

Hepatocellular Carcinoma Prediction and early Diagnosis of Hepatitis B and C viral infection using miR-122 and miR-223 in a sample of Iraqi patients.

Saja Khudhier Essa Al-Juboori  , *Shaymaa I K Al-Juboori**  

Department of Biology, College of Science for Women, University of Baghdad, Baghdad, Iraq.

*Corresponding Author

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Abstract

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death. Therefore, it is critical for researchers to understand molecular biology in greater depth. In several diseases including cancer, abnormal miRNA expression has been linked to apoptosis, proliferation, differentiation, and metastasis. Many miRNAs have been studied in relation to cancer, including miR-122, miR-223, and others. Hepatitis B and C viruses are the most important global risk factors for HCC. This study is intended to test whether serum miRNAs serve as a potential biomarker for both HCC and viral infections HBV and C. The expression of miRNA in 64 serum samples was analyzed by RT-qPCR. Compared to healthy volunteers, HCC patients' sera expressed significantly lower levels of miR-122 and miR-223. Furthermore, we compared the expression of these miRNAs between early-diagnosed HCC patients and healthy controls. There was a significant difference between miR-122 expression in HCC sera and healthy volunteers' sera (0.000 and 0.253, respectively), with a P value of <0.0001. Early diagnostic patients without treatment had completely deleted miR-122 expression levels, while those treated had slightly elevated levels. Clearly, miR-122 has been identified as a biomarker for early detection and follow-up of HCC treatment. HBV and HCV specimens expressed significantly lower levels of miRNA than normal samples with a P value of <0.0001. It is recommended that these findings be further investigated for diagnostic purposes. Further, these miRNAs are highly specific for diagnosing HCC, HBV, and HCV, making them valuable therapeutic indicators.

Keywords: Hepatocellular carcinoma, HBV, HCV, Micro RNA-122, Micro RNA-223.

Introduction

Liver cancer is a cancer that occurs within the liver's cells. A variety of kinds of cancer can occur in the liver. One of the most prevalent forms of liver cancer is hepatocellular carcinoma (HCC), which develops in the main type of liver cell (hepatocyte). There are approximately two million deaths caused by hepatocellular carcinoma (HCC) Worldwide¹. As the second leading cause of cancer-related death worldwide, hepatocellular carcinoma (HCC) has a very poor prognosis, making it the most prevalent primary liver malignancy. Since the beginning of

this century, there has only been a slight improvement in the prognosis of HCC patients in the United States. It is unclear which molecular mechanisms and other mechanisms contribute to HCC progression. Thus, a better understanding of these mechanisms is urgently required. Most patients who are diagnosed with HCC at an advanced stage will require systemic therapy, with sorafenib being the most commonly prescribed drug at present. However, sorafenib therapy only has a minimal effect on patient survival².

Hepatitis B virus (HBV) is a partially double-stranded DNA virus that belongs to the Hepadnaviridae family. There are over 250 million chronically infected individuals worldwide with HBV infection. The Hepatitis B virus is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma³. The pathogenesis of hepatitis B, which cause acute, chronic liver disease Worldwide and can lead to cirrhosis and hepatocellular carcinoma, is poorly understood⁴.

The hepatitis C virus is one of the leading causes of death in people because of terrible consequences on the liver. This virus has been classified into 67 subtypes with less than 15% nucleotide variation and seven main patterns with 30-35% nucleotide variation. The virus was identified in 1989 and is the cause of more than 50% of chronic hepatitis infections worldwide⁵. Although hepatitis viruses are the primary cause, other harmful chemicals, including certain medicines, alcohol, and certain autoimmune conditions can also cause infection. Patients with hepatitis C infections have a 15%–40% chance of developing other chronic liver conditions, including liver cancer and cirrhosis⁶.

MicroRNA is a small, single-stranded, non-coding RNA molecule containing 21 to 23 nucleotides. It is believed that miRNAs are found in plants, animals, and some viruses, and play a role in silencing RNA and regulating gene expression following transcription. A short non-coding RNA that is able to regulate the expression of target genes post-transcription and interact with mRNA-coding genes. Several illnesses, including cancer, have been linked to abnormal miRNA expression, which plays a vital role in a variety of biological functions including apoptosis, proliferation, differentiation and metastasis⁷. According to increasingly recent research, miRNAs play an important role in liver regeneration, development and metabolic functions^{7, 8}. Thus, changes in intrahepatic miRNA networks have been observed as a potential risk factor for liver diseases, such as hepatitis, steatosis, cirrhosis, and hepatocellular carcinoma. In the adult liver, miR-122 is the most prevalent miRNA, and it plays an important role in liver biology and disease. In addition, miR-122 has been demonstrated to be an important host factor for the transmission of the

hepatitis C virus, as well as a target for antivirals, which can be used in conjunction with current treatments such as direct-acting antivirals and interferons. According to recent studies, miR-122 plays a crucial role in the replication of HBV and HCV by differentially regulating the host gene. The microRNA-223 plays an important role in innate immunity, and dysregulation of its expression is responsible for the pathogenesis of several inflammatory diseases and cancers⁹. Many cancer types, including hepatocellular carcinoma (HCC), are associated with dysregulation of circulating miRNAs.

