INTRODUCTION

Hepatocellular carcinoma (HCC) is rapidly reduce quality of life and responsible for a large proportion of cancer deaths worldwide ***(Bertino et al., 2014).*** It is the sixth diagnosed cancer worldwide and the third cause of cancer death ***(Dai et al., 2014).*** This cancer varies widely in incidence throughout the world, with rising incidence in Egypt ***(Hafez et al., 2013).***

Infections with hepatitis B or C virus, as well as dietary aflatoxin exposure are considered major risk factors for the progression to liver cirrhosis and HCC ***(Finn, 2013).*** Egypt has the highest prevalence of HCV in the world ***(Hafez et al., 2013).*** There are no specific symptoms of HCC, making early diagnosis and detection of the disease difficult ***(John et al., 2014).*** When HCC presents with specific clinical symptoms, the tumor is typically very far advanced ***(Baffy et al., 2013)***.

Alpha-fetoprotein (AFP) is the most validated serological diagnostic marker for HCC ***(Hafez et al., 2013)***. However, its diagnostic value is more and more questioned. AFP levels drop markedly at birth, but are elevated in patients with HCC, cirrhosis, chronic hepatitis, liver necrosis, pregnancy, or gonadal tumors ***(Abdel-Rahman et al., 2010)***. The use of AFP as a diagnostic and screening test for HCC is limited. This is in part due to the poor performance of available tumor markers leading to delay in diagnosis ***(Huo et al., 2004).***

Therefore, new and more specific markers for early detection of HCC are critically needed to improve the survival of affected patients ***(Santambrogio et al., 2013).***

Simultaneously, angiogenesis is the process of formation of new capillaries from preexisting blood vessels ***(Linares et al., 2013)***, it represents an essential component of embryogenesis, normal physiological growth, repair, tumor expansion and progression of cancer, correlating with the metastatic potential of tumor cells ***(Zhu et al., 2013)***.

It is stimulated to provide oxygen and nutrients to injured tissue; unfortunately, chronic damage leading to capillarization of hepatic sinusoids, which restricts the blood supply, exacerbating tissue injury, fibrogenesis, and angiogenesis ***(Matsubara et al., 2013)***.

This sequence of events appears to govern the progression of CHC to cirrhosis and HCC ***(Sanz-Cameno et al., 2010)***. One of the most significant signaling pathways in pathological angiogenesis and HCC is the angiopoietin/Tie2 system ***(Cascone and Heymach, 2012)***.

The angiopoietins serve as ligands for the endothelium-specific receptor tyrosinekinases Tie1 and Tie2, which comprise 4 structurally related proteins, termed angiopoietin (Ang)-1, Ang-2, Ang-3 and Ang-4 ***(Sharma et al., 2013)***. Ang-2 (Ang-2; ANGPT2) is a member of the angiopoietin protein

family (angiopoietin-1, -2, -3, and -4 which plays an important role in the development and maintenance of blood vessels and the lymphatic system ***(Sallinen et al., 2014)***.

The gene for human angiopoietin-2 maps to chromosome 8p23 and the mature protein contains 496 amino acid residues. After signal sequence processing and maturation, Ang-2 becomes 478 amino acids long (from 19 to 496). In addition, several N-terminal truncated forms arise due to alternative splicing, including Ang-2 443, expressed by primary endothelial cells and tumor tissues ***(Fagiani and Christofori, 2013)***. High serum Ang-2 values are found in patients with inflammatory conditions as chronic HCV infection ***(Herna´ndez-Bartolome´ et al., 2013)***, inflammatory bowel disease and sepsis ***(David et al., 2012)***.

Also production of Ang-2 has been implicated in tumor development in human gastric and colon cancers ***(Engin et al., 2012)***, human prostate carcinoma, and human breast cancers ***(Ping et al., 2015)***. Ang-2 is over expressed in HCC and associated with portal infiltration, micro-vessel density, recurrence of HCC and decreased survival ***(Zhu et al., 2013)***.

Recent studies reported high serum Ang-2 values in patients with HCC suggesting that Ang-2 might represent a useful marker for HCC and a complementary diagnostic tool ***(Kelley et al., 2014).***

AIM OF THE WORK

This study aims to evaluate Angiopoietin 2 as novel diagnostic marker for the diagnosis of Hepatocellular Carcinoma in comparison with alpha feto protein.

