RESEARCH ARTICLE

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Clinical Utility of Cytokeratins for Accurate Diagnosis of Hepatocellular Carcinoma Among Hepatitis C Virus High-Risk Patients

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Abstract

Objectives: Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and a global health problem. It is often diagnosed at advanced stage where hopeless for effective therapies. Identification of more reliable biomarkers for early detection of HCC is urgently needed. Cytokeratins are a marker of hepatic progenitor cells and act as a key player in tumor invasion. Herein, we sought to develop a novel score based on the combination of cytokeratin 18 (CK18) and cytokeratin 19 (CK19) with routine laboratory tests for accurate detection of HCC. **Material & Methods:** Serum CK18, CK 19, α-fetoprotein, albumin and platelets count were assayed in HCC patients (75), liver cirrhosis patients (55) and healthy control (20). Areas under receiving operating curve (AUCs) were calculated and used for construction on novel score. A novel score named CK-HCC = CK 19 (ng/ml)×0.001+ CK18 (ng/ml)×0.004 + AFP (U/L)×5.4 - Platelets count ($\times10^9$)/L×0.003 – Albumin (g/L)×0.27–36 was developed. CK-HCC score produces AUC of 0.919 for differentiating patients with HCC from those with liver cirrhosis with sensitivity and specificity of a cut-off 1.3 (i.e., less than 1.3 the case is considered cirrhotic, whereas above 1.3 it is considered HCC. Conclusion: CK-HCC score could replace AFP during screening of HCV patients and early detection of HCC.

Keywords: Hepatocellular carcinoma- HCV- Cytokeratins- Diagnosis

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Introduction

Hepatocellular carcinoma (HCC) is considered the most common malignant primary liver cancer and the sixth common cause of cancer-related death worldwide [1]. Chronic hepatitis viruses (B and C) infections, alcoholic consumption, and metabolic disorders are considered the major leading HCC etiologies [2]. Detection of HCC at early stages is critical for good clinical outcomes as the prognosis of HCC patients is very poor when diagnosed at late stages. Although serum AFP (α-fetoprotein) is the most established tumor marker in HCC and is considered as the gold standard to which other markers are compared to, it was found to be normal in about 30% of the patients, especially in early stages [3]. Ultrasonography is an important tool for the diagnosis of HCC, however, it depends on the operator's experience, and accordingly the validity of other biomarkers in the diagnosis of HCC needs to be investigated [4]. In this regard, there was an urgent need to identify more accurate and sensitive biomarkers for early detection of HCC patents. Cytokeratins (CKs) are the major filament proteins in the breast tissue where any membrane integrity damage causes their release into the circulation [5]. Moreover, CKs have been known as cellular integrators in several neoplastic changes. Characteristic combinations of CKs are expressed by different epithelia according to the organ of origin and differentiation [6]. It has been confirmed that CK18 secretion occurs in parallel with DNA synthesis, protein synthesis, and cell division and this suggests an important role of Ck18 in carcinogenesis [7]. Cytokeratine-19 (Ck19) is an intermediate filament with a molecular weight of around 40 kDa. During the embryonic development, CK19 was detected in the primitive hepatic progenitor cells at the 4-10 weeks' gestation. CK19-positive breast cancer cells showed strong association with invasion, epithelial-mesenchymal transition (EMT) and angiogenesis. Moreover, knockdown of CK19 successfully inhibited the invasive capacity in human HCC cells [8].

Thus, herein, we performed a prospective clinical study in which non-invasive, simple and more accurate diagnostic score namely CK-HCC was developed. This

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score was based on a combination of CK19, CK18, albumin, platelets count and AFP for more accurate diagnosis of HCC patients compared with the conventional AFP alone.

Materials and Methods

Patients

The current study includes two groups of patients; the first one involved 75 patients with liver cirrhosis (LC group) who have developed hepatocellular carcinoma confirmed by liver biopsy, radiobiology investigation, and elevated AFP up 200 U/L. All HCC patients were recruited from Damietta Cancer Institute in the period from October 2017 till December 2018. The second group comprises 50 HCV patients (HCV group) with liver cirrhosis and bearing no evidence of malignancy, which is confirmed by golden standard test or liver biopsy. The diagnosis of HCV was first tested by HCV antibodies, and then confirmed by real-time PCR analysis. All patients were scored according to Metavair score [9]. In addition, a control healthy group which involved 20 individuals with matched age and sex and have no evidence of any hepatitis markers. Exclusion criteria of the study include infection with HBV virus, bilharzia, metabolic liver disorders and autoimmune disease. No chemotherapy was applied for HCC patients until the sampling of blood samples.

