Significance of Angiopoietin-2 as a Serum Marker for Hepatocellular Carcinoma

Thesis
Submitted for Fulfillment of the Master Degree in Tropical Medicine
Faculty of Medicine
Cairo University

By

Ahmad Mohamed Rashad Mashaal
(M.B., B.Ch. Cairo University)

Supervisors

Maha Sayed Hasab-Allah, MD
Assistant Professor of Tropical Medicine
Faculty of Medicine
Cairo University

Mahmoud Ahmad El-Ansary, MD
Assistant Professor of Tropical Medicine
Theodor Bilharz Research Institute

Shereen Shoukry Hunter, MD
Lecturer of Tropical Medicine
Faculty of Medicine
Cairo University

FACULTY OF MEDICINE
CAIRO UNIVERSITY
(2009)

ACKNOWLEDGMENT

"First and Foremost, Thanks are Due to GOD,

The Beneficent and Merciful of All"

It was an honor to work under the supervision of eminent professors, who gave me their whole hearted support and immense facilities as is their usual with their juniors. I owe them more than words can say.

I would like to express my deepest gratitude and sincere thanks to ***Dr. Maha Sayed Hasab-Allah,*** *Assistant professor of Tropical Medicine, Faculty of Medicine, Cairo University, for her instructive supervision, continuous guidance, valuable instructions and for offering all facilities for the completion of this work.*

My thanks and appreciation to ***Dr. Mahmoud Ahmed El-Ansary,*** *Assistant professor of Tropical Medicine, Theodor Bilharz Research Institute, for his strict supervision and revision of the work.*

Many thanks to ***Dr. Shereen Shoukry Hunter,*** *Lecturer of Tropical Medicine, Faculty of Medicine, Cairo University, for her continuous help, valuable suggestions, guidance and encouragement during the progress of this work. I would like to thank her for the meticulous and patient revision of the work.*

Whatever I say or write, I will never be able to express my deep feelings and profound gratitude to ***Prof. Dr. Mona Hassan,*** *Professor and Head of Clinical Chemistry Department, Theodor Bilharz Research Institute for planning the design of the work, devoting her intellectual energies and high sense of profession in the entire development of this*

thesis. Without her creative thinking, valuable suggestions, the completion of this work would have been difficult.

Many thanks to ***Dr. Faten Tharwat El-Shanaway,*** *Assistant Professor of Clinical Chemistry, Theodor Bilharz Research Institute, for offering all facilities for the laboratory work, standardization and interpretation of the results.*

I am extremely grateful to ***Dr. Tarek Mahmoud Diab****, Assistant Professor of Parasitology, Theodor Bilharz Research Institute, for his unlimited help in the statistical analysis of the data; he gave me much of his time advice and effort throughout this work.*

Finally, no words can express my deepest appreciation and gratitude to my family for their never ending support and care.

Ahmad Rashad Mashaal
2009

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and it is one of the major causes of death. HCC is now a rather common malignancy in Egypt which usually develops on top of liver cirrhosis secondary to viral infection.

AIM: The aim of this study was to investigate the potential role of Angiopoietin-2 (Ang-2) as a diagnostic non-invasive marker for HCC, in order to add a beneficial diagnostic value in patients with low levels of alpha-fetoprotein (AFP) and suspected to have HCC.

METHODS: This study was conducted on **30** patients with documented HCC using triphasic computerized tomography (CT) scan and histopathological assessment (when needed) and **30** cirrhotic patients with no evidence of HCC; as well as **30** healthy subjects who served as control group.

We determined the level of AFP and Ang-2 for all cases together with full clinical assessment, liver biochemical profile, viral markers, conventional ultrasound (US), abdominal triphasic CT scan and guided liver biopsy for HCC cases with atypical CT pattern.

