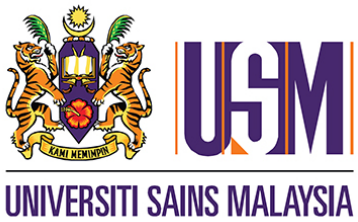
CORRELATION OF LIVER ELASTOGRAPHY WITH LIPID PROFILE, LIVER FUNCTION TEST, AND HbA1c IN TYPE II DIABETES WITH NON-ALCOHOLIC FATTY LIVER DISEASE IN HOSPITAL UNIVERSITI SAINS MALAYSIA

By

DR. SUBASHINI SUBBRAMANIAM

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE RADIOLOGY



SCHOOL OF MEDICAL SCIENCES

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2016

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Dissertation Submitted in Partial Fulfillment of the Degree of the Requirement for the Degree of Master of Medicine (Radiology)

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2016

SUPERVISOR:

DR. JUHARA BT HARON

DEDICATION

I would like to dedicate this thesis to my family and my husband Kasirajan who has shown an incredible amount of strength and faith in me.

ACKNOWLEDGEMENT

Firstly I would like to express my gratitude and utmost thanks to my main and co-supervisors, Dr. Juhara Bt Haron and Dr. Wan Mohd Izani bin Wan Mohamed, who have guided me throughout this study. I really appreciate their invaluable comments, advices, technical support, and patience for me till the completion of this study. I really in debt to all the staff in Radiology Department and Diabetic Clinic, Hospital Universiti Sains Malaysia for the technical help and allowing me to conduct my study in the department. I would like to thanks my friends that continuously motivate me from proposal writing, data collection to completion of thesis writing.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

ALT	Alanine transaminase
AST	Aspartate transaminase
AUROC	Area Under the Receiver Operating Characteristic
g/L	Gram per liter
HbA1c	Glycated hemoglobin
HDL	High density lipoprotein
HUSM	Hospital Universiti Sains Malaysia
LDL	Low density lipoprotein
METAVIR	Meta-analysis of Histological Data in Viral Hepatitis
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
T2DM	Type ii diabetic mellitus
TC	Total cholesterol
TG	Total glyceride

CORRELATION OF LIVER ELASTOGRAPHY WITH LIPID PROFILE, LIVER FUNCTION TEST, AND HbA1c IN TYPE II DIABETES IN HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRACT

Background

Non-alcoholic fatty liver disease (NAFLD) is a range of liver disorders which ranges from simple accumulation of fat in the hepatocytes (steatosis) to macrovescicular steatosis, periportal and lobular inflammation (steatohepatitis). The current gold standard to diagnose liver diseases is biopsy, however this has two major problems: it is dependent on the sampling area, which may cause an evaluation error in the case of mild fatty liver, and it is an invasive technique which carries a morbidity rate of 1-5% and a mortality rate of 0.01-0.1%. Non-invasive imaging tools such as shear wave elastrography has been proven to be useful for screening and categorizing of chronic liver disease, however, further evaluation of this tool is needed in different population.

Aim and Objective

This study aimed to assess the associations among liver elastography, lipid profile, liver function test and HbA1c among type II diabetic patient in Hospital Universiti Sains Malaysia, in which (i) the prevalence and incidence rate of NAFLD and liver fibrosis/cirrhosis among type II diabetic patient in Hospital Universiti Sains Malaysia was determined, respectively; (ii) the mean values of the blood lipid profile, liver functions test and HBA1c among patient with and without NAFLD were compared; and

(iii) the associations between degree of liver elasticity; and blood lipid profil, liver function test and HBA1c were investigated.

Method

Patients were recruited from Diabetic Clinic, HUSM. Only patients who fulfilled the selection criteria and provided written consent were enrolled into this study. The Medical record of the patient was provided by the clinic. Liver elastrography was performed in Radiology Department, HUSM by Supersonic Imagine Using Shear Wave Elastography (Siemens Acuson Antares -Premium Edition, Camberley, UK), equipped with Aixplorer Multiwave. Data were recorded and analysed using SPSS version 22.0.

Result

The prevalence of NAFLD among participant with DM type II was 87%, while the incidence rate of liver fibrosis among the patients with NAFLD was 11.7% (8/68). The distribution of METAVIR score among T2DM patients were F0 (60/68), F1 (7/68), and F2 (1/68). The result revealed very poor correlation between SWE values and part of the study parameters, which includes HDL, LDL, TG, AST and HbA1c ($P>0.05$). However, there were two parameters showed significant weak association between SWE values; TC ($r=0.283$; $P=0.019$) and ALT ($r=0.252$; $P=0.038$).

Conclusion

This study had determined to the prevalence of NAFLD and incidence rate of liver fibrosis among T2DM patients admitted to HUSM. The prevalence of NAFLD was higher but the incidence rate of liver fibrosis was lower compared to previous study.

Further research with inclusion of parameters like race, AST/ALT ratio and advanced fibrosis patients to strengthen the finding of current conclusion.

KORELASI ANTARA ELASTOGRAPHY HATI DENGAN PROFIL LIPID, UJIAN FUNGSI HATI, DAN HbA1c DALAM KALANGAN PESAKIT DIABETES JENIS II DI HOSPITAL UNIVERSITI SAINS MALAYSIA

ABSTRAK

Latar Belakang

Penyakit perlemakan hati bukan alcohol (NAFLD) adalah penyakit gangguan hati yang luas spectrum, bermula dari pengumpulan lemak dalam hepatosit (steatosis) sehingga menjadi steatosis macrovaskular, keradangan periportal dan seterusnya lobular (steatohepatitis). Standard diagnosis semasa untuk NAFLD ialah biopsi. Namun cara ini mempunyai dua kelemahan utama: (i) ia bergantung kepada kawasan persampelan, yang mungkin menyebabkan kesilapan penilaian dalam kes hati berlemak ringan, dan (ii) ia adalah satu teknik invasif yang mungkin membawa kepada kadar morbiditi 1-5% dan kadar kematian sebanyak 0,01-0,1%. Alat pengimejan tidak invasif seperti *shear wave elastrography* telah terbukti berguna untuk pemeriksaan dan mengkategorikan tahap penyakit hati kronik, bagaimanapun, penilaian lanjut alat ini diperlukan dalam populasi yang berbeza.

Matlamat dan Objektif

Kajian ini bertujuan untuk menilai perkaitan antara elastografi hati, profil lipid, ujian fungsi hati dan paras gula puasa dalam darah di kalangan pesakit kencing manis jenis II (T2DM) di Hospital Universiti Sains Malaysia, di mana (i) prevalens NAFLD dan kadar insiden cystic hati / sirosis di kalangan T2DM di Hospital Universiti Sains Malaysia

telah ditentukan; (ii) nilai min profil lipid darah, fungsi hati dan ujian HbA1c di kalangan pesakit dengan dan tanpa NAFLD dibandingkan; dan (iii) perkaitan antara tahap keanjalan hati; dan profil lipid darah, ujian fungsi hati dan HbA1c telah disiasat.

