Image analysis assessment of fibrosis in liver biopsy from chronic hepatitis patients

(Thesis)

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List of abbreviations

AAT	Alpha-1 antitrypsin
AIDS	Acquired immunodeficiency syndrome
AIH	Autoimmune hepatitis
ALT	Alanine aminotransferase
ANA	Antinuclear antibody
Anti LKM	Anti–liver-kidney microsomal antibody
AP-1	Activating protein type-1
APCs	Antigen presenting cells
ASMA	Anti–smooth muscle antibody
BCP	Basal core promoter
cAMP	Cyclic adenosine monophosphate
ccc	Covalently closed circular
CCL21	C-C chemokine ligand 21
CGs	Cryoglobulins
CTL	Cytotoxic T-lymphocyte
DC	Dendritic cells
E1	Envelope protein 1
E2	Envelope protein 2
ECM	Extracellular matrix
EHM	Extrahepatic manifestations
ER	Endoplasmic reticulum
HAI	Histologic activity index
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B envelope antigen
HBLAg	Hepatitis B large surface antigen
HBMAg	Hepatitis B middle surface antigen
HBSAg	Hepatitis B small surface antigen
HBsAg	Hepatitis B surface antigen

List of abbreviations

HBV	Hepatitis B Virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDAg	Hepatitis delta antigen
HDV	Hepatitis delta virus
HH	Hereditary haemochromatosis
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HSCs	Hepatic stellate cells
IAPs	Inhibitor of apoptosis proteins
IASL	International Association for the Study of the Liver
IFN	Interferon
IL	Interleukin
Kbp	Kilo base pair
LD	Lipid droplets
MAPK	Mitogen-activated protein kinase
MC	Mixed cryoglobulinaemia
MCP-1	Monocyte chemoattractant protein–1
MHC	Major histocompatibility complex
MIP-2	Macrophage inflammatory protein–2
MMPs	Matrix metalloproteinases
NADPH	Nicotinamide adenine dinucleotide phosphate
NANBH	Non-A, non-B hepatitis
NF-κB	Nuclear factor κB
NHL	Non-Hodgkin's lymphoma
NK	Natural killer
NS	Nonstructural
NTR	Nontranslated region
ORF	Open reading frame
PAF	Platelet-activating factor

List of abbreviations

PAI-1	Plasminogen activator inhibitor type 1
PDGF	Platelet derived growth factor
Pi	Protese inhibitor
PI3k	Phosphoinositol 3 kinase
PI3K/Akt/p70S6K	Phosphatidylinositol 3-kinase/Akt/ p70S6 kinase
PKC	Protein kinase C
RAS	Renin–angiotensin system
RdRp	RNA-dependent RNA polymerase
RNA	Ribonucleic acid
ROS	Reactive oxygen species
SERPINA1	SERine Proteinase Inhibitors, clade A, member 1
SOCS	Suppressor of cytokine signaling
SVR	Sustained viral response
TCR	T-cell receptor
TGF-β1	Transforming growth factor β1
Th	Helper T
TIMP-1	Tissue inhibitors of metalloproteinases
TNF-α	Tumor Necrosis Factor-α
UTR	Untranslated regions
WD	Wilson's disease
WHO	World health organization

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Abstract

Background: Liver fibrosis results from chronic inflammation of hepatic parenchyma. Progressive accumulation of fibrous tissue eventually leads to cirrhosis and its complications. The severity of liver fibrosis defines the stage of chronic hepatitis and carries with it important clinical implications.

Histological scoring systems such as Ishak provide descriptive evaluation of the liver tissue mainly in terms of architectural changes without measuring the amount of fibrosis.

Objective: To measure the severity of liver fibrosis quantitatively and to compare this with established methods, such as Ishak scoring system.

Materials and methods: Lieca Qwin 500 image analyzer with damaged area morphometry software was used by interactive method to measure the fibrous tissue area based on the different colors of hepatocytes and fibers following staining with Masson's trichrome stain. 43 cases (38 males, 5 females) recruited into the present study with a mean age of 45.5 years (range 15-58 years). Of these, 40 had chronic viral hepatitis and 3 had chronic non viral hepatitis.

Results: Computer morphometry values were highly correlated with results of the Ishak method. The correlation was statistically significant by Chi square (χ^2) test ($P < 0.0001$).

Conclusion: Quantitative image analysis estimation of liver fibrosis area percentage is simple and accurate method for fibrous tissue evaluation in patients with chronic hepatitis to help in therapeutic approaches.

Key words: Image analysis - Morphometry - Chronic hepatitis - Liver fibrosis

Aim of the work

The aim of this work is to evaluate the use of image analysis in assessing liver fibrosis in needle biopsy specimens from patients with chronic hepatitis. Although semi-quantitative scoring systems describe the pathological patterns of hepatic structure, the evaluation of fibrosis is not very precise. So this study aims to assess the image analysis morphometry theoretical advantage of providing truly quantitative data.

Liver fibrosis

Liver fibrosis is the excessive accumulation of extracellular matrix (ECM) proteins including collagen that occurs in most types of chronic liver diseases ***(Bataller & Brenner 2005).*** Both fibrosis and cirrhosis are the consequences of a sustained wound-healing response to chronic liver injury ***(Rockey & Friedman 2006).*** The accumulation of ECM proteins distorts the hepatic architecture by forming a fibrous scar, and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis. Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, which result in hepatic insufficiency and portal hypertension, respectively ***(Gines et al., 2004).***

Chronic liver disease and cirrhosis occur throughout the world, regardless of race, age or gender. However, there are marked geographic variations in incidence and prevalence, largely depending on the prevalence of causative factors ***(Guha & Iredale 2007).***

Early clinical reports in the 1970s suggested that advanced liver fibrosis is potentially reversible ***(Soyer et al., 1976).*** However, liver fibrosis received little attention until the 1980s, when hepatic stellate cells (HSCs) - formerly known as lipocytes, Ito cells, or perisinusoidal cells - were identified as the main collagen-producing cells in the liver ***(Friedman et al., 1985).***This cell type, first described by von Kupffer in 1876, undergoes a dramatic phenotypic activation in chronic liver diseases with the acquisition of fibrogenic properties ***(Geerts 2001).*** Besides HSCs, portal myofibroblasts and cells of bone marrow origin have been shown to exhibit fibrogenic potential ***(Forbes et al., 2004, Ramadori & Saile 2004).***

The onset of liver fibrosis is usually insidious, and most of the related morbidity and mortality occur after the development of cirrhosis ***(Bataller & Brenner 2005).*** Major clinical complications of cirrhosis include ascites, renal failure, hepatic encephalopathy, and variceal bleeding. Patients with cirrhosis can remain free of major complications for several years (compensated cirrhosis). Decompensated cirrhosis is associated with short survival, and liver transplantation is often indicated as the only effective therapy ***(Davis et al., 2003).***

Cirrhosis is also a risk factor for developing hepatocellular carcinoma. Liver fibrosis progresses rapidly to cirrhosis in several clinical settings, including repeated episodes of severe acute alcoholic hepatitis, subfulminant hepatitis, and fibrosing cholestasis in patients with hepatitis C virus (HCV) reinfection after liver transplantation ***(Davis et al., 2003).***

The natural history of liver fibrosis is influenced by both genetic and environmental factors. Epidemiological studies have identified polymorphisms in a number of candidate genes that may influence the progression of liver fibrosis in humans. These genetic factors may explain the broad spectrum of responses to the same etiological agent found in patients with chronic liver diseases. However, some studies have yielded contradictory results due to poor study design, and further research is required to clarify the actual role of genetic variants in liver fibrosis ***(Bataller & Brenner 2005).***

Extensive studies using models of hepatic fibrosis in transgenic mice have revealed key genes mediating liver fibrogenesis ***(Bataller et al., 2003a).*** Genes regulating hepatocellular apoptosis and/or necrosis (e.g., Bcl-xL, Fas) influence the extent of hepatic damage and the subsequent fibrogenic response ***(Takehara et al., 2004).*** Genes regulating the inflammatory response to injury

(e.g., IL-1β, IL-6, IL-10, and IL-13, Interferon γ {IFN-γ}, suppressor of cytokine signaling -1{SOCS-1}, and osteopontin) determine the fibrogenic response to injury ***(Safadi et al., 2004).*** Genes mediating reactive oxygen species (ROS) generation (e.g., Nicotinamide Adenine Dinucleotide Phosphate {NADPH} oxidase) regulate both inflammation and ECM deposition ***(Bataller et al., 2003b).*** Fibrogenic growth factors (e.g. Transforming growth factor β1 {TGF-β1}), vasoactive substances (Angiotensin II, Norepinephrine), and Adipokines (Leptin and Adiponectin) are each required for the development of fibrosis ***(Oben et al., 2004).*** Finally, removal of excess collagen after cessation of liver injury is regulated by tissue inhibitors of metalloproteinases-1 (TIMP-1) and TGF-β1 ***(Ueberham et al., 2003).***

Pathogenesis of liver fibrosis:

Fibrosis is the result of a complex interplay among resident hepatic cells, infiltrating inflammatory cells, and several locally acting peptides called cytokines ***(Saile & Ramadori 2007).***

Fibrosis is a dynamic process; in the healthy individual, although there is no change in the structure of the extracellular matrix on histology, there are simultaneous catabolic and metabolic processes that reach equilibrium with each other. In the fibrotic state, there is excessive ECM production, which outstrips the catabolism of ECM elements ***(Albanis & Friedman 2006).***In advanced stages, the liver contains approximately 6 times more ECM than normal, including collagens (I, III, and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Accumulation of ECM results from both increased synthesis and decreased degradation ***(Bataller & Brenner 2005).***

It has been shown that a major player in fibrosis is the hepatic stellate cell (HSC), which normally functions to store vitamin A and usually remains

morphologically stable. In liver injury, however, these cells become activated, wherein their morphology changes from spheroid cells to more elongated and spindle-shaped cells reminiscent of myofibroblasts. They have a reduction in the amount of vitamin A and begin to secrete dense forms of collagen, such as collagen I. They also express matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), which alter the makeup of the ECM ***(Parsons et al., 2007).***

Activated HSCs migrate and accumulate at the sites of tissue repair, secreting large amounts of ECM and regulating ECM degradation. Platelet derived growth factor (PDGF), mainly produced by Kupffer cells is the predominant mitogen for activated HSCs ***(Bataller & Brenner 2005).***

Hepatic cell types other than HSCs may also have fibrogenic potential. Myofibroblasts derived from small portal vessels proliferate around biliary tracts in cholestasis-induced liver fibrosis to initiate collagen deposition ***(Kinnman & Housset 2002, Magness et al., 2004).*** The relative importance of each cell type in liver fibrogenesis may depend on the origin of the liver injury. While HSCs are the main fibrogenic cell type in pericentral areas, portal myofibroblasts may predominate when liver injury occurs around portal tracts ***(Bataller & Brenner 2005).***

A complex interplay among different hepatic cell types takes place during hepatic fibrogenesis (Figure1). Different types of hepatotoxic agents produce mediators that induce inflammatory actions in hepatic cell types. Damaged hepatocytes and biliary cells release inflammatory cytokines and soluble factors that activate Kupffer cells and stimulate the recruitment of activated T cells. This inflammatory milieu stimulates the activation of resident HSCs into fibrogenic myofibroblasts. Activated HSCs also secrete cytokines that