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Serum clusterin as a marker for diagnosing hepatocellular carcinoma

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KEYWORDS

Clusterin;
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Abstract *Background:* Approximately 80% of patients with hepatocellular carcinoma (HCC) are untreatable because of advanced tumor stages at presentation. Therefore, finding newer markers for screening and diagnosing HCC is of utmost importance. Clusterin (CLU) is a 449 amino acid, heterodimeric glycoprotein with a plausible role in the regeneration, migration, and anti-apoptosis of tumor cells. It has been implicated in many malignancies such as prostate and pancreatic adenocarcinomas, but its role in HCC is not well defined.

Objective: We aimed to evaluate the diagnostic performance of serum CLU level in diagnosing HCC on top of hepatitis C virus-related liver cirrhosis, and comparing it to that of alpha fetoprotein (AFP).

Methods: Twenty cases of apparently healthy subjects, 27 cases of hepatitis C virus-related liver cirrhosis (CHC cases), and 44 HCC cases on top of hepatitis C virus-related liver cirrhosis were included in this study. Serum CLU concentration was determined using a quantitative sandwich enzyme immunoassay technique.

Results: Serum clusterin level showed a significant increase in the HCC group compared to the control group (151.96 ± 32.74 vs. 111.40 ± 27.46) and to the CHC group (151.96 ± 32.74 vs. 89.12 ± 31.62), while a significant decrease in serum clusterin level was found in the CHC group compared to the control group (89.12 ± 31.62 vs. 111.40 ± 27.46). Based on receiver operator

Abbreviations: CLU, clusterin; AFP, alpha fetoprotein; CHC, hepatitis C virus related liver cirrhosis; HCC, hepatocellular carcinoma.

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characteristic curve analysis, serum AFP still surpassed serum CLU in diagnostic sensitivity (77.3% vs. 70.5%), specificity (100% vs. 90%), and positive and negative predictive values (100% vs. 86.1% and 83.3% vs. 77.6% respectively). The use of a combined parallel approach improved the diagnostic sensitivity (95.5%) and negative predictive value (95.7%) over the single use of AFP.

Conclusions: Although the diagnostic performance of serum AFP outperformed that of serum CLU, their combined parallel approach improved the sensitivity which is required in screening high risk populations such as CHC patients.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide and the most common form of primary liver cancer.[1] In Egypt, the overall frequency of HCC is 2.3% among other types of cancer. Over a decade, there was nearly a twofold increase in the proportion of HCC among chronic liver disease patients in Egypt, where 48% of HCC cases were attributed to hepatitis C virus (HCV) related liver cirrhosis. In fact, it has now become widely accepted that HCC nearly exclusively arises in chronic HCV after cirrhosis is established.[2]

HCC is typically diagnosed late in its course. Indeed, patients who present with cancer symptoms and/or with vascular invasion or extra-hepatic spread have only 50% survival rate at one year. Therapeutic options are determined both by tumor extent and the severity of the underlying liver disease. Although the cornerstone of therapy is surgical resection, the majority of patients are not eligible because of tumor extent or underlying liver dysfunction.

The diagnosis of HCC can be radiological and/or laboratory. Radiological diagnosis depends largely on ultrasonography, triphasic computerized tomography (triphasic CT-scan) and dynamic magnetic resonance imaging (dynamic MRI). The sensitivity of US for the detection of HCC is directly related to tumor size. Another major drawback of US is that it is very much operator- dependent.[4,5] Laboratory diagnosis of HCC is established either by measurement of circulating biomarkers or by fine-needle cytology which is invasive with intra- or inter-observer variability.[6]

The American Association for the Study of Liver Diseases (AASLD) guidelines recommended that serum levels of AFP $\geqslant$200 ng/ml may be used instead of fine-needle cytology for diagnosis, especially in patients with liver cirrhosis.[3] Nevertheless, the diagnostic performance of AFP is moderate with a sensitivity of 39–65% and specificity of 76–94%, leaving about one-third of cases with early-stage HCC and small tumors ($<$3 cm) undiagnosed. Meanwhile, increased serum AFP concentration in several other types of cancer, chronic hepatitis, and liver cirrhosis should be taken into consideration. Newer markers are needed to overcome these problems and allow the diagnosis of HCC at an earlier stage.[1,6]

