

Expression of Hepatocyte Paraffin 1 antibody and Vascular Endothelial Growth Factor in Hepatocellular Carcinoma and Chronic Viral Hepatitis: An Immunohistochemical study

Thesis

Submitted for partial fulfillment of M.D degree in Tropical Medicine

By

Rabab Maamoun Salama

M.B.B.Ch , M.Sc.

Supervised

By

Soheir Zakaria Mohamed Eissa, M.D

Professor of Tropical Medicine

Cairo University

Nabil Mostafa Al Kadi, M.D

Professor of Tropical Medicine

Cairo University

Maha Mahmoud Akl , Ph.D

Professor of Pathology and head of clinical laboratories

Theodor Bilharz Research Institute

Mayssa EL- Said El-Raziky, M.D

Assistant Professor of tropical medicine

Cairo University

Faculty of Medicine

Cairo University

2007

ACKNOWLEDGEMENT

I would like to start by thanking GOD for his help during this work as a little part of his generous help throughout my life. It is nevertheless my privilege and honor to express my most sincere gratitude to all those who helped me in this work, in particular, my supervisors:

***Prof. Dr. Soheir Zakaria**, Professor of tropical medicine Cairo university, whatever I will say, my words will still stand short of my supreme gratitude for her, she always displayed a high degree of professionalism that is hard to emulate but such a privilege to follow. She is truly a rare example of excellence. She had, with her motherly attitude, set the plan, followed the steps of the work, critically discussed the results and taught me the scientific attitude. To her therefore I express my deep sense of gratitude.*

*My profound appreciation goes to **Prof. Dr. Nabeel El Kady** professor of Tropical Medicine, Cairo University, for his honest supervision, meticulous advice at all times and the ceaseless effort he expended with me throughout this study. No words can be sufficient to express my gratitude to him.*

*I wish to express my profound gratitude and sincere appreciation to **Prof. Dr. Maha Akl** professor of pathology and head of the clinical laboratories, Theodor Bilharz for her unlimited effort and time and her precious understanding she freely gave during the course of this study. No words can express my indebt to her.*

*I would like to express my sincere thanks and deepest gratitude to **Prof. Dr. Maissa El Raziky**, Assistant Professor of Tropical Medicine, Cairo University, who gave me much of the time experience,*

valuable directions, constant advice, guidance and kindness all through the work until its completion, so no words can express my gratitude to her.

*My profound appreciation goes to **Prof. Dr. Olfat Hammam**, assistant Professor of pathology, Theodor Bilharz, by whom, I was very much impressed by the noble characters and generous attitude, she gave me much of her time, sincere guidance and her technical help throughout the whole work. To her therefore I express my deep sense of gratitude which she will find ever lasting.*

*Special thanks to **Dr. Shereen Hunter** lecturer of Tropical Medicine Cairo University and **Dr. Motaz Seyam** Assistant professor of Tropical Medicine, Theodor Bilharz for their continuous help and support throughout this work.*

*I am greatly honored to express my deepest gratitude and faithfulness to **Prof. Dr. Serag Zakaria**, Head of the Tropical Medicine department and all the staff members for their generous attitude and kind help.*

I wish to thank all my colleagues in the Tropical Medicine for their continuous help and kind sympathy.

My deepest appreciation and no words can express my gratitude to my small family and my parents for their never ending support and care. Finally I would like to thank the patients, hoping that my research might help in alleviating their pains and suffering.

ABSTRACT

The prognosis of **HCC** patients is generally very poor, that's why early detection of patients with **HCC** became attractive and beneficial.

HCC is generally well known to be extensively vascularized and the occurrence of primary intrahepatic and lung metastases suggests its mainly hematogenous dissemination. Therefore, it is possible that angiogenesis plays a pivotal role during hepatocellular carcinogenesis.

VEGF expression is significantly associated with a higher proliferative index and may characterize progression towards higher proliferation in hepatocarcinogenesis. Using **immunohistochemistry**, the expression of **VEGF** is much stronger in tumor cells compared with hepatocytes in normal or cirrhotic liver and high expression of VEGF, especially under hypoxic conditions was reported in liver cirrhosis and viral hepatitis infection, which both predispose individuals to hepatocarcinogenesis.

Hep Par 1 antibody has been reported to be a sensitive marker for HCC in paraffin embedded sections; its expression is confined primarily to benign and malignant hepatocytes, it was shown to be up to 90% specific for hepatocytes in histologic specimens and is useful for differentiating HCC from liver metastases.

The aim of our study is to assess the expression of hepatocyte paraffin 1(**Hep Par 1**) and vascular endothelial growth factor (**VEGF**) in liver biopsy specimens of patients having hepatocellular carcinoma compared with patients having chronic viral hepatitis (**B or C**) and liver cirrhosis to study their clinical significance as possible markers for early prediction of malignancy in chronic viral hepatitis and liver cirrhosis patients.

