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CIRCULATING MIRNA-122, MIRNA-155, AND MIRNA- 200 AS PREDICTORS FOR HEPATOCELLULAR CARCINOMA OCCURRENCE IN EGYPTIAN PATIENTS WITH HCV RELATED LIVER CIRRHOSIS TREATED WITH DIRECT ACTING ANTIVIRAL DRUGS

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Background and aim: *Hepatocellular carcinoma (HCC) is commonly attributed to cirrhosis related to chronic HCV infection. We aimed to evaluate the occurrence and the risk factors of HCC as well as assessment of the novel markers for HCC in patients treated with direct-acting antiviral drugs (DAAs).* ***Patients and methods****: We conducted a prospective study on 300 patients with HCV related liver cirrhosis treated with DAAs. Calculation of the incidence rate for HCC was carried out on annual basis. Identification of risk factors correlated with HCC was performed using Cox regression model. The relative expressions of miRNA 122,155 and 200 were measured in patients with HCC and without HCC after DAAs therapy.* ***Results:*** *300 patients with HCV related liver cirrhosis were included in the study. The annual rate of incidence of HCC in our cohort was 5 per 100 patients. The predictors for development of HCC in such patients were miRNA-122 and failure to achieve SVR. Patients with HCC had remarkably greater expressions of miRNA-122 , miRNA-155 and miRNA-200. miRNA-122 expression had the best diagnostic accuracy (94.4%) for prediction of HCC in patients who received DAAs at cutoff point > 26.14 fold with 83.3% sensitivity and 95.45% specificity.* ***Conclusion:*** *DAAs do not appear to be correlated with promotion of HCC following eradication of HCV in cirrhotic patients. We found that DAAs-related SVR is accompanied with lower incidence of HCC. miRNA-122 is taken into consideration as a propitious serum biomarker for the diagnosis of HCC patient after DAAs therapy*

Keywords: *HCV; Hepatocellular carcinoma ; Direct acting antiviral drugs ; miRNA- 122; miRNA-155; miRNA-200*

INTRODUCTION

Hepatocellular carcinoma (HCC) is the seventh most widespread cancer in women and the fifth in men over the globe[1]. HCC is considered as the third among the most popular reasons for death amid cirrhotic patients[2]. In the Egyptian demographics, HCC is ranked as the second most prevalent cancer in men and ranked the sixth most prevalent in women[3].

By the time diagnosis of HCC takes place; several patients have already reached an advanced stage. Direct acting antiviral (DAAs) are an unprecedented therapy of hepatitis C and its route of administration is completely oral. In

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vast majority of patients who undergoing treatment for hepatitis C as well as those with decompensated cirrhosis, DAAs are the used treatment[4]. Interferon-based therapy has been completely substituted by this type of treatment. An induction of sustained virological response (SVR) is doable by treatment of chronic hepatitis C with DAAs exceeding 85% of cases, despite the existence of liver cirrhosis. Close monitoring of cases with cirrhosis should be carried out after therapy[4]. It is believed that liver exposure to inflammation and development of fibrosis has a role in development of HCC over time. Therefore, in case DAAs are capable of eliminating inflammation mediated by HCV, the HCC risk should decline. Nevertheless, it has been observed by several centers that this in fact may not be the situation[5].

The genesis of tumor involves a multistep, multifactorial process. Risk of HCC may not be reduced sufficiently by elimination of HCV-induced inflammation only. Nevertheless, the advantageous effect of DAA treatment on the existence or recurrence of HCC is still a controversy topic. In fact, it has been assumed by studies that a possible unforeseen occurrence of HCC in patients after treatment, both in those with no previous record of cancer and in those who have accomplished their HCC treatment and remained disease-free for diverse durations of time[6-10]. On contrary, it has been suggested by other studies that patients with SVR who underwent DAA therapy are not at higher danger and also a lowering of occurrence or recurrence of HCC[11-12].

Alpha fetoprotein (AFP) is widely recognized as an extremely often used hematological diagnostic marker of HCC in the clinic. But with early phase HCC, its rate of false negative could be 40%. Nevertheless, patients with other diseases including cirrhosis or hepatitis, for example, may have an increase in levels of serum APF[23].

Thus, the need for novel markers in order to predict and diagnose HCC became a necessary demand to be sought. In accordance with earlier mechanisms, MicroRNAs (miRNAs) may own the potential functions. They are a class of tiny, single-stranded, non-coding molecules of RNA (21–30 nucleotides) which control gene expression in a negative manner at the stage following transcription through degradation or translational repression of target RNA, according to the extent of complementary base pairing[23].

