INTRODUCTION

Worldwide, there is a growing incidence of hepatocellular carcinoma (HCC). It is the sixth most common cancer, the third cause of cancer related death, and accounts for 7% of all cancers. HCC represents more than 90% of primary liver cancers. Overall, the incidence and mortality rates were of 65,000 and 60,240 cases in Europe and 21,000 and 18,400 cases in the United States in 2008, respectively. It is estimated that by 2020 the number of cases will reach 78,000 and 27,000, respectively ***(Llovet et al., 2012).***

Egypt has the highest prevalence of HCV in the world and the prevalence of HCC is increasing in the last years ***(Shaker et al., 2013).***

Alpha fetoprotein may be elevated in chronic liver disease, especially in viral hepatitis, in the absence of HCC. It has been estimated that approximately 20% of patients with chronic hepatitis (of any cause) and 40% of patients with cirrhosis may have alpha fetoprotein levels between 10 and 400 ng/ml. The differential diagnosis of elevated alpha fetoprotein levels also includes gastric, biliary, pancreatic cancers and germ cell tumors ***(Colombo, 2002).***

The relatively poor specificity of conventional alpha fetoproteins levels has led to a search for more sensitive and

specific markers. Other tumor markers include serum Des-gamma-carboxyprothrombin (DCP) that has been used for screening of HCC. The failure of hepatoma cells to express prothrombin carboxylase leads to a higher DCP serum levels. The sensitivity of DCP is better than that of alpha fetoprotein only in larger tumors and hence is of limited benefit in clinical practice ***(Okuda et al., 1999).***

However, neither DCP nor AFP is optimal to complement ultrasound in the detection of early HCC. Development of novel biomarkers for the early detection of HCC thus remains an important target before a breakthrough appears on HCC surveillance and early intervention ***(Okuda et al., 1999).***

Osteopontin (OPN) is a phosphorylated glycoprotein secreted by activated macrophages, leukocytes, and activated T lymphocytes. Over-expression of OPN has been found in a variety of cancers, including carcinomas of stomach, breast, prostate, lung, colon, and liver. OPN over-expression tended to be associated with the presence of tumor vascular invasion and advanced tumor grade, thus, indicating poor prognosis for patients with HCC, it may also have predictive potential for HCC invasion and metastasis. Also it was found that Interference of osteopontin expression inhibits the invasion and metastasis of human hepatocellular carcinoma, this opens the potential for OPN directed treatment that could greatly enhance outcomes for HCC patients ***(Pan et al., 2003)***.

AIM OF THE WORK

To evaluate the plasma level of osteopontin in patients with different stages of hepatocellular carcinoma to verify the possibility of using its level as a potential biomarker for progression of HCC.

HEPATOCELLULAR CARCINOMA (HCC)

Introduction:

Hepatocellular carcinoma (HCC) is a major health problem worldwide as more than 700,000 cases are diagnosed yearly ***(Bazine et al., 2014).***

Hepatocellular carcinoma (HCC) has become the third most common malignancy worldwide with very poor prognosis, rendering it the fourth highest cause of cancer related deaths ***(Soliman et al., 2010)***.

Epidemiology:

Although HCC is the most common primary hepatic malignancy worldwide, there are striking variations in its incidence in various parts of the world, with the major burden of disease falling on the developing world ***(Dhanasekaran et al., 2012).***

The epidemiology of HCC is changing as a result of immigration to Europe and North America. Immigrants to these countries bring with them the prevalence of chronic viral hepatitis that exists in their home countries ***(Sherman, 2010).***

HCC is a major health problem in Egypt and its incidence is increasing. The high prevalence of HCV infection makes screening programs and surveillance of those patients a very important tool to early detect cases of small HCCs ***(Shaker et al., 2013).***

In Egypt, HCC has nearly doubled over the last decade from 4.0% in 1993 to 7.2% in 2002 among patients with chronic liver disease (CLD). The development of HCC is mainly due to the high rate of hepatitis B and C infections among Egyptian patients ***(El-Zayadi et al., 2005).***

Incidence:

The incidence of HCC varies across the world. More than 80% of HCCs develop in Asian and African countries, where between 40% and 90% of HCCs are attributable to chronic hepatitis B ***(Yang and Roberts, 2010a).***

The numbers of incident cases and liver cancer deaths are similar because most HCCs are detected at an advanced stage in patients with underlying liver dysfunction, making this a highly lethal cancer ***(Yang and Roberts, 2010b***).

