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Micro RNA 155 and Its Target Gene CTLA4 as a New Biomarker for Lymphocyte Activation in HCV-Induced Liver Fibrosis and Hepatocellular Carcinoma

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Abstract

The role of microRNA 155 (miRNA 155) and the immunological checkpoint cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) in chronic liver diseases and hepatocellular carcinoma (HCC) are complex. The alterations in the expression levels or functions of CTLA-4 and miRNA 155 could influence the outcome of HCV infection by affecting the quality and persistence of the antiviral immune response. Here, we tried to investigate the relationships of miRNA 155 gene expression with CTLA-4, the regulator gene of lymphocyte activation in patients with HCC and chronic HCV infection to assess the interactions between these genes and lymphocyte activation and its contribution in liver disease progression. A case-control study included 80 subjects (20 healthy controls, 20 HCV patients without cirrhosis, 20 with liver cirrhosis, and 20 patients with HCC). The expression of miRNA 155 and CTLA-4 gene were measured by quantitative real-time qRT-PCR. The percentage of circulating regulatory T (T reg) cells expressing CD4 and CD25 was done by flow cytometry. Results revealed that miRNA 155 and CTLA-4 gene were upregulated with higher expression in HCC and liver cirrhosis patients compared to HCV without liver cirrhosis and control groups (p value < 0.05). Together with a significant increase in CD4+CD25+ T reg in HCC and liver cirrhosis patients compared to the HCV group (p value <0.05). In conclusion, miRNA 155 and CTLA-4 gene could be utilized as new biomarkers in HCV- induced liver fibrosis and HCC for further exploration of immunomodulatory strategies targeting these molecules to enhance antiviral immune responses and prevent hepatocarcinogenesis.

Keywords: Micro RNA 155; CTLA-4; T lymphocytes; Liver cirrhosis; HCC.

1. Introduction

Hepatitis C virus (HCV) infection triggers chronic liver disease (CLD) with subsequent liver fibrosis and cirrhosis, as well as hepatocellular carcinoma (HCC) through persistent inflammation, cell necrosis, and regeneration [1, 2]. Moreover, the immune response is a critical component of the pathogenesis. Excessive or prolonged T lymphocyte activation can contribute to chronic inflammation and tissue damage, promoting fibrosis through a complex interplay involving the activation of hepatic stellate cells with subsequent excessive extracellular matrix deposition [3]. The formation and progression of hepatocellular carcinoma are attributed to many genetic alterations that lead to the expression of oncogenes, tumor suppressor genes, and other genes implicated in the regulation of oncogenesis pathways [4]. Activated T lymphocytes and regulatory T (Treg) cells express cytotoxic T lymphocyte associated antigen-4 (CTLA-4), an immunological checkpoint

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that downregulates T-cell activation through the inhibition of T-cell signaling when B7 (CD80/CD86) ligands bind to it [5]. Therefore, targeting CTLA-4 can affect activated T lymphocytes and Treg cells [6]. MicroRNAs are small non-coding RNA molecules that play a key role in controlling gene expression, which in turn affects a number of physiological and pathological processes such as cell division, signal transduction, growth, proliferation, and death. It is essential for inflammation and immunological responses [7]. It's important to note that the roles of CTLA-4 and miRNA 155 in CLD and HCC are complex, with variable effect according to the particular context, the stage of fibrosis, the cell types involved, and the tumor microenvironment. Research in this field is ongoing, and the understanding of the intricate mechanisms is continually evolving [2]. The most reliable method for diagnosing liver fibrosis is still a liver biopsy. Finding non-invasive biomarkers that can accurately identify people at increased risk of progression is increasingly important due to the hazards involved with liver biopsy and its impracticality for widespread use. In this respect, we tried in this study to investigate the relationships of miRNA 155 gene expression with the regulator gene of lymphocyte activation (CTLA-4) in CHC infection and HCC patients to evaluate the interactions between these genes and lymphocyte activation and its role in liver disease progression and HCC development.

2. Material and Methods

2.1. Participants

The study included 80 subjects (20 healthy controls, 20 HCV patients without cirrhosis, 20 with liver cirrhosis, and 20 patients with HCC). Patients were collected from the hepatogastroenterology department at Theodor Bilharz Research Institute, Giza, Egypt. A comprehensive medical history, physical examination, radiographic examinations, and laboratory evaluation, which included complete blood picture and liver function tests, as well as serological and HCV genotyping using HybProbe probes and the lightcycler carousel-based system, were done for proper diagnosis. Ethical approval was granted by the research ethics committee (REC) at Theodor Bilharz Research Institute (TBRI), study protocol No. (PT 814) after obtaining an informed consent from all participants. REC-TBRI operates under Federal Wide Assurance No. FWA00010609.

