ROLE OF HEPATIC STELLATE CELLS IN THE PATHOGENESIS OF HEPATIC FIBROSIS IN CHRONIC HEPATITIS C: AN IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Chronic hepatitis C is one of the most important health problems. End-stage liver disease due to hepatitis C is the leading indication for liver transplantation. Hepatic fibrosis, the common final manifestation of several chronic liver diseases, is the result of a prominent accumulation of extracellular matrix (ECM) materials and ultimately can lead to cirrhosis. HSCs have a crucial role in determining the pathogenesis and clinical course of liver fibrosis and cirrhosis. The alpha isotype of actin (α -SMA) expressed by hepatic stellate cells reflects their activation to myofibroblast-like cell and has been directly related to experimental liver fibrogenesis, and indirectly to human fibrosis in chronic liver disease. The role of GFAP expression in HSCs is currently unknown. Previous studies in rodents showed that, when rodent HSCs were activated, the expression of GFAP decreased. The decreased GFAP expression in an advanced stage of fibrosis suggested that GFAP could be a marker for quiescent cells in rodents.

This study was conducted on 69 chronic HCV patients, presented to the Department of Gastroenterology and Hepatology, Theodor Bilharz Research Institute, Egypt, in the period between 2004-2007. Amongst whom, there were 11 patients having mixed chronic HCV and schistosomal infections (served as pathological controls). In addition to 10 liver disease-free individuals serving as normal controls.

Clinical characteristics of the studied patients revealed the presence of fatigue as a common symptom among them, occurring in 87%. Rt hypochondrial dull aching pain was reported in 65.2%. None of the studied patients had clinically palpable spleen or ascites. Manifestations of liver cell failure in the form of Jaundice, spider nevi, palmer erythema, encephalopathy and bleeding tendency were totally absent in the study 69 patients. Quantitative PCR-HCV RNA performed to all patients revealed that, 30.4% had high viremia, 65.2% had low viremia. Only 3/69 (4.4%) had negative PCR test. Alpha-SMA-positive HSCs were detected in all stages of hepatic fibrosis. The α -SMA-positive cells were encountered mostly in the perisinusoidal region. Patients with low grade of necro-inflammatory activity showed a significantly higher α-SMA immunoexpression on *perisinusoidal* HSCs when compared with those having a high grade of activity. The control group had a significantly lower α-SMA expression when compared with the other groups. Patients with no evident fibrosis (F0 group) showed higher α-SMA immunoreactivity than both the cirrhotic and bilharzial groups. CHC group had the highest α-SMA immunoreactivity when compared with the bilharzial group and the cirrhotic groups. Alpha-SMA expression was significantly higher in the cirrhotic group when compared to the bilharzial group. Patients with low grade of necro-inflammatory activity showed a significantly higher expression of GFAP-positive perisinusoidal HSCs when compared to those with high grade of activity. The control group had a significantly lower GFAP expression when compared with the other groups. Also, F0 group showed the highest GFAP immunoreactivity than both the CHC and cirrhotic groups. Finally, cirrhotic group had lower GFAP expression than bilharzial group. Among the 69 studied patients, there was no statistically significant correlation between the immunoexpression of both markers and clinical and laboratory variables of those patients. Also, no statistical significant correlation was recorded between the immunoexpression of both markers and both stages of fibrosis and necro-inflammatory grading of those patients. On the other hand, the α -SMA expression correlated significantly with GFAP expression in the same studied patients.

Alpha-SMA could be a useful marker to identify the earliest grades of necro-inflammatory activity and also earliest stages of hepatic fibrosis. GFAP could represent a useful marker of early activation of HSCs in the HCV chronic hepatitis setting. In addition, GFAP-dependent activation of HSCs precedes fibrotic tissue deposition.

Kev words

Immunopathogenesis of hepatitis C, Hepatic stellate cells, Liver fibrosis, Alph-smooth muscle actin, Glial fibrillary acidic protein.