Kaedah

Pesakit telah dipilih dari Klinik Diabetik, HUSM. Hanya pesakit yang memenuhi kriteria pemilihan dan memberi persetujuan bertulis akan dipelawa untuk kajian ini. Rekod perubatan pesakit telah disediakan oleh klinik. Elastrografi hati telah dilakukan di Jabatan Radiologi, HUSM dengan menggunakan Supersonic Imagine Using Shear Wave Elastography (Siemens Acuson Antares -Premium Edition, Camberley, UK), equipped Aixplorer Multiwave. Data telah direkodkan dan dianalisis dengan menggunakan SPSS.

Keputusan

Prevalens NAFLD antara pesakit T2DM adalah 87% (68/78), manakala kadar insiden cystic hati / sirosis di kalangan pesakit NAFLD adalah 11.7% (8/68). Skor METAVIR pesakit-pesakit T2DM adalah F0 (60/68), F1 (7/68), dan F2 (1/68). Hasil kajian ini mendedahkan yang hubungan yang sangat lemah dan tidak ketara (P> 0.05) antara nilai-nilai SWE dengan sebahagian daripada parameter-parameter kajian, termasuk HDL, LDL, TG, AST dan HbA1c. Walau bagaimanapun, terdapat dua parameter menunjukkan perkaitan lemah yang ketara dengan nilai-nilai min SWE; TC (r = 0,283; P = 0.019) dan ALT (r = 0,252; P = 0.038).

Kesimpulan

Kajian ini telah menentukan prevalans NAFLD dan kadar insidens cystic hati / sirosis di kalangan pesakit T2DM di HUSM. Prevalens NAFLD adalah lebih tinggi berbanding dengan kajian lepas. Namun, kadar insiden cystic hati / sirosis adalah lebih rendah. Kajian seterusnya dengan pertimbangan unsur-unsur seperti etnik, nisbah AST/ALT dan pesakit fibrosis serius harus dikaji untuk memperkukuhkan hasil kajian sekarang.

CORRELATION OF LIVER ELASTOGRAPHY WITH LIPID PROFILE, LIVER FUNCTION TEST, AND HbA1c IN TYPE II DIABETES IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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Department of Radiology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia

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SECTION ONE

INTRODUCTION

CHAPTER 1

INTRODUCTION

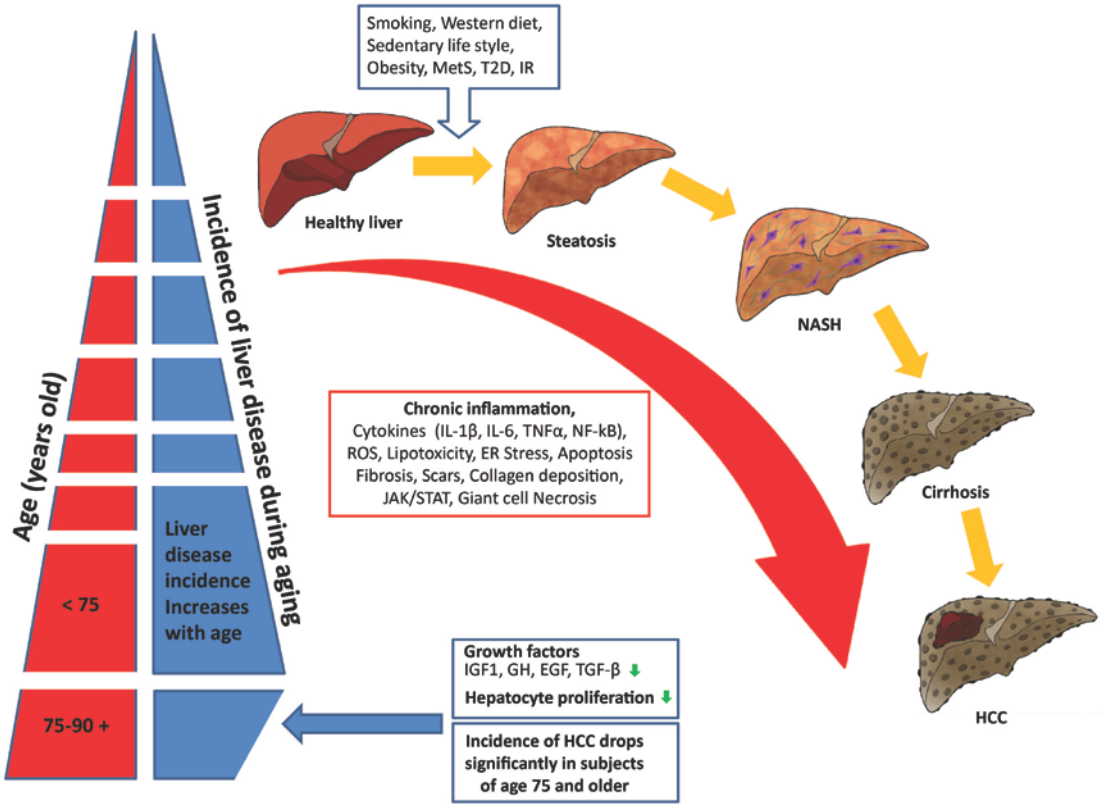
1.1 Background of the Study

In developing country such as Malaysia, metabolic syndrome such as NAFLD and DM has become major health problem in our community due to our sedentary life style and bad eating habits. NAFLD and T2DM are co-exist because they share the common risk factors (Macabuag-Oliva *et al.,* 2014). Obesity, insulin resistance and increased plasma fatty acid concentrations are considered to increase the risk of fatty liver, which are also the characteristic of T2DM. The incidence of NAFLD and fibrosis progression, including hepatocellular carcinoma, has been found to be significantly higher in diabetic patients compared with non-diabetic patients.

Paralleling the increasing prevalence of obesity, diabetes mellitus, and the metabolic syndrome in the general population, nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide (Browning *et al.,* 2004). NAFLD refers to the accumulation of hepatic steatosis, independent of excess alcohol consumption and its effects on the liver. It is a broad continuum of liver illnesses extending from the rather benign steatosis to the severe cryptogenic cirrhosis. Steatosis, or fatty infiltration of the liver, can progress to nonalcoholic steatohepatitis (NASH). Steatohepatitis, in turn, can progress to permanent liver damage in the form of cirrhosis or malignancy; 3% to 5% of patients with NAFLD progress to NASH, and 15% of those with NASH develop cirrhosis (Levinthal and Tavill, 1999; Smith and Adams, 2011)

Within the spectrum of NAFLD, simple bland steatosis often remains stable for a number of years and will probably never progress in many patients (Teli *et al.*, 1995; Dam-Larsen *et al.*, 2004). A subset of patients, however, particularly those with more advanced fibrosis, are at a higher risk for progressing to decompensated cirrhosis, portal hypertension, HCC, or death if liver transplantation is not accomplished (Dam-Larsen *et al.*, 2004; Ekstedt *et al.*, 2006). In contrast to patients with bland steatosis, patients with increased liver fibrosis require close follow-up with surveillance for the development of esophageal varices and HCC and enrollment into treatment trials. Thus, identifying the presence and severity of liver fibrosis in patients with NAFLD is of major importance in guiding the subsequent management of patients with this liver condition.

