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Serum level of cripto -1 in hepatitis B virus & hepatitis C virus induced hepatocellular carcinoma

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Abstract
Background: Cripto-1 is over expressed in tissues and closely correlated with the invasion and proliferation of Hepatocellular carcinoma (HCC) cells. The aim of this work is to measure the serum concentrations of Cripto-1 and its relation to the clinical aggressiveness of HCC in hepatocellular carcinoma (HBV) and hepatitis C virus (HCV) infected individuals.
Methods: This work was performed on 60 individuals with clinical criteria of newly diagnosed HCC, hepatitis related liver cirrhosis and HBV or HCV diagnosed by PCR. Participnts were split into equal five groups: Group I: HCC on top of HBV related cirrhosis. Group II: HCC on top of HCV-related cirrhosis. Group III: HBV with hepatic-cirrhosis and no HCC. Group IV: HCV with hepatic-cirrhosis and no HCC. Group V: healthy subjects as control group.
Results: a statistically substantial rise was exited in the mean values of cripto-1 of HCC and cirrhotic patient groups contrasted to the control group and HCC with HBV HCC with HBV (P=0.001), and there was also significant increase in HCC patients group values and cihrotic HBV patients compared to cirrhotic patient's groups (P=0.001). In HCC and cirrhotic patients groups a significant positive association was existed among cripto-1 and each of Alpha-fetoprotein (AFP), serum glutamic-pyruvic transaminase (SGPT), serum glutamic-oxaloacetic transaminase (SGOT), and prothrombin time (PT).
Conclusions: Serum levels of cripto-1 is greater in HCC on top of HBV patients than HCC on top of HCV patients. Serum levels of cripto-1 is greater in cihrotic HBV individuals than cihrotic HCV patients. Cripto-1 can be used as aguide in early diagnosis of HCC.

Keywords: Cripto -1, Hepatitis C Virus, Hepatitis B Virus, Hepatocellular Carcinoma

Introduction
The third most prevalent reason for cancer-related death is hepatocellular carcinoma (HCC), a common solid tumour that affects people all over the globe. HCC is more likely to develop in people with chronic hepatitis B virus (HBV) infections, particularly in those who also have cirrhosis and chronic liver disease [1].
The most frequent factor that predisposes to HCC is liver cirrhosis. Viral hepatitis C and B are the most prevalent causes of liver cirrhosis, and detecting HCC early in its progression is essential to improving the survival of afflicted individuals [2]. About 80% of instances of HCC occur in people with cirrhotic livers. In addition to starting various kinds of changes in the liver, persistent infection with HCV that is capable of replication also fosters the growth of liver cancer [3].
In sets of the globe where the hepatitis B virus (HBV) is widespread, chronic HBV infection is a significant etiological factor for HCC [4]. According to three recognised pathways, the hepatitis B virus induces carcinogenesis: (1) The proteins of HBV participate in a variety of hepatocyte signalling pathways, which influences the expression and activities of certain genes and causes liver diseases. The majority of these alterations are connected to HCC. (2) Endogenous genes' functions are changed or brought on by the integration of HBV DNA into the host genome. (3) The modification of certain signalling pathways brought on by inflammation aids in the development of tumours. Inflammation that persists over time is crucial to the occurrence of HCC. Hepatocarcinogenesis is made more likely by recurrent cycles of inflammation-induced apoptosis and hepatocyte regeneration [5].
The teratocarcinoma-derived growth factor-1 (TDGF-1) family, which includes human Cripto-1, is additionally recognised as epidermal growth factor (EGF)-Cripto-1, fibroblast

growth factor related ligand (FRL1)/Criptic (EGF-CFC) [6].
It is generally known that cripto-1 performs crucial biological tasks throughout embryogenesis. It is essential for coordinating the creation of primitive streaks, the determination of endoderm and mesoderm, and the direction of the anterior and posterior (A/P) axes. Normal adult tissues do not contain Cripto-1, although malignancies have been shown to re-express Cripto-1 [5].
Cripto-1 is regarded as a stem cell marker since it has been demonstrated to retain the ability and self-renewal of human and embryonic stem-cells from mice [7]. Cripto-1 is inappropriately re-expressed in a variety of epithelial malignancies in adults, indicating a connection between stem cells and tumour development [8].
The aim of this study was to measure the serum concentrations of Cripto-1 and its relation to the clinical aggressiveness of HCC in HBV and HCV infected patients.

