Introduction

Hepatocellular carcinoma (HCC) is the most common primary hepatic malignancy of adults. It is the sixth most common cancer worldwide and the third most common cause of cancer death **(Dai *et al*., 2014)**. In Egypt, rising incidence is mostly due to high prevalence of chronic viral hepatitis (Hepatitis C virus (HCV) & Hepatitis B virus (HBV)) and its complications mostly liver cirrhosis **(Rashed *et al*., 2020)**.

Angiogenesis is a critical step in the development and progression of HCC. Myeloid lineage cells, such as macrophages and monocytes, have been reported to regulate angiogenesis. The tyrosine kinase with Immunoglobulin (Ig) and endothelial growth factor (EGF) homology domains 2 (Tie-2), a receptor of angiopoietins, conveys pro-angiogenic signals and identifies a monocyte/macrophage subset (Tie-2-expressing monocytes (TEMs)) with pro-angiogenic activity **(Matsubara *et al*., 2013; Dapas *et al*., 2014)**. Angiopoietin-2 (Ang-2) was originally regarded as a specific ligand of Tie-2 **(Yu and Ye, 2020)**.

In the circumstances of hypoxia, endothelial cells are activated and secrete Ang-2 from Weibel-Palade bodies recruiting TEMs to tumor site where they stimulated

angiogenesis **(Cossutta *et al*., 2019)**. TEMs have been found in various human tumors, to form tumor blood vessels and express several proangiogenic factors **(Venneri *et al*., 2007; Coffelt *et al*., 2010)**. TEMs were reported to be increased in HCC patients **(Matsubara *et al*., 2013; Mao *et al*., 2017)**.

It was suggested that TEMs frequency can be used as a diagnostic marker for HCC since early stage HCC is difficult to detect by non-invasive imaging and alpha-fetoprotien (AFP) as "surveillance biomarker" has been dropped in current guidelines because of low sensitivity and specificity**. (Germano and Daniele, 2014)**.

Aim of the Work

The aim of this study is to assess the role of TEMs in diagnosis of Hepatitis C virus-related HCC. Also, investigation of the ability of TEMs in combination with other known HCC markers to improve diagnosis of the disease.

Chapter (1)
Overview of Hepatocellular Carcinoma

Hepatocellular carcinoma (HCC) is considered the sixth most common malignancy worldwide and the third cancer in terms of mortality. It is also considered the most common primary malignancy of the liver **(Rawla *et al*., 2018)**.

1-Epidemiology:

a-Sex and age

Hepatocellular carcinoma is more common in males than females with the ratio range between 2:1 and 4:1 this range differs according to each different risk area **(Zhu *et al*., 2016)**.

There are different age-based risk groups. In low-risk areas the age most susceptible age group is beyond 75 years old. In high-risk areas, the age group becomes 60 to 65 years old. **(Yang and Roberts, 2010)**.

b-Geographic distribution:

Hepatocellular carcinoma is most distributed in Asian countries especially China, Western and Eastern Africa. The prevalence of HCC in developed countries of the world is

lower, except for Japan, Italy and France **(Ghouri *et al*., 2017)**.

In Egypt, 11.75% of the malignancies of gastro-intestinal tract are liver cancers and contribute to 1.68% of overall malignancies. HCC prevalence among all liver tumors is 70.48% **(Ashmawy *et al*., 2019)**. This may be due to the high prevalence of hepatitis C virus (HCV) and increase importance of hepatitis B virus (HBV) in the past few years since they are primary risk factors to HCC along with the improvement in the screening and diagnostic tools **(Holah *et al*., 2015)**.

2-Risk Factors of HCC

Hepatocellular carcinoma has a wide range of etiological risk factors with strong association to its occurrence. The presence of more than one factor increases aggravates the risk **(Fattovich *et al*., 2004)**.

a-Hepatotropic viruses

Hepatitis B and C viruses are the most common risk factors for HCC. **(Ghouri *et al*., 2017)**. Chronic HCV infection is associated with a 15 to 20-fold increased risk of developing HCC as compared to uninfected individuals. In

addition, HCV and HBV coinfection increase risk of developing HCC varying from additive to synergistic effect. Thus, liver cancer resulting from viral infections is close to 90%. **(Zhu *et al*., 2016; Axley *et al*., 2018)**.

b-Cirrhosis

Cirrhosis reduces the mass of functional hepatocytes and leads to portosystemic shunting. Whatever its underlying cause, cirrhosis predisposes to HCC and is considered as a pre-neoplastic condition. The vast majority of HCC worldwide develops in patients with pre-existing cirrhosis **(Chettouh *et al*., 2015)**.

