THE EFFECT OF MODIFIED ALTERNATE DAY CALORIE RESTRICTION ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

DR KHAIRIAH BINTI MAT YUSOFF

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

UNIVERSITI SAINS MALAYSIA

2018

ACKNOWLEDGEMENT

The completion of this study would have not been possible without the participation, cooperation and assistance from so many people whose names may not all be enumerated. Their contributions are sincerely appreciated and gratefully acknowledged. However, I would like to express deep and utmost appreciation particularly to the following:

1. Dr Juhara Haron, Consultant Radiologist and lecturer at Radiology Department who is also my supervisor and the principle investigator of the RUT grant.
2. Professor Dr Lee Yong Yeh and Dr Chandran Nadarajan who are co-supervisors of this dissertation and Puan Khairun Nisah Ibrahim who is dietition involved.
3. Prof Madya Dr Mohd Shafie Abdullah, Prof Madya Dr Mohd Ezane Aziz, Dr Ahmad Tarmizi Musa, Dr Wan Aireene Wan Ahmed, Dr Ahmad Hadif Zaidin Samsudin and Prof Madya Dr Wan Ahmad Kamil, lecturers/radiologists all of whom directly or indirectly contributed their ideas and comments to the success of this study.
4. Colleagues and all the staff in the Department of Radiology, HUSM.

Table of Contents

ACKNOWLEDGEMENT .... **ii**

TABLE OF CONTENT .... **iii**

LIST OF TABLES.…… .... **vi**

LIST OF FIGURES .... **vii**

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS .... **viiii**

ABSTRAK .... **x**

ABSTRACT .... **xi**

2.1 Title: **6**

2.2 Objective **6**

2.2.1 General objective: 6

2.2.2 Specific objectives: 6

2.3 Methodology **7**

2.3.1 Study design: 7

2.3.2 Population and Sample: 7

2.3.2.1 Reference Population: 7

2.6.2 Diet protocol 12

2.7 Research Tools **13**

2.8.1 Fatty Liver Grading 13

2.8.2 Liver Stiffness Grading 14

2.9 Operational Definition **15**

2.9.1 Non-alcoholic fatty liver disease (NAFLD) 15

2.9.2 Non alcoholic Steatohepatitis (NASH) 15

2.9.3 Shear wave elastography (SWE) 15

2.10 Validation study **16**

2.11 Statistical analysis **16**

3.MANUSCRIPT **18**

3.1 INTRODUCTION **18**

3.2 METHODOLOGY **22**

3.2.1 Subjects 22

3.2.2 Diet protocol 22

3.2.3 Statistical analysis 23

3.3 RESULTS **24**

3.3.1 Demographic data 24

3.3.2 Changes of liver steatosis grading in study participants before and after intervention programme 24

3.3.3 Changes of liver elasticity in study participants before and after intervention programme 25

3.3.4 Correlation between liver elasticity and liver steatosis gradings 25

3.4 DISCUSSION **26**

3.5 REFERENCES **29**

3.6 TABLES AND FIGURES **31**

3.6.1 TABLES 31

Table 3.6.1.1. Liver stiffness measurement 31

Table 3.6.1.2: Demographic data of participants 32

Table 3.6.1.3: Changes in liver steatosis grading of study participants before and after intervention programme 33

Table 3.6.1.4 : Changes in liver elasticity level of study participants before and after intervention programme 33

Table 3.6.1.5 : Correlation between liver elasticity and liver steatosis gradings 35

3.6.2 FIGURES 35

Figure 3.6.2.1 Diagram depicting the process of shear wave-based ultrasound elastography 36

Figure 3.6.2.2.Diagram showed SWE production 37

Figures 3.6.2.3 Super Sonic Imagine's Aixplorer® by Super Sonic Image 38

Figure 3.6.2.4 Grades of fatty liver on visual analysis 39

Figure 3.6.2.5 shows SWE shear-wave elastography values obtained from offline quantification by placing a region of interest 40

Figure 3.6.2.6 Shear wave elastography in this study..41

Figure 3.6.2.7: Scatter plot of steatosis grade versus fibrosis SWE 42

3.7 APPENDICES.. 43

3.7.1 Appendix A: Data collection sheet... 43

3.7.2 Appendix B: Ethical approval 46

3.7.3 Appendix C: Journal format..47

LIST OF TABLES

Table		Page
3.6.1.1	Liver stiffness measurement	31
3.6.1.2	Demographic data	32
3.6.1.3	Changes in liver steatosis grading of study participants before and after intervention programme	33
3.6.1.4	Changes in liver fibrosis level of study participants before and after intervention programme	34
3.6.1.5	Correlation between SWE readings and liver steatosis gradings	35