According to several studies, miR-122 and miR-223 expression are decreased in a subset of patients with highly invasive and metastatic hepatocellular carcinoma (HCC) who are infected with the hepatitis B virus (HBV). In addition, it is reduced in patients with nonalcoholic steatohepatitis (NASH). The plasma miR-122, and miR-223 provide a high diagnostic accuracy for HCC that can be used to diagnose early-stage HCC¹⁰. In the adult liver, miR-122 is the most prevalent miRNA, and it plays an important role in liver biology and disease. In addition, miR-122 has been demonstrated to be an important host factor for the transmission of the hepatitis C virus, as well as a target for antivirals, which can be used in conjunction with current treatments such as direct-acting antivirals and interferons. The miRNA-223 plays an important role in innate immunity, and dysregulation of its expression is responsible for the pathogenesis of several inflammatory diseases and cancers⁸. However, miR-223 expression levels are usually higher in colorectal and recurrent ovarian cancers, whereas miR-223 is typically repressed in hepatocellular carcinoma and leukemia. MiR-223 downregulation is correlated with high tumor burden, disease aggressiveness, and poor patient prognosis in some cases. Therefore, understanding miR-223's complex role in cancer diagnosis and treatment is essential. It has been extensively studied which miRNAs are involved in various forms of cancer, including miR-122, miR-223, and others. However, much still remains to be discovered about miRNAs, particularly regarding their role in cancer therapies, despite the considerable growth of research into miRNAs over

the last decade. In several types of cancer, miR-122 and miR-223 have been shown to be dysregulated and abnormally expressed, suggesting that they may be useful diagnostic and prognostic biomarkers for HCC and both HBV and HCV¹⁰. The objective of

this study is to test the hypothesis that serum miRNAs (122 and 223) may serve as a potential biomarker for both HCC and viral infection HBV and C by performing quantitative real-time PCR.

Materials and Methods

The collection of samples

The study was approved by the Iraqi Ministry of Health's ethics committee. A total of 64 samples were collected, 20 samples of HCC (under treatment), 4 samples of HCC (before treatment), 10 samples of HBV, 10 samples of HCV, and 20 samples of healthy individuals. An extensive collection of samples was performed on Iraqi patients after an oncologist made a clinical diagnosis of hepatocellular carcinoma and a diagnosis of Hepatitis B and C infection by a blood test. Baghdad hospitals provided samples, including the following:

1. Medical City Hospital (Oncology Teaching) Hospital
2. Medical City Hospital (Gastroenterology and Hepatology Teaching) Hospital
3. Al-Yarmook Teaching Hospital

Between November 2022 and April 2023.

Total RNA extraction

A total of five milliliters of blood were taken by venipuncture using disposable syringes from each participant. Using disposable gel tubes and leaving it at room temperature for five minutes to allow it to clot, the blood will be separated by centrifugation for five minutes and, using a pipette. In an Eppendorf tube, 250 ml of serum was added to 500 ml of Trizol, mixed properly, and stored in the refrigerator at -20 °C until examination.

Following the separation of blood samples into serum, total RNA was isolated. The total RNA was extracted using TRIzol™ Reagent, 2023 (Invitrogen, USA), according to the manufacturer's instructions.

Assessment of RNA quantity and purity

To determine the quantity and purity of the extracted RNA, the Qubit™ RNA HS Assay Kit (Q32852) from ThermoFisher® (USA) was used according to the manufacturer's instructions. This is a highly reliable and selective method for quantifying low-abundance RNA samples. The concentration of miRNA in all samples is within the range of 3 ng/μL and 45 ng/μL, which indicates that miRNA is highly selective for miRNA over other forms of RNA.

Complementary DNA (cDNA) Synthesis, with Specific Primer

The Mirna cDNA synthesis kit with polymerase tailing kit provides a complete system for the synthesis of first-strand miRNA from total RNA templates. In accordance with the manufacturer's instructions, total RNA was reverse transcribed in order to obtain complementary DNA (cDNA). The kit includes the well-known protoScript® Reverse Transcriptase from NEB as well as all the chemicals required for miRNA synthesis (ProtoScript® First Strand cDNA Synthesis Kit (E6300S), NEB).

Quantification of microRNAs

According to the manufacturer's protocol, Qubit™ microRNA Assay Kits from ThermoFisher® (USA) were used to quantify small RNA (~20 nucleotides or base pairs). This miRNA quantification kit allows rapid detection of all types of small RNA, including microRNAs and siRNAs, as well as single-stranded and double-stranded RNAs. It is highly selective for small RNA over large mRNA or rRNA, and can tolerate contaminants such as salts, solvents, or detergents.

