Clinical utility of serum chromogranin A as a marker of hepatocellular carcinoma in chronic hepatitis patients

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Background
Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer deaths accounting for about 745 000 deaths per year globally. HCC is often diagnosed at an advanced stage where effective therapies are lacking.

Aim
Is to investigate the role of serum chromogranin A (CgA) as a marker for detection of HCC in patients with HCC complicating chronic hepatitis and correlate its serum levels with alpha fetoprotein (AFP).

This study was conducted on 90 HCC patients (group I), 30 patients with hepatic cirrhosis (group II), and 30 age-matched and sex-matched healthy-controls (group III). Group I was further subdivided into group Ia (30 patients) with HCC complicating chronic hepatitis B, and group Ib (60 patients) with HCC complicating chronic hepatitis C. All participants included in the study were subjected to full history taking and clinical examination, laboratory investigations, including complete blood count, liver-function tests, renal-function tests, and viral markers. Serum AFP and serum CgA levels were also determined.

Results
Our results revealed that there was a highly significant elevation ($P < 0.01$) in the median serum CgA in hepatitis-B virus -related HCC (200 ng/ml) and hepatitis-C virus-related HCC (190 ng/ml) when compared with the control group (30 ng/ml) and when compared with cirrhotic group (60 ng/ml) with no significant difference between the median serum CgA in hepatitis-B virus -related HCC and hepatitis-C virus-related HCC ($P > 0.05$). A highly significant positive correlation between chromogranin and AFP ($P < 0.01$) was found in HCC patients. On the other hand, no significant correlations were found between chromogranin and complete blood count, kidney functions, and liver-function tests in patient groups. Diagnostic reliability testing revealed that for CgA, the best cutoff for discriminating HCC patients was 100 ng/ml at which the sensitivity was 77.78%, the specificity 93.3% with positive predictive value 94.59% and negative predictive value 73.68%. Regarding the combination between CgA and AFP, it showed a sensitivity of 97.78% and a specificity of 86.67%.

Conclusion
Serum CgA is a promising sensitive and specific tumor marker for identification of HCC. Addition of serum CgA to the current standard tests will improve the sensitivity and accuracy of diagnosis of HCC patients and thus could allow them to benefit from earlier treatment.

Keywords:
Chromogranin A - Hepatocellular Carcinoma - Hepatitis

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world and the third most common cause of cancer deaths, accounting for about 745 000 deaths per year globally [1]. In Egypt, HCC is the third most frequent cancer in men with more than 8000 new cases detected annually [2].

Cirrhosis due to chronic hepatitis-B virus (HBV) or hepatitis-C virus (HCV) is the leading risk factor for HCC [3].

HCC is often diagnosed at an advanced stage where effective therapies are lacking [4]. The current diagnostic tools for HCC among high-risk patients include clinical and laboratory imaging and biopsies. The most common HCC biomarker used to screen patients with liver cirrhosis is serum alpha fetoprotein (AFP), which is measured at 6-month interval [5]. Serum AFP test has a low sensitivity, which makes the AFP test insufficient for early detection of HCC in at-risk population. In addition, AFP test has a high false-positive rate among patients with chronic hepatitis and among those with liver cirrhosis [6].

Chromogranin A (CgA) is a member of the granin family of neuroendocrine- secretory proteins, it is

located in secretory vesicles of neurons and endocrine cells, such as islet beta-cell secretory granules in pancreas [7].

The importance of increased CgA levels in serum was first shown in patients with pheochromocytoma, and then demonstrated in other endocrine cancers [8]. High serum levels of CgA have also been demonstrated in patients with other malignancies, including colon, lung, breast, prostate cancer, and in carcinoid syndrome [9]. Clusters of cells containing CgA have been demonstrated within HCC tissue and recent studies reported elevated levels of serum CgA in HCC patients, suggesting a possible diagnostic role of this marker [10,11].

The aim of this work was to investigate the role of serum CgA as a marker for detection of HCC in patients with HCC complicating chronic hepatitis B and C.

Patients and methods

Patients

This study was conducted on 90 HCC patients (group I), 30 patients with hepatic cirrhosis (group II), and 30 age-matched and sex-matched healthy-controls (group III). An informed consent was taken from all participants. The study protocol was approved by the Research Ethics Committee in Ain Shams University Faculty of Medicine.