Patients and Methods

This work was performed on 60 individuals aged more than 18 years old, both sexes, with clinical criteria of newly diagnosed HCC, hepatitis related liver cirrhosis and HBV or HCV diagnosed by PCR.
After receiving clearance from Tanta University Hospitals' Ethical Committee, the research was carried out. All patients provided written permission after being fully briefed.
Exclusion criteria were other malignant diseases, hepatic encephalopathy and other causes of liver diseases (drug-induced, autoimmune hepatitis, alcoholic liver diseases).
Patients were categorized into equal five groups: Group I: individuals with HCC upon hepatitis B-related cirrhosis. Group II: individuals with HCC upon hepatitis C-related cirrhosis. Group III: upon with hepatitis B with cirrhotic liver and no HCC. Group IV: individuals with hepatitis C with cirrhotic liver and no HCC. Group V: apparently healthy instances as control group.
All patients had been exposed to; taking of history, clinical assessment (a focus on the signs and symptoms of chronic liver illness), radiological examination (ultrasonographic scan of abdomen & pelvis and triphasic CT abdomen), routine investigations [liver and kidney function tests and viral markers testing (HCV, HBV by Polymerase Chain Reaction (PCR) and Alpha-fetoprotein (AFP)] and specific investigation **(s**erum Cripto 1 level using enzyme-linked immuno sorbent assay technique).
Liver functions done by automated chemistry analyzer, sample oriented, random access (Beckman caulter Au480), kidney functions done by automated chemistry analyzer, sample oriented, random access (Beckman caulter Au480), prothrombin time (pt): by semi-automated coagulation analyzer. AFP: done by automated analyzer (Accex II), HCV-RNA: by real time PCR, HBV-DNA: by real time PCR and Serum Cripto-1: by enzyme-linked immune-sorbent assay (Human Teratocarcinoma Derived Growth Factor 1(Cripto-1) ELISA kit catalogue no.201-12-2604).

Cripto-1 assessment by ELSA

The kit combines an enzyme-linked immunosorbent assay (ELISA) with a double-antibody sandwiches to measure the concentration of Human Teratocarcinoma Derived Growth Factor1 (Cripto-1) in specimens. In an antibody monoclonal enzyme well that has already been coated with human Cripto-1 monoclonal antibodies, add Cripto-1. Combining biotin-labeled Cripto-1 antibodies with streptavidin-HRP to create an immunological complex, followed by further incubation and washing to get rid of the uncombined enzyme. When Chromogen Solution A or B is incorporated, the liquid's colour alters to blue. When acid is present, the colour eventually turns yellow. The content of the human substance Cripto-1 in the specimen and the colour chroma had a positive correlation. Within 10 minutes Calculation, stop solutions were applied, and the OD value was measured.

Statistical analysis

SPSS v26 (IBM Inc., Chicago, IL, USA) was used for the statistical analysis. The ANOVA (F) test with the post hoc test (Tukey) was used for comparing the quantitative parameters across the five groups after they were reported as mean and standard deviation (SD). The Chi-square test was used to analyse qualitative parameters, which were reported as frequencies and percentages (%). A two tailed P value < 0.05 was considered statistically significant.

Results

No significant variation was existed in demographic data among the studied groups. (Table 1)

Table 1: Demographic data of the age and sex within the study groups

		Group I [N=15]	Group II [N= 15]	Group III [N=15]	Group IV [N= 15]	Group V [N=15]	P value
Age (years)		54.47 ± 7.94	53.87 ± 5.66	48.15 ± 6.57	49.27 ± 7.54	49.33 ± 5.77	0.137
Sex	Females	5 (33.3%)	5 (33.3%)	7 (46.7%)	7 (46.7%)	6 (40%)	0.126
	Males	10 (66.7%)	10 (66.7%)	8 (53.3%)	8 (53.3%)	9 (60%)	

Data are presented as mean ± SD or numbers of (%)

Albumin was significantly reduced in-patient groups compared to control groups (p<0.001). Albumin was substantially lowered in group II than other patient groups. Serum Total bilirubin, AST, ALT, prothrombin time, activity and AFP level is significantly higher in-patient groups than control groups (p<0.001). Level of AFP was substantially higher in group I & II than group III& IV (cirrhotic patient). Patients from group II had significantly higher levels of ALT, AST, prothrombin time, activity and levels of total bilirubin than other patient groups. no statically significant variation was existed in creatinine and urea level among studied groups. (Table 2)

Table 2: Comparison of the mean value of laboratory tests within the study groups

