INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and the third most common cause of cancer related deaths worldwide. One reason for the poor prognosis of HCC is that most therapies are effective only if HCC is diagnosed in its early stages ***(Bruix and Sherman, 2011)***. Hepatitis C virus (HCV) infection is one of main causes of hepatocellular carcinoma (HCC) and the prevalence of HCV-associated HCC is on the rise worldwide ***(Hou and Bonkovsky, 2013)***. Indeed, HCV infection increases the risk for HCC development by nearly 17-fold compared to healthy individuals ***(Bartosch et al., 2009 and Hou et al., 2010)***.

In Egypt, HCC account for about 4.7% of chronic liver disease patients with doubling of the incidence rate for the past 10 years. HCC epidemic in Egypt is associated with hepatitis C viral infection (HCV); Egypt has the highest prevalence of HCV in the world with ~13.8% of the population infected and seven million persons with chronic HCV liver disease ***(Anwar et al., 2008)***.

Since the detection of alpha-fetoprotein (AFP) in the serum of HCC patients in 1970s, it has been the only serologic marker widely used for diagnosing HCC patients ***(Mohamed et al., 2014)***. As a limitation, elevated serum AFP is observed in only 60% to 70% of HCC patients and, to a lesser extent (33-65%) in patients with smaller HCCs ***(Zhou et al., 2006)***.

Moreover, nonspecific elevation of serum AFP has been found in some patients with chronic hepatitis and patients with liver cirrhosis. Therefore, it was necessary to identify new HCC markers that have a sufficient sensitivity and specificity for the diagnosis of HCC patients, especially in AFP-normal and/or smaller HCC lesions ***(Saad et al., 2013)***.

The development of HCC, as with other solid tumors, is believed to require the dysregulation of at least three biochemical pathways (proliferation, cell cycle, apoptosis/cell survival) within the cell ***(Yamashita et al., 2011 and Whittaker et al., 2010).***

Non-coding RNAs (ncRNAs) are transcribed RNA molecules with little or non-protein coding capacity; they represent approximately 97% of RNAs in higher eukaryotic organisms ***(Bartel, 2009)***. ncRNAs include structural or housekeeping ncRNAs such as transfer RNA, ribosomal RNA,small nuclear RNA and small nucleolar RNA, as well as regulatory ncRNAs, which function to regulate gene expression ***(Ghildiyal and Zamore, 2009)***. Based on transcript size, regulatory ncRNAs are classified into two major groups, small ncRNAs such as microRNAs (miRNAs), approximately 22 nucleotides (nt) in length, and long non-coding RNAs (lncRNAs) with sizes longer than 200 nt ***(Ponting et al., 2009).***

With the development of next generation sequencing (NGS) techniques, a growing number of lncRNAs have been

identified, characterized and functionally annotated ***(Wilusz et al., 2009)***. lncRNAs are still among the least well-understood of transcripts. Several lines of evidence have suggested that lncRNAs are biologically functional rather than transcriptional "noise". The underlying mechanisms by which lncRNAs function remain largely unexplored and unifying theories regarding their actions are still vague ***(Zhang et al., 2012)***. The mechanisms through which they act are molecular scaffolds, which are involved in transcriptional machinery, as post-transcriptional regulators of splicing or as molecular decoys for miRNA ***(Wang and Chang, 2011)***.

Since RNAs are natural antisense interactors included in regulation of many genes connected to survival and proliferation. Research is directed in development of useful markers for diagnosis and prognosis in cancer and in developing new RNA-based cancer therapies, of which some are already in clinical trials ***(Sana et al., 2012).*** ncRNAs including miRNAs and lncRNAs have been reported to be associated with cancer, including hepatocellular carcinoma (HCC), a highly prevalent and deadly cancer because of its frequent recurrence and/or metastasis ***(Geng et al., 2011).***

Analysis of the differentially expressed lncRNAs in HCCs (underlying etiology not specified) has revealed that a number of lncRNAs such as HOTAIR, HEIH, MVIH, MALAT-1), HULC, H19, CUDR, YIYA, lncRNA-Dreh, lncRNA-LET and MEG3 are associated with HCC. Most of

these lncRNAs are upregulated in HCCs, but less expressed or undetectable in normal controls ***(Hou and Bonkovsky, 2013)***. Understanding and insight into unique lncRNAs involved in HCC may suggest new approaches for diagnosis, early detection and treatment of HCC ***(Tsai et al., 2010).***

AIM OF THE WORK

1- To retrieve potential lncRNA associated with hepatocellular carcinoma from curated databases followed by evaluation of clinical utility of lncRNA expression in detection of HCC.

