INTRODUCTION

Hepatocellular carcinoma is one of the most common malignancies in the world (***Verslype, Rosmordue and Rougier, 2012***). Currently, AFP has undoubtedly been the most widely used tumor marker for detection and monitoring of HCC. ***(Stefaniuk, Cianciara and Wiercinskadrapalo, 2010).***

Alpha-fetoprotein has long been used for the diagnosis of HCC. However, the AFP is insufficiently sensitive or specific for use as a surveillance assay. Recent data also suggest that its use as a diagnostic test is less specific than was once thought. AFP can be elevated in intrahepatic cholangiocarcinoma (ICC).and in some metastases from colon cancer **(Sato Y, et al., 1994) (Adachi Y, et al., 2003)** and that AFP level often increases in the absence of HCC ***(Di Bisceglie et al., 2005).***

To date, several tumor markers have been proposed as complement or substitute for AFP in HCC diagnosis. Recently, lens culinaris agglutinin-reactive fraction of AFP (AFP-L3) ***(Oka et al., 2001)*** and des gamma carboxy prothrombin ***(Okuda et al., 1987)*** have been approved by the FDA as plasma markers for HCC. AFP-L3 can be detected in the plasma of patients with small tumors ***(Li et al., 2001).*** However, for the diagnosis of early stage of HCC, AFP-L3 and DCP ***(Marrero, 2009)*** are less sensitive than AFP for the diagnosis of early and very early stage of HCC.

The identification of a biochemical marker with better sensitivity and/or specificity than alpha-feto protein (AFP) could be extremely helpful in improving early diagnosis of HCC **(Trerotoli et al., 2009).**

Another potential biomarker for HCC is Glypican 3 (GPC3) which is one of the cell surface heparan sulfate proteoglycans that bind to the cell membrane via glycosil phosphatidyl inositol anchors. (**Filmus *& Selleck, 2001***) It has been found that glypicans interact with growth factors and modulate their activities; hence they play an important role in cell growth, differentiation and migration **(Kandil et al., 2009)**.While studies on ovarian cancer cell lines, mesotheliomas, and breast tumors have demonstrated the down regulation of GPC3, (***Lin et al. 1999***) other investigations on hepatocellular carcinoma have shown a marked elevation of GPC3 mRNA over the level observed in corresponding normal tissues (***Hippo et al., 2004***).

So, GPC3 may be a useful tumour marker for HCC.

AIM OF THE WORK

This study aims to evaluate the use of GPC3 as anovel marker for the diagnosis of HCC compared to alpha feto protein.

CHAPTER (1)

HEPATOCELLULAR CARCINOMA

Introduction:

Hepatocellular carcinoma (HCC) is an increasingly prevalent clinical problem worldwide and is the fifth most common cause of cancer-related death, results in between 250, 000 and one million deaths globally per annum. HCC has unique geographic, sex, and age distributions that are likely determined by specific etiologic factors ***(Venook et al., 2010).***

Chronic hepatitis B virus (HBV), hepatitis C virus (HCV) infections, alcohol abuse and accumulation of fat referred as nonalcoholic steatohepatitis represent more than 90% of HCCs develop on a cirrhotic liver. As cirrhosis of any etiology is the most common risk factor for HCC development. ***(Sanyal et al., 2010).***

Patients at risk for developing HCC should be entered into surveillance programs. Alpha-fetoprotein (AFP) is widely used as a surveillance and detection test for HCC among patients with cirrhosis, despite its limited performance, particularly in early-stage HCC ***(Bruix and Sherman, 2005).***

Apart from AFP, other markers (e.g., lectin bound AFP [AFP-L3], des-gamma carboxyprothrombin [DCP])

have been proposed for HCC detection, However, recent studies showed that neither DCP nor AFP-L3 presented better performance characteristics than AFP for the diagnosis of early-stage HCC, 11 and that neither DCP nor AFP is optimal to complement ultrasound in the detection of early HCC ***(Lok et al., 2010).***

Development of novel biomarkers for the early detection of HCC thus remains an important target before a breakthrough appears on HCC surveillance and early intervention ***(Lok et al., 2010).***

Epidemiology:

Liver cancer in men is the fifth most frequently diagnosed cancer worldwide, and is the second leading cause of cancer-related death in the world. In women, it is the seventh most commonly diagnosed cancer and the sixth leading cause of cancer death. In the United States, liver cancer is the ninth leading cause of cancer death ***(Altekruse et al., 2009).*** The number of deaths per year in HCC is virtually identical to the incidence throughout the world, underscoring the high case fatality rate of this aggressive disease. Almost 80 percent of cases are due to underlying chronic hepatitis B and C virus infection ***(Perz et al., 2006).***

