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Study of MiRNA-155Gene Expression in Egyptian Patients with Chronic Hepatitis C Viral infection

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Keywords

- Expression
- HCC
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Abstract

Objective: to evaluate the miRNA -155 expression in patients with chronic HCV infection & correlate it with hepatitis C viral load and treatment response.

Background: MicroRNAs (miRNAs), are small, single-stranded, noncoding RNAs that consist of 20 to 25 base pair (bp).It is a class of small RNAs that regulate mRNA translation and function as oncogenes or tumor suppressor genes. In patients infected with HCV, miRNA-155 expression levels were markedly increased and promote hepatocyte proliferation and tumorigenesis by modulating Wnt signaling**.**

Subjects and Methods: This study was conducted on 20 HCC patients, 60chronic liver disease (CLD) patients due to HCV infection subdivided in to20 patients with HCVnaive treatment,20 patients responder treatment to interferon,20 patients non responder treatment to interferon patients and 20 healthy subjects matching age and gender. Serum AFP was measured for all participants. The relative expression of miRNA-155 was determined in whole blood samples using Real-time polymerase chain reaction.

Results: The results revealed over expression of miRNA-155 in each of HCC patient group, patients with HCVnaive treatment and patients non responder to interferon treatment. However, miRNA-155 showed down expression inpatients responder to interferon treatment.miRNA-155 expression was positively correlated with presence of cirrhosis, increased number of focal lesions, larger size of tumor, advanced tumor stage and presence of vascular invasion. From ROC curve analysis, the best cutoff of miRNA-155 chosen to differeniate HCC cases from non HCC subjects was 3.41RQs(Fold expression), and at this point the sensitivity, specificity, +ve predictive value (PPV), -ve predictive value (NPV) and Accuracy were 88.8%,91%, 92.4% ,89.5%,91.4% respectively. For AFP the best cutoff was 85.3ng/ml at this cutoff point the Sensitivity, Specificity , PPV, NPV, Accuracy were (76.2 % , 87.3 % ,, 90.2 % , 72.41 %, 81.0%). Furthermore, combined use of serum AFP and circulating miRNA-155for detection of HCC cases, had the advantage over the use of AFP alone as the sensitivity, specificity, PPV, NPP and overall accuracy were increased (89.32 %, 91.9 %, 93.5%, 90.71 % and 92 % respectively).

Conclusion: miRNA-155 expression might be correlated with hepatitis C viral load and treatment response. Also, miRNA-155could be a novel diagnostic and prognostic biomarker for detection of HCC in combination with AFP as well as it could serves as a potential therapeutic target for HCV and HCC infection

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INTRODUCTION

HCV was discovered in 1989 as the major causative agent of non-A non-B hepatitis (1) HCV is a major cause of post transfusion and community acquired hepatitis. Approximately 70–80% of HCV patients develop chronic hepatitis of which 20–30% leads to cirrhosis and HCC. Treatment options for chronic HCV infection are limited and a vaccine to prevent HCV infection is not available (2).

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and is associated with liver cirrhosis (LC) in 80% of cases(3).In Egypt, the incidence rate of HCC has increased sharply in the last decade(4).The development and progression of HCC is a complex process, which involves the dysregulation of oncogenes and tumor suppressor genes. It has previously been reported that microRNAs (miRNAs) are essential in oncogenesis by the regulation of oncogenes and tumor suppressor genes (5).MicroRNAs are approximately 22-nucleotide, noncoding, endogenous RNA molecules with an important role in various cellular biological processes, including embryonic development, cell differentiation, and tumorigenesis (6).. In humans miRNA-155 is encoded by the MIR155 host gene or MIR155HG.(7) miRNA-155 plays a role in various physiological and pathological processes (8). Exogenous molecular control in vivo of miRNA-155 expression may inhibit malignant growth (9), viral infections, (10) and enhance the progression of cardiovascular diseases.(11)In patients infected with HCV, miRNA-155 expression levels were markedly increased, and promote hepatocyte proliferation and tumorigenesis by modulating Wnt signaling **(**12**).** Chronic HCV infection induced liver fibrosis is mediated by upregulation of transforming growth factor (TGF)-β **(**13**).** recently miRNA-155 was found to be involved in TNF α and monocytes inflammatory activation which suggest a role for miRNA-155 in the immune response to HCV infection (14).

Better understanding of the molecular mechanisms involved in hepatocellular carcinogenesis contributes to identification of novel prognostic and diagnostic biomarkers and therapeutic targets for HCC. So this study aimed to evaluate the miRNA -155 expression in the patients with chronic HCV infection & HCC and correlate it with hepatitis C viral load and treatment response .

