INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, which cause approximately 600,000 to 1,000,000 deaths annually ***(El-Serag and Rudolph, 2007).***

As the disease is often advanced at the first manifestation, there is a 5-year survival rate of less than 5% without treatment. Surgical resection and liver transplantation are the only potentially curative therapies ***(Lewandowski et al., 2010).***

However, only few patients (11.9%-30.1%) at clinical presentation of tumors are suitable candidates for surgery because of multicentricity or poor hepatic functional reserve due to pre-existing cirrhosis. In clinical practice, therefore, transarterial chemoembolization (TACE) or transarterial embolization (TAE) is considered standard palliative treatment ***(Bruix and Sherman, 2010).***

Chemoembolization (TACE) is the most widely used primary treatment for unresectable HCC ***(Takayasu et al., 2006)***, and the recommended first line-therapy for patients at intermediate stage of the disease ***(Bruix and Sherman, 2010).***

HCC exhibits intense neo-angiogenic activity during its progression. The rationale for TACE is that the intra-

arterial infusion of a cytotoxic agent followed by embolization of the tumor-feeding blood vessels will result in a strong cytotoxic and ischemic effect. TACE should be distinguished from chemo-lipiodolization– delivery of an emulsion of chemotherapy mixed with lipiodol–, bland transcatheter embolization (TAE), where no chemotherapeutic agent is delivered, and intra-arterial chemotherapy, where no embolization is performed ***(Brown et al., 2009).***

Current determination of survival and prognosis in patients treated with TACE or TAE for unresectable HCC is mainly based on clinical assessment. Serum alpha-fetoprotein (AFP) as a well accepted tumor marker is only expressed by 60% of our patients. Thus, for more than one-third of the patients, AFP could not be linked to prognosis. Apart from well-known clinical factors related to tumor stage or liver function, remarkably few data are available upon other measurable prognostic or predictive factors for TACE or TAE treatment response in HCC ***(Huang et al., 2005).***

Because HCC is a highly vascular tumor, it has been studied screening patients for markers of increased angiogenesis, which may be detected even before HCC, is clinically significant. Vascular endothelial growth factor (VEGF) has successfully been correlated with stimulation of angiogenesis and does therefore reflect functional tumor

activity, which is otherwise often difficult to be assessed by conventional imaging modalities ***(Dzik-Jurasz, 2004).***

Tumor expression of VEGF has been shown to be related to microscopic venous invasion, metastasis spread and poor prognosis of HCC ***(Poon et al., 2003 (a)).***

The elevation of VEGF in blood implies a promotion of tumor angiogenesis, and several studies have shown that high serum VEGF levels predicted poor survival results independent of clinicopathological features in patients with various types of cancer undergoing resection or receiving chemotherapy ***(Sergio et al., 2008)***.

AIM OF THE WORK

1. To assess the clinical usefulness of pre-treatment P-VEGF level as a predictor of outcome in patients undergoing TACE therapy for HCC.
2. To assess the value of P-VEGF as an indicator of recurrence or residual of tumor after TACE.
3. To assess the value of P-VEGF as a marker for HCC.

HEPATOCELLULAR CARCINOMA

Hepatocellular Carcinoma (HCC) is a major health problem. It is the 5th most common type of cancer all over the world; after lung, prostate, colorectal, and stomach cancers and the 3rd leading cause of cancer-related deaths in the world exceeded only by cancers of the lung and stomach (***Attwa and El-Etreby, 2015***).

Incidence and Epidemiology:

HCC incidence is increasing all over the world, with estimated incidence of new cases is about 500 000-1 000 000 per year, causing 600, 000 deaths globally per year ***(Yeh et al., 2007).***

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 ***(IARC, 2011***).