It has been indicated by the recent studies that HCC cell apoptosis and proliferation were related to aberrant miRNAs expressions. There are miRNAs that induce HCC cell apoptosis and proliferation that were suppressive. Thus, the aforementioned miRNAs have the ability to act as cancer restraints to manage both progression and development of HCC by regulation of cell apoptosis and proliferation. Likewise, some miRNAs that are improperly expressed have been shown to suppress cell growth and death in HCC[24].

Within HCC cells, MiRNA-122 have been shown to be abundant and down regulated. Repression of proliferation as well as induction of apoptosis by picking out pyruvate kinase muscle 2 (PKM2) in HCC was attributable to the over expression of miRNA-122. According to another report, it has been figured out that miRNA-122 have the ability to downregulate the oncogenic distal-less 4 (DLX4) expression, suppression of this oncogene's activity may decrease the growth of HCC cells[25].

In recent times, miRNA-200 family members have been shown to have the prospective as diagnostic markers for identification of cirrhosis-associated HCC[26]. The MiRNA-155 importance is demonstrated in its role as an immunological modulator of pro-inflammatory actions, also, it is taken into consideration as linking element between inflammation and carcinogenesis[27]. As a result, the miRNA-155 expression analysis may aid in the prediction of prognosis of chronic hepatitis C in addition to the onset of fibrosis, cirrhosis and HCC[28].

Our present study was designed for evaluating the influence of treatment with DAAs on de novo HCC development by comparing some miRNA profiles after DAAs therapy in HCV cirrhotic patients with and without development of HCC.

PATIENTS AND METHODS

This is prospective observational study conducted on all patients with HCV related liver cirrhosis who attended Al-Rajhi Liver University Hospital -Assiut – Egypt in out-patient department and met the inclusion criteria from February 2019 to February

2020.The inclusion criteria were: liver cirrhosis with confirmed HCV infection, older than 18 years and eligible for treatment with DAAs. Exclusion criteria were: Patients refusing to participate in the study, patients with hepatitis B virus or other causes of cirrhosis. (Alcohol consumption, Biliary, Cardiac…), patients with cancers other than HCC or metastatic liver cancer, patients who developed HCC on transplanted liver, patients who were previously treated for HCC and patients who received any immunosuppression drugs. Drug regimens and treatment duration (12 or 24 weeks) were chosen by the clinicians according to the patients' clinical characteristics, literature recommendations and Egyptian guidelines for HCV treatment. All patients were followed up for one year. All subjects gave their informed written consent to participate in the study that was conducted according to the rules of Helsinki declaration and approved by the local ethics committee.

Three hundred cirrhotic patients with confirmed HCV infection were eligible for treatment with direct acting analogues, they were followed up for one year after the end of therapy. During the period of follow up, 15 patients (5%) developed HCC based on alpha fetoprotein and abdominal multi-slice computed tomography (MSCT), they were considered (**Study group 1**).They were matched according to age and sex to 30 patients from those who didn't develop HCC during the period of follow up, they were considered (**Control group 2**) as representative to those without HCC. So, the analysis was done between those groups.

Clinical and radiological follow up

Prior to receiving DAAs, all patients had their baseline clinical characteristics and laboratory data registered. Cirrhotic patients with no prior history of HCC underwent ultra-sound (US) screening by a specialized dedicated physician and alpha-fetoprotein (AFP) determination every 3 months to exclude the presence of active HCC after treatment with DAAs. When de novo HCC diagnosis was established, treatment was determined using a multidisciplinary approach according to Barcelona Clinic Liver Cancer (BCLC) schedule and European association for study of liver diseases (EASL) guidelines[29].

Conventional laboratory investigations include measurement of the following

Following laboratory tests were done: Liver function tests and serum alpha fetoprotein (AFP), HCV antibodies, quantitative HCV PCR using real-time PCR, kidney function test, HBs Ag, HBc total antibody, coagulation profile, complete blood picture and hbA1c.

Determination of serum level of miRNA-155, miRNA-200 and miRNA-122by RT-qPCR

RNAs Assay [novel markers: miRNA-122, miRNA-155, miRNA-200 assessed in all patients before and after DAA therapy]. Before starting therapy: samples preserved and stored in deep freezer (-80) for all patients with HCV-related liver cirrhosis (eligible for treatment) during enrolment in HCV treatment program.

Specimen collection

Five milliliters of venous blood were obtained from each contributor into an EDTA tube (BD, Franklin Lakes, NJ, and USA) and centrifuged for 10 minutes at 2000 rpm. Plasma was preserved at -80°C till the assay.