1. Subjects and Methods

1.1. Study population

The study was conducted on 100 subjects divided into 3 groups, selected from inpatient wards and outpatient clinic, National Liver Institute, Menoufia University .

The patients were grouped as following:

Group 1 (HCC on top of HCV infection group): This group included 20 newly diagnosed patients before receiving therapy. The diagnosis was based on clinical examination, laboratory tests, ultrasonography and spiral CT.

Group 2 (Chronic Viral Hepatitis C - HCV Group): This group included

- 20 patients with HCVnaive treatment matching age and gender..
- 20 patients(Responder treatment to Interferon)

- 20 patients(Non Responder treatment to Interferon)

They were diagnosed by ultrasonographical findings (shrunken liver, coarse echopattern, attenuated hepatic vein and fine nodular surface) and biochemical evidence of parenchymal damage as well as liver biopsy in some cases. the dose and duration of antiviral therapy is 180 mcg SC once weekly for 48 weeks

Group 3 (Control group): Included 20 apparently healthy subjects served as a control group,

The criteria for inclusion in this study:

1- The diagnosis of HCC was based on triphasic CT or contrast enhanced dynamic MRI.

The presence of typical features of arterial enhancement and rapid portal or delayed washout on one imaging technique was diagnostic of HCC for nodules >2cm in diameter in cirrhotic patients. In cases of uncertainty or atypical radiological finding, diagnosis was confirmed by biopsy .

The criteria for exclusion in this study:

None of the patients had bacterial or other viral infection, chronic renal damage, insulin-dependent diabetes mellitus (IDDM) and other malignant diseases. The patients undergoing immune-suppressive therapy were also excluded from this study.

All patients and control groups were subjected to the following:

1. Complete history taking.
2. Complete clinical examination.
3. Abdominal ultrasonography and or CT.

The study was approved by ethics committee of National Liver Institute, Menoufia University. Enrolment of individuals in the study was conditioned by an obtained written informed consent.

1.2. Laboratory investigations:

Ten ml venous blood samples were collected from patients and controls and divided into three aliquots; two aliquots used for routine laboratory investigations; liver function tests using fully automated auto analyzer SYNCHRON CX9ALX (Beckman Coulter Inc., CA, USA), CBC using Sysmex K-21, (Sysmex Corporation, Kobe, Japan) and immunoassay; serum HBs-Ag.by using (Abbott Laboratories, Abbott Park, IL, USA) and HCV-RNA by using Real time PCR . Serum AFP concentration was measured using the Automated Chemiluminescence System (ACS: 180 provided by Siemens Medical Solutions Diagnostics Corporation, USA). The third aliquot was collected in EDTA containing tube and used immediately for miRNA extraction and molecular testing.

2.3.Molecular testing:

Quantification of miRNA-155 using Real Time PCR Technology Using 7500 Fast Real Time PCR - TaqMan® microRNA Assay for: MiRNA-155 and its control gene (MiRNA-16)

3. Extraction and cDNA synthesis:

3.1. MiRNA extraction:

MiRNAs were extracted from fresh EDTA treated blood sample using QiagenmiRNA Extraction kit and QIAzol (Lysissoulution) according to the manufacturer's instructions. Single-stranded cDNAs were generated using TaqMan® MicroRNA Reverse Transcription Kit .

3.2.Amplification:

Quantitative PCR using the Applied Biosystems 7500 fast real time System (Applied

Biosystems, Foster City, CA) was used to determine levels of miRNA -155(by TaqMan miRNA Assay) using Universal TaqMan master mix according to the manufacturer's protocol. The primers for miRNA-155, miRNA-16, were supplied by Qiagen Germany.miRNA-155Forward Primer
CTCCTTCCTTTCAACAGAAAATGGA
Reverse
PrimerAAAACAAACATGGGCTTGACATTTAA
miRNA 16forward Primer
GCGAATCATTATTTGCTGCTCTAGAAA
Reverse
PrimerGCTCTGTAACAGCTCTGATACTTAACA

3.3.Quantification:

Quantification of gene expression of miRNA-155 was accomplished by measuring the fractional cycle number at which the amount of expression reached a fixed threshold (Ct), which was directly related to the amount of product. The relative quantification given by the Ct values was determined and the control gene Ct subtracted to achieve ΔCt [ΔCt = Ct (gene of interest) - Ct (control gene)]. Then relative expression level was determined as $\mathbf{2^{(-\Delta\Delta C_T)}}$, where ΔΔ Ct = ΔCt (target sample) - ΔCt (reference sample).