Group I (hepatocellular carcinoma group)

This group icluded 90 patients with HCC attending the Internal Medicine and Hepatology Outpatient's Clinics and Inpatient Wards of Ain Shams University Hospitals between January 2017 and January 2018. They were further subdivided into group Ia (30 patients) with HCC complicating chronic hepatitis B, they were 18 males and 12 females with mean age 55.13 ± 7.43, and group Ib (60 patients) with HCC complicating chronic hepatitis C, they were 36 males and 24 females with mean age of 59.80 ± 8.22 years.

Group II (cirrhotic group)

This group included 30 patients with liver cirrhosis, they were 18 males and 12 females with mean age 59.07 ± 8.10. Ten patients were HBV + ve and 20 patients were HCV + ve.

Group III (control group)

This group included 30 age-matched and sex-matched healthy participants with negative viral markers. They were attending the Outpatient Clinic for checkup.

All participants included in the study were subjected to:

(1) Full history taking with special emphasis on risk factors, duration of disease, and alcohol intake.
(2) Full clinical examination with special emphasis on the presence of signs of chronic liver disease (spider naevi, palmar erythema, level of consciousness, flapping tremors, ascites, splenomegaly, and jaundice).
(3) Laboratory investigations including:
    (a) Complete blood count.
    (b) Liver-function tests, including s. albumin, bilirubin (total and direct), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, and prothrombin time.
    (c) Renal-function tests, including serum creatinine and urea.
    (d) Viral markers including
        (i) HBsAg and HBcAb (total and IgM).
        (ii) HBV DNA–PCR.
        (iii) HCV Ab (ELISA).
        (iv) HCV–RNA by real-time PCR.
        (v) HIV antibody (ELISA).
    (e) Serum AFP.
    (f) Serum CgA
(4) Abdominal ultrasonography (US) (for patients only) using real-time scanning device Toshiba, just vision 200 (SSA, 320 A) with convex probe, 3–5 μHz to confirm the presence of liver cirrhosis, ascites, splenomegaly, and portal-vein diameter and detect portal-vein thrombosis.

The diagnosis of HCV infection was defined by positive tests for antibodies against HCV based on an enzyme immunoassay and was confirmed by the presence of detectable HCV RNA in the circulation by PCR. Diagnosis of HBV was determined by HBsAg commercial enzyme immunoassay kits and confirmed by measurement of HBV DNA in serum by PCR.

The diagnosis of HCC cases was done by:

(1) Focal lesion in the liver in abdominal sonography.
(2) Enhancement of focal lesion on abdominal triphasic computed tomography (CT).

The diagnosis of HCC was confirmed by triphasic CT and including staging of HCC (size, number, local metastasis, vascular invasion, and the size of the largest tumor nodule).

Blood samples

Ten milliliters of venous blood were withdrawn from each patient under complete aseptic condition. Blood was put into a sterile plain vacutainer, left to clot for

30 min, centrifuged (at 4000 rpm for 10 min), and the separated serum was divided into several aliquots for analysis of chemical and serological tests. An aliquot of the serum was stored at −80°C till used for measurements of CgA.

Methods

Analytical methods

(1) Liver-function tests and AFP:
AST, ALT, ALP, and albumin were analyzed on the Beckman Coulter AU 480 system (Beckman Coulter Inc., 250 s, Kraemer Blvd. Brea, California, USA). Serum AFP was measured by electrochemiluminescence on the Cobas system provided by Roche Diagnostics, GMBH, Mannheim, Germany.

(2) Viral markers:
Anti-HCV antibody and HBV markers were measured using commercially available ELISA kits (Murex, Italy). HBV DNA was detected by PCR and HCV RNA was detected by RT-PCR (QIAgen Scien Inc. 19300 Germantown Rd, Germantown, Maryland, United States (USA)).