Geographic variations:

The incidence of HCC varies widely according to geographic location ***(Altekruse and Reichman, 2009).*** The

distribution of HCC also differs among racial and ethnic groups within the same country, and between regions within the same country ***(Munoz and Bosch, 1990).*** These extreme differences in distribution of HCC are probably due to regional variations in exposure to hepatitis viruses and environmental pathogens. As an example, the frequency of hepatitis B virus carriers is relatively high in the high-incidence regions and low in the low-incidence regions ***(Altekruse et al., 2009).***

- High-incidence regions (more than 15 cases per 100, 000 populations per year) include sub-Saharan Africa, the People's Republic of China, Hong Kong, and Taiwan ***(Munoz and Bosch, 1990).*** The incidence is 24.2/100, 000 in parts of Africa, and 35.5/100, 000 in Eastern Asia. Over 40 percent of all cases of HCC occur in the People's Republic of China, which has an annual incidence of 137, 000 cases ***(Munoz and Bosch, 1990).*** Japan has had one of the highest incidence rates of HCC associated with chronic HCV infection; however, the incidence appears to be decreasing in recent years ***(Tanaka et al., 2008).***

- Intermediate-incidence areas include several countries in Eastern and Western Europe, Thailand, Indonesia, Jamaica, Haiti, New Zealand (Maoris), and Alaska (Eskimos) ***(Munoz and Bosch, 1990).***

- North and South America, most of Europe, Australia and parts of the Middle East are low-incidence areas with

fewer than three cases reported per 100, 000 populations per year. However, the incidence in the United States has increased during the past two decades, possibly due to a large pool of people with longstanding chronic hepatitis C ***(El-Serag et al., 2003)*** The rate began to accelerate in the mid 1980s, most likely because of the increased incidence of cirrhosis due to chronic HCV infection and nonalcoholic fatty liver disease, combined with a large influx of immigrants from East Asia and other geographic areas with high endemic rates of hepatitis B viral infection ***(El-Serag, 2004).***

Liver cancer incidence rates are increasing in many parts of the world, including the United States and central Europe ***(Jemal et al., 2013).*** A total of 48, 596 cases of HCC were reported in the United States between 2001 to 2006 according to data from the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute and the National Program of Cancer Registries from the Centers for Disease Control and Prevention ***(Center for disease control and prevention, 2010).***

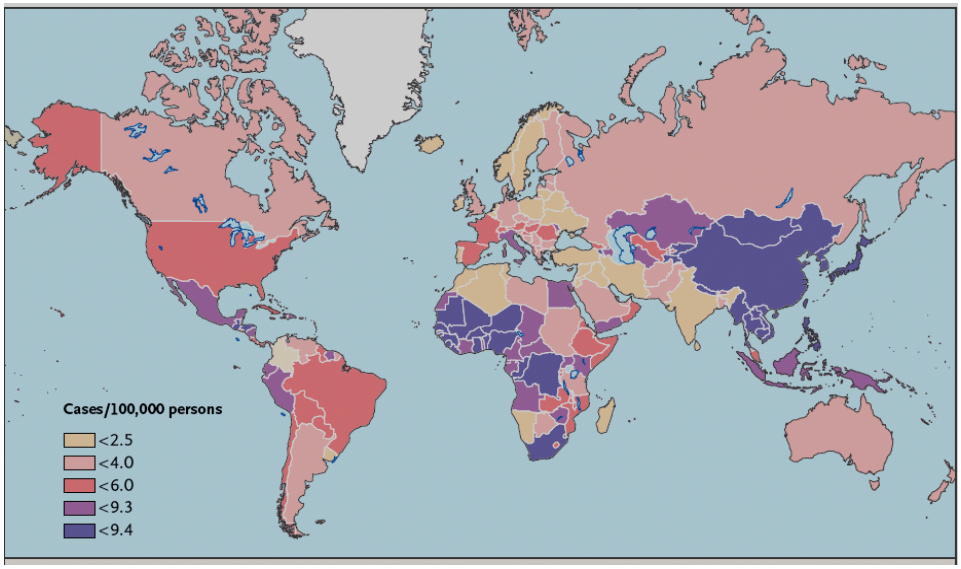

Fig. (1): Regional variation in the estimated Age-standardized incidence rates of liver cancer ***(WHO, 2008)***

Sex and age distribution:

In all parts of the world, men are more likely than women to develop HCC ***(Jemal et al., 2011).*** The disparity is more pronounced in high-incidence regions, where men are affected 2.1 to 5.7 times more frequently than women (mean 3.7:1). The ratio decreases to a mean of 2.4:1 in intermediate-incidence areas, and is lower in low-incidence regions. In Northern America, the incidence rates for males and females were 6.8 and 2.2 per 100, 000 persons, respectively, in 2008 ***(Jemal et al., 2011).*** Although not fully understood, the differences in sex distribution are thought to be due to variations in hepatitis carrier states, exposure to environmental toxins, and the trophic effect of androgens ***(Jemal et al., 2011).***

The majority of HCCs occur in patients with chronic liver disease or cirrhosis. Thus, older patients with longstanding liver disease are more likely to develop HCC ***(Jemal et al., 2013).***

Racial and ethnic variations: A population-based study in the United States identified racial and ethnic variations in the incidence of HCC. The incidence was highest among Asians, nearly double that of white Hispanics (11 versus 6.8 per 100, 000/year) and four times higher than Caucasians (2.6 per 100, 000/year) ***(Wong and Corley, 2008).***

Risk factors:

- ***Hepatitis B infection:*** The association between chronic HBV infection and HCC has been demonstrated in several studies ***(Sherman et al., 1995).***

HCC can develop in patients with chronic HBV, even in the absence of cirrhosis. However, 70 to 90 percent of patients with HBV who develop HCC will have cirrhosis. Because of the association of HBV with HCC, screening for HCC is recommended for many patients with hepatitis B, In addition to cirrhosis, a number of other factors have been associated with the risk of developing HCC among patients with chronic HBV, including the viral load, the presence of hepatitis B e antigen (HBeAg), and the presence of hepatitis B surface antigen (HBsAg) ***(Beasley, 1988).***

- **Viral load:** The risk of HCC is much greater in patients with high serum levels of HBV DNA compared with those who have low levels (<10, 000 copies/mL) ***(Chen et al., 2011).*** As an example, the risk of HCC in a 20-year-old woman who has a single serum HBV DNA measurement of 1 million copies/ mL is likely very different from that of a 50-year-old man with an HBV DNA level of 1 million copies/ mL persisting for five decades. Finally, the applicability of these data to HBsAg carriers who acquired HBV as adults is not clear. Currently, it is recommended that patients with high HBV DNA levels and signs of active inflammation (elevated ALT) for several years undergo surveillance for HCC ***(Chen et al., 2011).***

- **Active viral replication:** HBeAg positivity, which indicates active viral replication, is also associated with the development of HCC. One of the largest prospective studies to address this issue included 11, 893 Taiwanese men who were tested for HBsAg and HBeAg at enrollment and then followed for approximately 10 years. The incidence of HCC was significantly higher in patients who were HBsAg and HBeAg positive (1169 per 100, 000 patient-years) compared with patients who were only HBsAg positive (324 per 100, 000 patient-years) or who were negative for both HBsAg and HBeAg (39 per 100, 000 patient-years ***(Yang, 2002).***

- **Inactive carriers or resolved infection:** The risk of HCC is also elevated in patients who are HBsAg positive but HBeAg negative (inactive carriers) compared with the general population In addition, there is evidence that patients with serologically resolved infections remain at increased risk for HCC ***(Yang, 2002).*** Despite a generally favorable prognosis, clearance of HBsAg does not preclude the development of cirrhosis or HCC ***(Chen and Yang, 2005).*** The increased risk of HCC among patients with serologically resolved infection was demonstrated in a study that followed 1271 Alaskan Natives with chronic HBV for an average of 20 years. Among patients who cleared their HBV (i.e., became HBsAg negative), the incidence of HCC was lower than that of those who remained HBsAg positive, but still higher than among the general population.The likelihood of developing HCC is greater in those who clear HBsAg when older than age 50 years ***(Simonetti et al., 2010).***

- **Effect of HBV treatment:** the available data suggest that the relative risk is reduced by approximately 50 to 60 percent following treatment with interferon or nucleos(t)ide derivatives. However, treatment does not completely eliminate the risk, and the benefit was not seen in patients who developed nucleos(t)ide resistance ***(Singal, 2013).***

- **Co infection with HCV or HDV:** Co infection with HCV has also been associated with an increased risk of HCC. Some studies suggest that patients with dual HBV and HCV infection may have a higher rate of HCC compared with patients infected by either virus alone, particularly those who are anti-HCV and HBeAg positive ***(Huang et al., 2005).*** Co infection with hepatitis D virus (HDV) also appears to increase the risk of HCC among patients with HBV ***(Ji et al., 2012).***

