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Prospective evaluation of HCC-DETECT index in detection of small tumors and early stages of three common Child-Turcotte-Pugh, Okuda and CLIP staging systems

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The coexistence of hepatic inflammation and cirrhosis complicate hepatocellular carcinoma (HCC) early diagnosis. This highlights the need to identify valuable biomarkers for early disease detection. HCC diagnostic index (HCC-DETECT) based on the combination between cytoplasmic protein, cytokeratin-1, nuclear protein, nuclear matrix protein-52 and oncofetal protein, AFP, was developed with promising findings for the diagnosis of HCC patients. One hundred and twenty patients with chronic hepatitis C infection constituted the present study. HCC-DETECT was calculated and sensitivity, specificity and ROC curve analysis were evaluated in early HCC detection. Values of HCC-DETECT significantly (P<0.0001) increased with the progression in tumor size (<5cm (1.56±0.05) *vs.* ≥5cm (1.99±0.06)) and stages of Child (1.46±0.05, 1.70±0.06 and 2.13±0.07 in stage A, B and C, respectively), Okuda (1.41±0.05, 1.67±0.05 and 2.22±0.04 in stage 1, 2 and 3, respectively) and CLIP (1.47±0.06, 1.80±0.06 and 2.26±0.06 in stage 0-1, 2-3 and ≥4, respectively) systems. Interestingly, HCC-DETECT had good diagnostic performance in patients who had only single tumor, absent vascular invasion, and tumor size <5 cm with AUC of 0.93, 0.92, and 0.92, respectively. Moreover, AUCs for HCC-DETECT in the detection of early Child (stage A), Okuda (stage 1) and CLIP (stage 0-1) were 0.82, 0.76 and 0.80, respectively. In conclusion, HCC-DETECT can serve as promising index to detect HCC at an early stage and thus may facilitate definitive therapy.

Keywords: Hepatocellular carcinoma, Diagnosis, Serum biomarkers, early stage, HCC-DETECT.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a frequent human tumor and presently represents the 2nd commonest global cause of cancer related death. Tumorigenesis of HCC is relatively slow and patients experience late symptomatic display (Kim et al. 2016). Here lies the paramount importance of the early HCC detection for improving patient outcomes (Kalinich et al. 2017). From another view, however, curative HCC therapies such as

orthotopic liver transplant and hepatic resection offer good prognosis, they are limited to early disease stages (Kim et al. 2016). Thus, efficient HCC screening strategies for disease diagnosing at early stages is of extreme importance and may result in more effective treatment and extend patient survival (El-Serag, 2011). Despite pathological diagnosis, the HCC diagnosis can be obtained by estimating circulating levels of α-fetoprotein (AFP) along with diagnostic imaging, such as ultrasonography (US) and/or computed tomography (CT) and magnetic resonance imaging (MRI) (Kim et al. 2016). Unfortunately, AFP is inadequate tumor marker with low sensitivity, especially in early cancer stages, that is normal in up to 50% of HCC patients. Also, other HCC modalities still do not provide sufficient diagnostic performance for HCC detection at the early stage (Attallah et al. 2016b).

In an attempt to provide more sensitive HCC diagnostic test, a clinically useful index called HCC-DETECT based on the combination between cytoplasmic protein, cytokeratin-1 (CK1), nuclear protein, nuclear matrix protein-52 (NMP-52) and oncofetal protein, AFP, was developed for the diagnosis of HCC patients (Attallah et al. 2015). Interestingly, HCC-DETECT yielded 0.90 area under the curve (AUC) for identifying HCC from hepatic cirrhosis with 80% sensitivity, 92% specificity, 90% positive and 83% negative predictive values and 86% accuracy. Sequentially, this study aimed to validate HCC-DETECT in detection of HCC patients with small tumor size (<5cm) and to evaluate its clinical feasibility in the diagnosis of early HCC stages in three of widely common staging systems Child-Turcotte-Pugh (CTP) (Child and Turcotte, 1964; Pugh et al. 1973), Okuda (Okuda et al. 1985) and the Cancer of the Liver Italian Program (CLIP) (CLIP, 1998).

