INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and third most significant cause of cancer mortality in the world **(Liu et al., 2013)**. It is a common cancer with an estimated annual incidence of 600,000 world-wide **(Pracht et al., 2013)**.

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 and C infection, as it represents about 45.3% of the new cases of the digestive system cancer **(Miller and Abu-Raddad, 2010)**.

It is estimated that almost 20% of chronic viral hepatitis cases would progress to cirrhosis, and above 80% of tumors develop from cirrhotic livers. Therefore, cirrhosis is considered a premalignant condition of HCC **(El-Serag, 2011)**. Of particular importance is chronic infection with hepatitis B virus (HBV) or hepatitis C virus (HCV). Indeed, it has been estimated that HBV is responsible for 50%–80% of HCC cases worldwide, whereas 10%–25% of HCC cases are thought to be a result of HCV infection **(Venook et al., 2010)**.

There is considerable evidence strongly supporting the role of genetic predisposition in HCC patients, based on the results of genetic association studies, several loci are believed to be associated with HCC, such as MICA, HLA-DQA1/DRB1 and SATA4 **(Wang et al., 2013)**. In addition, tobacco smoking, alcohol drinking and aflatoxin exposure were also shown to be risk factors for HCC **(Lin et al., 2012)**. However, only a minority of individuals who are at risk will develop HCC, indicating that the theory of conventional risk factors can only explain part of the pathogenesis of HCC **(Nahon and Zucman-Rossi, 2012)**.

Due to the asymptomatic nature of early HCC and lack of effective screening strategies, most patients (>80%) present with overt advanced disease

(El-Serag, 2011). Currently, the most utilized surveillance methods for patients with cirrhosis are serum alpha-fetoprotein (AFP) level and ultrasonography **(Sun et al., 2012)**.

About 30% of HCC cases with normal serum AFP levels are hardly diagnosed before clinical manifestations appear, and this highlights the need for new early reliable detection biomarkers for HCC **(Malaguarnera et al., 2010)**.

Annexins are a family of proteins that bind anionic phospholipids in a calcium-dependent manner **(Clark et al., 2012)**. Annexins were first discovered in animal cells and were named for their ability to "annex" or aggregate membranes. The annexins are expressed in vertebrates (ANXA), invertebrates (ANXB), fungi and protozoa (ANXC), plants (ANXD) and protists e.g. algae (ANXE) **(Bharadwaj et al., 2013)**.

Annexin A2 exists both as a monomer and a heterotetramer. The annexin A2 monomer is an intracellular 36 kDa protein whilst, the annexin A2 heterotetramer consists of two subunits of annexin A2 monomers and two subunits of p11 proteins (also known as S100A10, is a member of the S100 family of Calcium-binding proteins that confers increased phospholipid binding affinity on annexin A2) localized on the plasma membrane **(Lokman et al., 2011)**.

The human annexin A2 (Anxa2) gene spans approximately 40 kb on the long arm of chromosome 15 (15q21) **(Lokman et al., 2011)**.

Annexin A2 is mainly expressed in human endothelial cells, mononuclear cells, macrophages, marrow cells and some tumor cells **(Zhang et al., 2012)**. Moreover, annexin A2 is an inducible, calcium-dependent phospholipid-binding protein which is overexpressed in a variety of human malignancies and has emerged as an attractive candidate receptor for increased plasmin generation on the tumor cell surface **(Zhai et al., 2011)**. It plays multiple roles in regu-

lating cellular functions, including angiogenesis, proliferation, apoptosis, cell migration, invasion and adhesion **(Yang et al., 2012)**.

Annexin A2 possesses an aberrated expression in digestive system-related tumors, such as hepatocellular carcinoma (HCC), esophageal, gastric and colorectal carcinoma. Also it is involved in other malignant tumors e.g. tumors of the lung and the breast **(Sun et al., 2012)**.

Annexin A2 is almost undetectable in normal liver and chronic hepatitis tissues, while highly positively detected in HCC, also serum levels of annexin A2 are elevated in early stage HCC patients which are AFP-negative **(Ji et al., 2009)**.