Clusterin (CLU) is a 449-amino acid, heterodimeric glycoprotein that is ubiquitously expressed and present in most body fluids. Functionally, CLU exerts a chaperone-like activity with action like small heat shock proteins, by binding to misfolded stressed proteins. In contrast to other heat shock proteins, it is present in the extracellular space, where its expression is altered in various diseases.[7–9]

So far, CLU is thought to play diverse functions both cytoprotective and cytotoxic, thus resulting in conflicting results.[9] For example, its involvement in numerous physiological processes important for carcinogenesis has been reported, including apoptotic cell death, cell adhesion, tissue remodeling, cell cycle regulation, DNA repair, lipid transportation, membrane recycling and immune system regulation.[10] Cytoplasmic CLU immunostaining was noted to correlate with poor prognosis in patients with renal cell carcinoma,[11] hepatocellular carcinoma,[12] urothelial bladder carcinoma,[13] and prostate adenocarcinoma.[14] Also increased expression of secreted CLU was associated with radioresistance, chemoresistance, and hormone resistance, making CLU a promising target for antitumor therapeutics.[15] Both preclinical and clinical phase studies demonstrated that inhibition of CLU expression using antisense oligonucleotides enhances the apoptosis induced by several chemotherapeutic treatments.[10] On the other hand, cytoplasmic CLU staining correlated with good prognosis in pancreatic adenocarcinoma and did not correlate with prognosis in breast carcinoma.[16,17]

As the data are still sporadic and only few studies have investigated CLU in serum, the aim of the present study was to determine serum CLU concentration in CHC and HCC, as well as assess the use of clusterin measurement vs. AFP in the diagnosis of HCC.

2. Materials and methods

A total of 127 adults at the Medical Research Institute Teaching Hospital, Alexandria University, Egypt between August 2010 and April 2012 were candidates for this study, but only 91 cases fulfilled our inclusion and exclusion criteria. All subjects (or their legal guardians) gave their informed consent to the study, which was approved by the local ethics committee of the institute in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. These subjects were set into three groups based on clinical and laboratory characteristics:

- Group 1(G1): Healthy subjects. This group included 20 apparently healthy blood donors with no history of liver disease.
- Group 2 (G2): 27 patients with chronic hepatitis-C virus infection-related cirrhosis (CHC).
- Group 3 (G3): 44 patients with hepatocellular carcinoma on top of chronic HCV infection-related cirrhosis (HCC group).

All subjects completed a medical history to retrieve information about health status, current medications, alcohol consumption, and history of viral or toxic hepatitis; had a physical examination and the Child-Pugh scoring system was used for staging the severity of liver disease. Conventional ultrasound and spiral CT-scans of the abdomen were performed in all cases.

Following an overnight eight-hour-fast, eight milliliters of whole venous blood samples were withdrawn from each subject; whole EDTA blood was used for complete blood picture, citrated plasma for prothrombin time determination, serum for routine clinical chemistry and AFP assays and finally the serum was stored in aliquots at −20 °C for the determination of CLU.

For all studied groups screening for serum schistosomal IgG antibodies was done using the indirect haemagglutination test. Serological testing for anti-HCV and hepatitis B virus surface antigen (HBs-Ag) was performed by sandwich enzyme linked immunosorbent assay (ELISA) technique according to the manufacturer's instructions (Murex Diagnostic Limited. Dartfold, England). HCV viral load was determined by means of the second generation branched DNA assay (Quantiplex HCV RNA 2.0; Bayer Diagnostics, Emeryville, California, USA). Serum AFP concentration was determined using a two site chemiluminometric immunoassay (ACS–180, Siemens Healthcare Diagnostics, Germany) by the Immulite 1000 Automated Analyzer (Diagnostic Products Corporation). Serum CLU concentration was determined using the ELISA technique according to the manufacturer's instructions (Biovendor-Laboratorni Medicina a.s., Cat. No. RD194034200R). The assay had a lowest detection limit of 0.5 ng/mL. The remaining biochemical parameters were measured using routine methods by fully automated chemistry analyzer Olympus AU400. The upper limit of alanine aminotransferase (ALT) activity was set at 38 IU/L, aspartate aminotransferase (AST) at 40 IU/L, alkaline phosphatase at 120 IU/L, and gamma glutamyl transferase at 55 and 38 IU/L for males and females respectively. Results of analyses were validated using internal and external quality control provided by Biorad USA.