LIST OF FIGURES

Figure		Page
3.6.2.1	Diagram depicting the process of SWE	36
3.6.2.2	Diagram showing SWE production	37
3.6.2.3	Super Sonic Imagine's Airexplorer® by Super Sonic Image	38
3.6.2.4	Grades of fatty liver on visual analysis	39
3.6.2.5	SWE shear-wave elastography values obtained from offline quantification by placing a region of interest on elastograms	40
3.6.2.6	Shear wave elastography image pre (A) and post (B) intervention	41
3.6.2.7	Scatter plot of steatosis grade versus fibrosis SWE	42

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

ARFI	Acoustic Radiation Force Impulse
ICR	Intermittent Calorie Restriction
kPa	kilopascal
MADCR	Modified Alternate Day Calories Restriction
NAFLD	Non alcoholic fatty liver disease
NASH	Non alcoholic steatohepatitis
PACS	Picture Archiving and Communication System
ROI	Region Of Interest
RTE	Real Time Elastography
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
SWE	Shear Wave Elastography
TE	Transient Elastography

ABSTRAK

KESAN DARIPADA SEKATAN MODIFIKASI KAWALAN KALORI SELANG SEHARI (MADCR) DALAM PESAKIT NON ALCOHOLIC FATTY LIVER DISEASE (NAFLD).

Pengenalan: NAFLD adalah isu perubatan yang serius di seluruh dunia. Pelbagai modifikasi diet telah dilaksanakan untuk mengatasi masalah steatosis hati ini. SWE adalah teknik baru yang menawarkan kaedah penilaian steatosis hati yang tidak invasif. Dalam kajian ini, kami membandingkan gred steatosis hati dan keanjalan hati di kalangan pesakit NAFLD yang menjalani 8 minggu MADCR.

Metodologi: Seramai 39 subjek telah menjalani pemeriksaan ultrasound hati menggunakan mesin Ultrasound Airexplorer. Gred steatosis dan fibrosis dilakukan. Bacaan elastografi gelombang ricih (SWE) juga telah direkodkan. Perbandingan bacaan sebelum dan selepas modifikasi diet dibuat. Korelasi antara steatosis dan keanjalan hati dianalisa menggunakan Kendall b tau analisis.

Keputusan: Tahap steatosis hati dan tahap fibrosis hati dari 30 peserta dalam kumpulan intervensi berkurang dengan ketara selepas MADCR. Keputusan kami menunjukkan pengurangan gred steatosis hati dalam 10 pesakit di mana 8 pesakit bertambah baik dari gred 2 ke gred 1 dan 2 pesakit dari gred 1 ke gred 0. Kajian kami menunjukkan perbezaan median yang signifikan (p value <0.001) keanjalan hati dalam kumpulan intervensi selepas MADCR. Dari analisis bacaan semua peserta (n = 78), nilai keanjalan hati menunjukkan signifikan korelasi dengan pengkelasan steatosis hati berlemak gred 0-3 (nilai p 0.013).

Kesimpulan: Program MADCR ini adalah bermanfaat untuk memperbaiki steatosis hati. SWE adalah kaedah yang berguna dan dipercayai untuk menilai keanjalan hati selepas program intervensi.

ABSTRACT

THE EFFECT OF MODIFIED ALTERNATE DAY CALORIE RESTRICTION (MADCR) ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Introduction: NAFLD is a serious medical issue worldwide. Various diet modifications had been implemented to improve liver steatosis. SWE is an emerging technique that offers non-invasive method of liver steatosis assessment. In this study, we aimed to compare the liver steatosis grading and liver elasticity among NAFLD patients who underwent 8 weeks of MADCR.

Methodology: Using the Aixplorer® ultrasound, liver ultrasound of 39 subjects (32 interventional and 9 control subjects) were performed. Liver steatosis grading, fibrosis grading and shear wave elastography of all patients were acquired. Liver steatosis and liver elasticity level pre and post pre intervention were compared. Correlation of the liver steatosis and gradings were analysed using Kendall b tau analysis.

Results: The mean liver steatosis grade and fibrosis level of the 30 participants in the intervention group were significantly reduced after MADCR (modified alternate day calorie restriction) programme. Our result showed that 10 patients had improved liver steatosis grading, whereby 8 patients improve from grade 2 to grade 1 and 2 patients from grade 1 to grade 0. Our study showed significant mean difference of liver elasticity in intervention group after MADCR (p value <0.001). From the analysis of the readings from all the participants (n=78), the SWE values showed significant weak correlation with the steatosis grading (0-3) of the fatty liver (P value 0.013).

Conclusions:

MADCR is beneficial to improve liver steatosis. SWE is a useful and reliable method to assess liver elasticity after intervention.

