Original Article

Non-Alcoholic Fatty Liver Disease in Metabolic Syndrome Patients in Serdang Hospital: Quantification by Contrast-Enhanced Computed Tomography

*Subapriya Suppiah,[1,2] Lee Roy-Ming Chow,[3] Nor Sharmin Binti Sazali,[3] Hasyma Abu Hassan[2]

[1]Centre for Diagnostic Nuclear Imaging, Universiti Putra Malaysia, 43000 UPM Serdang, Malaysia.
[2]Imaging Department, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, 43000 UPM Serdang, Malaysia.
[3]Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43000 UPM Serdang, Malaysia.

ABSTRACT

Introduction: Non-alcoholic Fatty Liver Disease (NAFLD) is one of the end organ damage detected in patients having metabolic syndrome X and it can lead to chronic liver failure. Therefore, it is important to be able to assess the condition in a quantifiable manner to help clinicians recognize and treat this disease. **Objective:** We aimed to determine the prevalence of NAFLD in patients with metabolic syndrome in Serdang Hospital, Malaysia using contrast-enhanced multidetector computed tomography (CECT) abdominal scan. The study also aimed to calculate the quantification of NAFLD using liver to spleen density CT Hounsfield Unit ratio, CTL/S or $CT_{L/S}$ measurement using abdominal CECT scans. Furthermore, we aimed to verify the correlation of dyslipidemia with NAFLD based on the $CT_{L/S}$ parameter. **Materials and Method:** We conducted a cross-sectional retrospective study in Hospital Serdang, Malaysia using data from January 2012 to December 2013. The sample size was 279 patients with metabolic syndrome who had undergone CECT abdominal scan. Patient demographics were descriptively analysed. Spearman's correlation test was used to look for association among lipid profile, blood sugar level and $CT_{L/S}$ ratio. **Results:** The prevalence of NAFLD in metabolic syndrome patients in our population was 82.8%. Prevalence of NAFLD was high among the elderly population ($\geq$ 57 years old). Additionally, Indian ethnics with metabolic syndrome had the highest risk of developing NAFLD (90.9%). There was a significant association between elevated LDL levels and $CT_{L/S}$ ratio ($p<0.05$); indicative of severity of NAFLD in metabolic syndrome patients. **Conclusion:** Contrast-enhanced CT scan can help to non-invasively detect and quantify the severity of non-alcoholic fatty liver disease using the $CT_{L/S}$ ratio in patients with metabolic syndrome who are referred for the scan for certain clinical indications. However, we do not recommend it as a stand-alone, first line investigation in the management of NAFLD in metabolic syndrome patients.

Keywords: Steatosis, Multidetector computed tomography, Liver Attenuation, Hyperlipidaemia

INTRODUCTION

Fatty liver is divided into alcoholic and non-alcoholic fatty liver disease (NAFLD). Fat deposition in the liver represents the hepatic manifestation of obesity and insulin resistance mediated by inflammatory processes manifesting in fatty liver disease, scarring and ultimately cirrhosis as well as liver failure (1). NAFLD is an independent correlation of metabolic syndrome X and its components, including abdominal obesity, insulin resistance and hypertriglyceridemia (2). Presence of NAFLD is associated with increased circulatory cytokines and development of systemic inflammation (3). Several studies have also shown an association between NAFLD and increased risk of cardiovascular disease and chronic kidney disease (4,5).

The prevalence of NAFLD is 20-30% of individuals in Western countries (6). In lean persons the prevalence is 16.5% and it increases to 75% in obese persons (6). In a Japanese study, the prevalence of NAFLD was increased in subjects with impaired glucose tolerance (43%) and in subjects with newly diagnosed diabetes mellitus (62%) (7). In addition, up to 80% of patients with dyslipidemia have NAFLD (8). A study performed in northwestern Peninsula Malaysia in 2010 by Magosso et al, detected fatty liver in 102 out of 180 subjects (56.7%) in Malaysia (9).