Samples and biomarkers

Ten milliliters blood was collected from each patient (prior applying any therapy protocol) from each patient included in the current study as well as corresponding control. Each sample is divided into three tubes, one with EDTA to keep cells without clotting for assessment of platelets count, a second tube with sodium citrate to separate citrate plasma to assess INR, and the last one with no additive to be clotted and serum was collected for biochemical assessments. Liver functions were performed by an automated instrument analyzer (A25, Biosystem, Barcelona, Spain). AFP as a routine tumor marker was assayed with full automated chemiluminescence analyzer (Vidas, Biomerieux, France). Many indices for liver function assessment were calculated from its original equations like aspartate aminotransferase/alanineaminotransferase (AST/ALT), AST platelets ratio index (APRI) [10]. Serum concentration of CK18 and CK19 were assayed by commercial ELISA kit (WKEA Med supplies Corp., Changchun, China). Informed consent was obtained from all individual participants included in the study.

Statistical analysis

Statistical analysis was performed with the Medcalc version 11.3.3.0 statistical software package. All data were presented as arithmetic mean ± standard deviation (X ± SD), and they were considered statistically significant if the two-sided p value was <0.05. Mann–Whitney U test was used for comparisons between independent groups. To assess and compare the diagnostic accuracy of biochemical markers for discriminating those with HCC from that with liver cirrhosis, we plotted receiver operating characteristic (ROC) curves. The best collection parameters were selected based on the significant difference between patients with cirrhosis versus HCC. The multivariant discriminate analysis (MDA) was carried out stepwise with the use of minimum Wilks' lambda. The discriminate model is designed by the standardized canonical discriminate coefficients. The sign (plus or minus) depicts whether it is a direct or inverse relation of the independent variables with the dependent variable (HCC or cirrhosis). In addition, sensitivity, specificity, efficiency, and positive predictive value (PPV) and negative predictive value (NPV) were calculated.

Results

Patient's characteristics

Clinico-pathological and demographic parameters for LC, HCC, and healthy control were illustrated in Table 1. Serum albumin as well as platelets count showed significant decrease in HCC patients compared with LC group. In contrast, there was a significant increase of INR, AST/ALT ratio, APRI, bilirubin, AFP, CK18 and CK19 in HCC patients compared with LC group. Moreover, HCC patients were divided into 30 patients (40%) with stage I+II and 45 patients (60%) with stage III+IV. Non-tumor capsulation was represented in 60 patients (80%) while 15 patients (20%) had complete encapsulation. As illustrated in Table 1, HCC patients with tumor grade I represent 56% of total patients and patients with high tumor grade (II+III) represent 44%. According to tumor size, patients were classified into 13 patients (17%) with small tumor size and 62 patients (83%) with high tumor size. Vascular invasion was represented in 55 patients (73%). Further, 42 patients showed multiple lesion (56%) whereas 33 patients (44%) showed single nodule.

Diagnostic performance using area under the ROC curves

ROC curve analysis was performed to assess and compare the diagnostic utility of multiple biomarkers in order to find the best biomarkers to be chosen in our combination score. Parameters include age, AST, ALT, AST/ALT, albumin, total bilirubin, platelets count, INR, AFP, APRI, CK18 and CK19. The most effective biomarkers with high area under curves were as follows in descending order: CK19 (0.864) > albumin (0.811) > CK18 (0.795) > platelets count (0.783) > AFP (0.692). On other hand, age, AST, ALT, AST/ALT, APRI, INR, bilirubin had a low AUCs and thus they were excluded from the score (0.543, 0.514, 0.612, 0.617, 0.589,0.531, 0.501, respectively) (Figure 1).