CHAPTER 1: INTRODUCTION & LITERATURE REVIEW

1. INTRODUCTION & LITERATURE REVIEW

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver diseases in Western countries, occurring in approximately 30% of the general population. The spectrum of this disease includes simple steatosis, nonalcoholic steatohepatitis (NASH), liver fibrosis, and liver cirrhosis. Patients with NAFLD show higher mortality rate as compared to the general population. (Mohammadi, Bazazi, Maleki-Miyandoab, & Ghasemi-rad, 2012).

Although the exact risk or incidence of progression from simple hepatic steatosis to advanced stages of fatty liver disease has yet to be determined, the progression of simple hepatic steatosis to cirrhosis through the development of steatohepatitis (NASH) and fibrosis has been established. NASH is characterized by hepatocyte injury, inflammation, and fibrosis. These are known risk factor for progression to cirrhosis, and such progression has been reported. NASH is additionally associated with an incremented risk of liver cancer and death from cardiovascular diseases or liver-related causes (Lee & Park, 2014).

Histopathological examination remains the gold standard for diagnosing liver fibrosis despite the intraobserver and interobserver variability in staging. Regev et al found approximately 25% of patients (30 of 124) to have a difference of at least one grade and 33% (41 of 124) to have at least one stage difference between the right and left liver lobes. Ultimately, they found an underdiagnosis of cirrhosis in approximately 15% of patients. (Regev et al., 2002). Other than that, liver biopsy is a painful technique despite local anaethesia. Generally it is not well accepted by most patients, it has morbidity and mortality risks, and is not an ideal method as routine reassessment. Thus, noninvasive methods for assessing liver fibrosis are of great clinical interest. In the last decade, techniques to noninvasively estimate the stage of liver fibrosis have become commercially available. They have the capability to evaluate differences in the elasticity of soft tissues by measuring tissue behavior when a mechanical stress is applied. Ultrasound and magnetic resonance imaging have been used for elasticity imaging. Magnetic resonance elastography, even though promising, has some disadvantages because it cannot be performed in a liver with an iron overload because of signal-to-noise limitations, the examination takes longer time as compared to ultrasound elastography, and it is a costly procedure (Ferraioli, Parekh, Levitov, & Filice, 2014).

Liver steatosis on ultrasound appears as a diffuse increase in hepatic echogenicity, or “bright liver”, due to increased reflection of ultrasound beam from the liver parenchyma, which is caused by intracellular accumulation of fat vacuoles. Ultrasound evaluation of hepatic steatosis typically consists of, a qualitative visual assessment of hepatic echogenicity, measurement of the difference between the liver and kidneys in echo amplitude, evaluation of echo penetration into the deep portion of the liver, and determination of the clarity of blood vessel structures in the liver (Lee & Park, 2014).

Ultrasound imaging is a well-established and cost effective imaging technique for the diagnosis of hepatic steatosis, specifically for screening a large population at risk of NAFLD. Ultrasonography has a reasonable accuracy in detecting moderate-to-severe hepatic steatosis although it is less accurate for detecting mild hepatic steatosis, operator-dependent, and rather qualitative(Lee & Park, 2014).Ultrasound also allows for reliable and accurate detection of moderate-severe fatty liver, compared to histology. Because of its low cost, safety, and accessibility, ultrasound is likely the imaging technique of choice for screening for fatty liver in clinical and population settings (Hernaez et al., 2011).

Ultrasound is still the first option for diagnosis; but its accuracy depends on the operators and patient's features. Computed tomography can detect hepatic fat content, but only at threshold of 30%, and it involves ionizing radiation which is hazardous to the patient. Magnetic resonance (MR) spectroscopy is probably the most accurate and fastest method of detecting fat but it is expensive and the necessary software is still not easily available in all MRI units. (Roldan-Valadez, Favila, Martínez-López, Uribe, & Méndez-Sánchez, 2008).

Tissue stiffness in liver is related to tissue composition, which is changed by cirrhosis, hepatocellular carcinoma or metastases. SWE is a new imaging technique by which the elasticity of soft tissue can be measured quantitatively. SWE is based on the automatic generation and analysis of transient shear waves. This method uses the acoustic radiation force of the ultrasound wave to push the tissue instead of using a compression force (called stress), as is used in conventional dynamic ultrasonographic elastography. The acoustic displacement of tissue is free of user dependence and is reproducible (Arda, Ciledag, Arıbas, Aktas, & Köse, 2013). Shear wave-based elastography applies a perpendicular stress force to a target organ in order to induce shear on the tissues. The information on the propagating shear wave including the velocity of the shear wave could be measured by obtaining radiofrequency images with a high frame rate, which can be used to generate a tissue displacement map. Then, the elastic property for quantitative estimation is calculated by propagating velocity of the shear wave. (Figure 3.6.2.1)(Jeong, Lim, Lee, Jo, & Kim, 2014).