Although the majority of the cases occur in Asia and Africa, the incidence has also been rising in the developed world. In the United States, the incidence has tripled over the last three decades with over 20,000 cases estimated to be

diagnosed in 2011 ***(Dhanasekaran et al., 2012)***. The incidence of HCC increases with age, reaching its highest prevalence among those aged over 65 years ***(El-Serag and Rudolph., 2007).***

The age at which HCC appears also varies according to gender, geographic area, and risk factors associated with cancer development. In most areas female age is higher than male ***(Bosch et al., 2004)***.

In high-incidence areas where HBV is the main etiologic agent, the peak age appears after 40 years, while in low-incidence areas such as the USA, the peak age appears beyond 75 years ***(Bosch et al., 2004)***.

The male predominance may be due to specific genetic and hormonal profiles together with a higher prevalence of risk factors such as viral infections, alcoholism and smoking ***(Bruix and Sherman, 2005)***.

Etiology:

1-Chronic hepatitis C virus infection (HCV):

Hepatitis C virus is a Hepaci-virus that infects hepatocytes and some lymphocytes [It chronically infects about 120–170 million people world-wide, resulting in about 350,000 deaths annually ***(Donlin et al., 2014).***

A direct role of hepatitis C virus (HCV) in hepatocarcinogenesis hasn't been clarified, however, it seems that cirrhosis is the common route through which several risk factors act and induce carcinogenesis ***(El-Garem et al., 2014)***. Once cirrhosis is established, patients are at risk for hepatocellular carcinoma (HCC) and decompensation, characterized by ascites, variceal hemorrhage, or hepatic encephalopathy (HE), and survival decreases from a median of 12 years to 2 year ***(King et al., 2014).***

2- Hepatitis B virus infection (HBV):

Chronic HBV infection is usually characterized by the presence of hepatitis B surface antigen (HBsAg) in the serum for at least 6 months after exposure to the virus. Patients with chronic HBV infection have a more than 100-fold increased risk of HCC occurrence compared with uninfected individual ***(Song et al., 2013).***

High HBV load and chronic hepatitis B (CHB) infection increase the risk of developing HCC. HBV is a DNA virus that can integrate DNA into host genome there by increase the yield of trans-activator protein HBsAg that may deregulate many pathways involving in metabolism of cells ***(Ayub et al., 2013).***

The viral genotype is another factor that affects cancer risk. Genotype C has a higher risk of causing HCC than

genotype B, and genotype D has a higher cancer risk than genotype A ***(Tan, 2011).***

3- Combined HCV and HBV infections:

Hepatitis B virus and (HCV) co-infection is not uncommon as a result of similar routes of infection. Patients who are co-infected represent a unique group with diverse serologic profiles ***(Crockett and Keeffe, 2005).***

Only a small number of HCV patients were co-infected with HBV, patients with documented HBV viremia were at a significantly higher risk for cirrhosis, HCC, and overall death than HCV mono-infected patients. Absence of HBV replication was associated with a clinical course similar to that of HCV mono-infected patients ***(Kruse et al., 2014).***

4-Combined HBV and HDV infection:

Hepatitis D virus (HDV) super-infection in patients with chronic hepatitis B leads to accelerated liver injury, early cirrhosis, and decompensation. It may be speculated that hepatocellular carcinoma (HCC) may differ in these patients from HBV mono-infectionwith more aggressive course in combined HBV and HDV infection ***(Yang and Roberts, 2010b).***

5- Human immunodeficiency virus (HIV):

The incidence of HCC in patients with HIV is rising. HCC in HIV almost invariably occurs in the context of HCV or HBV co-infection and, on account of shared modes of transmission ***(MacDonald et al., 2008).***

Several reports have outlined a more aggressive course of HCC in HIV-infected patients ***(Di Benedetto et al., 2013).***