Hepatitis C related cirrhosis patients (CHC) are those who had (1) positive serum anti-HCV antibodies; (2) cirrhosis compatible with HCV origin proved on ultrasound and spiral CT-scans; and (3) absence of HCC defined by the absence of a focal liver mass on ultrasonography or CT scan.

The diagnosis of HCC was based on the criteria published by the Egyptian Society of Liver Cancer (ESLC) in 2011. These included the presence of a hepatic focal lesion in high risk patients (cirrhotic patients) plus either serum AFP $\geqslant$200 ng/ml, or a triphasic CT-scan showing typical criteria for HCC. If in the presence of a focal lesion $\geqslant$1 cm the AFP level was $<$200 ng/ml or triphasic CT-scan of the abdomen showed a typical criteria for HCC, then either a dynamic contrast MRI or targeted liver biopsy was performed.[18]

"Early stage HCC" were identified according to the Milan criteria; those who either have one nodule $<$5 cm, or three nodules each $<$3 cm in diameter.[19] Meanwhile, vascular invasion, regional lymph node involvement, peritoneal deposits and/or distant metastases were designated as "extrahepatic spread of HCC".

Patients were excluded if they had (a) hepatitis B virus (HBV), human immunodeficiency virus (HIV), or schistosomal co-infection, (b) personal history of diabetes or fasting serum glucose $\geqslant$7.0 mmol/L, or a 2-h postprandial serum glucose $\geqslant$11.1 mmol/L, (c) history of ischemic heart disease during the previous 6 months, uncontrolled hypertension, unstable angina, or severe cardiac arrhythmia,[20] (d) alcohol consumption $>$30 g/day (e) inadequate kidney function (creatinine level $\geqslant$150 μmol/L), and (f) received concomitant antitumor treatment.[21]

2.1. Statistical analysis

Statistical analysis was done using SPSS version 18 (SPSS, Inc., Chicago, IL, USA). Normality test was done by the Shapiro-Wilk W test. Descriptive measures were done for each variable in every group. Data comparison between groups was done using the Mann–Whitney test. Spearman correlation coefficient (r) was applied to our results. A p-value less than 0.05 were considered statistically significant.

For choosing the best cut off value, receiver operator characteristic (ROC) curve was generated and the Youden's index [Youden Index = (sensitivity + specificity) −1] was calculated.[22]The best cut off values had the highest Youden indices. Diagnostic performance of each marker alone (diagnostic specificity, diagnostic sensitivity, positive and negative predictive values) was compared to the surrogate use of both markers (combined parallel approach) in HCC cases.

3. Results

The studied subjects comprised 91 individuals (20 apparently healthy volunteers: mean age 52.7 ± 3.9 years; 10 (50%) males and 10 (50%) females, 27 CHC cases: mean age 52.53 ± 4.27 years; 18 (66.7%) males and 9 (33.3%) females, and finally HCC cases: mean age 52.18 ± 4.09 years; 25 (56.8%) males and 19 (43.2%) females). According to Child-Pugh (CP) classes, CHC patients in the present study included 8 cases (29.6%) CP class A, 10 cases (37%) CP class B, and 9 cases (33.3%) CP class C. While HCC patients included 13 cases (29.5%) CP class A, 14 cases (31.8%) CP class B, and 17 cases (38.6%) CP class C. Based on the Milan criteria, the studied HCC cases can be sorted into early and late stage HCC; 9 cases (9.9%) and 35 cases (38.5%) respectively. Also the HCC group included 10 cases (11%) with extrahepatic spread and 34 cases (37.5%) without extrahepatic spread.

All subjects in the control group had normal liver biochemistry. As expected, serum AFP (ng/ml) was significantly higher in the HCC cases compared to both the control group (7243 ± 11613.6 vs. 2.79 ± 1.26) ($p < 0.001$), as well as to CHC patients (7243 ± 11613.6 vs. 19.7 ± 17.92) ($p < 0.001$), and still significantly higher in the CHC patients compared to the control group (19.7 ± 17.92 vs. 2.79 ± 1.26) ($p < 0.001$) (Table 1). Serum CLU (ng/ml) level showed a significant increase in the HCC group compared to the control group (151.96 ± 32.74 vs. 111.40 ± 27.46) ($p < 0.001$) and to the CHC group (151.96 ± 32.74 vs. 89.12 ± 31.62) ($p < 0.001$), while a significant decrease in serum CLU level was found in the CHC group compared to the control group (89.12 ± 31.62 vs. 111.40 ± 27.46) ($p = 0.019$) (Table 1) (Fig. 1).