There are several criterion for the diagnosis of metabolic syndrome. Based on the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III (NCEP: ATP III); the criteria for diagnosing metabolic syndrome is the presence of a minimum of

*Corresponding Author: Dr. Subapriya Suppiah
subapriya@upm.edu.my

three of these conditions ie. abdominal obesity, hypertrigliceridemia, low levels of high density lipoproteins (HDLs), elevated blood pressure or elevated fasting plasma glucose (10). On the other hand, the gold standard for diagnosing NAFLD is liver biopsy and histopathological examination (11,12). However, there are some limitations due to errors in sampling and possible complications of bleeding, infection and even death.

Inevitably, non-invasive methods such as blood tests and radiological imaging are being used as more practical tools in diagnosing and monitoring NAFLD. Ultrasound abdomen is a cheap, safe and reliable method to diagnose NAFLD (13,14). However, it has limitations when it comes to imaging morbidly obese patients and its sensitivity drops to about 40% (15). Ultrasound assessment of NAFLD is also operator dependant and is subject to intra- and interobserver variability (16). Conversely, computed tomography of the abdomen has a role to play in the objective and reproducible assessment of NAFLD.

The measurement of fatty liver in the abdomen using computed tomography (CT) scan is more precise and is reproducible (17). Fat in the liver appears to be homogenously deposited, and most of the variation in the measurement of CT beam attenuation can be captured by measuring just one axial slice. There are many methods for quantifying NAFLD on CT scan mostly using non contrast-enhanced CT images (18,19,20). Nevertheless, as most CT scans in clinical setting are performed in the porto-venous phase, it is more practical to assess fatty liver on contrast- enhanced CT scans. Quantitative methods of assessing NAFLD on CT scan images include differential CT value between liver and spleen as well as ratio of CT value between liver and spleen (21-23). These measurements provide a non-invasive and objective method of assessing NAFLD in particular for patients diagnosed with metabolic syndrome (24). We aimed to detect the prevalence of NAFLD in patients having metabolic syndrome in our population. We also tested the association between hyperlipidemia and quantitative contrast-enhanced CT measurements to assess NAFLD.

MATERIALS AND METHODS

Study Design
The study was conducted in Serdang Hospital, Selangor and approved by our local institutional Committee for Ethics in Research. Informed consent to our patient subjects was waived as it was a retrospective cross sectional population study. A retrospective stratified sampling method was used whereby all the patients who were referred to our radiology department for a contrast-enhanced computed tomography (CT) scan of the abdomen, between the period of January 2012 to December 2013, were recruited. Convenience sampling of this strata of patient population with metabolic syndrome was then performed; to select CT scan images from Serdang Hospital picture archiving and communications system (PACS).

Contrast-enhanced computed tomography (CECT) abdominal scans were performed on a multidetector computed tomography (MDCT) scanner (Siemens, SOMATOM® Definition Flash, 128-slice scanner, Munich, Germany). Regardless of the indication for the scan, we retrospectively recruited the patient population that fulfilled the study inclusion criteria ie. NCEP: ATP III criteria for metabolic syndrome. The criteria requires presence of three or more of the following: abdominal obesity, hypertriglyceridemia, regardless of whether or not patient is on medication (triglycerides ≥1.7 mmol/L); low HDL cholesterol (HDL cholesterol ≤1.03 mmol/L for men and ≤1.29 mmol/L for women); elevated blood pressure (systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥85 mmHg or current use of antihypertensive drugs); impaired fasting glucose (fasting plasma glucose ≥5.6 mmol/L).

Exclusion criteria for diagnosing patients with NAFLD were those with history of excessive alcohol intake of >30g/day and those with serologically proven viral hepatitis. We also excluded cases which did not have CECT scan imaging of the abdomen in the correct porto-venous phase. We collected the patients' demographic data which included age, gender and ethnic race. We also collected data on lipid profile, fasting plasma glucose and blood pressure and classified patients with abnormal readings according to our hospital normal value range as the diagnosis of metabolic syndrome was made based on our local laboratory calibrated values.