MATERIALS AND METHODS

Study patients

Consecutive Egyptian patients with chronic hepatitis C (n=120, 60 with hepatic cirrhosis and 60 with HCC) who were admitted at Mansoura University Hospitals, Mansoura, Egypt were included in this study. After informed consent, sera samples were collected from all patients. The HCC diagnosis was executed following the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines (Bruix and Sherman, 2005). None of the included patients had received any antiviral or tumor treatment therapy during or before this study. The ethical guidelines of the 1975 Declaration of Helsinki were followed in this study protocols.

Laboratory tests for HCC-DETECT candidate markers

From each patient, serum samples were obtained at the time of diagnosis. Immulite (1000) AFP kit (Diagnostic Products Corporation; Los Angeles, CA, USA) was used for AFP estimating by chemiluminescence. Also, all serum samples were tested for nuclear matrix protein (52 KDa) and CK1 according to Attallah et al. (Attallah et al. 2015).

Statistical analysis:

Statistical analyses were performed by SPSS (SPSS Inc., Chica go, IL) software version 17.0 and GraphPad (GraphPad Software, San Diego, CA) Prism package version 5.0. Differences in continuous variables were assessed using Student t-test or ANOVA and X^2 test for categorical variables. All tests were two-tailed and statistical significance assessed at the level of 0.05. HCC-DETECT (Attallah et al. 2015) index for discriminating HCC patients was calculated from the represented formula: HCC-DETECT= 0.004×CK-1 (µg/mL) + 0.007×NMP-52 (µg/mL) + 0.248×Log AFP (U/L) + 0.951. Area under receiver-operating characteristic curve (AUC) analysis was used for assessing the diagnostic efficacy of HCC-DETECT in different tumor stages and features. Sensitivity and specificity were calculated from a 2×2 contingency table.

RESULTS

Staging of HCC

The HCC patients were older than patients with liver cirrhosis. There were no significant differences in routine laboratory features, including liver enzymes, between HCC patients and cirrhotic patients except albumin and AFP (Table 1). As shown in Figure 1, HCC tumor stages were classified according to tumor size [small (<5cm) and large tumors (≥5cm)], CTP [stage A, B and C], Okuda [early (stage 1), intermediate (stage 2) and advanced (stage 3) and CLIP [early (stages 0-1), intermediate (stage 2-3) and advanced (stages ≥4)].

Index values increase with the severity of HCC and have good efficacy in early HCC detection

Values of HCC-DETECT increased with the progression in tumor size and stages of CTP, Okuda and CLIP systems (Figure 2). HCC-

Table 1: Characteristics of HCC patients and cirrhosis controls

Parameter	Cirrhosis	HCC	*P* value
Number	60	60	–
Male/female	41/19	44/16	0.962
Age (years)	51.2 ± 8.2	57.2 ± 9.5	0.042
AST (U/L)	71.5 ± 41.6	89.1 ± 47.0	0.091
ALT (U/L)	45.1 ± 23.1	53.7 ± 40.5	0.259
ALP (U /L)	120.4 ± 43.1	168.3 ± 101.6	0.074
Albumin (g/dL)	3.7 ± 0.4	3.3 ± 0.6	0.004
Total bilirubin (mg/dL)	1.13 ± 0.7	2.14 ± 1.41	<0.0001
PT-INR	1.35 ± 0.22	1.43 ± 0.12	0.428
Platelet count ($\times 10^9$/L)	149.6±81.0	122.9±82.4	0.608
Log AFP (U/L)	0.85±0.39	1.83±0.99	<0.0001
Single nodule/multiple nodules	–	40/20	–
Vascular invasion (absent/present)	–	14/46	–

Variables were expressed as mean±SD; Abbreviation and normal values: aspartate (AST) and alanine aminotransferase (ALT) (male up to 37 U/L, female up to 31 U/L), alkaline phosphatase (ALP) 22–92 U/ L, total bilirubin up to 1 mg/dL, albumin 3.8–5.4 g/dL, prothrombin time-international normalized ratio (INR) 1, platelet count 150-400 $\times 10^9$/L, alpha-fetoprotein (AFP) up to 10 U/L; *P* <0.05 is considered significant and *P* >0.05 is considered non-significant.