Multivariate analysis and predictive model

A predictive model was constructed using multivariate discriminant analysis. In order to enhance the diagnostic performance of AFP to be able to differentiate HCC patients from those with LC, we combined AFP with the other biomarkers of high AUC (mentioned previously). Simply, we started combination by combining two biomarkers (AFP and CK19), then combining three biomarkers (AFP, CK19 and CK18), then four biomarkers (AFP, CK19, CK18 and albumin) then five biomarkers

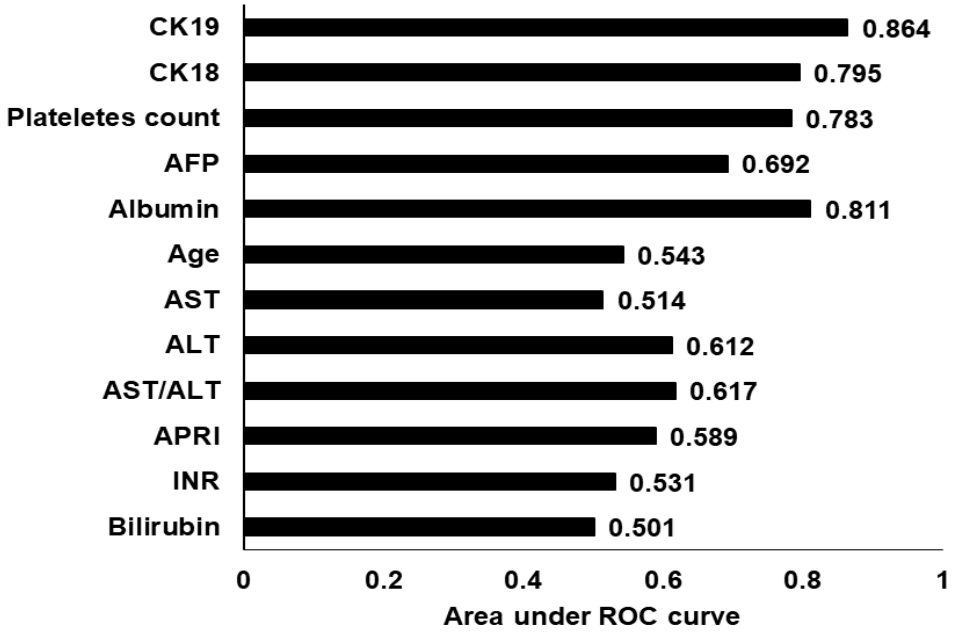

Figure 1. Area under Curve (AUC) of Biomarkers to Discriminate Patients with HCC from Cirrhotic Patients

(AFP, CK19, CK18, albumin and platelets count). Multivariate discriminate analysis selects the most potent model for early prediction of HCC among hepatitis C virus patients. Our proposed model is named CK-HCC = CK 19 (ng/ml)×0.001+ CK18 (ng/dl)×0.004 + AFP (U/L)×5.4 - Platelets count (×10^9)/L×0.003 – Albumin (g/L)×0.27–36. The score had a wide range from -0.68 to 4.2 and it showed high significance (P< 0.001. Figure 2) to differentiate patients with HCC from liver cirrhosis patients. CK-HCC score was calculated for each individual, it produced the highest AUC to differentiate HCC patients from those with liver cirrhosis (0.919) compared to AFP (0.692) as shown in Figure 3. The highest sensitivity (94%) and specificity (91%) was taken at a cut-off 1.3, where above 1.3, patient is considered with HCC and below 1.3, patient is considered with liver cirrhosis. Further, sensitivity of AFP for detection of HCC after implantation to the newly developed score was shifted from 55% to 94%.

Diagnostic performance of CK-HCC in comparison with AFP

The diagnostic utility of CK-HCC score and AFP against tumor burden were illustrated in Table 2 and Table 3. The diagnostic power of CK-HCC score was the better one for discriminating patients with HCC from those with liver cirrhosis compared to AFP alone (AUC were 0.919 and 0.692, respectively). The AUCs of CK-HCC for differentiating patients with low TNM

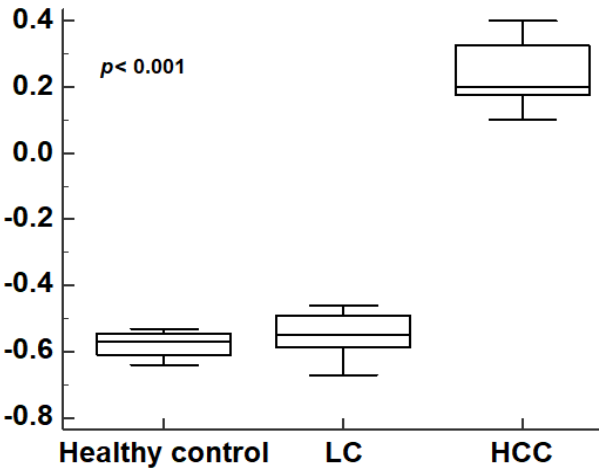

Figure 2. Box Plots of CK-HCC Score to Discriminate HCC Patients from Those with Liver Cirrhosis as well as Healthy Control. The box represents the interquartile rang. The whishkers indicates the highest and lowest values, and the line across the box indicates the medium value.