Extraction of miRNAs and reverse transcription into cDNA

We used Direct-zol™ RNA MiniPrep Kit (Zymoresearch, Catalog No. R2053, CA, USA) to extract total RNA, was including miRNA, from plasma. Biotech Nanodrop system was used to assess the RNA purity and concentration. To raise the poly a tail of small noncoding miRNAs, we used a Poly (A) polymerase enzyme (NEB, New England; Cat.no. M0276L).Then reverse transcription was carried out with a Thermo Scientific Revert Aid Reverse kit (Thermo, Waltham, MA, US) to yield cDNA.

The subsequent settings were used for quantitative real-time PCR (qRT-PCR) employing an Applied Biosystems (CA, USA) 7500 Fast Real-Time PCR System: 7-minute hot-start step at 95°C. Following that, for 40 cycles, initial denaturation at 95°C for 20 seconds, then annealing and extension at 59°C for 60 seconds. The relative expression levels of miRNA-122, miRNA-155, and miRNA-200 were computed using a particular formula: fold change= $2^{-\Delta\Delta CT}$.

Table 1: Sequence of primers used quantification of miRNA122, miRNA155, and miRNA200.

Primer name	Primer sequence
F-miRNA122	5'-GCTCTTCCCATTGCTCAAGATG-3′
R-miRNA122	5'-GTATGTAACAACAGCATGTG-3′
miR155-forward primer	5′-GACTGTTAATGCTAATCGTGATAG-3′
Reverse miR155	5′-GTGCAGGGTCCGAGGTATTC-3′
F miRNA 200	5′-GTAAATCGGTGTGTGTCGCGGGTC-3′
R miRNA 200	5′-CCGAGTCCCTGGGGACACTTC-3′
Forward U-6	5′-CGCTTCGGCAGCACATATAC-3′
Reverse U-6	5′-TTCACGAATTTGCGTGTCAT-3′

Statistical analysis

A SPSS 24.0 statistics software was employed (SPSS, Inc, Chicago, IL, USA). To gather, tabulate, and statistically analyses data, ANOVA (followed by LSD post-hoc test), Kruskal Wallis, and Pearson's correlation tests were used at the 5% significant level. ROC (Receiver Operating Characteristic) curve analysis was utilized to measure the diagnostic performance for miRNA-155 along with 122,200 to distinguish HCC cases from those without HCC. The PPV (positive predictive value), NPV (negative predictive value), specificity, and sensitivity were investigated for each marker.

RESULTS AND DISCUSSION

Results

Three hundred cirrhotic cases with confirmed HCV who received treatment with DAAs were monitored for one year after the therapy. Throughout the follow-up phase, 15 cases (5%) developed HCC based on alpha fetoprotein and abdominal multi-slice computed tomography (MSCT).

Patients' characteristics

Those patients who developed HCC (Study group1) were matched in accordance with age and sex to 30 patients from those who didn't develop HCC (Control group 2). So, the analysis was done between those groups.

Demographic data

Mean ages of HCC study group were 51.67 ± 6.38 years and the majority (80%) of them were less than 50 years old while the control group (without HCC) mean age was 49.63 ± 6.86 years and also, the great bulk of them (76.7%) were less than 50 years old.

Mean body mass index of HCC study group was 25.67 ± 3.09 kg/m^2 and 26.78 ± 2.98 kg/m^2 for the control group.

Majority of all patients in both groups were males and came from rural areas. Out of HCC study group, 2 (13.3%), 4 (26.7%), and 3 (20%) patients were smokers, diabetic and hypertensive, respectively while 3 (10%), 10 (33.3%) and 8 (26.7%) cases in the control group were smokers, diabetic and hypertensive, respectively, as illustrated in **Table (2)**

Laboratory data

In terms of baseline laboratory data, both groups were determined to have insignificant variations (P> 0.05). All enrolled patients were Child A5 with exception of two cases with HCC and three cases without HCC were Child A6. HCC group had significantly higher FIB-4 (2.74 ± 0.55 vs. 2.13 ± 0.13; P= 0.04) and APRI (6.87 ± 0.11 vs. 4.56 ± 0.32; P= 0.02) compared to those without HCC, as shown in **Table (3)**

Sustained virological response among enrolled groups

We noticed that all patients of the control group (without HCC) achieved SVR while only 5 patients (33.3%) of HCC study group achieved SVR and the majority (66.7%) of this group failed to achieve SVR.

