INTRODUCTION

Hepatocellular carcinoma (HCC) is among the most rapidly fatal malignancies worldwide. It is considered as the most commonly occurring primary liver cancer and ranks as the fifth most frequently occurring cancer worldwide with an estimated 0.5 – 1 million new cases per year ***(Frenette and Gish, 2012)***. HCC is also considered as the third leading cause of cancer deaths all over the world ***(Kumar et al., 2011)***.

In Egypt, HCC was reported to account for 70.48% of malignancies in the liver, which in turn represented 1.68% of all malignancies ***(Mokhtar et al., 2007)***. It was mentioned that the incidence of HCC has risen due to increased burden of chronic liver disease in Egypt, accounting for about 4.7% of such patients ***(Hassan et al., 2011)***.

Many risk factors are incriminated in the development of HCC including hepatitis B and C viruses, alcohol intake, non-alcoholic steatohepatitis (NASH) and dietary aflatoxin exposure ***(Song et al., 2012)***. Whatever the underlying liver disease is, cirrhosis is considered the major factor modulating HCC risk ***(Roncalli et al., 2011)***.

Metastatic tumors of the liver are reported to be more common than primary liver neoplasms and sometimes represent the initial clinical manifestations of primaries in the GI tract, breast, lung, or pancreas ***(Khadim et al., 2012)***.

Because of the wide spectrum of histologic differentiation with the great morphologic diversity of HCC, the differential diagnosis between HCC and other malignancies involving the liver can be difficult ***(Karabork et al., 2010)***.

Intrahepatic cholangiocarcinoma, metastatic tumors like renal cell carcinoma, adrenocortical tumors, carcinoid, malignant melanoma, and poorly differentiated adenocarcinoma, can closely mimic HCC and pose diagnostic problems. Immunohisto-chemical methods are often required to facilitate the diagnosis ***(Roncalli et al., 2011)***.

A well-recognized immunohistochemical marker, Hepatocyte paraffin antigen (HepPar-1), was established to be a fairly sensitive one to HCCs. This antibody is able to recognize a hepatocyte-specific epitope localized to mitochondria. Recently, the HepPar-1 antigen has been shown to correspond to the urea cycle enzyme carbamoyl phosphate synthetase. However, it was proved that HepPar-1 may also stain hepatoid adenocarcinomas of the gastrointestinal tract which mainly arise in the stomach and sporadically in the esophagus, papilla of Vater, pancreas, gallbladder, large bowel, as well as in several non-gastrointestinal sites, including lung and urinary bladder. HepPar-1 may rarely also stain adrenal gland carcinomas and other biliary tract and gastric carcinomas ***(Mantonakis et al., 2012)***.

Arg-1 (Arg-1) has been used as a specific marker for hepatocytes and hepatocellular carcinoma. When compared with HepPar1, Arg-1 was clearly superior from both the standpoints of sensitivity and specificity for HCC ***(Yan et al., 2010)***.

It was reported that Arginase -1 is a cytosolic enzyme which is expressed mainly in the liver. It is considered as a key enzyme of the urea cycle, which catalyzes the hydrolysis of L-arginine to urea and L-ornitnine ***(Chrzanowska et al., 2009)***.

AIM OF THE WORK

The aim of this work is to study the expression of Arg-1 in hepatocellular carcinoma, cholangiocarcinoma and metastatic tumors of the liver, and to compare its sensitivity and specificity for HCC with HepPar-1, as the latter may lose much of its sensitivity towards poorly differentiated HCCs, and is able to stain other different tumors.

HEPATOCELLULAR CARCINOMA

Epidemiology

Hepatocellular carcinoma (HCC) ranks as the fifth most common malignancy worldwide, with about (750,000) new cases diagnosed annually. It also represents the third leading cause of cancer-related death, exceeded only by cancers of lung and stomach ***(Li et al., 2014).***

In (2012), an estimated (782,000) new cases of liver cancer occurred and approximately (746,000) people died of this cancer worldwide, an increase from (748,000) new liver cancers and (696,000) deaths from liver cancer in (2008) ***(Ferlay et al., 2014).***

Gender:

Liver cancer is the fifth most common cancer in men and the seventh in women, with an overall ratio (male: female) being (2.4). Despite this male predilection, this ratio varies across the world (fig.1). The explanation for this sex difference might be firstly, men could have higher rates of environmental exposure to liver carcinogens (such as smoking or alcohol) and hepatitis virus infections; secondly, estrogen effects might suppress interleukin (IL)-6-mediated inflammation in women, reducing both liver injury and compensatory proliferation; & thirdly, testosterone effects could increase androgen receptor

signaling in men, promoting liver cell proliferation (***Naugler et al., 2007)***.

