Significance of Hepatocyte Growth Factor (HGF) Concentrations in Serum of Patients with Liver Cirrhosis and Patients with Hepatocellular Carcinoma

Thesis
Submitted for the Partial Fulfillment of the M.Sc Degree in Clinical and Chemical Pathology

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2010

ABSTRACT

Background: **HGF is a heterodimer composed of an alpha-chain subunit (69 kDa) and a beta-chain subunit (34 kDa), which are linked by a disulfide bond. Although HGF was initially identified as a potent mitogen for hepatocytes, considerable evidence indicates that intracellular signaling pathways driven by HGF-c-Met receptor coupling leads to multiple biological responses in a variety of cells, including mitogenic, motogenic, morphogenic, and antiapoptotic activities.**

Aim of the work: **This study tries to evaluate the role of serum HGF as a non invasive biomarker in diagnosis of liver cirrhosis and hepatocellular carcinoma.**

Subjects and Methods: **AFP and HGF were measured in 20 healthy control, 30 cirrhotic patients on top of HCV infection, 30 HCC patients on top of HCV infection and cirrhosis. Serum AFP was assayed by electro-chemiluminescence technique and serum HGF was assayed by the quantitative sandwich ELISA technique.**

Results: **HGF were highly significantly elevated in the HCC and cirrhotic groups (median=3709(2574.5-5128.75) pg/ml), (median=2843.5(2119-3721) pg/ml) respectively than that of the control group (median =913(770.7-1166.5) pg/ml) with (p=0.000) for both, and was elevated in the HCC group when compared to the cirrhotic group without reaching a statistically significant level . AFP levels were highly significantly elevated in the HCC group [median=128.5(81.75-239.5) ng/ml] than that of both the cirrhotic group [median=4.9(3.65-8.55) ng/ml)] and the control group [median=3.15(1.97-4.55) ng/ml)] (p=0.000) for both. The AFP levels of the cirrhotic group were higher than that of the control group but this difference did not reach a statistically significant level . We found a positive significant correlation between HGF and AFP for all studied subjects (r=0.561, p=0.000) . The sensitivity and specificity of HGF for the HCC group over the non-HCC group were 93.3% and 46%, respectively, at a cut-off value of 1451 pg/mL and AUC was 0.787, and that of AFP were 100% and 92%, respectively, at a cut-off value of 10 ng/mL and AUC was 0.999. The sensitivity and specificity of both parameters together were 100% and 66%, respectively, at the same cut-off values. The Odds ratio of HGF was 11.926 and 95% CI (2.56 - 55.55).**

Conclusion: **Serum HGF has the potential to be a novel complementary biomarker for assessment of the risk of the HCC development in the cirrhotic patients.**

Key words: Hepatocyte growth factor, alpha fetoprotein, liver cirrhosis and hepatocellular carcinoma .

ACKNOWLEDGEMENT

*I would like to thank **ALLAH** a lot for his kindness, patience and strength he gave to me to achieve this work and made me able to finish it.*

*I would like to express my deepest gratitude to **Prof. Dr Nermine Bahgat** Professor of Chemical Pathology, Cairo University, for her sincere guidance and her help and support throughout the work.*

*I am greatly honored to express my deep gratitude and faithfulness to **Dr. Heba Sharaf Eldeen,** Professor of Chemical Pathology, Cairo University, for her strict supervision and revision of this work, She gave me her valuable advices and support that cannot be expressed in words.*

*I am extremely grateful to **Dr. Dina Hesham ,** lecturer of Chemical Pathology, Cairo University, for giving me much of her time, experience and support, her valuable comments and efforts. Her encouragement were so supportive for the completion of this work.*

*Also I am deeply grateful to **Dr.Dalia Ramadan,** Ass.Professor of Chemical Pathology, Cairo University, for her support in the statistical analysis of the work.*

I would like to thank all my staff members of the Clinical and Chemical Pathology Department, faculty of medicine, Cairo University.

A special dedication to ***my family****, my father , mother, sisters and brothers for their never ending care. They were always supporting me and encouraging me to continue and finish this work. Really I can't do anything without them, so no words can express my gratitude to them, wishing them all the happiness and good health.*

Rehab Abdel Wadod

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List of abbreviation:

95%CI: Confidence interval
AAR(AST/ALT): Aspartate aminotransaminase/ Alanine aminotransaminase ratio
AF: Advanced fibrosis
AFP: Alpha (α) fetoprotein
ALP: Alkaline phosphatase
ALT: Alanine aminotransaminase
ANA: Anti-neutrophil antibody
APRI: AST to platelet ratio index
ASMA: Anti–smooth muscle antibody
AST: Aspartate aminotransaminase
AUC: Area under the curve
AUROC: The area under the receiver operating characteristic curve
CDS: Cirrhosis discriminant score
CLD: Chronic liver disease
CT: Computed tomography
CTHA: CT during hepatic arteriography
CTAP: CT arterial portography
D.Bil.: Direct bilirubin
DCP: Desgamma-carboxy prothrombin
ECD: Extracytoplasmic Domain
ECM: Extracellular matrix
EF: Early fibrosis
ELF: Enhanced liver fibrosis
ELISA: Enzyme linked immunosorbent Assay technique
GGT: Gamma-glutamyl transferase
HA: Hyaluronic acid
Hb: Haemoglobin
HbeAg: Hepatitis B e antigen
HbsAg: Hepatitis B surface antigen
HCC: Hepatocellular carcinoma
HGF: Hepatocyte growth factor
HHT: Hereditary hemorrhagic telangiectasia
HSC: Hepatic stellate cells
IEF: Iso-electric focusing
IL-10: Interleukin-10

