Evaluation of Serum Chromogranin A as a Useful Tumor Marker for Diagnosis of Hepatocellular Carcinoma

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Abstract: Background: In Egypt, HCC was reported to account for about 4.7% of chronic liver disease patients. Approximately 80% of HCCs are associated with cirrhosis, which is regarded as the most important precancerous etiological factor. Chromogranin A is a cellular marker for neuroendocrine tumors. High serum levels of CgA have also been demonstrated in patients with other malignancies including colon, lung, breast and prostate cancer. Objective: To evaluate serum CgA as a marker for HCC. Patients and Methods: Eighty cases (30 with HCC, 30 with liver cirrhosis and 20 apparently healthy controls) were subjected for estimation of Chromogranin A (CgA) and Alpha feto protein (AFP) by ELISA technique together with routine laboratory investigations including CBC, prothrombin time and concentration and INR and serum urea, creatinine, albumin, AST, ALT, alkaline phosphatase and bilirubin (total and direct). Results: There was a highly significant statistical difference between control group and HCC group and between liver cirrhosis group and HCC group as regard to AFP and Chromogrnin A ($P<0.01$). There was a significant statistical difference between control group and liver cirrhosis group as regard to AFP and Chromogrnin A ($P<0.05$). Conclusion: the results of the present study revealed that the application of CgA as a tumor marker in the diagnosis of HCC is to be considered especially in cases with low levels of AFP, as determination of CgA serum values represents a complementary diagnostic tool in monitoring chronic liver disease patients for detection of HCC. The combined use of both CgA and AFP to detect HCC increases their sensitivity and specificity.

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1. Introduction:

Hepatocellular carcinoma (HCC) is the most common cause of primary liver neoplasms and the fourth most frequent type of cancer worldwide following lung, breast and bowel cancers with an increasing incidence, causing one million deaths per year[(1)].

A study conducted at Cairo Liver Center, a specialized center for the study and management of liver diseases, revealed that HCC has nearly doubled over the last decade and there is a growing incidence of HCC in Egypt (10–120 cases/ 100,000), which represents the leading cause of death from all other cancer sites[(2)].

In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients[(3)] but there is a remarkable increase in the proportion of HCC among CLD patients from 4.0% to 7.2% over the last decade[(4)]. This rising proportion may be explained by the increasing risk factors such as the emergence of HCV over the same period of time, the contribution of HBV infection, improvement of the screening programs and diagnostic tools of HCC[(5)]. Age distribution among HCC patients revealed that the most predominant age is (40-59) years[(4)].

Approximately 80% of HCCs are associated with cirrhosis, which is regarded as the most important precancerous etiological factor. Hepatocyte necrosis and subsequent increased proliferation due to chronic hepatitis favor nodular regeneration, which might be followed by hepatocyte dysplasia and possibly HCC[(6)]. The causes for hepatocyte necrosis may be infectious, toxic, metabolic, and autoimmune. The majority of HCV-infected individuals become chronic carriers of the virus, and long term follow-up studies have demonstrated that a proportion (4%-25%) develops cirrhosis and ultimately HCC. Due to the silent progression of cirrhosis towards HCC, there is a clear need for a marker capable of detecting the transformation[(7,8)].

Alpha fetoprotein (AFP) is the most established tumor marker in HCC and the gold standard by which other markers for the disease are judged[(9)].

AFP is a fetal specific glycoprotein synthesized from fetal yolk sac, liver and intestines. Normally, its serum concentration falls rapidly after birth and its synthesis in adult life is depressed. However, more than 70% of HCC patients have high serum concentrations of AFP because of tumor excretion. Forty years after its discovery, serum AFP

remains the most useful marker for screening HCC patients. The normal range for serum AFP levels is up to 20 ng/ml [10].

Mild elevations of AFP can be seen in benign liver diseases such as virus related acute and chronic hepatitis[11].

Among patients with chronic hepatitis C, serum AFP values are frequently elevated, even in the absence of HCC. Factors associated with raised AFP include severity of liver diseases, female gender and black race[12].

Chromogranin A (CgA) is an acidic, hydrophilic protein of 439 amino acids (49 kDa), present in chromaffin granules of the neuroendocrine cells. CgA acts as a pro-hormone and its proteolysis constitutes a key element of its physiology. This degradation releases biologically active peptides (vasostatins, chromostatin, pancreastatin, paraststin, etc.) that have different paracrine and autocrine functions. The proteolysis is tissue specific, and the protein's fragmentation differs depending on its location. Although the function of CgA is not well known, it seems to be related to calcium binding activity[13].

