Investigating the Role of miR-223 and INF-α in Iraqi HBV Patients

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Abstract
Hepatitis B virus (HBV) causes acute or chronic infection in humans with long and variable incubation times ranging from eight weeks to six months. HBV has a unique replication strategy. Although HBV has been discovered for more than half a century, a cure for chronic hepatitis B (CHB) remains a challenging task. miRNAs are endogenous, single-stranded, non-coding small RNA with length of ~22 nucleotide (nt). This study aimed to determine the expression of miRNA-223 (miR-223) and Interferon-α (INF-α) level in HBV patients’ group compared with apparently healthy as a control group. The study consists of three groups as, Chronic Hepatitis B patient’s groups CHB (73), Inactive Carrier group IC (20) and control group CG (42). Interferon alpha was measured in all groups by ELISA. The results revealed that INF-α level was almost unchanged between groups as in CHB group the mean and standard deviation value was (9.932± 4.955) in IC group was (8.472 ± 2.185) and in control group (8.393 ± 1.794). Total cellular RNA was isolated and purified from serum sample according to the protocol of TRIzol™ Reagent, miR-223 gene expression was carried out by using specific primers the method of reverse transcription by using Promega RT kit involves the conversion of RNA to cDNA. It was found that the miR-223 levels was lower in chronic patientsthan in healthy control with highly significant difference (p<0.003) whereas it was low in inactive carrier patients (1.006 ± 1.73) in comparison with patients (1.249 ± 1.99).

Keywords: miR-223, INF-α, Iraqi HBV Patients

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Introduction
Hepatitis B virus (HBV) causes acute or chronic infection in humans with long and variable incubation times ranging from eight weeks to six months, the HBV belongs to the Orthohepadna genus of the Hepadnaviridae family of virus and has a unique replication strategy wherein it replicates its DNA genome using an RNA intermediate via reverse transcription. HBV infects hepatocytes to cause pathology in the liver as an acute or chronic infection[1]. The host-viral interplay has long been exciting, the field of research across the world conflicting efforts of the virusto manipulate the host for its successful propagation, as well as the hostto inhibit the virus, culminate in complex interactions resulting in either elimination of the virus or its persistence, the lack of a functional innate DNA sensing pathway in hepatocytes allows HBV to establish infection and to replicate as a consequence of co-evolution, viruses have acquired the ability to counter the antiviral response induced by type I IFN[2]. Although HBV has been discovered for more than half a century, a cure for chronic hepatitis B (CHB) remains a challenging task. Currently approved antiviral treatments for CHB include nucleos (t)ide analogues (NAs) and interferon[3]. The miRNAs are endogenous, single-stranded, non-coding small RNA with length of ~22 nucleotide (nt), the first discovered role of miRNA in Caenorhabditis elegans and participated in embryo development, then was found in plants, animals, protists, and viruses but not in bacteria, these small RNA molecules function as antisense RNA to negatively regulate their target genes at the post-transcription level[4]. The miR-223 was first identified in the hematopoietic system then increasing evidence has suggested that miR-223 is one of the key factors in the development and homeostasis of the immune system, and may also have an essential part in both inflammation disorders and various liver diseases[5]. Several exciting research studies suggested that miR-223 serves as a crucial regulator in innate immunity, ranging from myeloid differentiation to neutrophil and macrophage functions, various immune related targets of miR-223 have been identified in multiple inflammatory diseases conditions[6].
Subjects and Methods
The blood was collected from three groups to obtain the serum, firstly chronic hepatitis B patient’s group (CHBG) (73), Inactive Carrier group (ICG) a total of (20) patient who were admitted to Hepatology and Gastroenterology Teaching Hospital in Baghdad. Carrier state is diagnosed by absence of HBeAg and presence of anti-HBe, or undetectable to low levels of HBV DNA in PCR-based assays. In addition to control group (CG) a total of (42) apparently healthy individual were who attended blood bank for donation of blood and health check.

Serological Assay
Serum marker of HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc levels were quantitatively analyzed by Bioelisa ELISA kit were done for all three groups. Interferon-α measured using Interferon alpha-1 in vitro SimpleStep ELISA® (abcam) (Enzyme-Linked Immunosorbent Assay) this kit is designed for the quantitative measurement of Interferon alpha-1 protein in human serum.

Molecular Assay
Total cellular RNA was isolated and purified from serum sample according to the protocol of TRizol™ Reagent. The miR gene expression was done by using specific primers, the method of reverse transcription by using Promega RT kit involves the conversion of RNA to cDNA. All RNA types were converted into cDNA, using oligo-dT primers to reverse transcribed into cDNA. Total RNA containing miRNA was used as raw material for reverse-transcription reaction, the master mix for reverse transcription was added and it contains all materials required in cDNA first-strand synthesis except template RNA, template RNA was added to each tube. After the conversion of RNA into cDNA, SYBR Green reporter real time PCR reaction was used and the template this time is cDNA, mature miRNA detection using miRNA-specific primers and the SYBR Green PCR Kit. For accurate results in mRNA and miRNA quantification RNU was used as a reference gene. Primers using for miR-223 and RNU shown in (Table 1).