(3) Determination of CgA:
CgA was assayed by using the commercially available ELISA kit supplied by Quantikine® industry (Quantikine 614 Mckinley place NE, Minnesota, USA). The assay utilizes the two-site 'sandwich' ELISA technique with two selected antibodies. One of the antibodies is bound to a microtiter plate to create the solid phase. Patient samples, controls, and standards were incubated in microtiter-plate well. After washing the plate, the second antibody, which is horseradish peroxidase-labeled monoclonal antihuman CgA antibody, was added to the wells. After a second incubation and washing step, the wells were incubated with the substrate tetramethyl benzidine. An acidic stopping solution was then added, and the degree of the enzymatic turnover of the substrate was determined by dual-wavelength absorbance measurement at 405 and 620 nm. The absorbance measured is directly proportional to the concentration of human CgA [12].

Statistical methods

Statistical analysis was done using the Statistical Package SPSS is a software provided by IBM for Social Sciences, version 15. Data were expressed using mean, SD, and range for quantitative variables that are normally distributed, and as median and IQR (percentiles) in case of skewed data. Comparison between two groups was done using independent *t* test, analysis of variance for comparing more than two groups. Correlations were done to show the relation between quantitative variables. Linear regression was done to show the significant predictors affecting chromogranin. Receiver-operating characteristic curve analysis was done to show how well chromogranin result reflects HCC and the chromogranin cutoff level with the best diagnostic performance. *P* value less than 0.05 was considered as statistically significant and *P* value less than 0.01 was considered as highly significant.

Results

The results of the current study are presented in the following tables and figures (Tables 1 and 2).

In post-hoc test, AFP showed a highly significant difference between the control group and both HCC-complicating B group ($P < 0.01$) and HCC-complicating C group ($P < 0.01$), as well as between the cirrhosis group and both HCC-complicating B and C groups ($P < 0.01$, $P < 0.01$).

However, there was no statistically significant difference between AFP levels in HCC-complicating B group and HCC-complicating C group ($P > 0.05$) and between the control group and cirrhosis group ($P > 0.05$) (Fig. 1 and Table 3).

Regarding CgA, there was a highly significant difference between the control group and both HCC-complicating B group ($P < 0.01$) and HCC-complicating C group ($P < 0.01$), between the cirrhosis group and both HCC-complicating B and C groups ($P < 0.01$, $P < 0.01$), and between the control group and cirrhosis group ($P < 0.01$).

No statistically significant difference was found regarding CgA levels between HCC-complicating B group and HCC-complicated C group ($P > 0.05$) (Fig. 2 and Table 4).

In the present study, a highly significant positive correlation between CgA and AFP ($r = 0.618$, $P < 0.01$) was found. No significant correlation was found between chromogranin and total leukocyte count, hemoglobin, platelet count, prothrombin time, international normalized ratio, total bilirubin, direct bilirubin, serum albumin, ALT, AST, urea, and creatinine (Fig. 3).

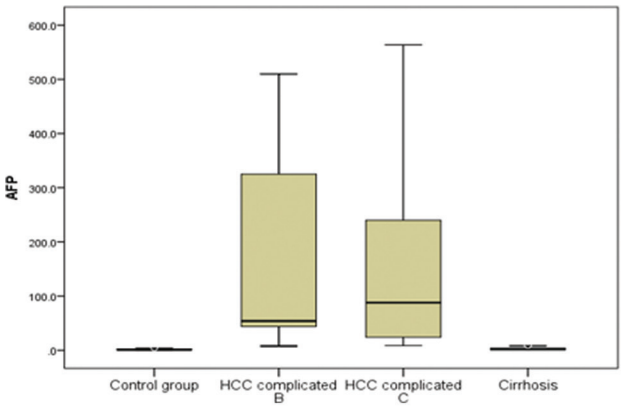
HCC patients were classified according to the number of focal lesions in the US and CT into three groups with one, two, and multiple focal lesions.

Table 1 Descriptive statistics of the various studied parameters in the different groups

