INTRODUCTION

The incidence of heapatocellular carcinoma (HCC) varies widely according to geographic location. The distribution of HCC also differs among groups within the same country these extreme differences in distribution of HCC are probably due to regional variation, in exposure to hepatitis and environmental pathogens ***(Kuda et al., 2011).***

Hepatocellular carcinoma (HCC) is strongly associated with either chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, and the third leading cause of cancer death worldwide ***(Shariff et al., 2009).***

Hepatocellular carcinoma is primary malignancy of the hepatocyte generally leading to death within 6-20 month. Hepatocellular carcinoma frequently arises in the setting of cirrhosis appearing 20-30 years following the initial insult to the liver. How ever, 25% of patients have no history or risk factors for the development of cirrhosis. Therefore, cirrhosis is considered the premalignant condition of HCC ***(Jemal et al., 2009).***

Due to the asymptomatic nature of early HCC and lack of effective screening strategies, most patients (>80%) present with overt advanced disease. Currently, the most utilized surveillance methods for patients with cirrhosis are serum

alpha-fetoprotein (AFP) level and ultrasonography with some limits ***(Marrero et al., 2009).***

Among the 49 identified proteins, annexin A2 was selected to further histological and serological validation. Annexin A2 belongs to a widely distributed, calcium-dependent, phospholipid-binding protein family. It is located on the surface of endothelial cells and most epithelial cells ***(Lokman et al., 2011).***

Annexin A2 has been implicated in many functions, for example, exocytosis, endocytosis, vesicle transport, regulating ion channels, immune response, cell–cell adhesions, mitogenic- and lipid-messenger-mediated signalling and fibrinolysis ***(Bandorowicz-Pikula et al., 2012).***

Regarding normal liver tissue, annexin A2 is consistently negative in hepatocytes but expressed in the biliary epithelial cells and endothelial cells. During hepatocarcinogenesis, it is expressed in limited hepatocytes of cirrhotic liver tissues and obviously elevated in the malignant hepatocytes ***(Kittaka et al., 2008).***

Moreover, it was found to upregulate in HCC tissues at the messenger RNA and protein levels. Recently, adding annexin A2 to the established histological diagnostic marker panel has been considered to improve the diagnostic accuracy in HCC. In addition, serum annexin A2 concentrations were frequently elevated in HCC patients ***(Longerich et al., 2011).***

AIM OF THE WORK

The aim of this study is to determine the value of serum annexin A2 in the diagnosis of hepatocellular carcinoma in high risk patient (cirrhotic patient)

Chapter 1
HEPATO CELLULAR CARCINOMA

Hepatitis C virus (HCV) is An blood borne pathogen that is endemic in most parts of the world, with an estimated overall prevalence of nearly 3% ***(Wasley & Alter, 2000).*** Approximately 80% of patients with hepatitis C virus develop chronic infection; also, progression to cirrhosis occurs in nearly 20% for these subjects ***(Alter, 1995)***.

Patients infected with hepatitis C virus (HCV) have different clinical outcomes, ranging from acute resolving hepatitis to chronic liver disease including liver cirrhosis or hepatocellular carcinoma ***(Lee et al., 2001).*** In most individuals, liver disease progresses gradually over several decades, but the rate of progression is highly variable. Ever since hepatitis C virus was discovered approximately 20 years ago, HCV infections have become the leading cause of chronic liver disease worldwide ***(Kiyosawa et al., 1990)***.

Egypt has the highest prevalence of HCV worldwide (15%) ***(Egyptian Ministry of Health, 2007)***, and the highest prevalence of HCV genotype 4, which are responsible for almost 90% of HCV infections ***(Abdel-Aziz et al., 2007)***. Studies assessing the relationship between serum viral titers and the severity of biochemical and histological abnormalities have produced conflicting results. Some found no correlation between HCV viral loads, and serum ALT values and the extent

of histological damage ***(Kao et al., 1996)*** ,and others found significant correlation between HCV RNA titers and both serum ALT and degree of hepatic inflammation degrees ***(Fanning et al., 1999).***

Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the 5th commonest fatal cancer worldwide with more than 90% in mortality. Hepatocellular carcinoma (HCC) represents the most important tumour, its prevalence approximately 85% of primary liver cancer, which usually develops in the setting of chronic liver disease, particularly viral hepatitis. It is one of the most common malignant tumors worldwide ***(Jemal et al., 2011)***.

