POSSIBLE DIAGNOSTIC AND PROGNOSTIC ROLE OF microRNA-122 IN HEPATOCELLULAR CARCINOMA

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The liver is the sixth most typical location for primary cancer in humans and is considered as one of the most prevalent malignant tumors, liver cancer is the fourth cause of mortality rate worldwide (Xiang *et al.,* 2023). By 2025, it is predicted that more than 1 million people would be diagnosed with liver cancer patients annually (Llovet *et al.,* 2021). Hepatocellular carcinoma (HCC), accounts for 80–90% of primary liver cancers (Ringelhan *et al.,* 2018), has a terrible prognosis and a relative five-year survival rate of 18% (Siegel *et al.,* 2022). Hepatitis B (HBV), hepatitis C (HCV), and alcoholism are the most prevalent aetiologies of liver cirrhosis and HCC (Sarin *et al.,* 2020; Singal *et al.,* 2020). There is a link between HCC and liver cirrhosis. Cirrhosis can occur together with HCC or it might evolve into HCC later on (Dhanasekaran *et al.,* 2016). Due to the lack of visible signs and symptoms when the tumorogenesis is mild, the diagnosis of HCC is sometimes delayed. The patient's life expectancy is also low as a result of the frequent delays in discovery and treatment (Gani, 2017). As a result, it's essential to create a diagnostic algorithm that uses improved techniques to identify and distinguish between cirrhosis and HCC. The right therapy will be chosen based on the accurate diagnosis.

The post-transcriptional level of messenger RNAs (mRNAs) is known to be significantly regulated by tiny noncoding RNAs called microRNAs (miRNAs),

which are short noncoding RNAs of around 22 nucleotides (nt) in length (Khasraghi *et al.*, 2023; Shirvani *et al.*, 2023). Although microRNA122 (miR-122) makes almost 70% of the total liver miRNA population, it is not present in other tissues (Fu and Calin, 2018). According to Fukuhara and Matsuura, (2013) as well as Szabo and Bala, (2013), MiR-122 regulates hepatocyte formation, differentiation, lipid metabolism, and stress response. By directly binding to the HCV 5′UTR of HCV RNA, miR-122 promotes hepatitis C virus (HCV) replication in the context of liver disorders (Fukuhara and Matsuura, 2013), however miR-122 inhibits replication of hepatitis B virus (HBV) by p53-mediated inhibition of HBV transcription (Wang *et al.*, 2012). By interacting with target genes involved in hepatocellular carcinoma (HCC) cell proliferation, migration, differentiation, apoptosis, and angiogenesis, miR-122 functions as a tumor suppressor and inhibits the growth of HCC (Szabo and Bala, 2013). Given that miR-122 is more accurate and stable in different specimens by RT-PCR, it is intriguing to be utilized as a marker for diagnosis. Since it can be measured, non-invasively, is both a promising biomarker for cancer screening and an intriguing marker for variations in HCV replication (Parizadeh *et al.*, 2019; Huang *et al.*, 2020).

Based on the above, this study was dedicated to construct a prognostic and diagnostic model for HCC patients using miR-122, which enhances patient outcomes, and enables a full understanding of the molecular pathways causing hepatic carcinogenesis.

SUBJECTS AND METHODS

Study design and population

The study protocol was approved by the committee of the National Liver Institute. This met the ethical requirements of the Declaration of Helsinki and the norms for good clinical practice. 150 individuals were recruited for this study, which were divided into the following three groups: 50 patients in Group I had cirrhosis of the liver, 50 patients in Group II had cirrhosis but no HCC, and 50 healthy subjects in Group III which were matched for age and sex and showed no symptoms of liver disease. Cirrhosis was confirmed using ultrasonography, biochemical, and clinical examination. The diagnosis of HCC was determined based on the AFP concentrations, ultrasonography, and triphasic CT criteria. The hepatology gastrointestinal department of the national liver institute included all of the patients from its outpatient clinic at Menoufia University. Severe infections, autoimmune diseases, cardiac, pulmonary, renal, long-term alcoholism, and various cancers rejected subjects.

All patients had the ensuing examinations done on them. A thorough medical history was to be taken, along with a clinical and general physical examination, ultrasonography, and CT. Haematological data, such as the complete blood count (CBC), were included. Tests for liver function include AST, ALT, total and di-

rect bilirubin, albumin, total protein, prothrombin time (PT), concentration, and INR, alkaline phosphatase (ALP), and glutamyl transferase (GGT). All samples were examined for tumor markers to identify the earliest stages of HCC, including AFP and alpha fucosidase (AFU). Using an RNA isolation kit in accordance with the manufacturer's instructions, RNA was extracted from the blood samples. The expression of miR-122 was then detected using qRT-PCR.

Sampling and Laboratory investigations

Ten millilitres of venous blood were to be taken from each person using three sterile vacationer tubes. For CBC and RNA extraction, one tube contained ethylene di amine tetra acetic acid (EDTA), the second tube contained sodium citrate, and the third tube lacked an anticoagulant and was used for standard liver function tests, AFP, and AFU (Kwo *et al.*, 2017; Tapper *et al.*, 2017). All biochemical test parameters were carried out in accordance with the manufacturer's instructions using the COBAS INTEGRA 800 chemistry auto analyzer (Roche Diagnostics Ltd., CH-6343 Rotkreuz, Switzerland). The Sysmex XP-300 automated cell blood counter was used to estimate the number of erythrocytes, haemoglobin concentration, hematocrit, blood indices, total leucocytes, and platelets. The Medonic automated haematology analyzer was used to confirm the results (Nah *et al.*, 2018). Thrombrel-S (human thromboplastin containing calcium) from Behring Diagnostic Inc. was used for PT, concentration, and international normalised ratio (INR) measurements (Dorgalaleh *et al.*, 2021). Alpha-l-fucosidase (AFU) was estimated utilising the spectrophotometric Stop Rate Determination technique and the chemiluminescent sandwich principle with the help of the fully automated analyzer Cobas e 411.