INR: International Normalised Ratio
LB: Liver biopsy
LCA: Lectin lens culinaris agglutin
mab: Monoclonal antibody
MF: Myofibroblast
MMPs: Matrix metalloproteinases
MRI: Magnetic resonance imaging
NAFLD: Nonalcoholic fatty liver disease
NO: Nitric oxide
NPV: Negative predictive value
NSGCT: non-seminomatous germ cell tumours
PC: Prothrombin concentration
PCR: Polymerase chain reaction
PDGF: Platelet derived growth factor
PICP: Procollagen type I carboxy terminal peptide
PIIINP: Procollagen III amino peptide
PIVKA-II: Protein induced by vitamin K absence/antagonist-II
PSC: Primary sclerosing cholangitis
PT: Prothrombin time
PVN: Predective value of negative
PVP: Predictive value of positive
RBCs: Red blood cells
RIA: Radioimmunoassay
ROC: Receiver operating characteristics
RTPCR: Reverse-transcription polymerase chain reaction
T.Bil: Total Bilirubin
TBRI: Theodor Bilharz Research Institute
TGF-β: Transforming growth factor β
TIMP: Tissue inhibitors of metalloproteinase
TNF-α: Tumour necrozing factor-α
WBCs: White blood cells

Liver Cirrhosis

Definition:

Cirrhosis represents the final stage of several chronic hepatic diseases **(Brandao et al. , 2006)** . It is a diffuse process of architectural disorganization characterized by fibrosis and the formation of structurally abnormal parenchymal nodules **(Anthony et al., 1978)** . This results in portal hypertension , portosystemic shunting , and a diminution of the effective parenchymal mass **(Groszmann and Atterbury 1982)**.

Furthermore, the accumulation of connective tissue within the space of Disse can impede the normal metabolic traffic between blood and hepatocytes , impairing the clearance of circulating macromolecules , disturbing the intercellular interactions , and resulting in liver cell dysfunction **(Orrego et al., 1987)**.

Fibrosis was staged by the METAVIR system as

F0: no fibrosis,

F1: portal fibrosis without septa,

F2: few septa,

F3: numerous septa without cirrhosis,

F4: cirrhosis .

The grading of activity **(The METAVIR Cooperative Group) .**

Etiology:

1. ***Hepatitis C virus***

HCV is a small , single-stranded RNA virus , remains a major cause of hepatic cirrhosis and hepatocellular carcinoma worldwide **(Alter 1999)**. In which its prevalence varies throughout the world, with the highest number of infections reported in Egypt **(Frank et al., 2000, Friedman and Schiano 2004, Crawford 2005)** . It has been estimated that 75% to 85% of individuals infected with HCV progress to chronic infection, persisting for at least 6 months after onset, with the rate of chronic infection varying by age, sex, race, and immune system status **(Hoofnagle JH,2002, Tsoulfas et al.,2009) .** Long-term infection has been associated with serious clinical sequelae, including development of hepatic fibrosis, cirrhosis of the liver, portal hypertension and HCC. **(Strader et al.,2004, Tsoulfas et al.,2009).**

2. ***Chronic hepatitis B***
3. ***Alcohol***
4. ***Biliary obstruction***
5. ***Biliary atresia/neonatal hepatitis***
6. ***Congenital biliary cysts***
7. ***Cystic fibrosis***
8. ***Primary and secondary biliary cirrhosis***
9. ***Primary sclerosing cholangitis (PSC)***
10. ***Haemochromatosis***

11. nonalcoholic fatty liver disease(NAFLD)

12. Autoimmune chronic hepatitis

***13. Drugs and toxins* as**

Alpha-methyldopa , Isoniazid , Methotrexate.

14. Genetic metabolic disease as :

α1-antitrypsin deficiency.

Glycogen storage diseases.

Wilson's disease.

15. Idiopathic/miscellaneous as :

Granulomatous liver disease (e.g. sarcoidosis), Idiopathic portal fibrosis ,Polycystic liver disease.

16. Infection as:

Brucellosis , Congenital or tertiary syphilis , Echinococcosis , Schistosomiasis.

17. Vascular abnormalities as :

Chronic passive hepatic congestion caused by right-sided heart failure, pericarditis, Hereditary hemorrhagic telangiectasia (Osler-Weber- Rendu disease).

18. Veno-occlusive disease

(Heidelbaugh and Bruderly, 2006).

Pathophysiology:

Cirrhosis is a dynamic situation where two extreme processes occur , fibrogenesis and fibrolysis **(Rocky and Bissell , 2006) .** This entails an accumulation of collagen , as well as other proteins in the extracellular matrix, in the tissue. Progressive deposition of these substances eventually results in disrupted liver morphology , parenchymal function impairment , and ultimately portal hypertension and its related sequels **(Martin Gabriel et al., 2009).**

The fibrosis , during the process of hepatic cirrhosis, is represented by connective tissue that separates the liver into multiple regeneration nodules . The fibrous septa vary considerably from delicate to extensive , and they may contain inflammatory cells and arterial, venous and biliary structures in varying numbers . In case of well established cirrhosis , the fibrosis surrounds the nodules completely. However, there are cases of incomplete septal cirrhosis, with partial involvement of the nodules **(Barnett et al., 1992)**.

The regenerative hyperplasia of hepatocytes is usually viewed as an attempt to restore parenchymal integrity , but it also contributes to the nodularity and overall architectural disorganization of cirrhosis **(Fausto and Mead 1989)**.

The normal regulation of hepatocyte growth appears to be controlled by various circulating growth factors . Following hepatocyte necrosis , the growth factors are secreted and trigger hepatocyte proliferation. Because of