Groups	Age (years)	TLC (10^9/l)	Hb (mg/dl)	PLAT (109/l)	PT (s)	Total bilirubin (mg/dl)	Albumin (g/dl)	AST (IU/l)	ALT (IU/l)	Urea (mg/dl)	Creatinine (mg/dl)
HCC complicated B group	55.13±7.43	5.37±3.25	12.18±1.79	144.4±103.5	14.61±1.83	1.3 (1.1-1.89)	2.70±0.64	47 (34-60)	36±21.26	31 (18-41)	1.01±0.26
HCC complicated C group	59.8±8.22	5.56±3.03	10.72±1.55	126.6±80.88	15.24±2.54	1.3 (0.95-2.1)	2.6±0.57	65 (44-104)	46±28.38	32 (22-58)	1.19±0.47
Cirrhosis group	59.07±8.10	7.57±3.65	10.91±1.73	166.07±111.71	17.44±5.24	1.6 (1.1-2.5)	2.85±0.77	42 (35-74)	40±23.32	48 (40-67)	1.42±0.77
Control group	55.27±6.69	6.88±2.06	12.38±1.48	281.07±63.86	12.32±0.48	0.8 (0.0-1.0)	4.27±0.35	24 (18-30)	24±7.38	22 (18-32)	0.84±0.32

Data are expressed as mean±SD or median and (interquartile range) in case of skewness. ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; HCC, hepatocellular carcinoma; PLAT, platelet; PT, prothrombin time; TLC, total leukocyte count.

Figure 1



Median values of AFP in the studied groups. AFP, alpha fetoprotein.

The median and the range of CgA according to the number of hepatic focal lesions in the US were 240 (90–660), 170 (63–330), and 200 ng/ml (72–710 ng/ml) for one, two, and multiple focal lesions, respectively.

The median levels of CgA according to the number of hepatic focal lesions in the CT were 225 (80–660), 140 (63–480), and 200 ng/ml (71–720 ng/ml) for one, two, and multiple focal lesions, respectively.

Statistical comparison between serum levels of CgA with the number of hepatic focal lesions in the US and CT shows no statistically significant difference ($P > 0.05$) (Table 5).

Moreover, no significant correlation was found between CgA levels and the size of hepatic focal lesion ($P > 0.05$) (Table 6).

Receiver-operating characteristic curve analysis was done to illustrate the diagnostic performance of both markers for discriminating patients with and without HCC. For CgA, having the best cutoff point of 100 ng/ml showed a sensitivity of 77.78%, specificity 93.33%, positive predictive value (PPV) 94.59%, and negative predictive value (NPV) 73.68% (Fig. 4). On the other hand, for AFP having the best cutoff at 20 ng/ml, the sensitivity was 66.67%, specificity was 90.0% with PPV 90.91% and NPV 64.29% (Table 7).

Regarding the combination between CgA and AFP, it showed a sensitivity of 97.78% and specificity of 86.76%.

Discussion

Primary liver cancer is one of the most common malignancies in the world, and about 90% of primary liver-cancer cases are HCC [13]. According to WHO, more than 6% of cancer incidence and 9% of cancer

Table 2 Statistical comparison between the studied groups regarding serum alpha fetoprotein

Groups	AFP (ng/ml)		ANOVA	
	Range	Median-IQR	*F*	*P*
HCC complicating B	7.8-580.0	47.5-(19-510)		
HCC complicating C	14.0-188.0	88-(17-260)	53.266	0.000*
Cirrhosis	0.66-9.0	4.2-(0.9-24)		
Control	3.0-5.0	0.98-(0.5-2.4)		
	Tukey's test			
HCC complicating B and C	HCC complicating B and control	HCC complicating C and control	Cirrhosis and control	
P>0.05	*P*<0.0001*	*P*<0.0001*	*P*>0.05	
HCC complicating B and cirrhosis		HCC complicating C and cirrhosis		
P<0.0001*		*P*<0.0001*		

AFP, alpha fetoprotein; ANOVA, analysis of variance; HCC, hepatocellular carcinoma; IQR, interquartile range.

Table 3 Statistical comparison between the studied groups regarding serum chromogranin A

Groups	CgA (ng/ml)		ANOVA	
	Range	Median-IQR	*F*	*P*
HCC complicating B	63-660	200 (160-251)		
HCC complicating C	27-710	190 (120-280)	49.128	<0.001
Cirrhosis	19.5-145	60 (30-90)		
Control	5-75	30 (20-50)		
	Tukey's test			
HCC complicating B and C	HCC complicating B and control	HCC complicating C and control	Cirrhosis and control	
P>0.05	*P*<0.0001	*P*<0.0001	*P*<0.01	
HCC complicating B and cirrhosis		HCC complicating C and cirrhosis		
P<0.0001		*P*<0.0001		