The reproducibility of the SWE method is very high, with intraobserver intraclass correlation coefficients of 0.95 and 0.93 for an expert and a novice operator, respectively, and interobserver agreement of 0.88. As for conventional sonography, it is user dependent; thus, it is recommended that at least 50 supervised scans and measurements should be performed by a novice operator to obtain consistent measurements. Values obtained in a small series of healthy participants ranged from 4.92 kPa (1.28 m/s) to 5.39 kPa (1.34 m/s).(Hernaez et al., 2011).

In a meta-analysis, they showed that SWE accuracy is good for staging of liver fibrosis as compared to the results of meta-analyses on RTE, TE, and ARFI, the performance of SWE seems to be nearly identical for the evaluation of cirrhosis. It was found that for significant liver fibrosis), SWE has accuracy that is to ARFI, however higher than RTE and TE (Singh, Das, & Baruah, 2013). Schematic image (Figure 3.6.2.2) showing physical principles of ultrasonographic shear wave elastography. Transmission of longitudinal acoustic pulse leads to tissue displacement, which results in propagation of shear waves away from region of interest (ROI) with ultrasound.

CHAPTER 2: STUDY PROTOCOL

2. STUDY PROTOCOL

2.1 Title:

THE EFFECT OF MODIFIED ALTERNATE DAY CALORIE RESTRICTION ON NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

2.2 Objective

2.2.1 General objective:

The aim of this study is to evaluate the effect of Modified Alternate Day Calorie Restriction on radiological changes in Ultrasound of the liver and biochemical changes in patient with NAFLD after 8 weeks of Modified Alternate Day Calorie Restriction.

2.2.2 Specific objectives:

1. To compare differences in mean of Liver Steatosis Grading after 8 weeks of Modified Alternate Day Calorie Restriction in NAFLD patients.
2. To compare liver elasticity using shear wave elastography (SWE) during pre and post Modified Alternate Day Calorie Restriction in NAFLD patients.
3. To correlate shear wave ultrasonographic elastography (SWE) result with grading of fatty liver.

2.3 Methodology

2.3.1 Study design: Intervention Study Design

2.3.2 Population and Sample:

2.3.2.1 Reference Population:

All patients attending Gastroenterology Clinic, HUSM

2.3.2.2 Source Population:

All Non-Alcoholic Fatty Liver Disease (NAFLD) patients attending Gastroenterology Clinic in Hospital USM, Kota Bharu, Kelantan.

2.3.3 Inclusion Criteria:

1. Age ranges 18 to 70 years old
2. ALT ≥41 U/L or AST ≥34 U/L
3. For diabetes mellitus and dyslipidaemia participants, they must be on a stable regimen for at least 6 months prior to study enrolment and initial liver imaging

2.3.4 Exclusion Criteria:

1. Medically or surgically ill patients who cannot consent
2. Complicated chronic liver disease with portal hypertension
3. Significant alcohol consumption (1 standard drink per day)
4. Contraindications to calorie restriction
5. Hepatitis B and C infection.
6. Pregnancy
7. Engagement in an active weight loss program
8. Taking weight-loss medication or substance abuse
9. Significant psychiatric problems

2.3.5 Withdrawal criteria

1. Subject who unable to tolerate alternate daily fasting intervention during the trial
2. Patient's own choice

2.3.6 Sampling Method

Non-alcoholic fatty liver disease patients who attended the Gastroenterology Clinic were screened from August 2015 till July 2016. A total of 105 participants were eligible but only 57 patients fulfilled inclusion criteria. Out of 57 participants, only 41 participants agreed to be enrolled in the trial. All participants were required to complete a 2 week run-in period consisting of maintaining and self-monitoring of usual dietary and daily activity. Participants had been explained regarding the trial and after fulfilling the selection criteria, informed consent were taken. Demographic data of the participants were recorded. Participants were given an appointment date for an ultrasound. An appointment to see dietitian in Dietitic Clinic HUSM also were arranged. Both appointments were done within 2 weeks prior to the initiation of the intervention programme. After 8 weeks of intervention programme, we repeat another ultrasound to grade liver steatosis and liver elasticity post intervention.

2.3.7 Ultrasound procedures

2.3.7.1 Fatty liver grading using B mode

Patients were put in supine position. Each patient underwent a conventional grey scale liver ultrasonography (B mode) using the Aixplorer ultrasound system (SuperSonic Imagine) with 5MHz linear transducer. Assessment of the liver parenchyma were performed and graded into fatty liver grading (Singh et al., 2013) whereby Grade 0 showed normal liver echogenicity, Grade 1 showed fatty liver with increased liver echogenicity, Grade 2 showed fatty liver with the echogenic liver obscuring the echogenic walls of the portal venous branches and Grade 3 which showed fatty liver in which the diaphragmatic outline is obscured.