The life span of human population in developed and developing countries has increased due to the improvement of health and welfare policy. Aging markedly increases the prevalence of the metabolic syndrome in the human population (Ford *et al.*, 2002). Accumulating evidence also points toward an increased prevalence of NAFLD with older age in humans (Floreani, 2007). Sheedfar *et al.* (2013) has described the association between NAFLD and aging. This disease has a trend that increase with aging process, but significantly reduced in very elderly population, as illustrated in Figure 1.1. Although it is much debated whether insulin resistance is a cause of age-associated metabolic disturbances or rather a protective adaptive mechanism (Barzilai and Ferrucci, 2012). It is generally believed that age is a risk factor for increased hepatic steatosis. Fat may accumulate in the liver as a result of multiple abnormalities of hepatic lipid metabolism, although the mechanisms that underlie this age-related liver steatosis are yet to be clearly defined. These mechanisms may include enhanced fat uptake, increased *de novo* lipogenesis, decreased β-oxidation, and/or decreased synthesis/secretion of very low-density lipoproteins (Cohen *et al.*, 2011).



Note: The incidence of liver diseases in humans increases with age up to 75 years. Interestingly, subjects aged more than 75 have a significantly reduced incidence of HCC (left). The different states of liver disease progression (NAFLD, NASH, cirrhosis and HCC) and the respective molecular changes are represented on the right. Adopted from Sheedfar *et al.* (2013).

Figure 1.1 Schematic illustration of the pattern of liver disease progression during aging

The prevalence of NAFLD is up to 30% in developed countries and nearly 10% in developing nations, making NAFLD the most common liver condition in the world. One in 3 adult Americans and 1 in 4 or 5 adult Italians suffer from NAFLD (Browning *et al.*, 2004; Bedogni *et al.*, 2005). NAFLD also has reached epidemic proportions among populations typically considered at low risk, with a prevalence of 15% in China and 14% in Japan (Nomura *et al.*, 1988; Fan *et al.*, 2005). The underlying relationship between NAFLD and DM is still under discovery, and pharmacogenetic factor might be considered as the contributing factor, suggested by Firneisz (2014).

In the absence of decompensated cirrhosis, liver biopsy remains the only reliable means to determine prognosis based on the severity of fibrosis. However, liver biopsy is an expensive and invasive procedure associated with a number of complications and prone to sampling error. Because of all this, efforts have been made to identify noninvasive indicators of liver fibrosis in patients with NAFLD. Noninvasive approaches for assessing the severity of fibrosis in NAFLD have included a combination of clinical features and routine laboratory investigations as well as some less readily available serum markers of fibrosis (Angulo *et al.*, 1999; Dixon *et al.*, 2001; Rosenberg *et al.*, 2004). These noninvasive approaches are, however, either insufficiently accurate in their prediction of liver fibrosis or their diagnostic accuracy has been evaluated in only a limited number of patients with NAFLD; further, most have not been validated in a separate population of NAFLD patients (Angulo *et al.*, 1999).

This study utilized SWE for screening of NAFLD among targeted population which is T2DM patient. Significant and useful finding were found from these analysis, as the authors found high correlation between SWE reading and the conventional METAVIR, the liver fibrosis scaling system. SWE cut-off value for METAVIR scoring system was established and can be used to categorize liver fibrosis status of a patient (Ferraioli *et al.*, 2014a). It is easy and produced fast result as compared to the gold standard, liver biopsy. This could improve the management of NAFLD as well as other chronic liver in HUSM. By the way, all noninvasive methods are ready to be used for detecting and staging liver fibrosis before therapy at a safe level of predictability. As with transient elastography, elastographic techniques based on shear waves generated by the acoustic beam are more accurate in detecting cirrhosis than significant fibrosis.

They have the advantage of B-mode guidance, which allows one to choose an area of liver parenchyma better suited for stiffness assessment (i.e. free of large vessels and focal lesions). Nevertheless, these methods are all valid when information about fibrosis is needed. Liver biopsy should still be performed when biochemical tests and imaging studies are inconclusive or information other than liver fibrosis is required (Ferraioli *et al.*, 2014a; Ferraioli *et al.*, 2014b).

1.2 Rationale of the Study

NAFLD has been reported among hypercholesterolaemic patient is Hospital Universiti Sains Malaysia with the incident rate of 56.7%. The NAFLD was defined by using visual scoring system using high sensitivity B-mode ultrasound, Pentax-Hitachi EUB6500 (Tokyo, Japan). Two experienced radiologists were blinded to the clinical and metabolic conditions of the subjects. The US evaluation consisted of a visual scoring system which assigned to focal hyperechoic hepatic areas of interest a value between 0 and 2. Hepatorenal echodiscrepancy, posterior echo penetration and portal vein wall clarity were the zones where echogenicity were evaluated. A total score >3 was interpreted as having a fatty liver, with grade 3 being mild, 4 moderate and 5-6 severe. The subjects were examined in the supine position. Anyhow, the data on the type II diabetic patients is still under-report (Magosso *et al.*, 2010). The incident of liver fibrosis and cirrhosis were also not reported. This is probably due to the conventional invasive liver biopsy for analysing of the degree of liver fibrosis progression. Shearwave elastography has been introduced for detection and grading of liver fibrosis and cirrhosis. This is a non-invasive technique for analysis of liver stiffness which offers easier clinical management for detection of NAFLD as compared to conventional liver biopsy. Thus far, this non-invasive technique was not applied for routine screening

purposes due to lack of supporting study of its usefulness in local population. In this study we would like to assess the correlation of liver elastography with lipid profile, liver function and HbA1c in T2DM patient in Hospital Universiti Sains Malaysia (HUSM). This information will provide preliminary data on prevalence of NAFLD and liver cirrhosis/fibrosis using elastography among the T2DM patient in HUSM using SWE method, and to look for its correlation with parameters that might affect the disease. These data might help to support the establishment of SWE method for routine liver fibrosis screening.

