INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, it accounts for 7% of all cancers and is considered the third cause of cancer-related deaths ***(Liovet and Ducreux, 2012).*** It is estimated that chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections account for 78% of the global HCC cases ***(Prieto and Cha, 2013).***The prognosis of patients with HCC is poor when diagnosed at an advanced stage, but when diagnosed and treated at early stage, the 5-year survival rate may reach up to 70-80% ***(Masatoshi, 2010).***

Although histo-pathological examination of tumor biopsy is considered the gold standard for diagnosis of HCC, it is considered an invasive technique with high risk of seeding the tumor along the biopsy tract ***(Gomaa et al., 2009).*** Non-invasive diagnosis includes radiological and laboratory methods. Radiological studies are operator-dependent. The quality of ultrasound examination is variable. It becomes even less reliable in the presence of cirrhosis, missing the diagnosis ofa significant number of cancer patients.However, spiral computerized tomography (CT) is still the routinely used method for diagnosis ***(Shariff et al., 2009).***

As regards serologic screening, α-fetoprotein (AFP) is the best serum biomarker for diagnosis ofHCC, but

sensitivity islow (25–65%) at the commonly used cut-off of 20 ng/mL,particularly in detection of early-stage HCC. Inaddition, many patients with non-malignant chronicliver disease have raised serum AFP concentrations,including 15–58% of patients with chronic hepatitis and 11–47% of those with liver cirrhosis ***(Shen et al., 2012).*** Also, AFPlevels are related to vascular invasion and tumor burden, and therefore can manifest late in presentation or sometimes not at all. Furthermore the most sensitive cut-off value byreceiver-operating characteristics (ROC) curve analysis has been a topic of great debate***(Giordano and Columbano, 2013).*** This highlights the need for a new more reliable non-invasive biomarker with better sensitivity and specificity for early diagnosis of HCC.

Dickkopf-1 (DKK-1), a secreted protein, is a known negative regulator of theWnt signaling pathway, which plays an important role in a varietyof cellular processes, including proliferation, differentiation,survival, apoptosis and cell motility***(Tao et al., 2013).***Itis a small secretory protein with 266 amino acids (35 kDa), and can be secreted into the bloodstream.It is hardly expressed in normal human adulttissues, except in placental and embryonic tissues***(Shen et al., 2012).***

Serum DKK-1 has been found to be increased in patients with various types of cancers and may be a useful biomarker ***(Tung et al., 2011).*** High levels of secreted DKK-1 could be specifically measured in culture media derived from multiple human-cancer cell lines such as lung cancer, breast cancer, glioma and cervical cancer ***(Shen et al., 2012).*** It has also been found to be a prognostic marker and predicts clinical outcome in oesophageal, ovarian and lung carcinomas. However, the clinical and prognostic significance of serum DKK-1 in HCC patients remains to be determined ***(Tung et al., 2011).***

AIM OF THE WORK

The aim of the present study is to evaluate the clinical utility of DKK-1 in the diagnosis and prognosis of HCC and to correlate its levels with AFP, the routinely used serological marker for diagnosis of the disease nowadays.

I. HEPATOCELLULAR CARCINOMA

A. Epidemiology:

1. Frequency:

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of death from cancer ***(Omata et al., 2010).*** More than 700,000 new cases are diagnosed each year throughout the world and also unfortunately more than 600,000 deaths are attributed to HCC each year. 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).***

2. Geographic Distribution:

Most cases of HCC (>80%) occur in sub-Saharan Africa and in Eastern Asia, with typical incidence rates of >20 per 100,000 individuals. Southern European countries (such as Spain, Italy and Greece) tend to have mid-incidence levels (10 to 20 per 100,000 individuals), whereas North America, South America, Northern Europe, and Oceania have a low incidence of HCC (<5 per 100,000 individuals). Lately, decreases in the incidence of HCC were reported among Chinese populations in Hong Kong, Shanghai, and

Singapore; the incidence in Japan is also decreasing. However, cases of HCC are increasing in low-incidence areas such as the United States and Canada ***(El-Serag, 2012).***In the United States, HCC is the most rapidly increasing cause of cancer-related mortality, and is ranked as third in men ages 40 to 60, clearly indicating its large socioeconomic impact ***(Hoshida et al., 2012).***

The prevalence of HCC worldwide parallels that of viral hepatitis, and the majority of cases are associated with hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Chronic HBV infection is a leading cause of HCC in most African and Asian countries except Japan. HCV predominantly contributes to HCC in some southern European countries (e.g., Italy and Spain) and Japan ***(Omata et al., 2010).***

Egypt has the highest prevalence of HCV worldwide (13.8%) and has rising rates of HCC. HCC has the second most frequent incidence and mortality among men in Egypt. Hospital-based studies in Egypt have reported an increase in the relative frequency of all liver-related cancers (95% as HCC), from 4% in 1993 to 7.3% in 2003 ***(Lehan and Wilson, 2009).***

3. Demographic Factors:

a) Age:

Age-specific incidence rates are strongly affected by the etiology of the background liver disease. Old age is an independent risk factor for HCC, especially in areas where HCV infection is endemic. On the other hand, the incidence rates increase after 20 years of age in countries where HBV-related carcinogenesis is dominant ***(Omata et al., 2010).***HCC is rarely seen during the first 4 decades of life, except in populations where HBV infection is hyperendemic. The mean ages of diagnosis of patients with HCC were 55–59 years in China and 63–65 years in Europe and North America ***(El-Serag, 2012).***

b) Sex:

Hepatocellular carcinoma occurs more commonly in men than in women. 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, they consume alcohol, smoke cigarettes and have increased iron stores. Androgenic hormones and increased genetic susceptibility may also increase the risk among males ***(Stuart and Stadler, 2009).***Overall, HCC displays a male predominance, occurring two to four times more often in males than in females ***(Dhanasekaran et al., 2012).***

B. Risk Factors:

The vast majority of cases of HCC occur in the setting of liver cirrhosis, and thus the etiology of HCC mirrors that of chronic liver disease and cirrhosis. Broadly, risk factors for HCC include viral infections, environmental toxins, co-morbid conditions, inherited errors of metabolism, and autoimmune disorders ***(Tinkle and Haas-Kogan, 2012).***

1. Chronic Hepatitis B Virus Infection:

Chronic infection with hepatitis B virus (HBV) is the most common global cause of HCC, affecting more than 350 million individuals (6% of world population), and is the dominant etiology especially in China and Africa ***(Hoshida et al., 2012).***

In many high risk areas—particularly those in Asia—HBV is transmitted from mother to newborn (vertical transmission); as many as 90% of infected babies develop chronic infections. This pattern is different in areas that have low incidence of HCC, HBV infection is usually acquired in adulthood, through sexual and parenteral routes (horizontal transmission). More than 90% of these cases of acute HBV infection resolve spontaneously. The higher frequency and longer period of chronic HBV infections contribute to a greater risk for HCC in areas where HBV infection is common ***(El-Serag, 2012).***

Most cases of HBV-related HCC (70%-90%) occur in patients with cirrhosis secondary to chronic necro-inflammation, but HBV itself can cause HCC in the absence of cirrhosis through direct carcinogenic mechanisms that have been related to the capacity of HBV to integrate into the host's genome and to produce proteins (X protein and truncated preS-S protein) with potential transforming properties ***(Della Corte et al., 2013).***

2. Chronic Hepatitis C Virus Infection:

Hepatitis C virus (HCV) affects 170 million individuals worldwide. It has been the major risk factor of HCC in many industrialized countries, and is contributing to the increasing HCC incidence in the US ***(Hoshida et al., 2012).***

The rate of HCC among HCV-infected persons ranges from 1% to 3% over 30 years. Similarly, HCV infection is associated with a 15- to 20-fold increase in risk for HCC compared with HCV-negative subjects in cross-sectional and case-control studies ***(El-Serag, 2012).***

Clinically, the incidence of HCV-related HCC increases according to the severity of liver fibrosis, and in contrast to HBV-related HCC, patients with minimal fibrosis rarely develop HCC, suggesting that cirrhotic microenvironment is the major driver.Some studies suggested that genotype 1 is associated with increased risk of HCC, although it may

simply reflect that this genotype is refractory to antiviral therapies and has more chance to progress into advanced disease ***(Hoshida et al., 2012).***

3. Co-Infection with Hepatitis B Virus and Hepatitis D Virus:

An estimated 15-20 million individuals with HBV worldwide are found infected with HDV, highlighting a need to exactly understand the pathogenesis and molecular biology of the virus. Chronic HDV/HBV infection causes a more severe liver disease than HBV monoinfection alone; the disease runs a rapidly progressive course, leading to early cirrhosis, decompensation, hepatocellular carcinoma and a shorter 5-year survival ***(Abbas and Afzal, 2013).***

4. Alcohol Intake:

Excessive alcohol consumption is known to have an influence on the development of cirrhosis and liver cancer. Furthermore, alcohol interacts in a complex way with other environmental or host factors, such as HBV or HCV infection, to induce HCC. Hepatic steatosis evolves in all excessive drinkers; however, only about one-third of them develop significant necro-inflammation and fibrosis, and only about 10% progress to cirrhosis. Among the latter, 1–2% per year develop HCC ***(Kim and Han, 2012).***

It has been suggested that heavy alcohol consumption of >80 g/day ethanol for at least 5 years increases the risk of

HCC by nearly 5-fold. The risk appears to be proportional to the amount of alcohol consumed ***(Hamed and Ali, 2013).***

5. Hereditary Hemochromatosis (Iron Overload Syndromes):

Hereditary hemochromatosis, a condition characterized by excess iron absorption, is caused by mutations in the hemochromatosis gene (HFE) gene and/or other mutations in the iron metabolism machinery. The altered iron metabolism seen in hereditary hemochromatosis leads to excess iron storage in the liver and the subsequent development of liver dysfunction. Although other organs/systems are also susceptible to iron overload, the liver bears the majority of malignant disease, with those patients with hereditary hemochromatosis being 20 times more likely to develop liver cancer than all other cancers combined ***(Hamed and Ali, 2013).***

