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HEPATOCELLULAR CARCINOMA;
BIOMARKERS: A MOLECULAR APPROACH FOR EARLY DETECTION

Dr. Amin Fahim[1] Prof. Dr. Mohammad Ahmed Azmi[2] Dr. Muhammad Hanif[3] Dr. Anila Qureshi[4]

1. Department of Pathology, Al-Tibri Medical College, Karachi
2. Department of Physiology, Al-Tibri Medical College, Karachi
3. Karachi Institute of Radiology and Nuclear Medicine,Kiran Hospital Karachi
4. Department of Pathology, Al-Tibri Medical College, Karachi

Correspondence Address:
Dr. Amin Fahim,
M.Phil. Assistant Professor
Deptt: of Pathology
Al-Tibri Medical College
Old Thana, Gadap Town,
Malir, Karachi
draminfahim@gmail.com

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ABSTRACT: Objectives: To determine the expression of miR-92-1 in HCC patients and correlate them with clinicopathological data. **Study Design:** A Cross sectional study. **Place of Study:** Samples were collected from Jinnah Postgraduate Medical Center Karachi, Civil Hospital Karachi and Asian Institute of Medical Sciences Hyderabad. **Duration of Study:** January 2014 to December 2014. **Materials and Methods:** 150 patients of hepatocellular carcinoma were divided into two groups on the basis of etiologic agent. HCC patients with chronic viral hepatitis B were labeled as group-I, whereas those with chronic viral hepatitis C in group-II, compared with 75 healthy control individuals in group-III. **Results:** The results showed that miR-92-1 expression was decreased in patients with HCC when compared with controls. Down regulation was seen more pronounced in HCC cases with chronic viral hepatitis C. A significant correlation was found between the expression of miR-92-1 and AFP level 20-200 ng/ml and Child Pugh score B. However no correlation was found between the expression of miR-92-1 and age, gender, fibro scan, tumor distribution, tumor size, tumor metastasis and BCLC stage. **Conclusion:** miR-92-1 is down regulated in HCC patients with significant correlation with serum AFP level between 20-200 ng/ml.

Keywords: Hepatocellular carcinoma, micro RNA, Child Pugh score, Viral hepatitis.

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INTRODUCTION

Hepatocellular carcinoma (HCC) represents a global health problem, ranks fifth in worldwide cancer incidence. In males it ranks as 2nd most common cause of mortality due to tumors whereas in females it is at 6th position. Approximately 626,000 new HCC cases and 600,000 mortalities due to HCC are reported worldwide annually.[1,2] Only between 30-40 percent of persons suffering from HCC are able to receive restorative cure, from the time when the cancer is diagnosed which is the main cause of excessive death rate.[2] Survival rate of HCC is only 5% in five years because of delayed diagnosis.[3]

The most documented and recognized reasons leading to liver cirrhosis and HCC are chronic hepatitis B and C. HCV leads at 31.1% and HBV 54.4% cases of HCC around the world.[4] It is estimated that around 520 to 570 million patients suffer from chronic hepatitis B in Sub-Saharan Africa and Asia-Pacific, marking these region on a very high scale of occurrence.[5,6] On the other side chronic hepatitis C is very evident in West in addition to Japan.[7,8]

Various reports on newly identified microRNA (miRNA), present in serum and plasma are a very promising area of research, also indicating their utilization in the detection of cancer.[1,9] Since the microRNA were initially noticed in 1993, more than 1500 miR have been recorded in miR Base database.[2,4] These miR are comprised of about 19-23 nucleotides (nt) which participate in various cellular homeostatic processes, pathogenic events and imbalances or dysfunctions which leads to various infectious diseases, cancer growth and existence of individuals suffering from cancer.[10,11,12]