Specific primers of miRNAs

Applied Biological Materials
(Macrogen)/South Korea designed and produced the primer sequences (miRNA-122, miRNA-223)

utilized in laboratory work with melting temperatures ranging from 60 to 95 ° C. Primer lengths range from 18 to 23 nucleotides and PCR amplicons are 75 to 150 base pairs long. The name and sequence are given below in Table 1:

Table 1. The name of primer, sequence used in the Molecular study

Primer	Sequence
miR-122 RT	GTCGTATCCAGTGCAGGGTCCGAGGTGCACTGGATACGACCAAACAC
miR-122F	TGCGGTTGGAGTGTGACAATGG
miR-122R	CAGTGCAGGGTCCGAGGT
U6-F	GAGAAGATTAGCATGGCCCCT
U6-R	ATATGGAACGCTTCACGAATTTGC
miR-223RT	GTCGTATCCAGTGCAGTGTCTCGTGGAGTCCGCAATTGCACTGGATACGACGGG GTA
miR-223F	TGTCAGTTTGTCAA
miR-223R	CAGTGCAGTGTCTCGTGGAGT

Real-time PCR (RT-qPCR) procedure for miRNA

A Luna Universal qPCR Master Mix (M3003S) from NEB (UK) was used in this study. It is an optimized 2X reaction mix for real-time qPCR detection and quantification of target DNA sequences using the SYBR®/FAM channel of most real-time qPCR instruments. The kit contains hot-start Taq DNA polymerase and a passive reference dye that can be used with a variety of instruments. The assay was conducted according to the instructions provided by the manufacturer.

The validation of miRNA ratios by RT-qPCR

To determine whether circulating miRNA expression could be used to diagnose HCC, serum samples from 64 participants were subjected to RT-qPCR. A comparison was made between healthy controls and different patient groups (HCC, HBV, and HCV).

Results and Discussion

Expression of miR-122 in HCC, HCV and HBV

According to an analysis of RT-qPCR data, miR-122 was downregulated in patients with HCC,

Calculate the gene expression

The expression of miR-122 and miR-223 in patient samples was measured using quantitative real-time PCR (qRT-PCR). Both miR-122 and miR-223 expression were measured using a relative cycle threshold ($2^{-\Delta\Delta C_t}$) methodology¹¹. In addition to healthy control samples, RNAU6 was used as an internal control (housekeeping gene). SnRNA plays a crucial role in the processing of premature mRNA within the spliceosome.

Statistical analysis

A combination of Microsoft Excel 2019 and GraphPad Prism software (version 8.0.0) was used to analyze the study's data. In order to compare two means, the unpaired t test was used, whereas a one-way analysis of variance (ANOVA) was used to compare more than two means. Furthermore, a two-way ANOVA followed by a Tukey multiple comparison test was used to compare more than two means. In this study, a p-value of 0.05 was used.

HBV, and HCV compared to healthy controls. The relative fold change average of miR-122 was (0.15, 0.59, and 0.36) in patients with HCC, HBV and HCV respectively compared to the control (Fig. 1

A, B, and C). For each sample, the fold change was calculated using the following equation: $FCH(p) = 2^{-(\Delta\Delta C_t)}$. To analyze the data, we used graph pad prism software (Unpaired T-test). Statistically significant differences were found between HCC samples and those collected from healthy controls P value (****) $P \leq 0.0001$. In accordance with this finding decreased expression of miR-122 is frequently observed in HCC and is associated with poor prognosis, larger tumor size, poor differentiation, invasion and metastasis. In addition, there has been a report that miR-122 is a tumor suppressor microRNA (miRNA) that is downregulated in HCC causing tumor progression, apoptosis evasion, drug resistance and metastasis¹². The results of another study showed that miR-122 promotes hepatitis C virus (HCV) replication by interacting with two binding sites located at the 5'-UTR of HCV genome, MiR-122 inhibits the expression and replication of HBV as well as the proliferation and malignant transformation of hepatocytes^{13,14}. HBV infection was also associated with the risk of non-liver cancer among Chinese adults, particularly cancers of the digestive system¹⁵.