Image / material analysis
Abdominal CECT scan images were interpreted by two experienced radiologists by consensus. Initial topogram acquisition was followed by abdominal scan performed 60 seconds after intravenous injection of the hydrosoluble low osmolar iodinated contrast media, by means of an automatic power injector at a rate of 2 – 3 ml/s and amount of 1.5 – 2.0 ml/kg body weight. This was done to acquire porto-venous phase of MDCT scan. The other acquisition parameters utilised were: collimation of 64 x 0.625 mm; gantry rotation time of 0.5 s; energy of 120 kVp and mAs depending on automatic modulation of the radiation dose based on body thickness; image reconstruction of 1 and 5 mm slice thickness and multiplanar reformatting in sagittal and coronal planes.

The images were analysed using the Region of Interest (ROI) markers available in the radiological diagnostic workstation application, Centricity DICOM viewer (GE Healthcare IT V3.0, Buckinghamshire, United Kingdom). The ROI used was standardized round in shape, measuring 0.2cm in diametre.

Computed tomography (CT) attenuation, Hounsfield unit (HU) of three different areas in the liver, avoiding areas of blood vessels, were sampled and on the same image slice. Three samples of the HU of spleen were also taken. The average value of each organ HU reading was calculated. Computed tomography (CT) attenuation, Hounsfield unit (HU) of Liver was divided by the CT attenuation, Hounsfield unit (HU) of Spleen to get the CT HU Liver to Spleen ratio, CTL/S.

Then, the ratio between the HU of liver and spleen, CTL/S measurement was tabulated. The CTL/S ratio method was utilized to standardise the interpretation of NAFLD in all patients. This is because certain parameters could not be standardised in this study, such as the exact amount of contrast media injected into each patient, the difference in distribution of contrast media in the patients' body; based on cardiac output and individual body habitus.

The interpretation of the CTL/S values is that ratio of > 0.90 as 'No' NAFLD, and values less than or equal to 0.90 ie. ≤ 0.90 as "Yes" NAFLD present. Therefore, it is interpreted as the smaller the ratio between HU of liver and spleen, the more severe the degree of fatty liver changes. We used the pro-forma document that covered all the socio-demographic data and the CTL/S ratio measurements that was necessary for the diagnosis of NAFLD in patients with metabolic syndrome.

Patients' lipid profile including the low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride (TG) as well as fasting plasma glucose and blood pressure readings were also recorded in the pro forma.

Definition of terminology

NAFLD – Non-alcoholic fatty liver disease: Infiltration of fat into liver cells due to causes related to neither influence of alcohol intact, hepatotoxic drugs ingestation nor inflammation caused by infection in the liver.
Parameters measured to assess for hyperlipidaemia:
TG – triglyceride
LDL – low density lipoprotein
HDL - high density lipoprotein
CT – computed tomography: a diagnostic imaging tool/ technology using ionizing radiation that is projected through the human body and processed to allow visualization of the internal organ structures
MDCT - multidetector computed tomography
CECT – contrast-enhanced computed tomography
HU – Hounsfield unit: arbitrarily assigned value for different densities seen on CT scans; a relative value based on density of water assigned as value zero
CTL/S ratio or $CT_{L/S}$ - the ratio value obtained by dividing Hounsfield unit of Liver with the Hounsfield unit of Spleen

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 21.0. Patient demographic data was descriptively analysed to determine the prevalence of NAFLD between different genders, among different age groups and among different ethnic races. Kruskal-Wallis test was used to identify the association between NAFLD (using CTL/S ratio method) and patients of different age as well as ethnic groups. Chi-square test was performed to test for the categorical variables to look for association of NAFLD with different genders. Spearman correlation test was performed to examine correlations between various continuous parameters that were not normally distributed ie. correlation between CTL/S and dyslipidemia as well as Type 2 diabetes mellitus. The results were considered statistically significant when p-value < 0.05.

RESULTS

There were 396 patients shortlisted initially. However, only 279 patients were selected to participate in this study because approximately 30% of subjects had to be excluded due to either incomplete blood test results, CT scan images that were no longer retrievable on PACS or images that were not in the correct porto-venous phase.