	Group I [N=15]	Group II [N= 15]	Group III [N=15]	Group IV [N= 15]	Group V [N=15]	P value
Albumin (gm/dl)	3.26 ± 0.34	2.96 ± 0.20	3.02 ± 0.46	3.21 ± 0.35	4.33 ± 0.48	<0.001
Total bilirubin (mg/dl)	1.70 ± 0.28	4.89 ± 0.52	1.62 ± 0.27	3.66 ± 1.96	0.92 ± 0.14	<0.001
AST (IU/ml)	44.27 ± 32.41	101.93 ± 16.71	41.87 ± 21.66	3.40 ± 21.70	20.53 ± 5.69	<0.001

ALT (IU/ml)	38.87 ± 30.99	108.07 ± 16.11	33.27 ± 13.78	3.20 ± 22.55	19.87 ± 6.74	<0.001
Prothrombin (sec)	17.2 ± 2.42	19.1 ± 1.88	18.68 ± 2.42	18.1± 2.52	13.47 ± 1.06	<0.001
Prothrombin activity	65.60 ± 2.72	56.93 ± 6.60	58.93 ± 4.18	60.80 ± 5.94	95.27 ± 2.05	<0.001
Creatinine (mg/dl)	1.01 ± 0.19	1.04 ± 0.18	0.99 ± 0.20	1.24 ± 0.29	0.97 ± 0.16	0.062
Urea (mg/dl)	24 ± 2.75	20.27 ± 2.49	19.93 ± 2.40	29.80 ± 7.86	22.87 ± 5.05	0.233
AFP (mg/dl)	1004.47± 736.64	824 ± 58.44	188.33 ±95.42	217.53 ± 07.81	5.73 ± 2.43	<0.001

Data are presented as mean ± SD, AST: aspartate transaminase, ALT: alanine transaminase, AFP: Alpha-fetoprotein.

Cripto-1 was elevated in patients who received triple therapy with (sofa+ dacla + ribavirin) for 3 months in comparison with those who received dual therapy (sofa + dacla) for 3 months but didn't reach statistically significant value. (Table 3)

Table 3: Comparison between level of cripto-1 within the study groups and in patients who received triple therapy for 3 months and who received dual therapy for 3 months

	Group I [N=15]	Group II [N= 15]	Group III [N=15]	Group IV [N= 15]	Group V [N=15]	P value
Cripto-1 (mg/dl)	1.90 ± 1	1.36 ± 0.61	1.81 ± 0.80	1.06 ± 0.40	0.12 ± 0.05	<0.001
Triple therapy for 3 months	1.3 ± 0.52					0.208
Dual therapy for 3 months	1.19 ± 0.72					

Data are presented as mean ± SD.

Regarding the correlation between cripto-1 and laboratory. Parameters show that there was a highly significant positive association. Between cripto-1 total bilirubin, AST, ALT, prothrombin time, and AFP. There was a high significance negative association among cripto-1 and abumin and urea. No significant association was existed among cripto-1 and creatinine and age. And no significant association was existed among cripto-1 and duration since treatment of hepatitis either HBV & HCV treatment (Table 4).

Table 4: Correlation between Cripto-1 and other variables of the study

Cripto-1	r	P value
Age	-0.166	0.156
Albumin	-0.481	<0.001*
Total bilirubin	0.287	0.013*
AST	0.338	0.003*
ALT	0.264	0.022*
Prothrombin	0.248	0.032*
CREAT	-0.049	0.677
Urea	-0.582	<0.001*
AFP	0.512	<0.001*
Duration between treatment of hepatitis and diagnosis of HCC	0.149	0.208

*Significant ≤0.0.5. AST: aspartate transaminase, ALT: alanine transaminase, AFP: Alpha-fetoprotein. HCC: hepatitis c virus.

Discussion

Hepatocellular carcinoma (HCC) is an issue for health across the world. It ranks 3rd in annual mortality from cancer and is the fifth most prevalent solid tumour. Seventy-five percent of malignant liver tumours in Egypt are HCCs. In terms of prevalence, liver cancer ranks fifth in both sexes, sixth in women (3.4% of all cancers), and second in men (2.5% of all cancers) following cancer of the urinary bladder constituting 11.5% of cancer cases. 2010 saw liver cancer rank third in both sexes (8.1%), first in men (12.1%), and fifth in women (4%) [9].

In the present study the age incidence of HCC patient ranged from (45-60) years. This in line with wang et al [10] work that demonstrated that no significance variation was existed in age among studied patients. As regard to sex in this study there was no difference between male and female in hepatitis infection or progression to cihrosis or HCC. This in disagreement with previous study that HCC and chronic viral hepatitis were more common in men than in women [11].