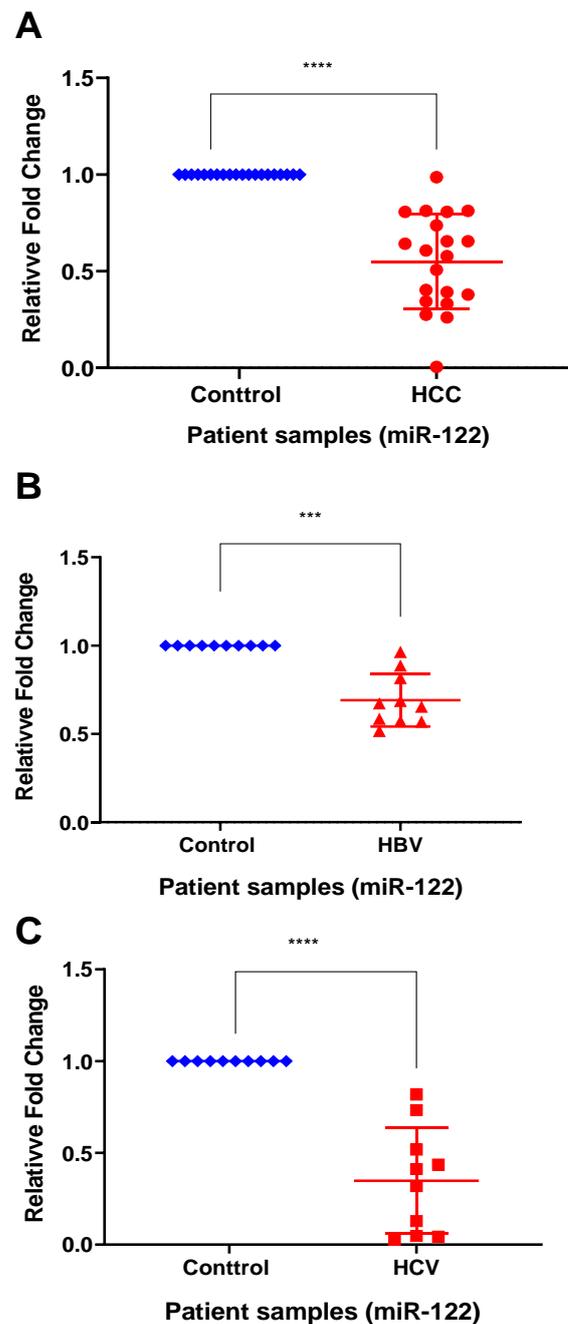


Figure 1. The graphs illustrates the relative fold change for patients with(A): HCC(n=20), (B): HBV(n=10) and (C): HCV (n=10) were compared to healthy control samples using (qRT-PCR). The relative fold change represents decrease in miR-122 expression. Also, the fold change for each sample was calculated using this Eq : $FCH(p) = 2^{-(\Delta\Delta C_t)}$. Unpaired t-test, P value (****) ≤ 0.0001 .

Additionally, the results were presented in the form of a heat map to provide a comprehensive picture of all HCC samples in one graph. The Hierarchical clustering heat maps were created using the GraphPad Prism software package. A heat map is a two-dimensional representation of data in which values are represented by colors. Fig. 2, presents a graphical representation of the total number of differentially expressed miR-122 and miR-223 (in which individual values are displayed in colors) based on the previously mentioned

approach (Relative Fold Change) for all HCC patient samples compared to healthy controls. Furthermore, the heat map illustrates the downregulation of miR-122 and miR-223 in different patient samples. Ordinary one-way ANOVA analysis was applied and the results showed that miR-122 and miR-223 levels in the HCC group ($P < 0.0001$) were significantly lower than the healthy controls.