Serum miRNAs expression (fold change) among enrolled groups

It was discovered that HCC cases had significantly greater expression of miRNA-122 (30.58 ± 1.22 vs. 14.52 ± 2.20 (fold); P< 0.001), miRNA-155 (25.67 ± 3.11 vs. 16.64 ± 2.01 (fold); P< 0.001) and micro-RNA-200 (24.66 ± 2.17 vs. 18.67 ± 1.50 (fold); P< 0.001), as observed in **Table (4)**

Table 2: Demographic data of studied groups.

Item	With HCC study group (n= 15)	Without-HCC control group (n= 30)	*P* value
Age (years)	51.67 ± 6.38	49.63 ± 6.86	0.45
Age group < 50 years > 50 years	 12 (80%) 3 (20%)	 23 (76.7%) 7 (23.3%)	0.91
Sex Male Female	 12 (80%) 3 (20%)	 21 (70%) 9 (30%)	0.36
BMI (kg/m^2)	25.67 ± 3.09	26.78 ± 2.98	0.53
Residence Rural Urban	 10 (66.7%) 5 (33.3%)	 22 (73.3%) 8 (26.7%)	0.76
Smoking	2 (13.3%)	3 (10%)	0.33
Diabetes mellitus	4 (26.7%)	10 (33.3%)	0.46
Hypertension	3 (20%)	8 (26.7%)	0.48
Previous therapy	5 (33.3%)	8 (26.7%)	0.76

Data illustrated as frequency (percentage), mean (SD). *P* value was significant if < 0.05. HCC: hepatocellular carcinoma; BMI: body mass index.

Table 3: Baseline laboratory data of studied groups.

Item	**With HCC study group (n= 15)**	**Without-HCC control group (n= 30)**	***P* value**
Hemoglobin (gm/dl)	12.45 ± 2.05	13.09 ± 3.33	0.10
Platelets (10^3/ul)	145.78 ± 45.67	149.01 ± 43.09	0.14
Leucocytes (10^3/ul)	4.09 ± 1.23	4.11 ± 1.54	0.53
Bilirubin (mg)dl	0.98 ± 0.13	1.01 ± 0.10	0.87
Albumin (mg/dl)	38.56 ± 2.87	39.87 ± 2.44	0.19
AST (u/l)	80.87 ± 10.34	79.01 ± 14.56	0.21
ALT (u/l)	84.13 ± 9.93	82.34 ± 14.45	0.17
Creatinine (mg/dl)	1.08 ± 0.13	1.01 ± 0.21	0.09
Urea (mg/dl)	4.34 ± 0.23	4.99 ± 1.14	0.19
INR	1.09 ± 0.01	1.08 ± 0.01	0.65
AFP (µg/dl)	14.56 ± 4.44	12.45 ± 2.10	0.16
Child Class **A5** **A6**	 13 (86.7%) 2 (13.3%)	 27 (90%) 3 (10%)	0.34
FIB-4	2.74 ± 0.55	2.13 ± 0.13	**0.04**
APRI	6.87 ± 0.11	4.56 ± 0.32	**0.02**
MELD	8.34 ± 2.22	7.34 ± 3.12	0.15

Data recorded as frequency (percentage), mean (SD). *P* value was significant if < 0.05. HCC: hepatocellular carcinoma; MELD: model for end-stage liver disease; APRI: AST to platelet ratio index; FIB-4: fibrosis-4; AST: aspartate transaminase; ALT; alanine transaminase; AFP: alpha fetoprotein

Table 4: Serum micro-RNAs expression (fold change) among enrolled groups.

Item	With HCC study group (n= 15)	Without-HCC control group (n= 30)	*P* value
micro-RNA-122	30.58 ± 1.22	14.52 ± 2.20	**< 0.001**
micro-RNA-155	25.67 ± 3.11	16.64 ± 2.01	**< 0.001**
micro-RNA-200	24.66 ± 2.17	18.67 ± 1.50	**< 0.001**

Data described as mean (SD). *P* value was significant if < 0.05. HCC: hepatocellular carcinoma; RNA: ribonucleic acid.

Pearson correlation of micro-RNAs with different variables in the study

Serum expression of miRNAs had insignificant correlations with different variables in the study with exception of positive significant correlation with FIB-4 and APRI, as described in **Table (5)**

Risk factors for hepatocellular carcinoma

Based on the current study the predictors for the progress of HCC in such patients were miRNA-122 (odd's ratio= 3.45, *P*< 0.001) and failure to achieve SVR (odd's ratio= 2.65, *P*= 0.02), as shown in **Table (6)**

Table 5: Correlation of micro-RNAs with different variables in the study.

