Introduction

Hepatocellular carcinoma (HCC) is the most common malignant hepatobiliary disease and is responsible for about 1 million deaths per year. It is more common in males and represents the 5th most frequent neoplastic disease. HCC globally accounts for 4.6% of all neoplasia and has a mortality rate of 94%. The mean age of diagnosis is between 50-60 years. Important risk factor responsible for the development of HCC is hepatitis C correlated cirrhosis that often manifests 20-30 years after the viral infection ***(Rossi et al., 2010)***.

Hepatocellular carcinoma (HCC) is one of the most vascular solid tumors, in which angiogenesis plays an important role. The status of angiogenesis in HCC correlates with the disease progression and prognosis. Until recently, for patients with advanced HCCs no therapy was available that prolonged overall survival (OS), therefore, the inhibition of angiogenesis represents a potential and promising therapeutic target in HCC ***(Christian et al., 2008).***

In recent years, knowledge regarding the biological processes of hepatocarcinogenesis has expanded significantly allowing the identification of the molecular processes involved in the onset of this neoplastic disorder. Among these molecules, growth factors and neoangiogenesis factors with their receptors and tyrosine kinase intracellular enzymatic pathways have been identified. All of these substances represent potential molecular targets of the so called targeted therapy ***(Rossi et al., 2010).***

Development of HCC is characterized by arterialization of its blood supply and sinusoidal capillarization ***(Zhen and Ronnie, 2008)***.Tumor cells release angiogenic factors to initiate the process of angiogenesis ***(Sun and Tang, 2004).*** Vascular endothelial growth factor **(VEGF)**, angiogenin **(ANG)** and basic fibroblast growth factor **(bFGF)**, are important angiogenic factors of neoangiogenesis ***(Zaghloul et al., 2006).***

Angiogenin or RNase 5 is a 14 124 Da soluble protein which is a member of the ribonuclease (RNase) super family, first isolated from the culture medium conditioned by colon carcinoma (HT-29) cells ***(Tello-Motoliu et al., 2006).*** Angiogenin is a normal constituent of the circulation and contained in a vasculature that rarely undergoes proliferation, but in some physiological and pathological conditions its levels increase in blood, promoting neovascularization. Hence, angiogenesis is a common pathophysiological attribute of angiogenin. In malignant disease, the most studied pathological state in regard to angionenin, abnormally high levels are seen, which may be of prognostic significance. Angiogenin has also been studied in other non-malignant pathological states ***(Tello-Motoliu et al., 2006).***

Previous reports have demonstrated that high expression of ANG in the tumor tissue or elevated serum ANG concentration was observed in patients with various malignancies, including gastric carcinoma ***(Shimoyama et al., 2003)***, Primary colorectal carcinoma ***(Etoh et al., 2000),***

hepatoma ***(Hisai et al., 2003)***, breast cancer ***(Sheen-Chen et al., 2000)*** and bladder carcinoma ***(Zhao et al., 2005).***

Angiogenin also play a role in a variety of non-malignant pathological conditions such as peripheral vascular disease, inflammatory bowel disease (IBD), rheumatoid arthritis, overweight and obesity, proliferative diabetic retinopathy and proliferative vitreopathy, and its levels were higher compared with healthy controls ***(Tello-Montoliu et al., 2006).***

Differentially expressed angiogenesis genes, including angiogenin gene, were observed between HCV cirrhotic patients with and without HCC. Soluble angiogenic factors might be useful for monitoring high-risk HCV cirrhotic patients ***(Mas et al., 2007).***

Aim of the Work

The aim of this study is to evaluate serum angiogenin level in Egyptian cirrhotic patients as well as in patients with HCC on top of cirrhosis, and to assess its clinical significance as a predictor factor for HCC.

I- Hepatocellular Carcinoma

A. Epidemiology

1. Frequency

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death worldwide with about 600, 000 patients dying from the disease annually ***(Semela et al., 2011)***.It accounts for 85 % to 90 % of primary liver cancers which include other categories as cholangocarcinoma, angiosarcoma and hepatoblastoma ***(Schutte et al., 2009)***. The incidence of HCC worldwide varies according to the prevalence of chronic hepatitis B virus infection and chronic hepatitis C virus infection ***(Braicu et al., 2009)***.

