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Type IV collagen as marker of fibrosis in nonalcoholic liver disease

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ABSTRACT

Currently nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are medical problems associated with the increasing prevalence of diabetes mellitus, obesity, hypertension and hypertriglyceridemia, usually designated as the metabolic syndrome associated with insulin resistance. One study demonstrated an increase in NAFLD prevalence of around 17-33% and in NASH prevalence of 5.7-16.5%. NAFLD comprises a range of mild to severe conditions, from simple steatosis to steatohepatitis, hepatic fibrosis and cirrhosis. The diagnosis of hepatic fibrosis is important for prognosis, stratification for treatment, and monitoring of treatment efficacy. Ultrasonography (USG) is a simple method for detecting fatty infiltrates in the liver. USG has a sensitivity of 82-89% and a specificity of 93%, but cannot differentiate between hepatic steatosis and fibrosis. The gold standard for evaluation of hepatic fibrosis is liver biopsy, which however is a painful and invasive procedure. Currently determination of serum type IV collagen has been suggested as an alternative to liver biopsy among the non-invasive methods for evaluation of hepatic fibrosis, as its serum concentration is closely correlated with advanced hepatic fibrosis in NASH. Type IV collagen is one of the components of basement membrane and its serum concentration is indicative of degradation of the extracellular matrix.

Keywords: NAFLD, NASH, type IV collagen, hepatic fibrosis

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) and its advanced stage, nonalcoholic steatohepatitis (NASH), currently are the subject of heated discussion in the field of medicine. The increasing prevalence of diabetes, obesity, hypertension, and hypertriglyceridemia is considered to be an important cause of NAFLD.[1] The World Health Organization (WHO) has estimated that there were 200 million patients with obesity worldwide in 1995 and 300 million in 2002.[2] On the basis of data published by the NAFLD/NASH 2006 concensus in Cebu, Indonesia has the highest percentage of NAFLD patients in Southeast Asia, namely 30%, in contrast with 17% for Malaysia and 5% for Singapore.[3]

NAFLD comprises a range of conditions, from simple steatosis to steatohepatitis, hepatic fibrosis and cirrhosis[4] and is histologically differentiated into four stages, where stages III

and IV are also known as NASH.[1]

Ultrasonographic imaging (USG) is a simple method for diagnosis of fatty liver, based on visualization of fatty infiltrates in the liver.[5,6] It has a sensitivity of 82%-89% and a specificity of 93%, but cannot properly differentiate between steatosis and hepatic fibrosis. For detecting hepatic fibrosis, the gold standard is liver biopsy, which however is an invasive procedure and has its limitations.[5-7] Currently several studies are in progress for detecting hepatic fibrosis by means of non-invasive procedures. It has been reported that the serum concentration of type IV collagen may be used as a non-invasive alternative to liver biopsy for evaluation of hepatic fibrosis.

Epidemiology

NAFLD, a clinical condition that may advance to terminal hepatic disease, is found in 10-24% of the general population of various countries.[4,8] According to population studies based on ultrasound and liver function tests, the prevalence of NAFLD tends to increase by 17-33%, while that of NASH increases with 5.7-16.5%.[9]

NAFLD is associated with insulin resistance and the metabolic syndrome.[10-12] The prevalence of NAFLD is 50% in diabetic patients and 76% in obese patients.[10] The risk factors associated with NAFLD are obesity, diabetes mellitus type 2, and hyperlipidemia.[4] Approximately 67% of patients with body mass index (BMI) $\geq$30 kg/m^2 and more than 90% of patients with BMI $\geq$39 kg/m^2 tend to have steatosis.[5] Many NAFLD patients are asymptomatic, although a number of patients reportedly suffer from fatigue, malaise, and a sensation of fullness in the right upper abdominal quadrant. Hepatomegaly may be found in 50% of patients on initial examination.[4,5,9]

Etiology

There are several causative factors of fatty liver, such as drugs and metabolic disorders. Among the causes of macrovesicular steatosis, which is associated with an imbalance of hepatic

Table 1. Causes of fatty liver disease[4]

Nutritional	Drugs*	Metabolic or genetic	Other
Protein-calorie malnutrition†	Glucocorticoids†	Lipodystrophy†	Inflammatory bowel disease†
Starvation†	Synthestic estrogens†	Dysbetalipoproteinemia†	Small-bowel diverticulosis with bacterial overgrowth†
Total parenteral nutrition†	Aspirin‡	Weber-Christian disease†	Human immunodeficiency virus infection†
Rapid weight loss†	Calcium-channel blockers†	Wolman's disease§	Environmental hepatotoxins
Gastrointestinal surgery for obesity†	Amiodarone§	Cholesterol ester storage§	Phosphorus‡
	Tamoxifen†	Acute fatty liver of pregnancy‡	Petrochemicals†‡
	Tetracycline‡		Toxic mushrooms†
	Methotrexate†		Organic solvents
	Perhexiline maleate§		Bacillus circus toxins‡
	Valproic acid‡		
	Cocaine‡		
	Antiviral agents		
	Zidovudine†		
	Didanosine‡		
	Fialuridine‡		

* This is a partial list of agents that produce fatty liver. Some drugs produce inflammation as well. The association of fatty liver with calcium-channel blockers and valproic acid is weak, whereas the association with amiodarone is strong. Drug-induced fatty liver may have no sequelae (e.g., cases caused by glucocorticoids) or can result in cirrhosis (e.g., cases caused by methotrexate and amiodarone).

† This factor predorminantly causes macrovesicular steatosis (mostly owing to imbalance in the hepatic synthesis and export of lipids).

‡ This factor predominantly causes microvesicular steatosis (mostly owing to defects in mitochondrial function).

§ This factor causes hepatic phospholipidosis (mostly owing to the accumulation of phospholipids in lysosomes).

lipid synthesis, are such agents as glucocorticoids, calcium channel blockers, tamoxifen, and methotrexate, and disorders such as lipodystrophy and dysbetalipoproteinemia. In contrast, microvesicular steatosis is mostly the result of mitochondrial functional defects, e.g. those caused by valproic acid, aspirin, tetracycline, and acute fatty liver of pregnancy. Wolman's disease causes hepatic phospholipidosis due to accumulation of phospholipids in lysosomes. (Table 1)[4]

The metabolic syndrome, associated with insulin resistance (IR), is a collection of symptoms comprising diabetes, central obesity, hypertension and dyslipidemia.[13] IR is associated with increased production of free fatty acids in the liver from glucose; in addition adipose tissue is also a source of TNF-α which is a regulator of insulin sensitivity. TNF-α presumably increases insulin resistance by decreasing regulation of peroxisome-proliferator-activated receptor gamma (PPARγ), which is the most important receptor for maintaining insulin sensitivity within normal limits.[10,14]

In obese patients there is an increased hepatic uptake of free fatty acids, which subsequently undergo β-oxidation or esterification with glycerol, forming triglycerides that play a role in the accumulation of fat in the liver.[15]

NAFLD classification

NAFLD may be classified into four histopathological stages, with stage I being characterized by fatty infiltration of the liver,

Table 2. Grading and staging the histopathological lesions of nonalcoholic fatty liver disease[4]

Grading for steatosis
Grade 1: <33% of hepatocytes affected
Grade 2: 33% to 66% of hepatocytes affected
Grade 3: >66% of hepatocytes affected
Grading for steatohepatitis
Grade 1, mild
Steatosis: predominantly macrovesicular, involves up to 66% of lobules
Ballooning: occasionally observed; zone 3 hepatocytes
Lobular inflammation: scattered and mild acute inflammation (polymorphonuclear cells) and occasional chronic inflammation (mononuclear cells)
Portal inflammation: none or mild
Grade 2, moderate
Steatosis: any degree; usually mixed macrovesicular and microvesicular
Ballooning: obvious and present in zone 3
Lobular inflammation: polymorphonuclear cells may be noted in association with ballooned hepatocytes; pericellular fibrosis; mild chronic inflammation may be seen
Portal inflammation: mild to moderate
Grade 3, severe
Steatosis: typically involves >66% of lobules (panacinar); commonly mixed steatosis
Ballooning: predominantly zone 3; marked
Lobular inflammation: scattered acute and chronic inflammation; polymorphonuclear cells may be concentrated in zone 3 areas of ballooning and perisinusoidal fibrosis
Portal inflammation: mild to moderate
Staging for fibrosis
Stage 1: zone 3 perivenular, perisinusoidal, or pericellular fibrosis; focal or extensive
Stage 2: as above, with focal or extensive periportal fibrosis
Stage 3: bridging fibrosis, focal or extensive
Stage 4: cirrhosis

stage II by fatty infiltration with inflammation, stage III by fatty infiltration with ballooning degeneration, and stage IV by fatty infiltration with lesions similar to alcoholic hepatitis and sinusoidal fibrosis and by polymorphonuclear infiltrates with or without Mallory's hyaline. NAFLD stages III and IV are known as NASH.[1] The term NASH was first introduced by Ludwig to describe the lesions of alcoholic steatohepatitis (ASH) in patients who had no history of regular alcohol consumption and did not have other liver diseases.[14]

The grading and staging for steatosis, steatohepatitis, and hepatic fibrosis are based on histopathological criteria. (Table 2)[4]

Pathogenesis of NAFLD

Fatty acids in the liver are normally esterified into triglycerides, some of which are excreted by the hepatocytes in the form of very low density lipoproteins (VLDL). The elevated level of lipids, particularly triglycerides, in the hepatocytes of patients with NAFLD is the result of an imbalance between enzyme systems that promote the uptake and synthesis of fatty acids and those that promote the oxidation and export of fatty acids. (Figure 1)[4]

IR also plays an important role in the accumulation of lipids in hepatocytes, via two pathways, i.e. lipolysis (which increases circulating fatty acids), and hyperinsulinemia. High fatty acid uptake results in mitochondrial β-oxidation overload leading to fatty acid accumulation within the hepatocytes. Fatty acids are substrates and inducers of the microsomal lipoxygenases cytochrome P-450 2E1 and 4A. Cytochrome P-450 2E1, the concentration of which is elevated in patients with steatohepatitis, produces free oxygen radicals that are capable of inducing lipid

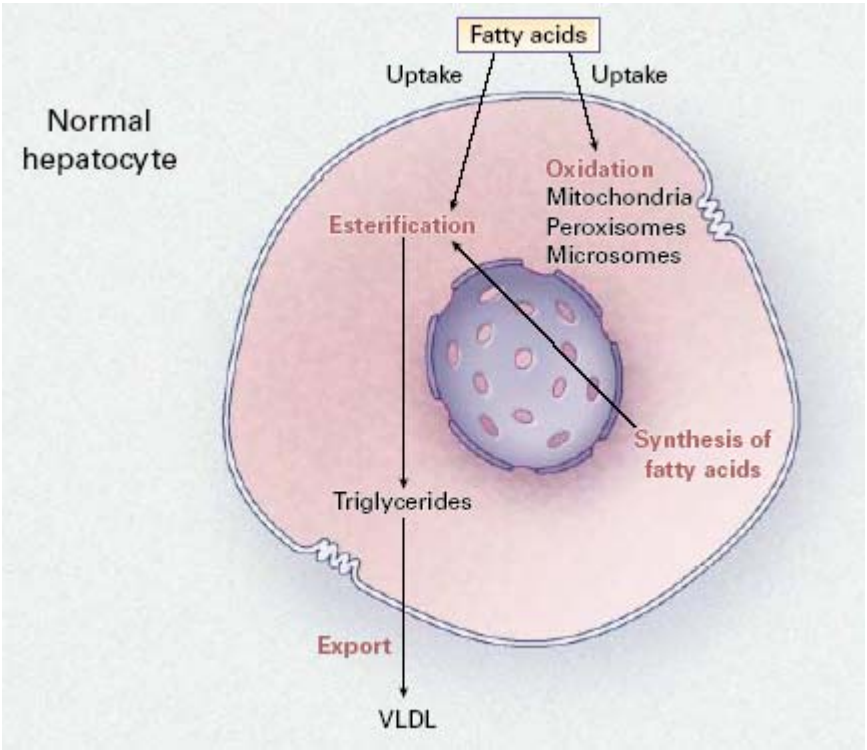

Figure 1. Pathogenesis of nonalcoholic fatty liver disease through imbalance between enzyme systems promoting uptake and synthesis of fatty acids and those promoting oxidation and export of fatty acids.[4]

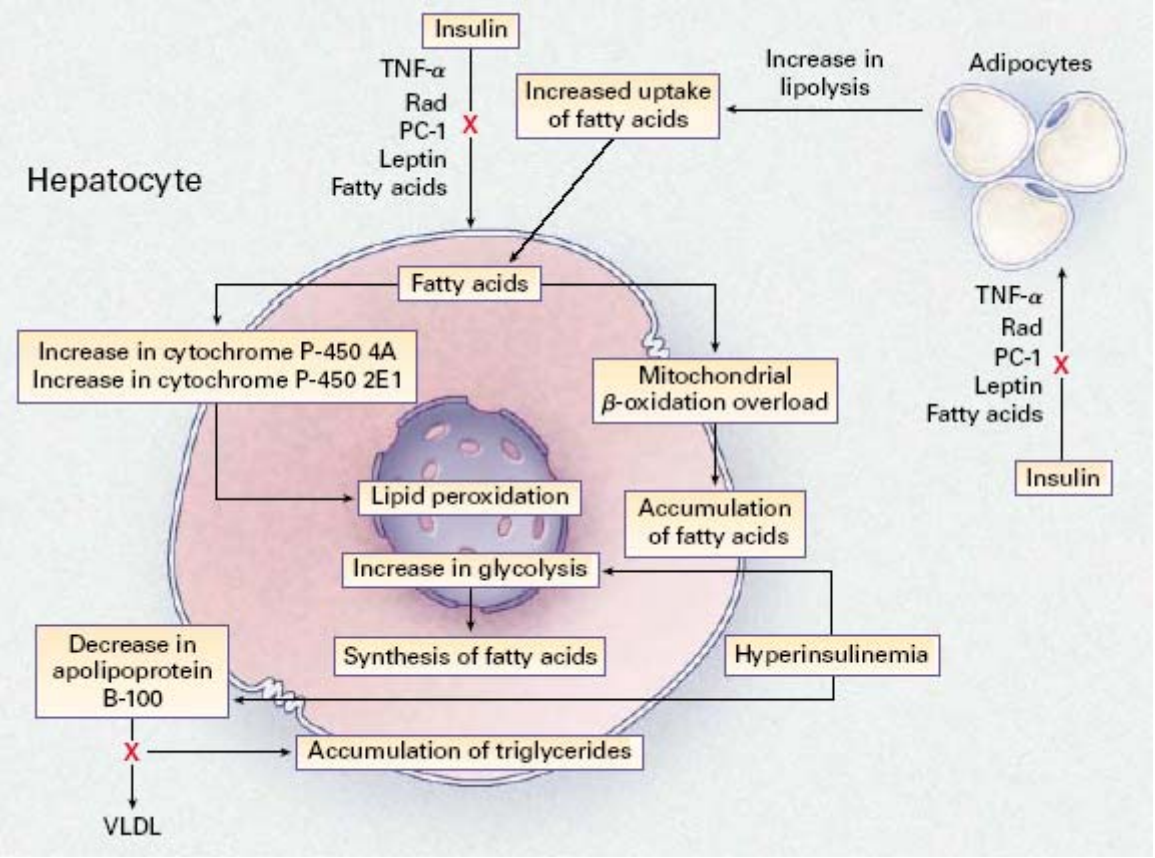

Figure 2. Pathogenesis of nonalcoholic fatty liver disease through insulin resistance.[4]

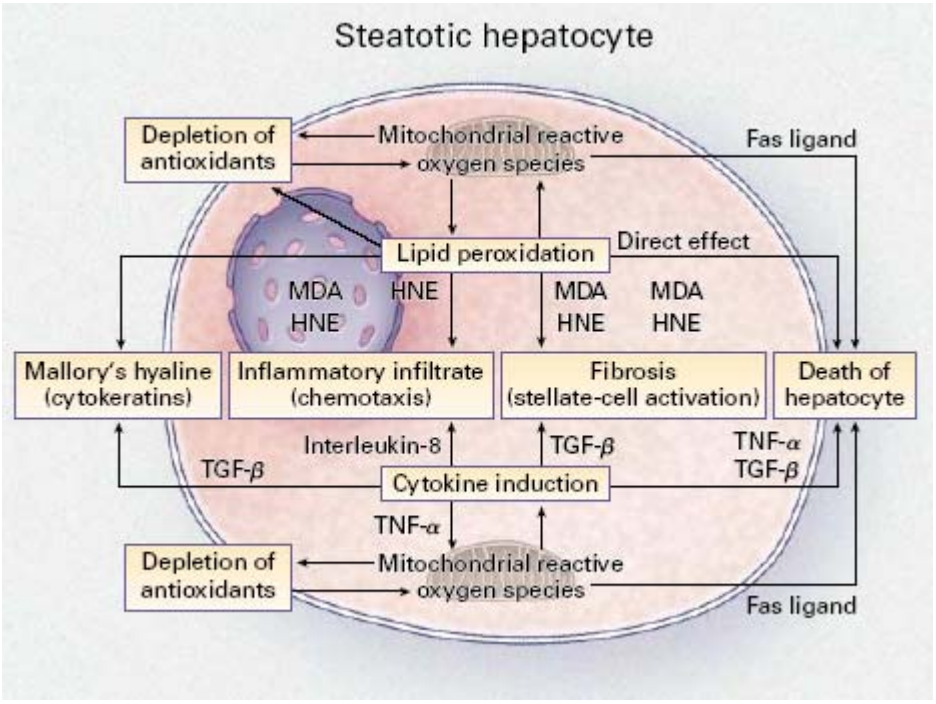

Figure 3. Pathogenesis of development of steatosis into steatohepatitis and fibrosis through lipid peroxidation, cytokine induction, and Fas ligand induction.[4]

peroxidation of hepatocyte membranes. Hyperinsulinemia due to IR increases glycolysis which subsequently increases fatty acid synthesis in hepatocytes and favors the accumulation of triglycerides within hepatocytes through decreased production of hepatic apolipoprotein B-100. (Figure 2)[4]

Mitochondrial reactive oxygen species promote progress of steatosis into steatohepatitis and fibrosis by three mechanisms, viz. lipid peroxidation, cytokine induction, and induction of Fas ligand. Reactive oxygen species (ROS) trigger lipid peroxidation, leading to cell death and production of malondialdehyde (MDA) and 4-hydroxynonenal (HNE). MDA and HNE cause cell death, are involved in the synthesis of Mallory's hyaline, and activate stellate cells, thus increasing collagen synthesis. ROS also induce the production of the cytokines TNF-α, transforming growth factor β (TGF-β) and interleukin-8. TGF-β activates stellate cells into synthesizing collagen, whereas interleukin-8 is a chemoattractant for neutrophils. Mitochondrial reactive oxygen species can activate Fas ligand in hepatocytes, which normally express the Fas receptor. Fas ligands may bind to Fas receptors on neighboring hepatocytes, thus killing these cells. (Figure 3)[4]

Hepatic fibrosis

Hepatic fibrosis is the excessive accumulation of extracellular matrix proteins in response to stimuli in acute or chronic liver disease.[16,17] Around 15%-40% of patients with NASH progress to hepatic fibrosis,[18] in which there occur changes in the number and quality of extracellular matrix proteins,[19] leading to structural changes in the liver through formation of fibrous scars.[16]

Pathogenesis of hepatic fibrosis

Fatty acid accumulation in the cytosol increases oxidation of the fatty acids in peroxisomes and endoplasmic reticulum. Peroxisomal oxidation reactions are catalyzed by acyl-Coa oxidase (AOX) with the formation of hydrogen peroxide. Oxidation in the endoplasmic reticulum is catalyzed by cytochromes P450 (CYP) 2E1, 4A10, and 4A14. Polyunsaturated fatty acids (PUFA) are the products of lipid peroxidation by reactive oxygen species (ROS), and the PUFA peroxidation products are trans-4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA). ROS and aldehydes can induce oxidative stress and cause cell death through depletion of ATP and NAD, damage to DNA and protein, and depletion of glutathione. ROS and lipid peroxidation products play a role in the formation of fibrosis through activation of collagen-synthesizing hepatic stellate cells. (Figure 4)[20]

Type IV collagen as noninvasive marker of hepatic fibrosis

Liver biopsy is the gold standard for determination of the severity of hepatic inflammation and fibrosis, while possessing limitations such as being painful and invasive to the patient.[19,21] In large studies, pain is a significant issue in 20% of cases and severe complications have been reported to occur in 0.57%.[22] The biopsy may represent only 1/50 000th of the liver, and sampling error has been shown to be an issue in a variety of liver diseases, including NAFLD.[23] All staging systems in widespread use share common failings that have been discussed at length.[24,25] Currently evaluation of hepatic fibrosis may be made through noninvasive methods, such as serology, imaging and genetics.[19,26] Non-invasive markers of liver fibrosis have been most extensively studied in the context of hepatitis C. There has been considerable interest in extending this work into the field of NAFLD because of the increasing prevalence of disease. The presumptive diagnosis of NAFLD is rapidly becoming the commonest cause for referral to hepatology outpatient clinics.[27] Markers of fibrosis in the

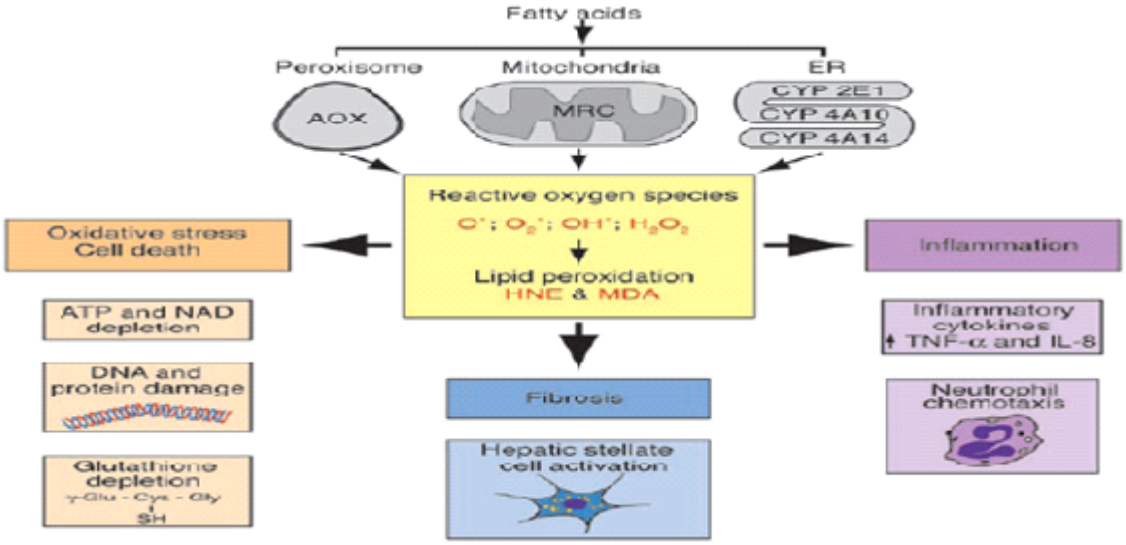

Figure 4. Mechanism of lipid-induced hepatic cell damage in NAFLD.[20]

blood may be divided into two types, i.e. direct markers that represent transformation of extracellular matrix and indirect markers that represent changes in hepatic functions. Direct serologic markers of fibrosis include those associated with matrix deposition—e.g. procollagen type III amino-terminal peptide (P3NP), type I and IV collagens, laminin, hyaluronic acid, and chondrex.[28]

Type IV collagen is a useful marker of hepatic fibrosis because its concentration is associated with the occurrence of hepatocellular decline and dysfunction, it plays a role in hepatocellular regeneration and repair and it is the first collagen to be synthesized by hepatocytes in experimental hepatic injury.[29]

Type IV collagen is composed of polypeptide α chains and is divided into 3 domains, i.e. the amino terminal 7S domain, the triple helical domain and the carboxy terminal globular non-collagenous (NC)-1 domain. The NC-1 domain is thought to play a role in the formation of the trimeric structure of type IV collagen.[30]

Type IV collagen is one of the main components of the basement membrane in addition to laminin (LN), heparan sulfate proteoglycan and nidogen/entactin, and is the principal component of all basement membrane proteins. Type IV collagen is capable of forming networks with itself and is thus also called network forming collagen; this property differentiates type IV collagen from other collagen types.[30] Type IV collagen is one of the essential components of the extracellular matrix, as it does not undergo proteolysis and is deposited intact in the matrix. The concentration of type IV collagen is indicative of matrix degradation. Type IV collagen is positively correlated with the stage of fibrosis in patients with chronic hepatitis. Patients with advanced hepatic fibrosis have elevated type IV collagen concentrations.[19,31]

The study by Yoneda et al. found that 7S collagen concentrations were increased in NASH patients with advanced fibrosis in comparison to NASH patients with moderate fibrosis.[31] Kubo et al. reported that in cases of hepatocellular carcinoma preoperative serum 7S collagen concentrations were correlated with post-operative hepatic failure and that a collagen concentration of ≥ 12 ng/mL was a relative contraindication for

correlated with stage of hepatic fibrosis and its determination may presumably be utilized for evaluation of hepatic fibrosis in patients with NASH.

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resection of the liver.[32] The study conducted by Sakugawa et al. also revealed that 7S collagen as well as hyaluronic acid (HA) may be used for differentiating NASH from simple fatty liver or patients with advanced fibrosis from those without advanced fibrosis.[33]

In other study, serum levels of HA, LN, and type IV collagen increased significantly in hepatic fibrosis, suggesting that detection of HA, LN, and type IV collagen level is an optimal choice.[34] Although various types of collagen like type I, III, IV, V and VI increase proportionally in the liver with the progression of fibrosis, type IV collagen, a constituent of the basement membrane, is particularly important and noteworthy for the following reasons: to relate the aggravation of hepatocellular damage and hepatocellular dysfunction, to play an important role in hepatocellular regeneration and the rearrangement of the lobular architecture and for being the earliest type of collagen to be synthesized by hepatocytes in experimental liver injuries.[35] Similar reports of elevated type IV collagen levels in serum were reported by Dos Santos et al.[36] One of the reasons why the field of non-invasive markers for NAFLD may lag behind hepatitis C is because of the uncertainty of which end point to measure. The current field is divided into studies attempting to distinguish between stages of fibrosis, fibrosis from NASH, and NASH from simple steatosis. Recently, investigators have published a panel test of non-invasive serum markers to diagnose steatosis alone within NAFLD.[37] The arguments of whether this is superior to ultrasound aside it highlights the uncertainty and open debate of which stages of NAFLD require diagnosis.

CONCLUSIONS

NAFLD and its advanced stage NASH can still progress to fibrosis and cirrhosis. Determination of the stage of hepatic fibrosis is performed by means of liver biopsy as the gold standard. Type IV collagen level is highly

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