MicroRNA 31 in activating granulocytes through regulation of fork head box protein P3 gene in hepatitis C virus related liver disease

Noha A. Amin[a], Rady E. El-Araby[b], Ayat S.M. Hassan[c], Khaled M. Ragab[d], Manal Y. Zahran[a], Ola B. Aboelnil[a]

Departments of [a]Haematolog, [c]Electron Microscopy, [d]HepatoGastroenterology, Theodor Bilharz Research Institute, Giza, Egypt, [b]Department of Basic and Clinical Translation Sciences, Tufts University, School of Dental Medicine, Boston, United States of America

Correspondence to Ola B. Aboelnil, MD, Department of Haematology, Theodor Bilharz Research Institute, Imbaba, Giza 12411, Egypt. Mob NO: +20 1005071442; e-mail: olabadr81@gmail.com

Received: 23 September 2024
Revised: 23 October 2024
Accepted: 27 October 2024
Published: 27 June 2025

Journal of The Arab Society for Medical Research 2025, 20:17–25

Background/aim
The role of microRNA 31 (miRNA-31) role and fork head box protein P3 (FOXP3) protein in pathogenesis of chronic liver diseases as well as hepatocellular carcinoma (HCC) is complex. However, the expression levels or functions alterations of miRNA-31and FOXP3 could affect hepatitis C virus (HCV) infection outcome. FOXP3 and miRNA-31 have emerged as pivotal molecules influencing immune tolerance, fibrosis, and tumor progression in this context. Moreover, miRNA-31 may also regulate the immunological response of the granulocytes, primarily neutrophils. The present work aims to evaluate the possible effect of miRNA-31 in activating granulocytes through the regulation of the FOXP3 gene in HCV related liver disease.

Patients and method
This case–control study included 60 patients with persistent HCV infection divided into three groups (20 each) as follows: HCV infection patients' group, HCV infection patients with cirrhosis group, and HCV infections patients with HCC group. In addition to 20 healthy patients served as a control group. Bioinformatics were used to select FOXP3 gene and its relations. miRNA-31 and FOXP3 gene expression were quantified using real-time PCR. Activated granulocyte percentage expressing CD16 was done by flow cytometry.

Results
After Bioinformatics selection of FOXP3 and its relation to miRNA-31, the present results disclosed that miRNA-31 was significantly upregulated ($P<0.05$) in both HCV with liver cirrhosis and without liver cirrhosis and in HCC groups in comparison with the control group. The expression of the FOXP3 gene was significantly downregulated ($P<0.05$) in both HCV with liver cirrhosis and without liver cirrhosis, whereas it was significantly upregulated ($P<0.05$) in the HCC group in comparison with the control group. Moreover, FOXP3 expression was significantly upregulated ($P<0.05$) in the HCV with cirrhosis and HCC groups when compared with HCV group without cirrhosis. In addition, there was a significant increase ($P<0.05$) in granulocyte CD16+ percentage in the HCV with cirrhosis and HCC groups, in comparing with HCV without cirrhosis group.

Conclusion
This work concluded that the miRNA-31 has a role in activating granulocytes (CD16+) through the regulation of FOXP3 gene in HCV with or without liver cirrhosis. Moreover, miRNA-31 and FOXP3 gene could be used as new biomarkers in HCV-induced liver cirrhosis and HCC.

Keywords:
activated granulocyte, fork head box protein P3, hepatitis C virus, microRNA 31

J Arab Soc Med Res 20:17–25

1687-4293

Introduction

Hepatitis C virus (HCV) is a positive-strand RNA virus. The overall prevalence of HCV is around 1.8% of the general population infecting more than 170 million patients globally. The highest prevalence of HCV in the African continent was 7.1% [1]. Chronic HCV infection induces the recurrent division of typically dormant hepatocytes, resulting in liver fibrosis, cirrhosis, and frequently advancing to hepatocellular carcinoma (HCC). The unrestraint accumulation of the extracellular matrix defines fibrosis [2]. The in-situ identification of HCV RNA in hepatocytes indicates that the liver damage is, at least partially, a consequence of HCV replication in the hepatocytes [3].

MicroRNAs (miRNAs) are conserved, noncoding short regulatory RNAs that facilitate translational repression and/or promote the degradation of their target mRNAs in animal cells, thereby regulating numerous biological processes [4]. miRNAs transcription creates a hairpin like secondary structure, which is subsequently processed sequentially by endo ribonucleases yield short double-stranded RNAs [5]. Single miRNA can target many genes, while one single gene can be controlled by various miRNAs. Chronic HCV infection induces the repetitive division of usually quiescent hepatocytes, resulting in fibrosis, cirrhosis, and occasionally advancing to HCC. Fibrosis is defined by the excessive accumulation of extracellular matrix [2]. The in-situ identification of HCV RNA in human hepatocytes indicates that the liver damage is attributed partially to HCV replication in hepatocytes [3].

