MicroRNAs biomarkers profiling in diagnosis and therapeutic management of hepatitis B virus infection

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Abstract

Introduction: Due to lack of unique method with high accurate and repeatable, assessment and even treatment of HBV infection and its complications such as cirrhosis and HCC has been with limitations. MicroRNAs (miRNAs) are small 19-24 nucleotide-long molecules with up-regulated and down-regulated expression. The present research provides a narrative review expression profiling biomarkers miRNAs in diagnosis, treatment and differentiated CHB from cirrhosis or HCC.

Methods: We search database google scholar, pubmed, scopus, SID on English Language article and also assess EASL and AASLD (2002-2016).

Results: Some of miRNAs are specifically more abundant in specific tissues, such as mir-122 in the liver. MiRNAs such as miRNA125a, miRNA141, miRNA197, miRNA122 and miRNA372, 373 have a major role in CBH and miRNA29a/b/c, miRNA200, miRNA199, miRNA133a, miRNA214 and miRNA181b have a major role in fibrosis/cirrhosis. miR-106b and miR-181b, have a significant clinical diagnostic value in liver cirrhosis, especially at its early stages. miR-122, miR-192, miR-92, miR-223, miR-26a, miR-27a and miR-801, has a highly accurate diagnostic power that can differentiate HCC from CHB and cirrhosis and from healthy people as well.

Conclusion: In the future, the miRNAs biomarkers provide researchers with a golden opportunity and can be used as early diagnostic and miRNAs based-therapeutic panels and current knowledge between miRNAs profiling biomarkers and progressive stage of HBV related diseases. Panels of miRNAs will play a significant role in decision-making about their proper course in both of treatment and diagnosis of diseases such as hepatitis B virus infection.

Keywords: Chronic hepatitis B; Epidemiology; Hepatocellular Carcinoma (HCC); cirrhosis; microRNA, HBV, Treatment

Abreviation: hepatitis B virus (HBV), MicroRNAs (miRNAs), chronic hepatitis B (CHB), hepatocellular carcinoma (HCC), European Association for the Study of Liver (EASL) and American Association for Study of Liver (AASLD)
1. Introduction

Hepatitis B viral (HBV) infection is considered the most common chronic viral infection and a major cause of acute and chronic liver disease and a significant health challenge throughout the world (1). According to the World Health Organization (WHO), almost one-third of the world's population is infected with hepatitis B virus and 240 million people suffer from chronic hepatitis B infection. More than 780,000 people die every year due to its complications, including cirrhosis and hepatocellular carcinoma (HCC). (2). Hepatitis B infection can be present asymptomatic, acute form, chronic or fulminant. The disease is diagnosed on the basis of an increase in liver enzymes including ALT and HBsAg positive or HBV DNA virology. This disease is treated with interferon and nucleotide/nucleoside analogues. Treatment with interferon aims to create an antiviral response by the host's immune system to permanently control the infection but treatment with nucleotide analogues aims only, to inhibit viral DNA production and reduce the number of infected hepatocytes (4). Despite the comprehensive vaccination programs and the favorable progress in the treatment of hepatitis B in many countries and the consequent dramatic decrease in its prevalence, the poor vaccination coverage and the failure to diagnose the infected in some countries have kept this disease a major global health concern and the global burden of hepatitis B thus remains high (5). In the early diagnosis of HBV and determining the time difference in the conversion of CHB complications to cirrhosis or HCC, one of the most important challenges is to be found. Laboratory tests such as ALT and HBV DNA have not always been effective. Liver biopsy is a golden standard method for liver pathological diagnosis with complications such as mortality and morbidity (6). Diagnostic tools such as CT/SCAN, MRI have not been used in all applications and have not been satisfactory. Therefore, attention has to be paid to methods that can be simpler and less risky and feasible with less error. In recent years, the microRNAs biomarkers profile has been widely studied in various diseases including HBV (7). MicroRNAs (miRNA) are small non-coding RNA molecules with 19 to 24 nucleotides that regulate the post-transcription expression of genes as up/down-regulation by the decomposition or inhibition of the translation of the target messenger RNA (mRNA). MiRNAs are stable in plasma or serum suggesting potential use of non-invasive biomarkers (8). These molecules can be found in humans, animals, viruses, etc. The first miRNA, called Line-4, was discovered in a Caenorhabditis elegans nematode in 1993 and was needed for the further conversion of larval stages (9). MicroRNAs (miRNAs) form almost 3% of the human genome and more than 2588 of them have been discovered to date (miRBase, Release 21). MiRNAs 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. Most miRNAs are coded in the inner parts of genes while some are coded in the opposite direction, i.e. antisense coding. A miRNA can sometimes target several genes (10).