There was no significant statistical difference in serum CLU among the different Child-Pugh classes in either the CHC or

Table 1 Comparison between the studied groups according to serum levels of alpha fetoprotein (AFP) and clusterin (CLU).

Variable	Control group G1 (n = 20)	CHC group G2 (n = 27)	HCC group G3 (n = 44)
AFP (ng/ml)			
Range	1.4–5.3	2.4–63.4	2.3–41865
Mean ± SD	2.79 ± 1.26	19.7 ± 17.92	7243 ± 11613.6
Median	2.35	13.1	1200
$Z_1(p)$		5.090* (<0.001)	6.084* (<0.001)
$Z_2(p)$			5.362* (<0.001)
CLU (ng/ml)			
Range	59–164.5	31.5–148.5	89.9–208.7
Mean ± SD	111.4 ± 27.46	89.12 ± 31.62	151.96 ± 32.74
Median	110.45	91.3	161.8
$Z_1(p)$		2.337*(p = 0.019)	4.179* (<0.001)
$Z_2(p)$			5.901* (<0.001)

G: group, CHC: chronic hepatitis-C virus infection related cirrhosis, HCC: hepatocellular carcinoma, AFP: alpha fetoprotein, CLU: clusterin.
Z_1: Z for Mann–Whitney test between controls and other groups.
Z_2: Z for Mann–Whitney test between CHC and HCC groups.
* Statistically significant at $p \leqslant 0.005$.

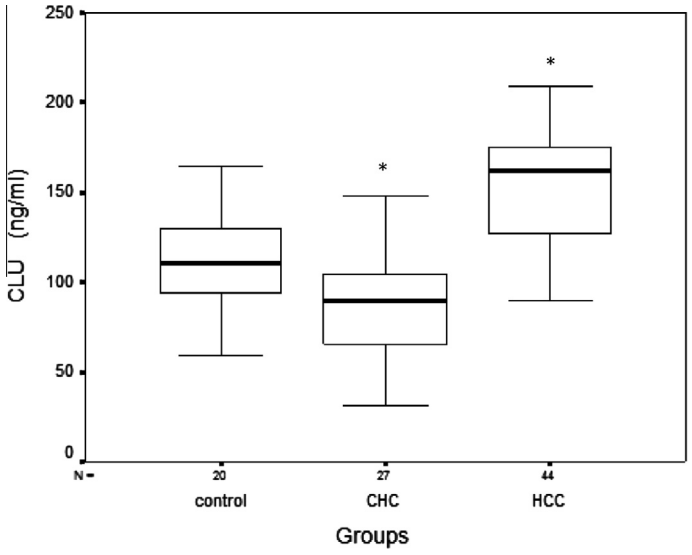

Figure 1 Boxplot of serum clusterin (CLU) in the studied groups. Box plot representing serum clusterin values in the studied groups: Control, CHC: chronic hepatitis-C virus infection-related cirrhosis, HCC: hepatocellular carcinoma. The box indicates the 25th and 75th percentile of the data and the middle line indicates the median.

HCC groups (Table 2). Also no significant correlation was found between serum CLU and Child-Pugh score (r = 0.09, p = 0.458).

Moreover, when sorting HCC patients into those with extrahepatic spread of HCC and those without, there was no significant increase in the level of both AFP (p = 0.710) and CLU (p = 0.88). But when HCC patients were categorized according to the Milan criteria, there was a significant increase in serum CLU in early stage HCC when compared to late stage (172.84 ± 24.63 vs. 146.59 ± 32.67) (p = 0.035), but such an increase was not present in serum AFP (p = 0.062) (Table 3).

When using the ROC curve for evaluating the diagnostic performance of serum CLU in relation to serum AFP in diagnosing HCC, the area under the curve of AFP (0.912, p < 0.001) was slightly bigger than that of CLU (0.874, p < 0.001) (Fig. 2).