Low levels of CgA in the circulation are present in healthy subjects and are independent of age and sex. The importance of increased CgA levels in serum was first shown in patients with pheochromocytoma, and then demonstrated in other endocrine cancers[14]. High serum levels of CgA have also been demonstrated in patients with other malignancies including colon, lung, breast and prostate cancer, possibly in relation to a neuroendocrine differentiation[15]. Interestingly, clusters of cells containing CgA have been demonstrated within HCC tissue[16] and recent studies reported elevated levels of serum CgA in HCC patients, suggesting a possible diagnostic role of this marker[17].

The present study was aimed at comparing serum CgA concentration in HCC patients and those with cirrhotic liver disease to assess the potential usefulness of this marker in diagnosis of HCC.

2. Subjects and Methods:

This study was conducted on 60 subjects admitted to Hepatology, Gastroenterology and Infectious Diseases Department, Benha University Hospital, during the period from April 2009 to April 2010. They were classified into 2 groups. The first group included 30 patients with hepatocellular carcinoma (HCC) on top of liver cirrhosis, 16/30 (53%) of them were males and 14/30 (46.7%) were females, their ages ranged from 42-70 years (mean of 54.27 ± 6.4 years). The second group included 30 patients with liver cirrhosis on top of chronic liver disease, 18/30 (60%) of them were males and 12/30 (40%) were females, their ages ranged from 40-70 years (mean of 54.2 ± 9 years). A third group of 20 apparently healthy subjects serving as control group were also included in this study, 10/20 (50%) of them were males and 10/20 (50%) of them were females, their ages ranged from 42-66 years (mean of 54.10± 6.9 years), they were clinically free with normal laboratory findings and negative viral hepatitis markers and normal abdominal ultrasonogrphic findings. Informed written consent was obtained from all participants. Patients with heart failure, kidney failure and carcinoma elsewhere were not included, since these conditions may be associated with increased levels of CgA.

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 C.T.
3. Typical hsitopathological findings. The lesions were of grade I histopathologically in 2 patients (6.7%), of grade II in 25 patients (83.3%) and of grade III in 3 patients (10%).

The diagnosis of HCV infection was defined by positive tests for antibodies against HCV, based on an enzyme immunoassay. The diagnosis was confirmed by the presence of detectable HCV RNA in the circulation by polymerase chain reaction (PCR). Diagnosis of HBV was determined by HBsAg commercial enzyme immunoassay kits and confirmed by measurement of HBV DNA in serum by PCR. Diagnosis of Bilharziasis was done by IHA and confirmed by rectal snip.

All studied individuals were subjected to the following:

I. Full history and clinical examination.

II. Laboratory investigations:

(A) Routine investigation.

1. CBC using automated blood counter (Sysmex KX. 21 N).
2. Prothrombin time and concentration and INR using (Option 4 coagulometer) and (DiaMed "Dia Plastin" reagent)
3. Serum chemistry by (Bs-300 automated chemistry analyzer) including: urea, creatinine, albumin, AST, ALT, alkaline phosphatase and bilirubin (total and direct)

(B) Tumor markers:

1. Chromogranin (A).
2. Alpha feto protein.

Samples:

- Blood samples were obtained by peripheral venipuncture from patients.
- One sample was taken.

Table (1): Characteristics of liver cirrhosis and HCC groups according to viral hepatitis markers and antibilharzial antibody:

Parameter		Liver cirrhosis		HCC		Total
		NO.	%	NO.	%	NO.
Hepatitis C	+ve	22	73	27	90	49
	-ve	8	27	3	10	11
Hepatitis B	+ve	11	37	7	23	18
	-ve	19	63	23	77	42
Bilharziasis	+ve	6	20	5	17	11
	-ve	24	80	25	83	49
Hepatitis C & B	+ve	3	10	4	13	7
Hepatitis C & Bilharziasis	+ve	2	6	5	17	7
Hepatitis B & Bilharziasis	+ve	4	13	0	0	4

- The blood sample obtained was divided as follow:
  1. 1 ml of blood on 15 µL EDTA to perform CBC.
  2. 2.25 ml of blood on 250 µL sodium citrate to perform prothrombin time.
  3. 5 ml of blood was taken in plain tube then put in water bath at 37 ^{o}C for 30 minutes then centrifuged for 10 minutes then the resultant serum was divided into two aliquots. The first aliquot was used for routine investigations. The second aliquot was kept frozen at -20 C° for measurements of chromogranin A and alph-feto protein.