Table 1: Primers used for miR-223 and RNU

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Primer Sequence</th>
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<tbody>
<tr>
<td>RNU RT-primer</td>
<td>5<code>-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAATCAG-3</code></td>
</tr>
<tr>
<td>RNU-F</td>
<td>5<code>-GTGAACCTTATTGACGGGCCG-3</code></td>
</tr>
<tr>
<td>Universal Revers</td>
<td>5<code>-GTCAGGGGTCCGAGGT-3</code></td>
</tr>
<tr>
<td>miR-223-3p-RT</td>
<td>5<code>-GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACCAGAGCCAACAATCAG-3</code></td>
</tr>
<tr>
<td>miR-223-3p-F</td>
<td>5<code>-GTTGGGTGTCAGTTTGTCAAAT-3</code></td>
</tr>
</tbody>
</table>

Results
Interferon-α (INF-α) level was slightly elevated in CHB group and almost nearly similar between IC and healthy groups, as in CHB group the mean and standard deviation value was (9.932± 4.955) , IC group was (8.472 ± 2.185) and in control group (8.393 ± 1.794). Whereas the miR-223 levels were variable in different groups, which was lower in patients than in healthy controls with highly significant difference ( P≤ 0.003), and miR-223 levels were lower in inactive carrier patients (1.006 ± 1.73) when compared with chronic patients (1.249 ± 1.99). The value of miR-223 expression and INF-α level of studied groups showed in Table (2).

Table 2: The mean and value of miR-223 expression and INF-α level in three studied groups

<table>
<thead>
<tr>
<th></th>
<th>Healthy Group</th>
<th>Chronic Patients Group</th>
<th>Carrier Patients Group</th>
</tr>
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<tbody>
<tr>
<td>miR-223</td>
<td>3.194 ± 4.684</td>
<td>1.249 ± 1.99</td>
<td>1.006 ± 1.73</td>
</tr>
<tr>
<td>INF-α</td>
<td>8.393 ± 1.794</td>
<td>9.932± 4.955</td>
<td>8.472 ± 2.185</td>
</tr>
</tbody>
</table>
The molecular experiment in present study was performed to detect the amplification plots of miR-223 gene and RNU (reference gene) to find the threshold cycle value for each (Figure 1).

Figure 1: Amplification plots obtained by real time PCR

The Ct values used to quantify real-time RT-PCR data that are inversely associated with amount of starting template and calculate melting temperature curve as in Figure (2).

Figure 2: The miR-223 expression Melting Curve

Discussion and Conclusion
MicroRNAs (miRNAs) form almost 3% of the human genome and more than 2588 of them have been discovered to date, miRs participate in many biological activities of the cells, such as cell growth, proliferation and differentiation, apoptosis (programmed cell death), inflammation, metabolism, suppression and disease or cancer miRNA can sometimes target several genes(7). miR-223 expression is shown to dysregulated in liver tissue of mice after induction of liver fibrosis, the levels were also found to concomitantly express highly in the serum during the fibrosis progression, the modulation of miR-223 has also been confirmed in clinical studies when correlated with liver cirrhosis patients(8).

The current study indicated that miR-223 has low expression in patients and inactive carrier when compared with healthy individual, and this maybe inhibit immune factors that help in fighting the viral, and make the virus more free in body this results conclude that as it is game of immunity when the virus is the culprit.The gained result revealed agree with Imura results ,when find that as miR-223 has been identified as one of the most downregulated miRNAs in HCC and low miR223 expression was also determined to be a risk factor of recurrence(9).Wong etal., reported a precise interaction between miR-223 and Statmin 1 (STMN1) when found the negative modulation effect by miR-223 was substantiated further by a strong inverse relationship between STMN1 mRNA and miR-223.
expressions and a significant reduced STMN1 protein level in HCC cell lines after re-expression of miR-223(10). Another study done in 2018 and found miR-223 was obviously decreased (P < 0.01) in the MicroViscels (MVs) took from normal people, which suggested these MVs-derived microRNA molecules may be used as important index of diagnosis CHB(11). Analysis conducted in China revealed that the expression of miR-223 was decreased in HCC and that explain miR-223 may inhibit HCC proliferation and cell cycle progression(12,13).

On contrary another result study conducted in China found unexpected results that the levels of miR-223 are were elevated in serum of HCC patients compared with healthy individuals, suggested that this serum miRNAs, maybe known to be liver specific, this miRNAs are also presented at significantly higher levels in serum of patients with chronic hepatitis, so the author suggested that its more likely to reflect liver injury caused by inflammation (14).

When highlight on the outcome of both miR-223 and INF-α values, it was appeared that INF-α level was almost unchanged between groups, while miR-223 was low in CHB and IC groups this indicated that,INF-α may regulated by miR-223, and suppress the miR-223 expression or the opposite is true. The current result conflict with study conducted in 2016 whichreferred to positive regulatory loop for type I IFN production,the study stated that infection could induce miR-223 up-regulation in a murine macrophage cell line and in murine primary macrophages, which was accompanied by a significant up-regulation of type I IFN(15).

Responses to IFN-α therapy vary greatly in CHB patients and the underlying mechanisms are almost unknown, IFN-α was found to suppress HBV replication in HBV transgenic mice when the viral load was high, whereas it enhanced HBV replication when the viral load was low, indicating its dual function for HBV taken, together the data showed that the precise mechanism of action of IFN-α has not been fully understood(16). The success rates of interferon (IFN)-based treatments are highly variable and hinge on both host and viral factors (17).

In conclusion miRNAs overall and miR-223 specially have vast fascinating roles as detection and therapy in many infectious diseases, but this need more researches and efforts to loosen the tangled threads and understanding how it really works, till now it’s clear that its level is lower in HBV infection and that’s could give a hint to use it as a biomarker for virus progression or use for HBV treatment. These multidisciplinary effects and multi targets make miR-223 the wonder molecules that is surprise us all the time and triggers more attention about its roles and effects. The studies showed many deregulated miRNAs associated with chronic liver disease and subsequent complications and IFN-α level also vary and not fully understood, so the relationship is very complex and need more efforts to solve this dilemma and make a clear picture that can help in cure the disease.

References