SECTION TWO

LITERATURE REVIEW

CHAPTER 2

LITERATURE RIVIEW

2.1 Diabetic Mellitus

Diabetes is an incurable chronic disease. It is a metabolic disorder in which the body unable to produce or produce too little or unable to properly use insulin, a hormone that is important in sugar metabolism. In diabetic patient, the body is unable to properly store and use the energy found in food. Human body cells need sugar as fuel for cell metabolism. Sugar or glucose cannot pass through cells directly. When blood sugar rises, beta cells in the pancreas secrete insulin. Insulin binds to cells and trigger the absorption of blood sugar; the blood sugar level in bloodstream decrease. Thus, insulin is generally known as the key factor that signal blood sugar absorption by body cells and be used for cell metabolism. The cause of diabetic is still under controversy. Genetics and environment factors is the two major contributing factors to this metabolic syndrome (Malaysian-Diabetes-Association, 2013). Generally, diabetes is divided into two categories, namely Type I or Type II diabetes.

2.2 Diabetes in Malaysia

According to International-Diabetes-Federation (2015), Malaysia is one of the 23 countries and territories of the IDF West Pacific region. There were 387 million people have diabetes in the world and almost 138 million people in the West Pacific region; by 2035 this will rise to 202 million. There were 3.2 million cases of diabetes in Malaysia in 2014. The prevalence of diabetes is increasing at 0.2% per year according to Ministry-of-Health-Malaysia (2010).

2.3 Types of Diabetes and Complications

Type I diabetes is also known as insulin-dependent diabetes mellitus. This disease is usually onset at child or young adult stage. It is also call juvenile-onset diabetes. This metabolic disorder onset when the body ceases to produce or produce too little insulin. Type II diabetes is also known as non-insulin-dependent diabetes mellitus. This disease is usually presented as maturity-onset diabetes, as most of the individuals who get this disease are over 40 years old. This is the most common diabetes, with the ratio os 10:1 with type I diabetes. In type II diabetes individuals, human body does not make sufficient insulin or human body could not use the insulin been produced (UMMC, 2015). Patients with diabetes have higher death rates than people who do not have diabetes regardless of sex, age, or other factors (UMMC, 2015). Heart disease and stroke are the leading causes of death in these patients. People with type 2 diabetes are also at risk for nerve damage (neuropathy) and abnormalities in both small and large blood vessels (vascular injuries) that occur as part of the diabetic disease process. Such abnormalities produce complications over time in many organs and structures in the body, e.g. kidney, foot, eyes, etc. Diabetes also increases the risk for developing other conditions, including:

- Hearing loss
- Periodontal disease
- Carpal tunnel syndrome
- Nonalcoholic fatty liver disease (NAFLD), also called nonalcoholic steatohepatitis (NASH), a particular danger for people who are obese
- Cancers of the liver, pancreas, and endometrium and, to a lesser extent, colon and rectum, breast, and bladder

2.4 Liver and Type II Diabetic Mellitus

According to Levinthal and Tavill (1999), the liver plays an important role in regulation of carbohydrate homeostasis. It is important to understand the alteration and changes in physical and biochemical component of liver in diabetes. The liver uses glucose as a fuel and also has the ability to store it as glycogen and synthesize it from non-carbohydrate precursors (gluconeogenesis). Glucose absorbed from the intestinal tract is transported via the portal vein to the liver. Although the absolute fate of this glucose is still controversial, some authors suggest that most of the absorbed glucose is retained by the liver so that the rise in peripheral glucose concentration reflects only a minor component of postprandial absorbed glucose. Diabetes mellitus is known to cause many complications to the liver such as glycogen deposition, fatty liver, and cirrhosis. Excess glycogen accumulation in the liver is seen in 80% of diabetic patients. Glycogen synthesis in the liver is impaired in diabetes due to defective activation of glycogen synthase. Almost 40 to 70% of diabetes patient have hepatic fat accumulation. Type 2 diabetes mellitus is strongly associated with fat accumulation regardless the glucose control. Fat is stored in the form of triglyceride and may be a manifestation of increased fat transport to the liver, enhanced hepatic fat synthesis, and decreased oxidation or removal of fat from the liver. Diabetes mellitus is also known to increase the risk of steatohepatitis subsequently leading to cirrhosis. The prevalence of Type 2 diabetes is expected to increase in parallel with obesity rates and the ageing population. Recent studies show that Type 2 diabetes is associated with a twofold increase in the risk of non-alcoholic fatty liver disease, a leading cause of chronic liver disease (Tai *et al.*, 2015). Individuals with non-alcoholic steatohepatitis, a more advanced stage of non-alcoholic fatty liver disease, are specifically at risk of developing fibrosis/cirrhosis (end-stage liver disease) and hepatocellular carcinoma; therefore, identifying individuals

(with Type 2 diabetes) who are likely to develop hepatic complications is paramount (Tai *et al.*, 2015).

2.5 Non-Alcoholic Fatty Liver Disease

Parallel with increase in obesity, there is an increase in obesity-associated diseases such as type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD). NAFLD has become the most common cause of liver disease worldwide. Non-alcoholic fatty liver disease (NAFLD) encompasses a spectrum of pathological conditions. The early stage of NAFLD starts with lipid accumulation in the liver also known as hepatic steatosis in the absence of excessive alcohol consumption (typically a threshold of 20 g/d for women and 30 g/d for men is adopted). This benign and reversible state of NAFLD may, however, evolve into non-alcoholic steatohepatitis (NASH), a condition of inflamed liver, which can further progress to liver fibrosis, cirrhosis and ultimately hepatocellular carcinoma (Ahmed *et al.*, 2010; Ratziu *et al.*, 2010; Anstee *et al.*, 2011). NAFLD has emerged as a growing public health problem worldwide that has reached epidemic proportions and is the most common cause of chronic liver disease in many developed countries. NAFLD increases the risk of end-stage liver disease, and NAFLD-induced liver failure is one of the most important reasons for liver transplantation. NAFLD is also strongly associated with overweight/obesity, insulin resistance, and type 2 diabetes (T2DM) (Ahmed *et al.*, 2010; Ratziu *et al.*, 2010; Anstee *et al.*, 2011). NAFLD is a manifestation of pathological ectopic fat accumulation combined with a low-grade chronic inflammatory state in an organ that is not able to accumulate fat. This condition is still poorly recognized by endocrinologists and general physicians, and recent work is now suggesting putative mechanisms by which NAFLD not only increases risk of developing T2DM, but also worsens glycemic control and contributes

to the pathogenesis of major chronic complications of diabetes, such as cardiovascular disease (CVD) and chronic kidney disease (CKD), in people with established T2DM.

2.6 Diagnosis of NAFLD

2.6.1 Liver biopsy

The diagnosis of chronic liver disease is often made from needle biopsy samples. Liver biopsy and histological analysis are considered the diagnostic reference standard for the assessment of fatty liver. Histological assessment provides information about the fat distribution within the hepatic lobules and allows for a semi-quantitative evaluation of steatosis (Joseph *et al.*, 1991; Chitturi *et al.*, 2007). Microscopically, the steatosis pattern can be divided into macro- and micro-vesicular steatosis with hepatocytes containing either one large vacuole of fat which is larger than the hepatocyte nucleus and displaces the nucleus or many small fatty cytoplasmatic inclusions without a significant nuclear displacement Macro-vesicular steatosis is more common and is found in NAFLD but also in alcoholic fatty liver disease (Oleszczuk *et al.*, 2007). In contrast, micro-vesicular steatosis is generally a more severe form of steatosis and is seen in a variety of conditions such as toxicity of several medications, defect in beta-oxidation of fatty acids, Reye's syndrome but also in alcoholism.