miRNA-31 is recognized to play significant contribution within various cellular processes, which includes inflammation, apoptosis, as well as cell proliferation. In the context of HCV infection, miRNA-31 is dysregulated. Studies have shown that miRNA-31 can influence the expression of genes entangled in the response of the immune system, contributing to liver pathogenesis disease caused by HCV [6–8]. In HCV-related chronic liver disease, miRNA-31 is not solely participate in fibrosis and tumorigenesis but additionally plays an important role in activating granulocytes, particularly neutrophils. The persistent activation of granulocytes through miRNA-31 signaling contributes to liver inflammation, fibrosis, and the progression to HCC [9].

miRNA-31 is known to regulate immune cells by influencing the pro-inflammatory cytokines expression encompassing Tumor Necrosis Factor- α (TNF-α), Interleukin 6 (IL-6), and Interleukin 1β (IL-1β). The aforementioned cytokines are critical for granulocyte activation and recruitment. In chronic HCV infection, miRNA-31 is upregulated, which enhances the production of these inflammatory mediators. Granulocytes, particularly neutrophils, are then activated and recruited to the liver, amplifying the inflammatory response. This process contributes to ongoing hepatocyte damage and the establishment of a chronic inflammatory microenvironment [10].

The overall impact of miRNA-31 on T-cell (Treg) development and frequency is contingent upon the interplay between its inhibitory and enhancing roles. Further investigation is necessary to identify the factors that determine this. Overall impact of miRNA-31 on Treg development and frequency is contingent upon the interplay between its inhibitory and enhancing roles [11].

In this study, we sought to assess the potential impact of miRNA-31 on the activation of granulocytes via the regulation of the fork head box protein P3 (FOXP3) gene in liver disease in HCV context to open new therapeutic pathways for HCV-related disease and decline the disease progression.

Patients and methods

Patients

This study is a case–control study enrolled 20 healthy patients, in addition to 60 patients recruited from Department of Hepatogastroenterology in the Research Institute of Theodor Bilharz, Giza, Egypt. The patient's inclusion criteria comprised patients from both sexes greater than 15 years and less than 65 years, with chronic liver disease who were infected with HCV (genotype 4), with or without cirrhosis and/or had HCC. Other causes of chronic liver diseases are considered exclusion criteria. Exclusion criteria encompassed other causes of chronic liver diseases, such as patients with a history of schistosomiasis, chronic viral diseases other than HCV, nonalcoholic steatohepatitis, autoimmune hepatitis, biliary disorders, regular hepatotoxic drugs, and alcohol abuse.

Study design

The present study encompassed 80 patients classified into four groups as follows:

Group 1: Including 20 healthy participants as a control group.

Group 2: Including 20 HCV infection patients without cirrhosis.

Group 3: Including 20 HCV infection patients with liver cirrhosis.

Group 4: Including 20 HCV infection patients with HCC patients.

A thorough medical history, physical examinations, radiographic assessments, and laboratory evaluations, encompassing a complete blood count and liver function tests, alongside serological analysis and HCV genotyping utilizing HybProbe probes and the light cycler carousel-based system, were conducted for accurate diagnosis.

Ethical consideration

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the Research Ethics Committee (REC) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt, with approval number FWA00010609. Written informed consent was provided by each participant before their inclusion in the study.

Samples collection

Five milliliters of blood were aseptically collected through venipuncture into two sterile EDTA-containing vacutainer tubes (Vacutainer; BD Biosciences) (2×2 ml), with one tube designated for flow cytometry analysis of CD16. The second EDTA tube was promptly utilized for RNA extraction for subsequent RT-PCR analysis. One milliliter was collected in plain tubes (Vacutainer; BD Biosciences) for liver function tests and the detection of serum anti-HCV antibodies.

Bioinformatic methods

FOXP3 gene was selected according to the gene cards database (https://www.genecards.org), which is a transcriptional regulator that plays a crucial role in regulating the maturation and suppressive role of regulatory Treg. The molecular interactions between the FOXP3 and the other gene were examined utilizing GeneMANIA, a multifaceted association network integration technique employed to forecast potential functional connections among them. (http://www.genemania.org). FOXP3 target miRNAs, specifically miRNA-31-5p, were anticipated using miRNet version 2.0, a miRNA-focused network visualization analytics tool (https://www.mirnet.ca). Furthermore, it forecasts FOXP3 target genes employing thoroughly annotated databases: miRTarBase v8.0, TarBase v8.0, and miRecords (Tartu, Estonia). Conserved region between FOXP3 and hsa-miRNA-31-5p were predicted using TargetScan V.8 database, which acts as a prediction tool for the miRNAs-targets (https://www.targetscan.org).