We discuss about these small, stable and conserved biomarkers provide researchers can be used as early diagnostic and miRNAs based-therapeutic panels and current knowledge between miRNAs profiling biomarkers and progressive stage of HBV related diseases.

2. Methods

2.1. Search strategy

A narrative review search was performed using citation databases of PubMed and Scopus, SID. Keywords included hepatitis B, epidemiology, transmission, virology, miRNA, CHB, HBV, alone and combined. We also searched European Association for the Study of Liver (EASL) and American Association for Study of Liver Disease (AASLD) on base English Language in period 2002 up to September 2017. The search strategy was evaluated using the search method of a professional library and using text keywords that were controlled by the
Medical Subject Heading (MESH) and key words. Key words include “HBV “Chronic hepatitis B (CHB) “Heatoctecellular Carcinoma (HCC) “MicroRNAs “,” miRNAs “Profiling microRNA “Treatment “ and articles that describe the microRNA profile in hepatitis B patients and their complications, such as CHB and cirrhosis, fibrosis and HCC. Papers on the importance of the plasma microRNAs in the diagnosis of HBV and the differentiation of the complications of the disease from each other or the therapeutic aspect was also studied.

2.2. Inclusion criteria
Inclusion criteria were English language studies, cross-sectional studies, cohort studies, randomized control trials, as well as reviews. All applicable studies were evaluated based on titles and abstracts.

3. The production and functions of microRNAs
MicroRNAs are produced in a series of stages: First, polymerase II is transcribed from the gene and thus produces 100-nucleotide hairpin-shaped precursors (pri-miRNAs). Next, a shorter structure of 60-70 nucleotides called the pre-miRNA is produced inside the nucleus by Drosha/RNase III and with the help of Pasha/DGCR8 (11). In the next stage, this structure enters the cytoplasm with the help of Exportin-5, a membrane protein; where the double-stranded molecule is converted into an miRNA of 22-24 nucleotides by another enzyme called Dicer/RNase III (12). Another complex called RNA Induced Silencing Complex (RISC) then makes the double-stranded pre-miRNA single-stranded. If this strand binds partially to a particular region of the gene in the three prime untranslated region (3'-UTR), it stops the translation of mRNA into protein, and mRNA is fully abrogated if miRNA is fully paired. Most studies argue that the other strand is lost, but in most cases, there is evidence that both strands remain active (13).

4. The natural history of chronic hepatitis
4.1. Acute hepatitis
Viral hepatitis emerges in chronic and acute forms. In acute mode, the incubation period often lasts between six weeks and six months. The symptoms of this disease include weakness, nausea, and vomiting, abdominal pain, loss of appetite, jaundice, dark urine and joint pain, which may last more than several weeks. Chronic hepatitis is defined as a long-term necroinflammatory disease caused by hepatitis B virus; the HBSAg test remains positive in patients for over six months and is divided into positive and negative HBeAg groups. HBV DNA titration reaches 10^5 copies per ml of serum, or the equivalent of 20,000 IU/ml in the HBeAg^+ group and between 2000 and 20,000 IU/ml in the HBeAg^- group, the ALT/AST ratio increases and signs of chronic hepatitis, inflammation and necrosis emerge in the liver biopsy. However, serum ALT drops significantly if the hepatocytes are severely damaged (14). As previously noted, more than 90% of infections occur in newborns less than one year old and about 5% of the adult cases of infection ultimately become chronic. Most chronic cases of this infection are transmitted from infected mothers to infants at birth or in the first year of life (15, 16). HBsAg positive patients has a wide range in countries between low (<2%) and high (>8%) (17). Iran is located in a low endemic area (<2%). The prevalence of hepatitis B has been reported as 1.3% in general population and among Iranian men is 3% and as 1.7% among Iranian women (18). Controlling the main risk factors of the infection and emphasizing the new ones emerging, such as tattoo, intravenous injections, the use of non-sterile medical tools and frequent injections in thalassemia and dialysis patients are highly important. Given the reduced immunity due to adult vaccination, it is highly recommended to extend hepatitis vaccination coverage to age 35 and to implement programs to increase awareness and better control the disease in endemic regions so as to obviate the burden of the disease. Statistical findings suggest that nearly 1.5 million people live with hepatitis in Iran. Cirrhosis and hepatocellular carcinoma
are the main complications of hepatitis B and perhaps the ultimate torturous outcome in patients. Nevertheless, early medical and medicinal interventions can help prevent the rapid progress of the disease toward HCC. (19-21). The likelihood of the disease becoming chronic is inversely associated with age. Almost 90% of children born to infected mothers will develop chronic infections if not vaccinated, but the rate drops to 30% in early childhood and to less than 5% in adulthood (22). Mir-106b, belonging to the miR-106B-25 cluster, is proven to have a major physiologic and pathophysiologic role in controlling the apoptosis of liver cells and performs this role as the negative post-transcription expression of several genes, such as TGF-β and p21/CDKNIA (23). Wen Chen conducted a study between 2008 and 2011 to assess the profile of micro in 104 patients diagnosed with Acute-Chronic Liver Failure (ACLF) and 76 patients diagnosed with Asymptomatic Carrier (AsC) and extracted miRNAs from PBMC samples. Out of the four miRNAs extracted (hsa-let-7a, hsa-let-7i, hsa-miR-16 and hsa-miR-17) with up-regulated expressions; hsa-miR-16 and hsa-let-7a had a greater up-regulated expression in patients with ACLF compared to in the AsCs. The results obtained showed a close relationship between PBMC-specific microRNAs and the miRNAs causing ACLF while no significant differences were observed between the two groups in terms of hsa-let-7i and hsa-miR-17 (24). Circulating miRNAs are non-invasive biomarkers of diagnostic tool, recently, in particular miRNA 122 was illustrated a new biomarker of acute liver injury in mice. Though miRNA are very stable molecules in serum or plasma, for better comparability and reproducibility in future studies, a well-standardized protocol is needed, in order to evaluate miRNAs as biomarkers for acute hepatitis.

