Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Insight on Circulating miRNA-106b and miRNA-198 Biomarkers for Prediction of HCC in Patients with HCV-Induced Liver Diseases

Alshaimaa F. Hegazy[1], Hisham Ismail[1], Amal A. Mohamed[2], Othman A. Othman[1]

[1]Biochemistry Division, Chemistry Dept., Faculty of Science, Minia University, Minia, Egypt.
[2]National Hepatology and Tropical Medicine Research Institute, Cairo, Egyp

ARTICLE INFO

Received :13/6/2024
Accepted : 30/8/2024
Available online : 31/8/2024

Keywords:
miR-106b, miR-198, Hsp27, P53, HCC, LC, Fibrosis, CHC.

ABSTRACT

Background: Many circulating microRNAs have been studied and analyzed for non-invasive prediction of hepatocellular carcinoma in HCV-infected Egyptians. However, both miR-106b and miR-198 have not received the adequate concern for research. **Aim of study:** The present study sought to create a novel model for diagnosis and predication of HCC using miRNA-106b and miR-198. **Material & Methods:** 100 patients with HCV-induced liver diseases and 32 normal volunteers as controls were included. The liver diseases include chronic hepatitis C (n = 32), liver cirrhosis (n=36), and HCC (n = 32). Real-time-PCR (qRT-PCR) used to asses serum microRNA-106b and microRNA-198 expression levels. Level of several traditional markers of liver function and ∝-fetoprotein were determined. Analysis data was determined using SPSS 20 and area under receiver operating characteristic curve utilized to assess variables diagnostic power. **Results:** The fold change of miR-106b expression (Median; IQR) was significantly ($P < 0.0001$) upregulated in HCC patients in comparison with LC patients and CHC patients. However, miR-198 expression was decreasing gradually with the progression of liver disorder from CHC to LC to HCC. The levels of AFP and other traditional HCC-associated markers showed significant differences ($p < 0.05$) between the investigated groups. The AUROC analysis showed that both miR-106b and miR198 had higher diagnostic values (AUROC = 0.920 and 0.888; respectively) than AFP (AUROC = 0.847) to differentiate between HCC and non-HCC subjects. **Conclusion:** miR-106b and miR-198 expression levels of can be utilized as non-invasive sensitive and specific biomarkers for early prediction of HCV-induced HCC in Egyptian patients.

Introduction:

Hepatocellular carcinoma (HCC) is the fifth utmost prevalent cancer type and the fourth greatest cause of cancer-related death worldwide [(1)]. HCC is the fourth most prevalent cancer in Egypt, making it ranks the third and fifteenth most populous country in Africa and the world, respectively [(2)]. In Egypt, HCC is one

***Corresponding author:**
Dr. Hisham Ismail, *PhD,* Biochemistry Division, Chemistry Dept., Faculty of Science, Minia University, Minia 61519, Egypt. ORCID ID: 0000-0002-7593-4532.

of the most serious health issues, with the highest annual incidence rate [3]. HCC is also sex related, with males being more affected than females. Most of HCC cases are caused by chronic liver disease, including cirrhosis caused by excessive alcohol consumption, prolonged infection with hepatitis B and C viruses, hepatotoxins exposure, and steatohepatitis [4]. According to reports, the highest incidence rate of hepatitis C virus infection is in Egypt (14.7%) [5]. Due to the ambiguous asymptomatic out set of HCC, several patients with advanced HCC receive their primary diagnosis at a point when it's too late for the optimum therapy [6]. As a result, accurate diagnostic markers for early HCC detection are urgently needed. Currently, medical personnel mostly use B ultrasound and serum AFP for initial screening. However, these two approaches have decreased sensitivity and are inadequate for prior alert of HCC susceptible populations [7]. Several studies have confirmed the main role of microRNAs (miRNAs) [8] in tumor biology. MiRNAs are short non-coding RNAs (almost 19-25 nt.) that could modulate several genes. MiRNAs mediate a diversity of biological processes, including the cell cycle, differentiation, proliferation, apoptosis, stress tolerance, energy metabolism, and immunological response [9]. Indeed, miRNAs can function as oncogenes or tumor suppressors [10]. These findings indicate that miRNAs might be utilized as biomarkers for featuring tumors. Tumor cells have the ability of releasing miRNA into the blood; therefore, circulating microRNAs are currently a popular concern. MiR-106b is one of these microRNAs. MiR-106b is a member of the miR-106b~25 cluster. It is encoded within intron 13 of the gene MCM7 on 7q22.11212 chromosome [11]. miR-198 expression was detected in tissues of 95 HCC patients, and miR-198 levels were found to be low [12]. Their investigation found that HCC tissue had lower levels of miRNA-198 than nonmalignant adjacent tissue. MiR-198 reduces HCC cell proliferation and migration by regulating mitosis and movement pathways [13]. MiR-198 expression is upregulated by FOXP3 via binding to its promoter sequence [14]; the proto-oncogene MYC was targeted by miR-198 which suppresses its expression. Eventually, high miR-198 levels enhance hepatic G2 cell death while suppressing growth. In this research, sera from examiners were selected randomly and evaluated the levels of miR-106b, miR-198. We investigated the impact of their combined utility on sensitivity and specificity in contrast to using each marker alone. We also examined the association of these markers with routine laboratory tests, AFP, and clinic pathological features and determined whether they are potentially predictive of early diagnosis and prognosis in HCC. The findings of this research supported the value of miR-106b and miR-198 in HCC diagnosis and offered additional data to confirm their application in clinical researches.

Materials And Methods

Blood samples

The current investigation uses blood samples collected during normal follow-up of 100 patients with HCV-induced liver diseases (46 males and 54 females; age range 34-80 yr) and 32 normal individuals as controls (11 males and 21 females; mean age 56.97 ± 6.49 yr) from June 2021 to December 2022 at the National Hepatology and Tropical Medicine Research Institute, Cairo and at the

Minia university Hospitals, Minia, Egypt. All participants received a thorough medical history check, clinical and physical examination. Blood specimen (10 mL) was collected from each individual via vein puncture in glass test tubes and divided into three parts: sodium citrate was added to the first part for INR-prothrombin testing, EDTA was added to the second part for complete blood count (CBC), and the final one without any additions for assessment of liver and kidney biochemical profiles, immunochemical detection of AFP, and qRT-PCR for miRNA-106b and miRNA-198 biomarkers.

