

**VALUE OF SERUM ALPHA-FETOPROTEIN IN THE
DIAGNOSIS AND FOLLOW UP OF
HEPATOCELLULAR CARCINOMA IN EGYPTIAN
PATIENTS INFECTED WITH HEPATITIS C VIRUS**

Thesis

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world and the third most common cause of cancer related death. Currently, it is the leading cause of death among cirrhotic patients (*Llovet and Bruix, 2004*). It represents more than 5% of all cancers in the world, and the estimated number of cancer-related deaths exceeds 500,000 per year (*Llovet and Beaugrand, 2003*).

HCC usually develops in patients with cirrhosis. Cirrhosis may be caused by viral hepatitis (primarily hepatitis B and C), alcohol, hereditary haemochromatosis, autoimmune liver diseases and actually any disease that results in chronic inflammation of the liver (*Martin and Dufour, 2008*). From a global perspective, the two most important risk factors for HCC are chronic hepatitis B and C infection (*But et al., 2008*).

The most important tumor marker for HCC is alpha-fetoprotein (AFP). The common method for screening high risk patients using AFP marker can detect more early tumors and prolong the survival of patients (*Lopez, 2005*).

The recent popularization of periodic surveillance and the development of diagnostic capabilities have resulted in the discovery of increasing numbers of patients with small HCC nodules (*Okuda, 2000*).

Massoud et al. (2006) reported that the only way for early detection of HCC which gives chance for possible

curative management is the use of screening programs. These should be composed of abdominal U/S as a corner stone, because it is the most sensitive imaging technique for detection of small focal lesions and is easy to perform, and also include the measurement of AFP. The rising AFP level in CLD patient is an important warning sign necessitating careful hepatic imaging for detection of very small focal lesions.

Although recent evidence indicates that the fucosylated fraction of alpha fetoprotein may be a more useful marker than total alpha-fetoprotein, yet it is a very expensive technique and the availability of this assay is still confined to few laboratories (**Trevisani et al., 2001**). Thus, alpha-fetoprotein remains the oncomarker universally utilized for monitoring high risk patients for hepatocellular carcinoma in clinical practice (**Pateron et al., 1994**).

The impact of virological status on the diagnostic accuracy of alpha-fetoprotein in hepatocellular carcinoma detection still remains largely unsettled (**Trevisani et al., 2001**). Most available data concerning AFP came from studies of patients with chronic hepatitis B or mixed etiologies. Studies concerning the diagnostic value of AFP for HCV-related liver cirrhosis are limited (**Kim et al., 2006**).

The role of serum AFP level in predicting the outcome of HCC patients remains controversial since many studies did not show a consistent result. The prognostic power of pre-treatment serum AFP level in relation to therapy and other clinical settings has not been specifically studied (**Huo et al., 2004**).

AIM OF THE WORK

The aim of this study is:

1. To investigate sensitivity and specificity of serum alpha-fetoprotein, its positive and negative predictive values and so the cut-off value for diagnosis of hepatocellular carcinoma in Egyptian patients with hepatitis C virus (HCV) infection.
2. The correlation between the serum level of alpha-fetoprotein and the size and multiplicity of hepatocellular carcinoma in HCV infected patients will be studied.
3. To study the prognostic value of serum alpha-fetoprotein level in short term non-surgical treatment of hepatocellular carcinoma.

EPIDEMIOLOGY OF HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is a major health problem throughout the world (*Chu et al., 2002 and Bae et al., 2005*). It ranks the fifth in frequency worldwide among all malignancies and causes one million deaths annually (*El-Serag and Mason, 2000 and Srivatanakul et al., 2004*). It is the second among cancers of the digestive tract after stomach cancer (*Bosch et al., 1999*).

It accounts for 7.4% of all cancers in males and 3.2% of all cancers in females (*Sherlock and Dooley, 2002d*), with a mean annual incidence of around 3–4% in patients with chronic liver disease (*Llovet and Beaugrand, 2003*). Worldwide, hepatocellular carcinoma is three times more frequent in males than females, which may be at least in part explained by differences in exposure to risk factors. However, sex hormones and other x-linked genetic factors may also be important (*Yu et al., 1991*). It has been speculated that estrogens and androgens could modulate hepatocarcinogenesis and explain the higher incidence of HCC in men (*Nagasue et al., 1985*).