The study was conducted on 20 **CH**, 30 **LC**, 30 **HCC** patients and 10 **metastatic** patients considered as control. They were subjected to liver biochemical profile, viral markers, abdominal US, together with immunohistochemical studying of their liver biopsies using **VEGF** and **Hep par 1**

We concluded that **VEGF** is intense (>75%) in **cirrhotic** and **HCC** patients, and **chronic hepatitis patients** can be considered to be predisposed to malignancy, if they have intense expression of **VEGF**. **VEGF** expression is less intense in **liver metastases** denoting that angiogenesis may be less in liver metastases than the original tumor.

Diffuse expression of **Hep Par 1** in chronic hepatitis and cirrhotic patients can predict their predisposition to malignancy, as most of **HCC** patients exhibit diffuse Hep par 1 expression. **Hep Par 1** plays an important role in the differentiation between **HCC** and **liver metastases** being negatively expressed in all metastatic patients.

Key words:

- **Hepatocellular carcinoma (HCC).**
- **Liver cirrhosis (LC).**
- **Chronic hepatitis (CH).**
- **Vascular endothelial growth factor (VEGF).**
- **Hepatocyte Paraffin antibody 1 (Hep par 1)**
- **Immunohistochemistry.**

TABLE OF CONTENTS

INTRODUCTION.....	1 -6
Review of literature	
<u>PART I: HCC and hepatocarcinogenesis:</u>	
<u>Introduction</u>	7
Age, Race and Sex.....	8
Risk factors of hepatocellular carcinoma.....	9
Chronic viral hepatitis B and C and liver cirrhosis.....	10
Chronic viral hepatitis B.....	11
Chronic viral hepatitis C	12
Liver cirrhosis.....	13
Mortality of HCC	14
<u>Hepatocarcinogenesis</u>	15
Sequential morphological changes in the liver leading to HCC.....	20
Role of viral Hepatitis and liver cirrhosis in hepatocarcinogenesis and the related molecular alterations	21
Hepatitis B Infection and Hepatocarcinogenesis.....	22
HBV Mechanisms of Hepatocarcinogenesis.....	22
Hepatitis C infection and Hepatocarcinogenesis.....	28
HCV Proteins and Hepatocarcinogenesis.....	29
Cirrhosis and Hepatocellular Carcinoma.....	31
<u>PART II: Diagnosis of HCC:</u>	
<u>CLINICAL DIAGNOSIS OF HCC</u>	35
Symptoms	35
Signs	36

Complications	37
Spontaneous rupture of HCC.....	37
Para neoplastic syndromes.....	37
<u>Methods of tumor detection</u>	39
Blood Chemistry.....	39
Serological tumor markers.....	40
Imaging diagnostic procedures	50
Plain X-Ray	50
Ultrasonography.....	50
Gray-scale ultrasonography.....	50
Basic Ultrasound patterns of solid mass lesions.....	50
Doppler ultrasound.....	52
Tissue Harmonic imaging.....	54
Three dimensional Ultrasound technology and Doppler.....	55
Four Dimensional US.....	56
Ultrasound Guided Fine Needle biopsy and Fine Needle Aspiration of Hepatic Focal Lesions	57
.....	
Fine needle biopsy.....	57
Fine needle aspiration.....	58
Computed tomography	59
Non-contrast enhanced CT scan	60
Contrast –enhanced CT scan.....	60
Dual-phase CECT (Spiral CT).....	61
Three-dimensional CT	63
Angiography assisted CT.....	63

Magnetic resonance imaging in HCC.....	64
Radio nucleotide screening.....	66
Positron Emission Tomography (PET).....	66
<u>Pathological diagnosis</u>.....	66
Pathology of Hepatocellular carcinoma.....	66
GROSS PATHOLOGY	66
MICROSCOPIC PATHOLOGY.....	68
HISTOLOGIC PATTERN OF HCC	68
CYTOLOGICAL VARIATIONS.....	69
Degree of differentiation and grading of HCC	70
Edmondson and Steiner's classification of HCC (1954).....	70
Sugihare et al. (1992) classification	70
Staging and scoring of HCC.....	73
AJCC STAGING SYSTEM FOR HCC	74
The Okuda staging system.....	75
CLIP scoring system	76
Tokyo scoring system.....	76
<u>SCREENING FOR HCC</u>	77
Groups of Patients Suitable for HCC Screening.....	81

Part III: IMMUNOHISTOCHEMISTRY IN CHRONIC LIVER

DISEASES:

<u>Immunohistochemistry</u>	82
Immunohistochemical protocol.....	84
<u>Pathology and immunohistochemistry of chronic viral hepatitis (B & C) and post hepatitis liver cirrhosis</u>	90
Pathology of chronic viral hepatitis (B&C) and post hepatitis liver cirrhosis.....	90
Staging, Grading, and Scoring of Liver Biopsies in Chronic Viral Hepatitis.....	90
Fibrosis and Fibrotic Progression.....	91
Inflammation.....	94
Steatosis and Steatohepatitis.....	97
Pathology of liver cirrhosis.....	98
Immunohistochemistry of chronic viral hepatitis B and C and cirrhosis for prediction of HCC.....	99
Hepatic Angiogenesis in chronic viral hepatitis (B&C) and post hepatitis liver cirrhosis.....	103
Molecular Insights into the Angiogenic Process.....	104
Angiogenesis in Chronic Inflammatory Liver Injury.....	109
Role of VEGF in hepatic angiogenesis.....	113
Immunohistochemical studying of VEGF in chronic viral hepatitis (B & C) and post hepatitis liver cirrhosis.....	118
<u>Immunohistochemistry of hepatocellular carcinoma</u>	119
Immunohistochemical markers of HCC.....	121

VEGF role in hepatic angiogenesis and tumor growth in HCC.....	122
Immunohistochemical studying of VEGF in HCC.....	128
Immunohistochemical studying of Hepatocyte paraffin 1 antibody in HCC.....	130
Immunohistochemical studying of VEGF and Hep Par 1 in liver metastases.....	133
VEGF role in angiogenesis in liver metastases.....	133
Hep par 1 in liver metastases.....	135
Patients and Methods.....	137
Results.....	146
Discussion.....	219
Summary and Conclusions.....	232
Recommendations	236
References.....	237

LIST OF ABBREVIATIONS

ABC	Avidin –Biotin complex.
ADH3	Alcohol dehydrogenase.
a FGF	Acidic fibroblast growth factor.
AFP	Alpha fetoprotein.
AFU	Alpha-1 fucosidase.
AJCC	The current American Joint Committee on Cancer.
ALK-5	Anaplastic lymphoma kinase (Ki-1).
Ang-1	Angiopoeitin-1.
ANGPTL3	Angiopoeitin like 3.
AP	Alkaline phosphatase(Calf intestinal).
BAX	BCL-2 associated X protein.
BCLC	Barcelona clinic liver cancer.
b FGF	Basic fibroblast growth factor.
BRCA2	Breast cancer gene 2.
CC	Cholangiocarcinoma.
CLIP	Cancer of the liver Italian program.
CECT	Contrast enhanced computed tomography.
CK	Cytokeratin.
C-Kit	Stem cell factor receptor.
COX-2	Cyclooxygenase 2.
CTAP	Computed tomography angio-portography.
CTHA	Computed tomography hepatic angiography.
DAB	Diaminobenzidine substrate.
DGCP	Des gamma carboxy prothrombin.
DPX	1,3-diethyl-8-phenylxanthine
DSA	Digital subtraction angiography.
2D US	Two dimension ultrasonography.
3D US	Three dimension ultrasonography.
4D US	Four dimension ultrasonography.
EASL	European association of study of liver diseases.
EC	Endothelial cell.

ECM	Extracellular matrix component.
ET	Endothelin.
FAS	TNF receptor superfamily member 6.
FGF	Fibroblast growth factor.
FLK-1	Fetal liver kinase1.
FLT-1	Fms-like tyrosine kinase receptor1.
18F-FDG	18 fluoro-deoxy-glucose.
FITC	Fluorescein Isothiocyanate.
FNH	Focal nodular hyperplasia.
GADD 45	Growth arrest and DNA damage gene.
GPC3	Glypican 3.
GST-II	Glutathione S transferase II.
HAC	Hepatoid adenocarcinoma.
HAI	Histological activity index.
HAP	Hepatic arterial phase.
HBc Ag	Hepatitis B core antigen.
HBe Ag	Hepatitis B e antigen.
HBs Ag	Hepatitis B virus surface antigen.
HBx	Hepatitis B x gene.
HGF	Hepatocyte growth factor.
Hep par I	Hepatocyte paraffin I.
HLA-DR	Human leukocyte antigen.
hMLH1	Human mutL homolog 1.
HME	Human macrophage metalloelastase.
hMSH2	Human mutS homolog 2.
HPA	Hepatocyte antigen.
HRP	Horse radish peroxidase enzyme.
hTERT	Human telomerase reverse transcriptase.
ICAM-1	Intercellular adhesion molecule 1.
IEF	Iso electric focusing.
IGF II	Insulin like growth factor.
IGF II r	Insulin like growth factor 2 receptor.
IGF BP I	Insulin like growth factor binding protein one.