Dysregulation in the several miRNAs, including miR- 122, miR-16, miR-21, miR-196b, miR-25, miR-375 and let-7f showing either over expression or down regulation in chronic hepatitis, liver fibrosis

and HCC individuals in comparison with controls have been reported.[13,14,15] For example most abundantly found miRNA is miR- 122, in liver, also modulated the fat metabolism beside controlling and modifying the HCV multiplication. In patients with HCC miR-122 was the most commonly down-regulated and its down-regulation was associated with poor outcome.[16] In addition to miR-122, role of various miR in chronic hepatitis due to viral etiology is being studied by many researchers. In addition miR-92-1 usefulness in treating patients while having hepatic necrosis, the progress to liver cirrhosis and subsequent carcinogenesis has been discussed widely.[16] Expression of miR-92-1 advances initiation and extension of both CD4 and CD8 Immune cells, white blood cells at the early phase of allo-response in vivo.[17] The polycistronic miR-17~92 cluster encodes miR-92-1 which is reported to be overexpressed in HCC. This has also been noticed in different tumors, including the expression involved in carcinogenesis of HCC cell line.[18] Furthermore miR-92-1 silencing group, in vitro brought about a half decrease in the cellular division and development of hepatocytes.[18] Its interface with the HCV genome has remained as an area of pivotal interest. This study is designed to observe the expression pattern of miR-92-1 in patients with HCC due to viral etiology by HBV and HCV infection in comparison to the healthy individuals.

MATERIALS AND METHODS

A purposive non probability sampling was carried out to include 150 patients of hepatocellular carcinoma divided into two groups on the basis of etiologic agent. HCC patients with chronic viral hepatitis B were labeled as group-I, whereas those with chronic viral hepatitis C were kept in group-II and compared with 75 healthy control individuals in group-III. The samples were collected from Jinnah Postgraduate Medical Center Karachi, Civil Hospital Karachi and Asian Institute of Medical Sciences Hyderabad during January 2014 to December 2014. Patients with HCC due to alcoholic hepatitis, non-alcoholic fatty liver disease, primary biliary cirrhosis, aflatoxin B1, exposure to vinyl chloride, xenobiotics, diabetes, hemochromatosis and α1-antitrypsin deficiency were excluded from the study.

The complete biodata regarding personal information, relevant history, clinical findings and other laboratory reports such as LFT, HBsAg, Anti HCV Ab, PCR results, serum AFP level, Ultrasonic findings, other imaging techniques and clinical staging employed were recorded in the well-designed Proforma. Total RNA was extracted from the serum samples using the RNA purification Kit (Trizol LS) and cDNA was synthesized by using specific primers of GAPDH forward, GAPDH reverse, 5S forward and 5S reverse as internal controls with Random hexamer primers. Initially extraction of total RNA from serum samples was followed by cDNA synthesis. Finally Real time PCR was performed using the specifically designed primers of miRNA 92-1.

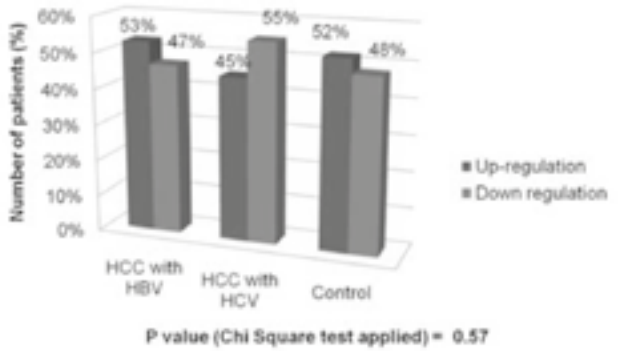
miR 92-1 forward
5'-TCTACACAGGTTGGGATCGG- 3'
miR 92-1 reverse
5'- CGGGACAAGTGCAATACCATA- 3'

The data was analyzed on SPSS version 21.0. The normality of the data for different variable was checked with Chi-square test. Sigmoid curves for expression of miRNA were taken by Bio-Rad CFX software. Correlation between the expression of miRNA and various clinicopathological parameters was done by univariate analysis using Odds ratio and 95% confidence interval. A p-value ≤ 0.05 was taken as statistically significant.

RESULTS

In the present study a total of 225 serum samples were evaluated for the expression of miRNA-92-1. All the information regarding personal bio-data and history, clinical based findings, laboratory-based findings, ultrasonic findings and imaging techniques were recorded and mentioned in the well-designed information proforma. The assessment in all group of persons were then reconfirmed and compared between the laboratory profile, clinical findings and clinical staging of cancer status with the expression of miR-92-1 using real time PCR.