c-Aflatoxins

Aflatoxin B1 (AFB1) is an extremely potent hepatocarcinogen that is a secondary metabolite produced by fungi, especially Aspergillus flavus. They are typically found in tropical and sub-tropical regions of the world in which grains such as rice stored in hot humid conditions promote growth of these toxin-producing fungi **(Rushing and Selim, 2019)**.

d-Alcohol

Chronic alcohol use of greater than 80 g/day for more than 10 years increases the risk for HCC approximately 5-fold. Furthermore, there may be synergism between alcohol and hepatitis C in the development of HCC **(Morgan *et al*., 2004)**.

e-Non-alcoholic steatohepatitis (NASH):

Non-alcoholic steatohepatitis is anticipated to account for a greater proportion of HCC incidence due to the growing epidemic of obesity and diabetes. **(Cholankeril *et al*., 2017)**.

f-Obesity:

Obesity is a significant risk for the development of HCC particularly in patients with NASH. Obese (body mass index > 30 kg/m2) patients have a reported 1.93-fold higher risk of developing primary liver cancer. **(Cholankeril *et al*., 2017)**.

3-Pathogenesis of HCC:

Liver microanatomy

Normal liver is organized in lobules segregated by interlobular connective tissue and containing 'cords' of hepatic parenchymal cells and hepatocytes, which surround a central vein and are separated by vascular sinusoids (Figure 1A) **(Krishna, 2013)**.

Sinusoidal liver endothelium is fenestrated and lacks a basement membrane. The fenestrations permit blood plasma to surround the exposed surfaces of the hepatocytes through the space between the fenestrated endothelium and the cells (the space of Disse) which contains collagen fibers and fibroblasts (Figure 1B) **(Poisson *et al*., 2017)**.

Hepatic stellate cells localized in the space of Disse have a major role in liver fibrosis in response to liver damage. Kupffer cells (liver macrophages) are also closely associated with the sinusoids (Figure1B). Blood from the portal vein and hepatic artery mixes together in the hepatic sinusoids and after 'filtration' by hepatocytes drains out of the lobule through the central hepatic vein (Figure 1A) **(Zhu *et al*., 2011)**.

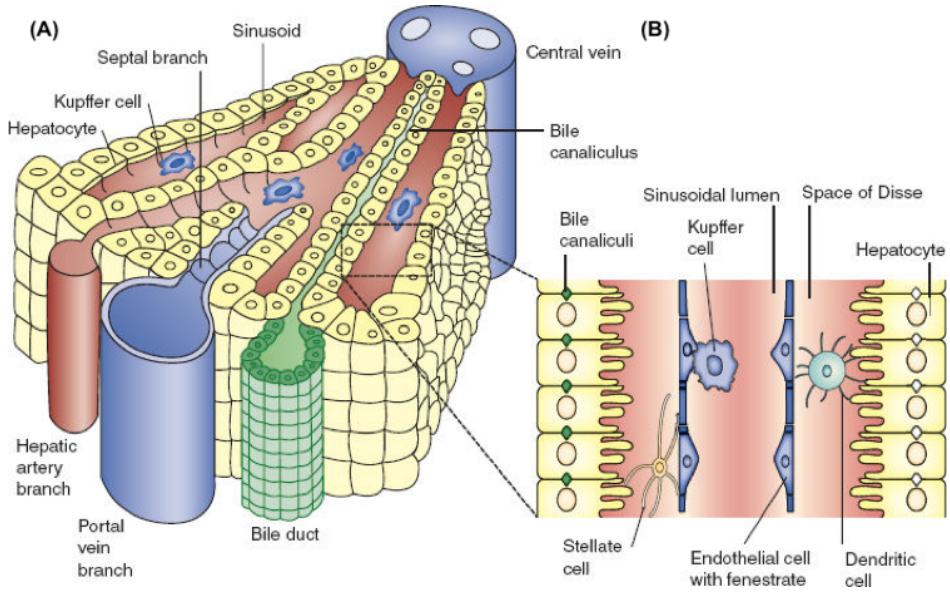

Figure (1): Liver micro anatomy **(Moore *et al.*, 2013)**.

The Role of Angiogenesis in HCC:

Although HCC follows diverse causes of liver damage, common associated findings are hypervascularity and marked vascular abnormalities such as arterialization and sinusoidal capillarization. Liver tumor vessels have an abnormal blood flow and are excessively leaky. In turn, this leads to hypovascular areas, necrosis and severe hypoxia which may promote HCC growth, progression and resistance to therapy **(Zhu *et al.*, 2011)**.