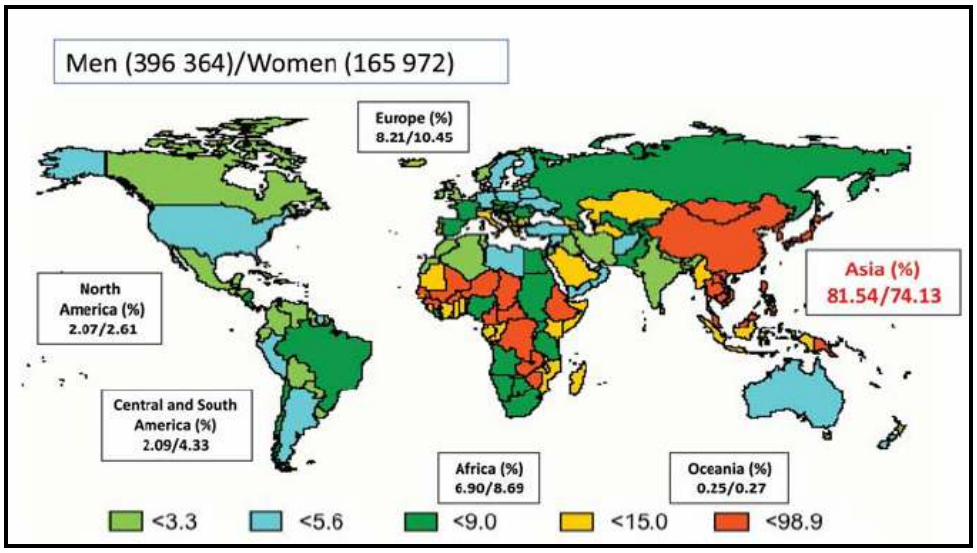

Figure (1): Age-adjusted incidence rates of liver cancer

Geographic Distribution:

The incidence of liver cancer varies around the world, most of the burden of HCC occurs in developing countries. The regions of high incidence are Eastern and South-Eastern Asia, Middle and Western Africa. By contrast, Low rates are estimated in developed regions such as North America, Europe (except for Southern Europe), Central and South America, Australia and New Zealand. The highest incidence of HCC occurs in Mongolia (116.6 cases per 100,000 person-years for men; 74.8 cases per 100,000 person-years for women) (fig. 2) ***(Ferlay et al., 2014)***.

Global variations in incidence rates of HCC closely reflect the variation in its risk factors; thus, countries with a high prevalence of HBV or HCV infections usually have a high incidence of HCC. This finding is consistent with the fact that about three-quarters of HCCs are attributed to chronic HBV and HCV infections. Interestingly, the incidence of HCC started to increase in the developed countries recently. The increase in number of patients infected with chronic hepatitis C in these regions is believed to drive this trend, which is expected to peak around (2015–2020) ***(Yang and Roberts, 2010)***

Also, the increasing prevalence of the metabolic syndrome, diabetes mellitus and non-alcoholic steatohepatitis (NASH) are all expected to continue to contribute to increased rates of HCC in the developed countries for the nearby future. This is in addition to increased immigration of people from populations at high risk of HCC into developed countries, which also contributes to their rising trend in incidence rates of HCC. In most of the countries, HCC accounts for (70-85%) of primary liver malignancy ***(Dahanasekaran et al., 2012).***

Hepatocellular Carcinoma in Egypt:

In Egypt, hospital-based studies have reported an overall increase in the frequency of all liver cancers from approximately (4%) in (1993) to (7.3%) in (2003). In a recent population based study, the age-standardized rates for liver

cancer for both genders were reported as (43.6/100,000). Among males the rates were reported as (61.8/100,000) and among females, the rates were (24.4/100,000). It was also reported that in Upper Egypt, liver cancer was still the most common cancer, this could be attributed to the high prevalence of hepatitis C viral infection (HCV), which is one of the highest prevalence rates worldwide and also due to the lack of HBV vaccination for a long period of time ***(Lehman et al., 2008) (Ibrahim et al., 2014).***

Investigations in Egypt suggested the HCV infection constitute a major risk factor in the development of HCC, accounting for (40 to 50%) of cases. The rising incidence of HCC in Egypt could be also explained by the improving screening measures and diagnostic tools, together with improved survival in cirrhotic patients giving some of them enough time to develop HCC (fig. 3)***(Omar et al., 2013).***