RT-PCR for FOX P3 gene expression and miRNA-31

Gene expression of the target was analyzed through the subsequent procedures: the total amount of RNA was extracted utilizing the High Pure RNA Isolation Kit from Roche (version 12, 2011), catalog number: 11828665001. In the reverse transcriptase (RT) step (BioRad-T 100, Singapore), 1 µg RNA with the FIREScript RT cDNA synthesis KIT from SOLIS BIODYNE, catalog number 06-15-00050 and specific primers (Qiagen, Hilden, Germany) for PCR amplification step. Gene expression was assessed utilizing HOT FIREPol EvaGreen qPCR Mix Plus (Rox), SOLIS BIODYNE, catalog number: (08-24-00001) was used. The results were analyzed using the SYBR Green I filter combination (465–510), integral to the LightCycler EvoScript RNA SYBR Green I Master. Comparative computed tomography methods were utilized for data analysis. Beta-actin served as an endogenous control for normalizing total mRNA levels of FOXP3 across samples, whereas U6 was utilized for miRNA-31-5p normalization. Gene expressions were quantified in proportion to control samples, designated as the calibrator, utilizing the formula $2^{-\Delta\Delta CT}$, and expressed as fold-change.

Flow cytometric analysis of peripheral blood granulocytes

The BD Accuri C6 flow cytometer, USA, was employed to determine the percentage of circulating CD16+ granulocytes. Five microliters of human CD16-FITC monoclonal antibody (BioLegend, cat. No. 335035) were added to 100 microliters of whole blood. The mixture was subsequently incubated in darkness for 20 min. Following the acquisition of 10 000 events, logarithmic amplification was employed for data collection. Granulocyte gating was performed based on their forward and side scatter characteristics.

Statistical analysis

Data were analyzed using Microsoft Excel 2016 and the statistical software IBM SPSS Statistics for Windows, version 28 (IBM Corp., Armonk, New York, USA). The normally distributed quantitative data were presented as mean ± SD, while those not normally distributed were expressed as median and interquartile range. One-way ANOVA test and Kruskal–Wallis test were used to compare normally distributed and not normally distributed quantitative data, respectively. A P value less than 0.05 was considered statistically significant and that of less than 0.01 as being statistically highly significant. The evaluation of the diagnostic performance of the studied genes was conducted using receiver operating characteristic analysis (ROC) curves. Accuracy index for prognostic performance of selected tests was calculated as the area under the ROC (AUC) curve. The study cohort diagnostic cut-off was determined at the point of optimal combination sensitivity (SN) and specificity (SP).

Results

Bioinformatics results

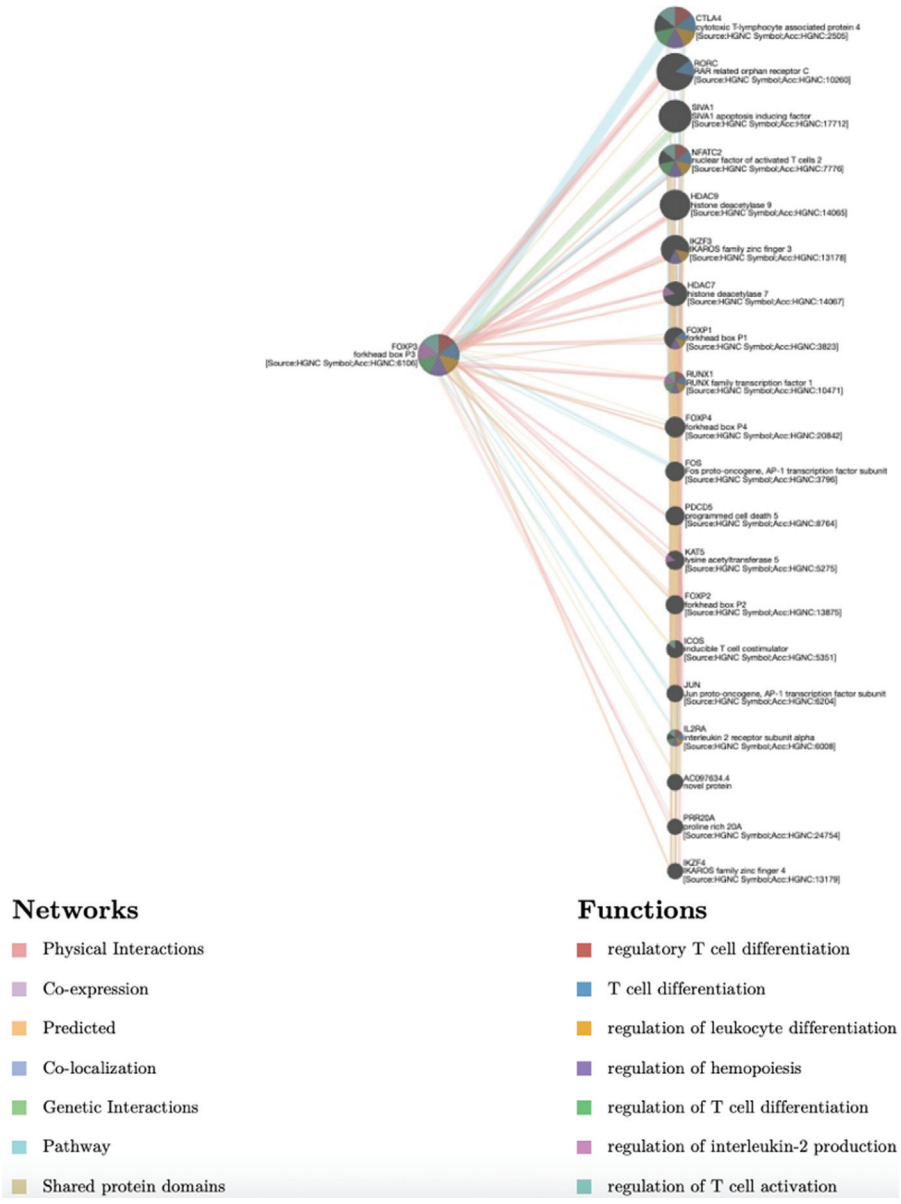
The motivation behind the selection of studied candidates:

It is important to understand why these candidates were selected. Based on the gene cards database (https://www.genecards.org), we found that The FOXP3 gene serves as a transcriptional regulator essential for the development and inhibitory function of regulatory Treg. Furthermore, the online database for gene-gene interactions GeneMANIA v3.6.0 (https://genemania.org). The results indicated that FOXP3 gene is the main regulator of Treg, which can act as a regulatory Treg differentiation, activation, leucocyte differentiation, hemopoiesis regulation, and regulation of IL-2 production. At the same time, the FOXP3 directly interact with the FOXP3 gene which is working as the main regulator of lymphocytes (Fig. 1).

To predict the outer effector of FOXP3, which can be uncontrolled, the miRNet database was used https://www.mirnet.ca, the results showed that the miRNA-31-5p and miRNA-21-5p directly targeted FOXP3 (Fig. 2). The binding site between FOXP3 and miRNA-31-5p was conserved with a 7mer-m8 seed region and a Context++ score of −0.11 (Table 1), according to the TargetScan V.8 database https://www.targetscan.org.

Based on previous bioinformatics online prediction results to identify this candidate, we are motivated to evaluate its validity as a diagnostic and prognostic biomarker for HCC.

Figure 1



Fork head box protein P3 gene interaction network, through the GenMANIA online database.

MiRNA-31 and FOX P3 gene expression pattern

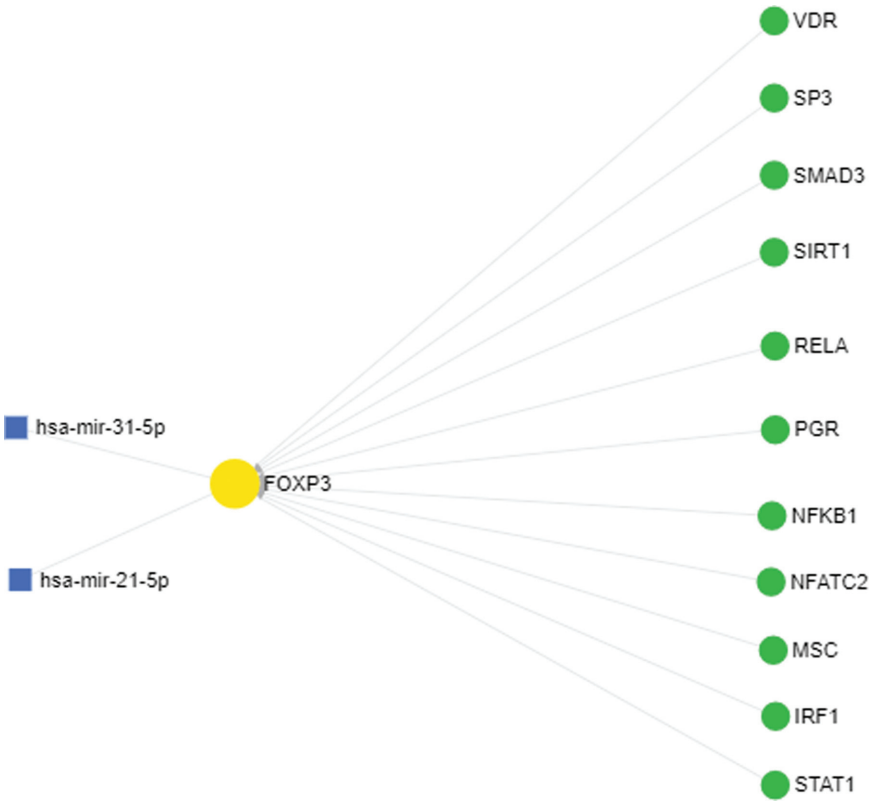
miRNA-31-5p gene expression results revealed that miRNA-31 expression was significantly downregulated in studied groups in comparison to control group, as well as compared with the group of HCV patients. Additionally, miRNA-31 expression was significantly decreased in HCC compared with cirrhosis as well as Chronic Liver Disease (CLD) groups (Table 2). However, FOXP3 expression was significantly downregulated in the HCV and cirrhosis groups, whereas it was significantly upregulated in the HCC group when compared with the control group. As compared with the HCV group, FOXP3 expression was significantly higher in the cirrhosis and HCC groups. Compared with cirrhosis or even CLD groups, FOXP3 expression was significantly upregulated in HCC groups (Table 2).