3.4.Statistical analysis:

Data were collected, tabulated and statistically analyzed by SPSS version 24.0 statistical package (SPSS, Inc, Chicago, IL, USA). ANOVA (followed by LSD post-hoc test), Kruskal Wallisand Pearson's correlation tests were performed at 5% level of significance. The diagnostic performance for miRNA-155 and AFP to discriminate HCC cases from those without HCC was evaluated using Receiver Operating Characteristic (ROC) curve analysis. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each marker were determined.

2. Results

A total of 100 subjects were enrolled in this study, 20 HCC patients (86% were males) with mean age of 46.35 ± 5.65years, 60 CLD patients (75% were males) with mean age of 42.00 ± 6.88 years, and 20 healthy volunteers (90% were males) with mean age of 40.40 ± 6.40years. The studied groups were homogenous in terms of age and gender (p>0.05).

- **Comparison of miRNA-155 and serumAFP among HCC, HCV (subgroups)and Control groups revealed** that There was significant increase of miRNA-155 in HCC group compared to each of(HCV NAÏVE,HCV responder ,HCV Non responder and control group) (p< 0.01).As well as there was significant increase of miRNA-155 in each of (HCV NAÏVE and HCV non-responder group compared to control group(p< 0.01).Mean while, there was no significant difference between control group and HCV Responder group regarding miRNA-155(p>0.05).Also, there was highly significant difference between all the studied group regarding AFP (p<0.01**).(Table1&fig1)**
- **Statistical comparison between clinical and pathological criteria of tumor regarding mean RQ of miRNA-155 in HCC group (No=20).**

The mean RQ of miRNA-155 showed significant increase, with presence of cirrhosis, increased number of focal lesions, larger size of tumor,

advanced tumor stage and presence of vascular invasion**(Table 2**)

- **Pearson Correlation Matrix Between miRNA-155 And different variables in HCC group**

There was significant correlation between miRNA-155 and each of (GGT and AFP)in HCC group. **(Table 3 & fig 4,5)**

- **Pearson Correlation Matrix Between miRNA-155 And different variables in HCV NAIVE group**
- There was significant correlation between miRNA-155 and DB in HCV NAÏVE group**. (Table 4&fig6)**
- **Pearson Correlation Matrix Between miRNA-155 And different variables in HCV NON Responder group(data not shown).**

There was significant correlation between miRNA-155 and each of (AST and prothrombin concentration) in HCV non responder group.

Receiver Operator of Characteristics (ROC) curve analysis of AFP and/or miRNA-155 in HCC group (No=20) versus non HCC: displayed that the best cut-off of serum AFP for differentiation of HCC cases from those without HCC was 85.3ng/ml at this cutoff point the Sensitivity , Specificity , +ve predictive value , -ve predictive value , Accuracy were (76.2 % , 87.3 % ,, 90.2 % , 72.41 %, 81.0 %).. For miRNA-155 the best cutoff was 3.41RQs and at this point the sensitivity, specificity, +PPV, -NPV and Accuracy were (88.8%91%, 92.4% ,89.5% ,91.4%) Respectively . Furthermore, combined use of serum AFP and circulating miRNA-155for detection of HCC cases, had the advantage over the use of AFP alone as the sensitivity, specificity, PPV, NPP and overall accuracy were increased (89.32 %, 91.9 %, 93.5%, 90.71 % and 92 % respectively).

Table (1):Comparison of MicroRNA -155 and Plasma AFP among HCC, HCV (Groups)and Control groups.

Studied variables	HCC (N=20) Mean ± SD	HCV NAIVE (N=20) Mean ± SD	HCV Responder (N=20) Mean ± SD	HCV Non Responder (N=20) Mean ± SD	Control (N=20) Mean ± SD	Kruskal-Wallis Test	P-Value	Tamhane Post Hoc p-value
Micro RNA 155 RQ (Folds)	**15.1± 7.62**	**2.05± 0. 53**	**1.11± 0.62**	**5.75 ± 1.8**	**1.06 ± 0.18**	**86.75**	**< 0.01****	P1= < 0.01** P2= < 0.01** P3= < 0.01** P4= < 0.01** P5 = < 0.01** P6 = > 0.05 P7 = < 0.01**
Plasma AFP (ng/ml)	**34.27 ± 22.28**	**6.27 ± 3.98**	**11.08 ± 9.34**	**13.6 ± 9.4**	**2.5± 0.79**	**53.4**	**< 0.01****	P1= < 0.01** P2= < 0.01** P3= < 0.01** P4= < 0.01** P5 = < 0.01** P6 = < 0.01** P7 = < 0.01**

RQ: relative quantity. p<0.01: significant. P1 between HCC and HCV NAÏVE. P2 between HCC and HCV Responder. P3 between HCC and HCV NonResponder. P4 between HCC and control. p5 between control and HCV NAÏVE. p6 between control and HCV Responder. p7 between control and HCV NonResponder

Table (2): Statistical comparison between clinical and pathological criteria of tumor regarding mean RQ of miRNA-155 in HCC group (No=20).