In the present study regarding albumin, it was lower in HCC patients than individuals with cirrhosis than control instances. This agreed with [10], which showed that Individuals with chronic viral hepatitis showed normal albumin levels. While cirrhotic individuals in the current research exhibited low albumin levels, those who have advanced cirrhosis virtually usually have hypo-albuminemia [12]. Majority of the individuals with HCC in this research had low blood albumin levels, however few had normal levels. High blood albumin levels are a key predictor of a good prognosis since prior studies found that individuals with HCC had a low recurrence rate and high serum albumin concentrations [13].

This work showed that serum total bilirubin is significantly greater in-patient groups compared to control groups, and group II (HCC on top of hepatitis C virus (HCV)) &IV (cihrosis on top of HCV) had significant higher levels of total bilirubin than other patient groups. Another study shows that bilirubin levels may be elevated in HCC patients and HCV patients, and the higher levels of T.bilirubin indicates more sever disease [14]. This agrees with the present study.

The current study showed an increased level of alanine transaminase (ALT) and AST levels in cirrhotic patients and HCC patients, and the highest level of ALT & aspartate transaminase (AST) was found in HCC related HCV patients than other patient and control subjects.

In earlier research, individuals with HCV and HBV

infections had persistently high ALT and AST values [15]. Most individuals with persistent infections have mild ALT and AST level increases [16]. This was consistent with the current research. Another research, however, revealed that individuals with persistent HCV infection had normal or borderline ALT readings [17]. Another research conducted on individuals with HCV infection revealed a substantial correlation between the prevalence of HCC and high ALT levels over 70u/L. [18].

As regards prothrombin time the present study showed that prothrombin time (PT) was prolonged in cirrhotic and HCC patients than control group. Lower plasma protein production by the liver results in lower serum concentrations of various clot-forming factors in severe hepatic disease. Due to thrombocytopenia, impaired platelet activity, or a combination of these causes, haemorrhage may happen as a side effect of chronic liver disease [18].

Unless cirrhosis exists and the liver fibrosis is very severe, the PT is often not raised. PT does not evaluate the impact of fibrinolysis agents; it solely evaluates the generation of fibrin from thrombin [19].

In the present work, AFP in HCC patients was higher than its level in cihrrotic patients and showed normal level in control group. HCC may result in AFP readings ranging from normal to more than 100 000 ng/ml, which is consistent with prior research by Wang et al. [10] who demonstrated that individuals with HCC also had greater AFP levels compared to patients with cirrhosis did. This is due to AFP is a foetal component protein which is produced throughout the embryonic phase by the visceral endoderm of the gestational sac and, subsequently, by the liver [20]. Elevations in blood blood levels of AFP have been linked to tumours developing in organs with endodermal lining similar to that of the hepatic diverticulum, such as malignancies of the pancreas, stomach, and biliary tract [21].

As contrasted to other groups of patients in this investigation, HCC patients' serum Cripto-1 levels were considerably higher than those of the control group, which indicated a normal level. Individuals with cirrhosis brought on by HBV had considerably higher serum Cripto-1 levels, which raises the possibility that they have a higher chance of developing HCC. It appears to be a potential novel biomarker for the detection of HCC linked to HBV. Due to the fact that HBV is an oncogenic virus, it may generate HCC even if there is no evidence of cirrhosis. In the present investigation, individuals who had HCC on top of HBV as well as those with HBV-related cirrhosis had blood levels of Cripto-1 that were greater than those with HCV-related HCC and cirrhosis.

And this agrees with previous study [6] found that serum cripto-1 is elevated in HCC on top of HBV patients than HCC on top of HCV and elevated in cihrotic HBV patients than cihrotic HCV patients.

This revealed that we can use serum level of cripto-1 examination as follow up marker in observation of cihrotic patient to follow up progression of HCC, as once there is increase in levels of cripto-1 it is suggestive to take more pericautions in following up those patients as they may have foci of HCC, and it will be good in early diagnosis of HCC.

The study recommended that measurement of cripto-1 in sera of large number of cihrotic individuals and follow-up might pave the way to pick up early stage of HCC and demonstrated its impact on prognosis.

Conclusions

According to the study's findings, it was determined that; Serum level of cripto-1 is higher in HCC on top of HBV patients than HCC on top of HCV patients. Serum levels of cripto-1 is higher in cihrotic HBV patients than cihrotic HCV patients. Cripto-1 can be used as aguide in early diagnosis of HCC.

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Conflict of Interest
Nil

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