Youden's index was calculated to get the best cut off value (COV). The optimal COV for serum CLU was 135 ng/ml and this offered a diagnostic sensitivity of 70.5%, and a diagnostic specificity of 90%. On the other hand, at a COV of 137 ng/ml, serum AFP gave a diagnostic sensitivity of 77.3%, and a diagnostic specificity of 100%. The combined parallel approach improved the diagnostic sensitivity to 95.5% and negative predictive value to 95.7% over the single use of serum AFP in HCC cases, but decreased the specificity to reach 88% and positive predictive value to 87.5% (Table 4).

4. Discussion

Hepatocellular carcinoma is a highly malignant and lethal tumor, with an estimated 5-year survival rate of 5–9% from the time of clinical diagnosis.[1] It is considered a health burden

Table 2 Comparison of serum clusterin (ng/ml) in chronic hepatitis-C virus infection-related cirrhosis (CHC) and hepatocellular carcinoma (HCC) groups according to Child-Pugh classes.

	Child-Pugh classes	A	B	C
CHC group (n = 27)	N (%)	8 (29.6)	10 (37)	9 (33.3)
	Range	42–148.5	31.5–145.3	50.7–142.9
	Mean ± SD	98.65 ± 33.50	80.04 ± 33.31	90.83 ± 28.81
	Median	95.8	75.4	89.7
	$Z_1(p)$		1.155 (p = 0.274)	0.577 (p = 0.606)
	$Z_2(p)$			0.858 (p = 0.4)
HCC group (n = 44)	N (%)	13 (29.5)	14 (31.8)	17 (38.6)
	Range	90.7–207.6	90.5–208.7	89.9–187.5
	Mean ± SD	152.23 ± 37.31	154.95 ± 31.62	149.29 ± 31.76
	Median	166.30	162.75	150.70
	$Z_1(p)$		0.291 (p = 0.793)	0.021 (p = 1)
	$Z_2(p)$			0.278 (p = 0.799)

G: group, CHC: chronic hepatitis-C virus infection related cirrhosis, HCC: hepatocellular carcinoma, n: number.
Z_1: Z for Mann–Whitney test between Child-Pugh class A and other groups.
Z_2: Z for Mann–Whitney test between Child-Pugh class B and Child-Pugh class C.

Table 3 Comparison of serum alpha fetoprotein (AFP) and clusterin (CLU) in the hepatocellular carcinoma (HCC) group according to different variables.

Staging according to Milan criteria	Early stage	Late stage
N (%)	9 (9.9)	35 (38.5)
AFP(ng/ml)		
Range	2.3–41,865	2.4–36,300
Mean ± SD	4985.94 ± 13838.63	7823.49 ± 1127.57
Median	236	1375
Z (p)		1.862 (p = 0.062)
CLU (ng/ml)		
Range	132.8–208.7	89.9–207.6
Mean ± SD	172.84 ± 24.63	146.59 ± 32.67
Median	175.9	146.2
Z (p)		2.109* (p = 0.035)
Extra-hepatic spread	Absent	Present
N (%)	34 (37.4)	10 (11)
AFP(ng/ml)		
Range	2.3–41,865	5.4–31,704
Mean ± SD	7320 ± 11830.69	6981.25 ± 11450.10
Median	1223	656.05
Z (p)		0.392 (p = 0.710)
CLU (ng/ml)		
Range	89.9–208.7	111.9–184.9
Mean ± SD	152 ± 34.91	151.82 ± 25.53
Median	161.8	156.25
Z (p)		0.168 (p = 0.88)

n: number.
Z: Z for Mann–Whitney test between the two subgroups.
* Statistically significant at $p \leqslant 0.005$.

in Egypt due to its rising incidence.[2] However, with the currently available diagnostic tools, HCC is frequently not diagnosed until it has reached an advanced stage when the remaining therapeutic modalities are less effective, leaving this disease with unfavorable prognosis.[6] Therefore, finding new markers for screening and diagnosing HCC at an early stage is highly recommended.