5.2 Chronic hepatitis

Chronic hepatitis is divided into four phases. A: The Immune Tolerant Phase: This phase occurs mostly after neonatal infection transmitted through a mother with positive HBsAg/HBeAg. ALT level is often normal in this phase, but HBVDNA is replicated to more than one million copies. Liver biopsy is either normal or slightly inflammatory or is reported with minimal fibrosis or without fibrosis. The duration of this phase is highly variable, but is longer in people who have been infected in their neonatal period. HBeAg helps inhibit the detection of the virus by the immune system and is mostly observed in genotype C. Due to the high proliferation rate of the virus, chances of transmission are high. B: The Immune Active Phase (spontaneous clearing): In this phase, there are laboratory signs of the immune tolerant phase; ALT and HBVDNA levels increase (≥20,000 IU/ml) and HBeAg is positive. The proliferation of the virus is relatively lower in this phase compared to in the immune tolerant phase. The spontaneous clearing of HBsAg may occur in 1% to 3% of the infected every year. Anti-HBe is positive and the immune system identifies the virus as a foreign invader that causes moderate to severe damage to the liver tissue. C: The Inactive Carrier Phase: Characteristics: ALT remains at the ultimate normal level (40 IU/ml) and HBVDNA (<2000 IU/ml) is reduced or undetectable, and anti-HBe becomes positive. In histological terms, the liver shows minimal necroinflammation. D: The Reactivation Phase (HBeAg): In this phase, ALT and HBVDNA increase (≥2000 IU/ml) and the liver tissue undergoes fibrosis and inflammation. In this phase, hepatitis B virus may become reactivated and thus exacerbate the disease in 10% to 20% of the inactive carriers. The liver undergoes fibrosis and inflammation and HBVDNA levels should be examined every three or four months and ALT levels every six months (after the first year) (25-27). MiRNAs potentially contribute to the diagnosis of chronic hepatitis complications, such as silent cirrhosis. Two miRNAs, namely miR-106b and miR-181b, have a significant clinical diagnostic value in liver cirrhosis, especially at its early stages (6). Studies conducted on serum miRNA in different phases of chronic hepatitis B and during treatment with anti-viral medications show that miRNAs are effective in both the normal course of the disease and during treatment as miR-B index is an effective biomarker for the early
diagnosis of patients with chronic hepatitis treated with anti-viral medications and progressing toward passive hepatitis (28). The down-regulated expression of miR-122 obtained from the liver biopsy of patients with advanced cirrhosis and its relationship with fibrosis stages and liver stiffness can be a particular characteristic of hepatic fibrosis caused by a number of reasons (29).