Blood samples of the third part were allowed to clot for 40 minutes at 37 °C. Then, the specimens were centrifuged at 3000 rpm and 4℃ for 10 minutes and consequently sera were stored at – 80 ^{0}C until thawed for testing. Based on clinical examinations, abdominal ultrasonography, and laboratory tests that follow the institutional procedure, the liver-diseased patients were classified into: CHC patients (n = 32, 14 males and 18 females; mean age 56.0 ± 9.99 yr), LC patients (n = 36, 16 males and 20 females; mean age 59.20 ± 5.47 yr), and HCC patients (n = 32, 16 males and 16 females; mean age 59.67 ± 8.98 yr).

The present study was granted permission by the Ethics Committee of Faculty of Science, Minia University, Minia, Egypt **(MPEC 240605)**. Informed consent was attained from all volunteers in compliance with ethical criteria outlined in the 1975 Helsinki Declaration. Exclusion criteria: patients with liver disease or HCC caused by factors other than HCV infection, hepatic encephalopathy, chronic kidney disease, hepato-renal syndrome, alcohol intake, bacterial peritonitis, or co-infection with HIV.

Routine laboratory investigations

The complete blood count (CBC), Liver profile including aspartate transaminase (AST) and alanine transaminase (ALT), serum albumin, total bilirubin, plasma prothrombin time (PT), and INR was investigated. The automated chemistry analyzer OLYMPUS AU 400 (Olympus America, Pennsylvania, USA) was used to perform routine biochemical tests. CanAg AFP EIA 600-10 (EIA-5396; DRG International Inc., Springfield, New Jersey, USA) was used to quantify Alfa fetoprotein (AFP) in accordance with product instruction.

Assessment of serum miR-106b and miR198 relative content using quantitative Real Time-PCR

A QIAamp RNA Mini kit (Qiagen, Hilden, Germany) utilized for the extraction of total RNA from the final sample mixture, following the manufacturer's instructions. To summarize, from each 140 µL specimen, 40 µL RNA solutions was extracted. Nanodrop spectrophotometer was used to evaluate the quality of RNA (A260/280 ratio). The extracted RNA was subsequently utilized to synthesize cDNA. 5 µL of RNA reverse transcribed using The ReverAid RT Kit (ThermoFisher Scientific, Waltham, USA), according to the instructions provided by the manufacturer.

Using the primer sets listed in **Table 1**, RT-PCR amplification was conducted on the Rotor Gene Q platform (Qiagen, Hilden, Germany) to measure microRNA expression. The housekeeping gene U6-MiR was utilized as a control. The cDNA was mixed with 20 µl of SYBER Green Master Mix (Qiagen, Hilden, Germany). Real-time PCR reactions were run for 5 minutes at 94°C, followed by 40 cycles of 15

seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C. All tests were performed in triplicates. The cycle threshold (CT) values were identified and utilized to report the expression, which was estimated by the subtraction of housekeeping CT values from target CT values. Target expression levels were quantified using the 2-ΔΔ (Ct) approach.

Statistical Analysis

Data was processed with IBM SPSS software version 20.0. (Armonk, New York: IBM Corporation). Numbers and percentages were used to explain the qualitative data. To check the distribution's normality, the Shapiro-Wilk test was utilized. Quantitative data were defined as range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The significance of the acquired results was assessed at the 5% level. The chi-square test was performed to compare categorical variables across groups. For comparing more than two groups, the one-way ANOVA test was used for normally distributed quantitative variables and the post-hoc Tukey test for comparisons of pairwise. For not normally distributed quantitative variables, the Kruskal-Wallis test was used for comparing more than two study groups, and the Post Hoc (Dunn's multiple comparisons test) was used for comparisons of pairwise. ROC is created by illustrating sensitivity (TP) on the Y axis and 1-specificity (FP) on the X axis at various cutoff values also used for the performance comparison between two tests. AUROC represents the diagnostic efficacy of the test. Areas greater than 50% provide adequate efficacy, whereas areas around 100% provide the optimum efficacy of the test.

Results

Traditional biomarkers of Liver profile and hematology

There is a significant variation between four groups in the ALT, AST, ALP, Albumin, Bilirubin and INR levels with $p < 0.001$ as indicated in **Table 2**. Serum creatinine levels were normal in the control and HCV groups, but increased in the cirrhosis and HCC groups. Platelet count levels were normal in the control group, while lower aberrant values were seen in the Cirrhosis, HCC, and CHC groups (**Table 2**). The investigation demonstrated that HCC patients had statistically significant higher serum AFP levels (Median 200) than LC patients (Median 120), while LC patients had higher levels than CHC patients (Median 29) in comparison with healthy volunteers (Median 5.0) with $P < 0.001$, **Table 2**.

Diagnostic potential of MicroRNA-106b (miR-106b)

The fold change in miRNA-106b expression levels in the sera of patients in comparison with the control group was found to be significantly higher in all research groups. The highest levels were shown in HCC patients, followed by cirrhosis and CHC patients, with median fold changes of 44.29, 8.30, and 2.33, respectively. In addition, significant difference was found between the HCC and cirrhotic groups ($P = 0.004$), as indicated in **Table 3**. In terms of expression, miR-106b was significantly up-regulated with the progression of the disease from CHC to HCC ($P < 0.001$) (**Figure 1A** and **Figure 2A**).

Diagnostic potential of MicroRNA-198 (miR-198)

The analysis of the miRNA-198 fold change in the sera of patients in

comparison with the control group indicated a substantial fold decrease in the HCC, cirrhotic, and CHC groups. In this research, miR-198 levels in HCC patients were significantly lower than those in the cirrhotic and CHC groups. There was a significant variation between the HCC and cirrhotic groups (P = 0.002). MiRNA-198 levels differed significantly between the cirrhosis and CHC groups (P < 0.001). However, there was no statistically significant difference between CHC patients and controls (P > 0.05), as indicated in **Table 3**. Our investigation found that miR-198 expression decreased dramatically as the illness progressed from CHC to HCC (P < 0.001). Regarding miR-198 expression, our study revealed that its levels were significantly down-regulated as the disease progress from CHC to HCC (P <0.001) **(Figure 1B** and **Figure 2**B).