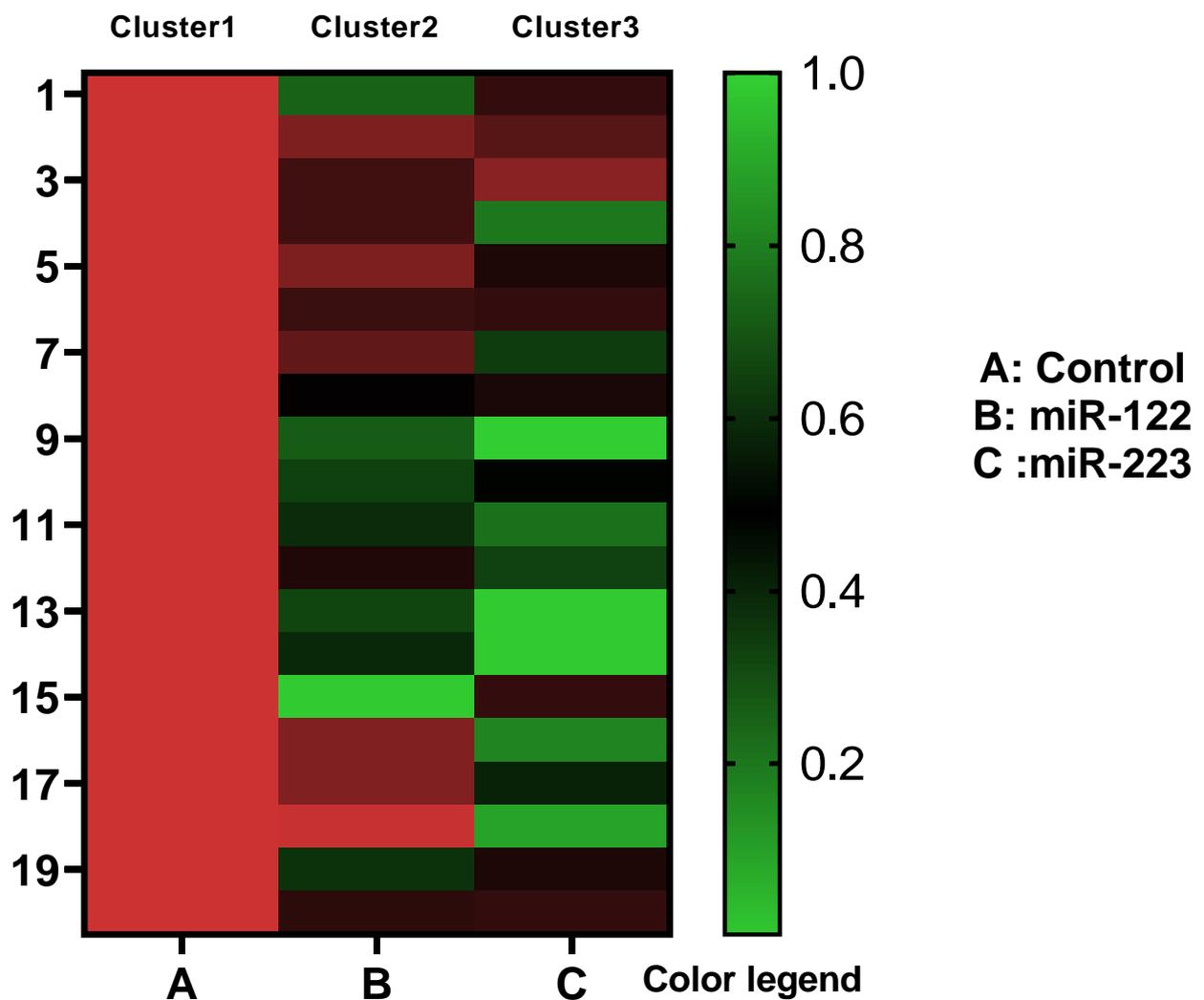


Figure 2. Heat map displaying the differential expression patterns of miR-122 and miR-223 between HCC patient samples compared to healthy control samples (n=20). The color gradient is between red and green with black in the middle. Ordinary one-way ANOVA analysis, P value (****) ≤ 0.0001 .

MiR-223 expression in HCC, HBV and HCV:

Results of miR-223 gene expression, as determined by qRT-PCR, are presented as a relative fold change between patients with HCC, HBV and HCV and healthy control samples. As shown in (Fig. 3:A,B, and C), the relative fold change indicates a decrease in miR-223 expression. Additionally, the fold change for each sample was calculated using the following Eq : $FCH(p) = 2^{-(\Delta\Delta Ct)}$. The data were analyzed using the unpaired t test, which demonstrated a highly significant difference in miR-223 expression between HCC,HBV and HCV samples and control samples ($P \leq 0.0001$). Several cancer types, including hepatocellular carcinoma (HCC), have been shown to have dysregulated miRNAs (miRNAs). In HCC, miR-223 is downregulated in association with the epigenetic regulation of highly expressed sulfatide and plays an important role in tumor metastasis¹⁶.

As reported by Pratedrat and colleagues in 2020, miR-223-3p, miR-199a-5p, and miR-451a were significantly less expressed in cancerous tissues compared to non-cancerous tissues. The expression of miR-223 is associated with hematopoiesis, apoptosis, cell proliferation, migration, and invasion. The miR-223 gene target includes the cytoplasmic activation and proliferation-associated protein-1 (Caprin-1), insulin-like growth factor-1 receptor (IGF-1R), and other genes involved in cell proliferation and cell cycle. The miR-223 has been proposed as an early cancer diagnostic biomarker in several studies. It is evident that miR-223 plays a significant role in the progression and development of cancers. There is evidence that miR-223 regulates multiple aspects of the immune response, and that abnormal miR-223 expression is associated with multiple infectious diseases, including viral hepatitis^{17,18}.