In addition to establishing a disease etiology, liver biopsy can determine the degree of inflammation present and can stage the degree of fibrosis. The degree of inflammation and fibrosis may be assessed by different scoring schemes. Histological (biopsy) diagnosis classifies the severity of fibrosis into five stages, S0 to S4. S0 means no fibrosis. S4 is cirrhosis. In between, S1 is a mild fibrosis only seen at the portal area. S2 is a moderate stage of fibrosis, between portal areas, but without the destruction of

the lobular structure. S3 is severe fibrosis. At this stage, there is fibrostic bridging between portal areas and between portal areas and center veins. At S4, in addition to S3's changes, there are pseudo-lobules formed and this stage is the final stage, cirrhosis.

The METAVIR score is an established method helps pathologist to interpret a liver biopsy (Kaplan and Bonis, 2009). When this biopsy is performed, clinician needs a reliable way to quantify what is seen under the microscope. This scoring system assigns two standardized numbers: one to represent the degree of inflammation and the other the degree of fibrosis. The activity, which is the amount of inflammation (specifically, the intensity of necro-inflammatory lesions), is graded on a 4-point scale from A0 to A3.

Fibrosis score:

- F0 = no fibrosis
- F1 = portal fibrosis without septa
- F2 = portal fibrosis with few septa
- F3 = numerous septa without cirrhosis
- F4 = cirrhosis

Activity score:

- A0 = no activity
- A1 = mild activity
- A2 = moderate activity
- A3 = severe activity

There are several limitations to liver biopsy, including its invasive nature, small tissue sample size, and subjective grading system. Regarding small tissue sample size, liver fibrosis can be patchy and thus missed on a biopsy sample, which includes only 0.002% of the liver tissue (Bedossa *et al.*, 2003; Colloredo *et al.*, 2003; Schiano *et al.*,

2005; Chitturi *et al.*, 2007). A noninvasive alternative to liver biopsy would be particularly helpful, both to initially assess patients and then as a monitoring tool to assess response to therapy (Schwenzer *et al.,* 2009).

2.6.2 Non-Invasive Method

2.6.2 1 Multi-analyte Assays

A variety of non-invasive laboratory tests are being evaluated as an alternative to liver biopsy. The rapid development of new medications for the treatment of some liver diseases, such as CHB, CHC, and nonalcoholic fatty liver disease (NAFLD), increases the requirement for more frequent evaluation of liver fibrosis to assess treatment response. Liver biopsies are not ideal for frequent evaluations (Fallatah, 2014). The typical mechanism underlying the development of hepatic fibrosis is an imbalance between the deposition and removal of extracellular matrix (ECM). Hepatic stellate cells are the predominant producers of ECM, and their activation and proliferation are mediated by different cytokines during the process of liver injury. The activation and proliferation of Hepatic stellate cells ultimately result in an excessive deposition of ECM. In advanced fibrosis, the ECM may increase sixfold compared with that in normal liver.

Biochemical tests can be broadly categorized into indirect and direct markers of liver fibrosis. The direct markers directly correlate with or are parts of the liver matrix produced by the Hepatic stellate cells during ECM turnover in the fibrosis process. Direct markers linked to matrix deposition include Procollagen type 1 and type III; Type IV collagen; Hyaluronic acid and Laminin. Direct markers linked to matrix degradation include MMP-1 (collagenases), MMP-2 (gelatinase-A), MMP-9

(gelatinase-B), and Tissue inhibitors of matrix metalloproteinases (TIMPs). Cytokines and chemokines linked to liver fibrosis include transforming growth factor-β (TGF-β1) and transforming growth factor alpha (TGF-α). Indirect markers include liver function tests such as ALT (alanine aminotransferase) as described by Sebastiani and Alberti (2006), AST (aspartate aminotransferase), the ALT/AST ratio, also referred to as the AAR proposed by McPherson *et al.* (2010), AST/platelet ratio (APRI) proposed by Wai *et al.* (2003), The Forns Index (Forns *et al.*, 2002), The PGA Index (CHAPUT, 1991), fibro test and fibrosure (Baranova *et al.*, 2011). The first three markers were frequently used for prediction of NAFLD, while the latter were commonly applied for prediction of alcoholic liver fibrosis. Besides serum markers, lipid profile such as HDL, LDL, TG and TC had found to be altered among individuals with NAFLD (Mahaling *et al.*, 2013).

2.6.2 2 Imaging Technologies

Non-invasive imaging technologies to detect liver fibrosis or cirrhosis among patients with chronic liver disease are also being evaluated as an alternative to liver biopsy. The noninvasive imaging technologies include Shear Wave Elastrography (SWE), transient elastography (eg, FibroScan®), magnetic resonance elastography (MRE), ARFI (eg, Acuson S2000™), and real-time tissue elastography (RTE; eg, HI VISION Preirus). Noninvasive imaging tests have been used in combination with multianalyte serum tests such as FibroTest or FibroSURE with FibroScan. Liver elasticity is the measurement of tissue stiffness in liver is related to tissue composition, which is changed by cirrhosis, hepatocellular carcinoma, or metastasis.

2.6.2.2.1 Ultrasound

In ultrasound (US) images, hepatic steatosis appears as a diffuse increase in echogenicity due to the increased parenchymal reflectivity caused by intracellular accumulation of fat inclusions. Ultrasound is accepted as an initial screening for fatty liver since it is non-invasive, inexpensive and widely available (Saverymuttu *et al.*, 1986; Ricci *et al.*, 1997; Palmentieri *et al.*, 2006; Roldan-Valadez *et al.*, 2008). Nevertheless, the sensitivity and specificity of ultrasound in detecting hepatic steatosis are a constant matter of debate. The previous studies demonstrated that ultrasonography has a sensitivity of 60–94% and a specificity of 66–95% in detecting fatty liver (Foster *et al.*, 1980; Debongnie *et al.*, 1981; Steinmaurer *et al.*, 1984; Saverymuttu *et al.*, 1986; Graif *et al.*, 2000). In most studies, liver biopsy was used as the gold standard. The specificity could be increased up to 100% if known liver disease, drug use or alcohol consumption were ruled out (Hamaguchi *et al.*, 2007). Several studies demonstrated that ultrasound cannot reliably distinguish between fibrosis and steatosis (Taylor *et al.*, 1981; Meek *et al.*, 1984). Others concluded that it is possible to differentiate between fibrosis and hepatic steatosis, at least at higher degrees of fibrosis leading to an increase in coarse echoes without posterior beam attenuation (Saverymuttu *et al.*, 1986; Palmentieri *et al.*, 2006). Although ultrasound has been shown to have an acceptable level of sensitivity it does not provide reproducible quantitative information.