2.2. Study design

A case-control study in which patients' enrollment criteria included chronic liver disease patients with HCV infection (genotype 4) and HCC. Exclusion criteria include other causes of chronic liver diseases, such as patients with a history of schistosomiasis, chronic viral diseases other than HCV, nonalcoholic steato-hepatitis, autoimmune hepatitis, biliary disorders, regular hepatotoxic drugs, and alcohol abuse.

2.3. Sample collection

Five milliliters of blood were aseptically withdrawn via venipuncture into three vacutainer tubes, two sterile ethylenediaminetetraacetic acid (EDTA)-containing tubes (Vacutainer; BD Biosciences) (2x2 ml), one tube was used to perform flow cytometry for CD3, CD4, and CD25. The 2nd EDTA tube was immediately used for RNA extraction for further RT-PCR. One ml was collected in a plain tube (Vacutainer; BD Biosciences) for liver function tests and the detection of serum anti-HCV antibodies.

2.4. RT- PCR for CTLA4 gene expression and miRNA 155

Extraction of total RNA was done using the high Pure RNA isolation kit (Roche ltd., cat. no. 11828665001). 1 ug RNA was used in the reverse transcriptase (RT) step by using FIREScript RT cDNA synthesis KIT, SOLIS BIODYNE, cat. no. (06-15-00050). Gene expression was detected using HOT FIREPol EvaGreen qPCR Mix Plus (Rox), SOLIS BIODYNE, cat. no. 08-24-00001. The gene expression was done using the Light Cycler EvoScript RNA SYBR Green I Master kit. All miRNAs and mRNAs expression values will be evaluated using relative quantification analysis by ΔΔCt method and will be normalized to beta-actin and U6 housekeeping genes for CTLA-4 and miRNA155, respectively.

2.5. Bioinformatics' tools

I-Gene-Gene interaction analysis with GeneMANIA:

Tool description: GeneMANIA (Version 3.6.0) (http://www.genemania.org) is an online database and tool used to predict gene functions based on multiple data sources, including protein and genetic interactions, pathways, co-expression, co-localization, and protein domain similarity.

Purpose: In this study, GeneMANIA was utilized to explore the interaction network surrounding the CTLA-4 gene. By inputting CTLA-4 as the query gene, the tool predicted interactions with other genes that regulate lymphocyte activation and differentiation.

II- Micro RNA-Target prediction with MiRNet

Tool description: MiRNet (Version 2.0) (https://www.mirnet.ca) is a web-based platform

designed for visual analytics of miRNA-target interactions, combining data from miRTarBase, TarBase, and miRecords. It allows for the exploration of miRNA networks based on experimentally validated interactions.

Purpose: MiRNet was used to predict and visualize the interactions between miRNA 155 and CTLA-4. The analysis identified a conserved binding site on the CTLA-4 gene for miRNA155, suggesting a regulatory relationship.

III- Micro RNA-Target Binding Site Prediction with TargetScan

Tool description: TargetScan (Version 8.0) (https://www.targetscan.org) is a database that predicts biological targets of miRNAs by searching for the presence of conserved 8mer, 7mer, and 6mer sites that match the seed region of each miRNA.

Databases and algorithms used: The tool leverages conserved sequences across species to predict miRNA binding sites. The predictions include a Context++ score that reflects site conservation and predicted repression strength.

Purpose: TargetScan was used to validate the predicted interaction between miRNA 155 and CTLA-4 by identifying specific binding sites within the CTLA-4 3' untranslated region (UTR).

2.6. Flow cytometric analysis of peripheral blood T lymphocytes

The BD Accuri C6 flow cytometer was used to calculate the percentage of circulating CD4+CD25+ Tregs. Briefly, 5 µl of human CD25-PE monoclonal antibody (BioLegend, cat No. 302606) and 5 µl of human CD4-FITC monoclonal antibody (BioLegend, cat. No. 317408) were added to 100 µl of whole blood. The mixture was then incubated in the dark for 20 minutes. After obtaining 10,000 events, a logarithmic amplification was used to collect the data. Gating for lymphocytes was done according to the forward and side scatter properties of lymphocytes.