2.3.7.2 Liver Elasticity Grading using SWE

Subsequently, shear wave elastography were performed. Elastography application selected. SWE was performed using a standard M probe. It uses a 5-MHz ultrasound transducer mounted on the axis of a vibrator. The vibrator generates a painless vibration generating shear waves that propagate through the skin and the subcutaneous tissue into the liver. The shear wave velocity is directly related to the liver stiffness. In all patients SWE measurements were performed in the right liver lobe, by intercostal approach, in supine position with the right arm in maximal abduction. Liver portion of at least 6 cm thick and free of large vascular structures was identified. Once the measurement area had been located, the operator pressed the probe button to start an acquisition. The Q box is placed over the area of interest and the quantitative elasticity values will be obtained automatically by moving the delineated Q box over the colour map.

Ten successful measurements were performed on each patient and a median value expressed in kPa was calculated. Images will be recorded in Picture Archiving Computer System (PACS) for retrieval.

2.3.8 Diet Protocol

A designated dietician taught and explained the participants in details regarding calorie restriction and monitored adherence to intervention. During the 2 weeks control period (before the intervention programme started), participants were required to keep their body weight stable by maintaining their usual eating and activity habits, then, during the 8 week modified alternate day calorie restriction period, all subjects consumed only 30% of their energy needs over 24 hours on each fast day and then consumed ad libitum on each alternate feed day. The feed and calorie restriction days began at 9am each day, and on calorie restriction day, meals were consumed between 2pm and 8pm to ensure that each participants were undergoing the same of time of taking a meal. On each calorie restriction day, the participants were allowed to consume energy-free beverages, tea, coffee, and sugar-free gum and were encouraged to drink plenty of water. Diets were not provided to participants but were self-selected using detailed individualised food portion lists, meal plans, and recipes. To maximise compliance participants received phone calls and 2 weekly appointments to ensure adherence. All participants were encouraged to use cognitive behavioral techniques such as self-monitoring, obtaining peer/family support and stimulus control to maintain diets. The researcher assessed participant's adherence to intervention programme via diet diary and also dietary recall during follow up.

2.4 Sampling technique:

Simple random sampling method was used.

2.5 Sample Size Calculation

Objective 1:

To compare differences in mean of Liver Steatosis Grading after 8 weeks of Modified Alternate Day Calorie Restriction in (NAFLD) patients

(There are no available data at the time of study design to estimate ultrasound changes with Modified Alternate day fasting (ADF) as intervention)

Objective 2:

To compare liver elasticity using shear wave elastography (SWE) during pre and post Modified Alternate Day Calorie Restriction in (NAFLD) patients

(There are no available data at the time of study design to estimate ultrasound changes with Modified Alternate day fasting (ADF) as intervention)

Objective 3:

To correlate Shear wave ultrasonographic elastography (SWE) result with grading of fatty liver.

(There are no available data at the time of study design to estimate correlation transient elastrography with grading fatty liver using Modified Alternate day fasting (ADF) as intervention)

2.6 Patient Recruitment and Study protocol

2.6.1 Subject

Non-alcoholic fatty liver disease patients who attended the Gastroenterology Clinic were screened from August 2015 till July 2016. A total of 105 participants were screened but only 57 patients fulfilled inclusion criteria. Out of 57 participants, only 41 participants agreed to be enrolled in the trial. All participants were required to complete a 2 week run-in period consisting of maintaining and self-monitoring of usual dietary and daily activity. Participants had been explained regarding the trial and after fulfilling the selection criteria, informed consent were taken. Demographic data of the participants were recorded. Participants were given an appointment date for an ultrasound and blood-taking pre and post intervention.

2.6.2 Diet protocol

A designated dietician gave advise to the participants regarding calorie restriction and monitored adherence to intervention. During the 2 weeks control period (before the intervention programme started), participants were required to keep their body weight stable by maintaining their usual eating and activity habits, then, during the 8 week modified alternate day calorie restriction period, all subjects consumed only 30% of their energy needs over 24 hours on each fast day and then consumed ad libitum on each alternate feed day. The feed and calorie restriction days began at 9am each day, and on calorie restriction day, meals were consumed between 2pm and 8pm to ensure that each participants were undergoing the same of time of taking a meal. On each calorie restriction day, the participants were allowed to consume energy-free beverages, tea, coffee, and sugar-free gum and were encouraged to drink plenty of water. Diets were not provided to participants but were self-selected using detailed individualised food portion lists, meal plans, and recipes. To maximise compliance participants received phone calls and 2 weekly appointments to ensure adherence. All participants were encouraged to use cognitive behavioral techniques such as self-monitoring, obtaining peer/family support and stimulus control to maintain diets. Adherence to intervention programme was assessed via diet diary and also dietary recall during follow up by the dietician.