Serum CgA was assayed by a commercial kit from DRG International Inc., USA. The assay utilizes the two-site "sandwich" ELISA technique with two selected antibodies that bind to different epitopes of human chromogranin A.

Assay standards, controls and patient samples were directly added to microtiter wells of microplate that was coated with a polyclonal chromogranin A antibody. After the first incubation period, the antibody on the wall of microtiter well captured human chromogranin A in the sample and unbound antibodies in each microtiter well was washed away. Then a horseradish peroxidase (HRP) labeled monoclonal anti-human chromogranin A antibody was added to each microtiter well and a "sandwich" of "monoclonal antibody - human chromogranin A – polyclonal antibody" was formed. The unbound monoclonal antibody was removed in the subsequent washing step. For the detection of this immunocomplex, the well was then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader at 450 nm.

The enzymatic activity of the immunocomplex bound to the chromogranin A on the wall of the microtiter well was directly proportional to the amount of chromogranin A in the sample.

A standard curve was generated by plotting the absorbance versus the respective human chromogranin A concentration for each standard on point-to-point curve fit. The concentration of human chromogranin A in test samples was determined directly from this standard curve.

Serum AFP was measured by enzyme immunoassay (EIA) using human AFP EIA kit provided by DIMA Company, Germany. The test is based on simultaneous binding of human AFP to two monoclonal antibodies, one immobilized on microwell plates, the other conjugated with horseradish peroxidase. After incubation, the bound/free separation was performed by a simple solid-phase washing, and then the substrate solution (TMB) was added. After an appropriate time was elapsed for maximum color development, the enzyme reaction was stopped and the absorbance was determined at 450 nm against blank. The AFP concentration in the sample was calculated based on a series of standards. The color intensity was proportional to the AFP concentration in the sample.

Statistical Methods:

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 16. For quantitative data, mean and standard deviation were

calculated. Student "t" test: used to test the significance of the difference between two groups. Qualitative data was expressed as frequency and percentage.

Chi square test: to compare between qualitative parameters.

The association of serum chromogranin A level with continuous variables was tested with Pearson's correlation. P value was considered significant if < 0.05 & not significant if > 0.05.

3. Results

The results of the present study are summarized, statistically analyzed and presented in the following tables and figures.

Table (2): Blood picture and INR in control, liver cirrhosis and HCC groups:

Parameter	Group	N	Median	t		p	
Hb (gm/dl)	control	20	12.6	t_1	5.9	P_1	<0.01
	Liver cirrhosis	30	10.5	t_2	4.9	P_2	<0.01
	HCC	30	11	t_3	1.7	p_3	>0.05
WBCs (x 10^9 /L)	control	20	7.1	t_1	5.3	P_1	<0.01
	Liver cirrhosis	30	4.0	t_2	4.7	P_2	<0.01
	HCC	30	4.1	t_3	0.3	p_3	>0.05
Platelets (x 10^9 /L)	control	20	104.5	t_1	4.03	P_1	<0.01
	Liver cirrhosis	30	172	t_2	7.4	P_2	<0.01
	HCC	30	108	t_3	5.1	p_3	<0.01
INR	control	20	1	t_1	4.6	P_1	<0.01
	Liver cirrhosis	30	1.5	t_3	6.5	P_2	<0.01
	HCC	30	1.6	t_3	2.03	p_3	>0.05

t_1 & p_1 between control and liver cirrhosis.
t_2 & p_2 between control and HCC.
t_3 & p_3 between liver cirrhosis and HCC.
p value > 0.05 is considered non significant.
p value < 0.05 is considered significant.
p value < 0.01 is considered highly significant.

There was a highly significant statistical difference between control group and liver cirrhosis group and between control group and HCC group as regard Hb concentration, WBCs count, platelet count and INR (P<0.01) (table 2).

There was a highly significant statistical difference between HCC group and liver cirrhosis group as regard to platelet count (P<0.01) (table 2).

There was non significant statistical difference between HCC group and liver cirrhosis group as regard Hb, WBCs count and INR (p>0.05) (table 2).