2.7. Statistical analysis

The statistical analysis of data was done using the IBM SPSS version 28 for Windows (IBM Corp., Armonk, N.Y., USA). Abnormally distributed parameters were represented as median and interquartile range (IQR) (25%–75%), with a 95% confidence interval; a p value < 0.05 was considered statistically significant. Comparison between medians of abnormally distributed variables was done using a Mann-Whitney U test. The receiver operating characteristic (ROC) curve was used to assess the diagnostic performance of the studied genes. The area under the curve (AUC) was calculated and presenting the accuracy index for the prognostic performance of selected test with a diagnostic cut-off values. The prognostic performance was assessed using logistic regression model.

3. Results

3.1. Bioinformatics results

Based on the gene-gene interaction online database GeneMANIA Version 3.6.0 https://genemania.org/search/homo-sapiens/CTLA4. The results indicated that CTLA4 is the main regulator of lymphocytes, which can act as a costimulation factor for lymphocytes, a positive regulator for lymphocyte activation, and a regulator for lymphocyte differentiation. In addition to T cell regulation and leukocyte activation. The network generated was visualized (Figure 1) to illustrate these interactions. In order to predict the outer effector of CTLA4, which can be uncontrolled, the miRNet database was used https://www.mirnet.ca, the results showed that the miRNA-155-5p directly targeted to CTLA4. The binding site between CTLA4 and miRNA-155-5p was conserved with a 7mer-m8 seed region and a Context++ score of -0.22 (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 biomarker for HCC diagnosis and prognosis.

3.2. MiRNA-155 and CTLA4 gene expression pattern

MiR-155 gene expression analysis showed a significant upregulation in all patients groups in comparison to the control group. Additionally, a significant upregulation was found between liver cirrhosis and HCC patients in comparison to the HCV patients (p value < 0.01) as shown in table 2 and figure 2.

CTLA4 gene showed a significant upregulation in liver cirrhosis and HCC patients in comparison to the control group. As compared to the HCV group, CTLA4 expression was significantly higher in the cirrhosis and HCC groups. However, no statistically significant difference was detected between HCV patients and the control group (Table 2 and Figure 2).

3.3. Flow cytometric assessment of CD4+/CD25+ T cell percentage

There was a significantly dramatic increase in circulating CD4+/CD25+ T cells in accordance with the progression of the disease in HCC and liver cirrhosis patients in comparison to the HCV patients, as well as when compared to the controls. However, no statistically significant difference could be detected between HCV patients and the control group (Table2, Figure 2 and 3).

3.4. The diagnostic performance of the miRNA 155 and CTLA-4 genes

The diagnostic performance of the studied biomarkers was assessed using a receiver operating characteristic (ROC) curve (Table 3 and Figure 4) and showed:

MiRNA-155-5p showed no significant discrimination between HCV vs. the control group (p value = 0.104), but a significant difference was found between the cirrhosis group and the HCV group with a cut-off value <0.295 (Sn = 83.3, SP = 66.7, p value = 0.012), the cirrhosis group and HCC group with a cut-off value <1.54 (Sn = 88.0, SP = 61.0, p value = 0.006), and the CLD group and the HCC group with a cut-off value 1.27 for diagnosis of HCC (Sn = 80.0, SP = 67.0, p value = 0.0001).

The CTLA4 gene showed significant discrimination between the cirrhosis and HCV groups (Sn=95.0, SP=100.0, p=0.0001), the cirrhosis group and HCC group (Sn=100.0, SP=66.0, p=0.0001), and the CLD group and the HCC group with a cut-off value 0.285 for predicting the diagnosis of HCC (Sn=94.0, SP=70.0, p=0.0001). Nevertheless, no significant difference was detected between the HCV and control groups (p = 0.367).

