DISCUSSION

Hepatocellular carcinoma (HCC) is the most common primary liver tumor. It represents the third leading cause of cancer death worldwide. It is the fifth most common cancer in men and seventh in women, accounting for 7% of all cancers. (151)

The burden of HCC has been increasing in Egypt, with a doubling in the incidence rate in the past 10 years. (152) Early detection of HCC opens doors for various effective treatments, which can subsequently lead to long term survivals in a great number of HCC patients. (153)

Clusterin (CLU) gene in human is a single-copy gene located on chromosome 8 p21-p12 which is expressed in almost all tissues. (154) Although many reports were done to explain CLU functions in various cell types and tissue, an understanding of CLU function has remained elusive, especially in term of apoptosis and tumorigenesis. (131)

The aim of the present work was to study the significance of serum CLU level as a serological tumor marker in HCC on top of HCV related hepatic cirrhosis, compared to serum AFP level and to correlate the serum level of clusterin in the studied groups with the clinical and the laboratory findings.

This study was conducted on 21 patients suffering from HCC on top of HCV related hepatic cirrhosis, 25 patients with chronic HCV related liver cirrhosis, 25 patients with chronic HCV with no evidence of liver cirrhosis and 10 healthy control subjects. All patients in this study were admitted to hepatobiliary unit, Internal Medicine Department. Alexandria Main University Hospital. HCC patients were diagnosed by abdominal ultrasonography and triphasic CT abdomen as well as serum AFP level.

In this study, males predominated females in all studied groups. The same finding was reported by Nafee AM et al (154), Comunale MA et al, (155) Abdel Aal HE. (156) The reasons for the disparity between men and women are obscure, may be environmental factors such as a higher prevalence of chronic HBV or HCV infection, alcohol abuse and smoking in men than in women. Genetic and hormonal factors may also be important, this has been reported in a study by Naugler WE et al. (157)

In the present study, right hypochondrial pain was a very common complain among HCC patients represented by 66.7%. This was reported by Abbasi A et al, (158) in 63.4% of HCC patients. This can be explained by hepatomegaly associated with the tumor and consequent stretching of its capsule which may cause aching pain in the right hypochondrium. Other causes of pain in enlarged liver are traction of the supporting ligaments when standing or walking, the liver pressing against the rib cage or pinching the wall of the abdomen, and straining the lumbar spine. The tumor may also infiltrate the liver's capsule, causing dull and sometimes stabbing pain. (159)

Jaundice was present in 33.3% of HCC patients, while ascites was found in 85.7% of patients. GIT bleeding, lower limb edema and splenomegaly were also very common representing 71.4%, 85.7% and 66.7% respectively. Also spider angioma and palmer erythema were found and were represented by 47.6 % and 57.1 % respectively. Presence of jaundice in HCC patients is explained by infiltration or extrinsic compression of the tumor

on biliary tree [160] while ascites, GIT bleeding and splenomegaly were attributed to portal hypertension. [161] Spider angioma and palmer erythema were explained by elevated estradiol levels. [162]

In the present study, there was a significant decrease of hemoglobin concentration among HCC patients. This was in agreement with Yosry A et al, [163] and may be attributed to loss of appetite, maldigestion or bone marrow suppression in patients with malignancy, as reported by Li XQ et al. [164]

There was also a significant decrease in platelet count in this group. This was in accordance with Franca A et al, [165] who reported that there are various theories about thrombocytopenia in chronic liver disease where portal hypertension, hypersplenism and bone marrow suppression are associated factors.

On analysis of liver biochemical profile, there was a significant increase in ALT, AST and ALP serum levels. Also, a significant decrease in albumin levels and prothrombin activity were found and this was in agreement with Abdel Aal HE, [156] Awadallah AM et al, [166] and Yorsy A et al. [163] on the other hand Nafee et al, [154] reported no significant difference in prothrombin activity. The elevated serum levels of ALT and AST are attributed to their direct release from injured or damaged hepatocytes into blood and their levels have been widely recognized as effective tools to detect liver diseases. [167] Low serum albumin concentration denotes poor liver function as albumin protein is synthesized only in the liver. Decreased serum albumin levels are not seen in acute liver failure because it takes long time of impaired albumin production until the serum albumin level drops. [168] Serum albumin concentration may be normal in chronic liver disease, until cirrhosis and significant liver damage develops. [168]

As regards total bilirubin level, it was increased in HCC patients but with no significant difference between the studied groups. This disagrees with results of Yosry A et al [163] and Nafee AM et al, [154] as both of them reported a significant difference in similar groups. Hyperbilirubinemia in HCC patients develops in later stages of the disease, and can be caused by diffuse cancer infiltration, invasion of the biliary ducts, extrinsic tumor compression on the extrahepatic biliary tree, progressive liver failure, and sever cirrhosis.[161]

In this study, serum AFP cut off value was calculated from its ROC curve to be 11ng/ml. This was in accordance with the cut off value of Choi JY et al [169] and Shashi BP et al. [170]

In HCC patients **(Group III)**, serum AFP level was significantly higher in comparison to other studied groups; 8.75± 7.27 ng/ml, 11.85± 9.9 ng/ml and 154.3± 191.7 ng/ml for **Group I**, **Group II** and **Group III** respectively. This was in agreement with Nafee AM et al, [154] Awadallah AM et al [166] and Yosry A et al. [163] On contrary Massironi S et al [171] reported no significant difference in AFP between HCC group and other similar groups. However AFP has a limited role in HCC diagnosis since it may be increased in some of benign chronic liver disease or it may be normal in patients with HCC. [154]

As regards correlation studies in this work, serum AFP was positively correlated with the progression of BCLC staging system. With no significant correlation between serum

AFP levels and any of its criteria (tumor size, number of nodules, capsular infiltration, portal vein invasion or L.N metastasis).

In this work, 6 out of 21 HCC patients **(Group III)** had serum AFP level below the cut off value, while 9 out of 25 HCV patients **(Group I)** and 13 out of 25 HCV related hepatic cirrhosis patients **(Group II)** had serum AFP levels above the cut off value. Thus, AFP had poor diagnostic performance where specificity, sensitivity, PPV, NPV and accuracy were 66.67%, 62.0%, 42.42%, 80.58% and 63.38% respectively. However, El-Zayedi AR et al [174] considered that AFP is the most widely used tumor marker but has poor diagnostic performance.

It has been reported by many studies that AFP-L3, one of the AFP isoforms is a more valuable index than total AFP for early diagnosis of HCC. It has been used as a marker for early diagnosis and assessment of the therapeutic effect as well as prognosis of HCC. AFP-L3 was found to be associated with liver dysfunction, poorly differentiated tumors, and other biologically malignant characteristics. [173]

CLU is a highly conserved multifunctional glycoprotein present in all mammalian tissues and almost human body fluid. CLU is implicated in various physiological processes. It has been reported to play a significant role in stress response including apoptosis [154] and tumorigenesis. [174] Some reports documented a decrease of CLU level in the serum of some human cancers, [175-177] others reported an increased levels in the majority of other human cancers. [178-180]

The present study demonstrated a significant increase in the level of serum CLU in HCC patients compared to all other groups. No significant difference was found between serum CLU level in HCV positive patients with or without cirrhosis. On the other hand, Wang Y et al [181] reported a significant decrease of serum CLU level in HCC patients when compared to those with chronic hepatitis and healthy subjects, but it was higher than those with cirrhosis. Li Y et al [182] also reported that serum expression of clusterin is significantly decreased in both serum and tissues of HCC patients.

Increased serum CLU has been reported in HCC patients in many other studies such as Chen D et al, [183] Lau SH et al, [184] Wang C et al, [185] Kang YK et al [186] and Chau GY et al. [188] Increased serum CLU might play a role in tumorigenesis and could be used as a marker for early detection of cirrhotic liver that progressed to HCC whereas significantly lower levels of serum CLU may be due to reduced liver cell mass or regenerating nodules that cannot express clusterin like normal cells. [154]

Kang YK et al [186] studied immune-reactive pattern of CLU in patients with HCC and found two distinct patterns, namely; cytoplasmic one and canalicular one. They also concluded that cytoplasmic overexpression is a predictor for poor survival compared to canalicular one. Also, Rodríguez-Piñeiro AM et al [188] pointed to the importance of measuring CLU isoforms rather than total serum level. They demonstrated an increase in some isoforms and a decrease or absence of others.