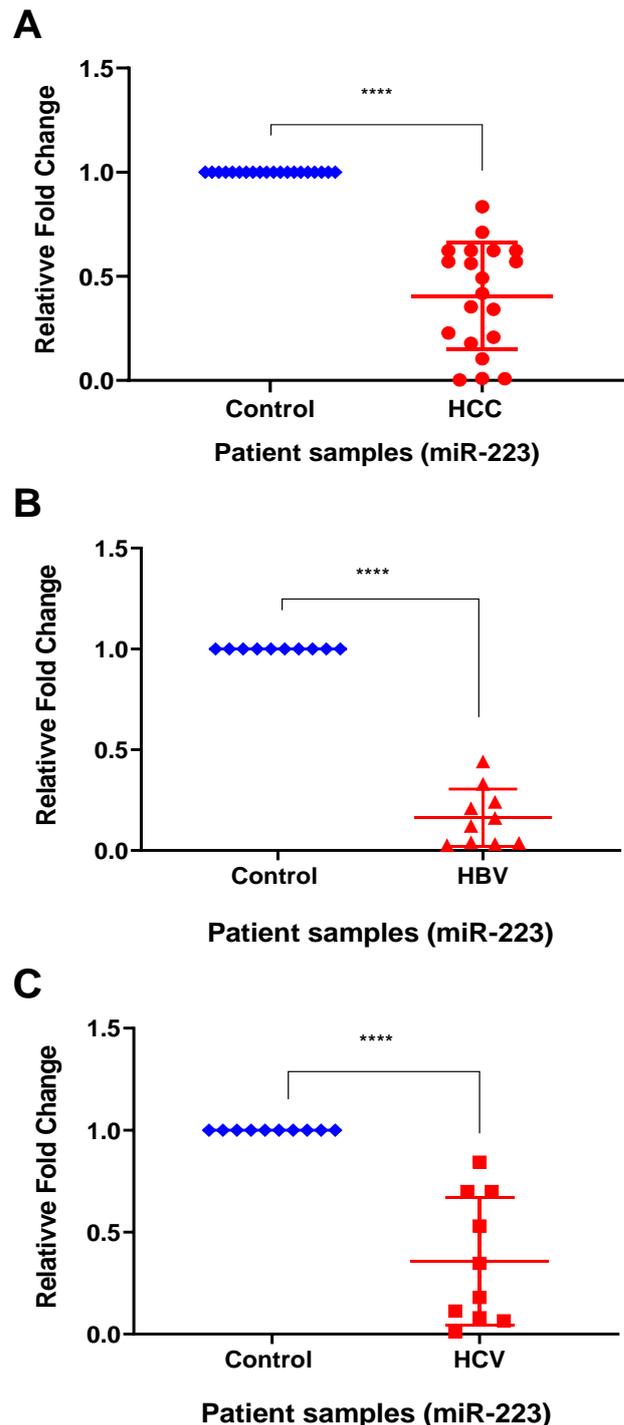


Figure 3. The graph illustrates the relative fold change for patients with (A): HCC(n=20), (B): HBV(n=10) and (C): HCV(n=10) compared to healthy control samples using (RT-qPCR). The relative fold change represents decrease in miR-223 expression. Also, the fold change for each sample was calculated using this Eq: $FCH(p) = 2^{-(\Delta\Delta Ct)}$. Unpaired t-test P value (****) ≤ 0.0001 .

In addition to providing an overview of the miR-122 and miR-223 levels in samples from patients with HBV, HCV, and healthy controls, the data was also presented as a heat map. GraphPad Prism 8.0 was used to create the Hierarchical clustering heat maps. Using the previously mentioned approach (Relative Fold Change), the total number of differentially expressed miR-122 and miR-223 (in which the individual values are displayed in color) for all patient samples comparing to healthy controls based on the differential expression of miR-122 and miR-223.

The heat map also illustrates how miR-223 and miR-122 are downregulated across different samples compared of patients as depicted in Fig. 4. Moreover, using conventional one-way ANOVA techniques, a significant difference was found between the miR-122 and miR-223 levels in the groups with HBV and HCV ($P \leq 0.0001$). This suggests that the downregulation of miR-122 and miR-223 could be used as reliable biomarkers to differentiate HBV and HCV infections from normal healthy samples.