Table 2. Predictive power of HCC-DETECT in HCC diagnosis according to tumor size, Child-Turcotte-Pugh, Okuda and CLIP staging systems.

Classification System	HCC-DETECT at cutoff ≥1.4		
	AUC	Sensitivity %	Specificity %
Tumor size			
<5 cm	0.93	88	92
≥5 cm	1.0	100	92
Child-Turcotte-Pugh			
Child A	0.82	70	92
Child B	0.94	86	92
Child C	1.0	100	92
Okuda stage			
Stage 1 (early)	0.76	50	92
Stage 2 (intermediate)	0.94	89	92
Stage3 (advanced)	1.0	100	92
CLIP stage			
CLIP 0-1 (early)	0.80	64	92
CLIP 2-3 (intermediate)	0.98	95	92
CLIP ≥4 (advanced)	1.000	100	92

AUC = Area under curve. AUC generated from comparing HCC to the liver cirrhotic patients.

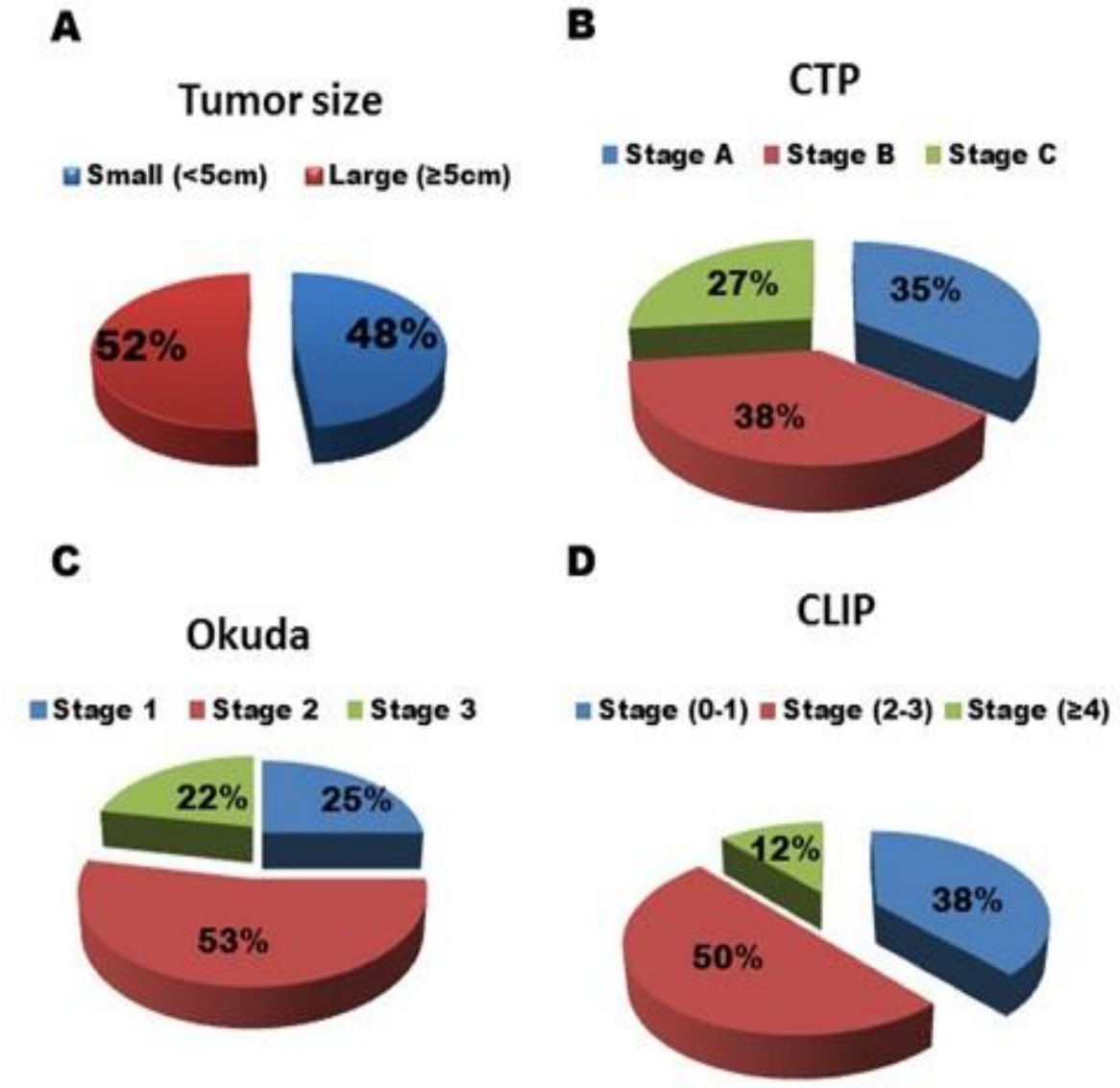

Figure 1: Staging of included HCC patients. Cases were classified according to (A) tumor size, (B) Child-Turcotte-Pugh (CTP), (C) Okuda and (D) the Cancer of the Liver Italian Program (CLIP).