Annexin A2 was reported to promote HCC metastasis and invasion through its interaction with HAb18G/CD147 (a member of immunoglobulin family) **(Zhang et al., 2012)**.

The change in serum levels of Annexin A2 in the early stages of HCC is still to be elucidated.

AIM OF THE WORK

The aim of the present study is to investigate the clinical utility of Annexin A2 serum level as a novel diagnostic marker of hepatocellular carcinoma (HCC) and to correlate its level with alpha fetoprotein the current marker of hepatocellular carcinoma (HCC).

HEPATOCELLULAR CARCINOMA

Primary hepatic cancer (PHC) is one of the most common malignant tumors with 90-95% of liver cancer being hepatocellular carcinoma (HCC) **(Zhao et al., 2013)**.

A. Epidemiology:

1-Global incidence of hepatocellular carcinoma:

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer death worldwide **(Semela et al., 2011)**. 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 **(Surveillance Research Program, National Cancer Institute. Fast stats, 2011)**.

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 **(Dhanasekaran et al., 2012)**.

2-Incidence of hepatocellular carcinoma in Egypt:

In 1998, the Egyptian Ministry of Health and Population in collaboration with the National Cancer Institute of Cairo University established a population-based Cancer Registry "The National Cancer Registry" (NCR).

The NCR data confirmed the high incidence of HCC in Egypt. HCC was reported to account for about 4.7% of chronic liver disease patients **(Hamed and Ali, 2013)**.

Egypt has high prevalence of hepatocellular carcinoma (HCC). It is the second most common cancer among males and seventh among females **(Abdelgawad et al., 2013)**. It represents about 45.3% of the new cases of the digestive system cancer. And 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 et al., 2010)**.

The rising rates of HCC in Egypt are due to the high prevalence of hepatitis B virus (HBV) and hepatitis C virus infection (HCV) among Egyptian population. There is a shift in the relative influence of these viruses in HCC etiology in Egypt, as HBV infection significantly decreased while HCV did not **(Abdelgawad et al., 2013)**.

In addition, the increased incidence of HCC in Egypt can be attributed to the improvements in the screening programs and diagnostic tools as well as the increased survival rate among patients with cirrhosis allowing time for some of them to develop HCC **(Anwar et al., 2008)**.

The role of exposure to aflatoxin in Egypt may also contribute to the development of HCC. A significant higher percent

of aflatoxins in the serum of Egyptian patients with HCC compared to their controls was detected; with a twofold increased risk **(Anwar et al., 2008)**.

3-Morbidity and mortality:

The majority of HCC are diagnosed at an intermediate or advanced stage. Curative treatments (resection, transplantation and radiofrequency) are only suitable for very early and early stage patients with compensated liver function. For patients who are not eligible for curative treatments, local ablative therapies play an important role in reducing tumor burden, providing palliation of symptoms, and increasing survival **(Weng et al., 2013)**.

Owing to its high morbidity, high malignancy, high rate of recurrence after curable treatments, and resistance to traditional therapies, the 5-year survival rate of patients with untreated HCC is <5%, placing it among cancers with the worst prognosis **(Wang et al., 2014)**. Surgical cure is possible in less than 5% of patients. The prognosis is affected by both the tumor severity, and the degree of pre-existing liver damage **(Kuwaki et al., 2011)**.

4-Demographic factors:

a. Age:

Age at diagnosis varies widely according to geographic distribution. In low incidence regions, the median age at diagno-

sis is 65 years. Hepatocellular carcinoma rarely occurs before the age of 40 years and reaches a peak at approximately 70 years of age except in patients with cirrhosis **(El-Serag, 2011)**.

In high incidence areas, age at diagnosis is substantially younger, occurring in the fourth and fifth decades of life. Diagnosis at a younger age is thought to reflect the natural history of hepatitis B virus and hepatitis C virus related HCC **(Winter, 2006)**.

b. Sex:

HCC displays a male predominance, occurring two to four times more often in males than in females **(Jemal et al., 2011)**.