5.3 Liver cirrhosis is another complication of chronic hepatitis in which the damaged liver loses its function. Cirrhosis is derived from a Greek word meaning ‘yellowish’; in histological terms, it is defined as the development of regenerative nodules surrounded by fibrous strands. Cirrhosis is divided into compensated and decompensated categories and leads to a wide range of symptoms and complications in patients, including ascites, jaundice, encephalopathy, splenomegaly and ultimately hepatocellular carcinoma (30). In spite of the risks involved (such as liver bleeding and inaccurate pathological diagnosis), liver biopsy is currently the best diagnostic tool that reports the grade and stage of the tissue inflammation, necrosis and fibrosis. Stage F4 is regarded as cirrhosis. Given the presence of ascites and bilirubin, albumin and encephalopathy, the severity of the cirrhosis, disease prognosis and potential need for liver transplantation are determined according to the Child-Pugh scale; this scale has three classes (A, B and C), with 5-6 points indicating class A, 7-9 points indicating class B and 10-15 points indicating class C (31). The mortality rate of cirrhotic patients is determined using the Model for End Stage Disease (MELD), which is based on a mathematical equation that helps calculate creatinine levels, the International Normal Ratio (INR) and bilirubin through laboratory tests (32). FibroScan measures liver stiffness and helps with the diagnosis of the disease, but it has a limited application in cases such as obesity, ascites and small intercostals spaces. Other commonly-used devices for the follow-up of patients include CT scan, ultrasound and MRI (33). The role that miR-181b plays in the progress of the disease and HBVDNA levels in patients with chronic hepatitis B demonstrates one of these capacities that eliminate the need for a liver biopsy. MiR-181b is said to activate the hepatic satellite cells and increase in the serum of cirrhotic patients; it is up-regulated in CHB patients compared to healthy people and is associated with the proliferation of HBVDNA virus and the progress of the disease, which indicates the potential importance of this marker in the independent prediction of the progress of chronic hepatitis B (34).

5.4 Hepatocellular carcinoma is a malignant liver tumor that develops mostly in cirrhotic patients. This carcinoma is the second and common leading cause of cancer-related deaths across the world, claiming almost 5210,000 lives every year (35). Viral infections with hepatitis B and C are the main causes of this carcinoma. Ultrasound, CT scan and MRI are used for diagnosing this disease. The α-fetoprotein test is also currently used as a diagnostic biomarker test, although it lacks full sensitivity and specificity. Cancerous tumorsof the liver are classified according to the Barcelona Clinic Liver Cancer (BCLC) staging system and using tumor grade, cirrhosis stage and liver function indices, which are later used in deciding the right course of treatment for the patient. Stage zero is the initial stage of cancer and has the best prognosis. Other stages include A, B, C and D and also the terminal stage, which has the worst prognosis (36, 37). The detection of plasma miRNAs in HCC patients caused by hepatitis B virus is also highly important. Zhou Jian et al. found a panel of miRNAs that was distinctively able to diagnose early-stage HCC patients; it may be therefore effective in the treatment of patients who may be missed in the window of cure. The results obtained from previous studies show that this panel, which includes miRNAs such as miR-122, miR-192, miR-92, miR-223, miR-26a, miR-27a and miR-801, has a highly accurate diagnostic power that can differentiate HCC from CHB and cirrhosis and from healthy people as well as (38).
can improve the survival rate, a novel diagnostic method to discriminate liver disease stages, circulating miRNAs have potential test as a tool of diagnosis.

6. Treatment

Hepatitis B virus is an enveloped hepatotropic virus with small double-stranded DNA of 42-nm-diameter and 3.2-kbp-length (equivalent of 3200 open pairs) belonging to the Hepadnaviridae family (39). Viruses proliferate by reverse transcription in the liver cells; however, they do not directly affect cells. DNA virus enters the nucleus, forming the ring-shaped Covalently Closed Circular DNA (cccDNA), which is similar to a small chromosome and is used as a template for mRNA synthesis. In the presence of cccDNA, antiviral medications that inhibit virus synthesis cannot create full recovery; rather, they delay damage to the liver cells (40). In the last few decades, α-interferon has been used for the treatment of patients; however, the new double formula of PEG-INF is considered a standard treatment. Six nucleotide and nucleoside medications have also been added to the treatment panel of chronic hepatitis, including Tenofovir, Telbivudine, Adefovir, Entecavir, Lamivudine and Emtricitabine, each with its different effectiveness. In most studies, the preferred duration of treatment with PEG-INF is 48 weeks, although it differs for HBeAg+ patients treated with oral anti-viral medications and depends on biochemical and virology markers and is recommended to be continued for 6 to 12 months even after the normalization of ALT, the disappearance of HBeAg, the emergence of anti-HBe and the non-detection of HBVDNA. Treating patients with analogue nucleotide medications requires greater attention to liver creatinine clearance. Surgical and non-surgical methods and ultimately transplantation are used for the treatment of complications such as hepatocellular carcinoma and end-stage cirrhosis (41, 42). (Table 1)

