Introduction

Hepatocellular carcinoma (HCC) is the fifth most common diagnosed solid tumor ***(Alacacioglu et al., 2008)*** and the third most common cause of cancer-related death in the world behind lung and gastric cancer, causing more than 500,000 deaths every year. It represents a major health challenge with significant and increasing global impact ***(Jemal et al., 2010 and Mittal and El-Serag, 2013).***

The prevalence of HCC in Egypt is extensively increasing. In 2001, HCC was reported to account for about4.7% of chronic liver disease (CLD) patients***(El-Zayadi and Abaza, 2001).*** The incidence of HCC has doubled in the past 10 years,now, it is the second most incident and lethal cancer in men ***(Lyer et al., 2010).*** The heavy burden of HCC parallels high rates of hepatitis C virus (HCV) while hepatitis B virus (HBV) rates have declined in Egypt after the introduction of the vaccine in 1992 ***(Lau and Lai, 2008 andLehman and Wilson, 2009)***.

The lack of HCC biomarkers prevents early detection so the hepatocellular carcinoma is often diagnosed at advanced stages where effective therapies are lacking resulting in a poor prognosis of the disease ***(Sakamoto et al., 2008a)***. The median survival for newly diagnosed cases classified as unresectable at diagnosis is 6-10 months in most

published reports ***(El-Serag, 2007 and Chaparro et al., 2008)***. Although surgical resection and liver transplantation are curative therapeutic options, they are applicable only in fewer than 20% of patients due to late presentation with advanced disease staging, poor hepatic function and limited organ availability ***(Alsina, 2010)***.

The screening for HCC in patients with cirrhosis or advanced hepatic fibrosis, irrespective of the cause is ultrasonography of the liver combined with measurement of serum alpha-fetoprotein levels every 6 to 12 months***(Singal etal., 2009)***. In North American studies, the combined measurement of alpha-fetoprotein and other biomarkers, such as des-gamma-carboxyprothrombin or lectin-bound alpha-fetoprotein, was shown to provide only a limited additional benefit as compared with the measurement of alpha-fetoprotein alone and thus was not be recommended ***(Lok et at., 2010 andMarrero and Feng, 2010)***.

In inconclusive cases, the gold standard still remains the histological studies of liver biopsy ***(Bruix andSherman, 2005)***. More difficult is the proper diagnosis of small HCC which frequently does not present with typical vascular patterns in imaging tests and acquisition of reliable histological specimens often requires repetitive biopsies and pathological interpretation is demanding ***(Park and Kim,2011)***.

Clusterin is a secretory heterodimeric glycoprotein (75–80 kDa)expressed in several tissues and present in all human fluids**.**It has been involved in a wide range of physiological and pathophysiological processes important for carcinogenesis and tumor growth, including lipid transportation and redistribution, apoptosis, cell cycle regulation, DNA repair, folding of damaged extracellular proteins, cell adhesion and aggregation, membrane recycling, complement regulation, tissue remodeling, tumorigenesis and immune system regulation ***(Wang et al., 2010a)***.

Incancer cells, sCLU exerts an anti-apoptotic function and itsexpression is elevated upon metastasis. However, no definitive mechanism has been proposedto account for the differential expression of CLU isoforms.Understanding how the expression of these isoforms is regulatedmay provide new strategies for the diagnosis, prevention, and treatment of cancers ***(Park et al., 2014).***

Some reports documented that in the majority of humancancers, such as prostate, breast, lung, bladder and colon cancers, upregulated expression of CLU was frequently detected ***(Wang et al., 2010a)***.***Chen et al. (2012)*** provided evidence that CLU plays an important role in HCC invasiveness.

This work aims to investigate the role of serum CLU as a biomarker for evaluating diagnosis and metastasis potential of HCC.

Aim of the Work

The aim of this study is to evaluate the clinical utility of serum clusterin as a biomarker for diagnosis and the metastasis potential of HCC compared with the available evaluatingmodalities, namely AFP.

I. Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is a major cause of morbidity and mortality; it is the fifth most common cancer worldwide and the third leading cause of cancer-related deaths(0.8 million, 9.1%)***(Lau and Lai, 2008 andFerlay et al., 2013).***

According to the National Institute of Cancer (NIC), HCC is considered to be one of the common malignancies in Egypt as a normal consequence of the high prevalence of hepatitis B and C infection, as it represents about 45.3% of the new cases of the digestive system cancer. Therefore, early detection of HCC is a critical goal to reduce health costs***(Miller et al., 2010*)**.

A. Epidemiology:

1. Frequency:

The incidence of HCC worldwide varies according to the prevalence of hepatitis B and C infections.An estimated 560,000 new cases are diagnosed annually. Areas such as Asia and sub-Saharan Africa with high rates ofinfectious hepatitis have incidences as high as 120 cases per 100,000, so HCC is more common in Asia and Africa with the highest incidence in Japan (4-5%)***(Schwartz et al., 2014b).***

Hepatocellular carcinoma (HCC) contributes to 14.8% of all cancer mortalities in Egypt, with a higher incidence in males (17.3%) than in females (11.5%). It is the second most frequent cancer type in Egyptian males after bladder cancer and the eighth most frequent in Egyptian females***(Anwar et al., 2008).***

The high incidence of HCC in Egypt is attributed to the high prevalence of hepatitis C virus (HCV). HCV is currently the most significant public health problem in Egypt with an overall prevalence of 17.4% in males and 12.2% in females, and it increases with age to a prevalence of 39.4% in 55-59 year-old population***(Sievert et al.,2011).***

Chronic hepatitis usually leads to the sequential occurrence of liver fibrosis and cirrhosis with a high risk of development of HCC.Hospital-based studies in Egypt have reported an increase in the relative frequency of all liver-related cancers in Egypt (95%being HCC), from 4% in 1993 to 7.3% in 2003 ***(Lehman and Wilson, 2009).***

2. Demographic Factors:

Worldwide, there is a clear predominance in males, with male to female ratio ranging from 1.5: 1 in countries with a low incidence of HCC, to approximately 3: 1 in populations with a high frequency ***(El-Serag and Rudolph, 2007 and Jemal et al., 2011).***

In most western low-risk populations, the highest age-specific rates occur among persons aged 75 and older. A similar pattern is seen among most high-risk Asian populations (Hong Kong). In contrast, male rates in high-risk African populations (Gambia and Mali) tend to peak between ages 60 and 65before declining; while female rates peak between 65 and 70 before declining***(Nordenstedt et al., 2010).***

3. Morbidity& Mortality:

More than 70% of HCC are considered unresectable at the time of presentation. However, chemotherapy and radiation control may improve the clinical course ***(Jelic, 2009).***

The prognosis of patients with HCC is generally very poor with a 5-year survival rate of less than 10–15% since most of them are diagnosed clinically at a late stage ***(Kawanoet al., 2008).***Most patients with HCC die within 1 year after diagnosis, patients with cirrhosis have a shorter survival rate with a median survival of 6 months***(Chaparro et al., 2008).***

B. Risk factors:

The major risk factor for the development ofHCC iscirrhosis ofthe liver.At one major referral center in the United States, the most commonly seen risk factors for HCC were HCV infection, HBV infection, alcohol use, and nonalcoholic fatty liver disease (**Figure 1**)***(Yang et al., 2011)***.

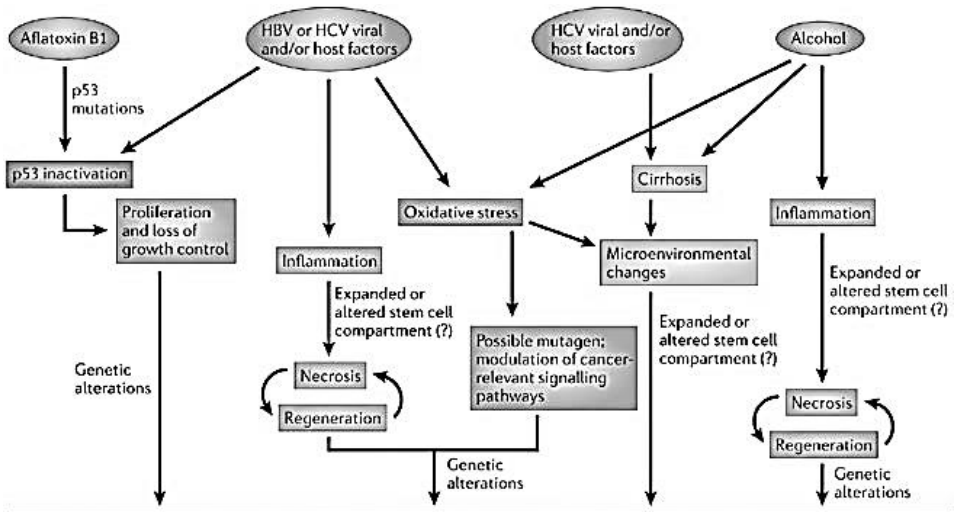

Figure (1): Risk factors of HCC ***(Paraskevi et al., 2006).***

1) Hepatitis BVirus (HBV):

Chronic HBV infection is the most important risk factor of HCC worldwide, accounting for 50% to 55% of all cases. Most cases occur in patients with cirrhosis, but a significant proportion, ranging from 30% to 50%, occur in non-cirrhotic patients ***(Scotto et al., 2010)***.

While HBV can cause HCC in the absence of cirrhosis due to its ability to integrate into the host genome, the majority of the HBV-related cases occur in the background of cirrhosis. In high-risk countries in Africa and some parts of Asia, HBV-related chronic liver disease is the most important risk factor, whereas in Japan, hepatitis C virus(HCV) related cirrhosis is the most common***(Chen et al., 2011)***.

2) Hepatitis C virus (HCV):

A strong association between chronic HCV infection and HCC has been observed, but the mechanisms involved in carcinogenesis remain unclear ***(Omland et al., 2012).***

The major risk factor for HCC in patients with chronic HCV is advanced hepatic fibrosis or cirrhosis***(Lok et al., 2009).***

Unlike HBV, HCV is a ribonucleic acid virus and hence, cannot integrate into the host genome. Similar to HBV, the carcinogenesis of HCV-associated HCC is thought to be a multistep process involving upregulation of inflammatory cytokines and induction of oxidative stress from chronic hepatitis, fibrosis, liverregeneration and ultimately, the development of cirrhosis***(Budhu and Wang, 2006).***

The association of HCV with the followingrisk factors has been shown to further increase the chances of developing HCC: alcohol use, coinfection with HBV or human immuno-deficiency virus, diabetes mellitus, older age, African American race, thrombocytopenia, elevated alkaline phosphatase, esophageal varices and smoking***(Lok et al.,2009).***

3) Co-infection of hepatitis B and hepatitis C:

Studies have shown that patients with combined HCV and HBV infection have a higher risk of developing HCC than those with a HCV or HBV alone ***(Ikeda et al., 2007).***

The two viruses may possibly act through common, as well as different, pathways in the carcinogenic process. Given that HBV acts as a cofactor in the development of HCV related cirrhosis and HCC, vaccination of patients with chronic hepatitis C against HBV has been recommended, aiming to avoid further liver injury***(Michielsen et al.,2005)***.

4) ***Nonalcoholic fatty liver disease (NAFLDs)*****:**

Non-alcoholic steatohepatitis (NASH), first described by Ludwig and co-workers in 1980, is a stage in the wide spectrum of non-alcoholic fatty liver diseases (NAFLDs), and one of the leading causes of chronic liver disease.NASH occurs due to abnormal fat deposition in the liver, which ranges in severity from simple hepatic steatosis with no inflammation, to steatohepatitis (NASH) which can progress to liver cirrhosis. NASH is also a known etiology for cryptogenic liver cirrhosis ***(Khedmat and Taheri, 2011).***

It is likely that NAFLDs causes HCC via cirrhosis, although the exact pathogenesis has not yet been determined. One study found that HCC in NASH was associated with obesity, diabetes, hypertension and male sex ***(Yasui et al., 2011).***

5) ***Aflatoxin:***

In Asia and Africa, exposure to dietary aflatoxin is an important risk factor for the observed high rate of HCC. Aflatoxin is well known to be both anindependent risk factor

for HCC and a cofactor in patient with chronic HBV infection***(Kensler et al.,2011).***

Aflatoxin causes DNA damage and mutations of the p53 gene. Humans are exposed to aflatoxin through the ingestion of moldy foods found in susceptible grains, so dietary levels in endemic areas correlate directly with incidence of HCC ***(Keith et al., 2006).***

6) Diabetes Mellitus (DM):

A meta-analysis of 14 prospective epidemiologic studies also found an increased risk of HCC among patients with diabetes ***(Yang et al., 2011)***. However, DM is associated with NAFLD which is associated with cirrhosis,an established risk factor for HCC***(Gomaa et al.,2008 and Pattullo and Heathcote,2010).***

7) Alcohol:

Alcohol adds to the risk of developing HCC in patients with chronic HCV or HBV infections ***(Schacherer et al., 2007)***, whereashigher levels of consumption are probably required for development of HCC in the absence of viral infection ***(Seitz and Stickel, 2006).***

Concomitant heavy alcohol use, diabetes mellitus, and obesity were found in various reports to increase the risk of HCC ***(Saunders et al., 2010 and Loomba et al., 2013).***