Diagnostic performance for miRNA-106b and miRNA-198 to discriminate Non-HCC individuals from HCC patients in comparison with AFP

The ROC curve for traditional AFP biomarker was used to differentiate HCC patients from patients without HCC. Serum AFP was higher in HCC than in non-HCC groups (AUROC = 0.847, P <0.001); see Figure 3A. AFP showed 80% sensitivity and 71% specificity (**Table 4**). The ROC curve for miRNA-106b used to differentiate patients with and without HCC demonstrates that serum miR-106b was significantly up-regulated in HCC than in non-HCC groups (P <0.001), with 93% sensitivity and 83% specificity. AUROC = 0.920, as reported in **Table 4** and **Figure 3B**.
The ROC curve for miRNA-198 to distinguish HCC patients from patients non- HCC shows that serum miR-198 levels were significantly down-regulated in HCC groups than in non-HCC groups (P <0.001), with 93 % sensitivity and 70 % specificity. AUROC = 0.888, as shown in **Table** 4 and **Figure 3C**.

Diagnostic performance for miRNA-106b, miRNA-198b and AFP to discriminate patients with liver cirrhosis from patients with HCC:

ROC curves were created, and the AUC was calculated to estimate the sensitivity and specificity for predicting HCC cases among CHC and cirrhotic patients based on each miRNA expression level. ROC curves showed that a cut-off value of >16.427 yielded 93% sensitivity and 70% specificity for miR-106b relative gene expression, while a cut-off value of ≤0.00056 resulted in 70% sensitivity and 93% specificity for miR-198 relative gene expression in discriminating HCC patients from cirrhosis patients. While the AFP ROC curve reported a cut-off value of >161, it gave a sensitivity of 60% and a specificity of 83%, discriminating HCC patients from those with cirrhosis, as shown in **Table 5**.

Discusion

HCC incidence varies greatly around the world, with Egypt experiencing a rise in incidence. The majority of HCC cases occur in patients with hepatitis, which is most popular in the Middle East and Africa [15]. In 2008, Egypt had the world's highest HCV prevalence [16,17]. Chronic hepatitis C (CHC) patients encounter a high danger of lethal complications, including cirrhosis and hepatocellular carcinoma (HCC) [18,19]. Multiple investigations on treating HCV-infected patients with DAAs have indicated a surprisingly

high incidence of recurrence of early HCC [20,21,22]).

In 2018, a program turned into a nationwide strategy for eliminating HCV as a national health issue. By 2030, WHO signatories stated to eliminate HCV as a public health concern [23]. Several studies have indicated the close association between HCV infection and HCC development [24,25]. Due to the silent and asymptomatic growth of liver pathology and late-stage diagnosis of liver failure, or HCC [26], early diagnosis is crucial for preventing and treating cirrhosis and HCC. Half-yearly abdominal ultrasonography for HCC surveillance, either alone or with alpha-fetoprotein (AFP) was recommended (EASL Guidelines 2018) [27]). Recent research, however, highlighted the limitations of ultrasound-based monitoring, such as its limited sensitivity to identify early HCC when used without AFP and the risk of screening-related challenges [28].

Serum AFP levels are markedly higher in HCC patients with HCV or HBV infection in certain Egyptian individuals [29]. Consequentially, reliable markers with high effectiveness for early detection and subsequent therapy surveillance of HCC are critically required [30]. Non-coding RNAs comprise a significant proportion of the genome of humans. MiRNAs have been demonstrated to have an important impact on modulating gene expression [31]. After transcription, miRNAs may regulate the rate of cell physiological processes that involve proliferation, differentiation, and death. miRNAs biogenesis in animal cells and the processes that regulate the expression of their target genes; in basic words, this process may be divided into many steps [32,33].

Several investigations have found that miRNAs have a direct impact on cancer initiation and progression by regulating post-transcriptional gene silencing through mRNA degradation and suppressing translation [34]. Tumor cell proliferation is regulated by the appropriate miRNA. Furthermore, miRNA causes alterations in the blood, which leads to tumour formation; so, this development is significant and its concentrations remain mostly stable after repeated freezing and thawing [35]. Therefore, the expression of miRNA can be used as a marker in pathological circumstances [36]. MiR-106b and other miRNAs from the miR-106b-25 cluster are increased in HCC, which promotes cell migration and metastasis and is correlated with a poor prognosis [37–39]. MiR-106b is also involved in hepatocarcinogenesis [37] and has been identified as a potential early HCC diagnostic marker [38]. The current study demonstrated a significant fold increase in miRNA-106b levels in HCC group patients compared to cirrhotic and CHC groups, based on a fold change methodology.

The findings of this investigation demonstrated a statistically significant variation in miR-106b levels within the four groups ($P > 0.001$). This indicates that it provides a certain value for diagnosis. However, to enhance the specificity of HCC diagnosis, further diagnostic indicators may be required. MiR-198 has the potential to be used as a tumor suppressor and in the treatment of liver cancer. MiR-198 expression is down-regulated in HCC patients. It was also discovered that miR-198 suppressed HCC cell proliferation via the HGF/c-MET pathway [40]. Additionally, our investigation found that miR-198 in HCC serum had a

significantly fold decrease than that in cirrhosis, CHC, and control groups. The HCC group showed a significant reduction in miR-198 (3.2± 2) compared to the CHC and control groups (P ≤0.0001). Furthermore, cirrhosis had higher miRNA-198 expression levels than the HCC group.

Regarding the correlation between miR-106b, miR-198, and AFP in liver disorders of Egyptian patients, this study results showed that, compared to non-HCC cases, serum miR-106b levels and AFP were elevated in the HCC cases group with a significant P ≤0.0001, and their levels were further increased in the cirrhosis group but still highest in the HCC group with a significant (P ≤0.0001) for both. MiR-198 levels decreased in the CHC and cirrhosis groups, with the lowest level in the HCC group with a significant p >0.001. Most importantly, the three markers values differ significantly between the cirrhotic and HCC patient groups with a P >0.001 for miR-106b and miR-198 and p = 0.007* for AFP. Indicating that serum miR-106b, miR-198, and AFP levels can significantly differentiate between CHC patients and cirrhotic subjects, as well as their ability to discriminate HCC patients from the cirrhosis patient group.