Extraction and Reverse Transcription (RT) of RNA

Following the manufacturer's instructions, total RNA (involving miRNAs) was extracted utilizing the RNeasy Mini Kit with Qiazol Reagent (Qiagen, USA). RNA quality was evaluated using the NanoDrop 1000 (Thermo Scientific, United States). RT was applied regarding the producer's instructions utilizing the miScript II RT Kit (Qiagen, USA).

Quantitative Real-Time PCR

The SYBR Green PCR Kit (Qiagen, USA) was used to measure the expression levels of miR-122 in sera. A 12.5 ul SYBR green master mix, 2.5 l of diluted cDNA, 2.5 l of the miScript universal primer (reverse primer), 2.5 l of the miScript primer assay, and 5 l of RNase-free water as required for the real-time PCR of each miRNA. According to Pan *et al.*, (2016), the forward primer sequence for miR-122 is 5'-UGGAGUGUGACAAUGGUGUUUG-3'. Using an Applied Biosystems 7500 Real-Time PCR System (Foster City, CA, USA), real-time PCR was performed as follows: After 15 minutes at 95°C, 40 cy-

cles of 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s were carried out. The number of cycles necessary for the fluorescent signal in real-time PCR to cross the threshold is known as the cycle threshold (Ct). The Ct value for miRNA expression was reported. Ct was obtained by deducting the target miR-122 value from those of the endogenous housekeeping gene miRNA-16. Relative gene expression= $(2)^{-[\Delta][\Delta]Ct}$ Where, $^{\Delta\Delta}Ct = {}^{\Delta}Ct$ sample $-{}^{\Delta}Ct$ reference.

Statistical Analysis

For the statistical analysis, IBM Inc., Chicago, IL, USA, used SPSS (Statistical Package for the Social Sciences) version 25. The Shapiro-Wilks normality test and histograms were used to examine the distribution of quantitative data in order to decide whether parametric or non-parametric statistical testing should be used. The F test was used to compare the parametric variables between the three groups, and the post hoc (Tukey) test was performed to compare each pair of groups independently. Mean and standard deviation (SD) were used as representations for parametric variables. To compare two variables within the same group, the paired T-test was utilized. Non-parametric variables were assessed using the Kruskal-Wallis test and were displayed as median and interquartile range (IQR). Then, each pair of groups was compared using the Mann-Whitney (U) test. Wilcoxon test was used to compare two variables within the same group. Categorical variables were statistically examined by Chi-square test and presented as frequency and percentage. A two-tailed P value ≤ 0.05 was considered statistically significant and the Receiver Operating Characteristic Curve (ROC) was used to evaluate the diagnostic performance of the markers. An area around 100% denotes the test's ideal performance, while an area higher than 50% denotes an acceptable level of performance.

RESULTS AND DISCUSSION

Demographic and clinicopathological characteristics of study subjects

Out of a total of 150 participants, there were 37 males and 13 females with a mean ± SD age of 59.52 ± 5.71 years who had HCC, 36 males and 14 females with a mean ± SD age of 58.36 ± 4.17 years who had LC; and 31 healthy males and 19 healthy females with a mean ± SD age of 59.74 ± 4.05 years. Between the three groups, there was no discernible difference in either age or sex ($p = 0.292$ and 0.378, respectively). Weight reduction and smoking status did not differ significantly between the two patient groups with HCC and LC (P=1.000, 0.052, respectively).

In regard with results of peripheral blood picture, parameters showed a significant decrease in the HCC group (P<0.001), while the mean level of serum transaminases, bilirubin serum, AFP, GGT, ALP, and AFU were significantly higher in the HCC group (P<0.001), in addition, albumin, total protein, and coagulation profile were significantly lower in HCC group than LC and healthy control groups (P<0.001) (Table 1).

Jaundice, hepatic encephalopathy, and splenomegaly were all greater in the HCC group but not significantly difference, although bilharzia was considerably higher (P=0.009). These clinicopathological characteristics were different between the HCC and LC groups. In contrast to the moderate ascites that were seen in HCC and LC patients (42% and 48%). Severe ascites was present in 22% of HCC patients with a significant correlation (P=0.005). Child-Pugh grades A, B, and C were present in both the HCC and LC groups, with B being more frequent and with a significant difference (P=0.040). With no discernible changes, the comorbidities of disorders like diabetes and hypertension were greater in the LC patient group (Table 2). In the HCC patient group, vascular invasion and LN Metastasis were both positive (24% and 30%, respectively).

Computed tomography and Survival analysis of HCC subjects

Multiple nodules were found in 62% of the cases, and large focal lesions (>5 cm in diameter) were found in 44% of cases based on nodule features. The right lobe had the highest rate of HCC (46%), followed by the left lobe's 28% and 26% rates in each lobe. TNM stage IIIa showed a higher prevalence (38%) than stages I, II, III b, and IV a. The prevalence of BCLC Staging-C is also greater than BCLC-B (60% vs. 40%).

The correlation between biochemical, clinicopathological characteristics and miRNA 122 in HCC subjects

The significant associations between mir-122 expression levels and clinical laboratory tests are displayed in Fig. (1). PLT, WBCs, and albumin showed a positive and significant association with mir-122 expression level, whereas ALT, T.bilirubin, d.bilirubin, GGT, AFP, and AFU showed a significant negative correlation. Similarly, (Table 3) displays the association between mir-122 and clinicopathological features in the HCC group. Figure (2) demonstrates a significant correlation between mir-122 and tumour size, number, and TNM staging.

Diagnostic Performance of HCC biomarkers and miRNA-122

Table (4) displays the predictive efficacy of the HCC biomarkers AFP, AFU, and miRNA 122. According to the ROC curve study, miRNA 122 has a high sensitivity (82%), specificity (74%), and ability to discriminate between instances of HCC and LC.

The relationship between clinicopathological characteristics and mortality of HCC patients

In order to determine if the computed prognostic value of the research variables may operate as an independent risk factor, both univariate and multivariate Cox regression analyses were used. A univariate examination of T. bilirubin, D. bilirubin, GGT, AFP, and BCLC revealed that prognostic value was a statistically

significant predictor of death (P<0.001). MiR-122 affected mortality in univariate analysis with a hazard ratio of 0.0(0.0-0.005) and in multivariate analysis with a hazard ratio of 0.0(0.0-0.121) that was statistically significant (P<0.05). A non-statistically significant connection between mortality and all demographic and clinicopathological characteristics was discovered using multivariate analysis (P>0.05) (Table 5).