We classified age groups according to several ranges ie. adolescent group were subjects aged from 10-17 years old, adults group from 18 – 39 years old (including young adults ranging from 18-25 years old, and older adults ranging from 25-39 years old), middle aged group were those from 40 – 64 and elderly group were those aged more than 65 years old based on Britannica dictionary definitions. The sociodemographic characteristics analysis of our 279 subjects identified 59.1% (n=165) were elderly, while 30.8% (n=86) were middle aged, 9.0% (n=25) were adults, and

1.1% (n=3) were adolescents. There were 113 (40.5%) males and 166 (59.5%) females. Out of which, 54.8% (n=153) of them were of Malay race, 29.4% (n=82) were Chinese and 15.8% (n=44) were Indian ethnics. This data was a fairly good representative of the normal population distribution in Serdang, Selangor region.

Out of the total number of NAFLD detected in this study (n= 231), 87.9% were from the elderly age group. The lowest prevalence was from adolescent group (n= 2) ie. two obese teenagers and one of them was also diabetic (Figure 1A).

Although the results showed that the prevalence of NAFLD was higher in female patients with metabolic syndrome, chi-square test had *p* value was > 0.05. Therefore, there was no significant association between NAFLD and metabolic syndrome patients of different genders.

The median $CT_{L/S}$ values were was not significantly different among ethnicity. Nevertheless, it was noted that Indian ethnics with metabolic syndrome had the highest predisposition (90.9%) to develop non-alcoholic fatty liver disease (Figure 1B).
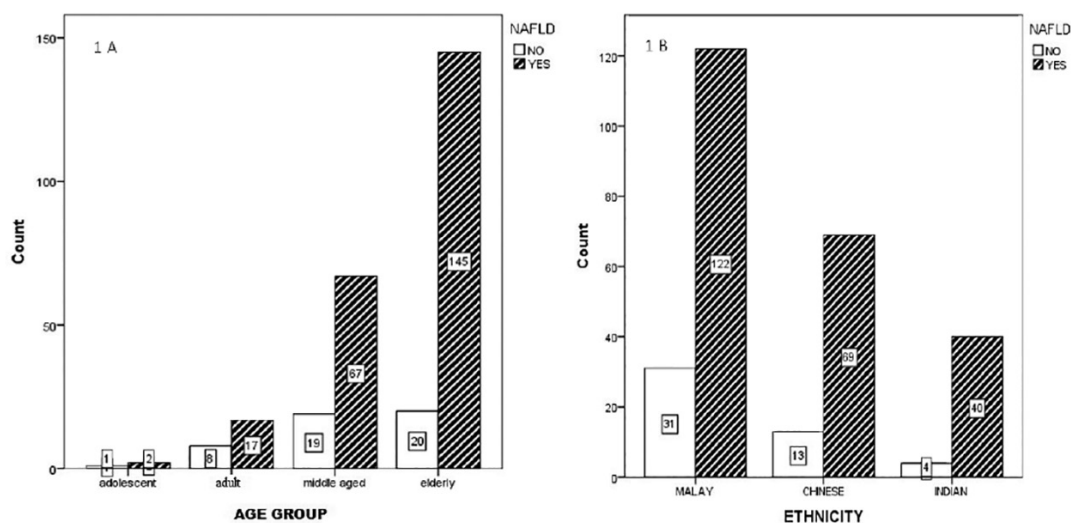

Figure 1

Figure 1: (A) Prevalence of NAFLD in metabolic syndrome patients of different age groups in Serdang Hospital. (B) Prevalence of NAFLD in metabolic syndrome patients of different ethnicity.

Based on laboratory results, the mean value of serum LDL in patients with NAFLD was higher than the mean of patients with normal liver density (Figure 2). We detected $CT_{L/S}$ values between 0.24 – 0.90 (mean = 0.78, standard deviation +/-0.14)for patients with NAFLD and between 0.98 – 1.44 (mean = 1.09, standard deviation +/-0.09) for patients with no evidence of fatty liver, using the cutoff value of $CT_{L/S} \leq 0.9$ as indicative of NAFLD. At an average, patients classified as having NAFLD has significantly higher serum LDL levels.
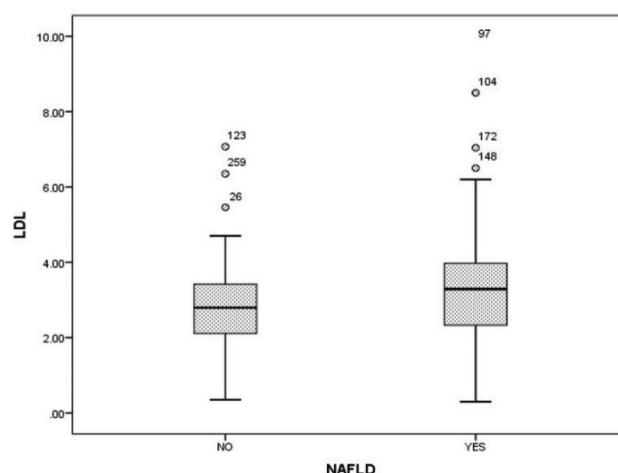
Figure 2

