K., Ali, M., Abd Elmageed, Z.Y. & Wahab, A.H.A. (2017). Circulating microRNAs panel as a diagnostic tool for discrimination of HCV-associated hepatocellular carcinoma. *Clinics and Research in Hepatology and Gastroenterology* **41**: e51-e62. https://doi.org/10.1016/j.clinre.2017.06.004

Bandiera, S., Pfeffer, S., Baumert, T.F. & Zeisel, M.B. (2015). miR-122 – a key factor and therapeutic target in liver disease. *Journal of Hepatology* **62**: 448-457. https://doi.org/10.1016/j.jhep.2014.10.004

Chen, E., Xu, X., Liu, R. & Liu, T. (2018a). Small but heavy role: microRNAs in hepatocellular carcinoma progression. *BioMed Research International* **2018**: 6784607. https://doi.org/10.1155/2018/6784607

Chen, J., Zaidi, S., Rao, S., Chen, J.S., Phan, L., Farci, P., Su, X., Shetty, K., White, J., Zamboni, F. *et al.* (2018b). Analysis of genomes and transcriptomes of hepatocellular carcinomas identifies mutations and gene expression changes in the transforming growth factor-β pathway. *Gastroenterology* **154**: 195-210. https://doi.org/10.1053/j.gastro.2017.09.007

Chen, J., Gingold, J.A. & Su, X. (2019). Immunomodulatory TGF-β signaling in hepatocellular carcinoma. *Trends in Molecular Medicine* **25**: 1010-1023. https://doi.org/10.1016/j.molmed.2019.06.007

Dai, M., Li, L. & Qin, X. (2019). Clinical value of miRNA-122 in the diagnosis and prognosis of various types of cancer. *Oncology Letters* **17**: 3919-3929. https://doi.org/10.3892/ol.2019.10024

Demerdash, H.M., Hussien, H.M., Hassouna, E. & Arida, E.A. (2017). Detection of microRNA in hepatic cirrhosis and hepatocellular carcinoma in hepatitis C genotype-4 in Egyptian patients. *BioMed Research International* **2017**: 1806069. https://doi.org/10.1155/2017/1806069

de Brito, W.B., Queiroz, M.A.F., Amoras, E.D.S.G., Lima, S.S., da Silva Conde, S.R.S., Dos Santos, E.J.M., Cayres-Vallinoto, I.M.V., Ishak, R. & Vallinoto, A.C.R. (2020). The *TGFB1*-509C/T polymorphism and elevated TGF-β1 levels are associated with chronic hepatitis C and cirrhosis. *Immunobiology* **225**: 152002. https://doi.org/10.1016/j.imbio.2020.152002

Dewidar, B., Soukupova, J., Fabregat, I. & Dooley, S. (2015). TGF-β in hepatic stellate cell activation and liver fibrogenesis: updated. *Current Pathobiology Reports* **3**: 291-305. http://doi.org/10.1007/s40139-015-0089-8

Dituri, F., Mancarella, S., Cigliano, A. & Giannelli, G. (2019). TGF-β as multifaceted orchestrator in HCC progression: signaling, EMT, immune microenvironment, and novel therapeutic perspectives. *Seminars in Liver Disease* **39**: 053-069. https://doi.org/10.1055/s-0038-1676121

El-Ahwany, E., Mourad, L., Zoheiry, M.M.K., Abu-Taleb, H., Hassan, M., Atta, R., Hassanien, M. & Zada, S. (2019). MicroRNA-122a as a non-invasive biomarker for HCV genotype 4-related hepatocellular carcinoma in Egyptian patients. *Archives of Medical Science* **15**: 1454-1461. https://doi.org/10.5114/aoms.2019.86621

El-Araby, R.E., Khalifa, M.A., Zoheiry, M.M., Zahran, M.Y., Rady, M.I., Ibrahim, R.A., El-Talkawy, M.D. & Essawy, F.M. (2020). The interaction between microRNA-152 and DNA methyltransferase-1 as an epigenetic prognostic biomarker in HCV-induced liver cirrhosis and HCC patients. *Cancer Gene Therapy* **27**: 486-497. https://doi.org/10.1038/s41417-019-0123-9

Farid, I.M., Hamza, I.M., El-Abd, D.M., Mohyi, A.M., AbdulLatif, M.M.A., Aref, A.T. & Hamza, D.M. (2014). Transforming growth factor-β1 gene expression in hepatocellular carcinoma: A preliminary report. *Arab Journal of Gastroenterology* **15**: 142-147. https://doi.org/10.1016/j.ajg.2014.10.007

Franck, M., Sch tte, K., Malfertheiner, P. & Link, A. (2020). Prognostic value of serum microRNA-122 in hepatocellular carcinoma is dependent on coexisting clinical and laboratory factors. *World Journal of Gastroenterology* **26**: 86-96. https://doi.org/10.3748/wjg.v26.i1.86

Gonzalez-Sanchez, E., Vaquero, J., Férnandez-Barrena, M.G., Lasarte, J.J., Avila, M.A., Sarobe, P., Reig, M., Calvo, M. & Fabregat, I. (2021). The TGF-β pathway: a pharmacological target in hepatocellular carcinoma? *Cancers* **13**: 3248. https://doi.org/10.3390/cancers13133248