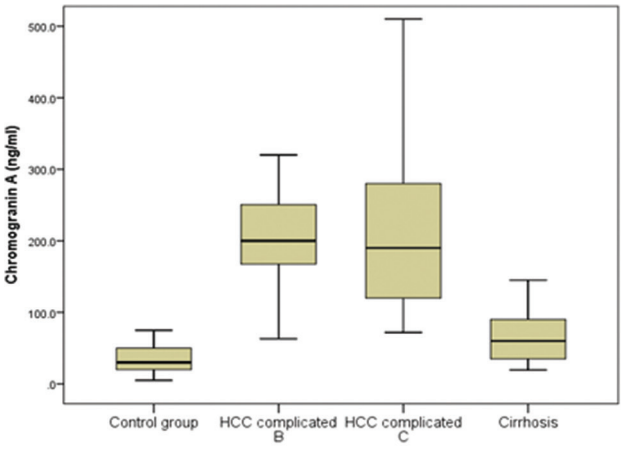
ANOVA, analysis of variance; CgA, chromogranin A; HCC, hepatocellular carcinoma; IQR, interquartile range. *p<0.001: Highly significant

Table 4 Correlation study between serum chromogranin A and the other studied parameters in patients groups

	Chromogranin A	
	r	*P*
TLC	−0.105	0.424
Hb	0.172	0.188
PLT	0.041	0.757
PT	−0.062	0.636
INR	−0.078	0.555
Total bilirubin	0.020	0.882
Direct bilirubin	−0.016	0.904
Serum albumin	0.140	0.285
AST	0.056	0.672
ALT	0.031	0.814
Urea	−0.179	0.171
Creatinine	−0.080	0.543
AFP	0.618**	0.000*

AFP, alpha fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Hb, hemoglobin; INR, international normalized ratio; PT, prothrombin time; TLC, total leukocyte count. *p<0.001: Highly significant; *p<0.5: Non significant.

Figure 2



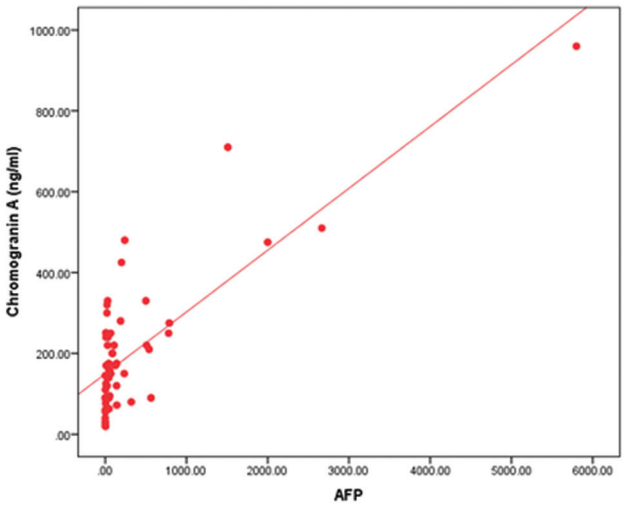
Median values of CgA in the studied groups. CgA, chromogranin A.

mortality can be attributed to HCC in the global range [14].

Early detection of HCC opens doors for various effective treatments, such as surgical resection, radiofrequency ablation, and transplantation, which can subsequently lead to long-term survivals in a great number of HCC patients [15]. Patients with symptomatic HCC have a survival rate of only 0–10%; however, early diagnosed patients can achieve 5-year survival rates of over 50% with liver transplantation or resection [16].

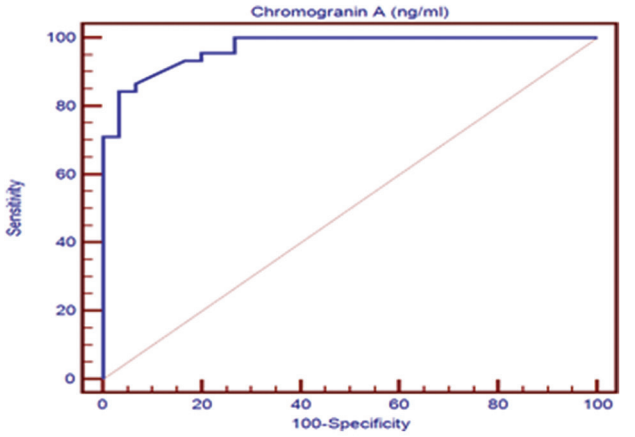
The best chance for early diagnosis comes from the surveillance of patients known to be at high risk [17]. Liver US is recommended as the primary surveillance modality for HCC. AFP measurement is also commonly used for HCC surveillance because it is relatively inexpensive, simple to perform, and is widely available. However, AFP alone is not recommended as an HCC-surveillance test due to its low sensitivity and specificity for detecting HCC [18].

