



Role of Micro RNA in Diagnosis of hepatitis b virus

Mohamed K. mohamed.a; Afaf D. Abdel mageed.a; Omnia A. Abdullah.b and Ahmed A. Al-sayed.c

a-Biochemistry Dep., Fac. Vet. Med., Benha Univ.

b- Medical Biochemistry and Molecular Biology Fac. of Med., Benha Univ.

c- National blood transfusion bank, Benha branch.

ABSTRACT

This study aimed to evaluate the role of estimated levels of miRNA 21 and 126 for differentiation between asymptomatic and symptomatic CHB patients. All subjects enrolled of this study included the small sample size, expensive technique and also the limited diversity of the patient's blood bank at Benha and University hospitals for blood donation attending Benha University Specialized Hospital, Such limitations can be overcome in further researches. The study included 75 patients diagnosed as CHC (Group I) and 25 healthy controls (Group II). Group (I) was further subdivided according to the last HBsAg RT-PCR into two groups: Group IA including 25 patients with HBV-PCR positive Group IB including 25 patients with HBV-PCR negative. All individuals included were subjected to measurement of AST, ALT, ALB, AFP and HBsAg load. The study showed high statistical significant difference. ($P > 0.001$) between CHC patients and control group regarding ALT, AST and AFP being higher in the former group. On the other hand, serum level ($P > 0.001$) between ALB showed a high statistical significant difference. Both groups being decreased in CHC patients. ($P > 0.005$) of serum similarly, there was statistical significant increase AST level and decrease of serum AFP in group IA when compared to group IB. Similarly, statistical significant difference of both miR 21 and miR126 expression levels in the peripheral blood being high in group IA when compared to group IB ($P > 0.001$). On the other hand, there was no significant difference between group IB and group II as regard peripheral blood miR- 21 and miR- 126 expressions. Regarding the correlation between miR- 21 and miR- 126 expression levels in peripheral blood and each ALT and AST, the study showed a significant positive correlation. On the other hand, there was statistical negative correlation with AFP level group I and group IA. In conclusion, miRNA 21 is more sensitive than miRNA 126 in diagnosis of hepatitis B virus infection.

Key word: Hepatitis B virus, miRNA 21, micro RNA 126, liver carcinoma.

(<http://www.bvmj.bu.edu.eg>)

BVMJ-36(1): 88-49, 2019)

1- INTRODUCTION

Hepatitis B virus (HBV) infection is one of the main causes of chronic liver disease worldwide. HBV infection is highly variable from minimal changes to chronic hepatitis, extensive fibrosis with or without

hepatocellular carcinoma (HCC) (WHO, 2009).

The prevalence of chronic HBV infection is highly variable, ranging from 0.1% in United States to 20-30% in some Pacific Island nations (Perzz et al., 2006).

Approximately 15-40% of patients with chronic HBV will develop cirrhosis, end-stage HCC or liver failure in their lifetime (Goldstein et al., 2005). Most of the deaths (94%) were attributed to complications of chronic infection, such as HCC and cirrhosis, and only 6% were attributed directly to acute hepatitis (Ferlay et al., 2010).

MicroRNAs (miRNAs) are approximately 22-nucleotide non-coding RNAs that regulate target genes at post-transcriptional levels. They not only play an important role in cell development, differentiation, and physiological function, but are also significant in the development of tumors, viral infections, and other closely related diseases. Furthermore, miRNA molecules have been increasingly regarded as a diagnostic and prognostic marker in the evaluation of certain diseases. Recent studies have found that miRNAs are

abundant in the liver and modulate a diverse range of liver functions. Several miRNAs in serum and tissue have been reported in the diagnosis and prognosis of tumors related to HBV infection (Li et al., 2010)

2- Material and methods:

This study included of 25 patients with advanced primary HCC, 25 patients with chronic type B hepatitis PCR positive, 25 patient with chronic type hepatitis PCR negative and 25 healthy controls. Patients with HCC and patients with chronic type B hepatitis were recruited at blood bank donors.

- Molecular analysis of micro RNA:

(1) Total micro RNA extraction

Total micro RNA was extracted from blood samples using RNeasy Protect Animal Blood kits (Qiagen kits) according to manufacture instructions.