2- To correlate lncRNA expression with different clinicopathological factors.

Chapter 1
HEPATOCELLULAR CARCINOMA

A- Epidemiology of Hepatocellular Carcinoma:

The American Cancer Society's estimates for primary liver cancer and intrahepatic bile duct cancer in the United States for 2016 are:

- About 39,230 new cases (28,410 in men and 10,820 in women) will be diagnosed
- About 27,170 people (18,280 men and 8,890 women) will die of these cancers

The percentage of Americans developing liver cancer has been rising slowly for several decades ***(American Cancer Society, 2016).***

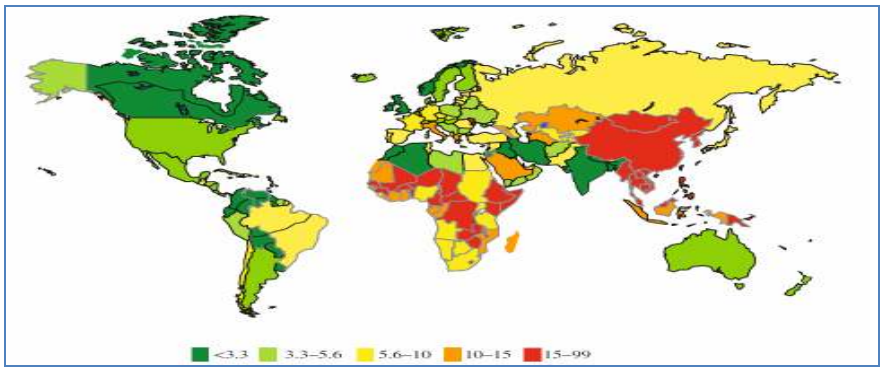

Figure (1): Regional variations in the incidence rates of hepatocellular carcinoma categorized by age-adjusted incidence rates ***(White et al., 2010)***.

Hepatocellular Carcinoma is a serious if not the most serious cancer problem in Egypt. It is ranked first among cancers in males (33.6%) and next to breast cancer among females the incidence of breast cancer was (32.0%) followed by liver cancer (13.5%) based upon results of National Cancer Registry Program ***(NCRP 2008-2011)***. The rising rates of HCC in Egypt are due to the high prevalence of hepatitis B virus (HBV) and hepatitis C virus infection (HCV) among Egyptian population. There is a shift in the relative influence of these viruses in HCC etiology in Egypt, as HBV infection significantly decreased while HCV did not. The role of exposure to aflatoxin in Egypt may also contribute to the development of HCC ***(Abdelgawad et al., 2013)***.

B- Risk Factors of HCC:

1. Personal History:

a) Gender:

In general, HCC is two to four times more common in men than in women. It can be suggested that sex hormones including progesterone may play some roles in HCC ***(Yeh et al., 2013)***.

b) Age

Hepatocellular carcinoma rarely occurs before the age of 40 years and reaches a peak at approximately 70 years of age ***(El-Serag, 2011).***

c) Race:

HCC incidence rates also vary greatly among different populations living in the same region ***(Kuntz and Kuntz, 2006).***

2. Family History:

Familial predisposition of hepatocellular carcinoma has been reported due to the high prevalence of chronic infection with HBV and the vertical transmission of HBV as a major source for viral transmission among Asians ***(Hassan et al., 2009)***.