Figure 3



Correlation between chromogranin A and AFP. AFP, alpha fetoprotein.

Figure 4



ROC-curve analysis showing the diagnostic performance of chromogranin A for discriminating HCC patients. HCC, hepatocellular carcinoma; ROC, receiver-operating characteristic.

Table 5 Statistical comparison between serum levels of chromogranin A and the number of focal lesions in the ultrasound and computed tomography

	CgA
US	
H	0.232
P[a]	>0.05
CT	
H	1.713
P	>0.05

CgA, chromogranin A; CT, computed tomography; U, ultrasound.
[a]Kruskal-Wallis test. *P*>0.05: nonsignificant.

Table 6 Correlation study between serum levels of chromogranin A and tumor size

	CgA	
	r_s	*P*
Size of hepatic focal lesion <3 cm	−0.149	>0.05
Size of hepatic focal lesion >3 cm	0.187	>0.05

In multiple hepatic focal lesions the largest size was considered in the statistics. CgA, chromogranin A. r_s: Spearman s correlation coefficient. *P* value more than or equal to 0.05: nonsignificant.

Several tumor markers have been proposed as a complement or substitute for AFP in HCC diagnosis. Lens culinaris agglutinin A, reactive fraction of alpha-fetoprotein (AFP-L3), and des-gamma-carboxy prothrombin have been established as HCC-specific tumor markers [19]. However, AFP-L3 and des-gamma-carboxy prothrombin are less sensitive than AFP for the diagnosis of early and very early stage of HCC [20].

CgA is a member of the granin family of neuroendocrine- secretory proteins, it is located in secretory vesicles of neurons and endocrine cells, such as islet beta-cell-secretory granules in pancreas. Low levels of CgA in the circulation are present in healthy participants and are independent of age and sex [7]. The importance of increased CgA levels in serum was first shown in patients with pheochromocytoma, and then demonstrated in other endocrine cancers [8]. Recent studies reported elevated levels of serum CgA in HCC patients, suggesting a possible diagnostic role of this marker [10,11].

The aim of this work was to investigate the role of serum CgA as a marker for detection of HCC comparing its level in patients with HCC complicating chronic hepatitis B and chronic hepatitis C.

In the present study, there was a highly significant elevation ($P < 0.01$) in median serum AFP in HBV-related HCC patients (54 ng/ml) and HCV-related HCC patients (88 ng/ml) when compared with the control group (0.89 ng/ml) and when compared with the cirrhotic group (2.2 ng/ml).

This was in agreement with Abbasi *et al.* [21], Shahid *et al.* [6], and Kabil *et al.*[22] who reported that HCC patients had AFP levels higher than the cirrhotic group.

However, Page *et al.*[23] declared that one of the limitations in the use of AFP for the diagnosis of HCC is its increase in CLD patients who do not have HCC. On the same hand, El-Serag[24] stated that hepatic injury and regeneration alone (as in chronic hepatitis) can increase the serum levels of AFP in patients who do not have HCC. In 2011, American Association for the Study of Liver Diseases (AASLD) practice guidelines omitted AFP from the algorithm for surveillance and diagnosis of HCC [25].

Our results revealed that there was a highly significant elevation ($P < 0.01$) in the median serum CgA in iHBV-related HCC (200 ng/ml) and HCV-related

Table 7 Diagnostic performance of serum chromogranin A and alpha fetoprotein for discriminating patients with and without hepatocellular carcinoma

	Cut off point	AUC	Diagnostic sensitivity	Diagnostic specificity	Positive predictive value	Negative predictive value
CgA	100 ng/ml	0.93	77.78	93.33	94.59	73.68
AFP	20 ng/ml	0.83	66.67	90.00	90.91	64.29

AFP, alpha fetoprotein; AUC, area under the curve; CgA, chromogranin A.