Age:

The incidence of HCC generally increases with age, although there are geographic differences. In Europe and the USA the peak age specific incidence is in the 7th decade, while in China the peak is in the 5th decade. In South Africa, the average age of patients with HCC is (35) years and (40%) are (30) or younger, whereas in Taiwan (an area of high incidence), the majority of patients are (40–60) years old with a peak incidence in the 8th decade. Nevertheless, HCC can occur in younger individuals and even young children ***(Goodman et al., 2012).***

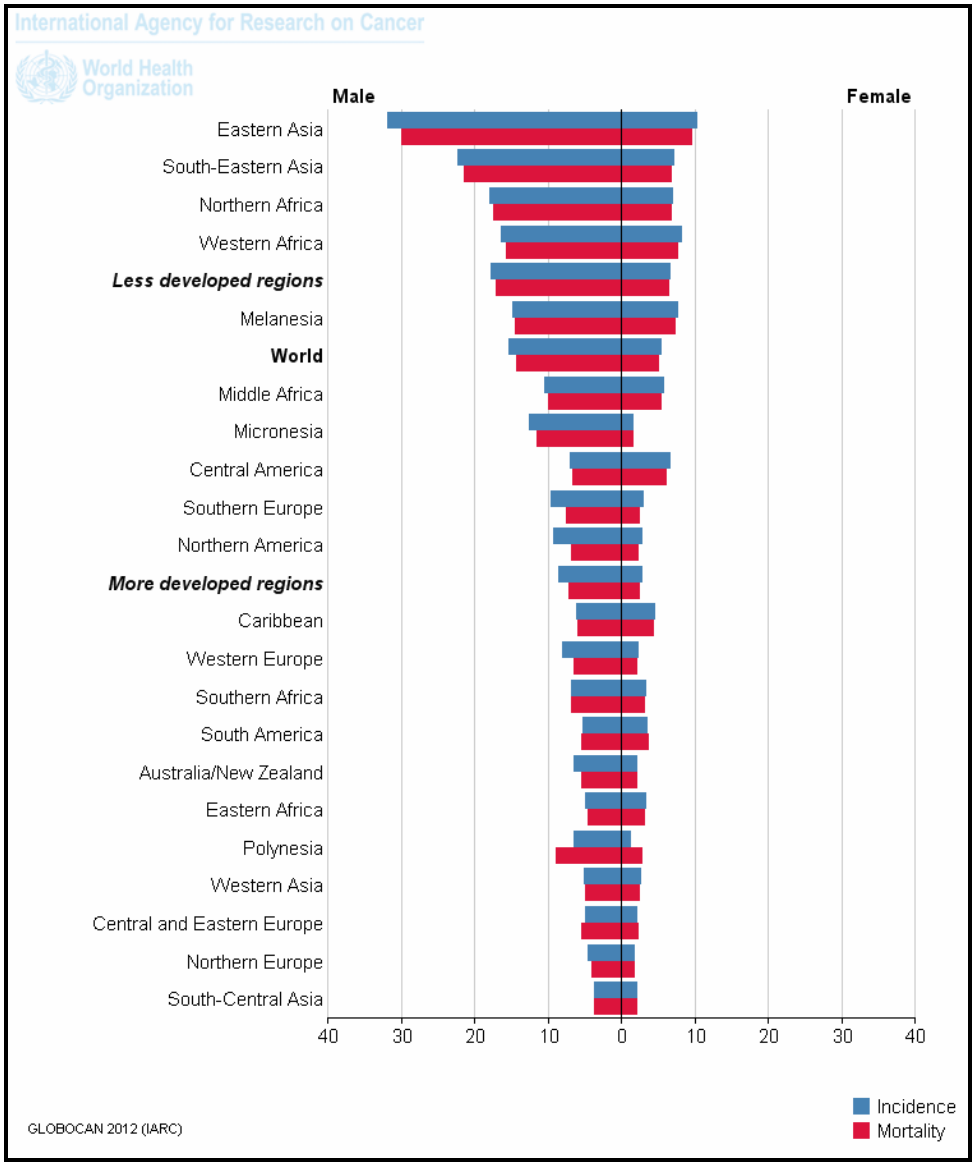

Figure (2): Estimated age-standardised rates (World) per 100,000
(Ferlay et al., 2014)