RESULTS: We found that the diagnostic sensitivity of AFP at a cutoff of**200** ng/ml was **24%** and the specificity was **100%**. The cutoff level of Ang-2 for diagnosis of HCC in this study was **8100** pg/ml, with a sensitivity and specificity of **70%** and **80%** respectively. Serum Ang-2 was significantly elevated in the HCC group than cirrhosis and control groups and in HCC patients with portal vein thrombosis than those without. There was a significant positive correlation between the number of hepatic focal lesions and the serum level of Ang-2. The combined use of the two markers (AFP & Ang-2) led to an increase in the sensitivity of AFP from **53.3%** to **83.3%.**

CONCLUSION: Serum Ang-2 is elevated in patients with cirrhosis and further elevated in patients with HCC, so its use as an independent tumor marker in the diagnosis of HCC is to be considered. Simultaneous measurement of serum AFP and Ang-2 may enhance the sensitivity of HCC detection.

Key words:

- Hepatocellular carcinoma (HCC)
- Alpha-fetoprotein (AFP)
- Angiopoietin-2 (Ang-2)

LIST OF CONTENTS

LIST OF ABBREVIATIONS

AASLD	American Association for the Study of Liver Disease
Ab	Antibody
AFB1	Aflatoxin B1
AFP	Alpha fetoprotein
Ag	Antigen
AIH	Autoimmune hepatitis
AJCC	American Joint Committee on Cancer
ALP	Alkaline phosphatase
ALT	Alanine transaminase
Ang-1	Angiopoietin-1
Ang-2	Angiopoietin-2
AR	Acyclic retinoid
AST	Aspartate transaminase
BCLC	Barcelona Clinic Liver Cancer
bFGF	basic fibroblast growth factor
CEA	Carcinoembryonic antigen
CLD	Chronic liver disease
CLIP	Cancer of the Liver Italian Program
CRG –L2	Cancer related gene-Liver 2
CT	Computerized tomography
CTA	Computerized tomography angiography
CTAP	Computerized tomography angioportography
CTHA	Computerized tomography hepatic angiography
DCP	Des-γ-carboxy prothrombin
EGF	Endothelial growth factor
ELISA	Enzyme linked immune sorbent assay
EMEA	European Medicine agency
ETs	Endothelins
FDA	Food & Drug Administration
FLC	Fibrolamellar carcinoma
GGT	Gamma glutamyl transpeptidase
GPC3	Glypican-3
GST-π	Glutathione-S-transferase-π

HAP	Hepatic arterial phase
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HFLs	Hepatic focal lesions
HGF	Hepatocyte growth factor
HGV	Hepatitis G virus
HIF	Hypoxia inducible factor
HIFU	High intensity focused ultrasound
IFN-α	Interferon-α
IGF-β	Insulin-like growth factor β
ILP	Interstitial laser photocoagulation
INR	International normalization ratio
IRS-1	Intracellular receptor substrate-1
LC	Liver cirrhosis
LCA	Lens-culinaris agglutinins
LDH	Lactic dehydrogenase
LH	Local hyperthermia
LTAE	Lipiodol trans arterial embolization
LTx	Liver transplantation
M	Mean
MRI	Magnetic resonance imaging
msAFP	Monosialyted alpha fetoprotein
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
PAI	Percutaneous acetic acid injection
PBC	Primary biliary cirrhosis
PC	Prothrombin concentration
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PEI	Percutaneous ethanol injection
PET	Positron emission tomography
PIVKA II	Prothrombin induced by vitamin K absence II
PLGF	Placental growth factor
PLTs	Platelets

PSI	Percutaneous hot saline injection
PT	Prothrombin time
PVP	Portal venous phase
RFA	Radiofrequency ablation
ROC	Receiver operating curve
SBP	Spontaneous bacterial peritonitis
SD	Standard deviation
TACE	Trans arterial chemoembolization
TAE	Trans arterial embolization
TGF	Transforming growth factor
THI	Tissue harmonic imaging
TKRs	Tyrosine kinase receptors
TNM	Tumor- nodes-metastasis
TSH	Thyroid stimulating hormone
UCSF	University of California at San Francisco
US	Ultrasound
VEGF	Vascular endothelial growth factor

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in men and the eighth in women ***(Parkin et al., 1999).*** HCC commonly develops in cirrhotic livers whatever the etiology, so that liver cirrhosis by itself represents the strongest risk factor ***(Colombo, 1999 & Giannelli et al., 2002).***

Both hepatitis C virus (HCV) and hepatitis B virus (HBV) infections increased the risk of HCC in Egyptian patients ***(Badawy & Micheal, 1999).*** Over a decade (1993-2002), there was nearly a twofold increase of the proportion of HCC among chronic liver disease (CLD) patients in Egypt with a significant decline of HBV and slight increase of HCV as risk factors ***(El-Zayadi et al., 2005)***.