Studied variables	MiRNA-155 expression (fold change) in HCC group (No = 20) (M ± SD)	Mann-Whitney Test	P value
Cirrhosis Positive (No=14) Negative (No=6)	**18.0 ± 7.1** **8.27 ± 3.11**	**8.0**	**< 0.01****
Number of focal lesions Single (No=7) Multiple (No=13)	**8.12 ± 2.86** **18.85 ± 6.64**	**4.0**	**< 0.01****
Size of tumor < 3 cm (No=5) ≥ 3cm (No=15)	**10.3± 6.3** **16.6 ± 7.5**	**16.0**	**< 0.05***
Vascular invasion Positive (No=12) Negative (No=8)	**19.7 ± 6.14** **8.18 ± 2.7**	**3.1**	**< 0.01****
		Kruskal Wallis	
Stage of tumor (TNM) 1 (No=3) 2 (No=3) 3 (No=3) 4 (No=11)	**7.7 ± 4.7** **8.6 ± 1.4** **8.0 ± 0.6**5 **20.7 ± 5.2**	**14.28**	****** **< 0.01**
Type of tumor Trabecular (No=10) Trabecular &acinar (No=8) Sclerotic (No=2)	**14.3 ± 8.6** **15.7 ± 7.5** **17.1 ± 4.2**	**1.054**	**>0.05**

p<0.05: significant.
p>0.05: non-significant

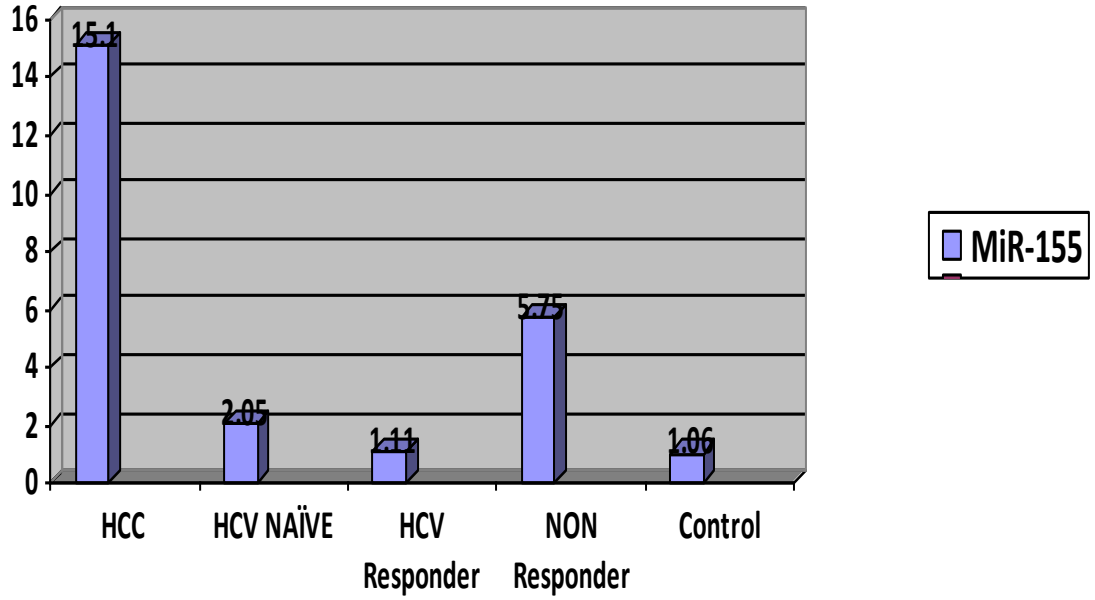

Figure 1:miRNA155 differences between Hepatocellular carcinoma, HCV(NAIVE , responder & non responder) and control groups

Table (3): Pearson Correlation Matrix Between miRNA-155 And different variables in HCC group

Studied variables	HCC (N=20) MiR-155 r*	P-Value
AST(IU/L) Up to 40	**0.284**	**>0.05**
ALT(IU/L) Up to 42	**0.271**	**>0.05**
ALP (IU/L) M Up to115 F Up to 104	**0.257**	**>0.05**
GGT (IU/L) 7 – 33	**-0.435**	**<0.05***
T.B (mg/dl) 0 – 1.0	**-0.93**	**> 0.05**
D.B (mg/dl) 0 – 0.25	**0.98**	**>0.05**
TP (g/dl) 6.5 – 8.5	**-0.31**	**>0.05**
Alb (g/dl) 3.5 - 5	**-0.205**	**> 0.05**
PC % 80 -120	**0.193**	**> 0.05**
AFP (ng/ml) Up to 10	**0.300**	**<0.01****