There was a highly significant statistical difference between control group and liver cirrhosis group as regard AST, ALT, alkaline phosphatase, total bilirubin, direct bilirubin and albumin (P<0.01) (table 3).

There was a highly significant statistical difference between control group and HCC group as regard AST, ALT, alkaline phosphatase and albumin (P<0.01) (table 3).

There was a significant statistical difference between control group and HCC group as regard total bilirubin and direct bilirubin (P<0.05) (table 3).

There was a highly significant statistical difference between HCC group and liver cirrhosis group as regard AST (P<0.01) (table 3).

There was a significant statistical difference between HCC group and liver cirrhosis group as regard ALT, alkaline phosphatase and albumin (P<0.05) (table 3).

There was non significant statistical difference between HCC group and liver cirrhosis group as regard total bilirubin and direct bilirubin (P>0.05) (table 3).

There was a significant statistical difference between control group and liver cirrhosis group as regard AFP and Chromogrnin A (P<0.05) (table 4).

There was a highly significant statistical difference between control group and HCC group and between liver cirrhosis group and HCC group as regard AFP and Chromogrnin A (P<0.01) (table 4).

Table (3): Levels of to AST, ALT, and Alk.phosphatase, albumin, total bilirubin and direct bilirubin in control, liver cirrhosis and HCC groups.

Parameter	Group	N	Median	t		p	
AST (U/L)	control	20	27.5	t_1	15.5	p_1	<0.01
	Liver cirrhosis	30	67.5	t_2	13.6	p_2	<0.01
	HCC	30	95	t_3	6.2	p_3	<0.01
ALT (U/L)	control	20	24	t_1	12.9	p_1	<0.01
	Liver cirrhosis	30	56	t_2	9.3	p_2	<0.01
	HCC	30	58	t_3	2.9	p_3	<0.05
Alkaline phosphatase (U/L)	control	20	90	t_1	2.9	p_1	<0.01
	Liver cirrhosis	30	114.5	t_2	3.3	p_2	<0.01
	HCC	30	122	t_3	2.6	p_3	<0.05
Albumin (g/dl)	control	20	4.25	t_1	3.9	p_1	<0.01
	Liver cirrhosis	30	3.6	t_2	4.8	p_2	<0.01
	HCC	30	3.4	t_3	3.5	p_3	<0.05
Total bilirubin (mg/dl)	control	20	0.9	t_1	6.4	p_1	<0.01
	Liver cirrhosis	30	1.5	t_2	3.6	p_2	<0.05
	HCC	30	1.05	t_3	1.8	p_3	>0.05
Direct bilirubin (mg/dl)	control	20	0.2	t_1	5.1	p_1	<0.01
	Liver cirrhosis	30	0.4	t_2	3.4	p_2	<0.05
	HCC	30	0.3	t_3	0.2	p_3	>0.05

Table (4): Comparison between control, liver cirrhosis and HCC groups according to AFP & Chromogranin A:

Parameter	Group	N	Median	t		p	
AFP (ng/ml)	control	20	2.75	t_1	2.8	p_1	<0.05
	Liver cirrhosis	30	5.35	t_2	3.6	p_2	<0.01
	HCC	30	26.5	t_3	4.04	p_3	<0.01
Chromogranin A (ng/ml)	control	20	15.8	t_1	2.1	p_1	<0.05
	Liver cirrhosis	30	19.5	t_2	5.1	p_2	<0.01
	HCC	30	71.7	t_3	5.2	p_3	<0.01

Table (5): Sensitivity, specificity, PPV and NPV of AFP, Chromogranin A and both:

Parameter	sensitivity	specificity	PPV	NPV
AFP	86.7%	80%	81.3%	85.7%
Chromogranin(A)	83.3%	76.7%	78.1%	82.1%
AFP and Chromogranin(A)	90%	83.3%	81.8%	89.3%

PPV= Positive predictive value. NPV= Negative predictive value.