2.6.2.2.1 (i) B-mode

In B-mode (brightness mode) ultrasound, a linear array of transducers simultaneously scans a plane through the body that can be viewed as a two-dimensional image on screen. It is commonly known as 2D mode. Toyoda *et al.* (2009) had performed a study on liver fibrosis using B-mode ultrasound with algorithm based on statistical analysis of

signals. The result revealed the grades of liver fibrosis in patients with chronic hepatitis C are well discriminated with the B-mode ultrasound–based analysis algorithm without discrimination between grades F0 and F1. This showed that findings on conventional ultrasound images may reflect progression of liver fibrosis even in the absence of cirrhosis.

2.6.2.2.1 (ii) Transient Elastography

Transient elastography (FibroScan®) uses a mechanical vibrator to produce mild amplitude and low-frequency (50 Hz) waves, inducing an elastic shear wave that propagates throughout the liver. US tracks the wave, measuring its speed, which correlates with liver stiffness. Increases in liver fibrosis also increase liver stiffness and resistance of liver blood flow. Transient elastography does not perform as well in patients with ascites, higher body mass index, or narrow intercostal margins. Although FibroScan may be used to measure fibrosis, unlike liver biopsy, it does not provide information on necroinflammatory activity and steatosis, nor is it accurate during acute hepatitis or hepatitis exacerbations.

2.6.2.2.1 (iii) Shear Wave Elastrography

It is a non-invasive technique to asses liver tissues stiffness. The principle of SWE was as shown in Figure 2.1. Shear wave elastography relies on the generation of shear waves determined by the displacement of tissues induced by the force of a focused ultrasound beam or by external pressure. The shear waves are lateral waves, with a motion perpendicular to the direction of the force that has generated them. They travel slowly (between 1 and 10 m/s) and are rapidly attenuated by tissue. The propagation velocity of the shear waves correlates with the elasticity of tissue; ie, it increases with increasing

stiffness of the liver parenchyma. Many studies (Arda *et al.*, 2013; Leung *et al.*, 2013; Deffieux *et al.*, 2014; Ferraioli *et al.*, 2014a; Ferraioli *et al.*, 2014b; Suh *et al.*, 2014) had correlate SWE values with the METAVIR liver fibrosis score, i.e. F0 (Figure 2.2), F1 (Figure 2.3), F2 (Figure 2.4), F3 (Figure 2.5), and F4 (Figure 2.6). These study showed promising correlation between SWE and liver fibrosis score, and it is highly reproducible.

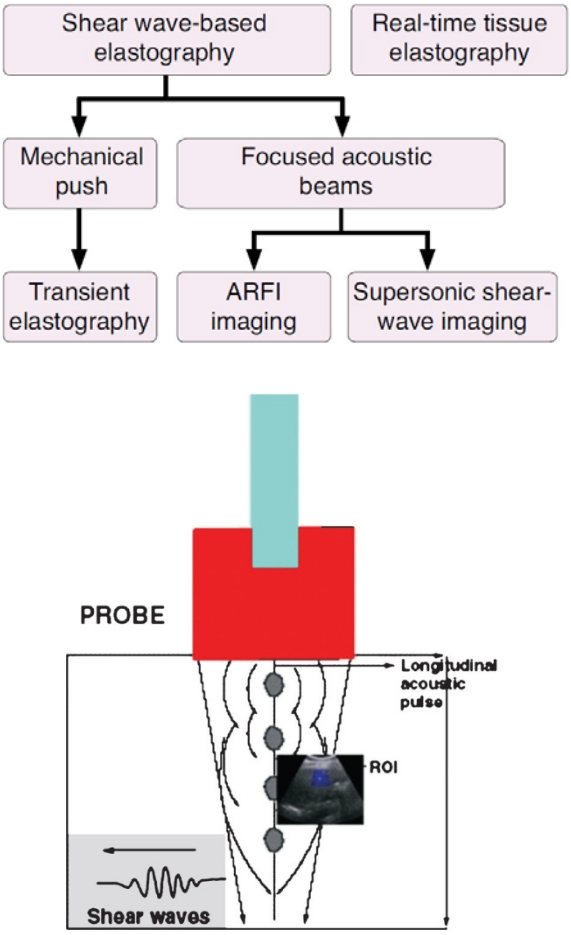
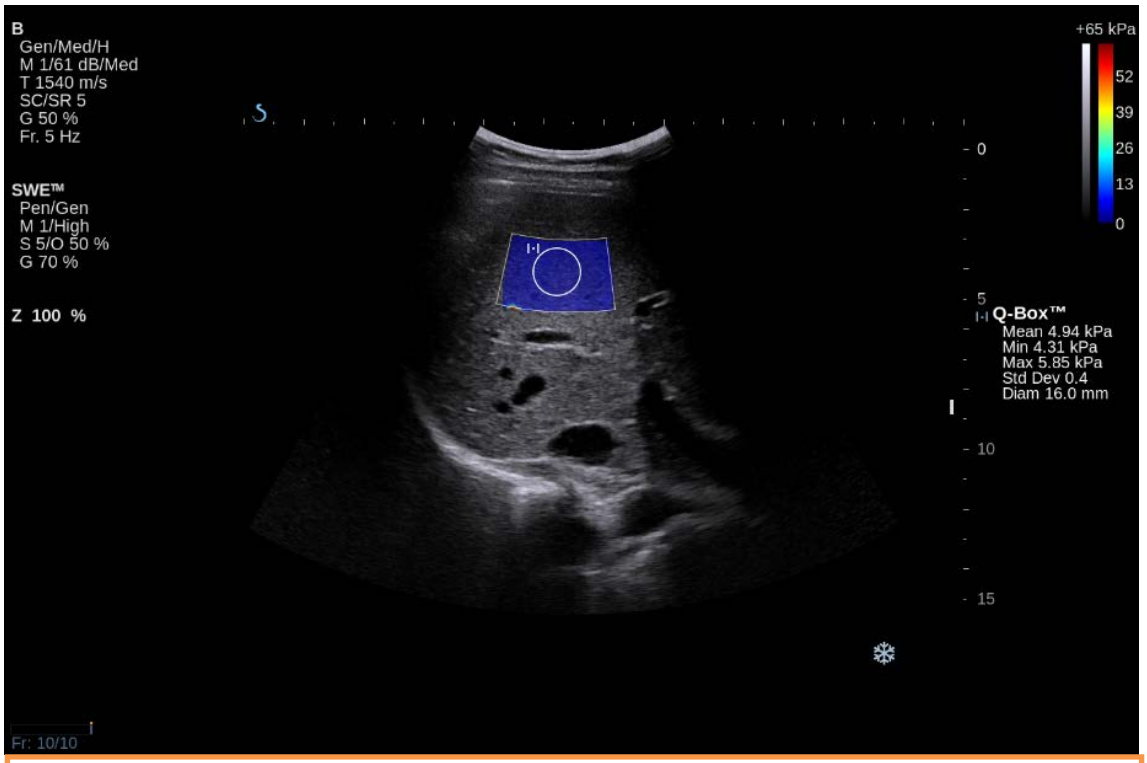


Figure 2.1 Schematic image showing physical principles of ultrasonographic shear wave elastography (Arda *et al.*, 2013)

Note: Transmission of longitudinal acoustic pulse leads to tissue displacement, which results in propagation of shear waves away from region of interest (ROI) with ultrasound.