HCC incidence has doubled in egypt in the past 10 years, which could be attributed to the high prevalence of hepatitis C infection (HCV) and hepatitis B infection (HBV). Although HBV is considered worldwide as amajor risk factor for liver cirrhosis and HCC, the prevalence of HBV infection in Egypt has been declining over the last two decades ***(Khattab et al., 2010).***

The HCC risk in patients with liver cirrhosis depends on the activity, duration, and the etiology of the underlying liver disease ***(Spangenberg et al., 2008).***

Epidemiology and risk factors:-

Geographic distribution:-

Hepatocellular carcinoma is the 5th most common cancer in men and the 7th in women. Most of the burden is in developing countries where almost 85% of the cases occur and particularly in men. The regions of high incidence rates are Eastern and South-Eastern Asia, Middle and Western Africa. Low rates are estimated in developed regions, with the exception of Southern Europe where the incidence in men (10.5 per 100,000) is significantly higher than in other developed regions. There were an estimated 694000 deaths from HCC in 2008 (477000 in men, 217 000 in women), and because of its high fatality; HCC is the third most common cause of death from cancer worldwide. The geographical distribution of the mortality rates is similar to that observed for incidence ***(Globocan, 2008).***

Over the last few decades, the incidence of HCC has increased in eastern Asia and sub-Saharan Africa (the estimated number of new cases diagnosed annually increased from 437,000 to 564,000 between 1990 and 2000 ***(Caldwell et al., 2009).***

Incidence of hepatocellular carcinoma in Egypt:-

HCC is a rather common malignancy in Egypt, which usually develops on top of liver cirrhosis of viral origin.

Because of the high prevalence rate of HCV in cirrhotic Egyptian patients, it accounts for most HCC cases in Egypt ***(Hassan et al., 2001).*** Over a decade (1993-2002), there was nearly a twofold increase of the proportion of HCC among chronic liver disease (CLD) patients in Egypt with a significant decline of HBV and slight increase of HCV as risk factors ***(El-Zayadi et al., 2005)***.

Sex distribution

In Egypt, hepatocellular carcinoma is the second most common cancer in men and the 6th most common cancers in women ***(Globocan, 2008).*** In almost all populations, males have higher liver cancer rates than females, with male: female ratios usually averaging between 2:1 and 4:1. At present, the largest discrepancies in rates (more than 4:1) are found in medium-risk European populations ***(El Serag and Rudolph, 2007).***

The reasons for higher rates of liver cancer in males may be related to differences in exposure to risk factors. Men are more likely to be infected with HBV and HCV, consume alcohol, smoke cigarettes, and have increased iron stores. However, experiments show a 2- to 8-fold increase in HCC development in male mice; these data support the hypothesis that androgens influence HCC progression rather than sex-specific exposure to risk factors ***(Rudolph et al., 2000)***.

Age distribution:

The global age distribution of HCC varies by region, incidence rate, sex and, possibly, by etiology. In almost all areas, female rates peak in the age group 5 years older than the peak age group for males. In low-risk populations (e.g., United States, Canada, and United Kingdom), the highest age-specific rate occur among persons aged 75 and older. Age-specific incidence rates are strongly affected by the etiology of the background liver disease. Old age isan independent risk factor for HCC, especially in areas where HCV infection is endemic ***(Yoshida et al., 2004).*** 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).***

These variable age specific patterns likely are related to differ -rences in the dominant hepatitis virus in the population, the age at viral infection, and the existence of other risk factors ***(El Serag and Rudolph, 2007).***