2.7 Research Tools

2.7.1 Ultrasound machine – Supersonic Ultrasound Machine (Airexplorer) by Supersonic Image, Air-en-Provence, France (Figure 3 Super Sonic Imagine's Aixplorer® by Super Sonic Image)

2.7.2 Picture Archiving and Communication System (PACS)

2.7.3 Statistical software (SPSS 22.0 for windows; SPSS Inc., Chicago , IL, USA)

2.8 Assessment of Liver on Ultrasound

2.8.1 Fatty Liver Grading

Patients were put in supine position. Each patient underwent a conventional grey scale liver ultrasonography (B mode) using the Aixplorer ultrasound system (SuperSonic Imagine) with 5MHz linear transducer. Assessment of the liver parenchyma were performed and graded into fatty liver grading (Singh et al., 2013). Fatty liver or liver steatosis was graded by comparing the echogencity of liver parenchyma to the adjacent structures. It was categorized into 4 grades as shown in Figure 4. Grade 0 (normal) showed normal liver echogencity, Grade 1 showed generalized increased in echogencity but the echogenic walls of portal venous branches was still demonstrated, Grade 2 showed echogenic liver parenchyma, obscuring the echogenic walls of the portal venous branches and Grade 3 in whch the diaphragmatic outline was obscured. (Singh et al., 2013)

2.8.2 Liver Stiffness Grading

Subsequently, shear wave elastography were performed. Elastography application was selected. SWE was performed using a standard M probe. It uses a 5-MHz ultrasound transducer, which was mounted on the axis of a vibrator. The vibrator generates a painless vibration generating shear waves that propagate through the skin and the subcutaneous tissue into the liver. The shear wave velocity is directly related to the liver stiffness. In all patients SWE measurements were performed in the right liver lobe, by intercostal approach, in supine position with the right arm in maximal abduction. Liver portion of at least 6 cm thick and free of large vascular structures was identified. Once the measurement area had been located, the operator pressed the probe button to start an acquisition. The Q box is placed over the area of interest and the quantitative elasticity values will be obtained automatically by moving the delineated Q box over the colour map. Ten successful measurements were performed on each patient and a median value expressed in kPa was calculated. Interpretation of liver fibrosis by shear wave elastography in kPa divided the entity into no fibrosis (F0), mild fibrosis (F1), severe fibrosis (F2), significant fibrosis (F3) and cirrhosis (F4). Automatic median value generated by the ultrasound software was used to establish the elastography grade as follows < 4.6 = F0, 4.6-5.6 = F1, 5.7-7.0 = F2, 7.1-12.0 = F3 and > 12 = F4 (adapted from (Sande, Verjee, Vinayak, Amersi, & Ghesani, 2017) . Images were recorded in Picture Archiving Computer System (PACS) for retrieval. Figure 3.6.2.5 shows SWE shear-wave elastography values obtained from offline quantification by placing a region of interest on elastograms obtained in upper right lobe (adapted from (Samir et al., 2015)) . Liver stiffness fibrosis stage is shown in Table 3.6.1.1 (adapted from (Sande et al., 2017)). Figure 3.6.2.6(A) and Figure 3.6.2.6(B) showed an example of SWE image taken in one of our patients.

2.9 Operational Definition

2.9.1 Non-alcoholic fatty liver disease (NAFLD)

It represents a spectrum of progressive liver disease ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis, and cirrhosis, in the absence of excessive alcohol consumption. NAFLD is regarded as a hepatic manifestation of metabolic syndrome

2.9.2 Non alcaholic Steatohepatitis (NASH)

It is defined as non alcaholic steatohepatitis, characterised by elevated transaminases and lipid level followed by hepatocyte injury, inflammation, and fibrosis, is a clear risk factor for progression to cirrhosis

2.9.3 Shear wave elastography (SWE)

It is a non-invasive technique to assess liver tissues stiffness. 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 10m/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.

2.10 Validation study

Validation study was performed to validate the technique of the researcher in performing conventional ultrasound and shearwave elastrography study. The technique was validated by a senior consultant radiologist with 10 years of working experience in radiology. The researcher performed conventional ultrasound and shearwave elastrography study on a patient and the examination was repeated in the same setting by the radiologist. Images were stored in PACS system. Data were entered in SPSS system and analysed.