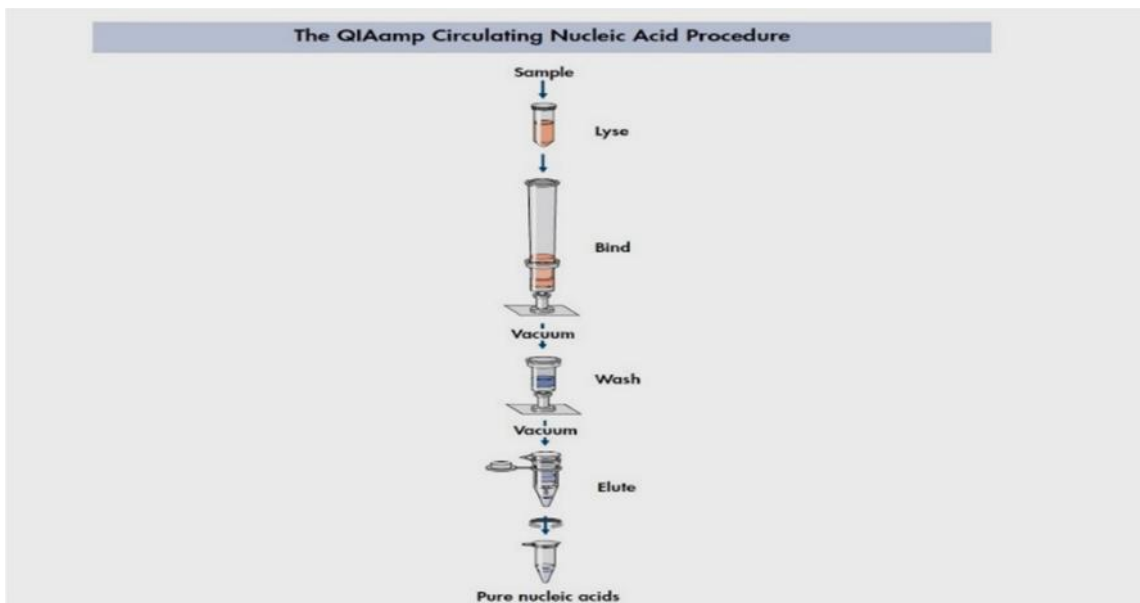


Figure 1: Procedure of purification of Circulating Nucleic Acids from blood

(1) Two-step RT-PCR for relative quantitation of miR-21 and miR-126 expression :

a- Reverse transcription (Wang et al., 2012).

Amplification and quantification of miR-21. Livak , K.J. and Schmittgen , T.D. (2001)

3-RESULTS

Comparative statistics between patients and controls as regards liver parameters revealed that the level of ALT,AST,ALB and AFP were higher in group I (chronic HBV patients) compared to group II (healthy control) with highly statistical significant difference between them (P>0.001). While serum albumin levels were shown to be lower in group I (chronic HCV patients), when compared to group II (healthy control) with statistically

significant difference between them (P>0.001) (Table2).

Comparative statistics between group IA (patients with last HCV PCR positive) and group IB (patients with last HBV PCR negative) as regards liver parameters revealed statistical significant increase inAST levels.In addition statistical significant decrease in AFP in group IA (P<0.05), while significant difference was found between both groups as regards ALT, albumin levels(P>0.05)

Table (1): Descriptive statistics of various assessed parameters in chronic HBV liver

Group Parameter	Group I (n= 25)	Group IA (n= 25)	Group IB (n= 25)	Group II (n= 25)
ALT(U/L)	25.6 ±10	28.5±12	27.7±12	22.8±14
AST(U/L)	28.9±12	34.7±11	27.7±13	22.8±14
BILI (Total) (mg/ dL)	0.9±0,4	0.9±0.4	0.8± 0.3	0.7 ±0.2
ALB(g/ dL)	3.77±1.5	3.22±1.1	3.5±1.1	4.3±1.2
Creat(mg/ dL)	0.9±0.2	1.3±0.3	1.2±0.3	0.75±0.3
AFP (ng/ L)	3.0± 1.5	11.9±3.5	15.1±65	0.6± 1.1
RQ 21	1.46±0.1	12.9± 0.3	1.35±0.3	1.0±0.1
RQ 126	1.10± 0.1	1.70±0.2	1.20±0.2	1.00±0.1

Group I : chronic HBV patient

Group II : control group

Group IA : Patient with last HBV PCR positive

Group IB : Patient with last HBV PCR negative

ALT : alanine leucine transaminase

AST : aspartate transaminase

Bili : bilirubin total

AFP : alpha feto protein

RQ 21 : relative quantitation of micro RNA 21

RQ 126 : relative quantitation of micro RNA 126

Table (2):Comparative statistics between group I and group II as regard liver parameters.