Hepatocellular carcinoma (hepatoma) accounts for 70-80% of all primary liver tumors (*Johnson, 2006*). Most cases of HCC are secondary to either hepatitis infection (usually hepatitis B or C) or cirrhosis (*Lau et al., 1999*).

The incidence of HCC varies considerably with the geographic area because of differences in the major causative factors (**Bosch et al., 1999**)

Geographically, HCC is rare in Western world and most common in Southeast Asia and parts of Sub-Saharan Africa (**Sherlock and Dooley, 2002d**). Marked increase has been shown in the USA over the last two decades. It has also increased in France and UK (**El-Serag and Mason, 1999**).

In Egypt, 4.7% of chronic liver disease patients suffer from HCC. The development of HCC is mainly due to high rates of hepatitis C infection among Egyptian patients (**El-Zayadi et al., 2001**).

In the Far East and sub-Saharan Africa, where HBV is highly endemic, HBV is the main cause of HCC. However, in areas with an intermediate rate of liver tumors such as Southern Europe, Egypt and Japan HCV is the predominant cause of HCC, where HCC is mostly discovered at an older age in patients with long-standing cirrhosis due to HCV. In regions with a low incidence of HCC such as Northern Europe and the United States, HCC related to HCV or HBV infection are found in a minority of cases and the tumor is often related to other factors such as alcoholic liver disease. In these low endemic areas, HCC is usually discovered at an older age in patients with longstanding cirrhosis due to alcohol abuse (**Donato et al., 1997**).

In France, alcohol consumption is still the leading cause of cirrhosis and was responsible for 60% of all HCC causes during the last decade (*Chevret et al., 1999*).

Hepatocellular carcinoma (HCC) is an aggressive hepatic neoplasm that most commonly affects adults. It is rarely seen in the first four decades of life except in areas where HBV infection is hyper endemic (*Simonetti et al., 1992*). Nevertheless, children who are affected with biliary atresia, infantile cholestasis, glycogen storage diseases, and a wide array of cirrhotic diseases of the liver are predisposed to developing HCC (*Bruix and Sherman, 2005*).

The incidence of HCC generally increases progressively with increasing age, although there is a tendency for incidence to stop changing in the oldest age group (*Colombo, 2003*). Peaks between 35 and 75 years, the peak being earlier in higher incidence areas (*Johnson, 2006*). There is shift to younger age group over the last two decades that may be attributed to emergence of HCV infection (*Hassan et al., 2002a and Montalto et al., 2002*), as well as to acquisition of both hepatitis B and C virus infection at younger age (*Velazquez et al., 2003*).

RISK FACTORS OF HEPATOCELLULAR CARCINOMA

Several factors have been identified as being related to the etiology of HCC. In many cases, these factors, such as chronic viral hepatitis, alcoholism, hemochromatosis (*Di Bisceglie, 1999*) and alpha-1-antitrypsin deficiency, cause chronic liver disease and cirrhosis (*Shiratori et al., 2001*).

In some instances, several etiologic factors may be identified in the same patient, suggesting a synergistic role (*Di Bisceglie, 1999*).

However, the most powerful risk factor for development of HCC is the existence of liver cirrhosis, regardless of its etiology (*Zaman et al., 1985*).

Hepatitis B Virus (HBV) Infection:

Hepatitis B virus (HBV) infection is a major public health problem. It is estimated that two billion people have been infected worldwide and 360 million suffer from chronic HBV infection. Over 520,000 die each year, 50,000 from acute hepatitis B, 470,000 from cirrhosis and liver cancer (*EASL, 2002*). However, the prevalence of HBV infection in Egypt has been declining over the last two decades (*El-Zayadi et al., 2001*). The decline of HBsAg may be partially attributed to successful control measures of blood transfusion introduced in mid-seventies and partially to development of mutant or occult

HBV infection, which requires costly assays for diagnosis. However, this decline could not be attributed to vaccination programs in children, which have been launched in Egypt about 10 years ago. It has been reported that the reduction of the incidence of HBV will be seen 30 – 40 years after the launch of the universal hepatitis B vaccination program (**Chang, 2003**).