Regarding the expression of miR-92-1 in healthy control group-III, 39(52.0%) individuals showed up-regulation in comparison with the down regulation shown in 35(46.7%) patients in group-I with HCC patients with HBV infection and 41(54.7%) patients in group-II with HCC patients with HCV infection. A non-significant (p-value = 0.57) was found between the groups (shown in graph-I and figure-1).



Graph-1. Expression of miR-92-1 in HCC pts and controls (n=225)

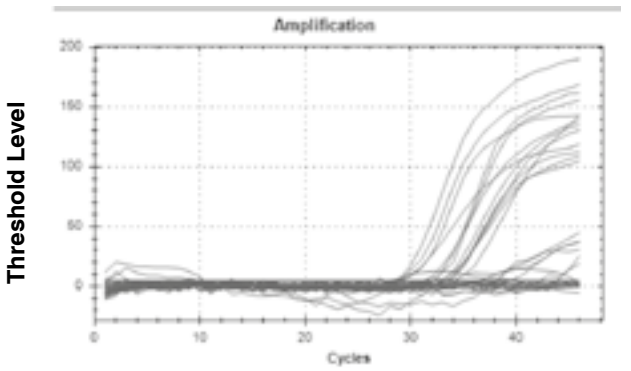


Figure-1. Expression of miR-92-1 in HCC patients and controls. Straight lines below the threshold level showing down regulation in patients with HCC and sigmoid curves showing up-regulation in healthy controls

Regarding the correlation of expression of miR-92-1, Upregulation was seen in 42(50.6%) male patients and down regulation was seen in 41(49.4%) male patients, whereas 32(47.8%) and 35(52.2%) female patients showed up-regulation and down regulation respectively. The p-value was found to be 0.42 which is non-significant (shown in table-I).

Variable	miR-92-1		x2 Test
	High	Low	P value
Gender			
Male	42(50.6%)	41(49.4%)	0.42
Female	32(47.8%)	35(52.2%)	
Age			
< 40 years	13(56.5%)	10(43.5%)	0.76
40-49 years	29(48.3%)	31(51.7%)	
50-59 years	22(44.9%)	27(55.1%)	
> 60 years	10(55.6%)	8(44.4%)	
AFP			
< 20	46(66.7%)	23(33.3%)	0.001
20-200	1(4.8%)	20(95.2%)	
> 200	27(45%)	33(55%)	
Fibro scan			
F0-F1	3(42.9%)	4(57.1%)	0.73
F2-F3	63(50.8%)	61(49.2%)	
F4	8(42.1%)	11 (57.9%)	
Tumor distribution			
Solitary	53(53.5%)	46(46.5%)	0.10
Multifocal	21(41.2%)	30(58.8%)	
Tumor size			
< 2 cm	20(55.6%)	16(44.4%)	0.68
2-5 cm	26(46.4%)	30(53.6%)	
> 5 cm	28(48.3%)	30(51.7%)	
Tumor metastasis			
Absent	63(46.0%)	74(54.0%)	0.02
Nodal metastasis	8(80.0%)	2(20.0%)	
Distant metastasis	3(100.0%)	0(0.0%)	
Child Pugh Score			
A	52(58.4%)	37(41.6%)	0.001
B	16(29.6%)	38(70.4%)	
C	6(85.7%)	1(14.3%)	
BCLC stage			
O (Very early)	15(71.4%)	6(28.6%)	0.001
A (Early)	38(79.2%)	10(20.8%)	
B (Intermediate)	3(6.2%)	45(93.8%)	
C (Advanced)	14(53.8%)	12(46.2%)	
D (Terminal)	4(57.1%)	3(42.9%)	

Table-I. Correlation of expression of miR-92-1 in HCC patients with clinicopathological parameters (n=150)

Regarding the comparison with expression of miR-92-1 with age groups, up-regulation was seen in 13(56.5%) patients below 40 years of age, 29(48.3%) patients between 40-49 years, 22(44.9%) patients between 50-59 years and 10(55.6%) patients above the age of 60 years. The down regulation was seen in 10(43.5%) patients below 40 years of age, 31(51.7%) patients between 40-49 years, 27(55.1%) patients between 50-59 years and 8(44.4%) patients above the age of 60 years. The p-value was found to be 0.76 which is non-significant (shown in table-I).