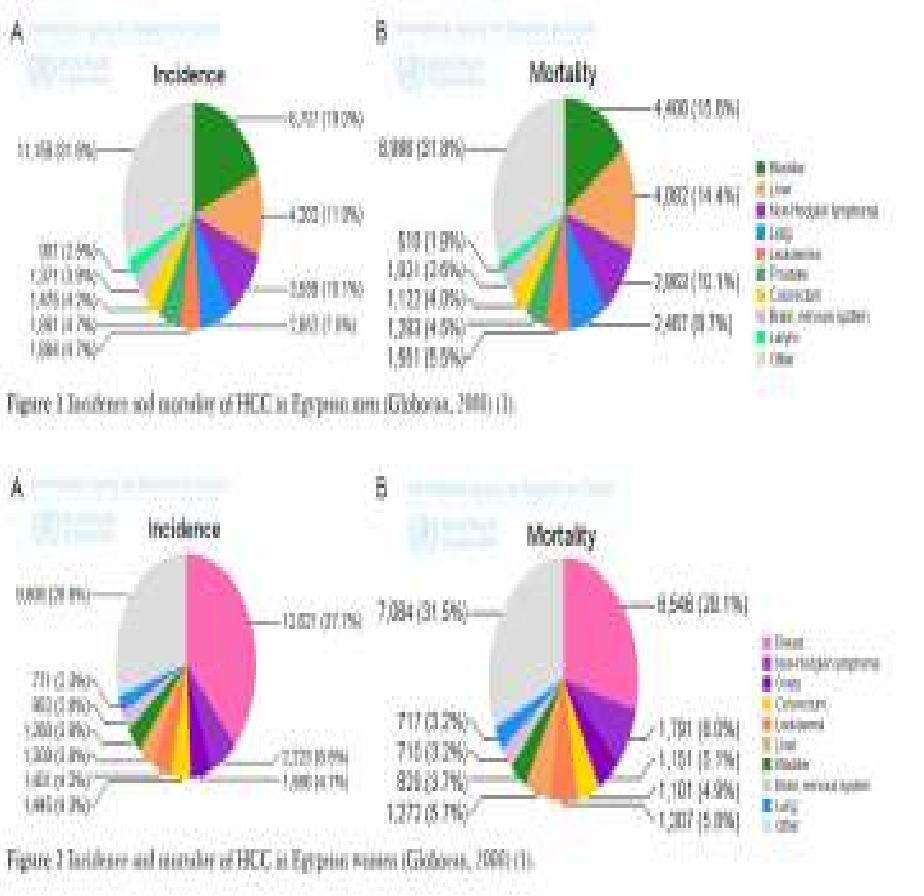

Figure (3): Incidence and mortality of HCC in Egyptian men and women ***(Omar et al., 2013).***

The age at which HCC develops in individuals infected with HBV or HCV is closely related to their age at acquisition of infection and the rate of active viral replication. As a consequence of viral infection at an early age, affected individuals (especially in African countries) develop cancer in their middle adulthood, during their most productive years of life. Conversely, in countries where viral hepatitis is not endemic, HBV and HCV infections are usually acquired in adulthood. In these countries,

HCC rarely develops before the age of (50) years and the highest age-specific incidence rates are observed in people over age (75) years ***(Yang and Roberts, 2010).***

Clinical Presentation

HCC is clinically silent and most of the patients present with advanced disease with diminished chance for effective treatment. Upper abdominal pain, weight loss, abdominal enlargement, hepatomegally with or without palpable masses are frequent on presentation. Some patients present primarily with hepatic decompensation manifested by rapidly accumulating ascites, variceal hemorrhage, hepatic encephalopathy and obstructive jaundice ***(Jaka et al., 2014).***

Unusual presentations of HCC may result from a number of complications of the tumor, such as tumor rupture causing an acute hemoperitoneum or Budd-Chiari syndrome and inferior venal caval obstruction from invasion of the hepatic venous system and inferior vena cava by the tumor ***(Kew, 2012).***

Rarely, some patients present with metastatic disease or paraneoplastic syndrome such as hypoglycemia, erythrocytosis, hypercholesterolaemia, hypercalcaemia, isosexual precocious puberty (in children), gynaecomastia (in the absence of cirrhosis), carcinoid syndrome, hypertrophic pulmonary osteoarthropathy, osteoporosis, hypertension, hyperthyroidism,

dysfibrinogenaemias, porphyria cutanea tarda and a variety of other cutaneous changes ***(Qiang et al., 2014).***