Serum albumin, T.B Total bilirubin , ALP Alkaline phosphatase. D.B Direct bilirubin, AST Serum aspartate aminotransferase, AFP Alpha fetoprotein , ALT Serum alanine aminotransferase, PC % Prothrombin concentration GGT Serum gama glutamyle transferase, TP Total protein

Table (4): Pearson Correlation Matrix Between miRNA-155 And different variables in HCV NAIVE group

Studied variables	HCV NAIVE (N=20) MiR-155 r*	P-Value
AST(IU/L) Up to 40	**0.218**	**>0.05**
ALT(IU/L) Up to 42	**0.132**	**>0.05**
ALP (IU/L) M Up to 115 F Up to 104	**0.425**	**>0.05**
GGT (IU/L) 7 – 33	**-0.014**	**>0.05**
T.B (mg/dl) 0 – 1.0	**0.447**	**>0.05**
D.B (mg/dl) 0 – 0.25	**-0.628**	**<0.05***
TP (g/dl) 6.5 – 8.5	**-0.445**	**>0.05**
Alb (g/dl) 3.5 - 5	**0.237**	**>0.05**
PC % 80 -120	**0.227**	**>0.05**
AFP (ng/ml) Up to 10	**-0.30**	**>0.05**

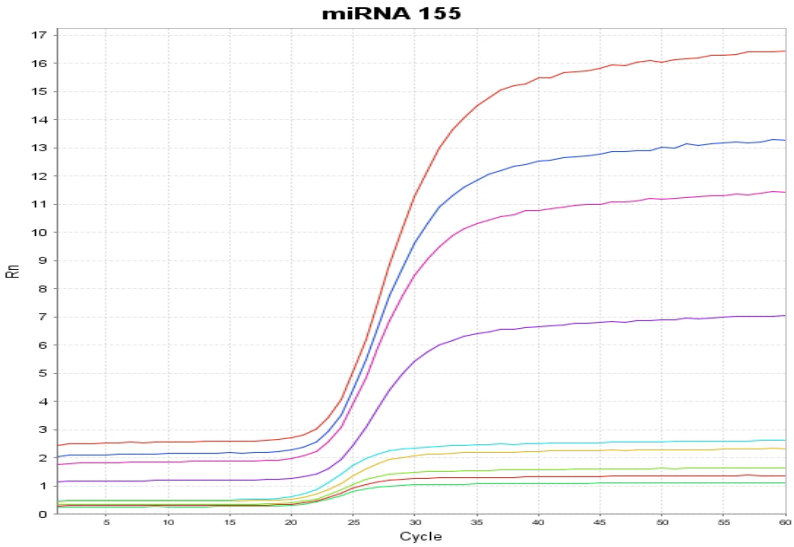

Figure 2: Amplification Plot (Rn vs. Cycle) of miRNA-155.

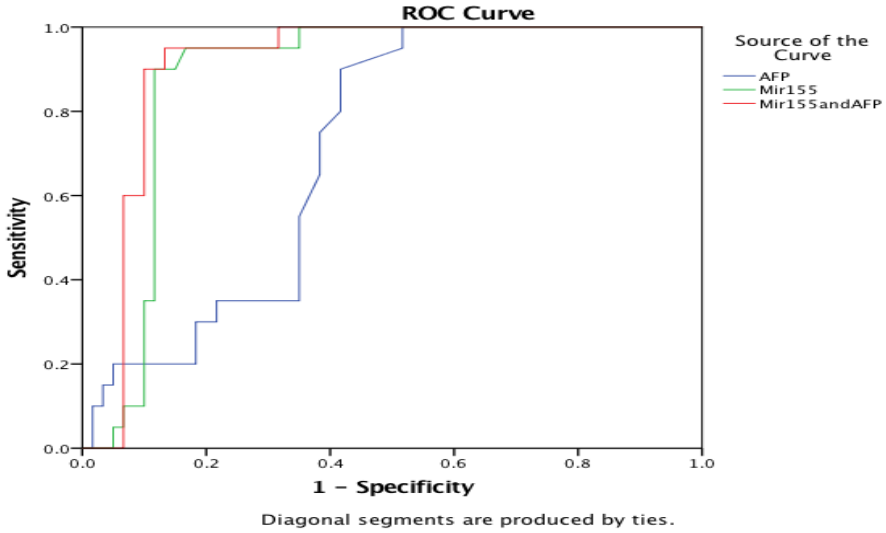

Figure 3: Receiver Operator of Characteristics (ROC) curve analysis of AFPand/or miRNA-155 in HCC group (No=20) versus non HCC .