Conclusion

In the current study, MiR-106b and AFP levels increased significantly as the disease progressed from control to HCC, although miR-198 levels decreased
significantly. Furthermore, our research suggests that the simultaneous combination of miR-106b, miR-198, and AFP in parallel or in series can enhance the specificity and sensitivity of HCC diagnosis, including early and advanced stages, versus using only one marker.

Acknowledgement:

The authors desire to express their heartfelt gratitude to the Academy of Scientific Research and Technology (ASRT) for financial support provided to the author AFH to achieve this work through the SNG scholarship and for continuous support and help provided by all ASRT staff all the way until this research was completed. Scholarship no 22, cycle 6, ASRT/SNG/BGM/2018-2, Molecular Biology.

Conflicts of interest: The authors declare that they have no conflicts of interest.

References:

1. **Augusto V. (2019).** Hepatocellular Carcinoma. *N Engl J Med*.;380(15):1450-1462. doi:10.1056/NEJMra1713263
2. **Rashed WM, Kandeil MAM, Mahmoud MO, Ezzat S. (2020)**. Hepatocellular Carcinoma (HCC) in Egypt: A comprehensive overview. *J Egypt Natl Canc Inst*. 32(1). doi:10.1186/s43046-020-0016-x
3. **Salim EI, Moore MA, Al-lawati JA, et al. (2009).** Cancer Epidemiology and Control in the Arab World - past, present and future. *Asian Pac J Cancer Prev*.;10(1):3-16..
4. **Pascual S, Miralles C, Bernabé JM, Irurzun J, Planells M. (2019).** Surveillance and diagnosis of hepatocellular carcinoma: A systematic review. *World J Clin Cases*. 7(16): 2269-22286.

doi:10.12998/wjcc.v7.i16.2269

5. **Mohamoud YA, Mumtaz GR, Riome S, Miller DW, Abu-Raddad LJ. (2013).** The epidemiology of hepatitis C virus in Egypt: A systematic review and data synthesis. *BMC Infect Dis*. 13(1). doi:10.1186/1471-2334-13-288
6. **Zong J, Fan Z, Zhang Y. (2020).** Serum Tumor Markers for Early Diagnosis of Primary Hepatocellular Carcinoma. *J Hepatocell Carcinoma*. Volume 7:413-422. doi:10.2147/jhc.s272762
7. **Zacharakis G, Aleid A, Aldossari KK. (2018).** New and old biomarkers of hepatocellular carcinoma. *Hepatoma Res*. 4(10):65. doi:10.20517/2394-5079.2018.76
8. **Shaker MK, Abdella HM, Khalifa MO, Dorry AKE. (2013).** Epidemiological characteristics of hepatocellular carcinoma in Egypt: A retrospective analysis of 1313 cases. *Liver Int*. 33(10):1601-1606. doi:10.1111/liv.12209
9. **Giovannetti E, Erozenci A, Smit J, Danesi R, Peters GJ. (2012).** Molecular mechanisms underlying the role of microRNAs (miRNAs) in anticancer drug resistance and implications for clinical practice. *Crit Rev Oncol Hematol*. 81(2):103-122. doi:10.1016/j.critrevonc.2011.03.010
10. **Zhang B, Pan X, Cobb GP, Anderson TA. (2007).** microRNAs as oncogenes and tumor suppressors. *Dev Biol*. 302(1):1-12. doi:10.1016/j.ydbio.2006.08.028
11. **Petrocca F, Vecchione A, Croce CM. (2008).** Emerging role of miR-106b-25/miR-17-92 clusters in the control of transforming growth factor β signaling. *Cancer Res*. 68(20):8191-8194. doi:10.1158/0008-5472.CAN-08-1768
12. **Huang WT, Wang HL, Yang H, et al. (2016).** Lower expressed mir-198 and its potential targets in hepatocellular carcinoma: A clinicopathological and in silico study. *Onco Targets Ther*. 9:5163-5180. doi:10.2147/OTT.S108828
13. **Elfimova N, Sievers E, Eischeid H, et al. (2013).** Control of mitogenic and motogenic pathways by miR-198, diminishing hepatoma cell growth and migration. *Biochim Biophys Acta - Mol Cell Res*. 1833(5):1190-1198. doi:10.1016/j.bbamcr.2013.01.023
14. **Duan X, Jiang B, Yang J, Zhou L, Tian B, Mao X. (2018).** FOXP3 inhibits MYC expression via regulating miR-198 and influences cell viability, proliferation and cell apoptosis in HepG2. *Cancer Med*. 7(12):6182-6192. doi:10.1002/cam4.1780
15. **Mankoula W. (2015).** Estimating economic and epidemiological burden of hepatitis C in Egypt, 2015-2025. Published online 2015.
16. **Kim CW, Chang KM. (2013).** Hepatitis C virus: virology and life cycle. *Clin Mol Hepatol*. 19(1):17-25. doi:10.3350/cmh.2013.19.1.17