Group Parameter	Group I (n= 25)	Group II (n= 25)	p	Sig
ALT (U/L)	25.6 ±10	22.8±14	<0.001	HS
AST (U/L)	28.9±12	22.8±14	<0.001	HS
BILI (Total) (mg/ dL)	0.9±0,4	0.7 ±0.2	<0.001	HS
ALB (g/ dL)	3.77±1.5	4.3±1.2	<0.001	HS
Creat (mg/ dL)	0.9±0.2	0.75±0.3	<0.001	HS
AFP (ng/ L)	3.0± 1.5	0.6± 1.3	<0.001	HS

HS: highly significant

4-DISCUSSION

Hepatitis B virus (HBV) infection is one of the main causes of chronic liver disease worldwide. HBV infection is highly variable from minimal changes to chronic hepatitis, extensive fibrosis with or without hepatocellular carcinoma (HCC) (WHO, 2009).

The global epidemiology of HBV is best reviewed according to the six regions defined by the World Health Organization (WHO): the Americas, Eastern Mediterranean, Europe, Africa, South-East Asia, and the Western Pacific. There are at least ten genotypes of HBV, (A through j) have been identified on basis of more than 8% difference in their genome sequences.

The HBV genotypes, except for genotype E and G, can be further divided into sub-genotypes. Genotype A is more prevalent in North America, Northern and Western Europe, India, Sub-Saharan Africa, and in some Regions of South America. Genotype B and C are most common in Asia. Genotype D is endemic to the Mediterranean region and Eastern Europe, Although it can also be found all over the world. Genotype E exists in Western

Africa. Genotype F is present in South America. Genotype G has been reported in France, Central America, Germany, Mexico and the United States. Lastly, genotype H can be found in Central America

MicroRNAs (miRNAs) are approximately 22-nucleotide non-coding RNAs that regulate target genes at post-transcriptional levels. They not only play an important role in cell development, differentiation, and physiological function, but are also significant in the development of tumors, viral infections, and other closely related diseases

Furthermore, miRNA molecules have been increasingly regarded as a diagnostic and prognostic marker in the evaluation of certain diseases. Recent studies have found that miRNAs are abundant in the liver and modulate a diverse range of liver functions. Several miRNAs in serum and tissue have been reported in the diagnosis and prognosis of tumors related to HBV infection (Li et al., 2010).

Circulating miRNAs are emerging as promising biomarkers for several pathological conditions. Some reports have

shown that the profiles of serum miRNA expression can specifically predict liver injury caused by chronic hepatitis B (CHB) (Chen et al., 2008).

MicRNA-21 was one of the first mammalian microRNAs identified. The mature miR-21 sequence is strongly conserved throughout evolution. The human microRNA-21 gene is located on plus strand of chromosome 17q23.2 within a coding gene (also called vacuole membrane protein). Despite being located in intrinsic regions of a coding gene in the direction of transcription, it has its own promoter regions and forms a ~3433-nt long primary transcript of miR-21 (known as pri-miR-21) which is independently transcribed. The stem-loop precursor of miR-21 (pre-miR-21) resides between nucleotides 2445 and 2516 of pri-miR-21. MicroRNA-21 has been explored as a potential biomarker for various hepatic conditions. The change in levels of miR-21 in the blood has been confirmed as an indicator for liver disease. This change is noted before increased liver aminotransferase activity, making it a preferable indicator for liver disease, which suggests that miR-21 can be a predictive injury (Wang et al. 2012). miRNA-126 is specifically and highly expressed in the endothelial cells (ECs), which regulates ECs migration, cytoskeleton reorganization, capillary network stability, cell survival and apoptosis (Agrawal S. et al. 2014). And microRNA-126 regulates cell survival or apoptosis, depending on different cell types. Furthermore, miR-126 is necessary for the maintenance of vascular structure in vivo (Fish et al., 2008). and reliable blood marker for viral-, alcohol- and chemical-induced liver Based on the literature and

available data for miR-126, its expression pattern generally exhibits down regulation in most cancers, including liver cancer.