Item	miRNA-122	miRNA-155	miRNA-200
Age (year)	0.34 (0.90)	0.11 (0.10)	0.10 (0.60)
BMI (kg/m^2)	0.11 (0.32)	0.10 (0.52)	0.10 (0.18)
Hemoglobin (gm/dl)	0.10 (0.09)	0.20 (0.30)	0.22 (0.19)
Platelets (10^3/ul)	-0.13 (0.13)	-0.10 (0.53)	0.10 (0.19)
Leucocytes (10^3/ul)	0.09 (0.10)	0.05 (0.23)	0.01 (0.22)
Bilirubin (mg)dl	0.19 (0.10)	0.20 (0.70)	0.09 (0.90)
Albumin (mg/dl)	0.17 (0.22)	0.20 (0.92)	0.13 (0.39)
AST (u/l)	0.21 (0.18)	0.10 (0.35)	0.04 (0.23)
ALT (u/l)	0.28 (0.19)	0.18 (0.09)	0.20 (0.45)
Creatinine (mg/dl)	-0.05 (0.19)	-0.10 (0.10)	-0.12 (0.10)
Urea (mg/dl)	0.07 (0.22)	0.11 (0.28)	0.17 (0.62)
INR	0.10 (0.20)	0.19 (0.45)	0.20 (0.80)
AFP (µg/dl)	0.13 (0.10)	0.03 (0.60)	0.10 (0.70)
FIB-4	**0.34 (0.03)**	**0.26 (0.04)**	**0.33 (0.04)**
APRI	**0.45 (0.01)**	**0.41(0.01)**	**0.43 (0.01)**
MELD	0.12 (0.21)	0.10 (0.41)	0.31 (0.09)

Data expressed as r value (strength of correlation) and P value (significance of correlation). MELD: model for end-stage liver disease; APRI: AST to platelet ratio index; FIB-4: fibrosis-4; AST: aspartate transaminase; ALT; alanine transaminase; AFP: alpha fetoprotein; BMI: body mass index.

Table 6: Predictors for development of HCC in patients received DAAs.

Variables	Odd's ratio	95% CI	*P* value
APRI	1.98	0.45-2.33	0.06
FIB-4	2.01	1.75-3.45	0.34
micro-RNA-122	3.45	2.34-6.76	< **0.001**
micro-RNA-155	1.45	0.98-2.34	0.23
micro-RNA-200	2.10	0.45-3.22	0.10
Failure to achieve SVR	2.65	1.65-3.78	**0.02**

P value was significant if < 0.05. CI: confidence interval; APRI: AST to platelet ratio index; FIB-4: fibrosis-4; AST: aspartate transaminase; RNA: ribonucleic acid; SVR: sustained virological response.

Diagnostic accuracy of miRNA-122 in prediction of HCC in cases received DAAs

According to our research; the serum expression of miRNA-122 had the best diagnostic accuracy (94.4%) for prediction of HCC in patients who received DAAs at cutoff point > 26.14fold with 83.3% sensitivity and 95.45% specificity with area under the curve 0.84 **(Fig. 1)**.

Characteristics of patients with hepatocellular carcinoma

Fifteen patients (5%) have developed HCC after DAA treatment, twelve patients (80%) are males below 50 years and 10 patients (66.7%) live in rural areas. Only 5 patients (33.3%) of HCC group accomplished SVR and the majority (66.7%) of this group failed to achieve SVI. Focal lesions sizes were (1.5 to 5.5 cm) and 14 patients (93.33%) had single hepatic focal lesion while only one patient had two hepatic focal lesions. Characteristics of HCC patients are more detailed illustrated in **Table (7)**. Considering our study, it was noticed that median time to onset of HCC following DAAs therapy was 7 months with 95% confidence interval was (6-10), as illustrated in **Fig. (2)**.