17. **Kandeel A, Genedy M, El-refai S, Funk AL, Fontanet A, Talaat M. (2017).** The prevalence of hepatitis C virus infection in Egypt 2015 : implications for future policy on prevention and treatment. (January 2016):45-53. doi:10.1111/liv.13186
18. **Waked I, Doss W, El-sayed MH, Estes C, Razavi H, Shiha G. (2014).** The current and future disease burden of chronic hepatitis C virus infection in Egypt. *Arab J Gastroenterol*. 2014:1-8. doi:10.1016/j.ajg.2014.04.003
19. **Elgharably A, Gomaa AI, Crossey MM, Norsworthy PJ, Waked I, Taylor-Robinson SD (2017)**. Hepatitis C in Egypt - past, present, and future. *Int J Gen Med*.;10:1-6. doi:10.2147/IJGM.S119301.
20. **Conti F, Buonfiglioli F, Scuteri A, et al. (2024).** Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. *J Hepatol*. (May). doi:10.1016/j.jhep.2016.06.015
21. **Reig M, Mariño Z, Perelló C, et al. (2016).** Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. *J Hepatol*.;65(4):719-726. doi:10.1016/j.jhep.2016.04.008
22. **Yang JD, Aqel BA, Pungpapong S, Gores GJ, Roberts LR, Leise MD. (2016).** Direct acting antiviral therapy and tumor recurrence after liver transplantation for hepatitis C-associated hepatocellular carcinoma. *J Hepatol*. 65(4):859-860. doi:10.1016/j.jhep.2016.06.023
23. **Organization WH. (*2016-2021).*** *Global Health Sector Strategy on Viral Hepatitis. Towards Ending Viral Hepatitis*. World Health Organization https://iris.who.int/handle/10665/246177.
24. **Pawlotsky JM. (2004).** Pathophysiology of hepatitis C virus infection and related liver disease. *Trends Microbiol*. 12(2):96-102. doi:10.1016/j.tim.2003.12.005
25. **Bartosch B, Thimme R, Blum HE, Zoulim F. (2009).** Hepatitis C virus-induced hepatocarcinogenesis. *J Hepatol*. 51(4):810-820. doi:10.1016/j.jhep.2009.05.008
26. **Forner A, Reig M, Bruix J. (2018).** Hepatocellular carcinoma. *Lancet*. 391(10127):1301-1314. doi:10.1016/S0140-6736(18)30010-2
27. **Association E. Corrigendum to ‘‘ EASL Clinical Practice Guidelines : (2018).** Management of hepatocellular carcinoma [J Hepatol 69 (2018) 182 – 236] q European Association for the Study of the Liver. *J Hepatol*. 70(4):817. doi:10.1016/j.jhep.2019.01.020
28. **Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA. (2018).** Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With

Cirrhosis : A Meta-analysis. Gastroenterology May;154(6):1706-1718.e1. doi:10.1053/j.gastro.2018.01.064. Epub 2018 Feb 6.

29. **Yameny AA, Alabd SF, Mansor MAM. (2023).** Evaluation of AFP for diagnosis of HCC in Egyptian patients. *J Med Life Sci*.; 5(1):43-48. doi:10.21608/jmals.2023.329306
30. **Sun S, Poon RTP, Lee NP, et al. (2010).** Proteomics of hepatocellular carcinoma: Serum vimentin as a surrogate marker for small tumors (≤2 cm). *J Proteome Res*. 9(4):1923-1930. doi:10.1021/pr901085z
31. **Ratti M, Lampis A, Ghidini M, et al. (2020).** MicroRNAs (miRNAs) and Long Non - Coding RNAs (lncRNAs) as New Tools for Cancer Therapy : First Steps from Bench to Bedside. *Target Oncol*. 15(3):261-278. doi:10.1007/s11523-020-00717-x
32. **Kim VN. (2005).** MicroRNA biogenesis: Coordinated cropping and dicing. *Nat Rev Mol Cell Biol*. 6(5):376-385. doi:10.1038/nrm1644
33. **O'Brien J, Hayder H, Zayed Y, Peng C. (2018).** Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Front Endocrinol (Lausanne)*. 9(AUG):1-12. doi:10.3389/fendo.2018.00402
34. **Winter J, Jung S, Keller S, Gregory RI, Diederichs S. (2009).** Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol*.; 11(3):228-234. doi:10.1038/ncb0309-228.
35. **Glinge C, Clauss S, Boddum K, et al. (2017).** Stability of circulating blood-based microRNAs-Pre-Analytic methodological considerations. *PLoS One*. 12(2):1-16. doi:10.1371/journal.pone.0167969
36. **Sohel MH. (2020).** Circulating microRNAs as biomarkers in cancer diagnosis. *Life Sci*. 248(February):117473. doi:10.1016/j.lfs.2020.117473
37. **Yau WL, Lam CSC, Ng L, et al. (2013).** Over-Expression of miR-106b Promotes Cell Migration and Metastasis in Hepatocellular Carcinoma by Activating Epithelial-Mesenchymal Transition Process. *PLoS One*. 8(3). doi:10.1371/journal.pone.0057882
38. **Li Y, Tan W, Neo TWL, et al. (2009).** Role of the miR-106b-25 microRNA cluster in hepatocellular carcinoma. *Cancer Sci*. 100(7):1234-1242. doi:10.1111/j.1349-7006.2009.01164.x
39. **Li BK, Huang PZ, Qiu JL, Liao Y Di, Hong J, Yuan YF. (2014).** Upregulation of microRNA-106b is associated with poor prognosis in hepatocellular carcinoma. *Diagn Pathol*. 9:226. doi:10.1186/s13000-014-0226-4
40. **Tan S, Li R, Ding K, Lobie PE, Zhu T. (2011).** MiR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/c-MET pathway. *FEBS Lett*. 585(14):2229-

2234.
doi:10.1016/j.febslet.2011.05.
042.

Table 1: Sequences of primers used in the present study.

Gene	Name	Sequence	Reference
U6	RT Primer	CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGAAAAATATG	Wang et al. 2017
	Forward Primer	CTCGCTTCGGCAGCACA	
	Reverse Primer	AACGCTTCACGAATTTGCGT	
Mir-106b	Forward Primer	5′-CCGGGGCTAAAGTGCTGACAG-3′	Liu et al., 2020
	Reverse Primer	5′-TGCTGGAGCAGCAAGTACCCA-3′	
Mir-198	Forward Primer	5’-CAACGGAAUCCCAAAAGCAGCU-3′	Fang et al., 2020
	Reverse Primer	5’-GGUCCAGAGGGGAGAUAGGUUC-3′	

Table 2. Comparison between the four studied groups according to liver function.