LIVER CIRRHOSIS

Definition:

Liver cirrhosis is a frequent consequence of the long clinical course of all chronic liver diseases and is characterized by tissue fibrosis and the conversion of normal liver architecture into structurally abnormal nodules ***(Pinzani et al., 2011).*** Histologically it is characterized by diffuse nodular regeneration surrounded by dense fibrotic septa with subsequent parenchymal extinction and collapse of liver structures, together causing pronounced distortion of hepatic vascular architecture ***(Dooley et al., 2011).*** This distortion results in increased resistance to portal blood flow and hence in portal hypertension and in hepatic synthetic dysfunction.

Epidemiology:

Liver cirrhosis is an increasing cause of morbidity and mortality in more developed countries. It is the 14th most common cause of death in adults worldwide but the fourth in central Europe, ***(Lozano et al., 2012)*** it results in 170 000 deaths per year in Europe, ***(Blachier et al., 2013)*** and 33 539 per year in the USA ***(Hoyert and Xu, 2012).***

In 2010, Egypt, followed by Moldova, had the highest age-standardized cirrhosis mortality rates, 72.7 and 71.2 deaths per 100,000, respectively. In Egypt, almost one-fifth (18.1%) of all deaths in males 45- to 54-years old were due to liver

cirrhosis ***(Mokdad et al., 2014).*** Cirrhosis is the main indication for 5500 liver transplants each year in Europe ***(Blachier et al., 2013).***

Causes of cirrhosis:

Main factors causing cirrhosis: Chronic Hepatits C, Alcoholic liver disease, Nonalcholic fatty liver disease and Chronic Hepatits B.

Other causes of cirrhosis:

Cholestatic and autoimmune liver disease (Primary biliary cirrhosis, Primary sclerosing cholangitis and Autoimmune hepatitis), Intrahepatic or extrahepatic biliary obstruction (Mechanical obstruction, Biliary atresia and Cystic fibrosis), Metabolic disorders (Hemochromatosis, Wilson's disease, Glycogen storage disease, α1 –antitrypsin deficiency, porphyria and Abetalipoprotienemia), Sinusoidal obstruction syndrome (Budd-chiari syndrome,Veno-occlusive disease and Right-side heart failure), Drugs and toxin, Intestinal bypass and Indian childhood cirrhosis.

Pathophysiology:

Liver Fibrosis/Cirrhosis:

During liver injury, stellate cells activate into alpha smooth muscle actin-expressing contractile myofibroblasts, which contribute to vascular distortion and increased vascular resistance, thereby promoting portal hypertension. Other features of stellate cell activation include mitogen-mediated

proliferation, increased fibrogenesis driven by connective tissue growth factor, and transforming growth factor beta 1, amplified inflammation and immunoregulation, and altered matrix degradation. Evolving areas of interest in stellate cell biology seek to understand mechanisms of their clearance during fibrosis resolution by either apoptosis, senescence, or reversion, and their contribution to hepatic stem cell amplification, regeneration, and hepatocellular cancer ***(Juan et al., 2013).***

Morphological classifications of liver cirrhosis:

Grossly, with the naked eye, a cirrhotic liver appears nodular, "hub-nailed", on the external surface and nodular on the cut surface. Variation in size, color, shape and consistency is relevant and may help in the identification of the etiology. The liver is usually indurated shrunken and yellowish-tan but it may be enlarged and yellow as in alcoholic fatty cirrhosis, rusty as in hemochromatosis or large and green as in biliary obstruction.

Micronodular cirrhosis:

Small rather uniform< 3mm nodules separated by thin fibrous septa usually due to a chemical agent as alcohol which diffuse uniformly thought the liver.

Macronodular cirrhosis:

Larger nodules >3 mm separated by wider scars and irregularly distributed throughout the liver usually due to an

infectious agent such as viral hepatitis which diffuse uniformly throughout the liver.

Microscopic changes:

Presence of nodules and fibrous septa with effacement of the lobular architecture. The nodules are of two types: Dissection type and Hyperplastic Regenerative type ***(Anthony et al., 1978).***

Clinical manifestations of liver cirrhosis:

The clinical manifestations of cirrhosis range widely, depending on the stage of cirrhosis,from an asymptomatic patient with no signs of chronic liver disease to a patient who is confused, jaundiced, has severe muscle wasting, and ascites. The natural history of cirrhosis is characterized by an initial phase, termed "compensated" cirrhosis, followed by a rapidly progressive phase marked by the development of complications of portal hypertension or liver dysfunction (or both), termed "decompensated" cirrhosis. In the compensated phase, portal pressure may be normal or below the threshold level identified for the development of varices or ascites. As the disease progresses, portal pressure increases and liver functions decreases, thereby resulting in the development of ascites, portal hypertensive gastrointestinal (GI) bleeding, encephalopathy, and jaundice. The development of any of these complications marks the transition from a compensated to decompensated phase. Progression to death may be accelerated by the development such other complications as recurrent GI bleeding,

renal impairment (refractory ascites, hepatorenal syndrome) hepatopulmonary syndrome, and sepsis (spontaneous bacterial peritonitis). The development hepatocellular carcinoma may accelerate the course of the disease at any stage**.**

The salient features are: Spider angiomata or spider nevi: One study found that spider angiomata occur in about 1/3 of cases ***(Li et al., 1999)***, Palmar erythema ***(James et al., 2005)***, Clubbing and Asterixis.

Other general features that may be seen include: Constitutional symptoms as weakness, fatigue, anorexia, and weight loss and features suggesting malnutrition, **Pigment gall stones** resulting from hemolysis ***(Bouchier, 1969),*** **Parotid gland enlargement** is usually secondary to fatty infiltration, fibrosis, and edema rather than a hyperfunctioning gland ***(Dutta et al., 1989),*** **Diabetes mellitus:** While up to 80% of cirrhotic patients are glucose intolerant, only 10 – 20% are truly diabetic. The prevalence of diabetes is greater among those with non – alcoholic liver disease - related cirrhosis, hepatitis C or alcohol -related cirrhosis compared with those with cholestatic cirrhosis ***(Zein et al., 2000),*** **Gynecomastia**, **Hypogonadism, Hepatomegaly:** The cirrhotic liver may be enlarged, normal sized, or small, **Fetor hepaticus*:*** The breath has a sweet, fecal smell to it and **Jaundice:** is a yellowish pigmentation of the skin, the conjunctival membranes, and other mucous membranes caused by high blood bilirubin levels ***(Click et al., 2013).***

Diagnosis of liver cirrhosis:

Laboratory findings:

AST (Aspartate Aminotransferase) and ALT (Alanine Aminotransferase): These transaminases are the most sensitive indicators of hepatic cell injury. Their highest levels are seen in acute hepatic necrosis. ALT is generally a more sensitive indicator of acute liver cell damage than AST.

Alkaline Phosphatase (ALP): Elevations of this enzyme in the blood are a sensitive indicator of a biliary process but they are also seen in liver cell damage. The general rule applies that the higher the alkaline phosphatase, the greater the chances for post-hepatic obstruction

GGT (Gamma GlutamylTransferase): GGT determinations are helpful in differentiating bone and liver sources of alkaline phosphatase, since there are no significant amounts of GGT in bone. GGT is also a sensitive indicator of alcohol-induced liver disease and of recent alcohol ingestion.

Bilirubin: Bilirubin levels may be normal in well compensated cirrhosis. However, they rise as cirrhosis progresses.

Plasma Proteins: Its concentrations in liver disease often are near normal

Albumin: Albumin levels fall as the synthetic function of the liver declines with worsening cirrhosis. Thus serum

albumin levels can be used to help grade the severity of cirrhosis.

Hematologic abnormalities:

Anaemia: Is usually multifactorial in origin; acute and chronic gastrointestinal blood loss, folate deficiency, direct bone marrow toxicity due to alcohol, hypersplenism, bone marrow suppression (as in hepatitis-associated aplastic anaemia), the anaemia of chronic disease (inflammation) and haemolysis may all contribute.

Thrombocytopenia: Is mainly caused by portal hypertension with attendant congestive splenomegaly.

Leukopenia and neutropenia: Are due to hypersplenism with splenic margination.

Blood Coagulation: A prolongation of the prothrombin time (PT) is an indicator of hepatic dysfunction as the synthesis of the coagulation factors is impaired in hepatocellular disease.

Blood Ammonia: Elevated blood ammonia is seen in severe liver disease and in actual or impending hepatic coma.

Radiographic findings:

Ultrasound:

- Ultrasonography is routinely used during the evaluation of the cirrhotic patient. It is noninvasive, well tolerated, widely available, and provides valuable information. In advanced cirrhosis, the liver may appear small and nodular. Surface

nodularity and increased echogenicity with irregular appearing areas are consistent with cirrhosis but can also be seen with hepatic steatosis ***(Sanford et al., 1985)*** and ***(Di Lelio et al., 1989).***

- Ultrasonography may be used as a screening test for hepatocellular carcinoma and portal hypertension. The finding of nodules on ultrasonography warrants further evaluation since benign and malignant nodules can have similar ultrasonographic appearance. Findings of portal hypertension include an increased diameter of the portal vein and the presence of collateral veins ***(Zwiebel, 1995).*** Ultrasonography is also useful for detecting splenomegaly, ascites, and portal vein thrombosis.