8. Discussion

MiRNAs have opened a promising new chapter for researchers in various diagnostic and treatment fields. These biomarkers act as a double-edged sword and will significantly contribute to science in the near future, since down-regulated and up-regulated expression are both associated with specific conditions in living creatures. Some miRNAs are more specifically present in certain tissues, such as miRNA-122 in the liver and miR-133a/b in the muscle cell. In addition to their intracellular presence, a number of miRNAs can also be found in bodily fluids such as urine, saliva and plasma. Characteristics such as conservation, endurance and stability and the ability to control hundreds of genes, which is considered an advantage, have favored them as a diagnostic test for some markers such as ALT in the assessment of liver or as miR-499 in the assessment of myocardial infarction (43, 44). Considering the extensive research currently being conducted on miRNAs, it should be admitted that, by way of unique characteristics such as mRNA inhibition or destruction, which affect gene expression through a negative control mechanism, these small, stable and conserved biomarkers provide researchers with a golden opportunity and can be used as early diagnostic panels and in decision-making about the treatment process to take before hepatitis progresses into deadly complications such as cirrhosis and liver carcinoma (45, 46). Their therapeutic importance has recently been proven with the synthesis of miRNA antagonim. Most powerful diagnostic tools currently existing, such as AST and ALT, which are also found in other diseases, cannot significantly help determine the grade or stage of complications related to hepatitis B or decide about treatment options. In many advanced cases, these enzymes are within the normal range or slightly deviate from it, and due to the AFP test's lack of adequate sensitivity and specificity, delays in diagnosing HCC are also considered a cause of the high
mortality rates associated with this disease. These tests cannot differentiate between someone with chronic hepatitis, someone who has recently developed cirrhosis, or a chronic hepatitis B carrier. Radiological techniques such as ultrasound, CT scan and MRI show little diagnostic accuracy when the lesions are very small. As stated earlier, although liver biopsy is considered the gold standard, its invasive nature and the associated risk of bleeding and infection and the likelihood of wrong pathology reports and wrong tissue samples make physicians more doubtful about the proper course of treatment. FibroScan does not have the necessary efficiency in every stage (44, 47, 48). Previous studies have shown that, in addition to their regulatory role in cells, miRNAs are also involved in many human diseases such as those associated with hepatitis B virus. By modulating the proliferation of the hepatitis virus, regulating the formation of extracellular matrix and silencing tumor-suppressor genes, these small molecules contribute to infection with chronic hepatitis B and the development of complications, including cirrhosis, fibrosis and hepatocellular carcinoma. As both diagnostic markers and fetal therapy, these molecules are considered appropriate tools for the diagnosis of hepatitis and the treatment of its complications.

