INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related death worldwide and, owing to changes in the prevalence of the two major risk factors, hepatitis B virus and hepatitis C virus, its overall incidence remains alarmingly high in the developing world and is steadily rising across most of the developed world **(*Yang and Robert, 2010*).**Early diagnosis remains the key to effective treatment and there have been recent advances in both the diagnosis and therapy of HCC, which have made important impacts on the disease ***(Jane et al., 2009).***

The relationship between HCV genotype and insulin resistance has been revealed where HCV genotypes 1, 3 and 4 (predominantly in Egypt) are associated with more severe insulin resistance **(*Duseja et al., 2009*).** IR in chronic HCV infection predicts faster progression diseases to fibrosis and cirrhosis, leading to liver failure and hepatocellular carcinoma (HCC) ***(Kiran et al., 2013).***

In recent years, the association of metabolic syndrome (MS), which is a series of conditions including insulin resistance (IR), obesity, hypertension, and hyperlipidemia, with malignancy attracted more and more attentions. As inevitable consequence ofIR, hyperinsulinemia plays an important role in occurrence and prognosis of cancer***(Chung et al.,2004).***

High fasting serum insulin was associated with significantly poorer overall survival and disease-free survival in patients with early stage hepatocellular carcinoma (HCC) ***(Miuma et al., 2009).***

Retinol-binding protein 4 (RBP4) has gained much attention after the first notion that its serum level was enhanced in insulin resistant humans and mice***(Yang et al., 2005).***

Preclinical trials demonstrated that RBP4 might be upstream regulator of IR and specific inducer of hyperinsulinemia ***(Yang et al.,2005).***

Accumulating evidence showed that RBP4 exert a pivotal function to accelerate pathogenesis in fatty liver disease and liver cirrhosis ***(Stefan et al., 2007; Petta et al.,Hepatology, 2008)***. However, limited number of studies was conducted to investigate the relationship between RBP4 and HCC.

AIM OF THE STUDY

The aim of this study was to determine the value of serum RBP4 level in Egyptian patients with hepatocellular carcinoma and to correlate this level with the metabolic profile in these patients.

Chapter 1

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is a primary tumor of the liver, which usually develops in the setting of chronic liver disease, particularly in patients with chronic hepatitis B and C. The diagnosis of HCC can be difficult and often requires the use of one or more imaging modalities ***(Bruix et al., 2011; Forner et al., 2012).***

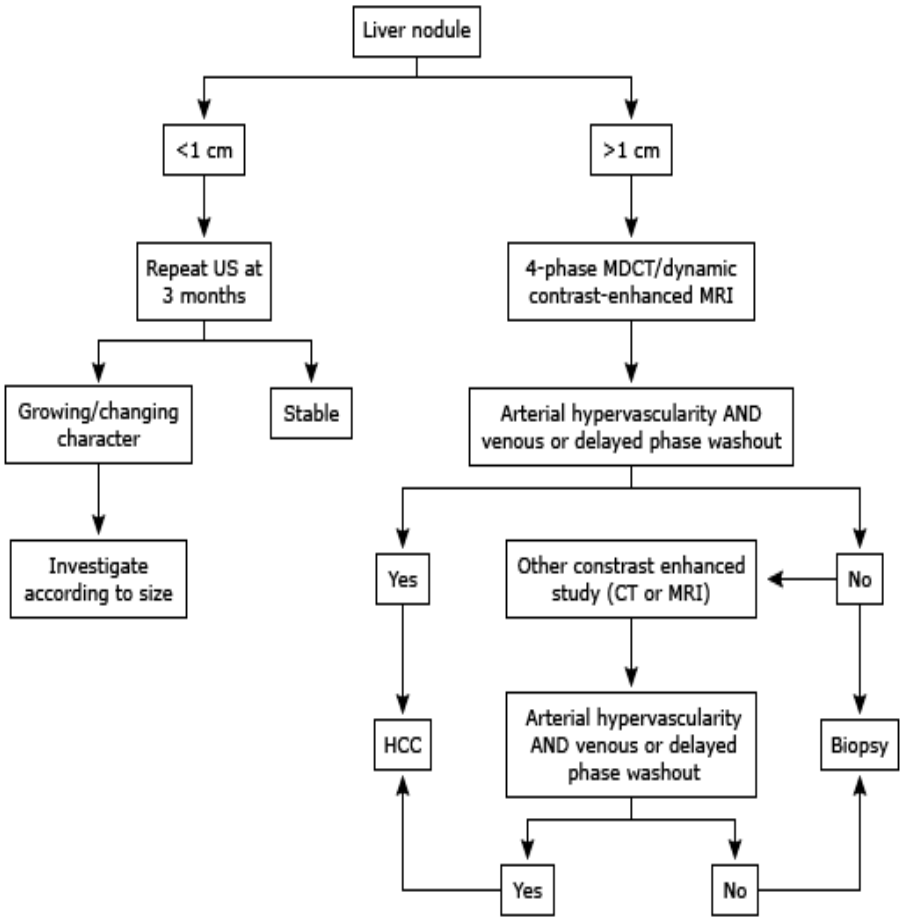
Diagnosis of HCC:

The consensus statement issued by the American Association for the Study of Liver Diseases (AASLD) (*algorithm 1)****(Bruix et al., 2011).*** is widely agreed upon for the diagnosis of HCC. Similar guidelines have been issued by the European Society for Gastrointestinal Endoscopy, though they differ in the evaluation of patients with nodules between 1 and 2 cm in size***(EASL-EORTC, 2012).***

The only way to effectively diagnose HCC in a timely fashion is to enter patients who are at high risk for development of this tumor in a regular surveillance program using ultrasound imaging every six months.In patients who are not in a routine surveillance program, the diagnosis of HCC may be first

entertained in a patient with underlying liver disease (i.e., cirrhosis, chronic viral hepatitis) who develops a rising serum alpha-fetoprotein (AFP) level. In such patients, a CT scan of the liver and/or magnetic resonance imaging (MRI) study is often the initial diagnostic maneuver (***Bruixet al., 2005***).

Algorithm 1: Algorithm for investigation of small nodules found on screening in patients at risk for hepatocellular carcinoma



MDCT: Multidetector CT scan.

AASLD guidelines:

The guidelines point out that a mass found incidentally or on screening in the setting of a patient with known hepatitis B or cirrhosis of other etiology is likely to be HCC. The sequence of tests used to establish the diagnosis in such patients should be guided by the size of the lesion:

- Nodules found on ultrasound surveillance that are smaller than 1 cm should be followed with ultrasound at intervals of three to six months. If there has been no growth over a period of up to two years, one can revert to routine surveillance.

- Lesions larger than 1 cm in diameter should be evaluated with dynamic MRI or CT. If the appearance is typical for HCC, no further investigation is required. If the characteristics are not typical for HCC (and do not suggest hemangioma), one of two strategies is acceptable: either a second study (CT or MRI, whichever was not performed) or a biopsy.

- Biopsies of small lesions should be evaluated by expert pathologists. Staining for tumor markers including CD34, CK7, glypican 3, HSP-70 and glutamine synthetase can help characterize lesions that are not clearly HCC on microscopy. If the biopsy is negative for HCC patients

should be followed by imaging at three to six month intervals until the nodule either disappears, enlarges, or displays diagnostic characteristics of HCC.

Some have argued that the data are insufficient to support the use of only one imaging modality for diagnosing HCC in patients with nodules between 1 and 2 cm and that in order to diagnose HCC in such patients, there should be concordant results from CT and MRI (***El-Serag et al., 2011; Yeh et al., 2012***).

EASL guidelines:

Guidelines from 2012 issued by the European Association for the Study of the Liver (EASL) are similar to the AASLD guidelines, though they differ with regard to the interval for obtaining follow-up ultrasounds in patients with small nodules and the number of radiographic tests needed to make a diagnosis for nodules between 1 and 2 cm. (***EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma, 2012***).

For nodules seen on ultrasound that are <1 cm, the EASL guidelines recommend:

- Repeat ultrasound at four months. If the nodule grows, additional investigation is required and dictated by the size

of the nodule. If the nodule is stable, ultrasound is repeated at four-month intervals for one year, after which time it can be performed every six months.

For nodules between 1 and 2 cm:

A. Obtain a 4-phase CT scan and/or dynamic contrast enhanced MRI. Only one imaging study is recommended in centers of excellence with high-end radiologic equipment. Otherwise, both studies should be obtained.

B. If there are radiologic hallmarks of HCC (arterial hypervascularity and venous/late phase wash out), then a diagnosis of HCC is made.