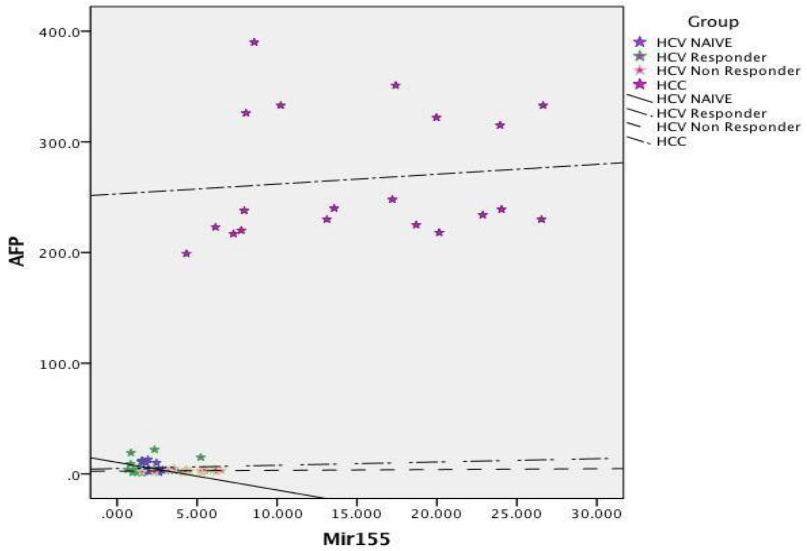

Figure 4:scatter plot with regression line showing correlation between miRNA-155 and AFP in HCC and HCV(NAÏVE,responder and non responder) groups

Discussion

HCV is considered the most common etiology of HCC in Egypt. HCC is the common primary liver cancer with increasing incidence to become the 5th common malignancy worldwide and the third leading cause of cancer-related death. In Egypt, there was an increase in HCC incidence among chronic liver patients, HCC was reported to account for about 4.7% of chronic liver disease patients **(15)**.

MicroRNAs (miRNAs) are approximately 22-nucleotide, noncoding, endogenous RNA molecules with an important role in a number of biological processes, including embryonic development, cell differentiation, and tumorigenesis**(16).** HCC as other malignancies is attributed to accumulated genetic alterations. As an oncomir, miRNA-155 gene was found to be over expressed in several solid tumors, such as thyroid carcinoma **(17)** breast cancer **(18,19),** cervical cancer **(20)** and lung cancer, where it is considered to be a marker of poor prognosis **(21)**. To date the world literature has revealed that among the presently known miRNAs, miRNA-155 is one of the miRNAs most consistently involved in neoplastic diseases. Indeed, the frequently detected up-regulation of miRNA-155 in malignant cells allows to consider this gene predominantly as an oncogene playing a role in the pathogenesis of many human cancers, such as malignancies of the haematopoietic system (i.e. Hodgkin's Lymphoma, some types of Non Hodgkin's Lymphoma, AML, and CLL).

This work demonstrated that miRNA-155 is up regulated in HCC where mean RQ of circulating miRNA-155 in patients with HCC was significantly higher than patients with HCV infected groups and healthy individuals, this was in agreement with Guan et al (22) and Hu et al (23).Who reported that miRNA-155 expression levels were enhanced in HCC tissues and this explained by

Circulating miRNAs originate from tumor tissues, and they are present in a certain form in the blood and are resistant to RNase activities (**24)**. However, it remains unclear how circulating miRNAs originate from tumor tissues. It was suggested that miRNAs could be derived from dying or lysed tumor cells, invasive lymphoma cells, cells from tissues affected by long-term disease, or the active secretion of tumor cells **(25)**.Other studies have investigated *miR-155* expression during hepatits C virus (HCV) infection, and a positive correlation was found between the posttreatment persistence of HCV RNA in the serum and peripheral blood mononuclear cells (PBMCs) of infected patients and the expression of the *miR-155* precursor (BIC) in PBMCs **(26).** Another study has shown that HCV replication was positively correlated to the increased expression of mature *miR-155* in PBMCs of HCV-infected patients **(27).** Furthermore, *miRNA-155* was upregulated in liver tissues, serum and PBMCs of genotypes 1, 2 and 3 HCV-infected patients **(28,29).**

In the current study it was found that treatment-naïve patients with chronic HCV infection have increased expression of circulating miRNA-155. Importantly, miRNA-155 levels were decreased in patients who have successfully cleared HCV infection after therapy, suggesting apossible correlation between increased miRNA-155 and HCV viral presence and/or replication and this is agree with **Bala et al (29).**

Table(5): Sensitivity, Specificity, PPV, NPV and Accuracy of AFP and/or miRNA-155 in HCC group (No=20) versus non HCC (CLD and Control groups (No=80).