Regarding the comparison of AFP in HCC patients positive for HBV and/or HCV infection with expression of miR-92-1, up-regulation was seen in 46(66.7%) patients with AFP below 20(ng/ml), 1(4.8%) with AFP between 20-200(ng/ml) and 27(45.0%) with elevated AFP above 200(ng/ml). The down regulation of miR-92-1 was seen in 23(33.3%) with AFP below 20(ng/ml), 20(95.2%) with AFP between 20-200(ng/ml) and 33(55.0%) with elevated AFP above 200(ng/ml). The p-value was found to be 0.001 which is highly significant (shown in table-I).

Regarding the comparison of fibro scan in patients with HCC positive for HBV and HCV infection with expression of miR-92-1, up-regulation was seen in 3(42.9%) patients with stage F0-F1, 63(50.8%) in stage F2-F3 and 8(42.1%) in stage F4. The down regulation was seen in 4(57.1%) patients with stage F0-F1, 61(49.2%) patients with stage F2-F3 and 11(57.9%) patients with stage F4. The p-value was found to be 0.73 which is non-significant (shown in table-I).

Regarding the comparison of tumor distribution in patients with HCC positive for HBV and HCV infection with expression of miR-92-1, up-regulation was seen in 53(53.5%) patients presenting with solitary tumor and 21(41.2%) patients with multifocal tumor. The down regulation was seen in 46(46.5%) patients presenting with solitary tumor and 30(58.8%) patients with multifocal tumor. The p-value was found to be 0.10 which is non-significant (shown in table-I).

Regarding the comparison of size of tumor in HCC patients positive for HBV and/or HCV infection with expression of miR-92-1, up-regulation was seen in 20(55.6%) patients with tumor size less than 2cm, 26(46.4%) with tumor size 2-5cm and 28(48.3%) patients with tumor size greater than 5cm. The down regulation was seen in 16(44.4%) patients with tumor size less than 2cm, 30(53.6%) patients with tumor size 2-5cm and 30(51.7%) patients with tumor size greater than 5cm. The p-value was found to be 0.68 which is non-significant (shown in table-I).

Regarding the comparison of tumor metastasis in patients with HCC positive for HBV and HCV infection with expression of miR-92-1, up-regulation was seen in 63(46.0%) patients without metastasis, 8(80.0%) patients with lymph node metastasis and 3(100.0%) patients with distant metastasis. The down regulation was seen in 74(54.0%) patients without metastasis, 2(20.0%) with nodal metastasis and no any patient with distant metastasis. The p-value was found to be 0.02 which is significant (shown in table-I).

Regarding the comparison of Child Pugh stage in patients with HCC positive for HBV and HCV infection with expression of miR-92-1, up-regulation was seen in 52(58.4%) patients in Child stage A, 16(29.6%) patients in Child stage B and 6(85.7%) patients in Child stage C. The down regulation was seen in 37(41.6%) patients in Child stage A, 38(70.4%) patients in Child stage B and 1(14.3%) patients in Child stage C. The p-value was found to be 0.001 which is highly significant (shown in table-I).

Regarding the comparison of BCLC stage in patients with HCC positive for HBV and HCV infection with expression of miR-92-1, up-regulation was seen in 15(71.4%) patients in BCLC stage O, 38(79.2%) patients in BCLC stage A, 3(6.2%) patients in BCLC stage B, 14(53.8%) patients in BCLC stage C and 4(57.1%) patients in BCLC stage D. The down regulation was seen in 6(28.6%) patients in BCLC stage O, 10(20.8%) patients in BCLC stage A, 45(93.8%) in stage B, 12(46.2%) in stage C and 3(42.9%) patients in

BCLC stage D. The p-value was found to be 0.001 which is highly significant (shown in table-I).