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LIST OF ABBREVIATIONS

ADCC Antibody-dependent cellular cytotoxicity

A-II Angiotensin II

ALT Alanine aminotransferase
ANA Anti-nuclear antibody

Ang-1 Angiopoietin 1

ANP Atrial natriuretic peptide
APRI (AST)-platelet ratio index

ARFP Alternate reading frame protein

A-SMA Alpha-smooth muscle actin
AST Aspartate aminotransferase
ATPase Adenosine triphosphatase
AUC Areas under the curve

BDNF Brain-derived neurotrophic factor Cyclic adenosine monophosphate

CBS Cystathionine- β -synthase

CCR Chemokine receptor

CD Crohn's disease

CD Cluster of differentiation

cGMP Cyclic quanosine monophosphate

CINC Cytokine-induced neutrophil chemoattractant

CLDs Chronic liver diseases

CMV Cytomegalovirus

CNS Central nervous system

CO Carbon monoxide
COX Cyclooxygenase
CRP C-reactive protein
CT Cysteinyl leukotrienes

CTGF Connective tissue growth factor

CTL Cytotoxic T-lymphocyte

DAG Diacylglycerol DCs Denderitic Cells

DPIV Dipeptidyl peptidase IV
ECM Extracellular matrix
ECM Extracellular matrix

EDCFs Endothelial derived contracting factors

EGF Epidermal growth factor

ELFG European Liver Fibrosis Group

ELISA Enzyme-linked immunosorbent assays

ENS Enteric nervous system
ER Endoplasmic reticulum

ET-1 Endothelin-1
ET-A Endothelin A
ET-B Endothelin B

FAK Focal adhesion kinase

FAP Fibroblast activation protein

FT FibroTest™

FXR Farnesoid X receptor

GFAP Glial fibrillary acidic protein

HBV Hepatitis B virus

HCC Hepatocellular carcinoma

HCV Hepatitis C virus

HCV-C Hepatitis C virus cirrhosis

HCV-CH Hepatitis C virus chronic hepatitis

HCV-PTR HCV-posttransplant recurrent hepatitis

HGF Hepatocyte growth factor
HIV Human immunodeficiency virus

HLA Human leukocyte antigen

HO-1 Heme oxygenase-1

HRT Hormone reposition therapy

HSCs Hepatic stellate cells

ICAM-1 Intracellular adhesion molecule 1

IF Intermediate filamentIFN- γ Interferon gammaIg Immunoglobulin

IGF Insulin-like growth factor

IHVR Intrahepatic vascular resistance

IL Interleukin

IP3 Inositol 1,4,5-trisphosphate
IRF Interferon regulatory factor
ISGF IFN- stimulated gene factor

ISGS IFN-stimulated genes

ISRE IFN-stimulated response elements

JAK Janus kinase

LDL Low-density lipoproteins
LMCs Liver mesenchymal cells
LPA Lysophosphatidic acid
LPS Lipopolysaccharide
MBL Mannose-binding lectin

MCP-1 Monocyte chemoattractant protein 1

M-CSF Macrophage colony-stimulating factor

MFBs Myofibroblasts
MFs Myofibroblasts

MHC Major histocompatibility complex MICA/B MHC class-I related chain A/B

MLC Myosin light chain

MLCK Myosin light chain kinase

MLCP Myosin light chain phosphatase MMP-2 Matrix metalloproteinase 2

MW Membranous web

NASH
Non-alcoholic steatohepatitis
NCAM
Neural cell adhesion molecule
NEC
Necrotising enterocolitis
NF-L
Neurofilament protein
NF-K B
Nuclear factor-kappa B
NGF
Nerve growth factor

NIH The National Institutes of Health

NK Natural killer NO Nitric oxide

NOs Nitric oxide synthase

NS Nonstructural

OLT Orthotopic liver transplantation

PA Phosphatidic acid

PAF Platelet activating factor

PAT Parentral anti-schistosomal therapy

PBC Primary biliary cirrhosis

PBMCs Peripheral blood mononuclear cells
PCNA Proliferating cell nuclear antigen

PCR Polymerase chain reaction
PCR Polymerase chain reaction

PDGF Platelet-derived growth factor PDL-1 Programmed death ligand-1

PG Prostaglandin

PGP9.5 Beta-tubulin, protein gene product 9.5

PGs Prostaglandins

PI 3-kinase Phosphoinositol 3-kinase

PIP2 Phosphatidylinositol 4,5-bisphosphate

PKC Protein kinase C
PKC Protein kinase C
PLC Phospholipase C
PLD Phospholipase D

PNS Post neuronal synapse

PPARs Peroxisome proliferators activated receptors

RANTES Regulated upon activation normal T-cell expressed and

secreted.