- **Sex:** Men who are HBsAg positive appear to be at increased risk for HCC compared with women ***(Tseng et al., 2012).***

- **Other factors associated with HCC in patients with HBV**: Other risk factors associated with HCC include older age, habitual alcohol consumption, cigarette smoking, elevated serum ALT levels, the presence of core and precore mutations, and co infection with HCV ***(Chen et al., 2011).*** In addition, a study from China suggested that compared with blood group O, male patients with blood group A or B may be at increased risk for HCC, whereas women with blood group AB or B may be at decreased risk ***(Li et al., 2012).***

- ***HCV infection:*** strong association between chronic HCV infection and HCC has been observed, but the mechanisms involved in carcinogenesis remain unclear. An important clinical observation is that HCC in patients

with HCV occurs almost exclusively in patients with advanced stages of hepatic fibrosis or cirrhosis. However, in up to 10 percent of patients with HCV infection who undergo resection for HCC, only mild degrees of fibrosis are found ***(Lewis et al., 2013).***

It is generally believed that HCC arises in the setting of rapid cellular turnover and the chronic inflammatory state induced by the hepatitis C virus. One theory is that there is an imbalance in the microenvironments and cytokines of livers infected with the hepatitis C virus, leading to increased inflammation and cell turnover, which ultimately causes cirrhosis. Poorly differentiated hepatocytes likely proliferate and develop into dysplastic nodules and HCC ***(Budhu and Wang, 2006).***

In support of this hypothesis is the observation that HCV-induced HCC correlates well with the degree of inflammation and necrosis, and seems to be caused by inflammation rather than specific oncogene activation. By contrast, hepatitis B-related HCC, does not correlate as well with inflammation, and there appear to be specific oncogenes induced by the virus that result in an increased risk of HCC ***(Kamegaya et al., 2005).*** The degree of inflammation in the liver of patients with HCV also correlates with prognosis once HCC is diagnosed. Several oxidative stress and inflammation markers, including CD68+ cells, 8-hydroxydeoxyguanosine (8-OHdG) DNA adducts, and 4-hydroxynonenal (HNE) protein adducts, have been examined

in noncancerous liver tissue in patients who had both HCV and HCC ***(Maki et al., 2007).*** Patients with higher levels of these markers were found to have a worse prognosis. The host immune response may also be an important factor associated with a risk for progression to cirrhosis and cancer ***(Maki et al., 2007).***

The use of interferon or combination therapy to treat hepatitis C has been associated with a decreased risk of HCC; benefits have been noted particularly among those achieving a sustained virologic response (i.e., viral clearance) ***(Morgan et al., 2013).***

- ***Chronic hepatitis and cirrhosis:*** Patients with chronic liver disease (chronic hepatitis or cirrhosis) of any cause have an increased risk of developing HCC. On the other hand, many patients (20 to 56 percent) with HCC have previously undiagnosed cirrhosis ***(Zaman et al., 1990).***

Patients with chronic hepatitis or cirrhosis who have hepatitis B, hepatitis C, or hereditary hemochromatosis (HH) have the highest risk of developing HCC. Among patients with HH, HCC is virtually limited to patients with cirrhosis; a similar relationship to cirrhosis has been reported in patients with primary biliary cirrhosis ***(Fargion et al., 1994).***

- ***Environmental toxins:***

  - *Aflatoxin:* Aflatoxin is a mycotoxin that commonly contaminates corn, soybeans, and peanuts. High rates of dietary aflatoxin intake have been associated with

HCC; Mutations of the p53 tumor suppressor gene have been demonstrated in patients with hepatocellular carcinoma who have chronically been exposed to aflatoxin ***(Unsal et al., 1994).***

- *Contaminated drinking water:* Several studies conducted in rural China have noted a higher mortality rate from HCC among people who drink pond-ditch water compared with those who drink well water (100 versus fewer than 20 deaths per 100, 000 populations per year). The blue-green algal toxin Microcystin commonly contaminates these ponds and is thought to be a strong promoter of HCC ***(Ueno et al., 1996).***

- ***Tobacco and alcohol abuse:*** Cigarette smoking has been shown to be a risk factor for HCC, Alcohol intake has been linked to HCC in many reports, although the threshold dose and duration of use are unclear. The relationship between ethanol and HCC could be a direct toxic effect, or an indirect one, since alcohol represents an important risk factor for cirrhosis, a predisposing factor for HCC ***(Trichopoulos et al., 2011).***

- ***Non alcoholic fatty liver disease:*** There is growing evidence that NAFLD 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 yet been determined ***(Yasui et al., 2011).***