The Univariate analysis of miR-92-1 showed significant correlation with AFP (p value = 0.006) and Child Pugh score B (p value=0.01) with OR showing higher risk. However no correlation was found between the down regulation of miR-92-1 with age, gender, fibro scan, tumor distribution, tumor size, tumor metastasis, and BCLC stage

DISCUSSION

The prognosis of the worldwide most common tumor hepatocellular carcinoma (HCC) depends on its early diagnosis. Long term disease free survival as well as 5 year survival can be improved with early detection and early intervention by the most appropriate curative treatment options, which include surgical removal of the tumor mass, percutaneous ablation and hepatic transplantation. Identifying the noninvasive approach for this purpose remains the area of interest for most of the researchers.[19]

In the present study the expression of miR-92-1, no any significant correlation was found among HCC cases associated with either HBV or HCV and healthy control. 47% HCC patients with HBV infection and 55% HCC patients with HCV infection showed down regulation in comparison to the 52% individuals showing up-regulation in healthy individuals. miR-92-1 belongs to cluster miR-17-92 showing up-regulation in many of the Myc gene induced tumors.[20] The down regulation of this cluster has been associated with developmental defects within the heart and lungs. Many of the white blood cell tumors reveals deregulation of same cluster.[21] Gupta et al[18] reported over expression of miR-92-1 in HCC patients. The up-regulation is associated with the increased tumor cell division in HCC patients. During the in vitro silencing of this miRNA cluster, results showed decreased hepatocyte proliferation and mitotic activity.

In present study miR-92-1 was comparatively more down regulated in female HCC patients. However in contrast to our findings male dominance in HCC patients has also been reported by Chen et al[22] for the expression of miR-365. Regarding the age distribution down regulation of miR-92-1was significantly correlated with patients of 50-59 years age. Chen et al[22] reported the higher expression of miR-365 in HCC patients above the age of 50 years in accordance to our findings. Regarding the correlation of miR-92-1 with serum albumin level it showed decreased expression even in normal albumin levels.

Also miR-92-1 showed significant association with AFP level between 20-200ng/ml. Qi et al [23] reported the up-regulation of miR-21 in chronic hepatitis than controls. Statistical analysis for both AUC and ROC of miR-21 was significant than the AFP values in predicting the HCC patients. Similarly in the HCC patients with low AFP, the expression of miR-15b, miR-16 and miR-130b was more sensitive.

Down regulated miR-92-1 showed no any association with degree of fibrosis. Down regulated miR-92-1 revealed no any significant correlation with solitary of multiple tumor foci of HCC masses. In a study conducted by Chen et al[22] reported strong association of expression profiles of miR-365 with solitary tumor nodules.

However in the present study miR-92-1 showed decreased expression irrespective of the size of primary tumor in HCC patients. In a study conducted by Chen et al[22] reported association of over expression of miR-365 with smaller tumor size of 5cm or below.

In comparison to the no correlation between the miR-92-1 expression and tumor metastasis Wong et al[24] studied the expression of various micro RNA in patients with HCC and found that down regulation of micro RNA plays role in the carcinogenesis. This was further aggravated with the HCC metastasis.

Down regulated miR-92-1 showed association with Child Pugh stage B. Similarly down regulated miR-92-1 showed independent expression of the BCLC stage. In accordance to our findings Zhou

et al[2] reported expression of various micro RNAs were not influenced by BCLC stage thus making them a good diagnostic biomarker.

CONCLUSION

miR-92-1 expression was decreased in patients with HCC when compared with controls. Down regulation was seen more pronounced in HCC cases with chronic viral hepatitis C. Univariate analysis revealed significant correlation between expression of miR-92-1 with AFP level 20-200 ng/ml and Child Pugh score B. However no correlation was found between the expression of miR-92-1 and age, gender, fibro scan, tumor distribution, tumor size, tumor metastasis and BCLC stage.

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PREVIOUS RELATED STUDY

Wasim Amer, Muhammad Akram, Muhammad Ashraf Majrooh. HEPATOCELLULAR CARCINOMA; CHARACTERISTICS A RETROSPECTIVE STUDY OF 34 PATIENTS AT JHL, LAHORE (Original) Prof Med Jour 16(3) 364-369 Jul, Aug, Sep, 2009.

"Happiness only real when shared."

Unknown

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Dr. Amin Fahim	Conception and design, Writing the article, Final approval of the article, Data collection	
2	Prof. Dr. M. Ahmed Azmi	Obtained funding, Analysis and interpretation	
3	Dr. M. Hanif	Critical revision of the article Statistical analysis	
4	Dr. Anila Qureshi	Writing the article, Data collection, Statistical analysis	