8.1: microRNAs as diagnostic and prognostic roles in CHB, HCC, Cirrhosis

MiRNAs such as miRNA125a, miRNA141, miRNA1, miRNA197, miRNA122 and miRNA372, 373 have a major role in CBH and miRNA29a/b/c, miRNA200, miRNA199, miRNA133a, miRNA214 and miRNA181b have a major role in fibrosis/cirrhosis. Given the stability of these molecules in the blood circulation and their specific detection in hepatic patients, discovering their relationship with the virus genome promises the use of this method as a better way of eliminating cccDNA, which is considered a major challenge in the treatment of patients (49). In many patients with hepatitis B infection, the expression of miRNAs in serum or tissue samples can be up-regulated or down-regulated. For instance, comparing the expression of the miRNA-101 profile in the serum and liver tissue samples of patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma and in the samples taken from a healthy control group shows a down-regulated expression of microRNA-101 in HCC patients compared to the other three groups. However, not only is the expression of miR-101 in patients with liver cirrhosis not down-regulated compared to in patients with CHB and healthy controls, it is also up-regulated in the serum and liver tissue samples. Researchers intending to use these panels in the future as a modern diagnostic technique in or even in treatment follow-up and disease prognosis should ensure that the test has an acceptable sensitivity and specificity. The validity of these tests is often assessed using the Receiver Operating Characteristics (ROC) and the Area under ROC Curve (AUC). The results of the present study showed that this biomarker has a favorable diagnostic power for monitoring HCC infection in cirrhotic patients and liver cirrhosis infection in chronic hepatitis patients (50). It should be noted that miRscan can be used as a potential marker in identifying liver pathologies and also in optimizing the clinical experience. The expression levels of miR-885-5b were found to be much higher in the serum of patients with cirrhosis, chronic hepatitis and hepatocellular carcinoma. It is possible that other laboratory markers such as GGT, AFP, AST and ALT are not related to the up-regulated expression of miR-885-5b in patients with liver damage. Nevertheless, this biomarker can be used as a supplementary biomarker in the assessment and detection of liver pathologies associated with hepatitis B (51). In a study conducted by Bo-XunJin et al. between 2009 and 2013 on 495 people divided into three groups of 165 and consisting of healthy controls and patients with chronic hepatitis and liver cirrhosis, which was performed in several phases, including the discovery phase, the training phase, the validation phase and the blinded test phase, the logistic regression analysis showed that the expression of some of these biomarkers was related to HBVDNA and liver function tests, including albumin (ALB), ALT and Cholinesterase (CHE) tests. Out of the 53 miRNAs in this study, 10 miRNAs were used for
determining cirrhosis, including miR-27a-3p, miR-451a, miR-1, miR-18a-5p, miR-29c-3p, miR-106b-5p and miR-185-5p, and three for both CHB and liver cirrhosis, including miR-21-5p, miR-122-5p and miR-146a. Based on the data obtained by the newly-designed panel, a new diagnostic tool was used for differentiating healthy people from patients with chronic hepatitis and cirrhosis. The sensitivity and specificity of this panel were reported as 85% and 70%, respectively (52). The use of the comprehensive expression of the miRNA present in peripheral blood exosome for the diagnosis of liver diseases has also been tested. The exosome in the endoplasmic reticulum network can carry mRNAs and miRNAs. RNA is extracted using microarray and real-time qPCR analysis. The results of the miRNA expression can then be used to compare with the grading and staging of chronic hepatitis, which are determined through blood and histology tests. The histological grading and staging of biopsies are performed using the METAVIR classification system. The differences between the initial phase of fibrosis and the final inflammation grade can be understood with the help of a group of miRNAs. These panels are even recommended to be used in the staging and grading of fibrosis (53). As mentioned earlier, for various reasons such as the patient’s unwillingness, bleeding, and the lack of sufficient tissue sample for pathology tests and poor results, gastroenterology and hepatology specialists do not recommend taking liver biopsies from patients in many cases. Certain miRNAs, such as miR-124, strongly appear in the necroinflammation of the liver tissue and can thus be used in grading and staging. MiR-124 has been found to serve as a non-invasive biomarker in the diagnosis of moderate to severe liver necroinflammation. Moreover, there is a positive relationship between interleukin-10 and up-regulated miR-124. It is worth noting that miR-124 is down-regulated in patients treated with entecavir for 48 weeks, which is also associated with histopathological recovery (54). One of the criteria for the treatment of chronic hepatitis and cirrhosis is the amount of virus in the patient’s serum and treatment is initiated or discontinued according to this amount. The capacities of miRNAs can also be used in relation to the progress of the disease. The ultimate question of whether or not plasma miRNAs have a diagnostic value in identifying diseases associated with hepatitis B can be answered by extensive research in the future, but the studies conducted to date are also promising and satisfactory. Two models of study, namely human and animal models, have used liver biopsy to determine necrosis and fibrosis levels. Of the eight miRNAs studied, miR-122 had the highest up-regulated expression in human and animal samples. The researchers have therefore concluded that this miRNA can have a major role as a new, reliable and predictive biomarker of the factors causing liver damage, such as alcohol, chemicals and viruses (55). By the same token, miR-143 and miR-215 are demonstrated to serve as diagnostic biomarkers in patients with CHB and HCC and in healthy people, and miR-215 has an up-regulated expression in CHB patients compared to healthy people. Statistical findings suggest that both these miRNAs have a significant sensitivity and specificity in patients with hepatocellular carcinoma and chronic hepatitis and can potentially be considered as diagnostic biomarkers (56). MicroRNAs panels are reliable and sensitive to discriminate HCC patients from non HCC individuals who infected with HBV OR HCV. Panels of miRNAs can produce results than single miRNA diagnosis (57). The main issue that remains is that these panels cannot have a perfect sensitivity and specificity in all populations, which may be due to genetic and physiological differences among people (58, 59). Table 2: Results of many Meta – Analysis

3. Conclusion

Given the noted differences, local studies need to be conducted in every region. Nevertheless, the identification and study of this miRNA in patients with hepatitis B virus will serve as a
good terminator or queen, since different studies have reported a promising role for it in the early diagnosis of different forms of chronic hepatitis and for grading and staging the disease and determining the patients’ clinical conditions. Overall, it appears that miRNAs will play a significant role in the future in both the diagnosis of diseases such as hepatitis B and in decision-making about their proper course of treatment.

**Conflict of Interest:** There is no conflict of interest to be declared.

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52. Bo-Xun Jin1 Y-HZ, Wen-Jing Jin2, Xiang-Ying Sun, Gui-Fang Qiao,, Wei Y-Y. MicroRNA panels as disease biomarkers distinguishing hepatitis B virus infection caused...