Important differences regarding HCC incidence have been noted between countries. The incidence of hepatocellular carcinoma worldwide varies according to the prevalence of hepatitis B and C infections. Areas such as Asia and sub-Saharan Africa with high rates of infectious hepatitis have incidences as high as 120 cases per

100,000.Also several countries, particularly in East Asia including China , have high incidence (over 20 cases/100 000 population) ***(Gomaa et al., 2008).***

While in most developed areas of the world, including North America and much of Europe, the liver cancer incidence is at low (<5 per 100,000) or intermediate (5–10 per 100,000) levels ***(Parkin et al., 2005).***

Besides the emergence of liver disease due to hepatitis C, this growth in incidence may be also due to an increase in HBV related HCC, particularly among immigrants from endemic countries ***(Tanaka et al., 2008).***

Situation in Egypt:

In Egypt, there is a growing incidence of HCC (10-120/100,000) which represents the leading cause of death from all other cancer sites ***(El-Zayadi et al., 2010*)*.***

Incidence of HCC has nearly doubled over the last decade from 4% in 1993 to 7.2% in 2002 among patients with chronic liver disease.This remarkable increase may be explained by an increase in risk factors, such as the hepatitis C virus (HCV) infection that emerges over the same period of time. Additionally, Egypt has a high prevalence of HCV affecting approximately 12% of the general population ***(El-Zayadi et al., 2010).***

Risk Factors for HCC:

Approximately 90% of HCCs are associated with a known underlying risk factor. The major risk factor for the development of HCC is cirrhosis of the liver. However, about one quarter of HCC cases diagnosed in the United States do not have any known predisposing risk factors ***(Parkin et al., 2005).***

The major known risk factors for HCC are:

1. Viral (chronic hepatitis B and hepatitis C),
2. Toxic (alcohol and aflatoxins),
3. Metabolic (diabetes and non-alcoholic fatty liver disease, hereditary haemochromatosis and Wilson disease).
4. Immune-related (primary biliary cirrhosis and autoimmune hepatitis).

(Parikh and Hyman, 2007)

Recently, the geographical variability in the incidence of HCC has been attributed to the changing distribution and the natural history of Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection **(*Liu and Kao, 2007).***

Cirrhosis:

Cirrhosis develops following long periods of chronic liver disease and is characterized by a decrease in hepatocyte proliferation, indicating an exhaustion of the regenerative capacity of the liver ***(Delhaye et al., 1996***). This is associated with an increase in fibrous tissue and a destruction of liver cells, which provides the soil for development of cancerous nodules ***(Caillot et al., 2009).***

A number of mechanisms thought to accelerate cancer formation in patients with cirrhosis have been identified, including telomere dysfunction and alterations in the micro- and macro-environment that stimulate cellular proliferation ***(El-Serag and Rudolph, 2007***).

Telomerase enzyme plays an important role in maintaining telomere length and chromosomal stability in proliferating cells such as hepatocytes ***(Wege and Brummendorf, 2007).***

Telomeres are an essential part of human cells that affect our cells age ***(Jaskelioff et al., 2011)***. Telomeres are the caps at the end of each strand of DNA that protect our chromosomes. So, without telomeres DNA strands become damaged ***(Blackburn and Epel, 2012).***

Shortening of telomere limits proliferation of these cells and is therefore thought to reduce the regenerative

capacity of organs during aging and chronic diseases. In hepatocytes of a cirrhotic liver, telomeres are significantly shorter than in non-cirrhotic tissue, and this shortening has been shown to correlate with fibrosis progression ***(Wiemann et al., 2002).***

The effect of telomere dysfunction appears to be dependent on other factors, such as cell type and p53 status. So with combination of both factors, tumor onset is accelerated ***(Farazi and DePinho, 2006).***

Another characteristic of cirrhosis is the activation of stellate cells. This leads to an increase in the production of cytokines, growth factors, and products of oxidative stress ***(Sanyal et al., 2010***), many of which have been shown to affect hepatocyte proliferation and so could play a role in tumor formation ***(El-Serag and Rudolph, 2007***).