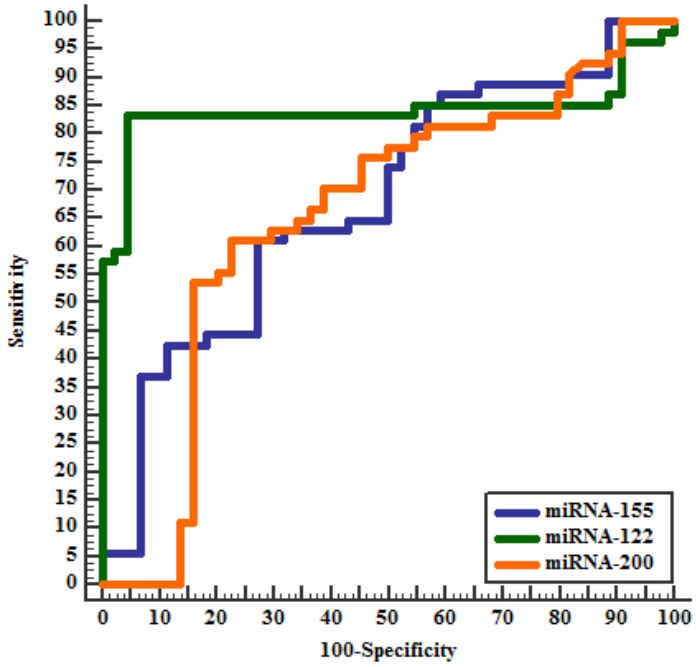

Fig. 1: Accuracy of micro-RNAs in prediction of HCC in patients received DAAs.

Table 7: Characteristics of studied cases with hepatocellular carcinoma after DAAs.

Case	Age	DM	SVR	Baseline AFP	Follow up AFP	Time	No of HCC	Size(cm)	micro-RNA-122	micro-RNA-155	micro-RNA-200
1	60		+	32	60.1	8m	2	2*4/2.5*4	55.46	14.00	27.13
2	43		+	12	21.5	4m	1	2.5*5	33.34	33.34	23.11
3	48		+	11	20	5m	1	3.3*4	32.76	32.76	24.40
4	49	+	+	13	75	6m	1	1.8*2.5	31.50	31.50	23.20
5	49		+	14	22.7	7m	1	2*4	31.20	31.20	22.00
6	45			12	1415	8m	1	2.5*4.5	30.99	30.99	24.66
7	37	+		23	336	6m	1	2.7*3.3	30.78	30.78	19
8	56	+		21	26.5	11m	1	1.5*3.5	30.36	30.36	24.11
9	48	+		11	33960	10m	1	2*4.5	30.33	30.33	24.91
10	44			11	36	11m	1	3*5.5	30.00	30.00	19.56
11	48	+		11	55	12m	1	2*5	30.00	30.00	27.00
12	45			12	22	6m	1	3*3	29.78	29.78	23.00
13	46			12	19.8	6m	1	3*2	29.76	29.76	24.96
14	44			11	21	6m	1	1.5*1.5	29.64	29.64	25.86
15	52			13	20	11m	1	2*4	29.46	15.38	23.32

No: number; HCC: hepatocellular carcinoma, SVR: sustained virological response, Non-SVR: non-sustained virological response, TTT: treatment; m: month, AFP: alpha-fetoprotein, DM: diabetes mellitus.

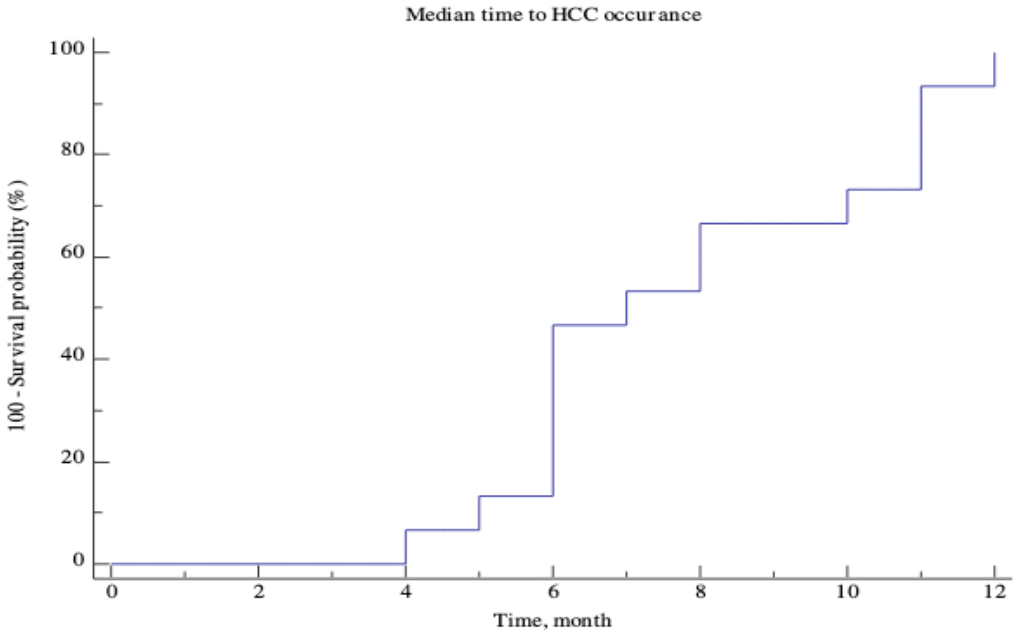

Fig. 2: Kaplan Meier curve: median time onset of HCC after DAAs.

Discussion

With the emergence of novel DAA therapies with higher SVR, anticipation of dropping HCC incidence has arisen[30]. In our study, the 5 % incidence rate for HCC per year was observed in HCV cirrhotic patients treated with DAAs. Our fifteen HCC patients were divided according to SVR: Five patients (33.3%) with SVR achievement and 10 patients (66.7%) with no SVR. It has been suggested by many recent studies that less rates of SVR in cases with HCV-related cirrhosis who developed HCC after DAA therapy. There are many suggested attributions for this,

comprising HCC functioning as a host for HCV replication, aberrant architecture of liver because of HCC, various resistant HCV strains infected cases with HCC, and immune dyes-regulation[31].

In accordance with our results, a prospective study reported 6.7% of HCC patients having SVR and 23.8% with no SVR[32]. On other hand, retrospective study from Italy incompatible with our findings, the investigation comprised 344 chronic HCV cases having cirrhosis and got various kinds of DAA; 91% of the cases had a SVR. After a 24 weeks follow-up, about 3.16% incidence rate was detected in cases without a history of prior HCC irrespective of DAA regimen used[5].

Other results similar to our study were found via Cardoso et al[10] and Kozbial et al[9]. Those investigations revealed 7.4% and 6.6% incidence rates of HCC in patients with cirrhosis who were treated with DAAs after one and two years of follow-up, respectively. Another study observed that in 242 Japanese patients, the incidence of HCC in patients with cirrhosis who were treated with DAA was 1.7% after one year follow up and 7% after two years follow up[33].

A crucial observation has been carried out in a European study in Belgium. Even though no difference was found in the rates of HCC incidence who were given DAAs with or without pegylated (Peg)-IFN therapy[17]. Moreover, important prospective study of large population carried in 2018 support our results. They mentioned a 2.9% incidence rate of HCC in 2249 cirrhotic cases after a one-year follow-up. In addition to that, they affirmed the fundamental impacts of eradication of HCV in cases with various phases of cirrhosis and stated that people with compensated cirrhosis without portal hypertension went through a remarkable decrease in the occurrence rate of HCC following SVR[22].

This was also confirmed by study which observed 3917 HCV cases were tracked up on following DAA treatment for an average of 536 (±192) days. They declared that in the first year, HCC prevalence rate was 0.46% in F3, 1.49% in CTP-A and 3.61% in CTP-B cirrhotic[34]. Besides, another trial found no increase in the incidence of HCC among individuals with HCV and HIV who were medicated with DAAs[35].

In spite of the fact that research with regards to the levels of expression and correlated actions of miRNA-122 in HCC and other liver disorders have been commonly conducted[36,37]. For example, miRNA-122 acted as a cancer deterrent gene and was shown to be diminished in HCC tissues and cells when compared to nearby normal cell lines as well as normal tissues[36,38]. On contrary, individuals with HCC, hepatitis C and other liver injury diseases all had a remarkable elevation in the serum expression measures of miRNA-122 in comparison with the healthy controls[39,40]. From this point of view, miRNA-122 may be touted as an onco-miRNA.

Our results revealed that individuals with HCC had substantially elevated expression of miRNA-122 (30.58 ± 1.22 vs. 14.52 ± 2.20 (fold);, miRNA-155 (25.67 ± 3.11 vs. 16.64 ± 2.01 (fold); and miRNA-200 (24.66 ± 2.17 vs. 18.67 ± 1.50 (fold) and it were also confirmed by the ROC curves analysis, in which miR-122 had greater values of AUC along with greater specificity and sensitivity in terms of certain cases developed HCC from individuals without HCC, after DAAs.

According to these evidences, miRNA-122 may serve as a serum biomarker candidate for identifying individuals susceptible to danger after DAAs treatment protocols especially in HCV-related liver cirrhotic cases. The raised prevalence of HCC observed in our cohort (5%) suggested that this biomarker could be utilized for improving surveillance program in DAA-treated patients[33,41]. Serum expression of miRNA-122 had the best diagnostic accuracy (94.4%) for prediction of HCC in patients received DAAs.

