INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and ranks as the second-leading cause of cancer-induced death in men ***(Torre et al., 2015)***.

Despite recent developments in surgery and medical therapy, which have significantly improved the outcome of patients with operable and advanced hepatocellular carcinoma (HCC), HCC remains a major health problem worldwide. The majority of HCC cases occur in patients with chronic liver disease, such as hepatitis B-virus (HBV), hepatitis C-virus (HCV) infection, alcoholic liver diseases and non-alcoholic fatty liver diseases ***(Shin et al., 2013).***

Owing to the characters of hepatocellular carcinoma, like insidious onset, high degree of malignancy, and nonspecific symptoms in early stage, the prognosis of HCC remains dismal with a 5-year overall survival rate of 0%–10% ***(Zhou et al., 2012).***

Surveillance strategies, including ultrasound imaging and serum α-fetoprotein (AFP) concentration measurements, have been recommended to detect HCC at earlier stages, without pathological confirmation.

AFP is the most commonly used serological marker worldwide to diagnose hepatocellular carcinoma. However Analysis of recent studies has indicated that AFP testing lacks

adequate sensitivity and specificity for effective surveillance, so identification of better early diagnostic biomarkers is crucial ***(Liu et al., 2013)***.

If HCC could be diagnosed at an early stage, potentially curative options, such as resection, ablation, and transplantation may be considered ***(Forner et al., 2010).***

Collagen is the main component of connective tissue. Deregulation of tissue collagen metabolism is one of the consequences of neoplastic transformation. Metalloproteinase initiate the breakdown of collagen; however, the final step of collagen degradation is mediated by prolidase ***(Palka et al., 2002).***

Prolidase, a member of the MMP (matrix Metalloproteinase) family, is a cytosolic imidodipeptidase, which specifically splits imidodipeptides with C-terminal proline or hydroxyproline. The enzyme plays an important role in the recycling of proline from imidodipeptides for resynthesis of collagen and other proline containing proteins ***(Surazynski et al., 2008).***

Prolidase enzyme activity has been shown in plasma, erythrocytes, leukocytes, dermal fibroblasts and various organs such as kidney, brain, heart, thymus, uterus, lung, spleen and pancreas ***(Liu et al., 2007).***

It is demonstrated that the activity of this enzyme may have a role in various disorders, such as chronic liver disease, osteoporosis, osteoarthritis, uremia, and hypertension ***(Demirbag et al., 2007).***

Prolidase seems a rate-limiting factor in the regulation of collagen biosynthesis because of its role in the last step of collagen degradation. The role of prolidase activity in neoplastic tissues is not yet known ***(Surazynski et al., 2008).***

AIM OF THE WORK

To assess the correlation between serum prolidase and alpha-fetoprotein levels in patients with hepatocellular carcinoma.

Chapter (1)

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is the fifth most common malignancy and the second leading reason of cancer-associated deaths around the world, and more than 600,000 deaths are reported internationally each year ***(Ferlay et al., 2015)***. HCC takes up 85%–90% of primary liver cancers with 500,000 new cases of HCC all over the world every year ***(Bozkaya et al., 2012)***.

Incidence rates of the disease are increasing globally. In the United States, the incidence has doubled over the past 20 years ***(El-Serag et al., 2014)***.

The risk for HCC is influenced by etiology, activity, and stage of underlying liver disease. Patients with liver cirrhosis due to chronic infection with hepatitis C virus (HCV) or hepatitis B virus (HBV) have the highest risk. The rising incidence in Western countries is mainly due to the still high prevalence of HCV-associated liver cirrhosis and the increasing number of patients with advanced steatohepatitis as hepatic manifestation of the metabolic syndrome ***(El-Serag et al., 2014)***.

Several reports indicate that HCC in the setting of metabolic syndrome may also arise in the absence of cirrhosis; however, the exact proportions of this condition or its risk factors are still under investigation ***(Younossi et al., 2015)***.

The main reasons for the high mortality rate of HCC patients are ascribed to the lack of effective treatments and the increasing resistance to conventional radiotherapy and chemotherapy ***(Whittaker et al., 2010)***.