6- Co-infection of HCV and *Schistosoma mansoni*:

Schistosomiasis is a common parasitic infestation in some parts of the world. In Egypt, Schistosomiasis is a major public health problem and infection with Schistosoma mansoni constitutes a major cause of liver disease ***(Gomaa et al., 2008).***

Schistosoma infection increased the risk of HCC, only in the presence of HCV, whereas isolated S. mansoni infection does not ***(Hassan et al., 2002).***

7- Alcohol:

Chronic alcohol use of greater than 80 g/day for more than 10 years increases the risk for HCC approximately 5-fold; alcohol use of less than 80 g/day is associated with a non-significant increased risk for HCC ***(Morgan et al., 2004).*** Although heavy alcohol intake is associated with the development of cirrhosis, there is still a controversy about a

direct effect of alcohol on the development of HCC ***(Kwon et al., 2010).***

8- Diabetes mellitus, non-alcoholic fatty liver disease and obesity:

Epidemiological studies have shown that obesity is a risk factor for hepatocellular carcinoma. Similar studies further indicate that diabetes is also a major risk factor. Both obesity and diabetes are frequently associated with nonalcoholic fatty liver disease ***(Caldwell et al., 2004).***

Nonalcoholic fatty liver disease (NAFLD) is intimately related to insulin resistance and ranges from a benign course to liver fibrosis and cirrhosis ***(Illnait et al., 2013).***

Nonalcoholic fatty liver disease is the hepatic manifestation of the metabolic syndrome, amongst the numerous patho-genetic factors, oxidative stress and apoptosis of hepatocytes initiate many inflammatory processes and are involved in the progression of disease, particularly in transformation of non-alcoholic steatohepatitis (NASH) to cirrhosis ***(Celinski et al., 2014).***

9-Aflatoxin:

Aflatoxins (AFT) are secondary metabolites produced by some Aspergillus species that contaminate food during storage, production and processing. Due to their high toxicity and

mutagenic, teratogenic and carcinogenic effects, they have long been suggested as possible an etiologic agent of HCC ***(Felizardo and Camara, 2013).***

Aflatoxin B is a mycotoxin that acts synergistically with HBV in the pathogenesis of HCC. Aflatoxin causes DNA mutations, particularly of the TP53 gene, that attenuate the tumor suppressor function of p53 ***(Yang and Roberts., 2010).***

Aflatoxin B1 (AFB1) is the most well-known bioaccumulative toxin involved in the development of HCC ***(Matsuda et al., 2013).***

10- Congenital disorders:

a) Hereditary hemochromatosis (HH) is a strong risk factor for hepatocellular cancer, and mutations in the ***HFE***gene associated with HH and iron overload ***(Agudo et al., 2013).*** However, a cross-sectional study showed that progression to HCC among hemochromatotic patients is mostly variable from one population to another, depending mainly on exposure to environmental factors that synergize the current underlying gene mutation ***(Willis et al., 2005).***

b) Alpha-1-antitrypsin deficiency (A1ATD) is one of the most common genetic causes of liver disease. It is characterized by accumulation of a misfolded secretory

protein in the endoplasmic reticulum of liver cells ***(Chu et al., 2014).***

Epidemiology studies revealed that severe A1ATD is a significant risk factor for cirrhosis and HCC unrelated to the presence of HBV or HCV infections. However, predisposition to HCC in moderate A1ATD is rare ***(Topic et al., 2012).***

El –Okabi et al. (1990) found that 2 \40 patients with HCC had Alpha –1-antitrypsin deficiency and 2\15patient with cirrhosis (12%). They concluded that cases with Alpha-1-antitrypsian deficiency should be followed up clinically and laboratory for their susceptibility to develop cirrhosis and HCC.

11-Hepatic venous disease

Budd-Chiari syndrome (BCS) is a diverse group of conditions associated with obstructions of hepatic venous outflow at the level of the large hepatic vein (HV) or the extrahepatic segment of the inferior vena cava (IVC) ***(Cai et al., 2015).***

Obstruction of hepatic venous outflow tract leads to sinusoidal congestion, ischemic injury to liver cells, and portal vein hypertension, subsequently leading to hepatic congestion with necrosis, regeneration, fibrosis, and liver cirrhosis.