METAVIR Score F0



B-Mode only:

- Good acoustic window even with lateral shadow
- Vessel free area

SWE™Mode:

- SWE box fully filled
- Homogeneous blue

Figure 2.2 SWE imaging on subjects liver with METAVIR Score F0

METAVIR Score F1

B-Mode only:

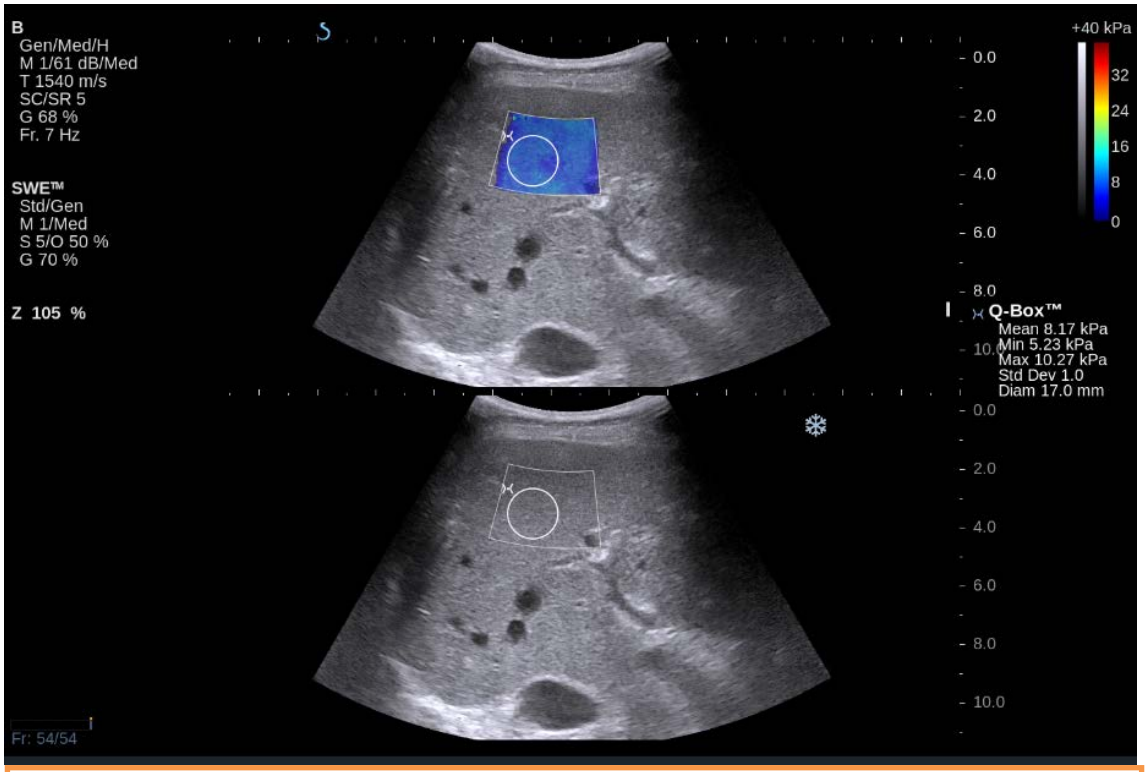
- Good acoustic window even with lateral shadow
- Vessel free area

SWE™Mode:

- SWE box fully filled
- Homogeneous blue

Figure 2.3 SWE imaging on subjects liver with METAVIR Score F1

METAVIR Score F2



B-Mode only:

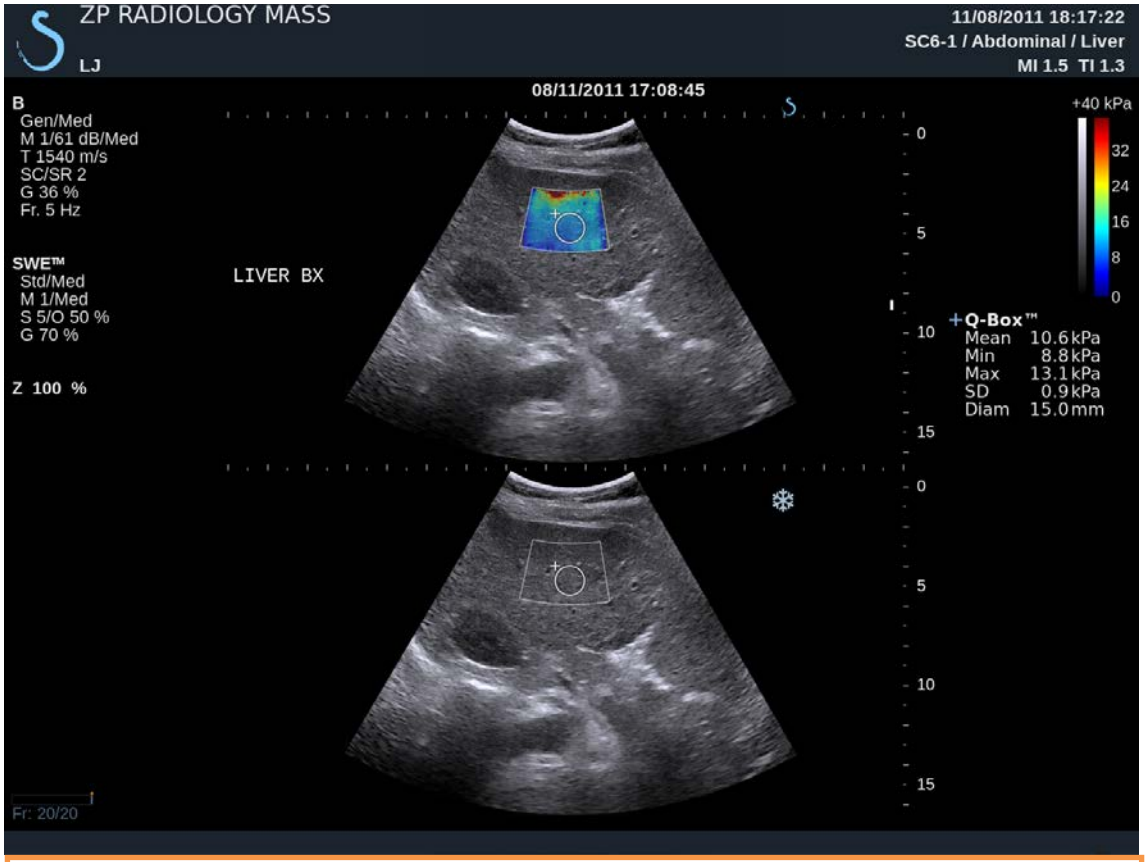
- Good acoustic window even with lateral shadow
- Vessel free area