Hereditary hemochromatosis is only one of the iron overload syndromes that lead to excessive iron deposition in the liver and other tissues. In fact, those patients with excess total body iron secondary to other etiologies have been shown to have a higher risk of HCC in the absence of genetic hemochromatosis. Studies have suggested that conditions such as β thalassemia or iron overload in people of African descent might be associated with an increased risk of HCC ***(Blonski et al., 2010).***

6. Aflatoxin:

Aflatoxin is believed to be a major causative agent in the high incidence of primary liver cancer seen in certain regions of the world. In some African and Asian regions, it is known to be a key risk factor of HCC. Aflatoxins are a group of approximately 20 related fungal metabolites with four major known as B1, B2, G1, and G2. Among them, B1 is the most potent naturally occurring chemical liver disease carcinogen known. They are produced by Aspergillusflavus and related fungi that grow on improperly stored foods, such as corn, rice, and peanuts. Effects of aflatoxin carcinogen result in p53 gene mutations ***(Raphael et al., 2012).***

7. Non-Alcoholic Fatty Liver Disease:

Several case reports and subsequent observational studies have proposed that non-alcoholic fatty liver disease (NALFD), and more specifically, non-alcoholic steatohepatitis (NASH), confers an elevated risk of developing HCC ***(Blonski et al., 2010).***

NAFLD can lead to cirrhosis in patients without a history of alcohol abuse. Its prevalence is increasing in Western countries because of the increasing obesity and diabetes mellitus incidence. Generally, 20% of NASH cases progress to cirrhosis and result in complications including HCC ***(Raphael et al., 2012).***

8. Diabetes Mellitus:

Diabetes mellitus (DM) is an independent risk factor for the development of HCC. Analysis of 2,061 patients with HCC showed a significant increase in the development of HCC in the background of DM regardless of the presence of other risk factors ***(Rahman et al., 2013).***

9.Precancerous Lesions Predisposing to HCC:

Several precancerous lesions were described as possible predisposing conditions for the development of HCC. These include liver cirrhosis and adenomatous hyperplasia ***(Raphael et al., 2012).***

a) Liver cirrhosis:

Cirrhosis is an end stage of chronic diffuse liver disease. It is characterized by alteration of the normal liver into structurally abnormal nodules of liver cells surrounded by fibrosis. The changes must be diffuse throughout the liver. The risk of cancer development from chronic liver disease or cirrhosis varies according to the degree of fibrosis. Main causes of liver cirrhosis are alcohol abuse, chronic hepatitis B and C, and non-alcoholic steatohepatitis ***(Raphael et al., 2012).***

Cirrhosis is present in 80–90% of HCC patients and is thereby the largest single risk factor. With the exception of HBV and aflatoxin, all other etiological risk factors are associated with cirrhosis ***(Raphael et al., 2012).***

Established cirrhosis serves as a milieu/microenvironment that fosters initiation and promotion of carcinogenesis by facilitating genetic aberrations and cellular transformation, which is often referred to as "field cancerization" or "field effect" ***(Hoshida et al., 2012).***

b) Liver cell dysplasia:

Emerging international guidelines classify clinically detectable pre-neoplastic lesions into low-grade dysplastic nodule (L-DN or LGDN) or high-grade dysplastic nodule (H-DN or HGDN), which provides the basis of molecular interrogation. L-DN, occasionally accompanied with large-cell change, shows more benign clinical behavior, whereas H-DN, as a potential HCC precursor, could exhibit cytologic atypia called small-cell change and nodule-in-nodule appearance, in which the subnodule represents de-differentiation of parent nodule (i.e., well-differentiated/early HCC) ***(Hoshida et al., 2012).***

C. Hepatocarcinogenesis:

Hepatocarcinogenesis is a complex multi-step process involving a number of genetic and epigenetic alterations, the activation of cellular oncogenes, the inactivation of tumor suppressor genes, and dysregulation of multiple signal transduction pathways ***(Tsai and Chung, 2010).***

1. Epigenetics Alteration Pathways:

Epigenetics is defined as heritable states of gene expression without altering DNA sequences. Epigenetic mechanisms encompass genomic DNA modifications (methylation of DNA cytosine bases), chemical modifications of histone tails, and non-coding miRNA regulation ***(Ma et al., 2014).***

The epigenetic phenomena regulate the chromatin structure modifications and the initiation of transcription in a manner that alters the availability of genes to transcription factors required for their expression ***(Lee et al., 2014).***

Epigenetic alterations have been identified as a major characteristic in human cancers. Recent findings suggest that epigenetic alterations occur at much higher rates and are more diverse in cancer cells compared to DNA mutations ***(Anwar and Lehmann, 2014).***

Global DNA hypomethylation, promoter methylation, aberrant expression of miRNAs, and dysregulated expression of other epigenetic regulatory genes such as enhancer of zeste 2 polycomb repressive complex 2 subunit gene (EZH2) are the best-known epigenetic abnormalities in HCC ***(Ma et al., 2014).***

DNA methylation is the most commonly studied epigenetic mechanism and is crucial in the development of