2. Geographic Distribution

The distribution of hepatocellular carcinoma varies widely according to geographic location and differs among ethnic groups within the same country and between regions within the same country. These extreme differences in distribution of HCC are probably due to regional variations in exposure to hepatitis viruses and environmental pathogens ***(Gomaa et al., 2009)***.

Most hepatocellular cases (more than 80%) occur in either sub-Saharan Africa or in Eastern Asia (over than 20 cases /100, 000 population), because of the endemic nature of hepatitis B and C in those regions. China alone accounts for more than 50% of the world's cases ***(Rampone et al., 2009)***.

Areas with moderately high risk (11-20 cases/100, 000) include Italy, Spain and Latin American countries, and those at intermediate risk (5- 10 cases/100, 000) include France, the United Kingdom and the Federal Republic of Germany. A relatively low incidence (less than 5 cases/ 100, 000) is found in the United States, Canada and in Scandinavia. Although currently relatively low, the incidence of hepatocellular is rising in the United States, tripling from 1.4 per 100, 000 persons during 1976 to 1980 to 4.7 per 100, 000 persons during 1996 to 1997 ***(Wong et al., 2011)***.

3. Incidence of hepatocellular carcinoma in Egypt

In Egypt, hepatocellular carcinoma was reported to account for about 4.7% of chronic liver disease (CLD) patients ***(El-Zayadi et al., 2005)***, the number of newly diagnosed patients with HCC increases annually. The burden of HCC has been increasing with doubling of the incidence rate in the past 10 years ***(Abdel-Wahab et al., 2007)***.

According to the national institute of cancer (NIC) in Egypt, HCC is considered to be one of the common malignancies in Egypt as a normal result of the high prevalence of hepatitis B, C infection, as it represents about 45.3% of the new cases of the digestive system cancer. About 10.6% of the new cases of malignancy among males and 3.6% among females, with 14.8% of total deaths from cancer ***(Miller and Abu-Raddad, 2010)***.

The prevalence of HCC is high in Nile Delta and is more common in males, farmers especially in hepatitis C virus and

hepatitis B virus patients, those suffering from Schistosomiasis and in rural residents due to the exposure to aflatoxin which is a common contaminant of foods in many developing countries ***(Anwar et al., 2008)***.

4. Mortality & Morbidity:

Most patients with hepatocellular carcinoma die within 1 year after diagnosis. Surgical cure is possible in less than 5% of patients. The median survival in unresectable cases is less than 4 months and under a year for untreated patients with less advanced disease ***(Llovet et al., 2008)***. The prognosis is affected by both the tumor severity, and the degree of pre-existing liver damage ***(Kuwaki et al., 2011)***.

5. Demographic Factors

a) Age

In general, the incidence increases with age in all populations and shows a slight decrease in the elderly ***(Saito et al., 2009)***. Age at diagnosis varies widely according to geographic distribution and the peak age in a given region tends to be inversely related to the frequency of the tumor, so in Western Hemisphere (low incidence regions) the median age at diagnosis is 65 years and the hepatocellular carcinoma is rarely diagnosed before age 40 years unless in patients with cirrhosis. In Asia, Africa (high incidence areas), age at diagnosis is substantially younger, occurring in the fourth and fifth decades of life, respectively ***(El Serag and Rudolph, 2007)***. Diagnosis at a younger age is thought to reflect the natural history of hepatitis B virus and hepatitis C virus related hepatocellular carcinoma ***(Winter, 2006)***.

b) Sex

Hepatocellular carcinoma shows a strong male predilection, being four to eight times more common in males than females ***(Goodman, 2007)***. This variation in the incidence rate is not completely understood but may be partly explained by sex specific prevalence of risk factors. Males are more likely to be infected with hepatitis B virus and hepatitis C virus, consume alcohol, smoke cigarettes and have increased iron stores. Androgenic hormones and increased genetic susceptibility may also increase risk among males ***(Stuart and Stadler, 2009)***.

c) Race:

Hepatocellular carcinoma incidence rates also vary greatly among different populations living in the same region ***(Kuntz and Kuntz, 2006)***. An example is the United States where, at all ages and among both sexes, HCC rates are 2 times higher in Asians than in African Americans, whose rates are 2 times higher than those in whites ***(El Serag and Rudolph, 2007)***.

B. Risk Factors

Cirrhosis, hepatitis B virus, hepatitis C viral infection, alcoholism, aflatoxins and sex hormone therapy are the most common risk factors of hepatocellular carcinoma ***(Chen et al., 2006)***.