The discrepancies of results between different studies may be attributed to differences in selection of cases, differences in technical methods (use of different antibodies, cell lines) as well as the diversity of CLU function and shift of pattern of its

isoforms production. Thus CLU is suggested to be lying at the crossroad of life and death and at the same time reflecting the fact that it may be a tumor suppressor or promoter. [95]

As regards correlation studies in this work, there was no correlation between serum CLU levels and the degree of deterioration of functional liver status assessed by Child-Pugh score and this was in agreement with Nafee AM et al [154] reports which suggested that increased serum CLU levels in HCC patients could be related to the process of carcinogenesis rather than cirrhosis or fibrosis.

Also, there was no significant correlation between serum CLU level and size of tumor or number of nodules in HCC patients, this is in agreement with Nafee AM et al [154] but disagree with Chau GY [187] who reported a higher serum CLU level in patients with tumor size > 5 cm and patients with multiple tumor nodules.

Also, no correlation was found between serum CLU levels and different laboratory findings (hematological and chemical).

As regard clinical examination and presenting signs and symptoms, a significant correlation was found between serum CLU level and fatigue, spider angioma and palmer erythema. Moreover, serum CLU level was positively correlated with the progression of BCLC staging system as well as portal vein invasion and L.N metastasis in HCC patients, this was in accordance with Chen D et al [183] and Nafee AM et al. [154] This finding suggests that CLU may promote HCC migration and metastasis, this may be explained by Wang C et al [185] who reported that overexpression of CLU increases the cell migratory ability via epithelial mesenchymal transition.

Diagnostic performance of CLU at a cut off value of 179 ng/ml (calculated from its ROC curve), showed sensitivity and specificity to be 95.24% and 79% respectively, PPV, NPV and accuracy were 57.1%, 97.22% and 77.46% respectively. The change of cut off value is followed by different diagnostic performance as shown by reports of Wang Y et al[181] with a cut off value of 50 ng/ml and Nafee AM et al [154] with a cut off value 128 ng/ml.

When performing the ROC curve, the AUC of serum CLU covers a greater area (0.898) than that of AFP (0.793). This suggested that serum CLU might be superior to AFP in diagnosing HCC and differentiating it from cirrhosis and this was clear from the diagnostic performance (sensitivity, specificity, PPV, NPV and accuracy) of both of them. These results agreed with that of Wang Y et al [181] who reported AUC for serum CLU and AFP to be 0.937 and 0.781 respectively and also in agreement with Nafee AM et al [154] reports where CLU covers a greater AUC (0.95) than that of AFP (0.85).

When using CLU and AFP in combination for diagnosis of HCC, sensitivity, specificity, PPV, NPV and accuracy were 71%, 100%, 100%, 89% and 91.5% respectively. So, it is better to use AFP and CLU in combination to get better diagnostic performance.

From the previously reported data of this work, it is concluded that serum CLU was up regulated in HCC and was more sensitive and more specific than AFP for differentiating HCC patients from those with cirrhosis.

CLU was positively correlated with BCLC staging, portal vein invasion and L.N metastasis suggesting that CLU is a promising useful marker for diagnosing HCC and predicting the prognosis and metastasis of HCC.

SUMMARY

HCC is one of the most common cancers worldwide. It has become the third most common malignancy worldwide with very poor prognosis, rendering it the third highest cause of cancer-related deaths.

In Egypt, there is a high incidence of the disease as a result of a high level of viral exposures. Egypt suffers from the world's highest prevalence of HCV infection. Moreover, the intra-familial spread of HCV has been documented as one of the most important routes of transmission, leading to the current estimated burden of up to 10 million infected Egyptians.

Risk factors of HCC include HBV, HCV, dietary aflatoxin, alcohol and obesity. Most risk factors promote formation of cirrhosis.

HCC classically arises and grows in a silent fashion, making its discovery challenging prior to the development of later stage disease. The various clinical presentations generally relate to the extent of hepatic reserve at time of diagnosis. Symptoms include malaise, anorexia, wasting, right upper quadrant abdominal pain, and distension. Physical examination may reveal an abdominal mass or hepatomegaly with hard and irregular borders that may demonstrate a vascular bruit.