The logistic regression analysis revealed:

MiRNA155 significantly predicts the progression of the disease in cirrhosis as compared with HCV, with odd ratio (OR) = 0.788, 95% confidence interval (C.I) (0.608-1.022), p=0.042. The same was found to be more significant in HCC than in the in the cirrhosis group, with OR = 0.159, 95% C.I (0.024-1.043), p = 0.045. It was also found to be more significant in HCC than CLD group with OR = 0.132, 95% C.I (0.023-0.767), p = 0.024. However, there was no statistical significance to predict progression between the HCV and control groups (p = 0.159) (Table 4). Although the CTLA4 gene showed significant predictive value for cirrhosis progression compared with HCV, the OR = 0.001, 95% C.I (0.0001- 0.212), p = 0.012. Additionally, the same outcome was found to be significant among patients with HCC compared to those with cirrhosis, with OR =1.237, 95% C.I (1.01-1.515), p=0.039. Moreover, a significant difference between CLD and HCC groups was found, with OR = 1.368, 95% C.I (1.096-1.706), p = 0.005. However, there was no statistical significance to predict progression between the chronic hepatitis C infection and control groups (p = 0.246) (Table 4).

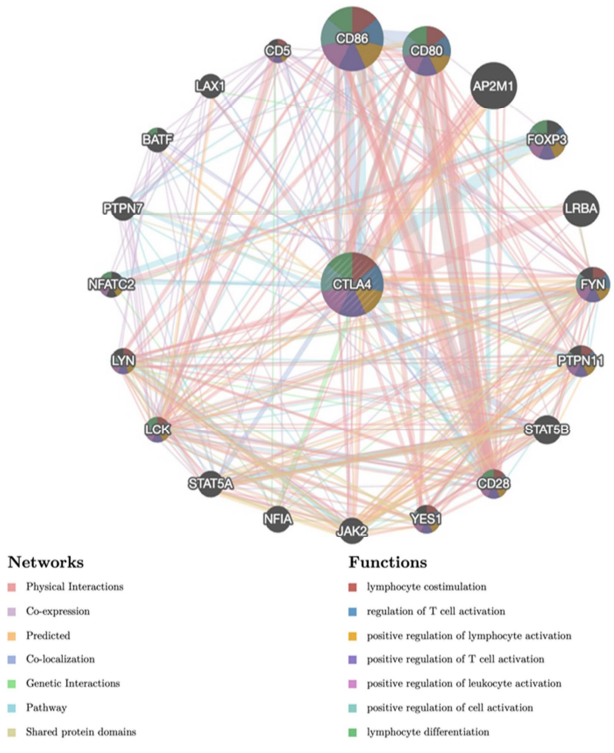

Figure 1: CTLA4 gene-gene interaction network, through the GenMANIA online database

Table 1: Conserved region between CTLA4 3' and hsa-miRNA155-5p

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	PCT	Predicted relative KD
Position 296-302 of CTLA4 3' UTR hsa-miRNA155-5p	5'...UUAAUAUGGGGAUGCAGCAUUAU... 3' UGGGGAUAGUGCUAAUCGUAAUU	7mer-m8	-0.22	90	-0.22	3.270	0.23	-3.687

Table 2: Studied biomarkers expression

Groups	Control	CLD		HCC
		HCV	Cirrhosis	
miR-155	1	1.86 (0.36- 31.89) *	3.78 (0.2-348) **††	13.9 (2.0-119) **††##, aa
CTLA4	1	0.95 (0.05- 4.89)	1.61(0.32- 16.10) *††	2.05 (0.17- 20.88) **††##, aa
T lymph CD4+CD25+ %	1.2 (0.4- 2.1)	1.8 (0.7- 3.6)	3.8 (1.7- 9.4)**, †	7.6 (4.7- 11.4)** , ††, ##, aa

Data were represented as median and interquartile range (25%–75%), with a 95% confidence interval; a p-value < 0.05 was considered statistically significant.
*: P-value is significantly different in comparison to control group.
†: P- value is significantly different in comparison to HCV group.
#: P- value is significantly different in comparison to Cirrhosis group.
a: P-value is significantly different in comparison to CLD cases.
One initial symbol: P- value < 0.05 is significant, two initial symbols: P- value < 0.01 is highly significant.