The used marker	Sensitivity	Specificity	+ve predictive value	-ve predictive value	Accuracy
Plasma AFP at a cutoff point of (85.3) (ng/mL)	76.2 %	87.3%	90.2 %	72.41 %	81.0 %
MicroRNA-155 at cutoff point of (3.41) RQ	88.8 %	91%	92.4 %	89.5 %	91.4 %
Plasma AFP + MicroRNA-155	89.32 %	91.9 %	93.5 %	90.71 %	92 %

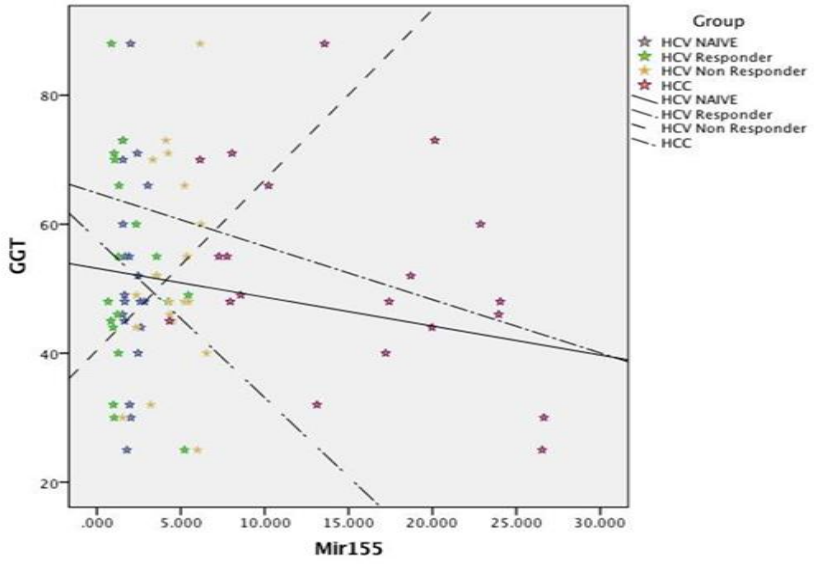

Figure 5:scatter plot with regression line showing correlation between miRNA-155 and GGT in HCC and HCV(NAÏVE,responder and non responder) groups

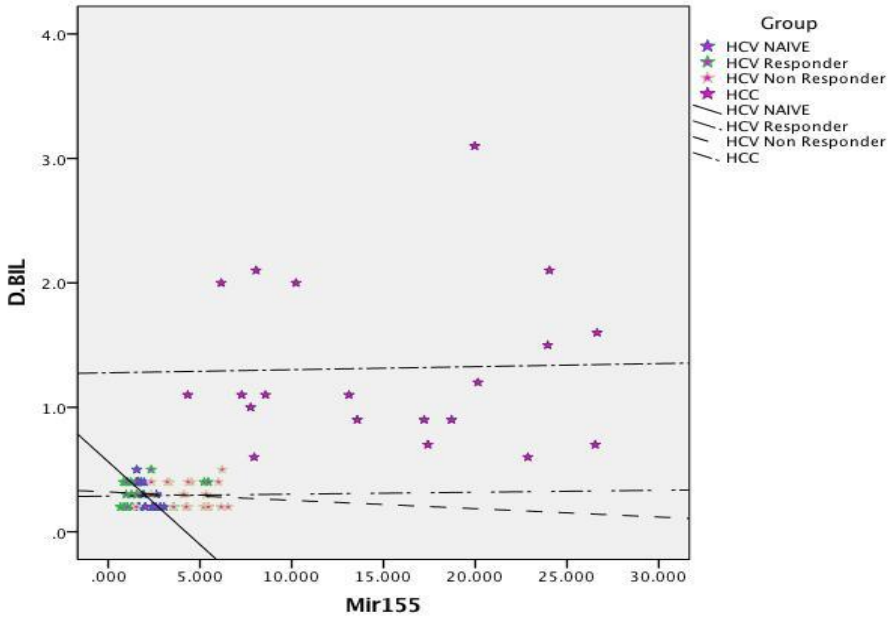

Figure 6:scatter plot with regression line showingcorrelation between miRNA-155 and D.Bil in HCC and HCV(NAÏVE, responder and non responder) groups

Recent studies suggest that miRNA-155 is not only limited to immune cells (dendritic cells, Kuffper cells, monocytes, NK cells, T cells), but also prevalent in non-immune cells (hepatocytes, endothelial cells) (29).