Carcinogenesis of HCC is a multi-factor, multi-step, and complex process. The cellular origin of HCC has long been debated, but whether HCC originates from mature hepatocytes, stem/progenitor cells, or both remain unclear. The fact that many liver tumors arise on top of cirrhosis, where hepatocyte senescence triggers the activation of liver progenitors, causes further confusion.

Cancer classification is intended to establish prognosis and enable the selection of the adequate treatment for the best candidates. In addition, it helps researchers to exchange information and design clinical and therapeutic trials with comparable criteria. There is no worldwide consensus on the use of any given HCC staging system. However, most major trials of HCC therapy have chosen the Barcelona clinic liver cancer (BCLC) staging system making it the reference staging system, as the BCLC has demonstrated superior survival stratification and prognosis prediction over the other classifications.

AFP has been considered for a long time the ideal serological marker for detecting HCC, and is still the most widely tested biomarker in HCC. However, AFP alone has a limited role in HCC surveillance as it may increase in some benign chronic liver disease or it may not increase in serum of some HCC patients. Therefore, there is a need for developing simple and reliable serum markers that will improve the detection of early HCC.

CLU is widely distributed in different tissues and highly conserved in species. There are two isoforms (1 and 2) with antagonistic actions regarding apoptosis. CLU is implicated in a number of biological processes, including lipid transport, membrane recycling, cell adhesion, programmed cell death, and complement cascade, representing a truely multifunctional protein.

In no other field has the function of CLU been more controversial than in cancer genetics. After more than 20 years of research, there is still uncertainty as regard as the role of CLU in human cancers. Some investigators believe that CLU is an oncogene, others - an inhibitor of tumorigenesis.

The aim of this work was to study the significance of serum clusterin (CLU) level as a serological tumor marker in hepatocellular carcinoma (HCC) on top of HCV related hepatic cirrhosis. Also, to correlate its level in different studied groups with clinical and laboratory findings.

This study was performed on 71 patients, who were admitted to Hepatobiliary Unit, Internal Medicine Department, Alexandria Main University Hospital. The patients were classified into three groups:

- **Group I (n=25):** HCV positive patients without liver cirrhosis.
- **Group II (n=25):** HCV positive patients with liver cirrhosis.
- **Group III (n=21):** HCV positive patients with liver cirrhosis and HCC.
- **Group IV:** 10 age and sex matched healthy subjects as a control group.

Serum CLU level was measured in all studied groups using the quantitative sandwich enzyme immunoassay technique.

In this study, males predominated females in all studied groups, right hypochondrial pain, upper GIT bleeding and fatigue were common symptom among HCC patients. Also, ascites and lower limb edema were very common among the same HCC group. Anemia and decreased platelet count were also found among HCC group.

In HCC patients clusterin at the best cut-off value of (179 ng/ml) had a sensitivity of 95.24%, specificity of 70%, while AFP showes much lower values at a cut-off value of 11 ng/ml, where the sensitivity was 66.67%, specificity was 62.0%.

When performing a ROC curve for clusterin and AFP, the AUC for clusterin was (0.898) which is greater than that of AFP (0.793).

When combining both markers, AFP and clusterin the diagnostic performance improves; where sensitivity, specificity, PPV, NPV and accuracy become 71%, 100%, 100%, 89.29% and 91.55% respectively and AUC become 0.946.

On studying correlation of serum clusterin level and different clinicopathological and laboratory parameters, it was found that there was a positive correlation with BCLC staging system and its criteria; portal vein invasion and L.N metastasis. Also, there was a significant correlation between serum clusterin levels and fatigue, palmer erythema and spider angioma. While there was no significant correlation found between its levels and other clinicopathological or laboratory findings.

CONCLUSION

Serum clusterin was increased in HCC and was more sensitive and specific than AFP for differentiating HCC patients with HCV related hepatic cirrhosis from those with HCV related hepatic cirrhosis alone.

Clusterin was also closely related to BCLC staging system, portal vein invasion and L.N metastasis. Therefore clusterin may be considered as a useful biomarker for diagnosis as well as predicting metastasis potential of HCC.