Race

HCC incidence rates also vary greatly among different populations living in the same region. For example, ethnic Indian, Chinese, and Malay populations of Singapore had age-adjusted rates ranging from 21.21/100,000 among Chinese males to 7.86/100,000 among Indian males between 1993 and 1997; the comparable rates for females were 5.13/100,000

among ethnic Chinese and 1.77/100,000 among ethnic Indians, another example is the United States where, at all ages and both sexes, HCC rates are twicely higher in Asians than in African Americans, whose rates are 2 times higher than those in whites. The reason for this ethnic variability likely includes differences in the prevalence and acquisition time of major risk factors for liver disease and HCC ***(El Serag and Rudolph, 2007)***.

Risk factors for HCC:-

Major risk factor:-

- Relation of hepatitis B virus to HCC.
- Chronic hepatitis C infection and HCC.
- HCC and dual infections with hepatitis B and C viruses.
- Cirrhosis and HCC.
- Dietry causes.

Chronic Hepatitis B

Chronic infection with HBV is the strongest risk factor for HCC in Asian countries**.** The risk of HCC is much higher in patients who are HBe Ag positive compared with those who are HBsAg positive but HBeAg negative, the risk of HCC also appears to be related to the serum level of HBV DNA, This was confirmed by showing a correlation between baseline HBV DNA levels in asymptomatic adult HBsAg carriers and the risk of HCC ***(Chan et al., 2008).***

Although cirrhosis is a strong risk factor for HCC, 30% to 50% of HCC associated with HBV occur in the absence of cirrhosis ***(Bosch et al., 2005).*** Non-Asian chronic carriers who are anti-HBe positive with long-term inactive viral replication and who do not have cirrhosis seem to have little risk of developing HCC. On the other hand Asian hepatitis B carriers without cirrhosis remain at risk for HCC regardless of replication status ***(Hsu et al., 2002).*** The risk of HCC persists in long term HBV carriers from Asia who lose HBsAg thus these patients should continue to undergo surveillance ***(Yuen et al., 2004).***

Studies have shown that HBV genotype correlates with the risk for HCC and that genotype C carries two- to three fold higher risk than genotype B in developing HCC. Other HBV variants, such as precore, basal core, and pre-S deletion mutants, may also influence the development of HCC in carriers ***(Yang et al., 2008).***

Not all patients with chronic HBV infection have the same risk of HCC. Those with more active liver disease (as indicated by the presence of hepatitis B e antigen (HBeAg) and high HBV-DNA levels) seem at greater risk of developing cancer, whereas healthy carriers of HBs Ag have little risk of HCC which could be explained by the age of acquisition of HBV infection ***(Chen et al., 2006).***

Ten years after the initiation of the immunization program, the incidence of HCC among 6- to 14-year-olds in Taiwan declined dramatically. The average incidence of HCC declined from 0.7 per 100,000 children from 1982 to 1986 to 0.36 from 1990 to 1994. There was a similar decline in the mortality associated with HCC ***(Kane, 2003).***

Chronic Hepatitis C:-

Chronic HCV infection with established cirrhosis is also strongly associated with HCC with 2-8% incidence/year ***(Sun et al., 2003).*** The increased incidence of HCC in the developed world is likely to be a direct result of the HCV epidemic occurring some 20–30 years ago in the target population. There is no clear evidence of the association between HCV genotype and HCC ***(Bruno et al., 2007)***. The significance of HCV viral titers in determining HCC risk needs further investigation ***(Omata et al., 2010).***

HBV and HCV coinfection, HIV coinfection with HBV or HCV:-

It has been suggested that co-infection with both HBV and HCV may lead to severe liver disease than with each virus alone and increase the risk of developing HCC ***(Freeman et al., 2001).*** Liver disease in HIV patients co-infected with HBV or HCV progresses faster and HCC develops in a more aggressive pattern than in mono-infected patients ***(Brau et al., 2007).***

Non viral causes of liver cirrhosis:-

Alcoholic cirrhosis**,** stage 4 primary biliary cirrhosis ***(Caballeria et al., 2001)*** have increased risk of HCC**.** It has been suggested that the risk of HCC in autoimmune hepatitis with cirrhosis is increased to degree enough to warrant surveillance ***(Yeoman et al., 2008).*** For cirrhosis due to alpha 1-antitrypsin (AAT) deficiency; there are insufficient data from cohort studies to accurately assess HCC incidence ***(Elzouki et al., 1996)***.