C. If the radiologic hallmarks of HCC are not seen, a biopsy should be obtained and assessed by an expert pathologist. If the biopsy results are inconclusive a second biopsy is recommended. If the results are still inconclusive, ultrasound should be repeated at four-month intervals to monitor for growth, with a repeat biopsy if there is growth or changes in the nodule's enhancement pattern.

For nodules >2 cm:

- Obtain a 4-phase CT scan or dynamic contrast enhanced MRI.
- If there are radiologic hallmarks of HCC, then a diagnosis of HCC is made.

- If the radiologic hallmarks of HCC are not seen then a biopsy should be obtained to confirm the diagnosis. If the biopsy results are inconclusive, ultrasound should be repeated at four-month intervals to monitor for growth.

Serum markers:

The most commonly used marker for HCC is the serum AFP concentration. Several other serologic markers (such as des-gamma-carboxy prothrombin) may indicate the presence of HCC, and used alone or in combination with the serum AFP may improve the diagnostic accuracy. Although these other markers are not used in routine clinical practice, they continue to be a topic of investigation.

A. Embryonic antigen

- ***Alpha-fetoprotein:***

Alpha-fetoprotein is a glycoprotein that is normally produced during gestation by the fetal liver and yolk sac, the serum concentration of which is often elevated in patients with HCC. Serum levels of AFP do not correlate well with other clinical features of HCC, such as size, stage, or prognosis. Elevated serum AFP occurs in pregnancy, with tumors of gonadal origin (both germ cell and non-germ cell ***(El-Bahrawy et al., 2010).*** and in a variety of other malignancies, of which gastric cancer is the most common ***(Liu et al., 2010).***

Despite the issues inherent in using AFP for the diagnosis of HCC, it has emerged as an important prognostic marker,especially in patients undergoing resection and those being considered for liver transplantation. Patients with AFP levels >1000 have an extremely high risk of recurrent disease following transplantation, irrespective of the tumor size ***(Ioannou et al., 2008; Pomfret et al., 2010).***

- ***Lens culinaris agglutinin-reactive AFP (AFP-L3):***

The development and applications of biological chemistry and related analysis, as well as additional study of AFP have revealed that AFP has three glycoforms (AFP-L1, AFP-L2 and AFP-L3), according to their binding ability to the lectin lens agglutinin (LCA). AFP-L1, as a non-LCA-bound heterogeneity, is a major glycoform in various benign liver diseases. AFP (AFP-L3) is a fucosylated fraction of AFP that may be a helpful diagnostic and prognostic maker of HCC, particularly in patients with low serum AFP levels ***(Toyoda et al., 2011; Oda et al., 2011; Morimoto et al., 2012; Kumada et al., 2011; Yamamoto et al., 2010; Sterling et al., 2009).***

The sensitivity and specificity of AFP-L3 are both relatively satisfactory as compared with AFP. Moreover, AFP-L3 does not correlate with AFP, thus the former can be used as an independent and significant factor for the early diagnosis of

HCC. In a study with 270 patients with newly diagnosed HCC and 396 patients with chronic liver disease, all of whom had AFP levels <20 ng/mL, the sensitivity and specificity of a highly sensitive AFP-L3 assay (using a cutoff of ≥5 percent) for HCC were 42 and 85 percent, respectively***(Toyoda et al., 2011).***

B. Proteantigen

- ***Glypican-3 (GPC3):***

The expression of GPC3 is upregulated in HCC tumor tissues compared with normal and benign liver diseases ***(Capurroet al., 2005).*** In addition, no correlation between GPC3 expression and tumor stage, size and AFP level has been observed. The sensitivity and specificity in the diagnosis of HCC was found to be 77 and 96%, respectively ***(Shirakawa et al., 2009).***

C. Enzymes and Isozymes

- ***Des-γ-carboxyprothrombin (DCP):***

Baek et al demonstrated that irrespective of whether the diameter of HCC is <3 cm, 3-5 cm or >5 cm, the diagnostic accuracy of DCP was higher than that of AFP***(Baek et al., 2009).*** In addition, the combined detection of DCP and AFP can improve the diagnostic sensitivity and can be used to predict the recurrence of HCC within 6 months after surgery ***(Yamamoto et al., 2009).*** By contrast, the level of DCP was closely associated

with a larger tumor, vascular invasion and it served as a more accurate tumor marker compared with AFP and AFP-L3 as reported by***(Yamamoto et al.,2010).***

