Alexandria Journal of Medicine (2014) **50**, 227–234

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Alexandria University Faculty of Medicine

Alexandria Journal of Medicine

http://www.elsevier.com/locate/ajme

Serum clusterin as a marker for diagnosing hepatocellular carcinoma

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Received 20 November 2013; accepted 7 May 2014
Available online 20 June 2014

KEYWORDS

Clusterin;
Alpha fetoprotein;
Tumor marker;
Hepatitis C virus related liver cirrhosis;
Hepatocellular carcinoma

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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Peer review under responsibility of Alexandria University Faculty of Medicine.

http://dx.doi.org/10.1016/j.ajme.2014.05.004

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).