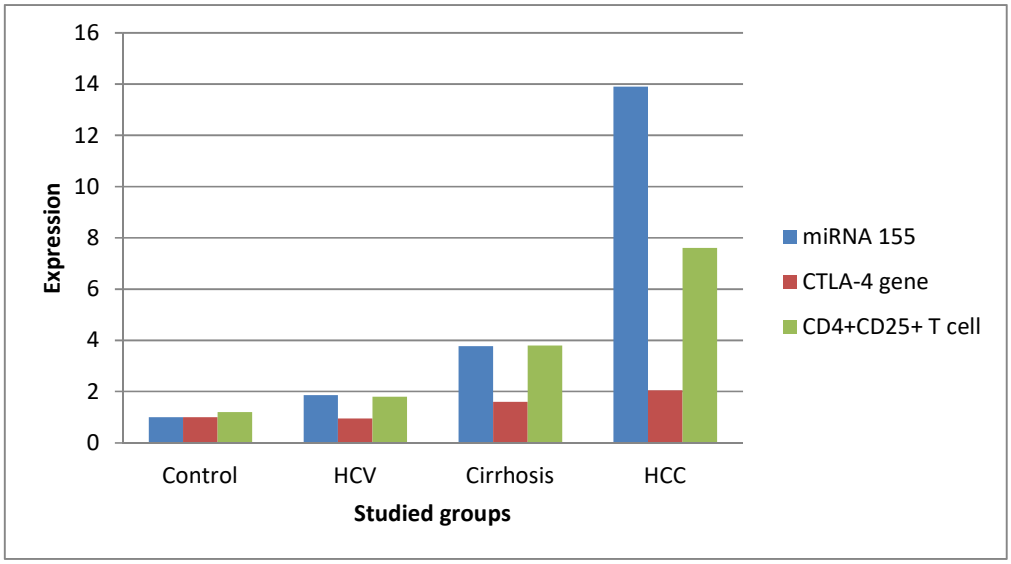

Figure 2: The studied biomarkers expression in the different groups

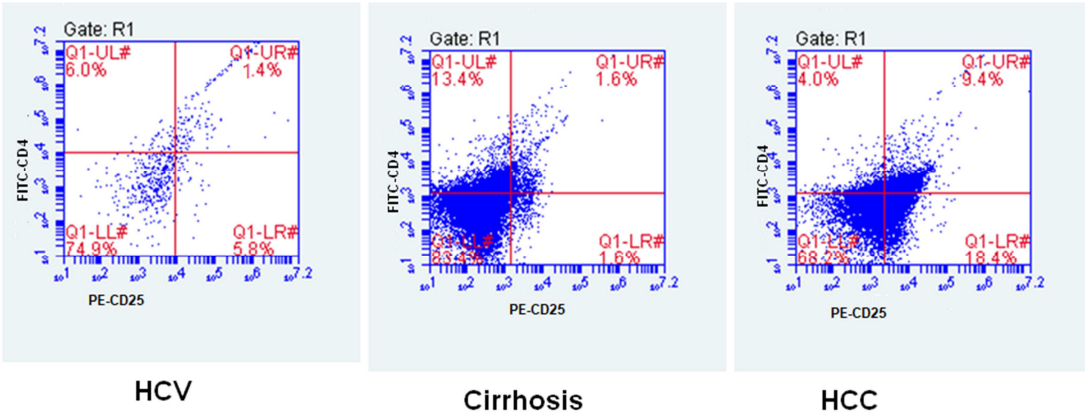

Figure 3: Flow cytometry of circulating regulatory T (Treg) cells expressing CD4 and CD25 in different patients groups. CD4 versus CD25 in biexponential scale plot showing the dual expression of CD4 and CD25 cells in quadrant Q1 UR#

Table 3: Diagnostic performance of the studied biomarkers

	Test Result Variable(s)	Cut-off	Sn.	Sp.	AUC	95% C.I		P value
						Lower Bound	Upper Bound	
HCV Vs Control group	miR-155-5p	<0.185	100.0	33.0	0.667	0.449	0.884	0.104
	CTLA4	1.11	95.0	100	0.083	0.000	0.204	0.0001**
Cirrhosis Vs HCV group	miR-155	<0.295	83.3	66.7	0.256	0.095	0.418	0.012*
	CTLA4	0.135	50.0	83.3	0.588	0.394	0.782	0.367
HCC Vs Cirrhosis group	miR-155	<1.54	88.0	61.0	0.231	0.079	0.384	0.006**
	CTLA4	0.175	100.0	66.0	0.846	0.717	0.975	<0.0001**
HCC Vs CLD group	miR-155	1.27	80.0	67.0	0.165	0.058	0.272	0.0001**
	CTLA4	0.285	94.0	70.0	0.909	0.833	0.985	0.0001**

Sn: Sensitivity, Sp: Specificity, AUC Area under curve and C.I: 95% Confidence Interval. * p value <0.05 is significant, ** p value <0.01 is highly significant.