Figure 2: Box plot showing the association between hyper-LDL-emia with non-alcoholic fatty liver disease.

Fisher's exact test showed that there was a significant association between hyper-LDL-emia with NAFLD ($p<0.05$). Man-Whitney U test also detected a significant association of distribution of low $CT_{L/S}$ ratios in the patients with hyperlipidaemia. Conversely, there was no statistically significant association between HDL levels or triglyceride levels with $CT_{L/S}$ values in our study. There was also no significant association between $CT_{L/S}$ values and patients having Type 2 diabetes mellitus.

DISCUSSION

Metabolic syndrome X is a condition characterised by the co-existence of several major risk factors for cardiovascular disease (CVD), mainly high blood pressure (hypertension), hyperglycemia, and dyslipidemia (21,22). It is strongly linked to genetic factors that predispose to Type 2 Diabetes Mellitus and dyslipidemia as well as body fat composition (23). Insulin resistance, which occurs when high plasma insulin concentrations fail to suppress plasma glucose normally, is portrayed by unresponsiveness to insulin at the cellular level. Due to changes in receptor binding or post-receptor mechanisms, metabolic changes occur that leads to exposure of high free non esterified fatty acid (NEFA) concentrations which cause an expanded intra-abdominal fat mass.The diagnostic criteria for metabolic syndrome in our study is based on the NCEP:ATPIII criteria (10). We interpreted the blood test results according to Hospital Serdang biochemical laboratory calibrated normal values range; as the metabolic syndrome patients' data interpretation and management were made accordingly by the hospital clinicians. Non-alcoholic fatty liver disease is one of the end organ damages that occur in the spectrum of metabolic syndrome X. It can occur in as high as 88% of metabolic syndrome patients (24).

A significant negative weak association was established between $CT_{L/S}$ ratio and LDL levels in metabolic syndrome patients in our study. As the serum level of LDL increased, the $CT_{L/S}$ ratio between liver and spleen decreased, thus indicative of the degree of severity of fatty liver.

Based on our demographic analysis, the prevalence of NAFLD in metabolic syndrome X patients in our population was 82.8%. The elderly age group (57 years old and above) had the highest prevalence of NAFLD (87.9%). The overall prevalence of NAFLD is highest among Malay ethnics with (52.8% of NAFLD subgroup) with 79.7% of metabolic syndrome X Malays being detected with NAFLD; which corresponded with the higher percentage of our subjects being Malay ethnics. Nevertheless, Indian ethnics with metabolic syndrome were more predisposed to develop NAFLD, whereby 90.9% of Indians with metabolic syndrome had NAFLD; out of which 78.6% of Indian males had fatty liver. This corresponds well with a previous Malaysian study that detected prevalence rate of NAFLD as high as 68.2% in male Indians (25). It is postulated that increased prevalence of insulin resistance and subsequently fatty liver in Asian-Indian men was associated with increased levels of interleukin-6 concentrations, a mediator of inflammation (26).

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There were also no significant association between NAFLD and hyperlipidemia in Type 2 diabetes mellitus subgroup of our study. This may be because diabetes mellitus is not a main factor for the presence of fatty liver in patients having dyslipidemia ie. dyslipidemia is an independant factor for development of NAFLD. Furthermore, most of the diabetic patients in our study were on treatment ie. either on oral hypoglycaemic agents or injected subcutaneous insulin. Therefore, diabetics on treatment would confound the effects of insulin resistance on development of NAFLD. There were several limitations that we encountered throughout the period of our research. The data that we collected were retrospective and only showcases data from the year 2012 and 2013. Next, only the metabolic syndrome patients who had undergone contrast-enhanced abdominal CT scans were chosen as the subjects can represent sampling bias for true prevalence of NAFLD in our population. Viral test for exclusion of viral hepatitis and exact alcohol intake measurements were not conducted for all subjects. We sourced the hospital data base and excluded patients with excessive alcohol intake when diagnosing presence of NAFLD wherever applicable.