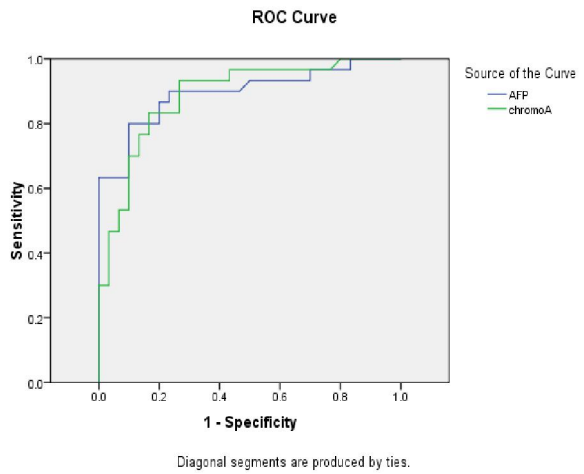

Figure 1: Comparison between AFP and Chromogrnin A by ROC curve.

Table (6): Area under the Curve for AFP and Chromogranin A

Test Result Variable(s)	AUC
AFP	0.895
Chromogranin A	0.886

* The more the area under the curve, the better is the test

Table (7): Correlation between AFP and different studied variables

Parameter	r	p
Chromogrnin A	-0.1	>0.05
Hb	-0.03	>0.05
WBCs	-0.03	>0.05
Platelets	0.2	>0.05
INR	0.5	<0.05
Creatinine	0.1	>0.05
Urea	0.4	>0.05
AST	0.1	>0.05
ALT	-0.02	>0.05
Alk.phosphatase	-0.1	>0.05
Total bilirubin	0.1	>0.05
Direct bilirubin	0.03	>0.05
Albumin	-0.1	>0.05

There was non significant correlation between AFP and Chromogranin A, Hb, WBCs, platelets, ALT, alkaline phosphatase, albumin, AST, total bilirubin and direct bilirubin, creatinine and urea (table 7).

There was a positive significant correlation between AFP and INR (table 7).

Table (8): Correlation between Chromogrnin A and different studied variables

Parameter	r	p
AFP	-0.1	>0.05
Hb	0.23	>0.05
WBCs	0.3	>0.05
Platelets	-0.04	>0.05
INR	0.14	>0.05
Creatinine	-0.06	>0.05
Urea	0.01	>0.05
AST	0.1	>0.05
ALT	-0.1	>0.05
Alk.phosphatase	0.85	<0.05
Total bilirubin	-0.1	>0.05
Direct bilirubin	0.3	>0.05
Albumin	-0.86	<0.05

There was non significant correlation between Chromogranin A and AFP, Hb, WBCs, platelets, INR, ALT, AST, total bilirubin and direct bilirubin, creatinine and urea (table 8).

There was a negative significant correlation between Chromogranin A and albumin (table 8).

There was a positive significant correlation between chromogranin A and alkaline phosphatase (table 8).

4. Discussion:

In Egypt, HCC is the third most frequent cancer in men with > 8000 new cases predicted by 2012[18]. 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[19].

In our study HCV as a cause of cirrhosis accounted for 90% of HCC patients reflecting the close relationship between HCV and HCC as one of prominent risk factors of developing HCC and this is in agreement with Montalto et al. [20] who reported that Liver cancer has a higher prevalence in patients with HCV-associated cirrhosis than in non-viral etiologies of chronic liver disease, while only a few cases of HCV-associated HCC have been reported in the non-cirrhotic liver, indicating that the virus possibly has a mutagenic effect.

In our study HBV carriers were 23% in HCC group and this is in agreement with Liu and Kao [21] who reported that HCC has been the first human cancer amenable to prevention using mass vaccination programs from global perspective. The burden of chronic HBV infection is expected to decline because of increasing utilization of HBV immunization since the early 1980 and this also in

agreement with El-Zayadi et al. [3] who stated that the relative risk of developing HCC for HBV carriers may be 100-200 fold higher than that for non-carriers however, the prevelance of HBV infection in Egypt has been declining over the last two decades.

Concerning hematological tests in this study, most of the patients with cirrhosis and HCC have significantly lower hemoglobin value and platelet count ($p < 0.01$) in comparison to the apparently healthy control group and this was in agreement with Franca et al. [22] who reported that there are various theories about thrombocytopenia in chronic liver diseases, portal hypertension, hypersplenism and bone marrow suppression are factors associated with thrombocytopenia.

On analysis of liver biochemical profile, there was significant deterioration in cirrhosis and HCC patients when compared to the control group and this was in agreement with Sleisenger and Fordtran [23] who reported that these tests will usually indicate the type of liver injury, whether hepatocellular or cholestatic, but cannot be expected to differentiate one form of hepatitis from another or to determine whether cholestasis is intra or extrahepatic.