2.11 Statistical analysis:

Descriptive analysis was used to calculate the mean and standard deviation of the participants' demographic data. Analysis of liver steatosis grading and mean liver elasticity were performed Super Sonic Airexplorer ultrasound machine. We used an independent t-test to investigate liver steatosis grading and mean liver elasiticity after 8 weeks modified alternate day calorie restriction. Kendall tau correlation was used to examine relationships between mean liver steatatosis and mean liver elasticity. Sensitivity, specificity, positive predictive value and negative predictive value of elastography were analysed using receiver operating curve (ROC).

CHAPTER 3. MANUSCRIPT

3. MANUSCRIPT

3.1 INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver diseases in Western countries, occurring in approximately 30% of the general population. The spectrum of this disease includes simple steatosis, nonalcoholic steatohepatitis (NASH), liver fibrosis, and liver cirrhosis. Patients with NAFLD shows higher mortality rate as compare to general population.(Mohammadi et al., 2012). The exact risk or incidence of progression from simple hepatic steatosis to advanced stages of fatty liver disease has yet to be determined, the progression of simple hepatic steatosis to cirrhosis through the development of steatohepatitis (NASH) and fibrosis has been established. NASH is characterised by hepatocyte injury, inflammation, and fibrosis. These are known risk factor for progression to cirrhosis, and such progression has been reported. NASH is additionally associated with an incremented risk of liver cancer and death from cardiovascular diseases or liver related causes (Lee & Park, 2014). It is very crucial for early detection of liver fibrosis as prevalence of obesity; hence fatty liver disease is high in our country.

Histopathological examination remains the gold standard for diagnosing liver fibrosis despite the intraobserver and interobserver variability in staging. Regev et al found approximately 25% of patients (30 of 124) to have a difference of at least one grade and 33% (41 of 124) to have at least one stage difference between the right and left liver lobes. Ultimately, they found an underdiagnosis of cirrhosis in approximately 15% of patients. (Regev et al., 2002). Furthermore, liver biopsy is a painful technique despite local anaethesia. Generally it is not well accepted by most patients, it has morbidity and mortality risks, and is not an ideal method as routine reasessment. Thus, noninvasive methods for assessing liver fibrosis are of great clinical interest. In the last decade, techniques to noninvasively estimate the stage of liver fibrosis have become commercially available. They have the capability to evaluate differences in the elasticity of soft tissues by measuring tissue behavior when a mechanical stress is applied. Ultrasound and magnetic resonance imaging have been used for elasticity imaging. Magnetic resonance elastography, even though promising, has some disadvantages because it cannot be performed in a liver with an iron overload because of signal-to-noise limitations, the examination takes longer time as compare to ultrasound elastography, and it is a costly procedure (Ferraioli et al., 2014).

With the increasing prevalence of NAFLD worldwide, there is a continuing need to develop noninvasive methods to assess fibrosis, NASH, and non-NASH NAFLD. In this issue of Hepatology, Cassinotto et al. added substantially to our understanding of the role of elastography-based imaging modalities on assessment of fibrosis in NAFLD. In the current state, elastography imaging modalities appear most useful to exclude significant fibrosis and cirrhosis with a high negative predictive value (Hannah & Harrison, 2016).

Ultrasound imaging is a well-established and cost effective imaging technique for the diagnosis of hepatic steatosis, specifically for screening a large population at risk of NAFLD. Ultrasonography has a reasonable accuracy in detecting moderate-to-severe hepatic steatosis although it is less accurate for detecting mild hepatic steatosis, operator-dependent, and rather qualitative(Lee & Park, 2014).Ultrasound also allows for reliable and accurate detection of moderate-severe fatty liver, compared to histology. Because of its low cost, safety, and accessibility, ultrasound is likely the imaging technique of choice for screening for fatty liver in clinical and population settings (Hernaez et al., 2011).

Tissue stiffness in liver is related to tissue composition, which is changed by cirrhosis, hepatocellular carcinoma or metastases. SWE is a new imaging technique by which the elasticity of soft tissue can be measured quantitatively. SWE is based on the automatic generation and analysis of transient shear waves. This method uses the acoustic radiation force of the ultrasound wave to push the tissue instead of using a compression force (called stress), as is used in conventional dynamic ultrasonographic elastography. The acoustic displacement of tissue is free of user dependence and is reproducible (Arda, Ciledag, Arıbas, et al., 2013). Shear wave-based elastography applies a perpendicular stress force to a target organ in order to induce shear on the tissues. The information on the propagating shear wave including the velocity of the shear wave could be measured by obtaining radiofrequency images with a high frame rate, which can be used to generate a tissue displacement map. Then, the elastic property for quantitave estimation is calculated by propagating velocity of the shear wave. ARFI, acoustic radiation force impulse. (Figure 3.6.2.1)(Jeong et al., 2014).