Chronic hepatitis B and C infection are responsible for the great majority of cases of HCC worldwide. They also account for the peculiar geographical distribution of the tumor (**Michielsen et al., 2005**).

The absence of serologic markers of either HBV or HCV infection cannot completely rule out a viral etiology of hepatocarcinogenesis as virus replication may persist in the hepatic tissue despite undetectable markers in serum (**Urashima et al., 1997**).

Infection at or around birth (as in Taiwan) predispose to earlier cancers than if people are infected later. The time between hepatitis B infection and development into HCC can be years even decades, but from diagnosis of HCC to death the average survival period is only 5.9 months, according to one Chinese study during the 1970-80s, or 3 months (median survival time) in Sub-Saharan Africa (**Lau et al., 1999**). The risk for HCC in persons chronically infected with HBV is 102 times greater than the risk in non-carriers (**Colombo, 2003**).

Evidence of infection with HBV may include serologic markers of active current infection, such as HBsAg or HBV DNA in serum, or the presence of antibodies to HBV antigens, such as anti-HBc and anti-HBs (**Kew, 1983 and Pan and Zhang, 2005**). The finding of HBV DNA in the serum of patients without HBsAg has been termed occult hepatitis B infection (**Torbenson and Thomas, 2002**). In some instances, HBV DNA can be isolated from liver or tumor tissue in patients with HCC who have no serologic evidence of HBV infection (occult HBV infection) (**Marrero and Lok, 2004**).

The entire nucleotide sequences of HBV genomes have been classified into 8 genotypes (A-H), with predominance of genotypes A and D in Western countries, and B and C in Southeast Asia and the Far East (**Norder et al., 1994, Stuyver et al., 2000 and Arauz-Ruiz et al., 2002**). Several studies from the Far East evaluated the association between distinct genotypes and severity of liver disease. Genotype C was shown to be associated with the development of liver cirrhosis and HCC in Taiwan (**Kao et al., 2000**), China (**Ding et al., 2001**), and Japan (**Orito et al., 2001**), whereas genotype B was shown rarely to be associated with the development of HCC in China and Japan. In contrast, in Taiwan genotype B is the predominant type in patients with HCC who are younger than 35 years (**Kao et al., 2000**). Another study from Taiwan showed that patients with genotype C had a greater tumor recurrence rate after curative resection of HCC compared with those with genotype B (**Chen et al., 2004**).

Hepatitis B virus induces hepatocarcinogenesis by a variety of mechanisms (**Kew, 2000**):

- Certainly, it results in chronic liver injury (in the form of inflammation, regeneration and fibrosis) which may predispose to HCC by itself (**Geissler et al., 1997**).
- However, a significant proportion of HBV-related HCCs arise in an otherwise normal liver, implicating that the virus can also be directly oncogenic through different mechanisms (**Michielsen et al., 2005**):

1. It has been demonstrated that HBV integrates into the DNA of the host cells. This integration may dysregulate the control mechanisms on the cell cycle by chromosomal abnormalities with production of viral proteins or alteration of human genes and proto-oncogenes. Several studies suggest that DNA integration sites are at random and that integration occurs at random times during the course of a chronic viral infection (**Chen et al., 1989**).

2. Chromosomal DNA instability:

Several studies have shown that HBV DNA integration enhances chromosomal instability. In many hepatic tumors large inverted duplication insertions, translocations and micro- and macrochromosomal deletions have been associated with HBV insertion (**Robinson, 1994**). These changes can result in loss of important cellular genes, sometimes involving tumor-

suppressor genes and other genes involved in the regulation of regeneration and growth processes.

3. Trans-activation of cellular genes:

Mammalian hepadnaviruses contain a gene (the HBX gene), of which the protein (HBX protein) can *trans*-activate several cellular promoters and upregulate their expression of different cellular and viral genes (**Twu and Schloemer, 1987 and Shirakata et al., 1989**). Integrated HBX, even when truncated, frequently encodes functionally active *trans*-activator proteins (**Paterlini et al., 1995**). This protein has been shown to transform mouse fetal hepatocytes into a full malignant phenotype (**Henkler and Koshy, 1996**).