On the basis of these findings, it is most likely that both immune cells and hepatocytes contribute to increase of miRNA-155 in the circulation in HCV infection (29). Further studies are warranted to investigate the cellular source of circulating miRNAs.

In the current study, it is also observed that, the high expression of miRNA-155 was closely correlated with tumor size, TNM stage and vascular invasion, indicating that miRNA-155 may be involved in HCC progression, this was consistent with Hu et al (30)and song et al (31) who showed that miRNA-155expression level were enhanced in HCC tissues also there was an association between miRNA-155 expression level ,vascular invasion and tumor stage.

This is explained by many studies which have supported that high expression levels of miRNA-155 may be associated with high degree of malignancy and invasion in HCC. According to these data ,miRNA-155 appears to be involved in tumor progression through the inhibition of multiple tumor suppressor genes ,such as sex-determining region Y-gene related high mobility-group box gene (32,33) and suppressor in cytokine signaling1(34) thus promoting proliferation and invasion in HCC.

There is a close association between miRNA-155 expression levels and HCC prognosis (35,36). Huang et al., (37) demonstrated that there was an association between miRNA-155 expression levels and 5-year relapse –free survival (RFS) of patients with HCC following radical surgical resection. The 5-year RFS of patients expressing high levels of miRNA-155 was reduced compared with patients exhibiting low levels of miRNA-155 .It remains to be fully elucidated whether miRNA-155 is associated with early recurrence of HCC.

Regarding correlation between the mean relative quantity (RQ) of miRNA-155 and some studied parameters in this study, it was found that, a significant positive correlation between the mean RQs of miRNA-155 and AFP in HCC group was noticed.

In the current study There was significant correlation between miRNA-155 and each of GGT and AFP in HCC group. However, other reports demonstrated that no association was observed between miRNA-155 expression and α-fetoprotein levels of patients ($P>0.05$) or the other studied variables such as the gender, age, tumor size, tumor number.

In the current study there was significant correlation between miRNA-155 and direct bilirubin in HCV naïve group. Also, there was significant correlation between miRNA-155and each of AST and prothrombin concentration in HCV non responder group.

This study revealed that there was no significant correlation between ALT and miRNA-155 expression in HCC group and HCV patients groups and this in agree with Bala et al (29).

On the contrary de Bruijne et al., (38) demonstrated that miRNA-155 expression showed positive correlation with ALT and AST levels and

he suggested that this correlation may highlight the role of miRNA-155 in hepatic inflammation during GT4-cHCV infection.

Also this study revealed a significantly higher levels of AFP in HCC patients compared to the HCV naive patients, HCV responder patients, HCV non responder patients and control group. This was in agreement with Spadaro et al., (39) & Anwar et al., (40) who reported a significant elevation in serum AFP in HCC group compared to chronic liver patients and control group (39,40).

In the current study, the cutoff values and validity of serum AFP and circulating miRNA-155 for differentiation of HCC patients from those without HCC (CLD and controls) were determined by ROC curves.

From ROC curve analysis, the best cutoff of miRNA-155 chosen to differeniate HCC cases from non HCC subjects was 3.41RQs (fold change). and at this point the sensitivity, specificity, +ve predictive value(PPV), -ve predictive value(NPV), and Accuracy were 88.8%,91%, 92.4% ,89.5% ,91.4.% respectively and Thus, ROC curve analysis confirmed miRNA-155 as a valuable marker capable of discriminating the CLD patients, HCC and healthy individuals.

For AFP the best cutoff was 85.3ng/ml. at this cutoff point the Sensitivity, Specificity, PPV, NPV, Accuracy were (76.2% , 87.3 % , 90.2 % , 72.41 %, 81.0 %) respectively.

Furthermore, combined use of serum AFP and circulating miRNA-155for detection of HCC cases, had the advantage over the use of AFP alone as the sensitivity, specificity, PPV, NPP and overall accuracy were increased (89.32 %, 91.9 %, 93.5%, 90.71 % and 92 % respectively).

Guan et al., (22)stated that compared with AFP, changes in circulating miRNA-155 are earlier and more accurately reflect the process of the formation of tumors than AFP. Also, circulating miRNA-155 was an independent significant factor for recurrence and was reported to be more sensitive than AFP for the detection of HCC.

The above results give an attention about the possibility of using miRNA-155 as a potential therapeutic target and this was previously stated by Guan et al (22) who suggested that miRNA-155 is related to the clinical characaticals of HCC and it may be a novel diagnostic marker and potential therapeutic target in HCC.

Conclusion:

miRNA-155 expression might be correlated with hepatitis C viral load and treatment response. Also, miRNA-155could be a novel diagnostic and prognostic biomarker for detection of HCC in combination with AFP as well as it could serve as a potential therapeutic target for HCV and HCC infection.

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