1. Cirrhosis:

In general, cirrhosis of any etiology is the major risk factor for hepatocellular carcinoma. About 80% of patients with

newly diagnosed hepatocellular carcinoma have preexisting cirrhosis ***(Tsai et al., 2010)***.

Many studies have investigated the possible mechanisms involved in the development of hepatocellular carcinoma in patients with cirrhosis including telomere dysfunction and alterations in the micro- and macro-environment that stimulate cellular proliferation ***(Sanyal et al., 2010)***.

2. Hepatotropic Viruses

There is a strong evidence of a pathogenic role of hepatotropic viruses in the development of liver cell carcinoma not only through the production of cirrhosis but also in non cirrhotic livers. As a matter of fact, viral hepatitis B and C is regarded as the leading cause of hepatocellular carcinoma ***(Liang et al., 2002)***.

a) Hepatitis B virus

Worldwide, chronic hepatitis B virus (HBV) infection is the most common cause of hepatocellular carcinoma. More than 50% of HCC cases worldwide and 70-80% of HCC cases in highly HBV endemic regions are attributable to HBV ***(Nguyen et al., 2009)***. In high endemic regions such as Asia and Africa early prenatal and horizontal infection, contact with infected family members may occur in communities with poor socioeconomic and hygienic conditions, in childhood is the main route of HBV transmission while in low endemic areas such as Western countries HBV is a predominant disease in

adolescents and adults due to high risk sexual behaviors or drug injections ***(Shi and Shi, 2009)***.

The mechanism by which the hepatitis B virus causes hepatocellular carcinoma is thought to be from a combination of chronic inflammation and integration of the viral genome into the host DNA ***(Kuntz and Kuntz, 2006)***. Integration is accompanied by chromosomal deletions and translocations, which affect cell growth and differentiation. The deletions are not related to sites of integration. Inconsistent patterns of integration have emerged and the viral genome may integrate in different sites in tumors from different subjects ***(Ha et al., 2010)***.

The HBV X protein (HBx), a small regulatory protein that is required for the establishment of viral infection, is believed to contribute to the development of hepatocellular carcinoma ***(Ha et al., 2010)***. HBx performs a variety of biological functions, including transcriptional repression of physiological proteolysis**,** interference with host DNA repair**,** interaction with p53**,** transactivation of various viral and cellular promoters**,** modulation of cell proliferation and apoptosis and induction of malignant cell migration. These functions may play important roles in the initiation and development of hepatocellular carcinoma associated with HBV infection ***(Feitelson and Lee, 2007)***.

It is generally shown that vaccination significantly decreases the incidence of hepatocellular carcinoma. Moreover, preventing the most severe HBV disease consequences in

infected people, such as cirrhosis and fibrosis, will require appropriate therapeutic agents and reduces the risk of developing hepatocellular carcinoma ***(Wu et al., 2010)***.

b) Hepatitis C virus

The incidence of hepatocellular carcinoma is expected to increase in the next two decades largely due to hepatitis C infection. Unlike with the HBV infection the majority of patients who develop HCC as a result of HCV will have liver cirrhosis. The average time to develop HCC after initial exposure to HCV is about 28 years and usually about 8-10 years after the development of liver cirrhosis ***(Vuotic et al., 2008)***.

HCV is a single strand of RNA that lacks a reverse transcriptase enzyme and does not become integrated into host DNA. Therefore, it is not certain whether the virus is directly carcinogenic itself or if its marked propensity to cause chronic necroinflammmatory disease is the real culprit ***(Foster et al., 2010)***.

The exact mechanism of hepato-carcinogenesis in patients with HCV-related cirrhosis is unknown; however, chronic inflammation, necrosis and regeneration of hepatocytes in chronic liver disease might be involved in the development of HCC ***(Van Der Poorten et al., 2010).*** It is possible that certain viral proteins may interact with oncogenes or tumor suppressor genes that regulate the cell cycle. At least four of these HCV gene products, HCV core, NS3, NS4B and NS5A,

have been shown to exhibit transformation potential in tissue culture, and several potentially oncogenic pathways have been shown to be altered by the expression of HCV proteins. The additive effects of oxidative stress caused by chronic inflammatory process may further enhance carcinogenesis in patients with chronic HCV infection ***(Suriawinata et al., 2007)***.