Patients who present with clinically evident disease are not good candidates for curative therapy. For those patients, the median survival is 1–3 months and survival beyond 1 year is exceptional. Survival rates in patients with earlier stage but still unresectable tumors are 17.5% at 1 year and 7.3% at 2 years, based on one clinical trial. Accordingly, the most encouraging trends in recent years have been in surveillance and early diagnosis of HCC in high risk populations combined with advances in surgical and other forms of definitive therapy ***(Cabibbo et al., 2010).***

Etiologic and risk factors

Many factors have been identified allover the years, increasing the risk for the development of HCC. Of them, the single most important one is liver cirrhosis, with the macronodular cirrhosis being more strongly associated with HCC than micronodular type. Most of the geographic variation reflects differences in the incidence of the underlying etiologic agent for cirrhosis ***(Goodman et al., 2012).***

The major known risk factors for HCC are hepatotropic viruses B and C, toxins such as alcohol and aflatoxin, metabolic diseases such as diabetes and obesity, hereditary hemochromatosis, and immune-related chronic liver diseases

such as primary biliary cirrhosis and autoimmune hepatitis ***(Dhanasekaran et al., 2012).***

HCC usually occurs in 10 years following the development of liver cirrhosis. This is highly consistent with the multistep process of hepatocarcinogenesis, which implies progressive accumulation of molecular and genetic defects leading to malignant transformation of preneoplastic lesions such as macroregenerative and dysplastic cirrhotic nodules ***(McGlynn and London, 2005).***

Viral hepatitis:

The majority of cases of HCC all over the world are due to hepatitis B virus (HBV), with a number of hepatitis C (HCV)-associated cases increasing in the western world. Globally, up to 80% of HCC is attributable to HBV or HCV and the risk of HCC is increased up to 15 folds and up to 17 folds in infection with HBV and HCV respectively. HBV and HCV are the main causal agents of chronic liver diseases, as approximately 5-10% of HBV and 75% of HCV infections become chronic ***(Seeff and Hoofnagle, 2006).***

- ***Hepatitis B:***

HBV is a prototype member of virus family "Hepadnaviridae", which has some biological charactersistics such as narrow host range, hepatotropism and tendency to chronicity ***(Sherman and Lovet, 2011).***

HBV consists of a partially double-stranded DNA genome enclosed by envelope proteins (HBsAg). The genome is packaged with a core protein (HBcAg) and a DNA polymerase. After penetration of the virus into the cell, its genome becomes a covalently closed, totally double-stranded molecule that can integrate into the host genome. The HBV polymerase, which reverse transcribes and replicates HBV DNA, lacks proofreading ability and is prone to generate mutations ***(McClune and Tong, 2010).***

Of all other risk factors, HBV has the strongest association with HCC. The relative risk of developing HCC in a serologically positive patient for hepatitis B surface antigen (HBsAg) is 98 times that of patients who are negative. Those who are also positive for e-antigen (HBeAg), indicating active viral replication, have 3.6 times the risk of those who are surface antigen positive only, suggesting that the activity of the disease does play a role in the pathogenesis ***(Sherman and Lovet, 2011)***.

Many evidences supported the direct oncogenic role of HBV in the development of HCC such as the integration of the HBV DNA into the chromosomal DNA of HCCs, the role of HBV X gene in the pathogenesis of HBV-associated HCC, in particular its binding to and inactivation of p53, and finally, HCC development in animal models chronically infected with hepadnaviruses. Additionally, the decline of HCC incidence

following HBV vaccination supports the etiological contribution ***(Hassan et al., 2011).***

The integration of HBV in sites within the host genome induces a wide range of genetic alterations including chromosomal deletions, translocations, production of fusion transcripts, amplification of cellular DNA and genetic instability. Common gene targets of integration include cyclin A2 gene, the retinoic acid receptor gene, human telomerase reverse transcriptase, PDGF receptor, and calcium signaling-related genes. A large proportion of HCC have integrated HBV sequences encoding HBV X (HBx) and/or truncated envelope pre-S2/S proteins, which both contribute in hepatocarcinogenesis. The proteins expressed from these integrated genes have intracellular effects, which account for their assosciation with HCC, including effects on cellular growth and apoptosis ***(Goodman et al., 2012).***

Chronic hepatitis D virus (HDV) infection does not increase the risk of HCC development over that of HBV infection alone, but the latency period between a superadded HDV infection and HCC development is 30-40 years, compared to 30-60 yeas for HBV infection alone ***(Hassan et al., 2011).***