Biomarkers	HCC (n = 30)	Cirrhosis (n = 30/)	CHC (n = 30)	Controls (n = 30)	Test of Sig.	P
ALT (U/L)						
Min. – Max.	17.0 – 105.0	17.0 – 160.0	20.0 – 122.0	20.0 – 50.0	H = 37.493*	< 0.001*
Mean ± SD.	58.60 ± 23.09	62.33 ± 33.87	45.93 ± 25.07	29.67 ± 7.71		
Median (IQR)	55.50 (47.0 – 68.0)	53.0 (42.0 – 81.0)	42.0 (29.0 – 60.0)	29.0 (23.0 – 30.0)		
p_0	<0.001*	<0.001*	0.003*			
Sig. bet. grps.	$p_1 = 0.818$, $p_2 = 0.015^*$, $p_3 = 0.027^*$					
AST (U/L)						
Min. – Max.	28.0 – 230.0	34.0 – 196.0	24.0 – 145.0	23.0 – 50.0	H = 59.657*	< 0.001*
Mean ± SD.	103.2 ± 50.74	85.03 ± 46.19	56.33 ± 29.16	31.53 ± 7.41		
Median (IQR)	88.0 (67.0 – 145.0)	75.50 (46.0 – 97.0)	56.0 (30.0 – 69.0)	30.0 (24.0 – 34.0)		
p_0	<0.001*	<0.001*	0.001*			
Sig. bet. grps.	$p_1 = 0.231$, $p_2 < 0.001^*$, $p_3 = 0.014^*$					
ALP						
Min. – Max.	63.0 – 337.0	18.0 – 800.0	61.0 – 286.0	61.0 – 164.0	H= 33.845*	<0.001*
Mean ± SD.	158.2 ± 66.35	174.7 ± 128.8	141.3 ± 58.08	87.23 ± 21.56		
Median (IQR)	161.0(104.0–194.0)	160.0(110.0–190.0)	130.0(87.0–172.0)	93.0(71.0–100.0)		
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	$p_1 = 0.761$, $p_2 = 0.284$, $p_3 = 0.169$					
Albumin (g/dl)						
Min. – Max.	2.10 – 3.70	2.40 – 44.0	2.10 – 4.70	2.80 – 4.0	F= 21.668*	<0.001*
Mean ± SD.	2.79 ± 0.42	4.61 ± 7.45	3.05 ± 0.62	3.72 ± 0.33		
Median (IQR)	2.70 (2.50 –3.10)	3.15 (3.0 – 3.50)	3.0 (2.70 – 3.20)	3.70 (3.60 – 4.0)		
p_0	<0.001*	0.002*	<0.001*			
Sig. bet. grps.	$p_1 < 0.001^*$, $p_2 = 0.122$, $p_3 = 0.233$					
Bilirubin (mg/dL)						
Min. – Max.	0.70 – 8.0	0.60 – 6.60	0.50 – 3.60	0.50 – 3.0	H = 45.148*	< 0.001*
Mean ± SD.	2.89 ± 1.71	2.94 ± 1.64	1.33 ± 0.78	1.10 ± 0.62		
Median (IQR)	2.60 (1.50 –3.70)	2.90 (1.50 – 4.10)	1.0 (0.80 – 1.80)	1.0 (0.70 – 1.0)		
p_0	<0.001*	<0.001*	0.322			
Sig. bet. grps.	$p_1 = 0.994$, $p_2 < 0.001^*$, $p_3 < 0.001^*$					
INR						
Min. – Max.	1.0 – 1.78	1.05 – 1.78	1.0 – 1.60	1.0 – 1.46	F = 11.395*	< 0.001*
Mean ± SD.	1.41 ± 0.23	1.46 ± 0.21	1.25 ± 0.19	1.20 ± 0.18		
Median (IQR)	1.40 (1.24 – 1.56)	1.49 (1.40 – 1.60)	1.27 (1.06 – 1.34)	1.28 (1.0 – 1.30)		
p_0	0.001*	<0.001*	0.815			
Sig. bet. grps.	$p_1 = 0.828$, $p_2 = 0.010^*$, $p_3 = 0.001^*$					
Serum Creatinine						
Min. – Max.	0.90 – 3.80	0.90 – 3.80	0.50 – 2.0	0.50 – 1.20	H= 60.198*	<0.001*
Mean ± SD.	2.05 ± 0.78	1.92 ± 0.73	1.04 ± 0.42	0.92 ± 0.21		
Median (IQR)	2.0 (1.50 – 2.50)	1.90 (1.40 – 2.0)	1.0 (0.60 – 1.20)	1.0 (0.80 – 1.0)		
p0	<0.001*	<0.001*	0.256			

Sig. bet. grps.	p1=0.493,p2<0.001*,p3<0.001*					
Platelets (×10³/µl) Min. – Max. Mean ± SD. Median (IQR)	42.0 – 247.0 120.2 ± 46.74 112.5 (88.0 – 152.0)	58.0 – 158.0 116.1 ± 26.97 114.0 (99.0 – 133.0)	48.0 – 256.0 134.5 ± 65.54 105.0 (79.0 – 200.0)	54.0 – 255.0 128.2 ± 50.59 126.0 (89.0 – 153.0)	H= 0.576	0.902
AFP (ng/mL) Min. – Max. Mean ± SD. Median (IQR)	25.0 – 480.0 190.6 ± 112.7 200.0 (120.0 – 230.0)	25.0 – 300.0 123.2 ± 65.85 120.0 (100.0 – 151.0)	12.0 – 330.0 63.13 ± 86.25 29.0 (20.0 – 70.0)	3.0 – 20.0 6.50 ± 4.17 5.0 (4.0 – 8.0)	81.800*	<0.001*
p0	< 0.001*	< 0.001*	< 0.001*			
Sig. bet. grps.	p1=0.139,p2<0.001*,p3=0.011*					

IQR: Inter quartile range SD: Standard deviation, H: H for Kruskal Wallis test, pairwise comparison between each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test), F: F for One-way ANOVA test, pairwise comparison between each 2 groups was done using Post Hoc Test (Tukey), p: p value for comparing between the four studied groups, p0: p value for comparing between control group and diseased groups., p_1: p value for comparing between HCC and Cirrhosis, p_2: p value for comparing between HCC and CHC, p_3: p value for comparing between Cirrhosis and CHC, *: Statistically significant at $p \leq 0.05$

Table 3. Comparison between fold change of miRNA-106b and miRNA-198 in the investigated groups.

Fold Change	HCC (n = 32)	LC (n = 36)	CHC (n = 32)	Controls (n = 32)	H	p
miRNA-106b						
Min. – Max.	4.75 – 1066.01	1.31 – 759.02	0.55 – 42.17	0.29 – 4.72		
Median (IQR)	44.29 (34.73 – 60.46)	8.30 (5.16 – 24.90)	2.33 (1.74 – 16.77)	0.96 (0.59 – 1.61)	78.875*	<0.001*
p_0	<0.001*	<0.001*	0.002*			
Sig. bet. grps.	$p_1 = 0.004^*$, $p_2 < 0.001^*$, $p_3 = 0.010^*$					
miRNA-198						
Min. – Max.	0.0001 – 0.0289	0.0005 – 0.0225	0.0002 – 1.8506	0.3204 – 2.7283		
Median (IQR)	0.0003 (0.0003 – 0.0012)	0.0014 (0.0010 – 0.0027)	0.0022 (0.0008 – 0.0219)	1.2728 (0.6868 – 1.4825)	78.041*	<0.001*
p_0	<0.001*	<0.001*	<0.001*			
Sig. bet. grps.	$p_1 = 0.002^*$, $p_2 < 0.001^*$, $p_3 = 0.459$					

IQR: Inter quartile range SD: Standard deviation
H: H for Kruskal Wallis test, pairwise comparison between each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test).
p_0: p value for comparing between **control** group and diseased groups.
p_1: p value for comparing between **HCC** and **Cirrhosis**
p_2: p value for comparing between **HCC** and **CHC**
p_3: p value for comparing between **Cirrhosis** and **CHC**
*: Statistically significant at $p \leq 0.05$

Table 4. Diagnostic performance of miRNA-106b, miRNA-198 in comparison with AFP to discriminate HCC patients (n = 32) from non-HCC patients (n = 100).

Biomarker	AUROC	P*	95% CI	Cut off#	Sensitivity	Specificity	Efficiency
Fold Change miRNA-106b	0.920	<0.001*	0.870 – 0.970	>16.77#	93	83	86
Fold Change miRNA-198	0.888	<0.001*	0.824 – 0.952	≤0.0017#	93	70	76
AFP (ng/mL)	0.847	<0.001*	0.773 – 0.920	>100	80	71	73

AUROC: Area Under ROC. p value: Probability value. CI: Confidence Intervals.

*Statistically significant at $p \leq 0.05$. # Cut off was chosen according to Youden index

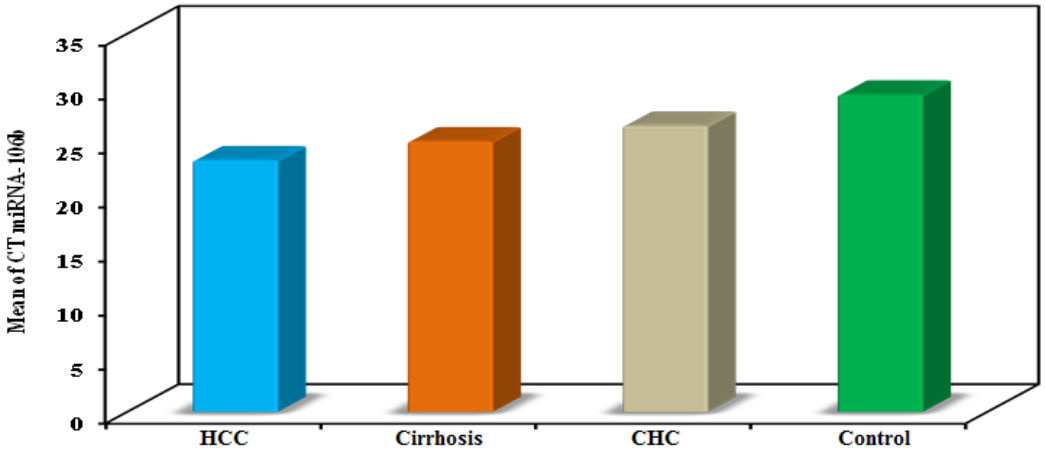
Table 5. Diagnostic performance for miRNA-106b, miRNA-198 and AFP to discriminate patients with HCC patients (n = 32) from liver cirrhosis patients (n = 36)

Biomarker	AUROC	p*	95% CI	Cut off#	Sensitivity	Specificity	Efficiency
Δct miRNA-106b	0.814	<0.001*	0.693 – 0.935	≤4.31	86.67	76.67	82
Δct miRNA-198	0.834	<0.001*	0.727 – 0.941	>11.82	70.0	93.33	82
AFP (ng/mL)	0.704	= 0.007*	0.567 – 0.842	> 161#	60	83	72

AUROC: Area Under ROC Curve p value: Probability value CI: Confidence Intervals

*: Statistically significant at $p \leq 0.05$. # Cut off was chosen according to Youden index

A.



B.

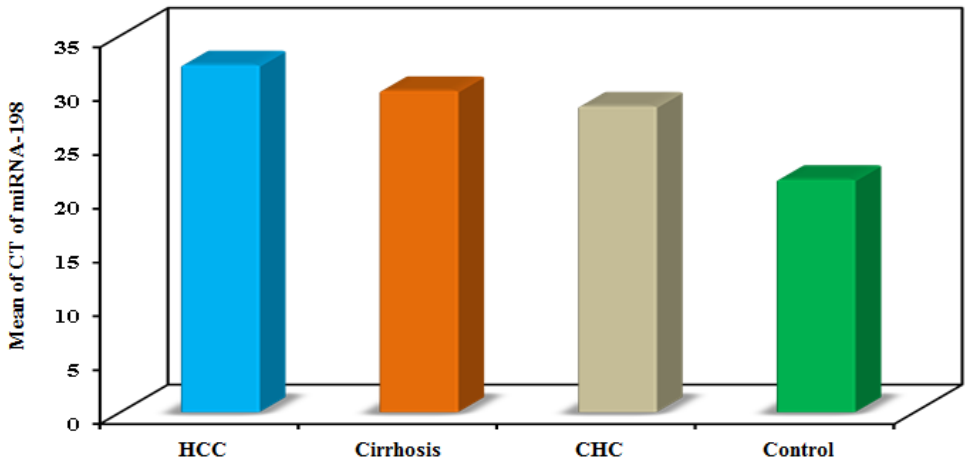
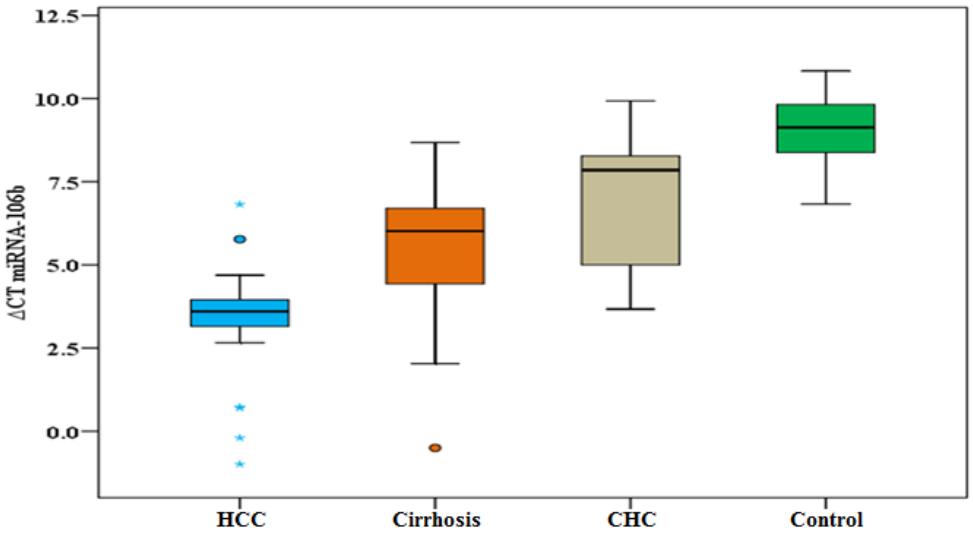