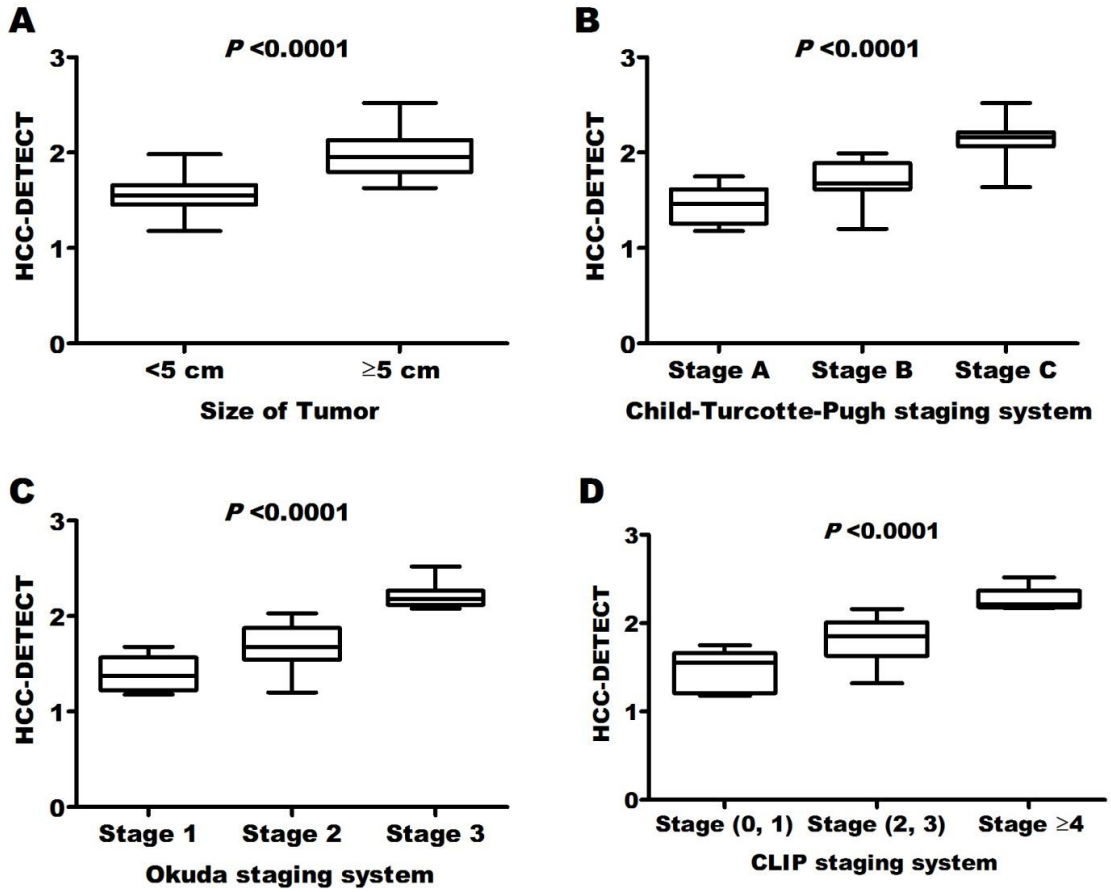

Figure 2: Distributions of HCC-DETECT values according to (A) tumor size, (B) Child-Turcotte-Pugh (CTP), (C) Okuda and (D) the Cancer of the Liver Italian Program. The whiskers indicate the highest and lowest values, and the line across the box indicates the median value. HCC-DETECT= 0.004×CK-1 (µg/mL) + 0.007×NMP-52 (µg/mL) + 0.248×Log AFP (U/L) + 0.951.