RAR Retinoic acid receptors
RAS Renin- angiotensin system
RBP Retinol-binding protein

rMLC Regulatory myosin light chain

ROS Reactive oxygen species
RT-PCR Reverse transcription-PCR

RT-PCR Real-time PCR

RXR Retinoid X receptors
SCF Stem cell factor

SHP Small heterodimer partner siRNA Small interfering RNA

SR-BI Scavenger receptor class B1

STAT Signal transducer and activation of transcription

SVR Sustained virologic response

TCR T-cell receptor

TGF-a Transforming growth factor alpha
TGF-B Transforming growth factor beta

Th Thelper

TLRs Toll-like receptors
TNF Tumor necrosis factor
Tollip Toll-interacting protein

TRAIL TNF-related apoptosis inducing ligand

TXA2 Thromboxane A2 TXs Thromboxanes

UTR Untranslated regions

VCAM-1 Vascular cell adhesion molecule VEGF Vascular endothelial growth factor

VEGFR-1&2 VEGF receptor-1 and -2

WBC White blood cells

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INTRODUCTION AND AIM OF THE WORK

Hepatitis C virus (HCV) is considered the most common etiology of chronic liver disease (CLD) in Egypt, where prevalence of antibodies to HCV (anti-HCV) is approximately 10-fold greater than in the United States and Europe (Strickland *et al.*, 2002).

Chronic hepatitis C is responsible for significant morbidity and mortality rates (Bataller and Brenner, 2005). Currently, the cirrhosis resulting from chronic virus C infection is the main cause of hepatic transplantation worldwide (Codes *et al.*, 2007).

The main injury caused by hepatitis C virus is the hepatic fibrosis, as a result of a chronic inflammatory process in the liver. The development of the chronic hepatitis C is better estimated by the fibrosis stage rather than by the necro-inflammatory activity level (Poynard *et al.*, 1997).

Fibrosis develops as a multicellular process involving paracrine signaling between the resident liver cells and inflammatory cells (Friedman, 2000).

Central events include the activation of hepatic stellate cells (HSCs) in association with tissue necrosis and inflammation (Friedman, 2004). In response to liver injury, human HSCs express alpha-smooth muscle actin (α -SMA), becoming "activated" and myofibroblast-like. Immunohistochemical staining for α -SMA correlates with HSC activation (Guido *et al.*, 1997).

So, the expression of $(\alpha$ -SMA) is a reliable and widely used marker of activation of HSCs to myofibroblast-like cells in patients with chronic hepatitis C

(Friedman, 2008). Although correlation between HSC activation and necro-inflammatory activity and/or fibrosis stage is a point of large depate (Carpino *et al.*, 2005).

GFAP is an intermediate filament (IF) protein that is found in glial cells (Fuchs and Weber, 1994).

GFAP is expressed in the central nervous system in astrocyte cells. It is involved in many cellular functioning processes, such as cell structure and movement, cell communication, and the functioning of the blood brain barrier (Tardy et al., 1990).

GFAP expression was reported in quiescent stellate cells in vivo, with an increased expression in the acute response to injury in the rat, and a down regulation in the chronic one (Morini et al., 2005).

Reports concerning GFAP expression in human liver are conflicting. Few studies have been performed in order to quantify the hepatic expression of GFAP at different stages of human chronic hepatitis (Martinelli et al., 2004).

GFAP becomes down-regulated in chronically activated HSCs and therefore is used as a unique marker for the activation of quiescent HSCs to proliferative myofibroblast-like HSCs, a critical step during inflammation and injury in the liver (Neubauer et al., 1996; Niki et al., 1996).

However, despite an increasing appreciation of the manner in which HSCs are regulated, the factors responsible for initiation of fibrogenesis and progression of fibrosis in chronic hepatitis C remain poorly understood (Lau et al., 2005). Hence,