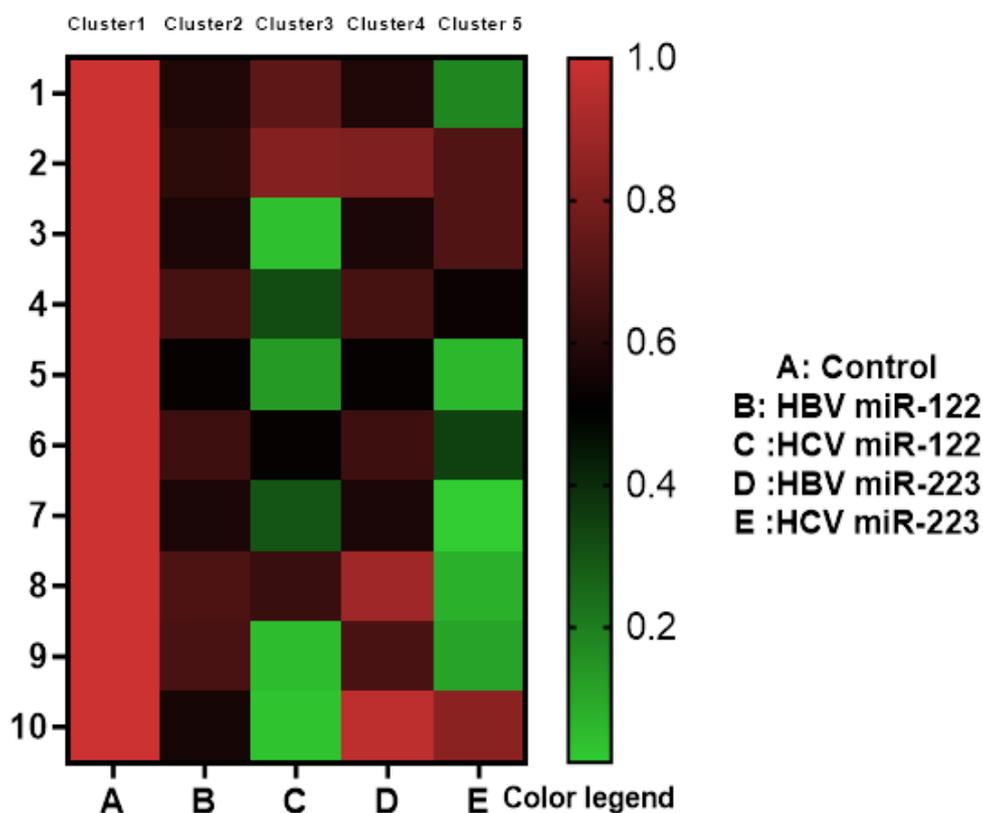


Figure 4. Heat map displaying the differential expression patterns of miR-122 and miR-223 between HBV, and HCV patient samples (n=10) compared to healthy control samples. The color gradient is between red and green with black in the middle. Ordinary one-way ANOVA analysis, P value (****) ≤ 0.0001 .

Expression of miR-122 and miR-223 in non-treated HCC

Four samples of non-treated HCC patients were collected and miR-122 and miR-223 expression was assessed in comparison to

previously analyzed samples of treated HCC patients. There are a limited number of samples due to the availability of samples. Fig. 5, illustrates the reduction of miR-223 and miR-122 expressions when compared to control samples. In accordance with the Two-way ANOVA analysis, there was a

highly significant difference between the two miR-223 and miR-122 patient samples and healthy control samples, $P \leq 0.0009$. Gene expression values were obtained using qRT-PCR and expressed as relative fold changes, which were calculated using the Eq : $FCH(p) = 2^{-(\Delta\Delta Ct)}$. According to findings from cancer cell lines, miR-122 functions as an oncogenic or tumor-suppressive miRNA. Examining patient tumor samples further supported the conclusion that miR-122 dysregulation plays a crucial role in the development of cancer. Furthermore, analysis of the levels of miR-122 expression in various tissues suggested that the physiologic function of miR-122 in carcinoma appears to vary depending upon the type of cancer. Several recent studies have demonstrated the effectiveness of miR-122 in the treatment of cancer 8 In fact, miR-122 has become a popular choice for cancer treatments due to its efficacy in improving patient outcomes. MiR-223 levels were reduced in cell lines with high metastatic potential and in HCC specimens, according to Dong et al.,2014¹⁹ . MiR-223 plays an important role in tumor metastasis by regulating the epigenetic regulation of highly expressed sulfatide in HCC. Therefore, miR-223 is considered to be a potential therapeutic target for inhibiting the progression of HCC. The results of this study are consistent with those of other studies^{20,17,8} . However, found that HCC patients exhibited significantly higher levels of miR-223 than healthy individuals in their study. Therefore, miR-223 has been proposed as a potential diagnostic biomarker for HCC. It is believed that tissue injury, rather than carcinogenesis, is the cause of miR-223 overexpression in hepatocellular

Conclusion

Based on our findings, both miR-122 and miR-223 expression decreases in HCC, HBV, and HCV patients compared to healthy controls. There was a reduction in expression in both HCC patients who had been treated and those who had not been treated. Consequently, we recommend that these microRNAs be used as biomarkers for early detection, disease progression, and recurrence markers following a treatment regime. Additionally,

disorders. Compared to patients with HCC-negative results and healthy subjects, patients with hepatitis B had higher serum levels of miR-223 than patients with HCC and healthy subjects. Patients with chronic hepatitis had greater hepatocyte damage than patients with HCC, and it is likely that hepatitis (tissue injury) is the cause of elevated miRNA-223 serum levels rather than HCC²⁰. Contrary to this, several studies have revealed that miR-122 and miR-223 levels are lower in-patient samples of HCC, HBV, and HCV when compared to healthy controls.

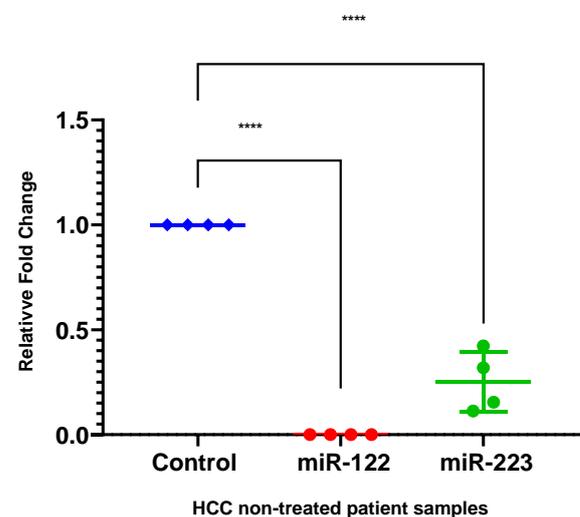


Figure 5. The graph illustrates the relative fold change for patients with non-treated HCC patients compared to healthy control samples using (RT-qPCR). The relative fold change represents decrease in miR-122 and miR-223 expression. Also, the fold change for each sample was calculated using this Eq: $FCH(p) = 2^{-(\Delta\Delta Ct)}$. Two-way ANOVA analysis, P value (*) ≤ 0.0009 .**

these biomarkers demonstrated a high level of sensitivity and accuracy and were recommended as follow-up tests for patients during and following treatment. It can also be considered a marker for HCC development in individuals with HBV or HCV. As such, these microRNAs can be seen as an invaluable resource for early diagnosis and tracking disease progression and recurrence. In addition, they provide a high degree of reliability and

accuracy. Furthermore, the presence of these biomarkers in individuals with a history of HBV or

HCV can be an effective predictor for HCC.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.

- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee in University of Baghdad and Medical City Code Number of Health Ministry Approval: [number 4437 on 30/1/2023].

Authors' Contribution Statement

This study was conducted by S. Kh. Essa Al. who performed most of the experiments and wrote the

manuscript, and Sh. I. K. Al. who designed the work, directed and corrected the paper.

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النتبؤ بسرطان الخلايا الكبدية والكشف المبكر عن العدوى الفيروسية لالتهاب الكبد B و C باستخدام miR-223 و miR-122 في عينات مرضى عراقيين

سجى خضير عيسى الجبوري، شيماء إسماعيل كاظم الجبوري

قسم علوم الحياة، كلية العلوم للبنات، جامعة بغداد، بغداد، العراق.

الخلاصة

يُعد سرطان الخلايا الكبدية (HCC) أحد أكثر أنواع سرطانات الكبد خطورة، وهو ثالث أكثر أسباب الوفيات المرتبطة بالسرطان انتشارًا. يتعلق العديد من الأمراض ومن ضمنها السرطان بالتعبير الجيني غير الطبيعي لجزيئات الرنا الصغيرة micro RNA والتي تلعب دورا مهما في الوظائف البيولوجية للخلية ومن اهمها الموت المبرمج والتكاثر والتمايز والايض الخلوي. هدفت الدراسة الى اختبار فرضية استخدام miR-122 و miR-223 كمؤشر حيوي محتمل في تشخيص وتطور سرطان الخلايا الكبدية HCC وكل من العدوى الفيروسية HBV و HCV عن طريق استخدام تقنية ال PCR الكمي . اجريت تقنية ال PCR الكمي لتحليل التعبير الجيني للمؤشرين الحيويين miR-122 و miR-223 ل (٦٤) عينة مصل تم الحصول عليها من مرضى مصابين بسرطان الكبد ممن كانوا يخضعون للعلاج ومجموعة لم يخضعوا للعلاج وفي بداية تشخيص المرض لهم. هذا فضلا عن عينات من مرضى مصابين بالتهاب الكبد الفيروسي بنوعيه B و C بالإضافة الى عينات مجموعة المقارنة من المتطوعين الاصحاء. أظهرت النتائج وجود فروق معنوية كبيرة بين مجموعات المرضى الثلاثة (سرطان الكبد، التهاب الكبد الفيروسي B و C) وبين مجموعة السيطرة (الاصحاء) في التعبير الجيني لكلا المؤشرين الحيويين , وبانخفاض معنوي في مستوى التعبير الجيني لكل من miR-122 و miR-223 على التوالي وبمستوى معنوي ($P \text{ value} \leq 0.0001$). علاوة على ذلك، قمنا بمقارنة التعبير الجيني لهذين المؤشرين بين عينات مصل المرضى الذين تم تشخيص مرض سرطان الكبد (HCC) لديهم ولم يبيد نظامهم العلاجي بعد وبين عينات المرضى المصابين بسرطان الكبد وهم تحت سير البروتوكول العلاجي لهم. أظهرت النتائج فرق معنوي كبير جدا بين العينات في مستوى التعبير الجيني حيث كان تعبير miR-122 معدوم تقريبا في عينات المرضى الذين لم يخضعوا الى العلاج بعد، مقارنة بالارتفاع النسبي الحاصل في التعبير الجيني لهذا المؤشر الحيوي في المرضى الخاضعين للعلاج بالمقارنة مع مجموعة الاصحاء. نتائج مشابهة ظهرت في المؤشر الحيوي miR-223 أيضا لنفس العينات وبفروق معنوية عالية $P \text{ value} \leq 0.0001$ لكلا المؤشرين على التوالي. بالإضافة إلى ذلك، أظهرت عينات المصابين بالتهاب الكبد الفيروسي بنوعيه B و C انخفاضا معنويا بمستويات التعبير الجيني لكلا المؤشرين الحيويين miR-122, miR-223 مقارنة بمجموعة الاصحاء $P \text{ value} \leq 0.0001$ على التوالي. هذه النتائج تستحق المزيد من التحقيق لأغراض التشخيص ومتابعة العلاج والنتبؤ بالمرض باستخدام كلا المؤشرين الحيويين.

الكلمات المفتاحية: سرطان الخلايا الكبدية، التهاب الكبد الفيروسي نوع B، التهاب الكبد الفيروسي نوع C، MiR223، miR122.