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. & Forman, D. (2011). Global cancer statistics. *CA: A Cancer Journal for Clinicians* **61**: 69-90. https://doi.org/10.3322/caac.20107

Lambrecht, J., Mannaerts, I. & van Grunsven, L.A. (2015). The role of miRNAs in stress-responsive hepatic stellate cells during liver fibrosis. *Frontiers in Physiology* **6**: 209. https://doi.org/10.3389/fphys.2015.00209

Lan, H., Lu, H., Wang, X. & Jin, H. (2015). MicroRNAs as potential biomarkers in cancer: opportunities and challenges. *BioMed Research International* **2015**: 125094. https://doi.org/10.1155/2015/125094

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **25**: 402-408. https://doi.org/10.1006/meth.2001.1262

Lohitesh, K., Chowdhury, R. & Mukherjee, S. (2018). Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: an insight. *Cancer Cell International* **18**: 44. https://doi.org/10.1186/s12935-018-0538-7

Mazzanti, R., Arena, U. & Tassi, R. (2016). Hepatocellular carcinoma: Where are we? *World Journal of Experimental Medicine* **6**: 21-36. https://doi.org/10.5493/wjem.v6.i1.21

Morikawa, M., Derynck, R. & Miyazono, K. (2016). TGF-β and the TGF-β family: context-dependent roles in cell and tissue physiology. *Cold Spring Harbor Perspectives in Biology* **8**: a021873. https://doi.org/10.1101/cshperspect.a021873

Ors-Kumoglu, G., Gulce-Iz, S. & Biray-Avci, C. (2019). Therapeutic microRNAs in human cancer. *Cytotechnology* **71**: 411-425. https://doi.org/10.1007/s10616-018-0291-8

Parizadeh, S.M., Jafarzadeh-Esfehani, R., Ghandehari, M., Goldani, F., Parizadeh, S.M., Hassanian, S.M., Ghayour-Mobarhan, M., Ferns, G.A. & Avan, A. (2019). MicroRNAs as potential diagnostic and prognostic biomarkers in hepatocellular carcinoma. *Current Drug Targets* **20**: 1129-1140. https://doi.org/10.2174/1389450120666190307095720

Qi, P., Cheng, S.Q., Wang, H., Li, N., Chen, Y.F. & Gao, C.F. (2011). Serum microRNAs as biomarkers for hepatocellular carcinoma in Chinese patients with chronic hepatitis B virus infection. *PLOS ONE* **6**: e28486. https://doi.org/10.1371/journal.pone.0028486

Ratnasari, N., Lestari, P., Renovaldi, D., Raditya Ningsih, J., Qoriansas, N., Wardana, T., Hakim, S., Gumilas, N.S.A., Indrarti, F., Triwikatmani, C. *et al.* (2022). Potential plasma biomarkers: miRNA-29c, miRNA-21, and miRNA-155 in clinical progression of Hepatocellular Carcinoma patients. *PLOS ONE* **17**: e0263298. https://doi.org/10.1371/journal.pone.0263298

Schanzer, D.L., Paquette, D. & Lix, L.M. (2014). Historical trends and projected hospital admissions for chronic hepatitis C infection in Canada: a birth cohort analysis. *Canadian Medical Association Journal Open* **2**: E139-E144. https://doi.org/10.9778/cmajo.20130087

Siegel, R.L., Miller, K.D. & Jemal, A. (2019). Cancer statistics, 2019. *CA: A Cancer Journal for Clinicians* **69**: 7-34. https://doi.org/10.3322/caac.21551

Suzuki, H.I. (2018). MicroRNA control of TGF-β signaling. *International Journal of Molecular Sciences* **19**: 1901. https://doi.org/10.3390/ijms19071901

Terentiev, A.A. & Moldogazieva, N.T. (2013). Alpha-fetoprotein: a renaissance. *Tumor Biology* **34**: 2075-2091. https://doi.org/10.1007/s13277-013-0904-y

Tu, S., Huang, W., Huang, C., Luo, Z. & Yan, X. (2019). Contextual regulation of TGF-β signaling in liver cancer. *Cells* **8**: 1235. https://doi.org/10.3390/cells8101235

World Health Organization (WHO). (2019). Hepatitis C. Geneva: World Health Organization.

Xu, M.Y., Hu, J.J., Shen, J., Wang, M.L., Zhang, Q.Q., Qu, Y. & Lu, L.G. (2014). Stat3 signaling activation crosslinking of TGF-β1 in hepatic stellate cell exacerbates liver injury and fibrosis. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1842**: 2237-2245. https://doi.org/10.1016/j.bbadis.2014.07.025

Xu, Y., Huang, J., Ma, L., Shan, J., Shen, J., Yang, Z., Liu, L., Luo, Y., Yao, C. & Qian, C. (2016). MicroRNA-122 confers sorafenib resistance to hepatocellular carcinoma cells by targeting IGF-1R to regulate RAS/RAF/ERK signaling pathways. *Cancer Letters* **371**: 171-181. https://doi.org/10.1016/j.canlet.2015.11.034

Yang, G., Zhang, M., Zhao, Y., Pan, Y., Kan, M., Li, J., He, K. & Zhang, X. (2020). HNF-4α inhibits hepatocellular carcinoma cell proliferation through mir-122-adam17 pathway. *PLOS ONE* **15**: e0230450. https://doi.org/10.1371/journal.pone.0230450