"Stiffness" measurement

Increasing scarring of the liver is associated with increasing "stiffness" of the tissue. A sonographic technique to assess liver stiffness has been developed (**Fibroscan**). A vibration of mild amplitude and low frequency is transmitted through the liver inducing an elastic shear wave that propagates through the tissue. A pulse-echo ultrasound follows the propagation of the wave; the harder the tissue (and hence the more dense the fibrosis) the faster the wave propagates. Initial assessment of the device suggests that it has excellent test characteristics in patients with advanced fibrosis ***(Kettaneh et al., 2007; Sandrin et al., 2003; Sandrin et al., 2002).***

Elastography is now widely used as a noninvasive test for staging fibrosis and is now being used in place of liver biopsy to investigate the natural history of chronic liver diseases; however, wide-scale outcome studies are not yet published. Its use is restricted in patients with acute hepatitis, obstructive cholestasis, and passive congestion, which can also alter liver stiffness ***(Cohen and Afdhal, 2010).***

- **Computed tomography**

Is not routinely used in the diagnosis and evaluation of cirrhosis. It provides similar information to ultrasonography, but at the expense of radiation and contrast exposure. CT findings may suggest the presence of cirrhosis, but they are not diagnostic.

- **Magnetic resonance imaging:**

The role of magnetic resonance imaging (MRI) in the diagnosis of cirrhosis is unclear. MRI may also reveal iron overload and provide an estimate of the hepatic iron concentration ***(Bonkovsky et al., 1999).*** MR angiography is more sensitive than ultrasonography in diagnosing complications of cirrhosis such as portal vein thrombosis ***(Finn et al., 1993).***

Liver fibrosis scoring techniques:

Table (1): Scoring Systems for Histologic Stage (Fibrosis):

Comparative Scoring Systems for Histologic Stage (Fibrosis)			
Score	**IASL**	**Batts-Ludwig**	**Metavir**
0	No Fibrosis	No Fibrosis	No Fibrosis
1	Mild fibrosis	Fibrous portal expansion	Periportal fibrotic expansion
2	Moderate fibrosis	Rare bridges or septae	Periportal septae (> 1 septum)
3	Severe fibrosis	Numerous bridges or septae	Portal-central septae
4	Cirrhosis	Cirrhosis	Cirrhosis

(Ghany et al., 2009)

Cause-specific treatments:

Patients with cirrhosis should be treated when possible for the underlying liver disease to stop disease progression; such treatment includes immunosuppression for autoimmune hepatitis, venesection for haemochromatosis, and copper chelators or zinc for Wilson's disease.

Patients with viral hepatitis should be assessed for antiviral treatment. All patients with cirrhosis who are positive for HBsAg should receive oral antiviral therapy with a potent antiviral (entecavir or tenofovir) irrespective of viral load ***(EASL, 2012).*** In patients with hepatitis-C-related cirrhosis without ascites, achievement of sustained virological response significantly reduced liver-related morbidity and mortality

(Morgan et al., 2010). In a subgroup of patients, there was also regression of cirrhosis.

Complications of liver cirrhosis:

1) Portal hypertension and varicel bleeding:

Portal hypertension, rather than hepatocyte failure per se, is the underlying cause of most of the complications of cirrhosis and subsequent mortality.

Hepatic venous pressure gradient (HVPG) is a good surrogate marker of portal hypertension and has robust prognostic power. Portal hypertension is present when the HVPG is more than 5 mmHg ***(Burroughs and Thalheimer, 2010)***. However, clinically significant portal hypertension and the threshold for development of oesophagealvarices is above 10mmHg ***(Groszmann et al., 2005).***

2) Ascites:

Ascites is the accumulation of free fluid within the peritoneal cavity.

In cirrhosis, portal hypertension and splanchnic vasodilation, resulting mainly from increased production of nitric oxide, is the main pathophysiological mechanism of ascites ***(Wiest and Groszmann, 2002).***

3) Infection:

Most frequently diagnosed are spontaneous bacterial peritonitis (ascetic neutrophil count is more than 250 per μL and