Table 4: Prognostic performance of the studied biomarkers

Studied groups	Studied biomarkers	OR	95% C.I		p value
			Lower	Upper	
HCV Vs Control group	**miR-155-5p**	1.725	0.807	3.685	0.159
	CTLA4	0.001	0.0001	0.212	0.012*
Cirrhosis Vs HCV group	**miR-155**	0.788	0.608	1.022	0.042*
	CTLA4	2.238	0.574	8.718	0.246
HCC Vs Cirrhosis group	**miR-155**	0.159	0.024	1.043	0.045*
	CTLA4	1.237	1.01	1.515	0.039*
HCC Vs CLD group	**miR-155**	0.132	0.023	0.767	0.024*
	CTLA4	1.368	1.096	1.706	0.005**

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

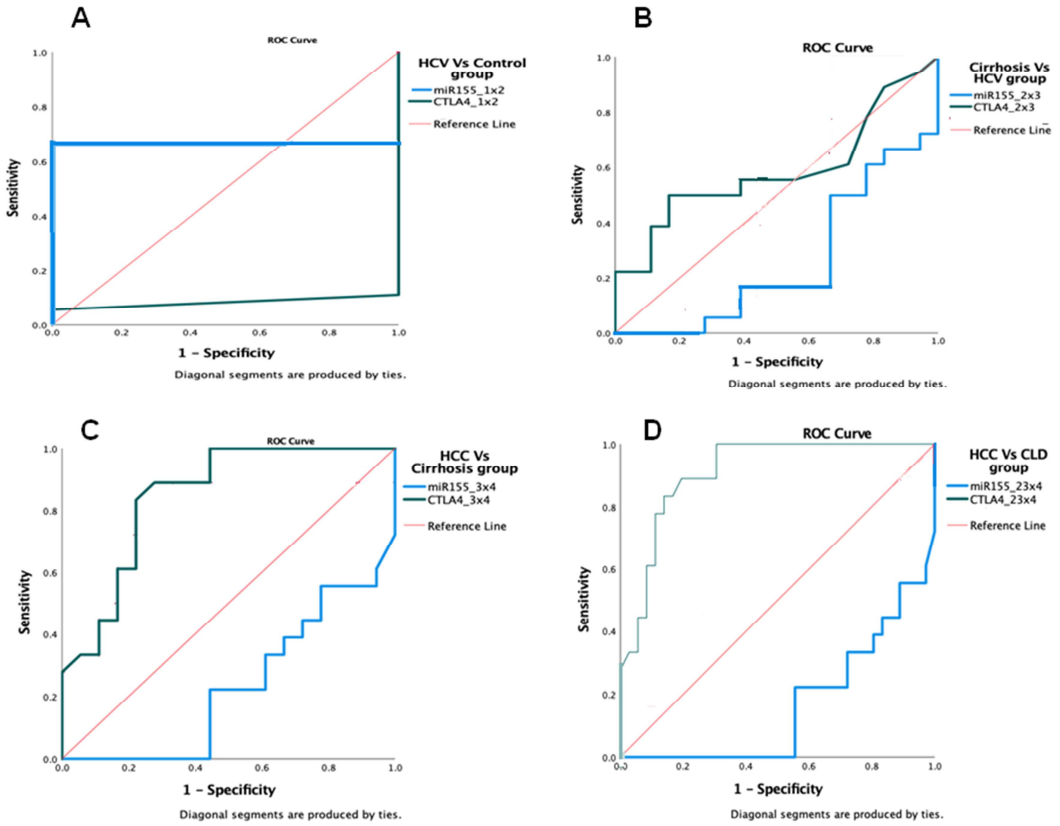

Figure 4: ROC Curve for the studied biomarkers in the studied groups. A) HCV Vs control group, B) HCV Vs Cirrhosis group, C) Cirrhosis Vs HCC group, D) CLD Vs HCC group

4. Discussion

MiRNA-155 is considered an important regulator of the immune response during inflammatory process. It is considered as a mediator between inflammation and the development of cancer [8]. Consequently, the evaluation of miRNA-155 expression could be beneficial for predicting the progression from chronic hepatitis C (CHC) virus infection towards liver cirrhosis and HCC [4, 9].