HCC is the fifth most frequent cancer throughout the world among men with estimated 442,000 new cases per year. Among women, HCC considered as the eighth most frequent cancer in world, with an estimated 184,000 new cases per year **(Zidan et al., 2012)**.

In Egypt HCC is the second most common cancer site among males and seventh among females **(Abdelgawad et al., 2013)**.

c. Race:

HCC incidence rates also vary greatly among different populations living in the same region **(Kuntz and Kuntz, 2006)**. An example is the United States where, African-American men are having higher incidence rates than USA Caucasian men **(Gomaa et al., 2008)**.

B. Risk Factors:

Any cause of liver disease that can result in cirrhosis should be considered a potential risk factor for HCC. Not surprisingly, the most common causes of cirrhosis, namely HBV, HCV and alcohol, are also the most common causes of HCC **(Davis et al., 2008)**.

1. Chronic Hepatitis B Virus Infection:

More than half of the world HCC burden has been attributed to HBV infection. The International Agency for Research on Cancer in 1994 reported that there was sufficient evidence for the carcinogenicity of chronic HBV infection **(Renumathy et al., 2012)**.

The etio-pathogenesis of HBV-related HCC is complex and multiple pathways of carcinogenesis have been proposed. The process involves HBV causing chronic hepatic injury with cirrhosis and hepatocyte regeneration leading to accumulation of

oncogenic mutations and also HBV DNA integration in the host genome causing transactivation of oncogenes of the host **(But et al., 2008)**.

While HBV can cause HCC in the absence of cirrhosis due to its ability to integrate into the host genome, the majority of the HBV related cases occur in the background of cirrhosis **(Amin et al., 2006)**.

The risk of HCC in patients with active chronic HBV infections is well established. But another study also showed that carriers of inactive chronic HBV are at risk for HCC and liver-related deaths **(Chen et al., 2010)**.

Among patients with chronic HBV, there are additional risk factors that further increase the risk of HCC including male gender, older age, family history of HCC, use of alcohol or tobacco, serum alanine aminotransferase level, co-infection with HCV, human immunodeficiency virus or hepatitis delta virus, persistent presence of HBe Ag, high serum levels of HBV DNA, HBV genotype C, and presence of cirrhosis **(Dhanasekaran et al., 2012)**.

2. Chronic Hepatitis C Virus Infection:

Hepatitis C virus related liver cirrhosis is also a risk factor for HCC. Worldwide, 25-30% of cases of HCC can currently be attributed to hepatitis C virus infection **(Malek et al., 2014)**.

HCC is a major health problem in Egypt where it accounts for about 4.7% of chronic liver disease patients. HCV infection is high in Egypt and its percentage in patients who develop HCC is higher than that of any other country **(Zekri et al., 2012)**.

The major risk factor for HCC in patients with chronic HCV is advanced hepatic fibrosis or cirrhosis. Unlike HBV, HCV is a ribonucleic acid virus and hence cannot integrate into the host genome. Similar to HBV, the carcinogenesis of HCV-associated HCC is thought to be a multistep process involving up-regulation of inflammatory cytokines and induction of oxidative stress from chronic hepatitis, fibrosis, liver regeneration, and, ultimately, the development of cirrhosis **(Dhanasekaran et al., 2012)**.

These steps are likely shared by the risk factors that transform the liver parenchyma into the fibrotic nodules characteristic of cirrhosis, which sets the stage for dysplastic nodules and genetic changes resulting in the development of HCC. A number of risk factors can have a synergistic effect on the risk of HCC in patients with chronic HCV when present in combination. The association of HCV with the following risk factors has been shown to further increase the chances of developing HCC: alcohol use, co-infection with HBV or human immunodeficiency virus, diabetes mellitus, older age, African American

race, thrombocytopenia, elevated alkaline phosphatase, esophageal varices, and smoking **(Davis et al., 2008)**.