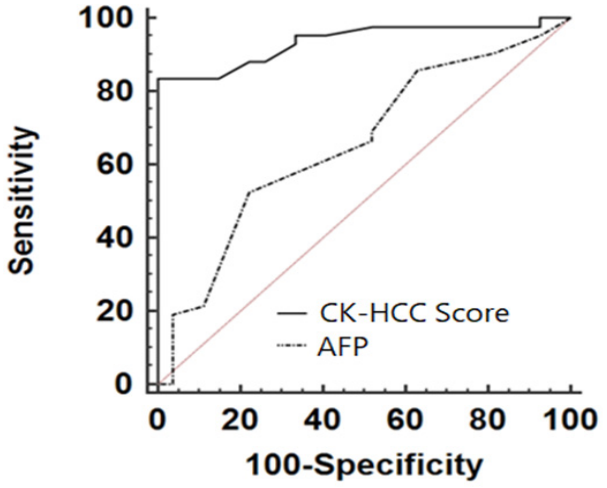

Figure 3. ROC Curve of CK-HCC Score and AFP for Discriminating Patients with Hepatocellular Carcinoma from those with Liver Cirrhosis (AUCs were 0.919 and 0.692, respectively.

Table 1. Clinico-Pathological Data of Healthy Individuals and Patients with Liver Cirrhosis and Hepatocellular Carcinoma

Variable	Healthy control (n= 20)	CH patients (n= 55)	HCC patients (n = 75)	*P value
Age (years)	42.3 ± 5.2	46.1 ± 8.7	60.4 ± 10.7	< 0.0001
AST (U/L)	13.4 ± 8.6	53.2 ± 18.4	89 ± 12.8	< 0.0001
ALT (U/L)	15.7 ± 10.5	57.4 ± 14.1	102 ± 11.4	< 0.0001
AST/ALT (AAR)	0.42 ±0.05	0.92 ± 0.05	0.88 ± 0.09	0.005
Albumin (g/dl)	4.7 ± 0.41	4.1 ± 0.53	2.9 ± 0.57	< 0.0001
Total Bilirubin (mg/dl)	0.92 ±0.34	1.07 ± 0.21	3.7 ± 0.31	< 0.0001
Platelets count (×109)/L	342 ± 56	151 ± 57	55 ± 14	< 0.0001
INR	0.98 ± 0.21	1.5 ± 0.34	2.7 ± 0.55	< 0.0001
AFP (U/L)	1.3 ± 3.06	7.5 ± 2.1	219 ± 23	< 0.0001
APRI	0.31 ± 0.07	1.9 ± 0.11	2.3 ± 0.71	< 0.0001
CK 18 (ng/ml)	87.7 ± 31.9	67.1 ± 10.3	253.9 ± 80.1	< 0.0001
CK 19 (ng/ml)	25.3 ± 3.9	24.8 ± 1.85	63.7 ± 3.6	< 0.0001
Tumor stage, n (%)				
I + II			30 (40)	
III + IV			45 (60)	
Tumor encapsulation, n (%)				
Non			60 (80)	
Complete			15 (20)	
Tumor grade, n (%)				
I			49 (65)	
II + III			26 (44)	
Tumor size, n (%)				
< 5cm			13 (17)	
>5 cm			62 (83)	
Vascular invasion, n (%)				
Absent			55 (73)	
Present			20 (26)	
Number of Lesion, n (%)				
Single			42 (56)	
Multiple			33 (44)	

*P> 0.05 considered not significant (NS), P< 0.05 considered significant. The reference group of p value were hepatocellular carcinoma (HCC) group and chronic hepatitis (CH) group. INR: international normalized ratio; AFP, alpha fetoprotein; APRI, [AST(U/L)/(40)]/[Platelet count $\times 10^9$/L]×100; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Variables were expressed as mean ± SD.

stage, complete capsulation, low grade, small tumor size, absence of vascular invasion and single focal lesions from patients with non-malignancy (0.712, 0.813, 0.815, 0.756, 0.891 and 0.761, respectively) which were better than AFP (0.544, 0.587, 0.685, 0.511, 0.578 and 0.611, respectively) (Figure 4). As illustrated in Table 3, AFP alone had a weak diagnostic power for differentiating HCC patients with small tumor size from those with liver cirrhosis where AUC was 0.512 versus CH-HCC score which produced AUC of 0.756. Noteworthy, AFP was unable to discriminate patients with low grade from those with LC (AUC of 0.501) compared with high AUC of 0.815 produced by our CK-HCC score.