MiRNAs may be useful clinically as pathological indicators for the classification, prognostic stratification, and early identification of HCC patients. A further indication of the therapeutic potential of miRNAs as a targeted molecular therapy for the treatment of HCC patients is the possible use of anti-miRNA oligonucleotides to modify HCC activities (Callegari *et al.,* 2015; Yerukala Sathipati and Ho, 2020; Morishita *et al.,* 2021). In contrast to suppressor miRNAs, HCC-related miRNAs are overexpressed (Oura *et al.,* 2020). According to various studies, a microRNA panel may distinguish between HCC and liver cirrhosis (Yamamoto *et al.,* 2019; Huang *et al.,* 2022). Recently, researchers focused onto MicroRNA-122 as a potential indicator of many liver disorders (El-Ahwany *et al.,* 2019; Franck *et al.,* 2020). The study was conducted to examine the clinical significance of circulating miR-122 as a novel predictive marker of HCC as soon as practical in order to improve the prognosis and diagnosis of the patients.

The risk of HCC among males increased slightly but significantly in the current study. According to Keng *et al.,* (2012), who found that the HCC is sexually dimorphic in both rats and humans, with a much greater prevalence in males, an effect that is reliant on sex hormones, this study's findings are in agreement with their findings. It is still unknown how androgens cause liver cancer whereas oestrogens protect against it at the molecular level.

When compared to the control group, the HCC and LC groups had considerably higher levels of the biochemical characteristics studied, liver functions (ALT, AST, bilirubin, ALP, GGT, AFP, AFU), and liver cirrhosis, with the HCC group being much higher than the LC group. This conclusion is consistent with those made by (Shaker *et al.,* 2020; Ma *et al.,* 2021 and Liu *et al.,* 2022), who found that HCC patients had higher blood concentrations of ALT, AST, bilirubin, GGT, and AFP than those with chronic liver disease. Moreover, individuals with HCC have their AFU elevated significantly. According to Fawzy Montaser *et al.,* (2012), who observed a very highly significant increase in the median serum AFU level in the HCC group, this result is consistent with their findings.

The HCC group was considerably higher as compared to the LC group, and the haematological parameters (HB, WBC, and PLT), albumin, total protein, PT, and miR-122 were all markedly decreased in the HCC and LC groups as compared to the control group. These findings were consistent with those of

Shaker *et al.,* (2020), who also reported a drop in Hb concentration, platelet count, and albumin. Franck *et al*., (2020), reported that HCC cases exhibited considerably lower levels of miR-122 than the LC group. According to El-Tonsy *et al.*, (2016), who found that the schistosomiasis insult on the liver is a slowly progressing one, bilharzia and HCC are highly associated. Therefore, it is challenging to start dysplastic alterations alone that might indicate a risk factor for the development of HCC within the normal life course of any patient. In this study, ascites also commonly occurred in HCC patients compared to LC patients, suggesting that ascites may be a predictor of LC progression to HCC. According to Hsu *et al.*, (2013), ascites is often seen in HCC patients and are associated with both tumor and cirrhosis factors as well as a worse long-term survival rate.

In the current study, the expression level of mir-122 was strongly inversely correlated with ALT, T.bilirubin, d.bilirubin, GGT, AFP, and AFU, but considerably positively correlated with PLT, WBCs, and albumin. According to Ahmed *et al.,* (2020), there is a substantial positive link between the levels of aminotransferase (ALT, AST) and miRNA-122 expression in people with liver illness. MiR-122 showed a strong association between tumor number, size, TNM staging, BCLC, and survival in terms of clinicopathological features. According to this study's findings, which concur with those of Zhan *et al.*, (2021), low miR-122 levels are to blame for the low 5-year patient survival rates. Additionally, it was related to the TNM and BCLC staging, vascular invasion, tumor size and number, and tumor size and number.

The identification of serum biomarkers has a significant clinical effect in the early detection and diagnosis of HCC due to the comparatively straightforward techniques. Although AFP is a frequently used serum marker in clinical settings, Shehab-Eldeen *et al.,* (2019), showed that it had suboptimal sensitivity and accuracy, with an AUC of 0.68, sensitivity of 70%, and specificity of 70%. According to the results of the current study, AFP has an AUC of 0.944, sensitivity of 88%, and specificity of 72% for differentiating between HCC patients and healthy individuals, and an AUC of 0.699, sensitivity of 60%, and specificity of 64% for differentiating between HCC and LC patients. Compared to other available biomarkers, miRNA-122 performed better in the diagnosis of HCC. The miR-122 expression level has an AUC of 0.903, sensitivity of 82%, and specificity of 96% for identifying HCC patients from healthy people. Additionally, serum miR-122 expression level for differentiating cirrhotic individuals with HCC from LC cases was found to be AUC 0.849, with sensitivity of 82% and specificity of 74% ($P \leq 0.001$), following Zhao *et al.,* (2020). After correcting for other clinical factors, univariate and/or multivariate Cox analyses revealed that miR-122 had a significant predictive value for the biochemical measures T. bilirubin, d. bilirubin, GGT, AFP, and BCLC ($P<0.001$). A hazard ratio of 0.0

(0.0-0.121) for multivariate miR-122 was statistically significant (P<0.05). These findings imply that grading systems based on miR-122 can be an effective independent predictor.

Conclusion: The current study validated the use of miR-122 as a special and important marker with high specificity and sensitivity for the diagnosis of HCC. To corroborate these findings, however, more thorough functional analyses and prospective population-based research with larger sample sizes and varied ethnic groups are required.

SUMMARY

Background and objectives: The sensitivity and specificity of the available biomarkers for the diagnosis of hepatocellular carcinoma (HCC) are inadequate. Consequently, there is a need to create trustworthy biomarkers that are sensitive, specific, and non-invasive for HCC early detection and quick management to boost patient survival rates. Circulatory miRNAs have received a lot of attention recently as potential non-invasive biomarkers for a number of illnesses. Their expression patterns reflect the state of the illness and/or the degree of therapeutic response, and they remain remarkably stable in blood. In order to improve patient prognosis, the goal of this study was to analyze the expression profile of miRNA-122 in HCC patients and to evaluate their clinical value as novel HCC prognostic indicators as early as practical.

