SERUM LEVEL OF miR-221 AS BIOMARKERS FOR IRAQI PATIENTS WITH CHRONIC HEPATITIS B VIRUS INFECTION

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ABSTRACT

Hepatitis B virus (HBV) infection is a critical problem in the public health and about 350 million people in the world are suffering from this infectious disease. The hepatic-specific microRNA-122 has been appear as a useful and promising marker of liver diseases. This study aims to evaluate the levels of blood miR-122 alteration in the CHB patients, inactive carrier compare with apparently healthy control. A total of 67 patient’s serum sample taken during the period from February 2019 till September 2019 at the Iraqi Hepatology and Gastroenterology Teaching Hospital Baghdad. Expression of miRNA was discovered by Real Time PCR. Our results show that serum miR-122 expression was decreased in the chronic HBV and inactive carrier compared with the healthy control our result showed there is highly significant difference \(P = 0.003\) between study groups.

Keywords: HBV, microRNA-122, Real Time PCR.

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INTRODUCTION

Extermination of hepatitis B virus contagion stills a world critical challenge \(^{(1)}\). About 350 million persons globally are carriers of chronic HBV about one-third of these patients living in China where the medium prevalence of HBV infection is more than 9\% \(^{(2)}\). Hepatitis B infection HBV can be present asymptomatic form or acute form, chronic or fulminant form. The Hepatitis disease is diagnosed depend on the basis of an increasing in liver enzymes including ALT, AST and HBsAg positive or HBV DNA viral load. This disease is treated with interferon and or nucleotide, nucleoside analogues \(^{(3,4)}\). Although the comprehensive programs of vaccination and the progress in the treatment of hepatitis BHBV in many countries and the consequent decrease in its expansion, the poor coverage of vaccination and the unable of diagnose the HBV infected in some countries have kept this infectious disease as a major worldwide health concern and the HBV global burden thus remains high \(^{(5)}\). HBV belongs to the family of Hepadnaviridae.
viruses. HBV has a genome highly compact about 3.2 kilobases (kB) in length; this viral genome has four overlapping open reading frames (ORF) that responsible for encoding the viral core protein capsid, surface proteins envelope, reverse transcriptase RT, and finally X protein HBx. The HBV genome is a partially double strand, circular relaxed DNA. The DNA replicates take place by reverse transcription RT of an RNA intermediate strand. Hepatic cell are the main cells that infected by HBV, and the host range of HBV are controlled by interactions between the cell surface receptor as well as hepatocyte-specific intracellular factors and the virus. Diagnostic ways like CTSCAN or MRI have not been satisfactory and not used in all applications so, attention has to be driven to tools that can be simpler, less risky and less error. The profile of microRNA (miRNA) biomarkers has been devilishly studied in many diseases like HBV. miRNAs are a family of short ~22 nucleotides, non-coding RNAs that play critical role in gene expression. miRNAs regulate expression of gene at level of posttranscriptional by binding to complementary sequences at 3’-UTR on target mRNA and either inhibit mRNA translation or catalyze the target degradation or. miRNA can be found in humans or animals and viruses. The first miRNA discovered, called Line-4, in a Caenorhabditis elegans nematode in 1993 and was critical for the further transform of larval stages. Many studies have been done in order to evaluate the cellular miRNAs role in viral life cycles. The most miRNAs widely studied to date is miR-122. It has been distinguish as one of the most considerable miRNAs in tissue, being present at more than 130,000 copies per cell in human hepatic cell and involve more than 70% of the entire liver miRNA pool. miR-122 a hopeful biomarker of liver cell injury, and liver diseases. Using of miR-122 in this line was first done by Wang, et al. in 2009. In contrast to hepatitis c virus, mir-122 inhibits hepatitis B virus (HBV) by engaged to the viral sequence. The purpose of the present study was to investigate serum miR-122 diagnostic values for Iraqi Patient with Chronic Hepatitis B Virus HBV Infection.