Discussion

Chronic HCV infection plays a major role in the development of cirrhosis and hepatocellular carcinoma. AFP is recommended for surveillance of HCV patients even alone or combined with radiological imaging of the liver and because to its low sensitivity for accurate diagnosis of HCC patient, many efforts were applied to improve its sensitivity for early detection of HCC among hepatitis high risk patients. In the current study, all patients of HCC were positive to HCV infection and they have previously developed cirrhotic texture of liver tissue. Multiple indices was designed to improve diagnosis of HCC, but all of them were depending on routine biomarkers which reflect liver impairment. Unfortunately, all of these indices were not considered ideal because all parameters involved scores that were affected by other pathological state rather than development of HCC itself. Thus, in our study we were concerned with biomarkers that directly reflect tumor progression. Our chosen group

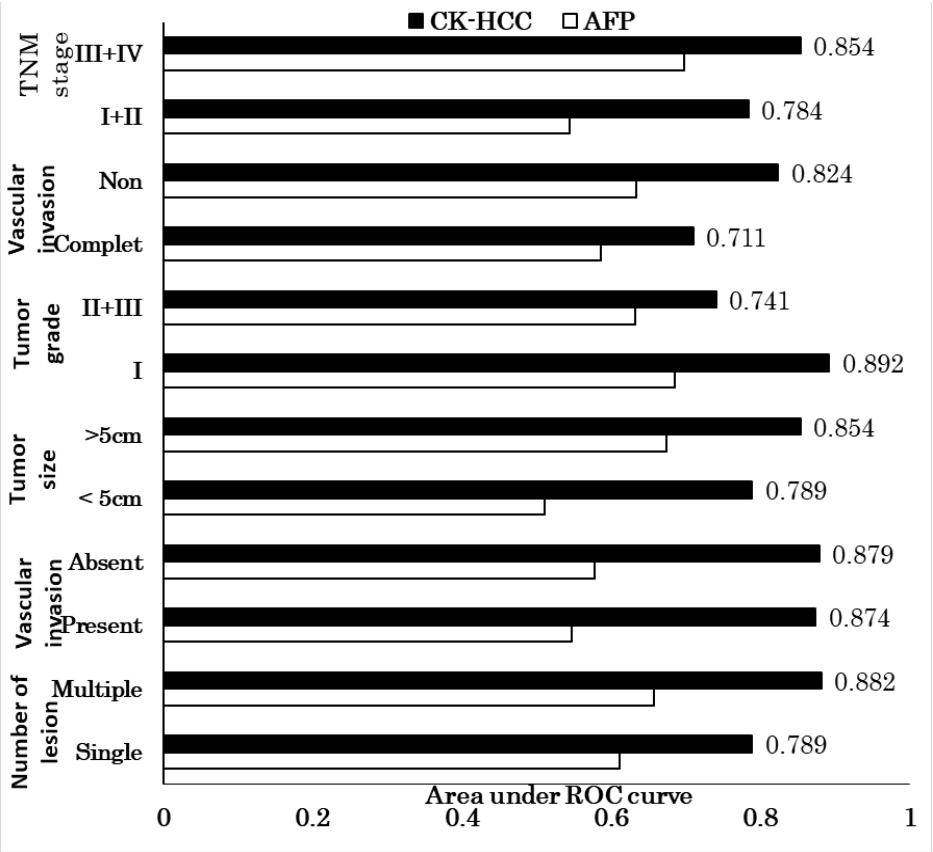

Figure 4. Area under ROC Curve (AUC) of CK-HCC Compared with AFP for early Diagnosis of HCC with Tumor Burden Feature Including Number of Hepatic Lesion, Vascular Invasion, Tumor Size, Tumor Grade, Tumor Encapsulation and TNM Stage

of markers are considered simple, non-invasive and offer more accurate diagnosis of HCC on top HCV infection [11]. An optimal biomarker for HCC must be specific and sensitive to HCC and is not expressed in nonmalignant cirrhotic status. Accumulating evidence suggested that the aggressive behavior of hepatocellular carcinoma