Finally, a number of molecular alterations that affect DNA, may promote tumor formation in the cirrhotic liver. These include loss of function of the p53 tumor suppressor gene ***(Sanyal et al., 2010).***Also inactivation of the p27 cell cycle regulator and loss of protein expression of the p16 cell cycle inhibitor ***(Matsuda, 2008***).

Cirrhosis is an important risk factor for HCC, and may be caused by chronic viral hepatitis, alcohol, inherited metabolic diseases such as hemochromatosis or alpha-1-antitrypsin deficiency, and non-alcoholic fatty liver disease.

All etiologic forms of cirrhosis may be complicated by tumor formation, but the risk is higher in patients with hepatitis infection. Overall, one-third of cirrhotic patients will develop HCC during their lifetime ***(Llovet et al., 2012).***

Long-term follow-up studies have demonstrated that approximately 1-8% per year of patients with cirrhosis develop HCC (e.g. 2% in HBV-infected cirrhotic patients and 3-8% in HCV infected cirrhotic patients)***(Ioannou et al., 2007).***

Hepatitis C virus infection:

The risk for developing HCC is 17-fold higher in HCV-infected patients and risk depends on degree of liver fibrosis ***(Sanyal et al., 2010)***.

Approximately 195,000 cases of liver cancer (31.1% of cases globally) are attributable to HCV, with northern and middle Africa being the areas of highest prevalence of HCV infection.While rates in North America, Europe and Australia have usually reported lower rates of HCV infection (***Parkin, 2006).***

Egypt has the highest prevalence of HCV in the world (predominantly genotype 4) (***Hassan et al., 2001***). So, HCV is considered as one of the main risk factors for development of HCC in Egypt (***Mabrouk, 1997***). It is

widely believed that the parenteral exposure to the virus is the most important route for acquiring infection in Egypt ***(Mohamed et al., 1996).***

Higher rates of HCV infection, up to 60%, have been reported among residents of rural areas such as the Nile delta, where schistosomiasis was endemic and where the previous public health eradication schemes for schistosomiasis had been applied***(Hassan et al., 2001***).

Up to 80% of HCV-infected individuals fail to eliminate the virus acutely and progress to chronic HCV infection. Continuous inflammation and hepatocyte regeneration in the setting of chronic hepatitis and subsequent progression to cirrhosis is thought to lead to chromosomal damage and possibly to initiate hepatic carcinogenesis (***Suruki et al., 2006).***

HCV, a RNA virus, cannot integrate into the host genome but it can also evade the host immune system and produce genomic instability like HBV ***(Zemel et al., 2011***). Its viral proteins can interfere with host defense mechanisms such as interferon (IFN) signaling pathway and the process of viral antigen presentation which then leads to chronic infection and eventually, malignant transformation ***(Ahmad et al., 2011)***. The HCV core protein, similar to HBx protein, can also cause genomic instability via its interactions with the mitotic spindle cell

checkpoint, thus contributing to the development of HCC ***(Zemel et al., 2011)***.

HCV yields chronic infection (60-80% of HCV cases versus 10% of HBV). The second key difference between HCV and HBV is the greater ability of HCV to promote liver cirrhosis compared with HBV: 5-10% of HCV-infected patients develop liver cirrhosis after 10 years of infection, approximately 10- to 20-fold higher than HBV ***(Farazi and DePinho, 2006).***

Regarding the HCV genotype, it was found genotype 1b was the most prevalent in patients with HCC followed by 2a than 2b***(Murakami et al., 1999).*** Patients infected with type 1b developed a more aggressive liver disease and have almost double the risk to develop HCC than those infected with other genotypes***(Raimondi et al., 2009)***.