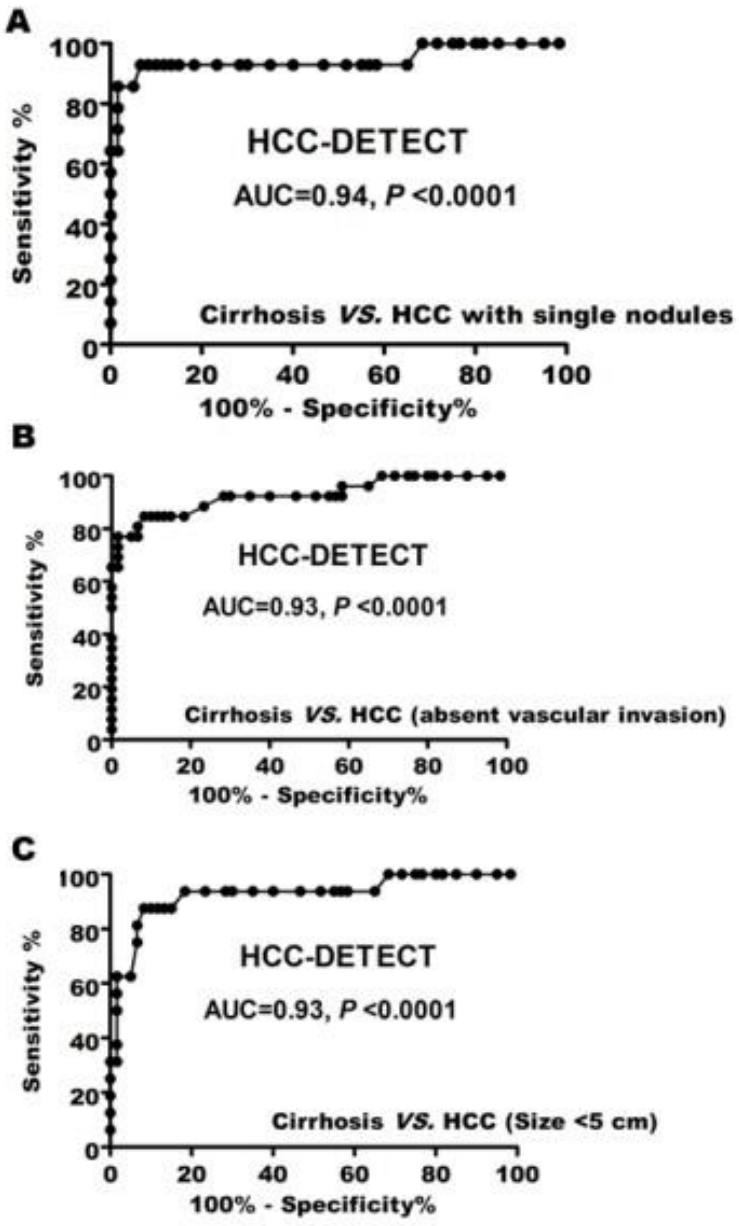

Figure 3: Area under receiver-operating characteristic curve of the HCC-DETECT model to discriminate HCC patients with (A) single nodule (B) absent vascular invasion (C) small tumor from patients with liver cirrhosis. HCC-DETECT= 0.004×CK-1 (µg/mL) + 0.007×NMP-52 (µg/mL) + 0.248×Log AFP (U/L) + 0.951.