It is somewhat unusual for an actively drinking alcoholic to develop HCC; the usual setting is an individual with alcoholic cirrhosis who has stopped drinking for ten years, and then develops HCC. When drinking is stopped, the liver cells try to heal by regenerating nodules. It is during this active regeneration that a cancer-producing genetic change (mutation) can occur ***(Blum and Spagenberg, 2007).***

8) Obesity:

Obesity may lead to HCC through the development of NAFLD, accumulation of fat in the liver to NASH, cirrhosis, and liver cancer. A recent study demonstratedthat obesity was a genuine promoter of HCC in a mice model depending on enhanced production of the tumor promoting cytokines IL-6 and tumour necrosis factor (TNF), which cause hepatic inflammationandactivationoftheoncogenic transcription factor signal transducer and activator of transcription 3 (STAT3)***(Park et al., 2010).***

9) Hemochromatosis:

Patients with hemochromatosis, especially in the presence of cirrhosis, are at an increased risk of developing HCC. HCC accounts for about 30% of all iron-related deaths in hemochromatosis ***(Allen, 2008).***

10) Immunodeficiency:

Human immunodeficiency virus(HIV) infection is recognized as a frequent cofactor that increases the risk of HCC in patients with chronic HBV or HCV infection***(Mallet et al.,2011)***.

The incidence of HCC in patients with HIV is rising. It is not clearly established whether HIV directly accelerates HCC pathogenesis or whether the rising incidence is an epiphenomenon of the highly active antiretroviral therapy (HAART) era, wherethe increased longevity of patients with HIV allows long-term complications of viral hepatitis and cirrhosis to develop ***(MacDonaldet al., 2008).***

11) Cigarette Smoking:

Cigarette smoking has been independently associated with HCC in several epidemiologic studies in Asia, but not all studies***(Trichopoulos et al., 2011).***

12)*Schistosomiasis:*

Several studies suggested that the presence of schistosomal infection may modify the course of HCV; co-infection may lead to significantly more complications, such as portal hypertension at an earlier stage, with accelerated progression to hepatitis C-associated fibrosis. This leads to quicker progression to HCC than those patients who do not have a parasite burden ***(Strickland, 2006).***

13) Precancerous Lesions Predisposing to HCC:

a) Liver cirrhosis:

Individuals with liver cirrhosis are at an increased risk of developing HCC whatever the cause is (hepatitis B, hepatitis C, alcohol, hemochromatosis, alpha 1anti-trypsin deficiency). In some areas with an intermediate incidence, notably in Japan, more than half of cases are associated with HCV and these patients usually have cirrhosis 15 years before the onset of HCC**.**Cirrhosis increases the susceptibility of the liver to malignant change. The normal liver is mitotically inactive but, when stimulated to divide rapidly, this prevents the repair of any damage to DNA. This abnormality is then 'fixed' and transmitted to the progeny, giving rise to an altered cell line and eventually HCC***(Sanyal et al., 2010).***

b) Adenomatous hyperplasia (AH):

Adenomatous hyperplasiawas the term traditionally applied to "atypical" nodular lesions occurring in a cirrhotic liver that did not fulfill the morphologic criteria for liver cell carcinomas, but were thought to possibly represent precursors for them.These atypical nodular lesions may be macro-regenerative nodules orborderline nodules:

i. *Macroregenerative nodule (MRN):*This term is applied to a nodule measuring >0.8cm with a generally intact reticular architecture, cell plates no more than two cells thick, and absence of infiltrative edge***(Trevisani et al., 2008).***

*ii. Borderline nodule(atypical adenomatous hyperplasia or dysplastic nodule):*This term is used for a nodule that contains foci of decreased reticulum staining, small cell dysplasia, isolated glandular structures and irregular edge. Dysplastic nodules (DNs) are frequently multiple, coexist with MRN, and are rarely larger than 2cm. Although the pre-malignant role of MRNs is still debatable, DNs are generally considered an important precursor to HCC ***(Takayasu, 2009).***

c) Liver cell dysplasia:

Two types of liver cell dysplasia have been identified,large cell dysplasiaandsmall cell dysplasia**:**

*i Large cell dysplasia:*There is cellular enlargement, nuclear pleomorphism, and multinucleation, but the nucleo-cytoplasmic ratio remains normal***(Chan et al., 2011).***

ii. Small cell dysplasia:It is characterized by decreased volume of hepatocytic cytoplasm associated with moderately enlarged nuclear size, resulting in an increased nucleo-cytoplasmic ratio. Hepatocellular carcinoma developed nearly equally in large or small cell dysplastic lesions(18% and17%,respectively)***(Chan et al., 2011).***