D. Cytokines

- **VEGF**: Xiang et al***(Xiang et al., 2011).***revealed that VEGF is a type of biomarker of lymph node metastasis in HCC. In addition, the expression of VEGF is closely correlated with tumor recurrence and prognosis. Of note, some VEGF receptor expression has been found to correlate with the development of tumor ***(Zhang et al., 2012).***

E. Genetic biomarkers

- **MicroRNAs:** Plasma microRNA expression has been studied as a possible marker of HCC ***(Borel et al., 2012; Zhou et al., 2011; Li L et al., 2012).*** One study examined 934 participants who were healthy, had chronic HBV, had cirrhosis, or had HBV-related HCC ***(Zhou et al., 2011).*** MicroRNA panel that included miR-122, miR-192, miR-21, miR-223, miR-26a, and miR-801 accurately identified patients with HCC, regardless of the stage of HCC. The panel also accurately differentiated patients with HCC from those who were healthy, had chronic HBV, or had cirrhosis.

Surveillance in adults with chronic liver disease

The 2010 AASLD guidelines on the management of HCC recommend that surveillance be performed using ultrasonography at six-month intervals ***(AASLD Practice Guideline, Management of Hepatocellular Carcinoma, 2010).***

The sensitivity of ultrasound for detecting HCC is 94 percent, though it drops to 63 percent for detecting early HCC***(Singalet al., 2009).*** The six-month interval is based primarily on observational data, the expected growth rates of HCC***(Singalet al., 2009; Trevisaniet al., 2002; Santagostinoet al., 2003),***and preliminary data suggesting that survival is better when surveillance is performed every six months rather than every 12 months***(Kim et al., 2007).***

The surveillance interval is a function of the tumor growth rate, not the degree of risk of developing HCC. One trial examined three-month screening intervals and found that while more lesions 10 mm in diameter or less were found with the shortened interval compared with a six-month screening interval, there were no differences in clinically relevant outcomes ***(Trinchet et al., 2011).***

Staging and Prognostic Scoring Systems

A number of systems have been proposed to predict the prognosis for HCC, none of which has been universally adopted

(Okuda et al., 1985; Prospective validation of the CLIP score, 2000; Farinatiet al., 2000; Yang et al., 2012). These schema variably incorporate four features that have been recognized as being important determinants of survival: the severity of underlying liver disease, the size of the tumor, extension of the tumor into adjacent structures, and the presence of metastases ***(Okuda et al., 1985; Prospective validation of the CLIP score, 2000).*** The four most commonly used systems are the TNM, Okuda and Barcelona systems, and the CLIP score.

A. Tumor, node, metastasis (TNM) staging:

The American Joint Committee on Cancer (AJCC) TNM staging system (identical to that of the Union InternationaleContre le Cancer (UICC)) was revised in 2010 (*table 1*) ***(American Joint Committee on Cancer, 2010).*** Like the 2002 classification, this system recognizes the most important predictors of prognosis: the number of tumors, and the presence and extent of vascular invasion within the tumor ***(Vautheyet al., 2002).*** However, compared to the 2002 staging system, there are some reclassification changes, primarily surrounding the better prognosis for multiple HCC versus HCC with major vascular invasion.

Table 1: TNM staging for hepatocellular cancer

Primary tumor (T)			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
T1	Solitary tumor without vascular invasion		
T2	Solitary tumor with vascular invasion or multiple tumors none more than 5 cm		
T3a	Multiple tumors more than 5 cm		
T3b	Single tumor or multiple tumors of any size involving a major branch of the portal vein or hepatic vein		
T4	Tumor(s) with direct invasion of adjacent organs other than the gallbladder or with perforation of visceral peritoneum		
Regional lymph nodes (N)			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Regional lymph node metastasis		
Distant metastasis (M)			
M0	No distant metastasis		
M1	Distant metastasis		
Fibrosis score (F)*			
F0	Fibrosis score 0-4 (none to moderate fibrosis)		
F1	Fibrosis score 5-6 (severe fibrosis or cirrhosis)		
Anatomic stage/prognostic groups			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage IIIA	T3a	N0	M0
Stage IIIB	T3b	N0	M0
Stage IIIC	T4	N0	M0
Stage IVA	Any T	N1	M0
Stage IVB	Any T	Any N	M1

* The fibrosis score as defined by Ishak is recommended because of its prognostic value in overall survival. This scoring system uses a 0-6 scale.