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EVALUATION OF SERUM LEVEL OF MICRO RNA 224 IN PATIENTS WITH HEPATOCELLULAR CARCINOMA.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, and the third most common cause of cancer-related death that kills more than 650,000 people around the world each year (1).

The burden of HCC has been increasing in Egypt with a doubling in the incidence rate in the past 10 years , This has been mainly attributed to viral infection (hepatitis B and C virus), and dietary factors (e.g. aflatoxin B1) (2).

HCV causes HCC mainly through continuous inflammation and hepatocyte regeneration in the setting of chronic hepatitis and subsequent progression to cirrhosis, which is thought to lead to chromosomal damage and possibly to initiate hepatic carcinogenesis (3).

MicroRNAs (miRNAs), single-stranded nocoding RNAs of 19–25 nucleotides, account for approximately 1% of the human genome but are assumed to regulate more than 50% of all protein-coding genes (4).

The regulation of miRNAs on their target genes is mainly negative. It is reported that about half of human miRNAs localized in fragile chromosomal regions, which are associated with the onset of various human cancers (5), indicating that miRNAs are crucial in cancer development and progression.

MiRNAs can act as both tumor suppressors (by binding to oncogenes and suppressing them) and oncogenes (by binding to tumor suppressor genes and suppressing them) (5).

Zhang et al. reported that miR-224 played a role in cell proliferation, migration, invasion, and antiapoptosis in HCC by directly binding to its gene targets (6).Thus, miR-224 might be a potential biomarker for predicting the aggressiveness of HCC.

Venous metastasis, with tumor thrombi in the portal vein and the inferior vena cava, is a major hallmark of metastatic HCC and represents poor survival. Cheng et al. reported that 40%–90.2% of advanced HCC patients had portal vein tumor thrombosis (PVTT). Even in patients with HCC tumor smaller than 2 cm, 40.5% of them had microscopic venous invasion (7).

It is an urgent need for new biomarkers to predict the development of PVTT; then precaution treatments can be taken. The results revealed that miR-224 is one of the elevated miRNAs in the serum of HCC patients with PVTT in comparison with HCC patients without PVTT(7).

Aim Of The Work

The aim of the work is to detect the prognostic value of new micro RNA(microRNA 224) in different stages of hepatocellular carcinoma and after local radiofrequency ablation.

Patients and methods

This study included thirty five patients with hepatocellular carcinoma (HCC) on top of hepatitis C virus (HCV) related cirrhosis and twenty patients with liver cirrhosis without hepatocellular carcinoma, matched for age and sex to studied group , as control group , all the patients were selected from the admittes to Tropical Medicine Department El- Minia university hospital .

Liver cirrhosis was diagnosed based on clinical , biochemical (as liver function tests, prothrombin time and concentration, etc…..) , and sonographic findings suggestive of liver cirrhosis (as shrunken coarse liver , irregular border ,portal venous dilatation , ascites , splenomegally, etc…) .

Hepatocellular carcinoma was diagnosed according to EASL clinical practice guidelines 2012 : which recommended the non invasive criteria in patients with liver cirrhosis only ,based on imaging techniques obtained by 4-phase MDCT scan or dynamic enhanced MRI .

The patients were classified according to Child-Pugh classification (Child et al , 1962 and Pugh et al , 1973) into Child s classes A , B , C .

All HCC patients were classified according Barcelona classification for liver cancer (BCLC) (Forner A , et al , 2010 and EASL , 2012) into

Group A :

15 patients with Bacelona classification stage A (BCLC stage A) : single nodule or not more than 3 nodules , their size not more than 5 cm ,diagnosed as HCC by 4-phase MDCT for whom local ablation

therapy in the form of radiofrequency ablation (RF) using micro catheter introduced trancutanaeus for ablation of tumor nodules was recommended.

This group of HCC was eligible for RF ablation done by team of radiology department – El Minia university hospital.

Serum sample was taken before local ablation and another sample was drawn one month after radiofrequency ablation.

Group B :
Ten patients with BCLC stage B: multiple nodules with size more than 5cm with no vascular invasion.