3. Liver cirrhosis:

Cirrhosis is the end stage of many chronic live r diseases characterized by loss of lobular architecture ***(Stacy et al., 2004)***. Cirrhosis of any etiology is the major risk factor for HCC. About 80% of patients with newly diagnosed HCC have preexisting cirrhosis ***(Tsai and Chang, 2010)***. Cirrhosis increases the susceptibility of the liver to malignant change. The normal liver is mitotically inactive. However, when stimulated to divide rapidly, this prevents the repair of any damage to DNA ***(Gomaa et al., 2009).***

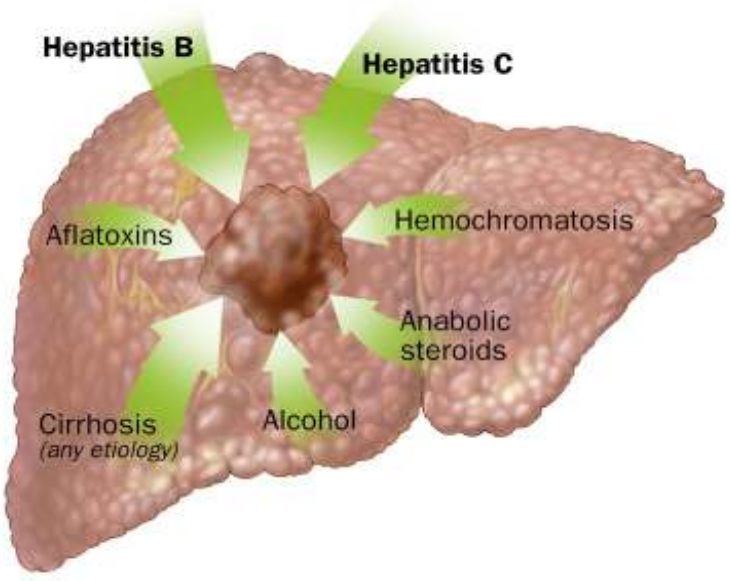

Figure (2): Multiple risk factors of Hepatocellular Carcinoma ***(Barghini et al., 2013).***

4. Hepatic viral infection:

a) Chronic hepatitis B virus infection:

Globally, HBV is the most frequent underlying cause of HCC with an estimated 300 million persons with chronic infection worldwide and accounting for 50% to 55% of all cases. Case–control studies have demonstrated that chronic HBV carriers have a 5 to 15 fold increased risk of HCC compared to the general population ***(Kew et al., 2008)***, Meanwhile, after the initiation of HBV vaccination, significant declines in the incidence of HCC have been documented ***(Zanetti et al., 2008)***.

b) Chronic hepatitis C virus infection:

Hepatitis C-related liver cirrhosis is also a risk factor for HCC. Worldwide, 25% to 30% of cases of HCC can currently be attributed to hepatitis C virus infection ***(Malek et al., 2014)***.

HCV is the most common cause of HCC in the USA, Europe, Japan and Egypt accounting for 48%, 56%, 75% and 94% of cases, respectively ***(Tatsuo et al., 2013 and Motawi et al., 2015).***

The exact mechanism of hepato-carcinogenesis in patients with HCV-related cirrhosis is unknown; however, chronic inflammation, necrosis and regeneration of hepatocytes in chronic liver disease might be involved in the development of HCC ***(van der Poorten et al., 2010).***

Chronic HCV infection is characterized by a silent onset in most infected individuals, a high rate of viral persistence, and the potential for development of worsening chronic liver disease ranging from chronic hepatitis to cirrhosis and occasionally to HCC ***(Castello et al., 2010).***

c) Co-infection of HBV and hepatitis D virus:

Hepatitis D virus (HDV) co-infection with HBV is associated with increased risk for HCC ***(Gomaa et al., 2008)***.

5. Schistosomiasis:

In Egypt, Schistosomiasis is a major public health problem and infection with Schistosoma Mansoni constitutes the major cause of liver disease ***(Strickland, 2006).***

6. Inherited liver disease:

HCC accounts for about 3% of all iron-related deaths in hemochromatosis ***(Allen, 2008).***

7. Environmental Risk Factors:

a) Alcohol intake:

Strong evidence suggests that the immune cells of the liver play a crucial role in alcoholic liver disease including steatosis, hepatitis, fibrosis and HCC development ***(Odile et al., 2012).***

b) Tobacco:

Smoking contributes to the induction and progression of HCC in both high and low risk geographical areas ***(Koh et al., 2011)***.

c) Aflatoxin exposure:

Hepatocellular carcinoma is associated with dietary exposure to aflatoxin in some regions of the world, where

fungal contamination of grain is common ***(Groopman and Wogan, 2011).***

C- Pathogenesis of HCC:

Hepatocarcinogenesis is a multistep process involving different genetic alterations that ultimately lead to malignant transformation of the hepatocyte ***(Ahmad and Rabinovitz, 2007).*** Genetic and molecular abnormalities generally associated with viral infection or due to the inflammatory condition represent an early step in hepatocarcinogenesis ***(Su et al., 2007)***

1. Disturbed Cell Cycle Regulation:

Cell cycle regulators include; multiple cell cyclins, cyclin-dependent kinases (Cdks) and cyclin-dependent kinase inhibitors which control the progression of eukaryotic cells through the division cycle. Disruption of the Gl/ S and G2/ M check points lead to uncontrolled cell growth, resulting in the development and progression of HCC ***(Braicu et al., 2009).***

Disturbed cell cycle regulation may be due to genetic or epigenetic alteration

- **Genetic alteration:**

  - ***Oncogenes and their receptors:***

Proto-oncogenes encode a wide range of protein products involved in the control of cell proliferation and differentiation including growth factors, growth factors' receptors, components of signal transduction pathways and transcription factors ***(Cui et al., 2001).*** The process of activation of proto-oncogenes to oncogenes including mutations or increased expression is identified in HCC and associated with poor prognosis ***(Anwar et al., 2008).***

  - ***Tumor suppressor genes:***

Tumor suppressor genes represent genes that are likely to negatively regulate cell growth. Loss or in- activation of these genes are associated with malignancy and carcinogenesis process. Apart from deletions and mutations, growing evidence has indicated that epigenetic alterations are implicated in inactivation of tumor suppressor genes ***(Munakata et al., 2007).***

- **Epigenetic alterations:**

Epigenetics alteration pathways refer to heritable changes in gene expression that occur without alteration in DNA sequence ***(Egger et al., 2004).*** It may be a consequence of the normal aging process, persistent viral infection and chronic inflammation. The epigenetic pathways of alteration

are characterized by these main mechanisms; DNA hypermethylation leading to gene inactivation, DNA hypomethylation causing genomic instability and histone modifications affecting chromatin conformation ***(Tischoff and Tannapfe, 2008).***

2. Disturbed Apoptosis:

Apoptosis is a highly regulated form of programmed cell death defined by distinct morphological and biochemical features. Apoptosis is a key mechanism causing cell death. Failure of apoptosis is understood to contribute to the development of HCC ***(Guo et al., 2002).***

3. Increased Angiogenesis:

Physiological angiogenesis occurs during liver regeneration leading to the formation of a new blood vessel from pre-existing vasculature; meanwhile pathological angiogenesis occurs in HCC ***(Zhu and Raymond, 2009).***

Hepatocellular carcinoma is typically a highly vascular tumor characterized by neovascularization. The development of neovasculature in the tumor is essential for the growth and metastasis of a cancer ***(Pang and Poon, 2006).***

D- Staging Systems of Hepatocellular Carcinoma:

The goal of tumor staging is to separate patients into different groups based on their predicted survival to determine the most appropriate treatment modality ***(Grieco et al., 2005)***.

1- Okuda Staging System:

It has been widely applied in HCC patients in the last decade. This classification properly stratifies patients when most of them are diagnosed at an advanced/ symptomatic stage (Table 1). Nowadays, the disease is diagnosed at an earlier stage and thus, this classification is not adequate to stratify patients prior to radical or palliative therapies ***(Marrero et al., 2010).***

Table (1): Okuda Staging System

Staging Criteria	Positive	Negative
Tumor size (percentage of liver volume)	>50%	<50%
Ascites	Detectable	Absent
Serum albumin	<3g/dL	>3g/dL
Serum bilirubin	>3mg/dL	<3mg/dL

Stage I: No positive

Stage II: One or two positive

Stage III: 3 or 4 positive.

(Johnson, 2000)