Table 1  
MicroRNAs function related with hepatitis B virus (CHB, fibrosis/cirrhosis, HCC)

<table>
<thead>
<tr>
<th>MicroRNAs in CHB</th>
<th>Up/Down regulated</th>
<th>Function</th>
<th>References</th>
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<tbody>
<tr>
<td>miR-122</td>
<td>Up</td>
<td>Inhibited viral production hepatic HBV DNA levels</td>
<td>(43)</td>
</tr>
<tr>
<td>miR-372/miR-373</td>
<td>Up</td>
<td>promoted HBV expression diagnostic tool occult hepatitis B virus infection</td>
<td>(44)</td>
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<tr>
<td>Panel miRNAs(let-7c, miR-23b, miR-122, and miR-150)</td>
<td>Down</td>
<td>reactivate liver inflammation</td>
<td>(45)</td>
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<tr>
<td>miR-125a-5p</td>
<td></td>
<td></td>
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<tr>
<td>miR-548, miR-548c5p</td>
<td>Up</td>
<td>involved in type I IFN signaling associated with the proliferation of HBVDNA virus and the progression of the disease and prediction of the progress of chronic hepatitis B as diagnostic biomarkers in patients with CHB from HCC</td>
<td>(46)</td>
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<tr>
<td>MiR-181b</td>
<td>Up</td>
<td></td>
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<tr>
<td>Panel(miR-21-5p, miR-122-5p and miR-146a)</td>
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<thead>
<tr>
<th>MicroRNAs in liver Fibrosis / Cirrhosis</th>
<th>Up/Down regulated</th>
<th>Function</th>
<th>References</th>
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<tbody>
<tr>
<td>miR-122</td>
<td>Down</td>
<td>Diagnostic advanced cirrhosis</td>
<td>(49)</td>
</tr>
<tr>
<td>miR-124</td>
<td>Down</td>
<td>Diagnostic biomarkers moderate to severe liver necroinflammation suppressed collagen synthesis, necroinflammation</td>
<td>(50)</td>
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<tr>
<td>MiR-133a</td>
<td>Down</td>
<td></td>
<td></td>
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<tr>
<td>(miR-199-200,miR-221/222, miR-214-5p, miR-181b)</td>
<td>Up</td>
<td>progression of liver fibrosis, liver fibrosis</td>
<td>(51)</td>
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<tr>
<td>hsa-miR-16 and hsa-let-7a</td>
<td>Down</td>
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<tr>
<td>miR-27a-3p, miR-451a, miR-1, miR-18a-5p, miR-29c-3p</td>
<td>Down</td>
<td></td>
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<tr>
<td>miR-106b and miR-181b</td>
<td>Down</td>
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Expression in patients with HBV Acute-on-chronic liver failure (ACLF) compared to in the Asymptomatic Carrier (AsCs) (24)

Differentiated healthy individual from cirrhosis (48)