Group C :
Ten Patients with BCLC stage C: multiple foci with vascular invasion (portal vein thrombosis).

Control group :
twenty patients with HCV related cirrhosis without HCC.

All patients were subjected to:
-thorough clinical history : e.g. age, sex, residence , special habits as smoking ,alcohol intake or drug abuse , past history of jaundice , operations , blood transfusion , parentral injections , history of diabetes or hypertension, etc…..

Present history with special analysis on history of abdominal pain , loss of appetite , loss of weight , abdominal distension , bleeding tendency , hematemesis or melena , change in color of urine or stool , bowel habits disturbances ,etc….

-clinical examination : -general examination as pallor , jaundice , oedema lower limb , finger clubbing , flapping tremors , palmar erythema , spider naevi,etc…

-Abdominal examination : stigmata of chronic liver disease as dilated veins , localized bulge , shrunken liver , hard liver , splenomegally , ascites , etc…….

- Laboratory investigations:
- Blood samples : 10cm of venous blood sample was taken , centrifuged and used for :
1- Complete blood picture using Minidry 3200 auto cell counter .
2- liver function tests (albumin , bilirubin , liver enzymes , alkaline phosphatase) determined by fully automated clinical chemistry Kone labe finloud .
3- prothrombin time and concentration.
4-blood urea and serum creatinine.
5- Fasting blood glucose level.

-serological assays :
1-HCV antibodies using ELISA technique.
2- alpha feto protein : done using enzyme linked immune fluorescence assay (ELIFA) biomerieux , France , with a cutoff value 400ng / ml for diagnosis of HCC (EASL ,2012) .

-Assay of microRNA 224:
-Small RNAs were extracted from 500 L of serum using a miR-PARIS kit (AM1556) according to the manufacturer's instructions.

- RNA Isolation:
- To allow for normalization of sample-to-sample variation in RNA isolation, synthetic Caenorhabditis elegans miRNAcel-miR-54 (purchased as a custom RNA oligo nucleotide from Qiagen) was added (50 pmol/L in a 5 L total volume) to each denatured sample.

- Quantitative Real-Time Reverse-Transcription- (RT-) PCR Assays:
-We used TaqMan miRNA probes (Applied Biosystems) to perform qRT-PCR assays according to the manufacturer's instructions.
-Briefly, 2 L aliquot of enriched small RNAs from serum samples was reverse transcribed using the Taq-Man MicroRNA Reverse Transcription Kit (Applied Biosystems, San Diego, CA).

-Then 2 L of the cDNA solution was used as template for the PCR stage.

-No-template controls for both RT step and PCR step were included to ensure targetspecific amplification.

-All reactions were run in duplicate.

-The CT(cycle threshold) (number of cycles required for the fluorescent signal to exceed back ground level) values of the different samples were compared using the ΔΔCT method (the difference between CT of MIR224 and CT of cel-miR -54) .The relative expression levels of target miRNAs were normalized by cel-miR-54.

Statistical analysis:
Statistical analysis was done using Statistical Package For Social Sciences Software (for windows 16.0, SPSS Inc. Chicago, IL , USA) . For comparison between parametric data, unpaired t-test was used to compare between two independent study groups . For comparison of non-parametric data, statistical chi-square test was used. Linear correlation between MIR 224 and other study variables was used using Pearson and Spearman rank correlation. P value less than 0.5 was considered statistically significant .

Results
This study included thirty five patients with hepatocellular carcinoma (HCC) on top of hepatitis C virus (HCV) related cirrhosis . patients divided into three groups according to Barcelona classification :

-group A : fivteen patients with BCLC A , in whom radiofrequency ablation was applied .

-group B: ten patients with BCLC B.
-group C : ten patients with BCLC C .

And twenty patients with liver cirrhosis without hepatocellular carcinoma , matched for age and sex were taken as control group .
All patients subjected to thorough clinical history , clinical examination , laboratory investigations , assay of serum AFP and MIR 224.