Diagnosis by Contrast-enhanced computed tomography abdominal scan

Non alcoholic fatty liver disease can be diagnosed by laboratory investigations such as liver biopsy and histopathology examination, which is the gold standard for diagnosis. Nevertheless, biopsy has certain disadvantages, whereby it samples a very small area of the liver and can miss sampling of the true region of fatty liver, thus leading to high false negative results. It is also associated with complications such as pain, haemorrhage / hematoma at biopsy site and infection. Therefore, radiological investigations such as ultrasound, CT scan and MRI are being more frequently used to non-invasively detect and grade NAFLD.

Ultrasound is a safe, cheap and reliable modality to assess NAFLD. In fact, a meta-analysing published in 2011 noted that ultrasound abdomen had a pooled sensitivity of 84.8% and specificity of 93.6% for detecting NAFLD (13). However, its interpretation is subjective and leads to variable specificity range for detecting fatty liver. It is also not reliable to be used in obese patients as the interpretation can be confounded by increased attenuation of the ultrasound beam by thick abdominal fat. Magnetic resonance imaging scanning in particular allows quantification of liver fat, a useful adjunct in clinical studies but expensive (27).

Computed tomography has certain advantages as there are several studies that have noted to have good sensitivity and specificity to predict fatty liver disease. Several studies have been conducted to qualitatively and quantitatively assess the severity of NAFLD by CT scan (17-20,28,29). There are three measurements which are frequently used to determine fatty liver /hepatic steatosis (20,30-32). These include (a) the absolute measurement of liver parenchymal attenuation values which are given in Hounsfield units. (b) the difference in attenuation values between liver and spleen, CT_{L-S} and (c) the calculation of the spleen-to-liver attenuation ratio, $CT_{L/S}$.

In a review done by Schwenzer et al, (20) it was stated that normal, healthy liver has an attenuation value of about 50–57 HU on non contrast-enhanced CT which is about 8–10 HU higher than the one of spleen. Attenuation values of the liver which are less than 40 HU or 10 HU smaller than those of the spleen are highly predictive of hepatic steatosis. The spleen was introduced as an internal control in order to reduce errors in CT attenuation caused by variations in CT parameters due to individual patient factors ie. body size, body shape and metallic instruments. Iwasaki et al (18) compared histopathological results of liver biopsy and CT scan images and proposed that CT spleen-to-liver attenuation ratios greater than 1.1 to be suggestive of at least a moderate fatty liver/ hepatic steatosis. They noted that mean $CT_{L/S}$ ratio of 0.9 (range: 0.7 – 0.99) to have significant correlation with severe hepatic steatosis. Park et al, (19) on the other hand recommended 0.9 as a cut off value to predict fatty liver, which inferred that the smaller the $CT_{L/S}$ value, the more severe was the fatty liver disease. Therefore, in our study we selected $CT_{L/S}$ values ≤ 0.9 to indicate presence of fatty liver, which was interpreted as positive for NAFLD (Fig. 3) and $CT_{L/S}$ > 0.9 as normal liver parenchyma ie. abscence of NAFLD (Fig. 4).
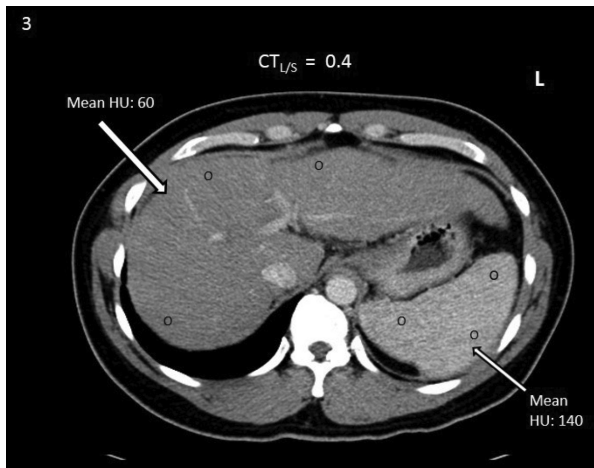
Figure 3

