

*Circulating mRNA of Lamin B1 for Detection
of Early Stages of Hepatocellular Carcinoma*

Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا
إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ
الْعَلِيمُ الْحَكِيمُ

صدق الله العظيم



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List of Abbreviations

2DE	Two dimensional electrophoresis
3D	Three dimension
ADLD	Adult-Onset Autosomal Dominant Leukodystrophy
AFP	Alpha fetoprotein
AFU	Alpha-L-fucosidase
ALT	Alanine amino transferase
AST	Aspartate aminotransferase
AUC	Area under curve
BCG	Bromocresol green
BCLC	Barcelona-Clinic Liver Cancer staging system
CA125	Carbohydrate antigen 125
CBC	Complete blood count
cDNA	Complementary DNA
CEA	Carcinoembryonic antigen
CLD	Chronic liver disease
CT	Computerized tomography
C_t	Cycle threshold
DCP	Des-gamma-carboxyprothrombin
DNA	Deoxynucleic acid
dNTP	Deoxyribose nucleotide triphosphate
EASL	European association for the study of the liver
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme linked immunosorbent assay
FN	False negative
FP	False positive

List of Abbreviations

GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDNA	Genomic DNA
GGT m RNA	Gamma glutamyl transferase
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HGF	Hepatocyte growth factor
HP1	heterochromatin protein 1
HRP	Horse radish peroxidase
hTERT mRNA	Human telomerase reverse transcriptase mRNA
IF	Intermediate filament
Ig	Immunoglobulin
IGFII mRNA	Insulin- like growth factor II-mRNA
INM	Inner nuclear membrane
INR	International normalized ratio
IQR	Interquartile range
LBR	Lamin B receptor
LDH	Lactate dehydrogenase
MALDI-TOF	Matrix-assisted laser desorption / ionization Time of flight mass spectrometry
MAPK	Mitogen activated protein kinase
MDH	Malate dehydrogenase
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid

List of Abbreviations

MS	Mass spectrometry
NAD	Nicotinamide adenine dinucleotide
NE	Nuclear envelope
NPC	Nuclear pore complexes
NPV	Negative predictive value
OCT1	Octamer 1 transcription factor
ONM	Outer nuclear membrane
OS	Oxidative stress
P5P	Pyridoxil 5 phosphate
PKCa	Protein kinase C alpha
PPV	Positive predictive value
PST	Performance status test
PT	Prothrombin time
ROC	Receiver operating characteristic curve
RT-PCR	Reverse transcription polymerase chain reaction
SCCA	Squamous cell carcinoma antigen
SDS	Sodium deodecyl sulphate
TGFB1	Transforming growth factor- β 1
TN	True negative
TNM	Primary tumor, lymphnode, metastasis staging system
TP	True positive
US	Ultrasonography
VEGF	Vascular endothelial growth factor

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of death worldwide, causing 600,000 deaths annually (*Terry and Copur, 2013*). In Egypt, HCC accounts for 4.7% of all liver diseases. Hospital-based studies have reported an overall increase in the relative frequency of all liver cancers in Egypt from approximately 4% in 1993 to 7.3% in 2003, more than 95% of which being HCC (*El-Garem et al., 2013*).

In an attempt to study the trend of HCC in Egypt and the possible associating risk factors, *El-Zayadi et al. (2005)* found that 86.9% and 28.4% of the studied HCC cases were positive for hepatitis C virus (HCV) antibodies and hepatitis B surface antigen (HBsAg) respectively. Indeed, liver cirrhosis is the major risk factor for HCC worldwide and is mainly due to chronic HCV or HBV infection and alcohol intake (*Gomaa et al., 2009*).

Patients diagnosed at an early stage of HCC have the best prognosis, where resection and transplantation achieve the best outcomes in well-selected candidates, with a 5-year-survival rate of 50 to 70% (*Llovet et al., 2004*). Unfortunately, only 10-20% of primary HCCs are

resectable at the time of diagnosis, therefore, surveillance, currently based on abdominal ultrasound (US) and serum alphafetoprotein (AFP), is recommended for all cirrhotic patients and other specific risk groups every 6 months. AFP is currently considered ‘the golden standard’ of serum markers for HCC. However, the usefulness of AFP testing for the population at risk is seriously questioned. It has shown poor diagnostic values for early HCC. Applying a cut-off value $> 100 \mu\text{g/L}$, its sensitivity ranges from 20%-30% for potentially resectable tumors of less than 3 cm in diameter. Moreover, the AFP cut-off level for the diagnosis of HCC is still a subject of debate. Assay of lens culinaris-reactive AFP, which is also known as AFP-L3, it is the main glycoform of AFP in the serum of HCC patients increased the sensitivity of AFP to $>50\%$ at the cut-off level of 15% of total AFP, yet it is not routinely measured (*Gomaa et al., 2009 and Sun et al., 2010*).

Currently, US represents the primary radiologic tool for HCC surveillance of at-risk populations. However, US has limited sensitivity ($<60\%$) to detect small HCC especially in obese patients and those with underlying cirrhosis. The gold standard test for diagnosis of HCC is spiral computerized tomography (C.T.) which has advanced sensitivity and specificity over US. Unfortunately, its use in HCC surveillances have been limited, since the diagnostic