Hepatitis B Virus

Hepatitis B virus (HBV) infection affects 400 million people worldwide and is the main risk factor for HCC in Eastern Asia and Africa. Approximately 54% of HCC cases worldwide can be attributed to HBV infection ***(Llovet et al., 2012).***

The annual incidence of HCC is 0.4% - 0.6% in HBV-infected non-cirrhotic patients, 2% in HBV-infected cirrhotic patients ***(Bruix and Sherman, 2005).***

HBV is a partially double-stranded DNA-containing virus. Infection with this virus is thought to cause HCC via both direct and indirect pathways. First, HBV infection causes hepatocyte injury and chronic necroinflammation, with subsequent hepatocyte proliferation, fibrosis, and cirrhosis. The continuous regeneration in cirrhosis leads to increased liver cell turnover and accumulation of mutations in the host genome that could result in genetic alterations, chromosomal rearrangements, activation of oncogenes, and inactivation of tumor suppressor genes ***(But et al., 2008).***

However, HBV can also cause HCC in the absence of cirrhosis ***(El-Serag and Rudolph, 2007).*** HBV is able to integrate its DNA into host cells and so may act as a mutagenic agent, causing secondary chromosomal rearrangement and increasing genomic instability ***(Sanyal et al., 2010).***

Also HBV may encode oncogenic viral proteins that contribute to hepatocarcinogenesis ***(Liu and Kao, 2007***).In addition, the regulatory protein HBx is thought to activate genes involved in deregulation of cell cycle control and interference with cellular DNA repair and apoptosis ***(Sanyal et al., 2010).***

Metabolic diseases:

Several inherited metabolic disease have been associated with the development of HCC. In most instances, it

seems to be the liver disease associated with the metabolic abnormality that results in HCC ***(Di Bisceglie et al., 2005).***

Haemochromatosis:

Genetic hemochromatosis is associated with an increased risk for hepatocellular carcinoma and the risk previously had been estimated to be as high as 200-fold increased. Recent studies suggest that HCC occurs predominantly in patients with cirrhosis***(Kowdley, 2004)***

Alpha-1-antitrypsin deficiency:

Chronic liver injury in Alpha-1-antitrypsin deficiency (A1AT) disease provides a sustained stimulus for hepatocellular proliferation that can lead to cirrhosis and hepatocellular carcinoma ***(Rudnick et al., 2004).***

Alpha-1-antitrypsin deficiency (AAT) is associated with the development of HCC, particularly its homozygous ZZ state. It occurs always with cirrhosis and may be diagnosed histologically by the presence of PAS-positive globules in liver tissue surrounding the tumor ***(Di Bisceglie et al., 2005).***

Wilson's disease:

Until recently, the association between Wilson disease (WD) and hepatocellular carcinoma (HCC) has been controversial ***(Riordan and Williams , 2001).***

All reported cases occurred in association with cirrhosis from Wilson's disease. Tumors have developed both before and after chelation therapy. The most important observation is that HCC tends to occur later in life, whereas the initial presentation of Wilson's disease occurs in the second or third decade of life. Perhaps HCC would occur more commonly if persons with untreated Wilson's disease survived beyond the fourth decade ***(Di Bisceglie et al., 2005).***

NASH & NAFLD:

Although it occurs in the absence of alcohol use, the hepatic histology appears consistent with alcoholic hepatitis (***Falck-Ytter et al., 2001)*** with changes in histology including hepatic steatosis, inflammation, hepatocyte injury as exemplified by cytologic ballooning and Mallory's hyaline, and fibrosis ***(Greenfield et al., 2008).***

Thus, NAFLD comprises a spectrum of conditions ranging from fat alone to fat plus inflammation, fat plus ballooning degeneration, and NASH, the latter being the most serious form of NAFLD ***(Falck-Ytter et al., 2001).***

Epidemiologic studies show that NAFLD is closely linked with the metabolic syndrome, particularly type 2 diabetes mellitus and obesity ***(Bugianesi et al., 2007)*** with NAFLD occurring almost universally among diabetic patients who are morbidly obese ***(Adams et al., 2005)***. Moreover, NASH in association with multiple components of the