Figure 4

Figure 3: Axial view contrast-enhanced computed tomography in soft tissue window (W:250, L:50) showing fatty liver, $CT_{L/S}$ ratio 0.4. Thick white arrow indicates the relatively hypodense liver and narrow white arrow indicating the spleen. Small black circles indicate the ROI for averaging the CT Hounsfield unit (HU).
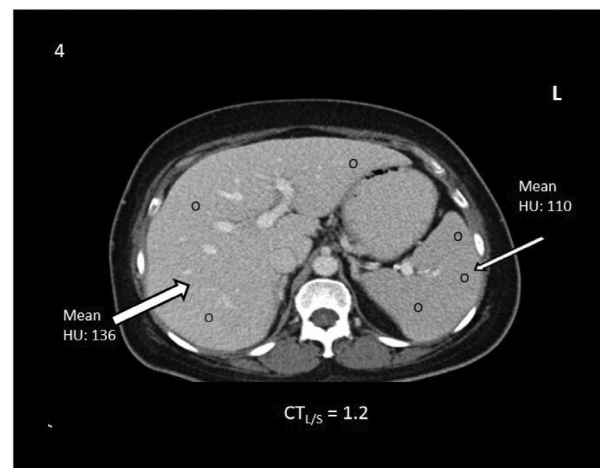
Figure 4: Axial view contrast-enhanced computed tomography in soft tissue window (W: 250, L:50) showing normal liver density, $CT_{L/S}$ ratio 1.2. Thick white arrow indicates the liver and narrow white arrow indicates the spleen. Small black circles indicate the ROI for averaging the CT Hounsfield unit (HU).

We opted for contrast-enhanced CT (CECT) scan for our study because many standard diagnostic abdominal CT scans are performed in the porto-venous phase. Therefore, many recent studies advocate the used of CECT scan to detect NAFLD (33,34). These studies have attempted to establish a criterion for diagnosis of fatty liver or hepatic steatosis by means of abdominal CECT scan using a proposed mathematical equation considering the liver density, incorporating density of the portal vein and the aorta in the calculation. They advocate the use of CECT in diagnosis of fatty liver, as it is the most diagnostically useful phase in most abdominal CT scan indications. CECT is also gaining acceptance as it shows good specificity (90 – 100%) for detecting NAFLD and is equally comparable to plain scans (35-37). Moreover, we used a quantitative method of analysis by calculating $CT_{L/S}$ ratio values to standardise our interpretation of NAFLD because CECT scan images are influenced by the type of contrast media, rate of contrast administration, dose of contrast medium, volume of contrast medium, and scanning delay time which influences the measured density of intra-abdominal organs (38). In addition, absolute Hounsfield unit measurements are influenced by patient-related factors which include cardiac output, body mass index, and presence of liver disease (39).

In our study, we found that $CT_{L/S}$ value had a negative association with severity of hyper-LDL-emia. Considering the severity of NAFLD was inversely related to $CT_{L/S}$ values, therefore severity of hyper-LDL-emia is proven to have a proportional relationship to NAFLD and that $CT_{L/S}$ can be used to non invasively detect and grade fatty liver.

CT HU of liver / CT HU of spleen = $CT_{L/S}$

$1/CT_{L/S}$ corresponds linearly to NAFLD

Severity of serum LDL corresponds linearly to $1/ CT_{L/S}$

CONCLUSION

Contrast-enhanced CT scan is an imaging tool that can help non-invasively detect and quantify the severity of non-alcoholic fatty liver disease using the CTL/S ratio in patients with metabolic syndrome who are referred for the scan for certain clinical indications. If required, it can be an optional alternative investigation to assess patients in the management of metabolic syndrome. Nevertheless, we do not advocate it as a routine stand-alone, first line investigation in the management of NAFLD in metabolic syndrome patients.

ACKNOWLEDGEMENT

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