Methodology: There were 150 Egyptian patients in this prospective case-control study, 50 with liver cirrhosis (LC), 50 had HCC, and 50 of whom were healthy. The miR-122 was measured using reverse transcription (RT) real-time PCR.

Results: HCC had considerably lower levels of miR-122 than the other two studied groups. MiR-122 distinguished HCC patients from healthy controls with a specificity and sensitivity of 96.0% against 82.0%, respectively AUC= 0.903, according to an examination of receiver operating characteristic curves. AUC=0.849 revealed a specificity and sensitivity of 74.0% and 82.0% respectively to distinguish cirrhotic patients from HCC participants. Univariate analysis of miR-122 affecting mortality was observed in this study.

Conclusions: Expression of miR-122 decreased in HCC patients can be used to differentiate HCC from LC. MicroRNA-122 may be utilized as early, non-invasive indicators for both diagnosis and prognosis of HCC.

REFERENCES

Ahmed T., Ahmad E. Ibrahim and Saeed A. M., (2020). Serum mirna-122 as a diagnostic marker in HCV related liver cirrhosis. Afro-Egyptian Journal of Infectious and Endemic Diseases, 10(4): 335-345. doi: 10.21608/aeji.2020. 30137 .1082.

Callegari E., Gramantieri L., Domenicali M., D'Abundo L., Sabbioni S. and

Negrini M., (2015). MicroRNAs in liver cancer: a model for investigating pathogenesis and novel therapeutic approaches. Cell Death Differ., 22(1):46-57. doi: 10.1038/cdd.2014.136.

Dhanasekaran R., Bandoh S. and Roberts L. R., (2016). Molecular pathogenesis of hepatocellular carcinoma and impact of therapeutic advances. F1000Res.;5:F1000 Faculty Rev-879. doi: 10.12688/f1000 research.6946.1.

Dorgalaleh A., Favaloro E. J., Bahraini M. and Rad F., (2021). Standardization of Prothrombin Time/International Normalized Ratio (PT/INR). Int J. Lab. Hematol., 43(1):21-28. doi: 10.1111/ijlh.13349.

El-Ahwany E. G. E., Mourad L., Zoheiry M. M. K., Abu-Taleb H., Hassan M., Atta R., Hassanien M. and Zada S., (2019). MicroRNA-122a as a non-invasive biomarker for HCV genotype 4-related hepatocellular carcinoma in Egyptian patients. Arch Med Sci. 2019, 15(6):1454-1461. doi: 10.5114/aoms.2019.86621.

El-Tonsy M. M., Hussein H. M., Helal Tel-S., Tawfik R. A., Koriem K. M. and Hussein H. M., (2016). Human *Schistosomiasis mansoni* associated with hepatocellular carcinoma in Egypt: current perspective. J Parasit Dis., 40(3):976-80. doi: 10.1007/s12639-014-0618-0.

Fawzy Montaser M., Amin Sakr M. and Omar Khalifa M., (2012). Alpha-L-fucosidase as a tumour marker of hepatocellular carcinoma. Arab J Gastroenterol., 13(1):9-13. doi: 10.1016/j.ajg.2012.03.006.

Franck M., Schütte K., Malfertheiner P. and Link A., (2020). Prognostic value of serum microRNA-122 in hepatocellular carcinoma is dependent on coexisting clinical and laboratory factors. World J. Gastroenterol., 26(1):86-96. doi: 10.3748/wjg. v26.i1.86.

Fu X. and Calin G. A., (2018). miR-122 and hepatocellular carcinoma: from molecular biology to therapeutics. EBioMedicine., 37:17-18. doi: 10.1016/j.ebiom.2018.10.032.

Fukuhara T. and Matsuura Y., (2013). Role of miR-122 and lipid metabolism in HCV infection. J Gastroenterol. 2013; 48:169-76.

Gani R. A., (2017). Hepatocellular Carcinoma (HCC) surveillance: comprehensive management in liver cirrhosis patients Indones. J. Gastroenterol. Hepatol. Dig. Endosc., 18, pp. 137-139.

Hsu C. Y., Lee Y. H., Huang Y. H., Hsia C. Y., Su C. W., Lin H. C. and Lee S. D., (2013). Ascites in patients with hepatocellular carcinoma: prevalence, associated factors, prognostic impact, and staging

strategy. Hepatology international., 7(1):88-198.

Huang Y., Chen Y., Tu S., Zhang J., Qiu Y. and Yu W., (2022). Diagnostic accuracy of circulating microRNAs for hepatitis C virus-associated hepatocellular carcinoma: a systematic review and meta-analysis. BMC Infect Dis., 22(1):323. doi: 10.1186/s 12879-022-07292-8.

Huang A., Yang X. R., Chung W. Y., Dennison A. R. and Zhou J., (2020). Targeted therapy for hepatocellular carcinoma. Signal Transduct Target Ther, 5(1):1-13.

Keng V. W., Largaespada D. A. and Villanueva A., (2012). Why men are at higher risk for hepatocellular carcinoma? J. Hepatol., 57(2):453-4. doi: 10.1016/j.jhep. 03.004.

Khasraghi L.B., Nouri M., Vazirzadeh M., Hashemipour N., Talebi M. and Zarch F.A., (2023). MicroRNA-206 in human cancer: mechanistic and clinical perspectives. Cell Signal., 101:110525.

Kwo P. Y., Cohen S. M. and Lim J. K., (2017). ACG Clinical Guideline: Evaluation of Abnormal Liver Chemistries. Am J Gastroenterol., 112(1):18-35. doi: 10.1038 /ajg. 2016.517.

Liu K., Huang Z., Yang S., Lin L., Zheng S., Zhang X., Xue Y. and Xie W., (2022). The Age, Gamma-Glutamyl Transpeptidase and Platelet Index: A Novel Noninvasive Model for Predicting Hepatocellular Carcinoma in Patients with Hepatitis B Virus-Related Liver Cirrhosis. J. Hepatocell Carcinoma., 9:1057-1063. doi: 10.2147/JHC.S386977.

Llovet J. M., Kelley R. K., Villanueva A., Singal A. G, Pikarsky E., Roayaie S., Lencioni R., Koike K., Zucman-Rossi J. and Finn R. S., (2021). Hepatocellular carcinoma. Nat Rev Dis Primers., 21;7(1):6. doi: 10.1038/s41572-020-00240-3.

Ma H. Y., Dong L., Quan S. Z., Li R. Y. and Wang X. R., (2021). Comparison of four markers of hepatic fibrosis and hepatic function indices in patients with liver cirrhosis and hepatoma. Ann Palliat Med., 10(4):4108-4121. doi: 10.21037/apm-20-1623.

Morishita A., Oura K., Tadokoro T., Fujita K., Tani J. and Masaki T., (2021). MicroRNAs in the Pathogenesis of Hepatocellular Carcinoma: A Review. Cancers (Basel)., 13 (3):514. doi: 10.3390 /cancers13030514.

Nah E.H., Kim S., Cho S. and Cho H. I., (2018). Complete Blood Count Reference Intervals and Patterns of Changes Across Pediatric, Adult, and Geriatric Ages in Korea. Ann Lab Med., 38(6):503-511. doi: 10.3343/alm.2018.38.6.503.

Oura K., Morishita A. and Masaki T., (2020). Molecular and Functional Roles of MicroRNAs in the Progression of Hepatocellular Carcinoma-A Review. Int J. Mol. Sci., 21(21):8362. doi: 10.3390 /ijms21218362.

Pan C., Wang X., Shi K., Zheng Y., Li J., Chen Y., Jin L. and Pan Z., (2016). MiR-122 Reverses the Doxorubicin-Resistance in Hepatocellular Carcinoma Cells through Regulating the Tumor Metabolism. PLoS One., 11(5): e0152090. doi: 10.1371/ journal.pone.0152090.

Parizadeh S. M., Jafarzadeh-Esfehani R., Ghandehari M., Goldani F., Parizadeh S. M., Hassanian S. M. and Ghayour-Mobarhan M., (2019). MicroRNAs as potential diagnostic and prognostic biomarkers in hepatocellular carcinoma. Current Drug Targets, 20 (11):1129-1140.

Ringelhan M., Pfister D., O'Connor T., Pikarsky E. and Heikenwalder M., (2018). The immunology of hepatocellular carcinoma. Nat Immunol., 19(3):222-232. doi: 10.1038/s41590-018-0044-z.

Sarin S. K., Kumar M., Eslam M., George J., Al Mahtab M. and Akbar S. M. F., (2020). Liver diseases in the Asia-Pacific region: a Lancet Gastroenterology & Hepatology Commission. Lancet Gastroenterol Hepatol., 5(2):167-228. doi: 10. 1016/S2468-1253(19)30342-5.

Shaker O., Mahfouz H., Salama A. and Medhat E., (2020). Long Non-Coding HULC and miRNA-372 as Diagnostic). Biomarkers in Hepatocellular Carcinoma. Rep Biochem Mol Biol., 9(2):230-240. doi: 10.29252/rbmb.9.2.230.

Shehab-Eldeen S., Nada A.and Abou-Elela D., (2019). Diagnostic performance of microRNA-122 and microRNA-224 in hepatitis C virus-induced hepatocellular carcinoma (HCC). Asian Pac J Cancer Prev., 20: 2515-2522.

Shirvani H., Ghanavi J., Aliabadi A., Mousavinasab F., Talebi M. and Majidpoor J., (2023). MiR-211 plays a dual role in cancer development: from tumor suppressor to tumor enhancer. Cell Signal., 101:110504.

Siegel R. L., Miller K. D., Fuchs H. E. and Jemal A., (2022). Cancer statistics, 2022. CA Cancer J. Clin., 72(1):7-33. doi: 10.3322/caac.21708.

Singal A. G., Lampertico P. and Nahon P., (2020). Epidemiology and surveillance for hepatocellular carcinoma: New trends. J. Hepatol., 72(2):250-261. doi: 10.1016/j. jhep.2019.08.025.

Szabo G. and Bala S., (2013). MicroRNAs in liver disease. Nat. Rev. Gastroenterol Hepatol., 10:542-52.

Tapper E. B., Saini S. D. and Sengupta N., (2017). Extensive testing or focused testing of patients with elevated liver enzymes. J. Hepatol., 66(2):313-319. doi: 10.1016/j. jhep.2016.09.017.

Wang S., Qiu L., Yan X., Jin W., Wang Y., Chen L., Wu E., Ye X., Gao G. F., Wang F., Chen Y., Duan Z. and Meng S., (2012). Loss of microRNA 122 expression in patients with hepatitis B enhances hepatitis B virus replication through cyclin G (1) -modulated P53 activity. Hepatology., 55:730-41.

Xiang Z., Wu J., Li J., Zheng S., Wei X. and Xu X., (2023). Gut microbiota modulation: a viable strategy to address medical needs in hepatocellular carcinoma and liver transplantation. *Engineering*. doi: 10.1016/j.eng. 2022.12.012

Yamamoto Y., Kondo S., Matsuzaki J., Esaki M., Okusaka T. and Shimada K., (2019). Highly Sensitive Circulating MicroRNA Panel for Accurate Detection of Hepatocellular Carcinoma in Patients With Liver Disease. Hepatol Commun., 4(2):284-297. doi: 10.1002/hep4.1451.

Yerukala Sathipati S. snd Ho S-Y., (2020). Novel miRNA signature for predicting the stage of hepatocellular carcinoma. Scientific reports., 10(1):1-12. 14452. doi: 10.1038/s41598-020-71324-z

Zhan G., Jiang H., Yang R. and Yang K., (2021). miR-122 and miR-197 expressions in hepatic carcinoma patients before and after chemotherapy and their effect on patient prognosis. Am J. Transl Res., 13(6):6731-6737.