Molecular pathways of HCC angiogenesis:

Inadequate intratumoral oxygen level is known to trigger a vast array of molecular and cellular responses which will influence tumor aggressiveness and therapeutic response. Hypoxia inducible factors (HIFs) are critical to sense intratumoral oxygen tension and mediate subsequently the activation of hypoxic response (Figure 2) **(Chen and Lou, 2017)**.

Upregulation of HIFs induce expression of proangiogenic factors that promote angiogenesis in HCC. At the molecular level, angiogenesis results from an imbalance between drivers of vessel growth and maturation and inhibitors. Proangiogenic factors activate endothelial cell tyrosine kinases and subsequent downstream intracellular signaling leading to angiogenesis (Figure 2) **(Morse *et al*., 2019)**.

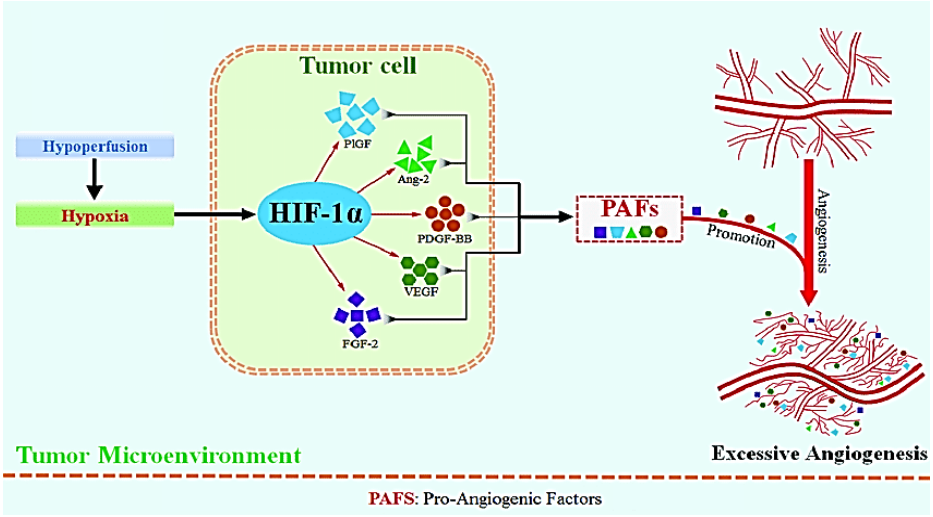

Figure (2): Hypoxia induced angiogenesis; PAFs: pro-angiogenic factors, HIF: hypoxia induced factor, PIGF: placental growth factor, Ang: angiopoietin, PDGF: platelet derived growth factor, VEGF: vascular endothelial growth factor, FGF: fibroblast growth factor **(Wang *et al*., 2017)**.

Angiogenesis drivers:

a) Vascular endothelial growth factor (VEGF)

Both VEGF and VEGF receptors (VEGFR), the most prominent regulators of angiogenesis, are critical for HCC growth and development. VEGFR-2 is stimulated by binding to VEGF leading to a phosphorylation cascade resulting in endothelial proliferation and formation of new leaky tumor blood vessels necessary for rapid tumor growth and dissemination. This leads to formation of areas of high interstitial pressure and severe hypoxia or necrosis, both of

which can further drive malignant potential **(Amini *et al*., 2012)**. Circulating VEGF levels are increased in HCC and have been shown to correlate with tumor angiogenesis and progression **(Zhang *et al*., 2012)**.

b) Platelet derived growth factor (PDGF)

Binding of PDGFs to the PDGF receptor (PDGFR)-α and β tyrosine kinase receptors expressed on other mesenchymal cells, such as fibroblasts, smooth muscle cells and pericytes, activates pathways that are the same as or similar to those stimulated by VEGF **(Heldin, 2013)**.

c) Fibroblast growth factor (FGF)

Both FGFs and FGF receptors (FGFR) are ubiquitously expressed and have numerous functions, including regulation of cell growth and differentiation of angiogenesis. Cross-talk between FGF-2 and VEGF-A during initial phases of tumor growth induces neovascularization and further tumor growth **(Tsunoda *et al*., 2007)**.

d) Endoglin (CD105)