A gene that may be affected by the HBX gene is the p53 tumor suppression gene. This gene has been shown to play an important role in hepatocarcinogenesis. It is considered to negatively regulate the cell cycle. The HBX protein has been shown to complex p53 protein and to inhibit its function (**Wang et al., 1994 and Truant et al., 1995**). In a transgenic mouse model it was shown that HCC development correlates with p53 binding to HBX (**Ueda et al., 1995**).

4. Oncogenes:

It has been proposed that HBV acts as an insertion mutagen by integrating into the host genome and activating the cellular proto-oncogenes *c-myc*, *ras* and *c-fos* (**Pasquinelli et al., 1992**).

5. Growth factors:

Growth factors and their receptors function as positive or negative modulators of cell proliferation and differentiation. Insulin-like growth factor-II and transforming growth factor- β expression correlate with HBX protein expression in animal models (*Fu et al., 1988 and Yoo et al., 1996*), suggesting *trans*-activation of these growth factors facilitating tumor formation (*Michielsen et al., 2005*).

6. Role of PreS mutations:

PreS deletion mutants accelerate the storage of large envelope proteins in hepatocyte cytoplasm, which could induce cytotoxic effects toward the development of end-stage liver disease (*Bock et al., 1999*). The accumulation of large envelope protein can activate cellular promoters by inducing endoplasmic reticulum stress (*Xu et al., 1997*). Furthermore, pre-S1 sequences can stimulate the transcription of transforming growth factor α (TGF α). Coexpression of TGF α and HBsAg could accelerate hepatocellular carcinogenesis by stimulation of hepatocyte proliferation (*Jakubczak et al., 1997*).

7. Allelic loss of chromosome 4q:

Allelic loss of chromosome 4q is one of the most frequent genetic aberrations found in HCC. It was found to be associated with HBV-related hepatocarcinogenesis, probably by inactivation of a putative tumor suppressor gene included in it (*Yeh et al., 2004*).

Coinfection of HBV and HDV:

Infection with HDV occurs exclusively among patients with HBV infection. Because HBV infection is associated with HCC, there has been speculation about the role of HDV in hepatocarcinogenesis. Evidence of HDV infection includes the presence of anti-HDV in serum. The prevalence of HDV infection varies considerably around the world and even among patients with HBV infection (*Kew, 1996a*).

Thus in southern Africa, HDV infection is rare, even though as much as 10 to 15% of the population may have HBsAg in serum. This variability has made it difficult to assess the role of HDV in causing HCC accurately (*Di Bisceglie, 1999*).

Verme and his colleagues (1991) suggested that HBsAg positive patients with HDV superinfection develop cirrhosis and HCC at an earlier age (mean age 48 year) than HBsAg carriers without HDV infection (mean age 62 years).

Hepatitis C Virus (HCV) Infection:

Hepatitis C is a major public health problem. More than 170 million people worldwide are chronically infected with HCV, which is responsible for more than 100,000 cases of liver cancer per year (*Madhava et al., 2002 and Poynard et al., 2003*). In Egypt, there is a high incidence of anti-HCV seropositivity in the population, with an overall age-adjusted

prevalence of HCV antibodies of 21.9% (*Frank et al., 2000*). The prevalent genotype in Egypt is type 4, with the presence of other genotypes (*Zekri et al., 2000*).



Figure (1): Estimated HCV prevalence by region (*Fabiani, 2007*).

HCV is a spherical, enveloped, single-stranded RNA virus with a diameter of about 50 nm, classified as a separate genus (Hepacivirus) within the Flaviviridae family. HCV is closely related to hepatitis G, dengue, and yellow fever viruses (*Walker, 1999*).

The genome of HCV is highly mutable, by constant mutation, HCV may be able to escape host immunologic detection and elimination (*Lemon and Brown, 1995*). As a consequence, most HCV-infected people develop chronic infection. HCV also knocks out the host's innate immunity (*Foy et al., 2003*).

Natural History

The incubation period after acute exposure to HCV ranges from 2 weeks to 26 weeks. Only 30-40% of adults who are acutely infected develop symptoms, and these symptoms are