In general, multiple experiments had confirmed the predictive importance of serum miRNA-122 in HCC individuals, despite conflicting outcomes[42,43]. Matching our outcomes, Liu et al found that down regulations of miRNA-122 was related to a better prognosis in a group of HCC patients with BCLC B and BCLC C stages[43]. One study has showed incompatible conclusions[44].

The miRNA-122 expression was diversified in various categories and stages of liver malignant focal lesions as well as liver injury disorders. From a broad perspective of categorization, the miRNA-122 levels of expression were remarkably elevated in

individuals' serum with HCC when brought in comparison to healthy controls[45,46]. The miRNA-122 expression stages in the individuals' serum who were HBV-positive were repeatedly declined when brought in comparison with patients with benign liver lesions[47].

As far as we know, this study is one among of few case-control studies with repeated evaluations to determine the circulating miRNAs in HCV cases following therapy of DAAs to achieve identification of possible bio-indicators for the early detection of HCC. In spite of fundamental limitations of our research; the small number of cases; limited time of follow up, we have a belief that our results might take part in the current debate about occurrence of HCC after DAAs therapy as well as providing a new insight in terms of circulating biomarkers in translational medicine.

Conclusion

DAAs do not appear to be correlated with progression of HCC following elimination of HCV in cirrhotic patients. We discovered that DAAs -related SVR is accompanied with a drop in HCC incidence. It is considered that miRNA-122 is showing a potential as a serum biomarker for HCC diagnosis after DAAs therapy. However, surveillance of HCC should be continued even after HCV eradication. Larger population studies in the future may give established criteria for HCC surveillance following HCV eradication.

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نشرة العلوم الصيدلية
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ميكرو ار ان أي ١٢٢ و١٥٥و ٢٠٠ كمتنبئات لحدوث الأورام السرطانيه الكبديه في مرضى التليف الكبدى في مصر الناتج من الفيروس الكبدى سى الذين يتم علاجهم بالعقاقير المضاده للفيروسات ذات التاثير المباشر

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أجريت دراستنا الاستطلاعية على جميع المرضى الذين يعانون من تليف الكبد المرتبط بالتهاب الكبد الفيروسي والذين حضروا إلى مستشفى الراجحي للكبد بجامعة أسيوط واستوفوا معايير الانضمام للعلاج من فبراير ٢٠١٩ إلى فبراير ٢٠٢٠ . تمت متابعتهم لمدة عام واحد بعد انتهاء العلاج في دراستنا، كان متوسط عمر مجموعة سرطان الخلايا الكبدية ٦.٣٨ ±٥١.٦٧ سنة وكانت الأغلبية(٨٠%) منهم أقل من ٥٠ عامًا بينما كان متوسط عمر المجموعة غير المصابة بسرطان الكبد٦.٨٦ ± ٤٩.٦٣ عاماً، وأيضًا الأغلبية(٧٦.٧%) منهم كان عمره أقل من 50 عامًا، وكان غالبية المرضى من الذكور وجاءوا من المناطق الريفية.

في دراستنا، لاحظنا معدل حدوث سرطان الكبد بنسبة (٥%) سنويًا، وقد اصيب خمسة مرضى(٣٣.٣%) من الذين حققوا الاستجابة الفيروسية المستمرة لمضادات الفيروسات المباشرة بسرطان الكبد وكذلك اصيب٦٦.٧ (١٠%) مرضى لم يحققوا الاستجابة الفيروسية المستمرة لمضادات الفيروسات المباشرة بسرطان الكبد.

وجدنا في دراستنا أن المرضى الذين يعانون من سرطان الكبد لديهم قيم اعلى، من MIR-122 معدل حساسية ومعدل خصوصية أعلى في التمييز بين المرضى الذين طوروا سرطان الكبد من المرضى الذين لا يعانون من سرطان الكبد بعد العلاج.

وجدنا في دراستنا أن التعبير المصل MIR-122- له ارتباطات غير مهمة مع المتغيرات المختلفة في الدراسة باستثناء الارتباط الإيجابي المعنوي مع عوامل التليف الكبدىAPRI and FIB-4.

لقد توصلنا في دراستنا أن الأدوية المضادة للفيروسات ذات التأثير المباشر غير مرتبطة بتعزيزسرطان الكبد بعد علاج فيروس سى لدى مرضى التليف الكبدى.

لقد أكدنا أن الاستجابة الفيروسية المستمرة لمضادات الفيروسات المباشرة المرتبط تتربط مع انخفاض في حدوث سرطان الكبد .يعتبر MiR-122 علامة حيوية واعدة لتشخيص مريض سرطان الكبد بعد العلاج بالأدوية المضادة للفيروسات ذات التأثير المباشر.