Endoglin (CD105) expression is up-regulated in actively proliferating endothelial cells, including that of HCC **(Nassiri *et al*., 2011)**. It promotes the invasion and metastasis

of liver cancer cells by increasing VEGF expression, therefore expression of endoglin correlated with stage, tumor differentiation and aggressive tumor behavior of HCC **(Li *et al*., 2014)**.

e) Angiopoetin (Ang) / Tie pathway

In normal tissue, Ang-1 has vasculo-protective effects to stabilize blood vessels and inhibits vascular permeability induced by several inflammatory cytokines. In contrast, increased Ang-2 expression in areas of remodeling inhibit this interaction and destabilizes blood vessel support cells, a step necessary to facilitate vessel proliferation or sprouting in the presence of VEGF. During tumor angiogenesis, endothelial Ang-2 secretion is upregulated that its levels are observed to be increased in the serum of cancer patients such as HCC **(Fagiani and Christofori, 2013)**.

Angiopoietin ligands (Ang-1–4) and the Tie (Tie-1 and Tie-2) receptor tyrosine kinases form an endothelial signaling pathway regulating vascular homeostasis and controls vessel permeability, inflammation and angiogenic responses (Figure 3) **(Saharinen *et al*., 2017)**.

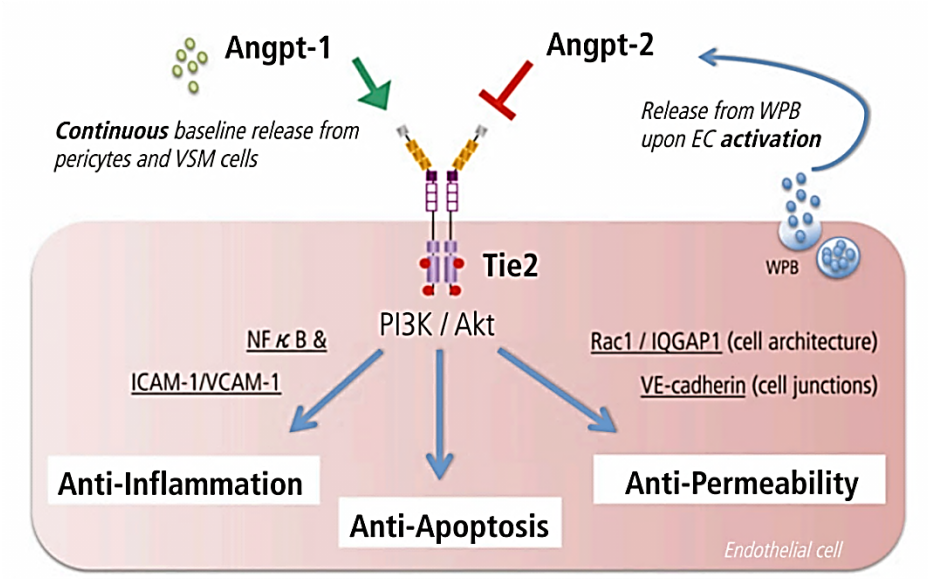

Figure (3): A schematic model of the angiopoietin-Tie2 ligand-receptor system **(David *et al.*, 2013)**.

4-Clinical presentation of HCC:

Hepatocellular carcinoma may progress silently in patients with sufficient liver function and escape early diagnosis due to vague complaints and non-specific symptoms. This is why in developing countries with limited surveillance resources HCC diagnosis is usually delayed **(Dimitroulis *et al.*, 2017)**.

In advanced stages, symptoms and clinical findings include vague right upper quadrant abdominal pain, hepatomegaly, obstructive jaundice and fever of unknown

origin. Non-specific symptoms of advanced malignant disease such as anorexia, nausea, lethargy and weight loss often co-exist **(Desai *et al*., 2019)**.

Hepatocellular carcinoma patients may initially appear with a paraneoplastic syndrome; the most common paraneoplastic syndromes associated with HCC are hypercholesterolemia, hypercalcemia, hypoglycemia and erythrocytosis **(Dimitroulis *et al*., 2017)**.

5-Diagnosis of HCC

Early diagnosis is the key to obtain the best treatment result for HCC. Diagnosis is based on laboratory tests and imaging studies as well **(Llovet and Bruix, 2000)**.

A-Laboratory diagnosis

a) Alpha-fetoprotein (AFP)

It is the most frequent used serological marker. AFP serum concentration increases in parallel with HCC tumor size. For this reason, AFP had to be considered 'the golden standard' for HCC serum markers **(Stefaniuk *et al*., 2010)**.

Alpha-fetoprotein has also a role in monitoring the response to treatment in HCC. The current recommended