Table 2. Diagnostic Performance of CK-HCC Score to Discriminate HCC Patients from Those with Liver Cirrhosis

Clinical data	CTCs-HCC score			
	Sensitivity (%)	Specificity (%)	Accuracy (%)	AUC
Tumor stage				
I + II	82	83	73	0.712
III + IV	79	81	72	0.773
Tumor encapsulation				
Non	84	78	71	0.868
Complete	78	79	86	0.813
Tumor grade				
I	76	78	84	0.815
II + III	80	73	81	0.756
Tumor size				
< 5cm	82	79	88	0.756
> 5 cm	84	74	76	0.735
Vascular invasion				
Absent	78	88	83	0.891
Present	81	91	76	0.761
Number of Lesion				
Single	81	75	78	0.761
Multiple	78	71	84	0.811

Table 3. Diagnostic Performance of AFP to Discriminate HCC Patients from those with Liver Cirrhosis

Clinical data	AFP			
	Sensitivity (%)	Specificity (%)	Accuracy (%)	AUC
Tumor stage				
I + II	61	81	69	0.613
III + IV	72	79	73	0.527
Tumor encapsulation				
Non	74	67	68	0.578
Complete	81	71	84	0.599
Tumor grade				
I	78	74	73	0.501
II + III	83	69	79	0.622
Tumor size				
< 5cm	79	73	81	0.512
> 5 cm	85	71	75	0.617
Vascular invasion				
Absent	74	87	75	0.589
Present	76	89	68	0.532
Number of Lesion,				
Single	88	61	77	0.603
Multiple	79	83	81	0.578

could be partially attributed to the presence of malignant hepatocytes that gained entry into circulation, either before or during surgery. Therefore, identification of these small populations of cells in patients' blood together with the search for sensitive biological biomarkers are highly recommended for better patient management [12]. In the present study, we validated the utility of immunological technique as a rapid and highly sensitive technique for the follow-up of HCV infected patients at different disease stages. This was achieved through detecting the interaction of CK antibodies with its antigens, which are present in the cytoplasm of hepatocytes. Our data indicated that flow cytometry was able to identify a significant concentration of CK18 and CK19 in the serum of HCC patients compared to CH and control groups. In an attempt to identify sensitive diagnostic markers that can help to differentiate between CH and HCC in HCV-infected patients and thus permit early detection of HCC, we construct a simple score based on combination of cytokeratins and routine available biochemical markers which associated with liver impairment. This provides evidence that biomarkers could be used as indicators to predict HCC in CH patients. In current study, CK19 was significantly elevated in HCC patients compared to CH patients that, which is in agreement with previous reports [13]. Recently it was reported that, CK19 can predict HCC with high sensitivity (87%) and specificity (100%), and can thus be used as a prognostic factor which is associated with increased metastatic potential and early recurrence [14] so, it was chosen as the basic index for construction of our score. As a tumor marker, CK18 has been well studied in different cancers as esophageal squamous cell carcinoma, renal cell carcinoma, oral cavity carcinoma, lung cancer, human breast and colorectal cancer [15]. Moreover, it was reported that both circulating and hepatic CK18 were significantly elevated in patients of chronic hepatitis compared to healthy controls which in line with our findings [7]. That elevation may be due to liver apoptosis and consequently could be useful for monitoring disease activity in chronic HCV and liver cirrhosis patients. In agreement with previous reports, our result showed a significant elevation of CK18 in HCC patient compared to CH patients and this suggests that CK18 measurement may improve non-invasive diagnosis of HCC [16, 17]. In addition, an in vitro study showed that cytokeratin-18 expression was significantly higher in six HCC cell lines examined than in the control cells using immunofluorescence staining and microscopic examination [18]. Consequently, that CK18 may drive neoplastic transformation of glutathione S-transferase in rat hepatocytes, causing HCC [19]. So, we could suggest that cytokeratins play important roles in the development and progression of HCV-associated HCC. In HCC patients, thrombocytopenia may occur due to a reduction in synthesis of thrombopoietin, which in turn increased slenic sequestration of platelets secondary to portal hypertension or the myelosuppressive action of HCV infection [20]. In the current study, it was observed that platelets count was significantly reduced in HCC patients. Further activation of platelets ensues from the original tumor; triggering enhanced growth of the tumor as a result of the release of platelets granules [21]. Release of the contents of the granules from platelets hinders the ability of the immune surveillance system against malignancy through cloaking tumor cells and protecting the tumor cells from natural killer (NK) cells by providing a physical barrier and also placing major histocompatibility complex (MHC) class I antigen into the vicinity of the tumor cell surface [22, 23].