Also the virus likely plays some role in the process. This concept is supported by the observation that the risk of HCC remains about 2.5 folds higher in cirrhotic patients who fail to clear HCV with antiviral therapy than in those who eradicate infection **(Davis et al., 2008)**.

There are strong suggestions that HCV genotype 1b is associated with the risk of developing hepatocellular carcinoma (almost double the risk of developing hepatocellular carcinoma than those infected with other genotypes). The risk is higher in the early stages of liver disease when compared with patients affected by liver cirrhosis. Several authors have suggested that this association could be due to a role of HCV genotype 1b in the early stage of liver disease progression prior to the establishment of liver cirrhosis, with genotype 1b playing a major role in carcinogenesis and the development of hepatocellular carcinoma through its association with chronic inflammation, liver cell necrosis, regeneration, and extensive fibrosis **(Bruno et al., 2011)**.

Clinical differences among HCV genotypes and specific host/virus interactions depending on the infecting genotype also exist, as suggested by the association of genotype 2 with mild

liver disease and better responses to antiviral treatment **(Bruno et al., 2011)**.

3. 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 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)**.

4. Alcohol Intake:

The mechanism by which alcohol consumption increases the risk of HCC is primarily through the development of cirrhosis **(Hamed and Ali, 2013)**.

It has been suggested that 10 years consumption of more than 80 mL of alcohol per day for men and 20 mL per day for women is generally required before there is a significant risk of HCC. This difference is related to gender-specific nutritional behavior and food preferences **(Donato et al., 2002)**. But the main factor that accounts for this gender difference is that women have less alcohol dehydrogenase enzyme in their gastric mucosa.

The latter is responsible for breakdown of alcohol into less toxic products. Therefore, women will absorb more non-

metabolized alcohol from their stomach lining, needing less dose of alcohol to be subjected to its toxic effects than their counterparts **(Poschl and Seitz, 2004)**.

The pathogenic mechanisms by which alcohol predisposes to HCC and other cancers in patients with heavy alcohol exposure are not known. It is not known whether alcohol is directly carcinogenic for these tumors or acts as a co-carcinogen. Acetaldehyde, the main metabolite of alcohol, causes hepatocellular injury and is an important factor in causing increased oxidant stress, which damages DNA. Malnutrition associated with alcohol intake is associated with defects in DNA methylation, an essential pathway in gene control. Defects in methylation lead to genomic instability and inactivation of tumor suppressor genes **(Fukushima et al., 2006)**.

5. Hepatic Iron Overload:

Excessive iron appears to be directly toxic to host tissues through the interactions of reactive oxygen species, and of iron itself with DNA leading to lethal injury or predisposition to HCC **(Kumar et al., 2004).** Patients with genetic hemochromatosis have a risk of HCC that may be increased as much as 200-fold compared with that of the general population. The risk of developing HCC is approximately 1% per year in the patients with genetic hemochromatosis accompanied with cirrhosis;

however, occasional cases occur in patients without known cirrhosis. Depletion of iron stores by phlebotomy does not decrease the risk of HCC once cirrhosis is established. Besides hemochromatosis, iron overload from other causes like chronic blood transfusion or ineffective erythropoiesis may also increase the risk of HCC **(Kowdley, 2004)**.

6. Aflatoxin Exposure:

Aflatoxins are naturally occurring potent hepatocarcinogenic mycotoxins produced by some Aspergillus species. They are weedy molds that grow on a large number of substrates, including grains, corn, peanuts, and fermented soya beans, particularly under high moisture conditions in parts of sub-Saharan Africa and eastern Asia. In areas where ingestion of aflatoxin contaminated food is common, the incidence rates of HCC tend to be high, most likely due to a characteristic mutation in the p53 tumor suppressor gene that has been found in 30–60% of all HCCs in these areas **(Polychronaki et al., 2007)**.

Furthermore, it has been found that individuals infected by HBV and who are exposed to aflatoxin have an even higher risk of liver cancer, suggesting a synergistic effect between HBV and aflatoxin. A prospective cohort study from China found that among individuals with chronic HBV infection the adjusted rela-