Despite substantial progress in understanding of the molecular pathogenesis of HCC, imaging techniques, and novel therapies (including targeted drugs), the overall prognosis of HCC patients is still poor. Tumor multiplicity, frequent vascular invasion, and accompanying cirrhosis are clinical features of HCC that lead to unsatisfactory outcomes. High rates of tumor recurrence and resistance to chemotherapeutic agents also make the management of HCC challenging. The poor outcome of patients with HCC is attributed to late detection, with more than two-thirds of patients diagnosed at advanced stages of the disease ***(Stravitz et al., 2008)***. However, a considerable improvement in survival has been observed (5-year survival up from 40% to 70%) when patients are diagnosed at an early stage and receive potentially curative therapy in the form of liver transplantation, surgical resection, or tumor ablation ***(Liu et al., 2004)***.

Epidemiology

Hepatocellular carcinoma in men is the fifth most frequently diagnosed cancer worldwide. 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. The number of deaths per year in HCC is directly related to the incidence throughout the world, underscoring the high case fatality rate of this aggressive disease ***(Jemal et al., 2011).***

Almost 80 percent of cases are due to underlying chronic hepatitis B and C virus infection ***(Perz et al., 2006).***

Geographic variation: The incidence of HCC varies widely according to geographic location. These differences in distribution of HCC are probably due to regional variations in exposure to hepatitis viruses and environmental pathogens ***(Jemal et al., 2011).***

The pattern of HCC occurrence has a clear geographical distribution, with the highest incidence rates in East Asia, sub-Saharan Africa, and Melanesia, where around 85% of cases occur ***(IARC, 2011).***

Liver cancer incidence rates are increasing in many parts of the world, including the United States and central Europe ***(Jemal et al., 2013).***

Sex and age distribution*:* In all parts of the world, men are more likely than women to develop HCC.

The incidence of HCC increases progressively with advancing age in all populations, reaching a peak at 70 years ***(Jemal et al., 2011).***

Racial and ethnic variations*:* A population-based study in the United States identified racial and ethnic variations in the incidence of HCC where the incidence was highest among Asians ***(Wong et al., 2008).***

Risk factors

A variety of important risk factors for the development of hepatocellular carcinoma (HCC) have been identified. These include hepatitis B viral (HBV) infection, chronic hepatitis C virus (HCV) infection, hereditary hemochromatosis, and cirrhosis of almost any cause ***(Llovet et al., 2012).***

- ***Hepatitis C***

A strong association between chronic HCV infection and HCC has been observed, but the mechanisms involved in carcinogenesis remain unclear ***(Omland et al., 2012).***

Hepatitis C accounts for at least one-third of the cases of HCC in the United States. An important clinical observation is that HCC in patients with HCV occurs almost exclusively in patients with advanced stages of hepatic fibrosis or cirrhosis ***(Lok et al., 2009).***

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).***

Among patients with HCV, the differences in HCC rates between men and women were not significantly different. In addition, higher viral loads were associated with an increased risk of HCC. A 186-gene signature on gene expression profile analysis has also been associated with an increased risk of HCC among patients with HCV and Child-Pugh class A cirrhosis ***(Hoshida 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 et al., 2006).***

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. Patients with higher levels of these markers were found to have a worse prognosis ***(Maki et al., 2007).***

The host immune response may also be an important factor associated with a risk for progression to cirrhosis and cancer ***(Suruki et al., 2006).***

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 (ie, viral clearance) ***(Morgan et al., 2013).***

- ***Hepatitis B***

The association between chronic HBV infection and HCC has been demonstrated in several studies. HCC can develop in patients with chronic HBV, even in the absence of cirrhosis ***(Chen et al., 2010).***

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, where 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) ***(Tseng et al., 2012).***

In addition; active viral replication indicated by HBeAg positivity, is also associated with the development of HCC ***(Yang et al., 2002).***

Also, The risk of HCC is also elevated in patients who are HBsAg positive but HBeAg negative (inactive carriers) compared with the general population ***(Tseng et al., 2012).***

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-HCVAb 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. A retrospective study involving 200 patients with compensated HBV-related cirrhosis, of whom 20 percent were anti-HDV positive, found that HDV infection increased the risk of HCC threefold and mortality twofold ***(Fattovich et al., 2000).***

Several studies have evaluated the impact of treatment for chronic HBV on the risk of HCC. Systematic reviews of the available data suggest that the relative risk is reduced by approximately 50 to 60 percent following treatment with interferon or nucleo tide derivatives (***Singal et al., 2013***).