Zhao XF., Li N., Lin D. D. and Sun L. B., (2020). Circulating MicroRNA-122 for the Diagnosis of Hepatocellular Carcinoma: A Meta-Analysis. Biomed Res. Int., 2020:5353695. doi: 10.1155 /2020/5353695.

Table (1): Biochemical characteristics of all studied groups .

Variables	HCC (n = 50)	LC (n = 50)	Control (n = 50)	p
Hb (gm/dl)	9.93 ± 1.20	11.30 ± 1.29	13.49 ± 0.98	<0.001*
Platelets (x10^3/mm^3)	107.36 ± 48.01	131.72 ± 66.09	266.9 ± 66.28	<0.001*
WBCS (x10^3/mm^3)	3.41 ± 0.83	4.07 ± 1.27	6.56 ± 1.76	<0.001*
ALT (U/L)	56.62 ± 26.40	49.82 ± 22.74	25.46 ± 8.04	<0.001*
AST (U/L)	55.60 ± 22.42	50.24 ± 22.72	25.48 ± 7.55	<0.001*
Serum albumin (gm/dL)	3.17 ± 0.46	3.49 ± 0.43	4.39 ± 0.40	<0.001*
T. protein (gm/dL)	5.70 ± 0.87	6.29 ± 0.69	7.11 ± 0.46	<0.001*
Total bilirubin (mg/dL)	3.43 ± 3.83	1.47 ± 1.06	0.58 ± 0.22	<0.001*
Direct bilirubin (mg/dL)	1.93 ± 2.76	0.71 ± 0.67	0.16 ± 0.07	<0.001*
GGT (U/L)	71.36 ± 21.52	50.72 ± 13.56	19.88 ± 6.52	<0.001*
ALP (U/L)	119.9 ± 35.39	83.92 ± 22.99	51.04 ± 14.41	<0.001*
PT (%)	72.79 ± 9.94	78.84 ± 8.86	98.06 ± 2.09	<0.001*
INR	1.33 ± 0.16	1.24 ± 0.13	1.02 ± 0.02	<0.001*
AFP (ng/mL)	377.35 ± 520.59	51.99 ± 78.40	5.12 ± 2.12	<0.001*
AFU (µmol/L)	142.28 ± 123.60	23.17 ± 18.53	4.80 ± 2.51	<0.001*

Hb: Hemoglobin; WBC: White blood count; PLT: Platelets; ALT: Alanine Aminotransferase;
AST: Aspartate Aminotransferase; ALB: Albumin; T. protein: Total protein.

Table (2): The clinicopathological characteristics of the HCC and LC groups.

Variables	HCC (n = 50)	LC (n = 50)	P
Jaundice	18 (36%)	11 (22%)	0.123
Bilharzia	34 (68%)	21 (42%)	0.009*
Hepatic encephalopathy	10 (20%)	4 (8%)	0.084
Splenomegaly	34 (68%)	32 (64%)	0.673
Ascites			
No	12 (24%)	19 (38%)	0.005*
Mild	21 (42%)	24 (48%)	
Moderate	6 (12%)	7 (14%)	
Tense	11 (22%)	0 (0%)	
Child PUGH class			
A	12 (24%)	19 (38%)	0.040*
B	25 (50%)	27 (54%)	
C	13 (26%)	4 (8%)	
Comorbidities			
DM	11 (22%)	20 (40%)	0.052
HTN	13 (26%)	16 (32%)	0.509
Heart diseases	0 (0%)	0 (0%)	

DM: Diabetes mellitus; HTN: Hypertension; SD: Standard deviation; RQ: Relative quantification.

Table (3): Relation between Micro RNA 122 and clinicopathological variables in the HCC group .

Clinico-pathological variables	N	Micro RNA 122	
		Median (Min. – Max.)	Mean ± SD.
Tumor number			
Single	19	0.68(0.01 – 1.46)	0.65 ± 0.45
Multiple	31	0.23(0.01 – 0.87)	0.30 ± 0.25
U(p)		**157.0*(0.006*)**	
Tumor Size			
Small (<3 cm)	9	0.68(0.12 – 1.23)	0.75 ± 0.38
Medium (3 - 5 cm)	19	0.30(0.01 – 1.46)	0.37 ± 0.36
Large (>5 cm)	22	0.27(0.01 – 1.20)	0.35 ± 0.33
H(p)		**7.333*(0.026*)**	
Tumor Site			
Rt Lobe	23	0.35(0.01 – 1.23)	0.45 ± 0.36
Lt Lobe	13	0.39(0.01 – 1.20)	0.48 ± 0.40
Both	14	0.27(0.01 – 1.46)	0.36 ± 0.40
H(p)		**1.641(0.440)**	
TNM staging			
I	7	0.68(0.35 – 1.00)	0.72 ± 0.23
II	3	1.20(0.36 – 1.23)	0.93 ± 0.50
III a	19	0.34(0.04 – 1.20)	0.40 ± 0.31
III b	9	0.12(0.04 – 1.46)	0.40 ± 0.47
IV a	12	0.09(0.01 – 0.77)	0.21 ± 0.26
H(p)		**15.240*(0.004*)**	
BCLC			
B	30	0.38(0.01 – 1.46)	0.49 ± 0.36
C	20	0.10(0.01 – 1.23)	0.34 ± 0.39
U(p)		**199.0*(0.045*)**	
Fate			
Live	35	0.56(0.04 – 1.46)	0.58 ± 0.35
Dead	15	0.08(0.01 – 0.12)	0.07 ± 0.04
U(p)		**20.50*(<0.001*)**	

U: Mann Whitney test H: H for Kruskal Wallis test. p: p-value for comparison between the studied categories. *: Statistically significant at $p \leq 0.05$

Table (4): Prognostic performance for tumor markers and miRNA 122 among studied groups.

Groups	Parameters	AUC	p	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
HCC vs. control	**AFP (ng/mL)**	0.944	<0.001*	0.898 – 0.989	>6.1	88.0	72.0	75.9	85.7
	AFU (µmol/L)	0.910	<0.001*	0.852 – 0.967	>7.1	80.0	70.0	72.7	77.8
	Micro RNA 122 RQ	0.903	<0.001*	0.838 – 0.966	≤0.765	82.0	96.0	95.3	84.2
LC vs control	**AFP (ng/mL)**	0.870	<0.001*	0.793 – 0.948	>6	82.0	72.0	74.5	80.0
	AFU (µmol/L)	0.904	<0.001*	0.846 – 0.963	>7.2	80.0	72.0	74.1	78.3
	Micro RNA 122 RQ	0.703	<0.001*	0.601 – 0.804	≤0.95	62.0	60.0	60.8	61.2
HCC vs LC	**AFP (ng/mL)**	0.699	<0.001*	0.507 – 0.727	>29	60.0	64.0	62.5	61.5
	AFU (µmol/L)	0.699	0.001*	0.590 – 0.807	>31.1	58.0	68.0	64.4	61.8
	Micro RNA 122 RQ	0.849	<0.001*	0.765 – 0.933	≤0.76	82.0	74.0	75.9	80.4

AUC: Area Under a Curve p-value: Probability value CI: Confidence Intervals
NPV: Negative predictive value PPV: Positive predictive value *: Statistically significant at $p \leq 0.05$

Table (5): The relationship between clinicopathological characteristics and mortality of HCC group.

Variables	Univariate		#Multivariate	
	p	HR (LL – UL 95%C.I)	p	HR (LL – UL 95%C.I)
Sex (female)	0.465	0.624(0.176 – 2.211)		
Age (years)	0.556	1.029(0.936 – 1.132)		
Loss of weight	0.668	0.790(0.270 – 2.314)		
Smoking	0.223	1.879(0.681 – 5.186)		
Jaundice	0.112	2.279(0.825 – 6.291)		
Bilharzia	0.797	1.162(0.370 – 3.649)		
Abdominal pain	0.394	0.643(0.233 – 1.773)		
Hepatic encephalopathy	0.443	1.566(0.498 – 4.920)		
Splenomegaly	0.298	1.984(0.560 – 7.033)		
Ascites	0.703	0.800(0.255 – 2.514)		
Vascular Invasion	0.360	1.651(0.564 – 4.830)		
LN Metastasis	0.039*	2.913(1.056 – 8.037)	0.439	0.472(0.071 – 3.151)
Child PUGH class (C)	0.486	1.465(0.501 – 4.286)		
Comorbidities				
DM	0.824	1.139(0.362 – 3.577)		
HTN	0.065	2.603(0.944 – 7.181)		
Hb (gm/dl)	0.922	0.979(0.636 – 1.505)		
Platelets ($x10^3/mm^3$)	0.245	0.993(0.980 – 1.005)		
WBCS ($x10^3/mm^3$)	0.127	0.364(0.099 – 1.333)		
ALT (U/L)	0.055	1.015(1.0 – 1.031)		
AST (U/L)	0.430	1.008(0.988 – 1.029)		
Serum albumin (mg/dL)	0.330	0.580(0.194 – 1.735)		
T. protein (mg/dL)	0.389	1.299(0.717 – 2.352)		
Total bilirubin (mg/dL)	0.002*	1.176(1.059 – 1.306)	0.601	1.045(0.071 – 3.151)
Direct bilirubin (mg/dL)	<0.001*	1.314(1.131 – 1.526)	0.971	0.995(0.769 – 1.289)
GGT (U/L)	0.004*	1.027(1.008 – 1.045)	0.122	0.952(0.894 – 1.013)
ALP (U/L)	0.582	1.004(0.990 – 1.017)		
PT (%)	0.652	1.012(0.962 – 1.064)		
INR	0.595	0.411(0.015 – 10.933)		
AFP (ng/mL)	0.008*	1.001(1.0 – 1.002)	0.343	1.001(0.999 – 1.002)
AFU (umol/L)	0.085	1.004(0.999 – 1.009)		
Tumor number (Multiple)	0.298	1.835(0.584 – 5.765)		
Tumor Size(Large)	0.209	1.940(0.691 – 5.451)		
Tumor Site				
Rt Lobe				
Lt Lobe	0.767	1.204(0.352 – 4.114)		
Both	0.964	1.029(0.301 – 3.515)		
TNM staging(III+IV)	0.204	29.610(0.158 – 5540.034)		
BCLC (C)	0.007*	4.902(1.559 – 15.416)	0.129	3.583(0.690 – 18.606)
Micro RNA 122	0.002*	0.0(0.0 – 0.015)	0.069	0.0(0.0 – 6.264)