In our study most of the HCC lesions on ultrasound (60%) were found in the right lobe of the liver and this was in agreement with that reported by Nihal et al. [24].

As regard the CT pattern of the HCC lesions in triphasic CT scan 26 lesions (86.7%) showed typical enhancement features of HCC (typical specific pattern of arterial uptake followed by venous washout in the delayed venous phase) this was in agreement with Peterson et al. [25] who performed a screening test on large populations of HCC patients before transplantation revealing that CT scanning showed typical enhancement pattern in 68% of HCC patients.

In this study histopathological assessment was done for patients with HCC revealing that 6.7% of patients were grade I, 10 % grade III and 83.3% were grade II and this was in agreement with that reported by Darwish [26] who noticed that grade II was more detected than the other two grades in biopsied HCC lesions.

Current diagnosis of HCC relies on clinical information, liver imaging and measurement of serum alpha-fetoprotein [27, 28].

In this study, there was statistically highly significant elevation ($p < 0.01$) in the median serum AFP in HCC group (26.5 ng/ml) when compared with control group (2.75 ng/ml) and highly significant elevation ($p<0.01$) when compared with cirrhotic group (5.35 ng/ml) and this is not in agreement with Massironi et al. [17], who reported no significant difference in AFP between HCC and liver cirrhosis and healthy subjects. In our study considering cut off value of 7.295 ng/ml (mean ± 2SD), the sensitivity of AFP was (86.7%) and the specificity was (80%). These results are in agreement with Massironi et al. [17] who reported a sensitivity of (75%) and specificity of (80%).

Our results revealed that there was a statistically highly significant elevation ($p<0.01$) in the median serum CgA in HCC group (71.7 ng/ml) when compared with control group (15.8 ng/ml) and highly significant elevation ($p <0.01$) when compared with cirrhotic group (19.5 ng/ml).

These results are in agreement with Leone et al. [17], Spadaro et al. [29] and Massironi et al. [17] who reported statistically significant elevation of CgA serum levels in HCC when compared to those in cirrhotic patients. Although it is not clear why a non neuroendocrinal tumor such as HCC express CgA, Bosman [30] noted that neuroendocrine differentiation can occur in carcinomas that lack neuroendocrine cells in their normal epithelial counterparts, such as hepatocellular carcinoma.

In our study considering the cut off value of 28.78 ng/ml the sensitivity of CgA was (83.3%) and the specificity was (76.7%). These results are in agreement with Massironi et al. [17] who reported a sensitivity of (70%) and specificity of (67%). The area under the rock curve for CgA was 0.886 compared to 0.895 for AFP.

In our results the combined use of the two markers AFP and CgA led to increase in the specificity of AFP and CgA from (80%) and (76.7%) respectively to (83.3%) and increase in the sensitivity of AFP and CgA from (86.7%) and (83.3%) respectively to (90%). These results were in agreement with Spadaro et al. [29] who concluded that when AFP is normal or < 200 ng/ml and in the presence of suspicious clinical, laboratory and or imaging signs of HCC, the evaluation of CgA levels becomes of particular importance in the follow up of chronic liver disease patients. This showed that simultaneous measurements of serum AFP and CgA are of value in detecting HCC.

Concerning the correlation between the levels of AFP and CgA there was no correlation between serum CgA and AFP in patients of HCC group. This was in agreement with Spadaro et al. [29] who reported no correlation between both markers in patients with HCC.

In conclusion, the results of the present study in keeping with evidences from literature revealed that the application of CgA as a tumor marker in the diagnosis of HCC is to be considered especially in cases with low levels of AFP, as determination of CgA serum values represents a

complementary diagnostic tool in monitoring chronic liver disease patients for detection of HCC. CgA could be combined with AFP to detect HCC, as the combined use of both markers increases their sensitivity and specificity.

A screening program for HCC detection is recommended to all patients with cirrhotic liver by tumor markers such as combined use of alpha-fetoprotein and chromogranin A and ultrasonography. However triphasic C.T and/or liver biopsy may be needed especially if the previous methods were not conclusive. On basis of these findings we are recommending further studies including a large number of patients to ascertain whether circulating CgA is useful as a prognostic marker and to evaluate its significance in the diagnosis of HCC. A follow-up of CgA serum values after treatment of HCC is also recommended in order to define the utility of the marker for the detection of recurrent tumor.

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