The present result found that, there was a significant increase ($P<0.05$) in granulocyte CD16+ percentage in the HCV with cirrhosis and HCC groups, in comparison with the control or HCV without cirrhosis groups, in addition there were significant increases ($P<0.05$) in HCC group than the cirrhotic group (Table 2).

The evaluation of the diagnostic performance of the analyzed biomarkers (miRNA-31, FOXP3 and CD16) was conducted through the application of a ROC curve, as represented in Table 3 and shown in Fig. 3.

The diagnostic performance for miRNA-31 showed that there was a significant discrimination between HCV versus control group with a Sn of 83.0% and Sp 100.0% ($P=0.001$), while the Sensitivity and Specificity in the cirrhosis group comparing to HCV group were 94.0% and 55.0%, respectively, ($P=0.025$), 94.0% and 61.0%, respectively, ($P=0.015$) in HCC group comparing to cirrhosis group and 97.0 and 67.0%, respectively, ($P<0.0001$) in HCC group

Figure 2



Targeted microRNAs for fork head box protein P3 gene, through the microRNet database (https://genemania.org).

Table 1 Conserved region between fork head box protein P3 and hsa-microRNA-31-5p

	Anticipated consequential association of target region (above) and microRNA (below)	Site type	Context++ score	Percentile of Context++ score	Score of weighted context++	Length of Conserved branch	PCT	Predicted relative KD
Position 125-131 of FOXP3 3' UTR hsa-miR-31-5p	5'-GUGAGGUUUCCACUGUCUUGCCU . . . ||||||| 3'-UCGAUACGGUCGUAGAACGGA	7mer-m8	−0.11	65	−0.11	2.422	0.26	−3.433

Table 2 MicroRNA-31, fork head box protein P3 and CD16+ granulocyte expression

	Group 1 control	Group 2 HCV	Group 3 Cirrhosis	Group 4 HCC
miRNA-31^{+}	1[a]	2.21 (1.14–20.4)[b]	1.03 (0.34–4.8)[c]	0.19 (0.02–0.79)[d]
FoxP3^{+}	1[a]	0.04 (0.016–0.27)[b]	0.9 (0.26–3.2)[c]	2.17 (1.46–13.4)[d]
CD16+ granulocyte^{++}	7.6±3.4[a]	7.36±3.1[a]	53.0±22.5[b]	89.2±45.7[c]

$^{+}$: Data are represented as median (interquartile range) and comparing using Kruskal–Wallis test. $^{++}$: Data are represented as mean±SD and comparing using One-way ANOVA test. Data with different superscript number (a, b, c) within the same raw are significantly changed at *P* value less than 0.05.

Table 3 Diagnostic performance of MicroRNA-31, fork head box protein P3, and CD16+ granulocyte expression in hepatitis C virus related liver disease

							95% CI		
	Variable (s)	Cut-off	Sensitivity	Specificity	AUC	SE	Lower bound	Upper bound	*P* value
HCV vs Control group	miRNA-31	>1.02	83.0	100.0	0.833	0.088	0.661	1	0.001**
	FoxP3	<0.011	78.0	100.0	0.167	0.088	0.001	0.339	0.001**
	CD16+ granulocyte	<5.2	83.0	87.0	0.807	0.09	0.63	0.984	0.003**
Cirrhosis vs HCV group	miRNA-31	>0.86	94.0	55.0	0.281	0.086	0.112	0.45	0.025*
	FoxP3	<0.145	83.0	72.0	0.765	0.085	0.598	0.933	0.007**
	CD16+ granulocyte	<14.9	100.0	100.0	1.0	0.0	1.0	1.0	<0.0001**
HCC vs Cirrhosis group	miRNA-31	>0.115	94.0	61.0	0.262	0.084	0.098	0.427	0.015*
	FoxP3	<1.03	100.0	62.0	0.735	0.089	0.56	0.909	0.016*
	CD16+ granulocyte	<43.01	88.0	44.0	0.605	0.101	0.408	0.802	0.282
HCC vs CLD group (HCV with and without Cirrhosis)	miRNA-31	>0.111	97.0	67.0	0.174	0.061	0.055	0.294	<0.0001**
	FoxP3	<1.08	94.0	72.0	0.816	0.058	0.703	0.929	<0.0001**
	CD16+ granulocyte	<42.8	89.0	78.0	0.748	0.079	0.594	0.903	0.003**

AUC, Area under curve, CI: 95% confidence interval. * *P* less than 0.05: significant, ** *P* less than 0.01: highly significant.

comparing to CLD group (that including HCV without Cirrhosis and HCV with Cirrhosis).