The present study was conducted on 60 patients with chronic HCV infection, cirrhosis and HCC, besides 20 healthy individuals as a control group. The findings from our study highlight the complex interplay between miRNA 155 and the immune checkpoint protein CTLA-4 in the context of CHC and HCC. Our results revealed a significant upregulation of both miRNA-155 and CTLA-4 in patients with chronic liver disease, particularly in those with cirrhosis and HCC, compared to healthy controls and individuals with HCV infection without cirrhosis. This aligns with previous studies that have suggested the role of both miR-155 and CTLA-4 as crucial players in immune regulation and tumorigenesis in liver diseases [4], [10-12].

MiRNA 155 plays a pivotal role in the differentiation and function of various T-cell subsets. It is known to be involved in the activation of T-helper cells (Th1, Th17) and the repression of regulatory T cells (Tregs), thereby influencing the balance between pro-inflammatory and anti-inflammatory responses. This dual role of miRNA-155 is critical in chronic inflammatory diseases, including HCV-induced liver fibrosis and HCC [7, 8]. MiRNA 155 exerts its effects by targeting multiple genes involved in the T-cell receptor (TCR) signaling pathway, including SOCS1 (Suppressor of Cytokine Signaling 1), which is a negative regulator of cytokine signaling. By downregulating SOCS1, miRNA-155 enhances T-cell activation and proliferation, leading to sustained immune responses. This prolonged activation can

contribute to the chronic inflammation observed in liver diseases and the eventual progression to cirrhosis and HCC [10-12].

In our study, we observed a significant upregulation of miRNA-155 in patients with liver cirrhosis and HCC compared to controls. This increase in miRNA-155 levels was accompanied by elevated percentages of CD4+CD25+ regulatory T cells, suggesting a complex interplay between miRNA-155 and T-cell activation. The upregulation of miRNA-155 likely contributes to the dysregulated immune environment, where continuous T-cell activation fosters chronic inflammation and fibrosis, creating an environment conducive to the development of HCC [11].

Our findings align with recent studies, such as those by Xue et al., which demonstrated that miRNA-155 is upregulated in chronic liver diseases and plays a crucial role in promoting inflammatory responses through T-cell activation [10]. Additionally, studies by O'Connell et al. have shown that miRNA-155 can enhance inflammatory T-cell development, further supporting its role in the progression of HCV-related liver disease [7]. Also, our observations corroborate findings from earlier studies, which have reported that high expression of miRNA 155 is associated with bad prognosis in various tumors, including HCC [4], [10], [13, 14].

Our findings demonstrated a significant upregulation in miRNA 155 in HCV patients compared to the controls. Our results were consistent with other research showing increased miRNA155 expression in individuals with chronic HCV infection [9], [15, 16].

One of the factors that is believed to aid in the development of chronic HCV infection is T-cell exhaustion, which is a state of dysfunction that occurs in T cells after prolonged exposure to antigens, often associated with chronic infections or cancers. In this state, T cells lose their ability to proliferate and produce cytokines, leading to diminished immune responses. This is characterized by the upregulation of inhibitory receptors, such as CTLA-4, and a change in metabolic activity, ultimately impairing the T cells' ability to effectively combat the pathogen or tumor [17]. CTLA-4 is an immunological checkpoint that down regulates T-cell activation through the inhibition of T-cell signaling when B7 (CD80/CD86) ligands bind to it through its expression on regulatory T (Treg) cells and activated T lymphocytes [5]. However, its significance in acute HCV infection is still debatable [17].

In our study, we demonstrated upregulation of CTLA-4 in HCV population with advanced liver disease with simultaneous a significant increase in circulating CD4+/ CD25+ regulatory T lymphocytes (Tregs) among the cirrhosis and HCC patients, however, no significant difference in CTLA4 expression and Treg cells could be detected between HCV patients without cirrhosis and the controls. In alignment with our findings, previous studies reported a significant increased in CD4+/CD25+ T reg cells in peripheral blood of patients with liver cirrhosis and HCC in comparison to the controls [18-20]. However, Lian et al found that there were no differences in T reg cells between those with liver cirrhosis and controls [21].

The simultaneous increase in the expression of CTLA4 in HCV population with liver cirrhosis when compared to the HCV without cirrhosis and controls in our study was in agreement with previous studies reported that prolonged HCV viremia induces a continuous high expression of CTLA-4, which in turn leads to suppression of CD8+cytotoxic T cells, viral escape mechanism and ultimately result in persistent infection [22-25].