Numerous HCV prevalence studies in Egypt suggesting that Egypt, relative to the other nations of the world has the highest prevalence of antibodies to hepatitis C virus (HCV) in the world. HCV-antibody prevalence was reported to be 70% in adults suffering from schistosomiasis and without a history of blood transfusion. Some authors showed that patients with concomitant HCV and schistosomiasis infection had a higher incidence of cirrhosis and HCC ***(Miller et al., 2010)***.

A recent meta-analysis showed that prevalence for HBV and HCV are 6.7% and 13.9% among healthy populations, and 25.9% and 78.5%, respectively among HCC cases. Among HCC cases, HBV significantly decreased over time while HCV did not, suggesting a shift in the relative influences of these viruses in HCC etiology in Egypt. The meta-analysis results highlighted large amounts of heterogeneity among the epidemiological factors associated with liver disease in Egypt and underscore the necessity of an integrated strategy for the successful prevention of viral hepatitis infections and chronic liver disease ***(Lehman et al., 2009)***.

c) Co-infection of HBV and hepatitis D virus

Hepatitis D virus (HDV) co-infection with HBV is associated with increased liver damage. Some studies showed that hepatitis B surface antigen (HBs Ag) positive patients with HDV superinfection develop cirrhosis and HCC at an earlier stage compared to HBs Ag carriers without HDV infection ***(Gomaa et al., 2008)***.

3. Schistosomiasis

In Egypt, Schistosomiasis is a major public health problem and infection with Schistosoma Mansoni constitutes the major cause of liver disease. From 1950s until 1980s, the Egyptian Ministry of Health (MOH) conducted a community wide therapy campaign using parenteral tarter emetic to control the Schistosomiasis infestation. However, this unfortunately established a large reservoir of HCV infection in the country through needle re-usage at the time of treatment ***(Strickland, 2006)***. There is some epidemiological evidence that the presence of Schistosomal infection may modify the course HCV co-infection and may lead to significantly more complications such as portal hypertension at an earlier stage with accelerated progression to hepatitis C-associated fibrosis and thus quicker progression to HCC than those patients who do not have a parasite burden ***(Gomaa et al., 2008)***.

4. Aflatoxin

Aflatoxin is the most known potent liver cancer forming chemical. Aflatoxin is a mycotoxin released by Aspergillus

species. The fungi grow rapidly on foodstuffs as corn, peanuts and legumes stored in warm damp conditions. Although there are 4 principle types (B1, B2, G1, G2), Aflatoxin B1(AFB1) is the most potent in animal studies. It has been suggested that AFB1 can lead to HCC through inducing a specific mutation of codon 249 of the p53 tumor suppressor gene ***(Polychronaki et al., 2007)***.

5. Pesticides

Pesticides exposure is one of the environmental factors hypothesized to increase the risk of HCC. Pesticides are considered to be possible epigenetic carcinogens through one or several mechanisms such as spontaneous initiation of genetic changes, cytotoxicity with persistent cell proliferation, oxidative stress, inhibition of apoptosis, suppression of intracellular communication and construction of activated receptors ***(Ezzat et al., 2005)***.

6. Disturbed iron and copper metabolism

Iron and copper are potentially mutagenic through a number of mechanisms related to oxidative stress. Theoretically, both metals can cause point mutations, cross linking of DNA strands and other rearrangements. 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 patients ***(Blonski et al., 2010)***.

7. Obesity

In the past several years, evidence supporting an association between obesity and HCC has been growing. It has been suggested that obesity may be responsible for a significant proportion of cryptogenic cirrhosis associated with HCC ***(Stuart and Stadler, 2009)***.

8. Oral contraceptives

Prior to the widespread use of oral contraceptives (OCs), benign liver tumors in young women were rarely observed. Oral contraceptives appear to be associated with the development of benign liver tumors such as hepatic hemangioma, hepatocellular adenoma or focal nodular hyperplasia. Although not well researched, it has been proposed that OCs might also be associated with malignant liver tumors including HCC ***(Blonski et al., 2010)***.

9. Alcoholism:

Chronic alcohol consumption has long been associated with progressive liver disease because the liver is the major site of ethanol metabolism and thus sustains the most injury from alcohol consumption. Moreover, alcohol mediated enzyme induction increases the conversion of co-carcinogens to carcinogens, also it promotes carcinogenesis through depression of immune responses and through impairment of carcinogen-mediated DNA alkylation ***(Ha and Yu, 2010)***.

Cirrhosis caused by chronic alcohol consumption is the most common association of HCC in the developed world. In