Clusterin (CLU) is a highly conserved glycoprotein with a wide tissue distribution. Many physiological processes have been attributed to CLU such as cell adhesion, tissue remodeling, and immune system regulation.[10]

In the present study, serum CLU was measured by the ELISA technique in a cohort of Egyptian patients (healthy controls, HCV related liver cirrhosis, and HCC cases on top of HCV related liver cirrhosis). We found a significant decrease in serum CLU level in the HCV related cirrhosis patients when compared to the control group. Also, CLU level was higher in Child class A patients compared to Child class C patients, as summarized in Table 2. The difference, however, was statistically non-significant. This may point to a possible protective role of CLU against liver cell fibrogenesis which ultimately ends in cirrhosis. Such an assumption was similarly postulated in renal fibrosis by Jung et al, who suggested that up regulation of clusterin during renal injury, in a mouse model, has a protective response against the development of renal fibrosis.[23] Likewise, Hogasen et al. and Wang et al. reported a decrease in serum CLU in alcoholic liver cirrhosis and hepatitis B viral liver cirrhosis, respectively.[24,25]

Clusterin was suggested to be either a pro-apoptotic or a pro-survival factor, rendering it an attractive biomarker for cancer studies as a diagnostic or prognostic or even surprisingly a therapeutic tool.[26,27] We found higher serum CLU in the HCC group than both control and HCV related liver cirrhosis, denoting its role in carcinogenesis. Such an increase was similarly reported in HCC in serum level as well as tissue level by previous studies. The former was done by Nafee et al.[28] who reported the significant rise of serum CLU in viral related HCC patients, and the latter was done by Kang et al with the use of a tissue microarray method which revealed CLU overexpression immunohistochemically in surgically resected HCCs.[12] Furthermore, the increase of CLU level was demonstrated in other tumors; such as bladder cancer,[29] colorectal adenocarcinomas,[30] and prostate cancer.[31] Our finding leads to hypothesize that CLU secretion occurs from tumor cells

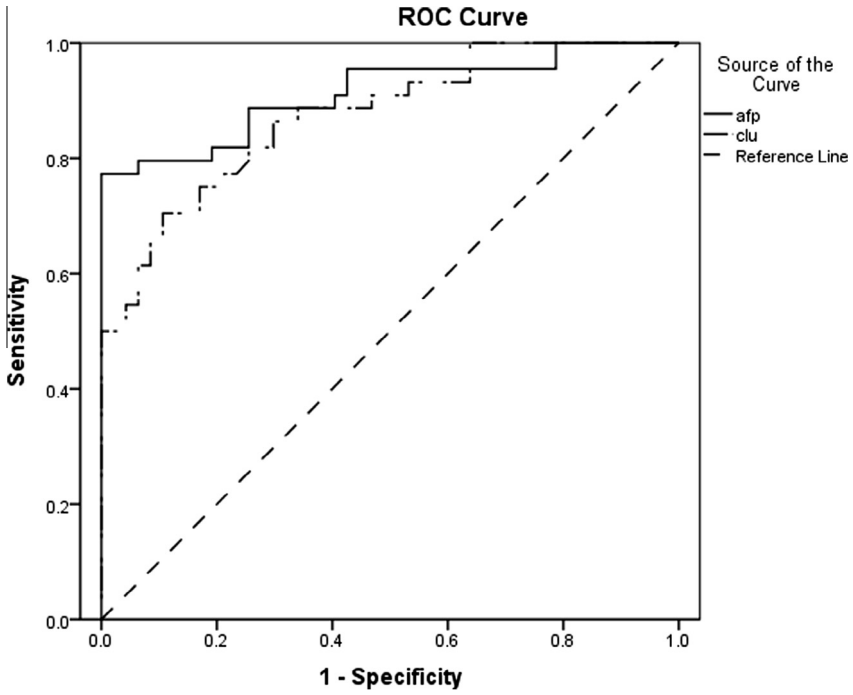

Figure 2 Receiver operating curves (ROCs) comparing clusterin and alpha fetoprotein in diagnosing hepatocellular carcinoma. Receiver operator curves (ROCs) comparing clusterin (CLU) and alpha fetoprotein (AFP) in patients with hepatocellular carcinoma (HCC) (G3) vs. those with and without HCC (G1 and G2). The solid line (-) denotes AFP and the dotted line (—) denotes CLUS. The curves show the optimal cutoff value for clusterin to be135 mg/mL and for AFP to be 136 ng/mL. Diagonal segments are produced by ties.