In our study, CTLA4 expression was significantly higher in HCC patients than in CLD patients and the controls. Our results aligned with previous research that reported a high expression of CTLA-4 in HCC patients [26]. In addition, other studies demonstrated its association with the poor outcome of the disease [27]. More over Liu et al reported an association between elevated CTLA4 expression and the higher risk for HCC development [28].

The role of Tregs in the tumor microenvironment has gained attention, given their potential to suppress anti-tumor immunity [29]. This underscores the adaptive immune evasion strategies employed by tumors. CTLA-4 is known to inhibit T cell activation, suggesting that its increased expression may allow HCC cells to escape immune surveillance [28], [30]. This presents a dual challenge in treating patients with HCV-related liver diseases: the need to bolster antiviral responses while also managing the tumor-promoting effects of immune checkpoint upregulation.

Based on our findings, together with the previous studies, the correlation between elevated miRNA 155 and CTLA-4 levels and the increase in Tregs is noteworthy. It suggests a pathway where the immunomodulatory effects of miRNA 155 could facilitate Treg expansion, which in turn may contribute to an immunosuppressive milieu that favors tumor progression in HCC.

Anti-miRNA155 therapies have demonstrated significant reductions in tumor growth and improved immune responses. For instance, studies in non-small-cell lung cancer have shown that anti-miRNA155 can effectively suppress miRNA155 levels, leading to reduced tumor growth and increased immune system activation, particularly

when combined with standard therapies or immune checkpoint inhibitors [31]. In liver disease, inhibiting miR-155 may help in reducing the inflammation associated with HCV, limiting fibrosis, and preventing progression to HCC [10].

CTLA-4 blockade has emerged as a successful strategy in oncology, with several anti-CTLA-4 monoclonal antibodies such as ipilimumab that has been shown to enhance anti-tumor immune responses by preventing immune evasion mechanisms that tumors often exploit [32]. Given our findings of its upregulation in advanced liver disease, targeting CTLA-4 could help re-activate T cells, overcoming the immune evasion tactics employed by HCC cells. This approach is supported by studies demonstrating that anti-CTLA-4 therapies can improve patient survival in various cancers, including melanoma [33].

The concurrent targeting of both miR-155 and CTLA-4 could yield more profound effects than targeting either pathway alone. By inhibiting miR-155 to revitalize antiviral and anti-tumor immunity while also blocking CTLA-4 to enhance T cell activation, there is potential for a more robust therapeutic response. Additionally, combining these strategies with other immune checkpoint inhibitors, such as PD-1/PD-L1 blockade, could create a multifaceted approach to overcoming resistance mechanisms in HCC [34].

Our understanding of the molecular mechanisms underlying HCV-induced liver fibrosis and HCC could open new avenues for therapeutic interventions. One of the promising strategies involves the dual targeting of miRNA 155 and CTLA-4. This combination approach holds potential not only for reducing chronic inflammation but also for enhancing anti-tumor immunity.

5. Conclusion

Our findings indicate that both CTLA-4 and miRNA 155 could serve as promising biomarkers for the progression of liver disease in the context of HCV infection. The potential for these molecules to be targets for immunomodulatory therapies is particularly intriguing. By enhancing antiviral immune responses and simultaneously addressing the mechanisms of immune escape from HCC, therapeutic strategies could be designed to improve patient outcomes. Future studies should focus on exploring the combined therapeutic effects of miRNA-155 inhibition and CTLA-4 blockade to improve outcomes for patients with advanced liver diseases and HCC. The intricate relationship between miRNA-155 and CTLA-4 provides valuable insights into the immune landscape of chronic liver diseases and HCC. Future studies should focus on delineating the mechanistic pathways underlying this relationship and exploring therapeutic interventions that couldexploit these findings to improve antiviral and anti-tumor immunity.

List of abbreviations

MiRNA 155: Micro RNA 155
CTLA-4: Cytotoxic T-lymphocyte associated antigen 4
HCC: Hepatocellular carcinoma
HCV: Hepatitis C Virus
T reg: Regulatory T cell
CLD: chronic liver disease
CHC: Chronic Hepatitis C
EDTA: ethylenediaminetetraacetic acid
Sn: Sensitivity
Sp: Specificity
AUC: Area under curve
C.I: Confidence Interval
OR: Odd ratio

Conflict of interest: No conflict of interest was reported by the authors

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