Figure 1. Comparison between the four studied groups according to CT. A. miRNA-106b. B. miRNA-198.

A.



B.

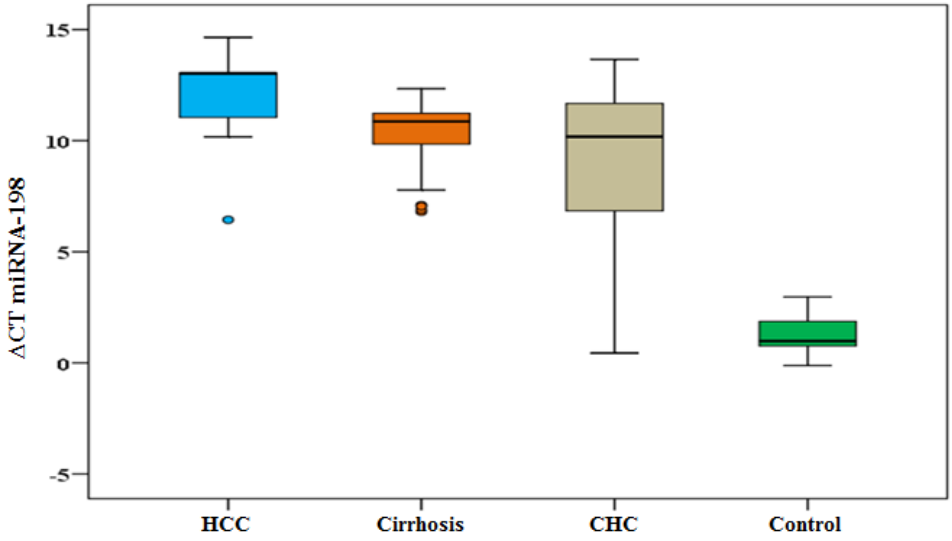
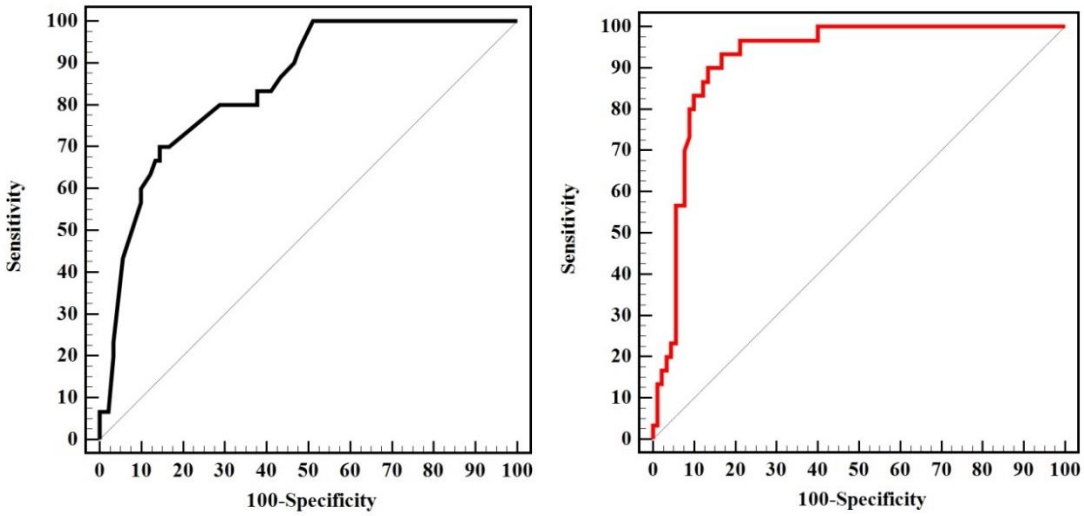


Figure 2. Comparison between the four studied groups according to ΔCT. A. miRNA 106b. B. miRNA-198.

A. **B.**



C.

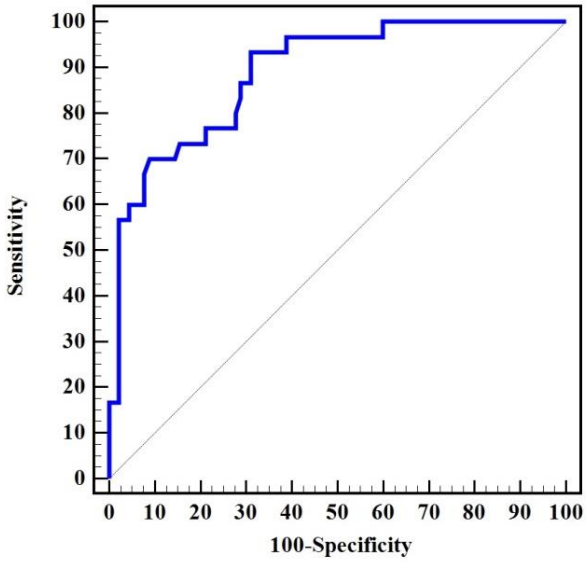


Figure 3. The use of ROC curve to discriminate patients with HCC (n = 32) from patients without HCC (n = 100). A. AFP. B. miRNA-106b. C. miRNA-198.