Thus, platelets count was taken in our consideration for monitoring liver status and development of malignancy. Liver is considered a huge reserve for albumin, so its decreased levels may reflect liver impairment and is considered main liver function monitor and thus albumin is used in liver assessment during development of HCC [24]. It was reported that, serum albumin is an important factor for use in several scoring systems, such as the Child–Pugh and CLIP score systems [25]. In the current study, serum albumin was significantly decreased in HCC patients compared to cirrhotic patents, so it was taken in our consideration during construction of our score. Herein, for the first time, we report the clinical validation of four biomarkers (CK19, CK18, albumin and platelets count) in combination with AFP to improve the accuracy for diagnosis of HCC among hepatitis C high risk patients. CK-HCC score could potentially be used to diagnose HCC, especially in the early stages and will help to resolve the deficiencies of AFP in the testing of AFP negative patients. The possibility of discriminating HCC from healthy individuals and patients with cirrhosis offers hope for the early detection of HCC. Our score could be used as blood tests for the noninvasive diagnosis of HCC to reduce the need for the invasive liver biopsy.

Author Contribution Statement

MA as postgraduate students, perform experimental methods, MA and AE supply us with clinical samples, RN, perform statistical analysis FM and HE prepare the manuscript. All authors review and approve the manuscript.

Acknowledgements

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

All procedures performed in in the study followed the relevant ethical standards of the institutional or national research committee (Ethics Board of Helwan University with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of Interests

The authors declare that they have no conflicts of interests.

References

1. Wang H, Liddell CA, Coates MM, Mooney MD, Levitz CE, Schumacher AE, et al. Global, regional, and national levels of neonatal, infant, and under-5 mortality during 1990-2013: A systematic analysis for the global burden of disease study 2013. Lancet. 2014;384(9947):957-79. https://doi.org/S0140-6736(14)60497-9
2. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. Lancet. 2012;379(9822):1245-55. https://doi.org/S0140-6736(11)61347-0
3. Chen L, Ho DW, Lee NP, Sun S, Lam B, Wong KF, et al. Enhanced detection of early hepatocellular carcinoma by serum seldi-tof proteomic signature combined with alpha-fetoprotein marker. Ann Surg Oncol. 2010;17(9):2518-25. https://doi.org/10.1245/s10434-010-1038-8.
4. Hao K, Luk JM, Lee NP, Mao M, Zhang C, Ferguson MD, et al. Predicting prognosis in hepatocellular carcinoma after curative surgery with common clinicopathologic parameters. BMC Cancer. 2009;9:389. https://doi.org/1471-2407-9-389
5. Bateman AC, Hubscher SG. Cytokeratin expression as an aid to diagnosis in medical liver biopsies. Histopathology. 2010;56(4):415-25. https://doi.org/10.1111/j.1365-2559.2009.03391.x.
6. Turley EA, Veiseh M, Radisky DC, Bissell MJ. Mechanisms of disease: Epithelial-mesenchymal transition--does cellular plasticity fuel neoplastic progression? Nat Clin Pract Oncol. 2008;5(5):280-90. https://doi.org/10.1038/ncponc1089.
7. Ismail SA, El Saadany S, Ziada DH, Zakaria SS, Mayah WW, Elashry H, et al. Cytokeratin-18 in diagnosis of hcc in patients with liver cirrhosis. Asian Pac J Cancer Prev. 2017;18(4):1105-11. https://doi.org/10.22034/APJCP.2017.18.4.1105.
8. Zhuo JY, Lu D, Tan WY, Zheng SS, Shen YQ, Xu X. Ck19-positive hepatocellular carcinoma is a characteristic subtype. J Cancer. 2020;11(17):5069-77. https://doi.org/10.7150/jca.44697.
9. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis c. The obsvirc, metavir, clinivir, and dosvirc groups. Lancet. 1997;349(9055):825-32. https://doi.org/S0140673696076428
10. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis c. Hepatology. 2003;38(2):518-26. https://doi.org/10.1053/jhep.2003.50346
11. El-mezayen HA, Darwish H. Development of a novel score for early detection of hepatocellular carcinoma among high-risk hepatitis c virus patients. Tumour Biol. 2014;35(7):6501-9. https://doi.org/10.1007/s13277-014-1858-4.
12. Bahnassy AA, Zekri AR, El-Bastawisy A, Fawzy A, Shetta M, Hussein N, et al. Circulating tumor and cancer stem cells in hepatitis c virus-associated liver disease. World J Gastroenterol. 2014;20(48):18240-8. https://doi.org/10.3748/wjg.v20.i48.18240.
13. Cai X, Feng L, Liu H, Xu M, Qu Y, Wan X, et al. Cytokeratin19 positive hepatocellular carcinoma is associated with increased peritumoral ductular reaction. Ann Hepatol. 2016;15(3):386-93. https://doi.org/10.5604/16652681.1198813.
14. Xu J, Chen C, Xiong J, Wang H, Linghu H. Predictive value of serum cytokeratin 19 level for the feasibility of conserving ovaries in endometrial cancer. Front Med (Lausanne). 2021;8:670109. https://doi.org/10.3389/fmed.2021.670109.
15. Menz A, Weitbrecht T, Gorbokon N, Buscheck F, Luebke AM, Kluth M, et al. Diagnostic and prognostic impact of cytokeratin 18 expression in human tumors: A tissue microarray study on 11,952 tumors. Mol Med. 2021;27(1):16. https://doi.org/10.1186/s10020-021-00274-7.
16. Waidmann O, Brunner F, Herrmann E, Zeuzem S, Piiper A, Kronenberger B. Cytokeratin 18-based cell death markers indicate severity of liver disease and prognosis of cirrhotic patients. Liver Int. 2016;36(10):1464-72. https://doi.org/10.1111/liv.13117.
17. Gonzalez-Quintela A, Garcia J, Campos J, Perez LF, Alende MR, Otero E, et al. Serum cytokeratins in alcoholic liver disease: Contrasting levels of cytokeratin-18 and cytokeratin-19. Alcohol. 2006;38(1):45-9. https://doi.org/10.1016/j.alcohol.2006.01.003.