Tobacco and alcohol intake:-

It is still controversial whether cigarette smoking is a risk factor for HCC. Alcohol also increases the risk for HCC in chronic hepatitis B and C ***(Chiesa et al., 2000).***

Environmental toxins:-

Aflatoxin is a mycotoxin that commonly contaminates corn, soy beans and peanuts. Exposure to aflatoxin has been associated with HCC it has also been shown that a synergistic effect exists between chronic HBV infection and aflatoxin exposure for hepatocarcinogenesis. Mutations of the p53 tumor suppressor gene have been demonstrated in patients with HCC who have chronically been exposed to aflatoxin ***(Wang et al., 2001)***.

Few studies have fully measured the presence of aflatoxins and their impact on liver disease in Egypt. The presence of aflatoxin B in 17% of the HCC cases compared to 9.4% of the healthy controls ***(El-Zayadi et al., 2001).*** As in many developing countries, Egypt is undergoing an epidemiologic transition. With increasing urbanization, smoking rates, environmental exposures, and aging, in addition to the maturing HCV epidemic, it is likely that HCC will continue to rise in the next few decades ***(Lehman et al., 2008).***

Metabolic factors:-

It has been shown that both obesity and diabetes are independent risk factors for HCC, depending on HBV and HCV infection status. There is growing evidence that non-alcoholic fatty liver disease **(NAFLD)** & non-alcoholic steatohepatitis **(NASH)** represents an increasingly frequent underlying liver disease in patients with HCC. It is likely that NAFLD causes HCC via cirrhosis, although the exact pathogenesis has not been determined ***(Chen et al., 2008).***

Iron accumulation in the liver is considered to be a co-factor for progression of liver disease. Iron overload can enhance the effects of oxidative stress and influence the natural history of patients with cirrhosis and HCC ***(Nahon et al., 2010).*** Patients affected with hereditary hemochromatosis **(**HH**),** a genetic disease of iron overload, were found to lead to cirrhosis and eventually an increased risk of HCC**.** In addition

to HH, the hepatic iron overload owing to other causes, such as homozygous beta thalassemia and the dietary form observed in South African blacks has been also associated with an increased risk of HCC ***(Borgna-Pignatti et al., 2004)***.

Epidermal growth factor (EGF) polymorphisms:-

EGF is a polypeptide growth factor that stimulates DNA synthesis in vitro in a variety of different cells. Genetic polymorphisms of the EGF gene have been associated with an increased risk of HCC in patients with cirrhosis. Over expression of EGF in the liver has been associated with HCC in animal models, providing a rationale for the observed association ***(Tanabe et al., 2008).***

Family history:-

Family history is associated with a moderately increased risk of HCC. The risk increases as the number of affected relatives increases ***(Yu et al., 2000).***

Red meat and saturated fat:-

Consumption of red meat and saturated fat has been associated with an increased risk of HCC ***(Freedman et al., 2010).***

Minor risk factors:-

- Relation to Alcohol
- Relation to oral contraceptives
- Relation to non alcoholic fatty liver disease **(NAFLD)**
- Relation to diabetes mellitus
- Relation to disturbed iron and copper metabolism.

Prevention of HCC:-

In the past decade HCC has gone from being an almost universal death sentence to a cancer that can be prevented, detected at an early stage, and effectively treated. Physicians caring for patients at risk need to provide high-quality screening, proper management of screen-detected lesions, and provision of therapy that is most appropriate for the stage of disease. HCC prevention can be achieved by: