SIGNIFICANCE OF ENDOGLIN (CD105) CONCENTRATIONS IN SERUM OF PATIENTS WITH LIVER CIRRHOSIS AND PATIENTS WITH HEPATOCELLULAR CARCINOMA

Thesis Submitted in Partial fulfillment for the Master Degree in Clinical and Chemical Pathology

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2009

أهمية تركيز الإندوجلين (CD 105) فى مصل الدم فى مرضى تليف الكبد و مرضى أورام الكبد

رسالة مقدمة من

الطبيبة/ نهى كمال عبد المؤمن

توطئة للحصول على درجة الماجستير
فى الباثولوجيا الإكلينيكيه و الكيميائيه

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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 am greatly honored to express my deep gratitude and faithfulness to **Dr. Heba Sharaf Eldeen,** Ass.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. Her encouragement were so supportive for the completion of this work.*

*I would like to express my deepest gratitude to **Dr. Azza Saleh Radwan**, Professor of Chemical Pathology, Theodor Bilharz Research Institute, for her sincere guidance and her help and support throughout the work.*

*I am extremely grateful to **Dr. Asmaa Esmail** , lecturer of Chemical Pathology, Cairo University, for giving me much of her time, experience and support, her valuable comments and efforts*

*I express my deep sense of gratitude **Dr. Mohe Atya**, Ass.Professor of Tropical Medicine, Theodor Bilharz Research Institute, for his continuous support, guidance and help in collecting data.*

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

I would like to thank all my staff members of the Chemical Pathology Department in Theodor Bilharz Research Institute, especially ***Dr. Afaf Abdel Hade,*** *the head of the department, for her continuous guidance, faithful advices encouragement and support for me and my colleagues.*

My deep thanks and appreciation to ***Dr.Mona Hassan****, Professor of Chemical Pathology, Theodor Bilharz Research Institute, for her sincere guidance and her help and support throughout the work. She gave me her valuable advices and support that cannot be expressed in words. Her motherhood attitude and encouragement were so supportive for the completion of this work.*

A special dedication to ***my family****, my father my, mother and my sisters 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.*

My deep thanks to ***my husband*** *for his strong support and continuous guidance that cannot be expressed in words. He was always standing by me when I needed his help and advice.*

My special dedication, to the best gift from ALLAh, ***my son, Yassin.***

Noha Kamal

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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
ALK: Activin receptor-like kinases
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: Areas under the curve
AUROC: The area under the receiver operating characteristic curve
BMP-2: Bone morphogenetic protein-2
BMP-7: Bone morphogenetic protein-7
CD: Cytoplasmic Domain
CD105: Endoglin
CDS: Cirrhosis discriminant score
co-SMAD: Common-mediator SMAD
CT: Computed tomography
D.Bil.: Direct bilirubin
DAB: Diaminobenzidine tetrahydrochloride
DCP: Desgamma-carboxy prothrombin
ECD: Extracytoplasmic Domain
ECM: Extracellular matrix
EF: Early fibrosis
EGR-1: Early growth response-1
ELF: Enhanced liver fibrosis
ELISA: Enzyme linked immunosorbent Assay technique
GGT: Gamma-glutamyl transferase
GS domain: rich in glycine and serine residues
HA: Hyaluronic acid
Hb: Haemoglobin
HbeAg: Hepatitis B e antigen
HbsAg: Hepatitis B surface antigen

HCC: Hepatocellular carcinoma
HHT: Hereditary hemorrhagic telangiectasia
HMEC-1: Human umbilical vein endothelial cells-1
HSC: Hepatic stellate cells
IEF: Iso-electric focusing
IL-10: Interleukin-10
IMVD: Intratumoural microvascular density
INR: International Normalised Ratio
I-SMAD: Inhibitory Smads
KD: Kinase Domain
LAP: Latency-associated peptide
LCA: Lectin lens culinaris agglutin
mab: Monoclonal antibody
MF: Myofibroblast
MMPs: Matrix metalloproteinases
MRI: Magnetic resonance imaging
MVD: Microvascular density
NAFLD: Nonalcoholic fatty liver disease
NC1:Carboxyterminal cross-linking domain
NO: Nitric oxide
NPV: Negative predictive value
NSGCT: Non-seminomatous germ cell tumours
OPN: Osteopontin
PAI-1: Plasminogin activator inhibitor-1
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
RGD: The tripeptide arginine-glycine-aspartic acid
RIA: Radioimmunoassay