Many surveillance programs aimed at detecting early stage HCC have been widely recommended. These programs are based on the use of ultrasound and alphafetoprotein (AFP). The reliability of imaging techniques has greatly improved in the last years but such diagnostic procedures are expensive and subject to mis-interpretation. On the other hand, the only diagnostic serologic test currently available in clinical practice is AFP. Receiver operating curve (ROC) analysis of AFP suggests that a value of about 20 ng/ml provides the optimal balance between sensitivity and specificity**.** However, at this level the sensitivity is only 60%, i.e., AFP surveillance would miss 40% of HCC if a value of 20 ng/ml is used as the trigger for further investigation. This is inadequately sensitive for general use. If a higher cut-off is used a progressively smaller proportion of HCCs will be detected. If the AFP cut-off is raised to, e.g., 200ng/ml the sensitivity drops to 22% ***(Trevisani et al., 2001).*** An elevated concentration may be

found in certain benign liver diseases. Furthermore, 20% to 30% of patients with HCC had a negative AFP result, while some cases of chronic hepatitis and cirrhosis showed a positive AFP result **(*Chen & Sung, 1997).*** In addition, the serum AFP level does not always correspond to the clinical stage of HCC ***(Nakagawa et al., 1999).*** Thus, AFP appears to have limited utility as a screening test **(*Bruix & Sherman, 2005).***

It is to be mentioned that the mortality rate due to HCC has not improved over the last 20 years. This is in part due to the poor performance of available tumor markers leading to delay in diagnosis. Therefore, new and more specific markers for HCC are critically needed ***(El-Serag & Manson, 1999).***

Angiogenesis is the process of formation of new capillaries from preexisting blood vessels ***(Risau, 1997)***, it represents an essential component of embryogenesis, normal physiological growth, repair, and tumor expansion. Although a variety of factors can modulate endothelial cell responses in vitro and blood vessel growth in vivo, only vascular endothelial growth factor (VEGF) family members and the angiopoietins are believed to act almost exclusively on vascular endothelium ***(Loughna & Sato, 2001).***

Angiopoietins 1 and 2 (Ang1 and Ang2) were originally identified in tissue culture experiments as agonist and antagonist, respectively. Ang1 was shown to support endothelial cells survival and to promote endothelium integrity, whereas Ang2 (a 66 kDa protein consisting of 496 amino acid residues) had the opposite effect promoting blood vessel destabilization and regression ***(Maisonpierre et al., 1997)***.

Induction of Ang-2 was proposed to be a survival signal to thwart the tumor's growth process. However, VEGF up regulation coincident with Ang-2 expression induces angiogenesis at the margins of the tumor,

allowing the tumor to thrive. Late expression of tumor-derived VEGF may serve to repress the signal for vessel regression by Ang-2, which is consistent with the observation that VEGF is necessary for promoting tumor vessel survival ***(Holash et al., 1999).***

High serum Ang-2 values are found in patients with inflammatory conditions as chronic HCV infection ***(Salcedo et al., 2005)***, inflammatory bowel disease ***(Koutroubakis et al., 2006)*** & sepsis ***(Parikh et al., 2006);*** also production of Ang-2 has been implicated in tumor development in human gastric cancers ***(Etoh et al., 2001)***, human prostate carcinoma ***(Wurmbach et al., 2000)***, and human breast cancers ***(Currie et al., 2001).***

Ang-2 is overexpressed in HCC-as measured by immunohistochemistry-especially of the highly vascular type ***(Sugimachi et al., 2003);*** Ang-2 expression is associated with portal infiltration, microvessel density, recurrence of HCC & decreased survival ***(Wada et al., 2006)***. Recent studies reported high serum Ang-2 values in patients with HCC suggesting that Ang-2 might represent a useful marker for HCC and a complementary diagnostic tool ***(Scholz et al., 2007).***