MATERIAL AND METHODS

The study was performed from February 2019 to September 2019 on 67 complete blood samples subjects (43 men and 25 women) collected from patients and 25 healthy controls. The patients were divided into two groups made of 51 chronic patients and 16 inactive carriers which were attend to Medical City -the Hepatology and Gastroenterology Teaching Hospital in Iraq. All the patients were HBs Ag positive and did not have any other diseases depend on clinical reports.

About 5 ml whole blood samples was taken from each participant, and then collected in EDTA tubes for serum separation that was used in detecting all serological markers for HBV as well as RNA quantitation by real time PCR. Serum was stored at –80°C and maintained it in 0.75 ml TRIzol reagent to use for micro-RNA extraction and identification of expression change for the microRNAs-122 using, real time PCR. RNA was extraction from sample depend on the protocol of TRIzol™ Reagent (Qiagene).

Molecular detection of gene expression of miRNA-122
cDNAs for miRNA-122 and RNU (reference gene) genes were produced by using of SYBRR Green, TRIzol® LS Reagent (Qiagene) and RT-qPCR System from Promega-USA, primers (Table-1) and depend on the manufacturer’s protocol. Quantus Florometer used for detection of the cDNA concentration in order to evaluate the goodness of samples for the applications downstream. For 1μl of cDNA, 199μl of diluted Quanty Flour Dye was mixed then after 5mints of incubation at room temperature in dark place the RNA
concentration amount were detected. miRNA-122 and RNU reference genes Amplification was done by two
Step RT-PCR: first step : 16°C for 30 min, 42C for 30min, 85°C for 5 min and 4°C for 10 min for 1 cycle.
second step 95°C for 5 min for 1 cycle. , 95°C for 15 sec, 55°C for 30 sec and 72°C for 30 sec for 40 cycles.

Table 1: Sequences of primers used for amplification miR-122 and RNU reference gene

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>RNU -RT</td>
<td>5’- GTTGGCTCTGGTGAGGGTCCGAGTGATTCGACACCAGAGCCAACAATCGAGG-3’</td>
</tr>
<tr>
<td>RNU-F</td>
<td>5’-GTAAGCTATTGACGGGCG-3’</td>
</tr>
<tr>
<td>RNU- R</td>
<td>5’-GTGCAGGGTCCGAGGT-3’</td>
</tr>
<tr>
<td>miR-122-3p-RT</td>
<td>5’- GTTGGCTCTGGTGAGGGTCCGAGGTATTGCACCAGAGCCAACATTGTA-3’</td>
</tr>
<tr>
<td>miR-122-F</td>
<td>5’-GGGAACGCCATTACAC-3’</td>
</tr>
<tr>
<td>miR-101- R</td>
<td>5’-GTGCAGGGTCCGAGGT-3’</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

It was found in these study that most of the patients were located within (20-30) years old with
(33.8%) table 2. These outcome correspond with studies done in Iraq as(16, 17) adduced that the mutual age
group for hepatitis B was in Third decade age. In republic of Iraq most infections occur young about (15-29
years old)(18). The infected patients likely will taken antiviral drug and the possibility for gene mutations take
place will be high in these patients in any age if the treatment persist.
The ages of many patients who cooperate in these study extend from 20-30 years, due to the verity that most
of the Iraqi populations who's infection with hepatitis B virus are youth and this age is vivid and more fearful
to HBV infections.

Table 2: patient’s age groups

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Frequency</th>
<th>Percent%</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>10</td>
<td>14.7</td>
</tr>
<tr>
<td>20-29</td>
<td>23</td>
<td>33.8</td>
</tr>
<tr>
<td>30-39</td>
<td>10</td>
<td>14.7</td>
</tr>
<tr>
<td>40-49</td>
<td>7</td>
<td>10.3</td>
</tr>
<tr>
<td>50-59</td>
<td>12</td>
<td>17.6</td>
</tr>
<tr>
<td>&gt;=60</td>
<td>6</td>
<td>8.8</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100.0</td>
</tr>
</tbody>
</table>
This study showed as in table 3 that 63.2% of patients males and 36.8% females. The sex apportionment of patients in these study was like to other studies in Iraq\(^{16, 17}\). This may be due to the truth that Iraqi males in general are more active and exposed to hazard factors than Iraqi females.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>43</td>
<td>63.2</td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>36.8</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**Expression profile of miRNA-122**

To investigate the circulating of miR-122 levels alteration in patients with CHB and inactive carrier compare with healthy control, the expression of miR-122 were detected in the serum from patients and controls using real-time PCR. Real-time RTPCR data were quantified based on Ct values that are inversely related with amount of starting template so, high Ct values equivalent with low levels of genes expression, and vice versa figure 1\&2.