Table 4 Predictive performance of serum alpha fetoprotein (AFP) and clusterin (CLU) as biomarkers for the detection of hepatocellular carcinoma (HCC).

Statistical parameter	AFP	CLU
Cut off value (ng/ml)	>137	>135
Sensitivity (%)	77.3	70.5
Specificity (%)	100	90
Positive predictive value (%)	100	86.1
Negative predictive value (%)	83.3	77.6
Youden's index	0.7727	0.6045
Area under the receiver operator curve	0.912	0.874
p Value	<0.001	<0.001
95% confidence interval	0.85–0.975	0.804–0.943

in HCC and is reflected in its serum level. This is further supported by the fact that clusterin exists as both an intracellular truncated form and an extracellular heterodimeric secreted glycoprotein, making clusterin the only known chaperone protein to be secreted.[32]

A handful of studies tackled the issue of the diagnostic performance of biomarkers in HCC. In the present study, serum AFP with a cutoff level of 137 ng/ml outperformed serum CLU with a cutoff level of 135 ng/ml in diagnostic sensitivity (77.3% vs. 70.5%), specificity (100% vs. 90%), and positive and negative predictive values (100% vs. 86.1% and 83.3% vs. 77.6%, respectively). Moreover, the use of a combined parallel approach improved the diagnostic sensitivity (95.5%) and negative predictive value (95.7%) over the single use of AFP but decreased the specificity to reach 88% and positive predictive value to be 87.5%, thus improving the sensitivity at the expense of the specificity. On the contrary, Wang et al.,[25] demonstrated that serum CLU with a cutoff level of 50 ug/ml outperformed serum AFP with a cutoff level of 15 ng/ml in diagnostic sensitivity (91% vs. 67%), specificity (83% vs. 76%), positive and negative predictive values (93% vs. 88% and 77% vs. 47% respectively). It is worth mentioning that such a low cutoff level of AFP (15 ng/ml) is somehow not applicable in countries with a high prevalence of hepatic diseases especially HCV as in our country. The sensitivity and specificity of the diagnostic biomarker are strongly dependent on the cutoff value above which it is considered positive, and such a cutoff value is affected by the criteria of the studied sample (demographic, clinical, biochemical and statistical) as well as implicated methodological assays, thus explaining the wide variation in diagnostic performance of biomarkers in different studies due to different sample criteria.

The relation of CLU to HCC progression was also variable in different reports. In vitro study using HCC cell lines by Lau et al.[33] demonstrated that overexpression of CLU increased cell migration and formation of metastatic tumor nodules, but Wang et al.[25] and Nafaa et al.[28] found no significant difference of CLU serum levels between different tumor sizes. In our study we did not find a significant increase in serum CLU in HCCs with extrahepatic spread when compared to HCC without extrahepatic spread. Interestingly, our study revealed a significant increase in serum CLU level in early stage HCC when compared to late stage HCC, such an increase was not found in serum AFP. Although this subgroup of early stage HCC

comprised only 9 cases, this can be attributed to rarity of such patients due to delayed diagnosis of HCC. We cannot deny the possibility of a random effect due to the small size of this subgroup rendering it difficult for establishing reliable statistical results that could be applied in clinical use. Therefore, we recommend investigating CLU level on a larger cohort to ascertain such an association. But a possible explanation may be that the CLU increase in late stages is directed to the nuclear form rather than the secreted form. Moreover, the complex nature of CLU and its multiple isoforms not only in tissues[34] but also in serum should be considered. Pucci et al.[35] explained the diversity of CLU function in colorectal carcinoma by a shift in the pattern of its isoform production. Also Rodríguez-Piñeiro et al.[34] pointed to the importance of measuring CLU isoforms over that of total serum level, when they demonstrated the increase of some isoforms and the decrease or absence of others in colorectal carcinoma.

Finally, we can conclude that serum AFP did better than serum CLU in all aspects of diagnostic performance for diagnosing HCC, but still the combined parallel approach improved the sensitivity which is required in screening high risk populations such as CHC patients. But still, further research of larger study populations and with various liver functions status, will be required to examine whether serum CLU is associated with specific liver disease etiologies.

Conflict of interest

We have no conflict of interest to declare.

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