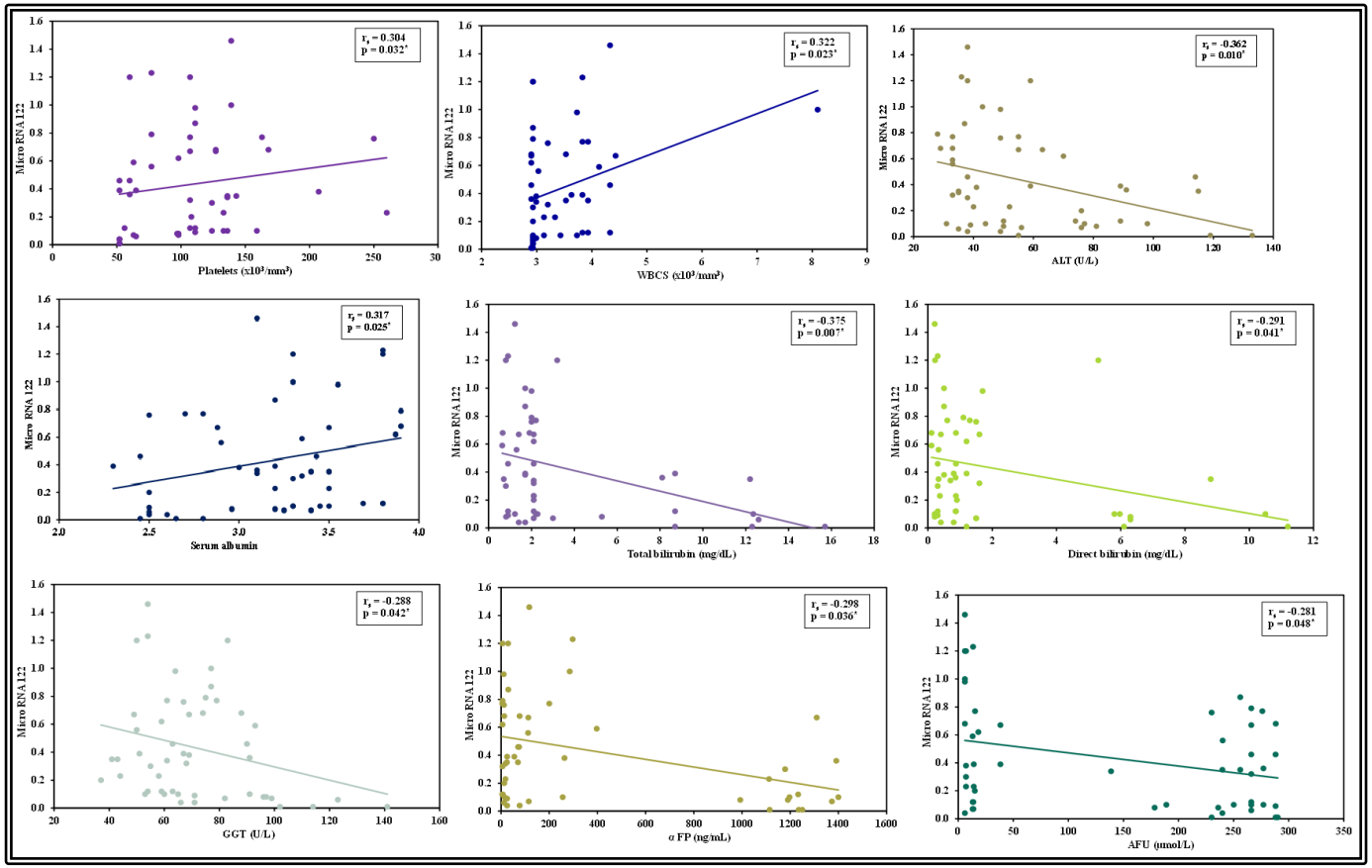

Fig. (1): Significant Correlation between Micro RNA 122 and biochemical parameters in the HCC group.

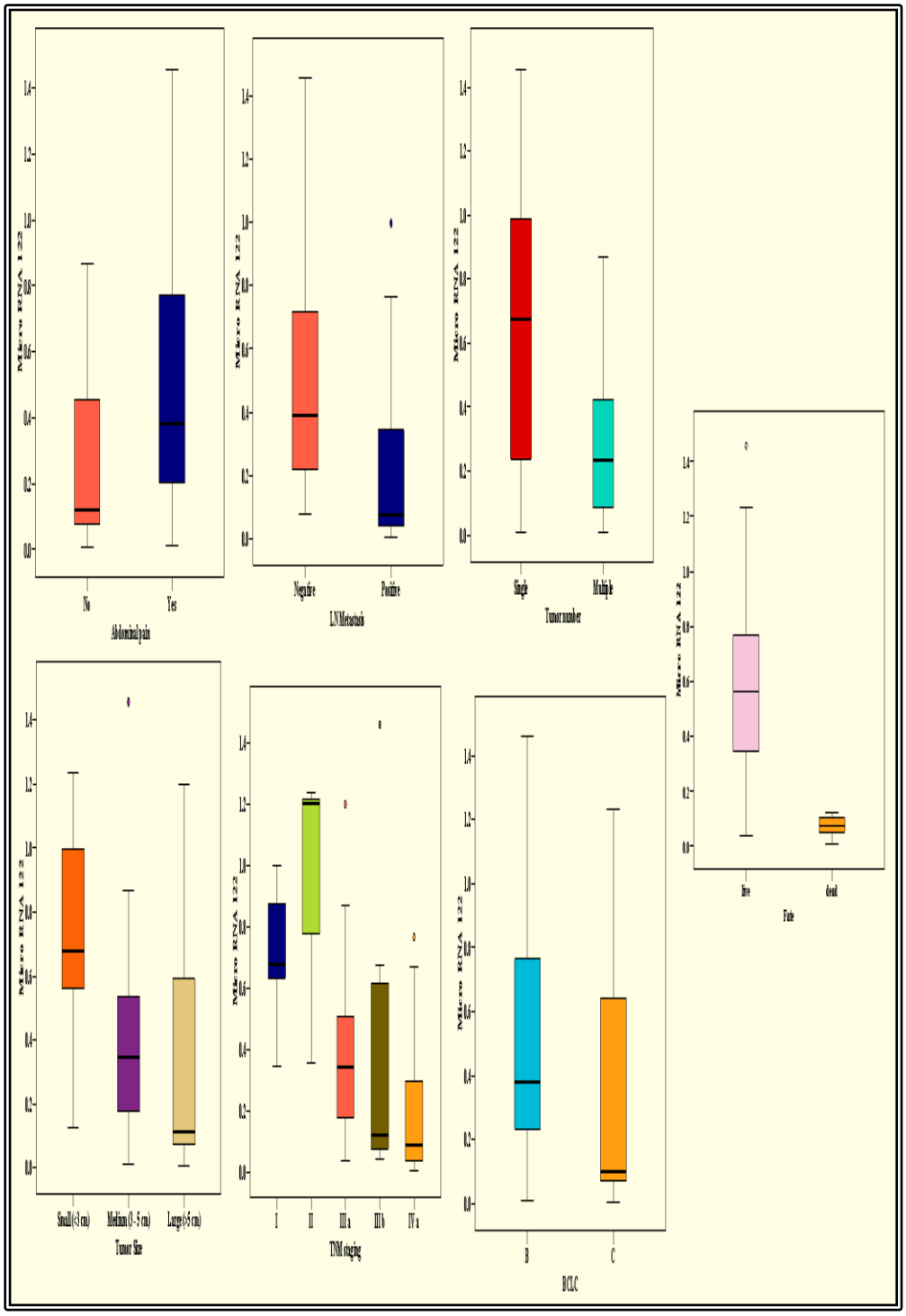

Fig. (2): Significant association between tumor number, size, TNM staging, BCLC with miRNA-122.