IGF BP 3	Insulin like growth factor binding protein three.
IHC	Immunohistochemistry.
ISH	In Situ hybridization.
IV	Intravenous.
KDR	Kinase insert domain receptor.
Ki 67	Monoclonal antibody Ki 67.
LAB	Labeled Avidin –Biotin.
LOH	Loss of heterozygosity.
MA	Metastatic adenocarcinoma.
M6P/IGF2R	Mannose 6 phosphate / IGF II receptor.
Mdm2	Transformed 3T3 cell double minute 2.
METAVIR	Meta analysis virology.
MHz	Mega hertz..
MIB1	Mind bomb homolog 1.
MMPs	Matrix metalloproteinases.
MOC 31	Mesothelioma cell 31.
MRA	Magnetic resonance angiography.
MRI	Magnetic resonance imaging.
mRNA	Messenger RNA.
MT1-MMP	Membrane type 1 matrix metalloproteinase.
MXR 7	Mitoxantrone resistance associated gene.
NCECT	Non contrast enhanced computed tomography.
NCI	Northern Cancer Institute.
NF-kB	Nuclear factor kappa B.
NO	Nitric oxide.
NS	Non Structural.
NSGCT	Non seminomatous germ cell tumors.
8-OH-dg	8 Hydroxy- 2'deoxy- guanosine.
ORF	Open reading frame.
ORFV2-VEGF	Orf virus 2 vascular endothelial growth factor.
P161 NK4	Cyclin dependent kinase inhibitor.
P21 waf1/CIP1	Cyclin kinase inhibitor protein.
PAI-1	Plasminogen activator inhibitor 1.

PBS	Phosphate buffer solution
PCNA	Proliferative cell nuclear antigen.
PDGF	Platelet derived growth factor.
PET	Positron emission tomography.
PHC	Primary hepatocellular carcinoma.
PIVKA	Protein induced by vitamin K antagonism.
PLGF	Placental growth factor.
PVP	Portal venous phase.
PVT	Portal vein thrombosis.
ROS	Reactive oxygen species.
RB	Retinoblastoma.
RF	Radio frequency.
SCT	Spiral computed tomography.
SERCA 1	Sarcoplasmic endoplasmic reticulum calcium ATP ase.
α-SMA	Alpha smooth muscle actin.
Src	Kinases encoded by Rous Sarcoma virus.
TAPA1	Target of anti proliferative antibody 1.
TESPA	3-aminopropyl-trimethoxysilane
TGF-β	Transforming growth factor beta.
TGF-α	Transforming growth factor α .
THI	Tissue harmonic imaging.
TIMPs	Tissue inhibitor metalloproteinases.
TNM	Tumor- nodes-metastasis.
tPA	Tissue plasminogen activator.
TRAP 1	Tumor necrosis factor associated protein 1.
TTF1	Thyroid transcription factor 1.
uPA	Urokinase plasminogen activator.
u PAR	Urokinase plasminogen activator receptor.
VEGF	Vascular endothelial growth factor.
VEGFR	Vascular endothelial growth factor receptor.
VPF	Vascular permeability factor.
XPB	Xeroderma pigmentosum complement group B.
XPD	Xeroderma pigmentosum D.

LIST OF TABLES

• Review of literature

Table	Title of table	Page
Table (1):	Molecular alterations in hepatocellular carcinoma	18
Table (2):	The chromosomal location of potential and candidate tumor suppressor genes for hepatocellular carcinoma.	19
Table (3):	Classification system of HCC	67
Table (4):	Important Features of the ABC Method for Immunohistochemical Staining.	87
Table (5):	Various staging and scoring systems of fibrosis.	93
Table (6):	The histological activity index.	96
Table (7):	Histologic scoring system of inflammation introduced by Batts and Ludwig in 1995.	96
Table (8):	Molecules Involved in Angiogenesis.	108

• Results

Table	Title of table	Page
Table (1):	Characteristic features of the studied groups.	147
Table (1a):	Demographic features of the studied groups.	147
Table (1b):	Risk factors for hepatitis C and B in the studied groups.	148
Table (1c):	Clinical characteristics of the studied groups.	149
Table (2):	Hematological findings and ESR of the studied groups.	151
Table (3):	Liver biochemical profile of the studied groups	152
Table (4):	Pattern of liver transaminases in the different studied groups.	154
Table (5a):	Interpretation of the results of viral hepatitis seromarkers in the studied groups	157
Table (5b):	The HCV RNA level in the studied chronic hepatitis C patients and the post hepatitis C cirrhotic patients	157
Table (6):	Correlation of HCV RNA level and the pattern of liver transaminases in the Chronic hepatitis C (19) and the cirrhotic C patients (23).	159
Table (7a):	Serum alpha Feto-protein levels of the studied groups.	162
Table (7b):	Pattern of alpha feto protein levels in the studied groups.	162