18. Mu H, Lin KX, Zhao H, Xing S, Li C, Liu F, et al. Identification of biomarkers for hepatocellular carcinoma by semiquantitative immunocytochemistry. World J Gastroenterol. 2014;20(19):5826-38. https://doi.org/10.3748/wjg.v20.i19.5826.
19. Yilmaz Y. Systematic review: Caspase-cleaved fragments of cytokeratin 18 - the promises and challenges of a biomarker for chronic liver disease. Aliment Pharmacol Ther. 2009;30(11-12):1103-9. https://doi.org/10.1111/j.1365-2036.2009.04148.x.
20. Dai CY, Ho CK, Huang JF, Hsieh MY, Hou NJ, Lin ZY, et al. Hepatitis c virus viremia and low platelet count: A study in a hepatitis b & c endemic area in taiwan. J Hepatol.52(2):160-6. https://doi.org/S0168-8278(09)00793-4 [pii]
10.1016/j.jhep.2009.11.017.
21. Nash GF, Turner LF, Scully MF, Kakkar AK. Platelets and cancer. Lancet Oncol. 2002;3(7):425-30. https://doi.org/S1470204502007891 [pii].
22. Ntziachristos P, Mullenders J, Trimarchi T, Aifantis I. Mechanisms of epigenetic regulation of leukemia onset and progression. Adv Immunol.117:1-38. https://doi.org/B978-0-12-410524-9.00001-3
23. Sabrkhany S, Griffioen AW, Oude Egbrink MG. The role of blood platelets in tumor angiogenesis. Biochim Biophys Acta.1815(2):189-96. https://doi.org/S0304-419X(10)00082-X
24. Attallah AM, Omran MM, Attallah AA, Abdallah SO, Farid K, Darwish H, et al. Hcc-art score, a simple, highly sensitive and specific test for early diagnosis of hepatocellular carcinoma: A large-scale, multicentre study. Br J Cancer.109(6):1657-65. https://doi.org/bjc2013481
25. Ishizuka M, Kubota K, Kita J, Shimoda M, Kato M, Sawada T. Impact of an inflammation-based prognostic system on patients undergoing surgery for hepatocellular carcinoma: A retrospective study of 398 japanese patients. Am J Surg.203(1):101-6. https://doi.org/S0002-9610(11)00038-9