In the present study aimed to evaluate the role of estimated levels of miRNA 21 and 126 for differentiation between asymptomatic and symptomatic CHB patients.

The data present in table (1) statistical comparative analysis between patients and controls as regards laboratory data shows significant higher level ($P < 0.001$) of ALT, AST, ALP and AFP and significant lower level of serum ALB in group I compared to group II. These results are in accordance with, (Subodh Kumar et al. 2014) who demonstrated significant higher level of serum ALT, AST, bilirubin and AFP in CHC patients when compared to control group and significant lower level of the serum albumin in the patient group, which as they suggested could be attributed to the effect of chronic HBV liver disease in group I which affect the liver function

The result given in table (2) reflect that statistical significant difference ($P < 0.05$) in the serum ALT level between group IA and IB being higher in group IA. This was in accordance with (Anderson et al. 2007) who report increase in AST level among more cirrhotic CHC patient suggesting that the AST level predict the liver pathology. The results were slightly similar to those of (Noreldin et al. 2015), where significant increase in AST and ALT levels in (HBVRNA- PCR) positive group when compared to (HBVRNA- PCR) negative grouping a group of (100) Egyptian CHC patients. The partial differences between our results and the results of their study might be explained by the larger sample size of their study according to table (2).

5- CONCLUSION

Our results demonstrated significant high expression of miR-21 and miR-126 in adult Egyptian CHC patient compared to controls and strong associate of miR-21 and miR-126 with hepatocyte inflammation markers (AST , ALT .Bilirubin) indication that it may play an important role in active state of HBV and introduce it as a potential predictor of disease . there find lead us to conclusion that HBV infection result in modulation of miR-21 and miR- 126 gene expression , find that has drawn to possible role of micr-21 and micr-126 .The biological function of the miRNAs requires further investigation if serum miR-21 and miR-126 may interesting biomarker for early hepatic inflammation. Elevated serum miR-21 and miR-126 level may require further understanding role of liver injury of mechanism of active synthesis of miR-21 and miR-126 in the most chronic HBV infection and accounts for the high levels of circulation miR-21 and miR-126.

Understanding miR-21 and miR-126 elevation in the hepatitis B setting might lead to new antiviral strategies .

6- REFERENCES

- Agrawal, S and Chaqour, B(2014). MicroRNA signature and function in retinal neovascularization. *Hepatology J*; 56: 1946.
- Anderson, A, Baohong , Z and Xiaoping , P(2007): micrRNA as oncogenes and tumor supressors. *Nature*; 431: 350.
- Chen X, Ba, Y, Ma, L and Cai , X (2008): Characterization of microRNAs inserum: a novel class of biomarkers for diagnosis of cancer and other diseases. *Report J*; 6: 215.
- Fish ,JE, Santoro, MM, Morton , SU, Yu ,S, Yeh , RF, Wythe , JD, Ivey , KN, Bruneau , BG, Stainier ,DYZ and Srivastava , D(2008): miR-126 regulates angiogenic signaling and vascular integrity. *Nat Immunol*; 9:839.
- Jonel T, Eviim , A and Natalia , E (2012): Hepatic and serum levels of miR-21 after chronic HBV-induced fibrosis. *Cell Press*; 17: 554
- Li LM, Hu, ZB, Zhou , ZX and Chen , X. (2010): Serum microRNA profile serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Semin Liver Dis*; 30: 333.
- Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-⁻ C T method. *Mol. Psychiatry*; 15:1176.
- Noreldin , A , Mohamed ,A and Abdelaziz , A (2015): correlation between liver function tests and polymerase chain reaction in chronic hepatitis b patients .
- Perz , J, Armstrong ,G and Farrington , L (2006):The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer world wide .*J Hepatology . Hepatology J*; 56: 1946.
- Subodh Kumar et al. (2014) and Shaobo et al. (2015): micRNAs role in HBV pathogenesis. *Test Mol Biomarkers*; 15: 807.
- Waidmann , B , Koberle , V and Waidmann , O(2014) : serum microRNA – 21 kinetics in patients

with chronic hepatitis B virus infection during antiviral therapy .
PLOS; 7: 1009

Wang, Shu-hao , H and Janaiah , K. (2012):
Essential metabolic, anti-

inflammatory, and anti-tumorigenic
function of miR-122 in liver.

World Health Organization.,(2009):
Hepatitis B vaccines Weekly
Epidemiological Record.40:405-4.