The results of our study were tabulated and illustrated in the following tables and figures:

Table 1 and 2 : shows the demographic data of studied groups of HCC and control group of LC , it was found that the mean age for HCC patients was52±4 , the mean age for control group was48± 10. As regard HCC patients , the mean age for group A was 45±11 , the mean age for group B was 55± 8 , the mean age for group C was 57±9.

Also the percentage of males in HCC group was 48.6 % while females 51.4% , in control group the percentage of males was 35% while females 65% .

There was no statistically significant differences between different groups.

Table 3 : shows the mean level of laboratory parameters in studied HCC patients , it was found that there were statistically significant difference between the mean level of HB, with the highest level in group A 11.3±1.3 and lowest level in group C 9.7±0.9 , ALT level with the highest level in group C 114±1.4 and lowest level in group A 28±7.8 , and bilirubin level with the highest level in group C 5.6 ±0.1 and lowest level in group A 1.1±0.29

Also there was no statistically significant difference between the

mean level of platelet count among different HCC groups.

Table 4 : shows the Child –pugh classes in different HCC groups , there was statistically significant differences between Child classes among HCC groups , in group A 60% of patients had Child class A while 40% had Child class B, in group B , 80% of patients had Child class B , while 20% had child class C , in group C ,100% of patients had Child class C.

Table 5: shows Child class in HCC patients versus control group of LC , there was no statistically significant difference between HCC patients and LC patients .

Table 6 and figure 1 : shows The mean of level of MIR 224 in studied patients of HCC and control groups, there was statistically significant difference between the mean level of MIR 224 between different HCC groups and between HCC patients and control group of LC , with the lowest concentration found in control group while the highest concentration found in HCC group C .

Table 7 : shows the mean level of MIR 224 among HCC patients versus control group of LC , there was statistically significant difference between the mean level of MIR 224 in HCC patients (11.6± 0.5) versus control group of LC (6.1±1.3).

Table 8 : the mean and median for level of AFP in studied patients , there was statistically significant difference between the mean and median of the level of AFP among different HCC groups and control group .

Table 9 : the mean of level of AFP in HCC patients versus control group of LC , there was statistically significant difference between the mean level of AFP in HCC patients (2680±3873) versus control group of LC (47.6 ± 88.6).

Table (10) : shows Correlations between level of MIR 224 and laboratory parameters in studied groups of patients with HCC and those with LC , there was statistically significant correlation between level of MIR 224 and both levels of AFP , HB , total bilirubin and ALT level , however there was no statistically significant difference between level of MIR 224 and platelet count.

Table (11) : shows The MDCT data of patients with HCC . The percentage of solitary nodules was 42.8% , of multicentric lesion 28.6 , and of multiple nodules 28.6%. the percentage of lesions less than 2 cm was 46.6% while more than 2cm was 53.4% . presence of portal vein thrombosis in 28.6 % of patients .

Table 12 : shows the Correlation between level of MIR 224 and triphasic multislice CT data in studied groups. This table shows statistically significant correlation between the level of MIR 224 and the data obtained by multislice CT abdomen as regard , prescence of portal vein thrombosis , multiplicity of nodules and large size of nodules in all studied HCC groups .

Table 13 : shows the Sensitivity, specificity, positive predicted value and negative predicted value of MiR 224 when performed in HCC and cirrhotic groups : the sensitivity was 97.1% , specificity was 95%, PPV was 95% and NPV was 97% and this was statistically significant .

Table (14) : shows the Sensitivity, specificity, positive predicted value and negative predicted value of AFP when performed in HCC and cirrhotic groups, the sensitivity was 45.7% , specificity was 95% , PPV was 90% and NPV was 63.6% and this was statistically significant.

Figure (2): shows the diagnostic performance of MIR 224 in discriminating patients with HCC from cirrhotic patients without HCC . ROC curve obtained by plot at different cut-offs for MIR 224 in HCC versus all controls; The area under the curve is 0.994 for MIR 224 with Std. Error=0.007 and 95% Confidence Interval.