RLUs: Relative light units
ROC: Receiver operating characteristics
R-SMADs: Receptor-regulated SMADs
RTPCR: Reverse-transcription polymerase chain reaction
SMCs: Smooth muscle cells
SSc: Systemic sclerosis
sCD105: Soluble CD105
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
vWF: von Willebrand factor
WBCs: White blood cells
α-SMA: Alpha smooth muscle actin

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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 Bedossa et al., 1994, Bedossa and Poynard 1996)**

Etiology:

1. ***Hepatitis C virus***

   HCV is a small, single-stranded RNA virus classified in the Flaviviridae family, 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)**.
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

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 cases 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 the disrupted vascular supply, this process occurs in an irregular rather than orderly fashion and the structural and functional integrity of the liver is consequently not maintained **(Callea et al., 1991)**.

Hepatic stellate cells (HSC)

- HSC also referred to as Ito cells, fatstoring cells and lipocytes **(Knook, 1982)**. They are mesenchymal cells adjacent to the space of Disse, being more frequently observed in proximities of the centrolobular veins (zone 3 of the hepatic acinus),
- HSC correspond to about 15% of the total number of cells in the liver **(Friedman, 2000)**.
- These cells maintain the main stock of vitamin A in lipidic granules. HSC have contractile cytoplasmic processes in intimate contact with the sinusoids. They can interfere in the intra-hepatic blood flow due to their privileged location, in simultaneous contact with the sinusoids and with the hepatocytes. The paracrine regulation of several hepatic functions and local neurotransmitter capacity were attributed to the stellate cells **(Kawada, 1997)**.
- The stellate cells play a crucial role in the offense mechanisms, regeneration and fibrosis in the hepatic tissue in response to noxious agents, and this is besides a variety of growth factors, cytokines, prostaglandins and other bioactive substances **(Rippe, 1998)**.
- They also participate in the maintenance of the extracellular matrix (ECM) and of the space of Disse **(Geerts, 2001)**.
- HSC acquire particularly important function when the liver is submitted to a harmful process. In which it can alter their morphology and physiology through a process known as activation **(Pinzani and Marra., 2001)**.In other words, the quiescent HSC (not activated HSC) are induced to activate themselves by paracrine signals **(Friedman, 2000, Pinzani et al., 2001)**.

This process can be subdivided into three parts:

Initiation

HSC receive numerous paracrine stimuli provided by harmed hepatocytes, endothelial cells, Kupffer cells and

ECM altered by the aggression. After that, HSC become more responsive to cytokines **(Friedman, 2000, Pinzani et al., 2001)**.

Perpetuation

HSC begin to produce cytokines that attract and stimulate themselves and leukocytes (autocrine and paracrine mechanisms), start to proliferate, lose their vitamin A deposits, acquire contractile capacity and start to produce great amount of fibrillar collagen (type I); because of that, activated HSC are also called "myofibroblast (MF) like" **(Friedman, 2000, Pinzani et al., 2001)**. The result is hepatic fibrosis and, with time, cirrhosis **(Brandao et al., 2006)**.

Resolution

After the removal of the harmful factors and if a terminal cirrhosis has not been installed, the fibrosis can be reverted by increase of the collagenases activity and reduction of the number of activated HSC. It is probable that the interleukin-10 (IL-10) has participation in that stage **(Friedman, 2000)**.

In spite of the association of HSC with cirrhosis, their activation can have beneficial function in the cases of acute aggression, because it results in the formation of an appropriate stromal outline for the hepatic regeneration with maintenance of hepatic architecture **(Pinzani et al., 1998)**.

The fibrogenesis corresponds to the increase of production of ECM by the HSC, resulting in fibrosis. The fibrogenesis is stimulated mainly by transforming growth factor β_1 (TGF-β_1), which is mainly autocrine. The production and secretion of cytokines by activated HSC result in increase of the inflammation **(Kawada, 1997, Pinzani et al., 1998, Friedman, 2000, Pinzani et al., 2001)**.

Endoglin is a transmembrane accessory receptor for the 'fibrogenic' key cytokines (TGF-β_1 and TGF- β_3) (**Westphal et al., 1993, Burrows et al., 1995**). CD105 modulates TGF-β signalling by interacting with the TGF-β receptor complexes **(Meurer et al., 2005, Miller et al., 1999)**. Endoglin may be important for the regulation of epithelial-mesenchymal