Patients with BCS have been reported to be associated with hepatocellular carcinoma (HCC) ***(Liu et al., 2013).***

Management of hepatocellular carcinoma:

1) Screening of HCC:

Surveillance for HCC is considered a standard of care for patients with chronic liver disease who are at risk of developing this malignancy. Several studies have shown that surveillance can improve the prognosis of patients diagnosed with HCC through an increased likelihood of application of curative or effective treatment ***(Giannini et al., 2013).***

The most commonly used surveillance tests for HCC are the alpha-fetoprotein (AFP) and hepatic ultrasound (US) ***(Flores and Marrero, 2014).***

US is performed in populations at risk such as cirrhotic patients, non-cirrhotic HBV carriers with active hepatitis or a family history of HCC, non-cirrhotic patients with chronic hepatitis C or advanced liver fibrosis ***(Hennedige and Venkatesh, 2013).***

Hepatocellular carcinoma surveillance lacks a reliable biomarker. Alpha-fetoprotein (AFP) is the most widely used. However, not all HCCs secrete AFP ***(Zhou et al., 2012).***

The combination of ultrasonography (US) and AFP is commonly used for surveillance of HCC. However, it has been recognized that AFP has limited sensitivity and specificity for HCC while US is an indirect diagnostic method depending on

operator skill and has limited ability to differentiate HCC from non-neoplastic nodules ***(Cheng et al., 2014).***

AFP has a sensitivity and specificity for detecting HCC in the range of 41–65% and 80–90%, respectively, when an AFP cut-off value at 20 ng/ml is used. However, up to 50% of patients with HCC have an AFP level below 20 ng/ml ***(Song et al., 2013).***

But the clinical value of AFP is challenged in recent years due to low sensitivity and specificity. In addition, AFP levels greater than 500 ng/ml are correlated with the tumor size: 80% of small HCC show no increase of AFP concentration ***(Zhou et al., 2012).***

The surveillance interval should be dictated by the expected doubling volume time of the surveyed tumor, and not by the degree of the inherent risk of HCC. Median doubling volume time of untreated HCC is around 170 days ***(Giannini et al., 2013).***

Screening should be performed by expert person in all risky population using abdominal US every 6 months ***(EASAL, 2012).*** Screening should be performed to all high risk groups: All cirrhotic patients: Non-cirrhotic patients: HBV infection (carrier) HCV (Metavir score 3 or 4) NASH, Alcoholic and Haemochromatosis, However, according Egyptian society of liver cancer Screening for HCC should be done for all high risk

patients with AFP and abdominal U/S with time interval every 4 months ***(ESLC, 2011).***

Small nodules less than 1 cm detected on US should be followed every 3–4 months in the first year and every 6 months thereafter ***(Hennedige and Venkatesh, 2013).***

Target population

Table (1): Groups for whom HCC surveillance is recommended or in whom the risk of HCC is increased ***(Bruix and Sherman, 2011)***

Surveillance recommended	
Population group	**Incidence of HCC**
Asian male hepatitis B carriers over age 40	0.4-0.6% per year
Asian female hepatitis B carriers over age 50	0.3-0.6% per year
Hepatitis B carrier with family history of HCC	Incidence higher than without family history
African/North American Blacks with hepatitis B	HCC occurs at a younger age
Cirrhotic hepatitis B carriers	3-8% per year
Hepatitis C cirrhosis	3-5% per year
Stage IV primary biliary cirrhosis	3-5% per year
Genetic hemochromatosis and cirrhosis	Unknown, but probably > 1.5% per year
Alpha 1-antitrypsin deficiency and cirrhosis	Unknown, but probably > 1.5% per year
Other cirrhosis	Unknown
Surveillance benefit uncertain	
Hepatitis B carriers younger than 40 (males) or 50 (females)	< 0.2% per year
Hepatitis C and stage 3 fibrosis	< 1.5% per year
Non-cirrhotic NAFLD	< 1.5% per year