![Figure 1: RNUexpression Melt on Green Melt from 72°C to 95°C.](image1)

![Figure 2: miR-122 expression Melt on Green Melt from 72°C to 95°C.](image2)
The results showed that serum miR-122 was downregulated in the chronic HBV and inactive carrier compared with the healthy control (Fig. 3).

![Figure 3: miR-122 expression](image)

Highly significant difference ($P = 0.003$) was observed in the miR-122 serum levels between the chronic HBV, inactive carrier and healthy control (table 4).

<table>
<thead>
<tr>
<th>miR-122</th>
<th>Chronic</th>
<th>Inactive carrier</th>
<th>Control</th>
<th>p-value ©</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.742</td>
<td>1.899</td>
<td>2.718</td>
<td>0.003 **</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.520</td>
<td>3.068</td>
<td>3.01</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.069</td>
<td>0.238</td>
<td>1.129</td>
<td></td>
</tr>
<tr>
<td>Standard Error</td>
<td>0.215</td>
<td>0.767</td>
<td>0.600</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>8.648</td>
<td>9.129</td>
<td>9.88</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>50</td>
<td>16</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

© Oneway ANOVA used to test means of the three groups, ** Highly significant ($P < 0.01$)

The last decade has witnessed growing interest in the invention and development of serum markers. So circulating microRNAs are critical biological serum markers because of their firmness and presence in all body fluids (19). There is a great transact of researches by use of miR-122 as a HBV and HCV biomarker. (20). Depend on the outcome of this study, the miR-122 serum levels were highly significantly different between chronic HBV and inactive carrier compare with healthy control. This finding is in agreement with a many previous studies which showed that serum miRNA-122 reflects liver diseases (21). The downregulation of

miRNA-122 expression in HBV infection can similarly upregulate the expression of cyclin G1 gene. Then, cyclin G1 can decline the P53 activity which raises HBV replication so loss expression of miRNA-122 in HBV patients may induce modulating cyclin G1 and raise HBV replication (22). Chen et al. have expound that miR-122 can engaged to the conserved region of a bicistronic mRNA named HBV pregenomic RNA, that encode the HBV polymerase beside core protein, thereby eventually leading to inhibition of HBV replication and gene expression.(19).other study found that four HBV mRNAs have a binding site that is miR-122 complementary and may be work as a sponge to sequester and bind endogenous miR-122, participate to HBV-induced miR-122 repression (15, 20, 23). Level of miR-122 were negatively related with levels of viral mRNA advocate that the binding sites of miR-122 in the HBV mRNAs segregate endogenous miR-122. These results are harmonious with many studies showing that miR-122 levels are negatively associated with liver cell HBV DNA load in CHB (24). Wu et al. (25) explain that levels of serum miR-122 steadily downregulation through antiviral treatment and upregulation level of miR-122 was correlated with suboptimal therapy response. Serum level of miR-122 at week (12) and week (24) were founded to be favorable predictors to viral response at week (96). Our Results demonstrated that miR-122 could be used as a biomarker of liver infection and might have better accurate sensitivity than liver enzymes test. This study correlates with previous studies demonstrated that miR-122 serum level is convenient in diagnosing of HBV infection (26).

CONCLUSION

Our results suggest that circulating serum levemiR-122 have a comparatively high diagnostic account for viral hepatitis detection, especially in the patients with HBV-related chronic viral hepatitis. Furthermore, large studies are still need to confirm our results.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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