Diagnostic biomarkers in liver cirrhosis, especially at its
<table>
<thead>
<tr>
<th>MicroRNAs in Hepatocellular Carcinoma</th>
<th>Up/down regulated</th>
<th>Function</th>
<th>References</th>
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<tbody>
<tr>
<td>miR-122</td>
<td>UP</td>
<td>inhibits replication of the HBV, suppress proliferation and invasion of HCC cells cancer</td>
<td>(57, 58)</td>
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<tr>
<td>miR-122</td>
<td>Down</td>
<td>associated with hepatocellular carcinoma</td>
<td>(58)</td>
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<tr>
<td>miR-375,372</td>
<td>Up</td>
<td>Initiation of hepatocellular carcinoma</td>
<td>(59)</td>
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<tr>
<td>miR-143 and miR-215</td>
<td>UP</td>
<td>as diagnostic biomarkers in patients with CHB</td>
<td>(60)</td>
</tr>
<tr>
<td>miR-101</td>
<td>UP(serum), Down(tissue)</td>
<td>as diagnostic biomarkers in patients with CHB and HCC</td>
<td>(61)</td>
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<tr>
<td>miR-18/miR-195/miR-199a/miR-200a/miR-125a</td>
<td></td>
<td>provides a promising biochemical marker of HBV-related HCC</td>
<td>(55, 62)</td>
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<tr>
<td>miR-192/miR-223/miR-26a/miR-27a/miR-801</td>
<td></td>
<td>highly accurate diagnostic, differentiate HCC from CHB and cirrhosis from healthy people</td>
<td>(38, 63)</td>
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<tr>
<th>MicroRNAs in therapeutic uses</th>
<th>Up/Down regulated</th>
<th>Function</th>
<th>References</th>
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<tr>
<td>miR-199a and miR-210</td>
<td>down</td>
<td>miRNA-binding to viral genome or transcript, bind to HBsAg mRNA leading to reduced HBsAg expression</td>
<td>(64, 65)</td>
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<td>miR-122 mimics</td>
<td></td>
<td>may provide a novel strategy to slow down liver disease progression and to prevent and treat HCC</td>
<td>(66)</td>
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<tr>
<td>Author</td>
<td>year</td>
<td>conclusion</td>
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<td>Zhang LH1, Zhang CY2, Dai XZ2, Zhang J2, Zhang F2.</td>
<td>2017</td>
<td>There is no significant association between miR-146a rs2910164 polymorphism and the risk of HCC, but miR-146a rs2910164 polymorphism may increase the risk of HBV-positive HCC.</td>
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<tr>
<td>Li CY1, Pang YY1, Yang H2, Li J1, Lu HX1.3, Wang HL1, Mo WJ1, Huang LS1, Feng ZB1, Chen G1</td>
<td>2017</td>
<td>MiR-101-1 may be a prospective biomarker for diagnosis and prognosis of HCC. Potential targets of miR-101-3p could regulate genesis and development of HCC and potential therapies in HCC.</td>
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</tr>
<tr>
<td>Zheng L1, Zhuang C1, Zhao J1, Ming L2.</td>
<td>2017</td>
<td>Our results suggest that miR-146a and miR-196a2 polymorphisms are associated with increased risk of HCC, especially in Asian.</td>
<td></td>
</tr>
<tr>
<td>Li G1, Shen Q, Li C, Li D, Chen J, He M.</td>
<td>2015</td>
<td>Circulating miR-21 has highest level of diagnostic efficiency among three miRNAs candidate biomarkers (miR-21, miR-122, and miR-223) for detection of HCC.</td>
<td></td>
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<tr>
<td>Wen Y1,2, Han J1,2, Chen J1,4, Dong J1, Xia Y5, Liu J4, Jiang</td>
<td>2015</td>
<td>meta-analysis revealed that four miRNAs (miR-20a-5p, miR-320a, miR-324-3p and miR-375) could be used as preclinical biomarkers (pmeta &lt; 0.05) for HCC</td>
<td></td>
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<tr>
<td>Xing TJ1, Jiang DF2, Huang JX2, Xu ZL2.</td>
<td>2014</td>
<td>The detection of miR-122 and miR-29 may be useful in evaluating the inflammatory liver injury and fibrosis associated with chronic HBV infection.</td>
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<tr>
<td>Sirio Fiorino, Maria Letizia Bacchi-Reggiani, Michela Visani, Giorgia Acquaviva</td>
<td>2016</td>
<td>miR-21, miR-122, miR-125a/b, miR199a/b, miR-221, miR-222, miR-223,miR-224, as biomarkers for an early diagnosis of HCC development as well as for the assessment of its prognosis in HBV- or HCV-positive patients with this type of malignancy,</td>
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<tr>
<td>Jingcheng Yang1, Shuai Han1, Wenwen Huang1, Ting Chen2</td>
<td>2014</td>
<td>five up regulated (miR-221, miR-222, miR-223 and miR-224) and four down regulated (miR-130a, miR-195, miR-199a and miR-375) miRNAs. These miRNAs may involve in the onset and progression of liver cancer and serve as potential diagnostic and therapeutic targets of this malignancy.</td>
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<td>2017</td>
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<tr>
<td>Authors</td>
<td>Year</td>
<td>Summary</td>
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<td>Yan Ding1,<em>, Jia-Lai Yan2,</em>, An-Ning Fang3, Wei-Feng Zhou4 and Ling Huang1</td>
<td>2015</td>
<td>The high frequency expression miRNAs (miR-21, miR-199 and miR-122) might be more specific for the diagnosis of hepatocellular carcinoma.</td>
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<td>G.Q. Zhou, H. Meng, J.R. Wang, F.X. Sun, X.J. Wang, R.B. Wang and X.B. Wang</td>
<td>2015</td>
<td>The meta-analysis results indicated that the miR-196a-2<em>T, miR-122</em>del, miR-106b-25<em>A, and miR-let-7c</em>del alleles/carriers increase the risk of hepatitis B among the Asian population. However, the miR-146a, miR-499, miR-149, miR-218, and miR-34b/c polymorphisms may not be linked with the risk of hepatitis B.</td>
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<tr>
<td>Shao-Liang Zhu1,<em>,Jian-Hong Zhong1,</em>,Wen-Feng Gong1,*,Hang Li2 Le-Qun Li1</td>
<td>2016</td>
<td>The polymorphism miR-196a2 C.T, but not miR-499 A.G, may be associated with decreased HBV-related HCC risk. These conclusions should be verified in large, well-designed studies.</td>
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