HCC (190 ng/ml) when compared with the control group (30 ng/ml) and when compared with the cirrhotic group (60 ng/ml). These results are in agreement with Massironi *et al.* [26], Biondi *et al.* [10], and Kabil *et al.* [22], who reported a statistically significant elevation of CgA serum levels in HCC patients when compared with those in cirrhotic patients and controls.

There was a nonsignificant difference ($P > 0.05$) between the median serum CgA in HBV-related HCC (200 ng/ml) and HCV-related HCC (190 ng/ml) in our study, which may be explained by the fact that CgA levels increase or decrease according to the degree of neuroendocrinal activity (differentiation) of HCC and not according to the causative agent.

No significant correlations were found between chromogranin and complete blood count, kidney functions, and liver-function tests. This is in agreement with Wafaa *et al.*[11] who reported the same findings.

Our results show a highly significant positive correlation between chromogranin and AFP ($P < 0.01$) in HCC patients and this is in agreement with Biondi *et al.*[10] and Massironi *et al.* [26], who reported that serum levels of CgA were significantly correlated with AFP ($P < 0.05$), but this was not in agreement with Spadaro *et al.* [27], who reported no correlation between both markers in patients with HCC.

Regarding the HCC size, there was no significant correlation between the levels of CgA with tumor size ($P > 0.05$). Also, regarding the tumor number in the US and CT, no significant correlation was found between the levels of CgA and the number of tumors in both CT and US ($P > 0.05$).

Diagnostic reliability testing revealed that for AFP, the best cutoff was 20 ng/ml with a sensitivity of 66.67%, specificity 90.0%, PPV 90.91%, and NPV 64.25%. At a cutoff level of 200 ng/ml, the sensitivity was 33.3% and the specificity was 100% with PPV 100% and NPV 60%.

Our results were comparable to those reported by Shahid *et al.* [6], who reported that at a cutoff of 20 ng/ml, the sensitivity of AFP was 72.2%, specificity was 86.2% with PPV 89.9% and NPV 64.7%, while at a cutoff of 200 ng/ml, the sensitivity was 45.6% and the specificity was 100% with PPV 100% and NPV 52.04%.

Our finding was also comparable to those reported by Park *et al.* [28], who reported that at a cutoff of 20 ng/ml, the sensitivity of AFP was 62.03% and the specificity was 92.21% with PPV of 89.09% and NPV of 70.3%.

For CgA, the best cutoff was at 100 ng/ml; the sensitivity was 77.78% and the specificity was 93.3% with PPV 94.59% and NPV 73.68%. At the same cutoff value, this finding was comparable to those reported by Biondi *et al.* [10], who reported that CgA serum levels were elevated in 72/96 patients with HCC (sensitivity was 75%), and those of Wafaa *et al.* [11], who reported a sensitivity of 100% and specificity of 100% for the same cutoff value.

Regarding the combination between CgA and AFP, at a cutoff level of 100 ng/ml for CgA and a cutoff level of 20 ng/ml for AFP, it showed sensitivity 97.78% and specificity 86.67% (increasing the sensitivity of AFP alone from 66.7 to 97.78% but decreasing the specificity from 90 to 86.67%).

These results are consistent with those of Kabil *et al.* [22], who reported that CgA level showed the best cutoff of 99.95 nmol/l with 90% sensitivity, 90% specificity, 90% PPV, and 90% NPV with 95.8% accuracy for the diagnosis of HCC, while when combined with AFP, it had 90% sensitivity, 67.5 specificity, 73.5% PPV, and 87.1% NPV with 78.8% accuracy for the diagnosis of HCC.

These results also agreed with Biondi *et al.* [10], who concluded that when AFP is normal or less than 200 ng/ml and in the presence of suspicious clinical, laboratory, or imaging signs of HCC, the combined use of both markers has a significant additional value to their diagnostic performance and becomes of particular importance in the follow-up of chronic liver-disease patients. In conclusion, serum CgA is a promising sensitive and specific tumor marker for identification of HCC. Addition of serum CgA to the current standard tests as a new diagnostic and screening tool for HCC will improve the sensitivity and accuracy of diagnosis of HCC patients and thus could allow them to benefit from earlier treatment.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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