DETECT had good diagnostic performance in detection of mild (early) HCC features with AUC of 0.93, 0.92, and 0.92 in patients who had only single tumor, absent vascular invasion, and tumor size less than 5 cm, respectively (Figure 3). Moreover, HCC-DETECT model has a good diagnostic ability in the detection of early stages of different HCC staging systems which reach optimal in the advanced stages (Table 2).

DISCUSSION

Effective modalities that offer the best potential for HCC treating are only available in patients whose tumors are diagnosed early (Song et al. 2013a). Generally, the screening tests for HCC around the world depend on imaging techniques and biomarkers (Attallah et al. 2016a). The imaging method most often in HCC screening is US because it is simple, noninvasive and inexpensive and allows real-time monitoring. Nevertheless, its success depends on apparatus available, the hepatic echo texture and the expertise of the physician; so, the assessing of US true sensitivity is complicated due to the lack of a definitive HCC standard (Song et al. 2013b). Also, AFP, the most frequent biomarker used in HCC detection, and its glycated isoforms are still failed to be used as the sole tool in detecting early stage HCCs (Attallah et al. 2016b). Thus, the discovery of reliable and effective tool for early HCC diagnosis will play a pivotal role in improving the disease prognosis and to increase the number of patients who are suitable for curative therapy (Zhu et al. 2013).

Recently, an interesting study suggests that HCC-DETECT, predictive HCC index, could improve the efficacy of HCC diagnosis (Attallah et al. 2015). Here, we have made worthy improvements in this index reliability by verification of its use in diagnosing small and early tumors. We found that significantly ($P<0.0001$) elevated HCC-DETECT values were associated with large tumors (≥5 cm) and advanced stages of three common staging systems, CTP, Okuda and CLIP. Concerning small tumors (<5 cm), HCC-DETECT has adequate predictive power with AUC of 0.93, sensitivity of 88% and specificity of 92%. HCC staging systems are serious in not only providing prognostic data but also in management designing (Attallah et al. 2016b). Another attractive aspect of HCC-DETECT is that it has a good diagnostic power for detecting HCC at early CTP (child A; AUC=0.82, sensitivity=70%), Okuda (stage 1; AUC=0.76, sensitivity=50%) and CLIP (0-1; AUC=0.80, sensitivity=64%) tumor stages.

Three candidate markers CK1, NMP-52 and AFP developed the HCC-DETECT. CK1 is one of basal type, high molecular weight cytokeratins. In epithelial tissues, the expression of such proteins can change with differentiation or malignant transformation. For example, not enumeration, the acquisition of CK1, 5, 6, 8, 19 in squamous cell carcinoma (Makino et al. 2009). During carcinogenesis and tumor progression of breast cancer, alterations in cytokeratin expression and partial loss of the normal regulation of these proteins expression has been demonstrated (Su et al. 1996). The cell transformation from normal to tumor condition was detected as apparent nuclear morphology changes. Thus NMPs emerged as an integral component of genetic processing and became serious components for studying the malignant transformation (Davido and Getzenberg, 2000). Differences in NMPs composition are found in different human tumors, including bladder (Getzenberg et al. 1996), breast (Luftner and Possinger, 2002), renal (Kaya et al. 2005), cervical (Keesee et al. 1998), prostate (Leman and Getzenberg, 2002) and colon (Keesee et al. 1994) cancer. Although, using AFP alone cannot achieved valuable HCC diagnosis, its combined detection with other circulating complement markers could improve the early diagnostic rate (Attallah et al. 2016b).

Findings derived from this work compared favorably with other markers used in early HCC diagnosis. For instance, in patients with small HCCs, AFP-L3 has a sensitivity of only 35% (Li et al. 2001). Elevated des-γ-carboxy prothrombin (DCP) circulating levels are present in 50-60% of all patients, but in only 15-30% of early HCC stages (Weitz and Liebman, 1993).

CONCLUSION

In conclusion, this work supports that HCC-DETECT can serve as promising index to detect HCC at an early stage and thus may facilitate definitive therapy.

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