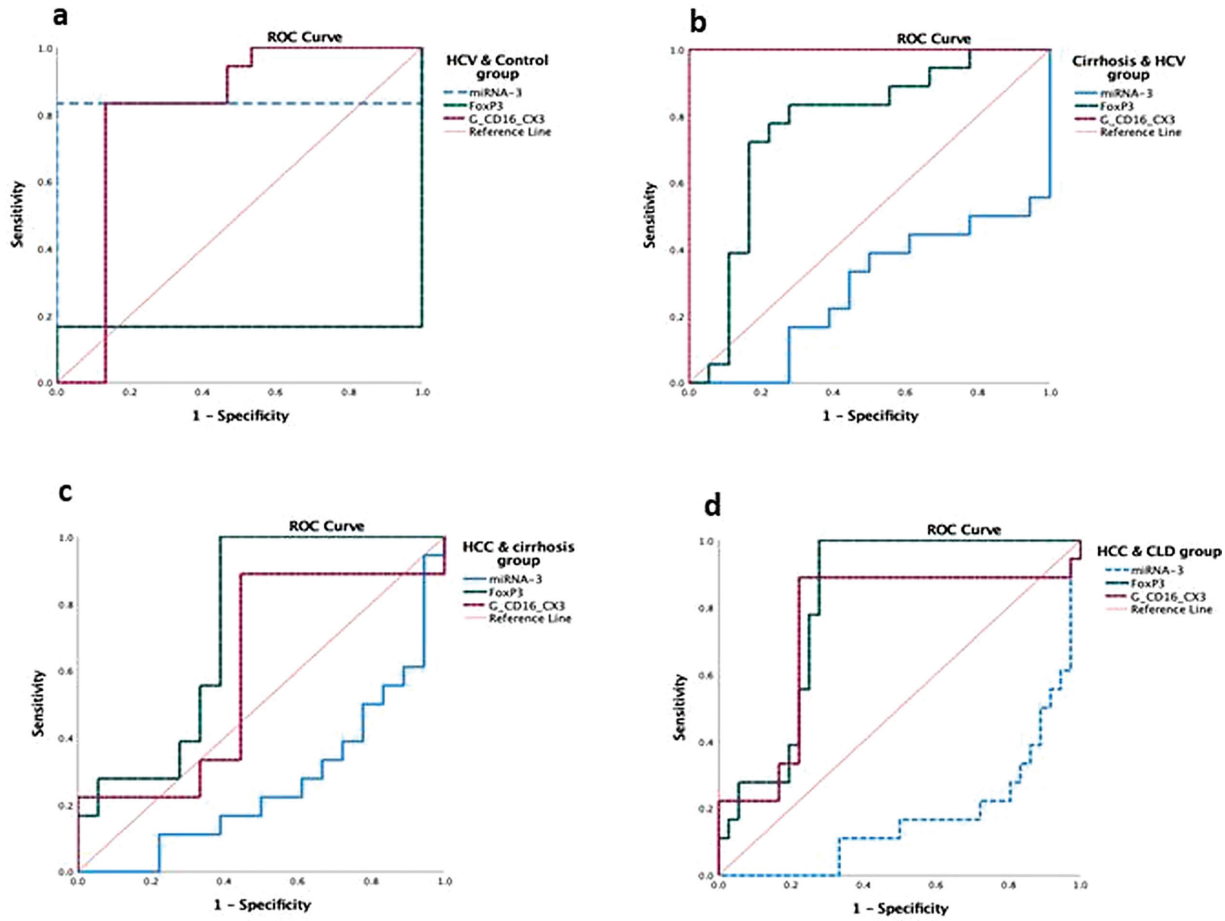
The FOXP3 gene showed significant discrimination between the HCV versus control groups with Sn of 78.0% and SP of 100.0% (*P*=0.0001), the cirrhosis group versus the HCV group reported Sn of 83.0%, and SP of 72.0% (*P*=0.007), while the HCC group versus Cirrhosis group reported Sn of 100.0% and SP of 62.0% (*P*=0.016), and HCC group versus CLD group (that including HCV without Cirrhosis and HCV with Cirrhosis) with Sn of 94.0% and Sp of 72.0% (*P*<0.0001).

It is noteworthy that the granulocyte expressing CD16 showed significant discrimination between the HCV versus control groups with Sn of 83.0% and Sp of 87.0% (*P*=0.003), between the Cirrhosis and HCV group with Sn of 100.0% and Sp of 100.0% (*P*<0.0001), and between the HCC group and the CLD group with Sn of 89.0% and Sp of 78.0% (*P*=0.003). Despite this, there was no significant difference between the HCC group and the cirrhosis groups (*P*=0.282), as represented in Table 3 and shown in Fig. 3.

Discussion

The present study involved 60 patients with persistent HCV infection, cirrhosis, and HCC, in addition to 20 healthy individuals acting as a control group. Our study shows the interplay between miRNA-31, activated granulocytes, and FOXP3, which play a critical role in shaping the immune landscape in patients with HCV-related cirrhosis and HCC. The present study revealed that miRNA-31 expression was significantly upregulated in the HCV group when compared with the control group, as well as compared with the HCC group. That is consistent with Bandiera *et al.* [6], where miRNA-31 is significantly upregulated in the context of HCV-related liver disease, contributing to the dysregulation of immune responses. miRNA-31 has been implicated in promoting inflammatory processes, which are central to the progression of HCV-related cirrhosis and the development of HCC. Additionally, our results are simultaneous with studies that have demonstrated that miRNA-31 enhances collagen production and the deposition of extracellular matrix, thus accelerating fibrosis [12]. This overexpression of miRNA-31 correlates with the severity of fibrosis and can drive the liver toward

Figure 3



Receiver operating characteristic Curve for studied biomarkers in the study groups. (a) The discrimination between hepatitis C virus versus control group, (b) The discrimination between Cirrhosis versus hepatitis C virus group, (c) The discrimination between hepatocellular carcinoma versus Cirrhosis group, (d) The discrimination between hepatocellular carcinoma versus CLD group.

cirrhosis proving that miRNA-31 directly targets proteins involved in inflammation resolution, such as suppressors of cytokine signaling, thereby prolonging the inflammatory response in the liver.