SWE™Mode:

- SWE box too close to the capsule (red superficial area)
- Q-box well positioned in the reasonably homogeneous area

Figure 2.4 SWE imaging on subjects liver with METAVIR Score F2

METAVIR Score F3



B-Mode only:

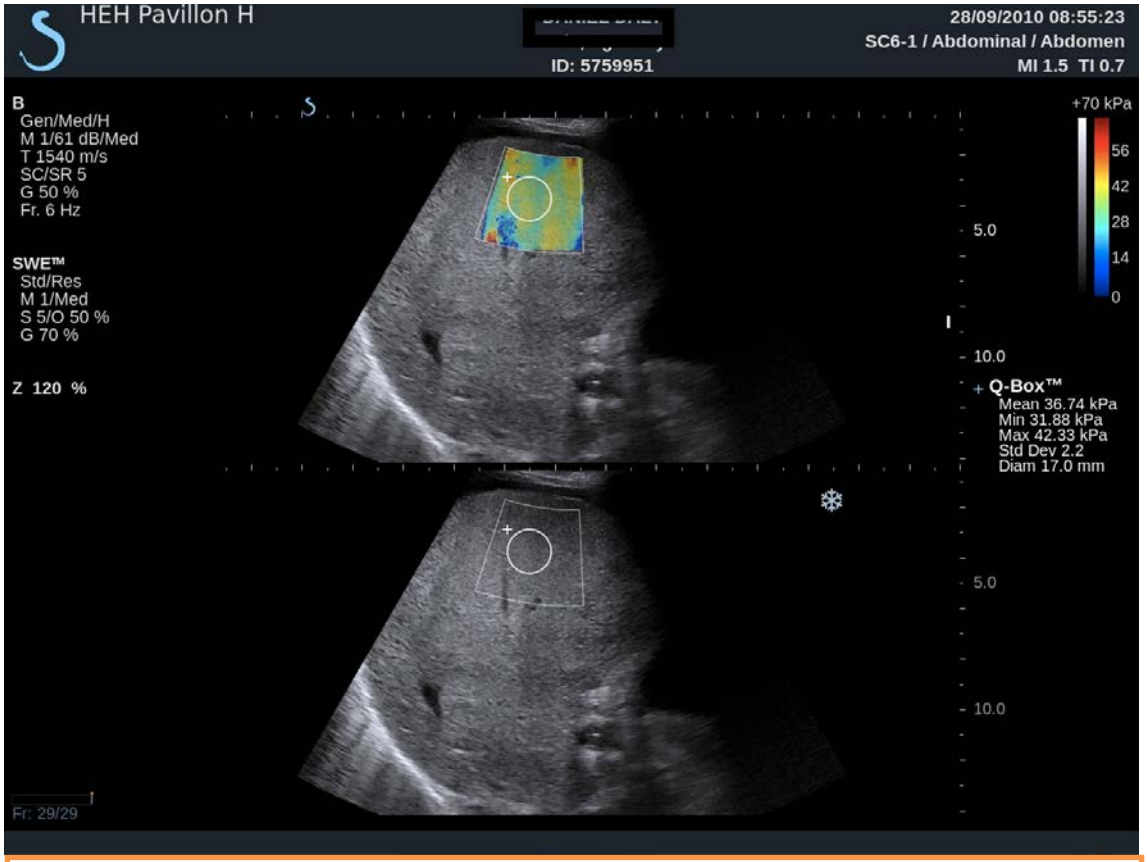
- Good acoustic window even with lateral shadow
- Vessel free area

SWE™Mode:

- SWE box fully filled
- Hues of blue

Figure 2.5 SWE imaging on subjects liver with METAVIR Score F3

METAVIR Score F4



B-Mode only:

- Good acoustic window even with lateral shadow
- Vessel free area

SWE™Mode:

- A small vessel at the bottom left
- Q-box well positioned outside the vessel zone

Figure 2.6 SWE imaging on subjects liver with METAVIR Score F4

2.6.2.2.1 (iv) Acoustic Radiation Force Impulse Imaging

ARFI uses an US probe to produce an acoustic “push” pulse, which generates shear waves that propagate in tissue to assess liver stiffness. ARFI elastography evaluates the wave propagation speed to assess liver stiffness. The faster the shear wave speed, the harder the object. ARFI technologies include Virtual Touch™ Quantification and Siemens Acuson S2000™ system. ARFI elastography can be performed at the same time as a liver sonographic evaluation, even in patients with a significant amount of ascites.

2.6.2.2.1 (v) Real-Time Elastography

Real-time elastography is an imaging technique that directly reveals the physical property of tissues using conventional US probes (Paparo *et al.*, 2014). RTE is a type of strain elastography which uses a combined autocorrelation method to measure tissue strain caused by manual compression or a person’s heartbeat. The relative tissue strain is displayed on conventional color B mode US images in real time. Hitachi manufacturers the RTE devices, including one called HI VISION Preirus. The challenge is to identify a region of interest while avoiding areas likely to introduce artifacts, such as large blood vessels, the area near the ribs, and the surface of the liver. Areas of low strain increase as fibrosis progresses and strain distribution becomes more complex. Various subjective and quantitative methods have been developed to evaluate the results. RTE can be performed in patients with ascites or inflammation. This technology does not perform as well in severely obese individuals.

2.6.2.2.2 Magnetic Resonance Elastography

Magnetic Resonance Elastography (MRE) is a non-invasive MRI based technique for quantitatively assessing the mechanical properties of tissues in vivo (Venkatesh *et al.*, 2013). MRE is performed by using a vibration source to generate low frequency mechanical waves in tissue, imaging the propagating waves using a phase contrast MRI technique, and then processing the wave information to generate quantitative images showing mechanical properties such as tissue stiffness.MRE uses a driver to generate 60-Hz mechanical waves on the patient's chest well. The MRI equipment creates elastograms by processing the acquired images of propagating shear waves in the liver using an inversion algorithm. These elastograms represent the shear stiffness as a pixel value in kilopascals. MRE has several advantages over US elastography, including: (1) analyzing larger liver volumes; (2) analyzing liver volumes of obese patients or patients with ascites; and (3) precise analysis of viscoelasticity using a 3-dimensional displacement vector.