- ***Tobacco and alcohol abuse***

Cigarette smoking has been shown to be a risk factor for HCC in some studies. 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).***

Concomitant heavy alcohol use, diabetes mellitus, and obesity were found in various reports to increase the risk of HCC ***(Loomba et al., 2013).***

- ***Non alcoholic fatty liver disease***

The pathogenesis of HCC in NAFLD is related to obesity and diabetes. It is believed that obesity plays an important role in HCC development by generating a chronic general low-grade inflammatory response due to an increased level of leptin and a relatively decreased level of adiponectin in obese patients ***(Margini and Dufour, 2016).***

Increased lipid storage in the liver leads to lipotoxicity that interferes with cellular signaling mechanisms and regulation of gene transcription promoting gene transcription alterations, which may result in HCC. Furthermore, there is supporting evidence that compensatory hyperinsulinemia and insulin-like growth factor (IGF) in obesity may promote the development of HCC by activating various oncogenic pathways ***(Margini and Dufour, 2016).***

The mechanisms of hepatocarcinogenesis in steatosis might be different from the classic mechanisms involved in

cirrhosis, and this could explain the high number of reported HCC in non-cirrhotic NAFLD ***(Torres and Harrison, 2012).***

It has, in fact, been reported that a significant number of patients with NAFLD-related HCC have no extensive fibrosis at presentation. A recent multicenter study described that steatohepatitis was more prevalent in the HCC cohort in comparison to the cholangiocellular cohort that showed an incidence similar to the general population, suggesting that steatohepatitis could play an important role in the development of non-cirrhotic HCC. It raised the hypothesis that HCC in NAFLD may arise in the absence of histologically evident inflammation ***(Alexander et al., 2013).***

- ***Diabetes mellitus***

Epidemiologic studies suggest a possible link between diabetes mellitus and HCC, and multiple systemic reviews and meta-analyses have also found an association ***(Wang et al., 2012).***

A systematic review that included a total of 49 case-control and cohort studies estimated that the risk was increased by approximately 2.2-fold, although few studies adjusted for diet and obesity ***(Wang et al., 2012).***

In addition, a study found that the presence of the metabolic syndrome (defined by the presence of three of the following: elevated waist circumference/ central obesity,

dyslipidemia, hypertension, and impaired fasting glucose) was a risk factor for HCC ***(Welzel et al., 2011).***

Clinical picture:

As most solid cancers, HCC is asymptomatic for several months and sometimes for years. This means that HCC should be searched for in all known chronic liver disease patients with adequate diagnostic tools at regular intervals. When HCC occurs in patients with an apparently healthy liver, the diagnosis is usually late and the patient has advanced disease ***(Mazzanti et al., 2016).***

A- Symptoms:

The clinical picture is very variable. The patient may be completely asymptomatic with no physical signs other than those of cirrhosis, and the tumor may have been diagnosed incidentally. Alternatively, the presentation may be so florid and liver failure so great with no improvement when ascites, bleeding varices or pre-coma is adequately treated ***(Sherlock and Dooly, 2011).***

- **Pain:**

The most common mode of presentation of HCC is with the triad of abdominal pain, weight loss and hepatomegaly while the most common and often the first complaint is upper abdominal pain or discomfort. The pain is felt most commonly

in the right hypochondrium or epigastrium, in the left hypochondrium or in the back. Rarely, it radiates to the right shoulder. The pain is usually a dull aching. Spontaneous rupture is a particularly dramatic presentation of the tumor. There is a sudden onset of abdominal pain and swelling ***(Roberts, 2011)***.

- **Gastrointestinal symptoms:**

The second most common symptom is weight loss together with anorexia ***(Johnson, 2006).***

- **Dyspnea:**

It is late and is due to the large size of the tumor compressing or directly involving the diaphragm, or due to pulmonary metastases ***(Johnson, 2006).***

B- Signs:

- **Hepatomegaly**

The liver is enlarged, and a hard irregular lump may be felt in the right upper quadrant, continuous with the liver. A vascular bruit may be heard over the liver because of the increased vascularity of the tumor. The spleen is also enlarged in most of the cases due to portal hypertension ***(Sherlock and Dooly, 2011).***