Additionally, our study demonstrated miRNA-31 downregulation in HCC group compared with control and HCV related chronic liver disease, that is in contrary to Xie *et al.* [13] whom adopt miRNA-31 being has oncogenic properties in HCC by targeting tumor suppressor pathways and promotes cell proliferation, resistance to apoptosis, and metastasis. However, our result aligns to Zhao *et al.* [14], who endorse that downregulation of miRNA-31 in HCC suggests that miRNA-31 may play a role in the suppression of tumor development and progression. Lower levels of miRNA-31 could be linked to more aggressive cancer behavior and poorer prognosis. These findings indicate that miRNA-31 might serve as a potential biomarker for diagnosing and monitoring HCC. These before mentioned findings suggest that miRNA-31-5p could serve as a valuable biomarker for distinguishing between different stages of liver disease, especially HCV and HCC. The high sensitivity and specificity values indicate that miRNA-31-5p has strong diagnostic potential, which could lead to earlier and more accurate detection of liver cancer.

In our study, FOXP3 expression was significantly downregulated in HCV and cirrhosis groups when compared with control group, that is accordant with Zhang *et al.* [15] and Liu *et al.* [16] whereas significant reduction in FOXP3 expression weas revealed, suggesting that the overexpression of miRNA-31 which has been observed in HCV patients, may impair the function of Tregs, leading to an uncontrolled immune response and further liver damage. Moreover, regarding our study, FOXP3 was significantly upregulated in HCC group when compared with control group and the other research groups. That is supported by the studies that have shown that FOXP3 expression is significantly higher in

HCC patients compared with those with just chronic liver disease or cirrhosis [17,18]. This upregulation of FOXP3 in HCC is thought to be attributed to the tumor microenvironment, which often promotes immunosuppressive conditions. Additionally, chronic inflammation and the presence of regulatory Tregs could further contribute to elevated FOXP3 expression [19]. Another potential mechanism is the alteration in signaling pathways, such as TGF-β, which can enhance FOXP3 transcription in cancerous tissues.

Based on our study, Granulocyte G_CD16 analysis, highly substantial differences were seen in the cirrhosis and HCC groups relative to the control group and that is compatible with Naeim [20], revealing that in the context of chronic HCV infection, elevated levels of activated granulocytes expressing CD16 have been affiliated with increased liver inflammation and accelerated fibrosis.

Additionally, significant differences were observed within the HCC group and the cirrhosis or CLD groups, that is concordant with recent studies that have demonstrated that granulocytes expressing CD16 exhibit immunosuppressive properties within the tumor microenvironment, thereby facilitating immune evasion and tumor progression, involving complex network of pro-inflammatory and pro-fibrotic signals, wherein activated granulocytes play a pivotal role [21]. Additionally, these cells secrete cytokines and growth factors that support angiogenesis and metastatic potential of HCC cells [9]. These findings suggest that G_CD16 can effectively differentiate between various stages of liver disease, including HCV and cirrhosis.

These data finding imply that the progression from HCV to more severe liver conditions such as cirrhosis and HCC is associated with marked changes in Granulocyte expressing CD16 levels. This suggests that Granulocyte expressing CD16 could potentially function as an indicator for recognizing and tracking the intensity of liver disease. Furthermore, the significant differences between the HCC group and other groups highlight its potential role in distinguishing between advanced stages of liver disease.

Conclusions

Understanding how miRNA-31 activates granulocytes in HCV-related diseases could open up new therapeutic avenues. Targeting miRNA-31 may help modulate the excessive inflammatory response and reduce liver damage in HCV-infected patients. Moreover, our research findings advocate that FOXP3 gene may have crucial involvement in the immune response as well as disease progression in liver conditions. Its significant discrimination between different groups indicates that FOXP3 could be a valuable biomarker for the diagnosis and monitoring of hepatic disease progression. Further research could elucidate its specific mechanisms and potential as a therapeutic target.

Acknowledgments

Author contributions: N.A.A.: project preparation, study design, conducting the laboratory work, and manuscript preparation. R.E.E.A.: project preparation, study design, performing the molecular methodology, and statistical analysis. A.S.M.H.: Data collection and revision, conducted the laboratory work, participated in reviewing statistical analysis, and final manuscript proofing. K.M.R.: patient clinical examination, recruiting, and patients diagnosis. M.Y. Z.: Manuscript reviewing. O.B.A.: project preparation, conducted the laboratory work, reviewing, final manuscript proofing, and writing the initial draft of the manuscript and prepare it for publishing submission. All authors revised and approved the final manuscript.