This ROC curves indicated that MIR224 at a CT value of 8.25 yielded the best sensitivity and specificity for differentiating patients with HCC from those without HCC as whole, with sensitivity 97.1 % and specificity 95% .

Figure (3) : shows the diagnostic performance of AFP in discriminating patients with HCC from cirrhotic patients without HCC. The area under the curve is 0.812 for AFP with Std. Error=0.059 and 95% Confidence Interval. This ROC curves indicated that AFP at a value of 405 ng/ml yielded the best sensitivity and specificity for differentiating patients with HCC from those without HCC as whole, with sensitivity 45.7 % and specificity 95% .

Figure (4) : shows comparison between the diagnostic performance of MIR 224 and AFP in discriminating patients with HCC from cirrhotic patients without HCC:

This curve shows that the sensitivity of MIR 224 is much significant than the sensitivity of AFP in diagnosis of patients with HCC , while they have approximately the same spesificity .

Figure (5) : Shows relation between the level of MIR 224 in HCC patients without portal vein thrombosis (PVT) and those with portal vein thrombosis.

The graph shows that there is a significant difference between the level of MIR 224 in HCC patients without PVT (low levels) and those with PVT (high levels) , with p value less than 0.001 which is statistically significant.

Table (15) and figure (6) : Show the change occurring in level of MIR 224 before and after radiofrequency ablation in group A (BCLC A).

It shows that there is a significant difference between the mean of level MIR 224 in group A (15 patients) before and after one month of treatment with radiofrequency ablation ,in which successful ablation was confirmed by multislice CT abdomen after one month of ablation , this was statistically significant with p value less than 0.001.

Table (1), (2) : Demographic data of the studied groups:

		Patients with HCC (n=35)			Patients with LC(n=20)	
Patients Groups		Group A (n= 15)	Group B (n= 10)	Group C (n=10)	Control group (n=20)	P value
Age in years (mean ± SD)		45±11	55±8	57± 9	48± 10	0.776
Sex	Male	8(55%)	4(40%)	5(50%)	8(35%)	0.545
	Female	7(45%)	6 (60%)	5(50%)	12(65%)	0.667

		HCC(n=35)	LC (n=20)	P value
Age in years (mean ± SD)		52±4	48± 10	0.544
Sex	Male	17(48.6%)	8(35%)	0.889
	Female	18(51.4%)	12(65%)	0.887

Table 3 : The mean levels of laboratory data in different HCC groups :

	Mean ± SD			P value	P value
Lab parameters mean±SD	Group A	Group B	Group C		
HB(gm%)	11.3±1.3	10.3± 1.4	9.7±.95	P1 0.001 P2 0.001 P3 0.001	0.001

Platelets	1658001 ±6515	1108003±8159	904564±8657	P10.001 P20.001 P30.001	0.001
ALT(IU|ml)	28.8±7.8	63.8±6.3	114.6±1.4	P10.001 P20.001 P30.001	0.001
Bilirubin(mg| dl)	1.1±0.29	2.9±.003	5.6±0.1	P10.001 P20.001 P30.001	0.001

P1=group A versus group B
P2=group A versus group C
P3=group B versus group C

Table (4) : Child-Pugh scoring in different groups of HCC patients;

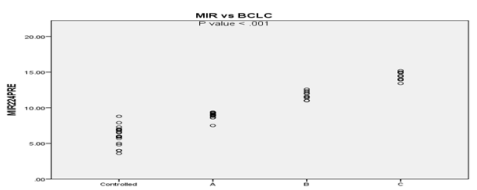
variants	Group A(n=15)	Group B(n=10)	Group C(n=10)	P value
Child scoring	9A (60%) 6 B (40%)	8 B (80%) 2 C (20%)	10 C (100%)	0.001

Table (5) : Child-Pugh scoring in HCC patients versus control ;

Child scoring	HCC (n=35)	Control Group B(n=20)	P value
Child A	9 (25.7%)	0(0%)	0.63
Child B	14(40%)	7(35%)	0.66
Child C	12(34.3%)	13(65%)	0.778

Table(6) , and figure (1) ; The mean of level of MIR 224 in studied patients of HCC and control groups :

	Group A	Group B	Group C	Control group	P value
MIR 224(CT value) (Mean ± SD)	8.9±0.4	11.7± 0.5	14.4±.0.6	6.1±1.3	0.001



Table(7) ; The mean of level of MIR 224 in HCC patients versus control :

variants	HCC (n=35)	Control group(n=20)	P value
MIR 224(CT value) (Mean ± SD)	11.66±0.5	6.1±1.3	0.001

Table(8); The mean and median of level of AFP in studied groups:

	Group A	Group B	Group C	Control group	P value
AFP(ng\ml) (median)	35	510	688	19	0.001
AFP(ng\ml) (mean± SD)	134.2± 183.4	615± 426.2	1931 ± 3264	47.6 ± 88.6	0.001

Table(9) ; The mean of level of AFP in HCC patients versus control :

variants	HCC (n=35)	Control group(n=20)	P value
AFP(ng\ ml) (Mean ± SD)	2680±3873	47.6 ± 88.6	0.001

Table (10) : Correlation between level of MIR 224 and laboratory parameters in studied groups:

	MIR 224(CT value)	
	R	P value
AFP(ng\ml)	0.667	< 0.001*
HB%	-0.532	< 0.01*
Bilirubin(mg|dl)	0.737	< 0.001*
ALT(IU)	0.544	0.001
Platelets count	0.07	0.7

Table (11) : The MDCT data of patients with HCC :

C T data		No(%)
No. of lesions	Solitary	15(42.8%)
	Multicentric	10(28.6%)
	Multiple	10(28.6%)
Size of solitary lesion	Less than 2 cm	7(46.6%)
	More than 2 cm	8(53.4%)
Portal vein	Patent	25(71.4%)
	Thrombosed	10(28.6%)

Table (12) : Correlation between level of MIR 224 and triphasic multislice CT data in studied groups :

	R	P value
PVT	0.784	< 0.001*
Large size of nodules	0.645	< 0.001*
Multiplicity of nodules	0.637	< 0.001*

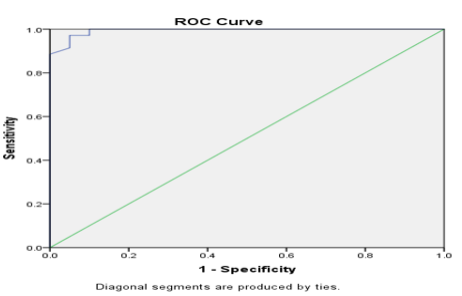
Table (13) : shows the Sensitivity, specificity, positive predicted value and negative predicted value of MiR 224 when performed in HCC and cirrhotic groups :

Variable	Cut off value	Sensitivity	specificity	P P V	N P V	P value
MIR 224(CT value)	8.25	97.1%	95%	95%	97%	0.001

Table (14) : shows the Sensitivity, specificity, positive predicted value and negative predicted value of AFP when performed in HCC and cirrhotic groups :

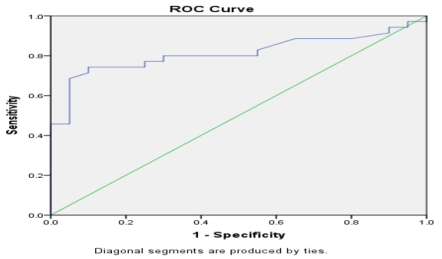
Variable	Cut off value	Sensitivity	Specificity	P P V	N P V	P value
AFP(ng\ml)	405	45.7%	95%	90%	63.6%	0.001

Figure (2): shows the diagnostic performance of MIR 224 in discriminating patients with HCC from cirrhotic patients without HCC



Area	Std. Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.994	.007	.000	.980	1.000

Figure (3) : shows the diagnostic performance of AFP in discriminating patients with HCC from cirrhotic patients without HCC.



Area	Std. Errora	Asymptotic Sig.b	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.812	.059	.000	.697	.927

Figure (4) : shows comparison between the diagnostic performance of MIR 224 and AFP in discriminating patients with HCC from cirrhotic patients without HCC:

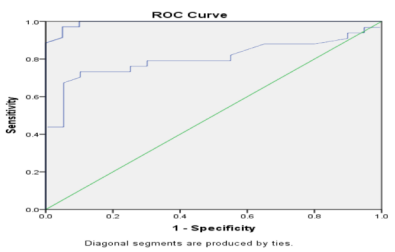


Figure (5) : Shows relation between the level of MIR 224 in HCC patients without portal vein thrombosis (PVT) and those with portal vein thrombosis

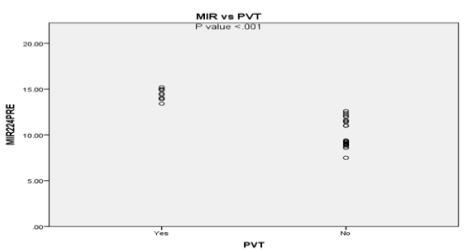
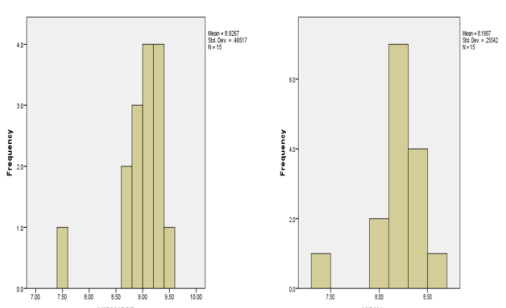


Table (15) and figure (6) : Show the change occurring in level of MIR 224 before and after radiofrequency ablation in group A (BCLC A) :

	Before(Mean ± SD)	After(Mean ± SD)	P value
(1)MIR (A)	9.40±3.26	8.16±.25	< 0.001*

(1) Wilcoxon Signed Ranks Test between the two groups
*: Significant difference at p value < 0.0



Discussion

Hepatocellular carcinoma is the fifth most common cancer and the second most frequent cause of cancer-related death globally, with 854,000 new cases and 810,000 deaths per year, accounting for 7% of all cancers. Hepatocellular carcinoma (HCC) represents about 90% of primary liver cancers and constitutes a major global health problem (8).

MicroRNAs (miRNAs), single-stranded nocoding RNAs of 19–25 nucleotides, account for approximately 1% of the human genome but are assumed to regulate more than 50% of all protein-coding genes (9)

It is reported that about half of human miRNAs localized in fragile chromosomal regions, which are associated with the onset of various human cancers , indicating that miRNAs are crucial in cancer development and progression (3) .

MiRNAs can act as both tumor suppressors (by binding to oncogenes and suppressing them) and oncogenes (by binding to tumor suppressor genes and suppressing them) . MiR-224 is one of the most commonly overexpressed miRNAs in hepatocellular carcinoma (HCC) tissues (10) .

The upregulation of miR-224 starts form the precancerous stage and persists throughout HCC development (11).

MiR-224 played a role in cell proliferation, migration, invasion, and antiapoptosis in HCC by directly binding to its gene targets, thus, miR-224might be a potential biomarker for predicting the aggressiveness of HCC (6) .

Our study included thirty five patients with hepatocellular carcinoma on top of HCV induced cirrhosis divided into 3 groups according to BCLC classification , in addition to twenty cirrhotic patients without HCC as a control group .

Interestingly, the statistical ROC curve analysis showed that miR-224 had excellent sensitivity (97.1%) , specificity (95%) and positive predictive value (95%) as compared to AFP (45%) , (90%) , (90%) respectively, and could serve as potential biomarker to predict HCC at the early stage.

The area under the curve was 0.994 at cut off value 8(CT value) with p value 0.001 while for AFP it was 0.812 at cut off value 400 ng | ml with p value 0.001.

These findings was consistent with Wataru Okajima and his colleges who found that the plasma level of miR-224 was significantly higher in the HCC patients than in the healthy volunteers (P < 0.0001), The AUC value for the plasma miR-224 analysis was 0.908.

The optimal cut-off point was 8.0 in relative expression using the miR-224/cel-miR-39 ratio with a sensitivity of 93.1%, a specificity of 80.0% in his large scale analysis including 87 HCC patients and 55 healthy volunteers (12).

Also this findings was consistent with Khalda , et al , 2017,(13) who studied 40 HCC patients and 40 chronic HCV patients , and found that MiRNA 224 had sensitivity 92.5% , specificity 90% with area under the curve 0.94 with p value 0.001 .

Venous metastasis, with tumor thrombi in the portal vein and the inferior vena cava, is a major hallmark of metastatic HCC and represents poor survival , it is reported that 40%–90.2% of advanced HCC patients had portal vein tumor thrombosis (PVTT), even in patients with HCC tumor smaller than 2 cm, 40.5% of them had microscopic venous invasion (14).

We found that serum level of MiRNA 224 was higher in HCC patients with portal vein thrombosis and other multislice CT abdomen parameters of poor prognosis as multiplicity , large size and presence of satellites , than in patients with HCC without portal vein thrombosis and this finding was statistically significant with P value 0.001.

This was consistent with Li-Ping Zhnang , et al , 2016, (15) who found that serum level of MiRNA 224 was higher in HCC patients with portal vein thrombosis than in patients without portal vein

thrombosis in his study which included 182 patients and these results was significant (P value 0.005).

Also these results were consistent with results of Samah Mamdouh , et al , 2017, (16) who studied 50 HCC patients and 20 healthy volunteers and found that serum level of MiRNA 224 was higher in BCLC C sub group (presence of portal vein thrombosis) than in other sub groups and these results were significant (P value 0.002).

When evaluating the ability of MiRNA 224 in predicting grade of HCC, we found that the serum level of MiRNA 224 was increased as the tumor grade increase with its lowest level in BCLC A and its highest level in BCLC C , and these results was significant (P value 0.001), denoting the ability of MiRNA 224 to predict grade of HCC.

These results were consistent with results of(Samah Mamdouh , et al , 2017) ,(16) who found the same results in her study and it was statistically significant (P value 0.02).

Variables associated with liver function and inflammation in liver were significantly different in patients with low serum miR-224 level when compared with high serum miR-224 level. The results showed that serum levels miR-224 showed significant correlation with parameters of liver damage, such as ALT, AST, and bilirubin level (P values were 0.001). In addition, significant correlation between serum level of miR-224 and AFP was also evident (p value 0.001).

This was consistent with study of(Li-ping Zhuang , et al ,2015) ,(15) who found a significant correlation between level of MIR 224 and liver related parameters (ALT , bilirubin level) and also between level of MIR 224 and AFP level in his studied patients (p value 0.001), also this was consistent with results of Samah Mamdouh , et al , 2017(16) who studied 50 HCC patients, and found strong correlation between MIR 224 level and liver related parameters such as ALT ,and bilirubin levels (P value 0.001).

Our study showed that there is a significant difference between the mean of level MIR 224 in group A (15 patients) before and after one month of treatment with radiofrequency ablation ,in which successful ablation was confirmed by multislice CT abdomen after one month of ablation , this was statistically significant with p value less than 0.001, denoting the dynamic action of MIR 224 in predicting response to treatment .

This was consistent with Wataru , et al ,2017,(12) who analyzed 10 patients with
subsequently resected tumors after local therapies, such as percutaneous ablation therapy and TACE, Pathologically, 2 patients had no remaining cancer cells, whereas the remaining 8 patients had residual tumors.

The plasma miR-224 levels were significantly higher in the 8 patients with residual tumors than in patients without remaining cancer cells (P = 0.0318).

Unfortunately there was a limited data about role of MIR 224 in predicting recurrence after local ablation therapy and more studies are needed about this topic of interest.

In conclusion , MIR 224 can play an important role in diagnosis of HCC especially in early stages in comparison to traditional diagnostic biomarkers as AFP, it also serves as a prognostic marker to occurrence of venous metastases especially Portal vein thrombosis ,and also as an indicator of successful ablation after local ablative therapy as radiofrequency ablation .

REFERENCES

1- Z.-Y. Tang, "Hepatocellular carcinoma—cause, treatment and metastasis," World Journal of Gastroenterology, vol. 7, no. 4, pp. 445–454, 2016.
2- Elgharably A, Gomaa AI, Crossey MME, Norsworthy PJ, Waked I, Taylor-Robinson SD. Hepatitis C in Egypt – past, present, and future. Int J Gen Med. ;10:1–6 , 2017.
3- M.Mitsunobu, A. Toyosaka, T. Oriyama, E. Okamoto, and N. Nakao, "Intrahepatic metastases in hepatocellular carcinoma: the role of the portal vein as an efferent vessel," Clinical & Experimental Metastasis, vol. 14, no. 6, pp. 520–529, 2015.
2. 4-D. P. Bartel, "MicrRNAs: genomics, biogensis, mechanism and function," Cell, vol. 116, pp. 281–297, 2004.
3. 5-G. A. Calin, C. Sevignani, C. D. Dumitru et al., "Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers," Proceedings of the National Academy of Sciences of the United States of America, vol. 101, no. 9, pp. 2999–3004, 2017.
4. 6-Y. Zhang, S. Takahashi, A. Tasaka, T. Yoshima,H.Ochi, and K. Chayama, "Involvement of microRNA-224 in cell proliferation, migration, invasion, and anti-apoptosis in hepatocellular carcinoma,"
5. Journal of Gastroenterology and Hepatology, vol. 28, no. 3, pp. 565–575, 2016.
6. 7-S. Cheng, M. Wu, H. Chen et al., "Tumor thrombus types influence the prognosis of hepatocellular carcinoma with the tumor thrombi in the portal vein," Hepato-Gastroenterology, vol. 54, no. 74, pp. 499–502, 2007.
7. 8-EASL guidelines , 2018.
8. 9-J. Krol, I. Loedige, and W. Filipowicz, "The widespread regulation of microRNA biogenesis, function and decay," Nature Reviews Genetics, vol. 11, no. 9, pp. 597–610, 2016.
9. 10-Y. Wang, H. C. Toh, P. Chow et al., "MicroRNA-224 is up-regulated in hepatocellular carcinoma through epigenetic mechanisms," The FASEB Journal, vol. 26, no. 7, pp. 3032–3041, 2017.
10. 11- P. Gao, C. C.-L. Wong, E. K.-K. Tung, J. M.-F. Lee, C.-M. Wong, and I. O.-L. Ng, "Deregulation of microRNA expression occurs early and accumulates in early stages of HBV-associated multistep hepatocarcinogenesis," Journal of Hepatology, vol. 54, no. 6, pp. 1177–1184, 2016.
11. 12-Wataru Okajima , Shuhei Komatsu , et al , " circulating micro RNA profiles in plasma : identification of miR – 224 as anovel diagnostic biomarker in hepatocellular carcinoma independent of hepatic function " , Oncotarget , vol. 7, No. 33 , 2017.
12. 13-KhaldaS.Amr , Hanan Abd El mawgoud Atia , et al , " early diagnostic evaluation of miR – 122 and miR – 224 as biomarkers for hepatocellular carcinoma " , Genes & diseases , volume 4 , P 215-221 , 2017.
13. 14- G.-Y. Chau, W.-Y. Lui, K.-L. King, and C.-W. Wu, "Evaluation of effect of hemihepatic vascular occlusion and the pringle maneuver during hepatic resection for patients with hepatocellular carcinoma and impaired liver function," World Journal of Surgery, vol. 29, no. 11, pp. 1374–1383, 2018.
14. 15- Li-ping Zhuang , Zhi-Qiangmeng , serum mIR 224 reflect stage of hepatocellular carcinoma and predict survival , Biomed research international , volume 2015, article ID 731781 , 2016.
15. 16- Samah Mamdouh et al , " Evaluation of Mir – 224 , Mir – 215, Mir – 143 as serum biomarkers for HCV associated hepatocellular carcinoma" , Asian Pac J cancer pev, 18(11), 3167-3171 , 2017