Financial support and sponsorship

This work has been supported by Theodor Bilharz Research Institute, Giza, Egypt.

Conflicts of interest

There are no conflicts of interest.

References

1 Salari N, Kazeminia M, Hemati N, Ammari-Allahyari M, Mohammadi M, Shohaimi S. Global prevalence of hepatitis C in general population: A systematic review and meta-analysis. Travel Med Infect Dis 2022; 46:102255.

2 Guyot C, Lepreux S, Combe C, Doudnikoff E, Bioulac-Sage P, Balabaud C, Desmoulière A. Hepatic fibrosis and cirrhosis: the (myo)fibroblastic cell subpopulations involved. Int J Biochem Cell Biol 2006; 38:135–151.

3 Chang M, Williams O, Mittler J, Quintanilla A, Carithers RLJr, Perkins J, *et al.* Dynamics of hepatitis C virus replication in human liver. Am J Pathol 2003; 163:433–444.

4 O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol (Lausanne) 2018; 9:402.

5 Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136:215–233.

6 Bandiera S, Pernot S, El Saghire H, Durand SC, Thumann C, Crouchet E, *et al*. Hepatitis C virus-induced upregulation of microRNA miR-146a-5p in hepatocytes promotes viral infection and deregulates metabolic pathways associated with liver disease pathogenesis. J Virol 2016; 90: 6387–6400.

7 Stepicheva NA, Song JL. Function and regulation of microRNA-31 in development and disease. Mol Reprod Dev 2016; 83:654–674.

8 Xu P, Wu Q, Yu J, Rao Y, Kou Z, Fang G, *et al.* A systematic way to infer the regulation relations of miRNAs on target genes and critical miRNAs in cancers. Front Genet 2020; 11:278.

9 Liu K, Wang FS, Xu R. Neutrophils in liver diseases: pathogenesis and therapeutic targets. Cell Mol Immunol 2021; 18:38–44.

10 Kim HS, Lee KS, Bae HJ, Eun JW, Shen Q, Park SJ, *et al.* MicroRNA-31 functions as a tumor suppressor by regulating cell cycle and epithelial-mesenchymal transition regulatory proteins in liver cancer. Oncotarget 2015; 6:8089–8102.

11 Liu Y, Li C, Yang Y, Li T, Xu Y, Zhang W, *et al.* The TGF-β/miR-31/CEACAM1-S axis inhibits $CD4^+$ $CD25^+$ Treg differentiation in systemic lupus erythematosus. Immunol Cell Biol 2021; 99:697–710.

12 Ajith A, Merimi M, Arki MK, Hossein-Khannazer N, Najar M, Vosough M, *et al.* Immune regulation and therapeutic application of T regulatory cells in liver diseases. Front Immunol 2024; 15:1371089.

13 Xie B, Zhao Z, Liu Q, Wang X, Ma Z, Li H. CircRNA has circ 0078710 acts as the sponge of microRNA-31 involved in hepatocellular carcinoma progression. Gene 2019; 683:253–261.

14 Zhao G, Han C, Zhang Z, Wang L, Xu J. Increased expression of microRNA-31-5p inhibits cell proliferation, migration, and invasion via regulating Sp1 transcription factor in HepG2 hepatocellular carcinoma cell line. Biochem biophys res commun 2017; 490:371–377.

15 Zhang L, Ke F, Liu Z, Bai J, Liu J, Yan S, *et al.* MicroRNA-31 negatively regulates peripherally derived regulatory T-cell generation by repressing retinoic acid-inducible protein 3. Nat Commun 2015; 6:7639.

16 Liu C, Li N, Liu G. The Role of MicroRNAs in Regulatory T Cells. J Immunol Res 2020; 2020:3232061.

17 El-Refaei KER, Zaky DSE, Attia FAK, Hendy OM, Kawzae AAM. Role of FoxP3+T regulatory cells in chronic HCV and its relation to disease severity, clinical medicine and diagnostics. Clinical Medicine and Diagnostics 2019; 9:74–78.

18 Zhang CY, Liu S, Yang M. Regulatory T cells and their associated factors in hepatocellular carcinoma development and therapy. World J Gastroenterol 2022; 28:3346–3358.

19 Qiu Y, Ke S, Chen J, Qin Z, Zhang W, Yuan Y, *et al.* FOXP3+ regulatory T cells and the immune escape in solid tumors. Front Immunol 2022; 13:982986.

20 Naeim F. Principles of Immunophenotyping. In Hematopathology, Faramarz Naeim, P. Nagesh Rao, Wayne W. Grody (eds). Los Anglos, USA: Academic Press 2008; 27–55.

21 Shen KY, Zhu Y, Xie SZ, Qin LX. Immunosuppressive tumor microenvironment and immunotherapy of hepatocellular carcinoma: current status and prospectives. J Hematol Oncol 2024; 17:25.