The reproducibility of the SWE method is very high, with intraobserver intraclass correlation coefficients of 0.95 and 0.93 for an expert and a novice operator, respectively, and interobserver agreement of 0.88. As for conventional sonography, it is user dependent; thus, it is recommended that at least 50 supervised scans and measurements should be performed by a novice operator to obtain consistent measurements. Values obtained in a small series of healthy participants ranged from 4.92 kPa (1.28 m/s) to 5.39 kPa (1.34 m/s).(Hernaez et al., 2011).

The purpose of this study is to compare liver steatosis grading and liver elasticity grading after 8 week of Modified Alternate Day Calorie Restriction in

NAFLD patientsas well as to determine the correlation between liver elasticity and liver steatosis grading among the patients. We found that this dietary method is easier and acceptable to most of the patients as compared to continous daily calories restriction because the patients were only required to limit the calories intake only three days in a week and can take normal diet the rest of the days. Previous study proven that even short term caloric restriction can rapidly improve, and hypercaloric feeding can rapidly worsen NAFLD and NAFLD-associated hyperlipidemia and insulin resistance. Both low carbohydrate and low-fat diets that cause weight loss are associated with decreases in intrahepatic triglyceride and improved metabolic phenotype(Sullivan, 2010). With the new advancement, a new technique of shear wave elastography was developed but still under limited study especially in Asian population. Therefore, the purpose of this study was to detect liver fibrosis earlier, hence the progression of liver steatosis to steatohepatitis, fibrosis and eventually hepatocellular carcinoma is reduced. Other than that, this method is noninvasive which makes it more acceptable to patients as compared to liver biopsy. By doing this study, we can diagnose and intervene the patient earlier.

3.2 METHODOLOGY

3.2.1 Subjects

Non-alcoholic fatty liver disease patients who attended the Gastroenterology Clinic were screened from August 2015 till July 2016. A total of 105 participants were eligible but only 57 patients fulfilled the inclusion criteria. Out of 57 participants, only 41 participants agreed to be enrolled in the trial. All participants were required to complete a 2 week run-in period consisting of maintaining and self-monitoring of usual dietary and daily activity. Participants were explained regarding the trial and after fulfilling the selection criteria, informed consent were taken. Demographic data of the participants were recorded. Participants had been given an appointment date for an ultrasound and blood-taking pre and post intervention.

3.2.2 Diet protocol

The dietition involved in this study designed a dietary protocol for the participants. She advised participants regarding calorie restriction and monitored adherence to the programme. During the 2 weeks control period, participants were required to keep their body weight stable by maintaining their usual eating and activity habits, then, during the 8 week modified alternate day calorie restriction period, all subjects consumed only 30% of their energy needs over 24 hours on each fast day and then consumed ad libitum on each alternate feed day. The calorie restriction days began at 9am each day, and on calorie restriction day, meals were consumed between 2pm and 8pm to ensure that each participants were undergoing the same of time of taking a meal. On each calorie restriction day, the participants were allowed to consume energy-free beverages, tea, coffee, and sugar-free gum and were encouraged to drink plenty of water. Diets were not provided to participants but were self-selected using detailed individualised food portion lists, meal plans, and recipes. To maximise compliance participants received phone calls and 2 weekly appointments to ensure adherence. All participants were encouraged to use cognitive behavioral techniques such as self-monitoring, obtaining peer/family support and stimulus control to maintain diets. The reseacher assessed adherence to intervention via diet diary and also dietary recall during follow up.

3.2.3 Statistical analysis

Descriptive analysis was used to calculate the mean and standard deviation of the participants' demographic data. Analysis of liver steatosis grading and measurement of liver elasticity were performed using Super Sonic Airexplorer ultrasound machine. We used an independent t-test to analyse the liver steatosis grading and mean liver elasiticity after 8 weeks modified alternate day calorie restriction. Kendall tau correlation was used to examine relationships between mean liver stetatosis and mean liver elasticity. Sensitivity, specificity, positive predictive value and negative predictive value of elastography was analysed using receiver operating curve (ROC).

3.3 RESULTS

3.3.1 Demographic data

A total of 39 participants were recruited for the study. The demographic data of the participants for control and intervention groups are as shown in Table 3.6.1.2. In the control group, they are 9 participants with 8 males and 1 female. As for the intervention group, they are 30 participants with 21 males and 9 females. The mean age for control and intervention groups are 50 and 44 years old respectively.

3.3.2 Changes of liver steatosis grading in study participants before and after intervention programme

The median liver steatosis grade of the 30 participants in the intervention group were significantly reduced (p value <0.001) after the modified alternate day calorie restriction programme (Tables 3.6.1.3). Our result showed that 10 patients had improved liver steatosis grading whereby 8 patients improved from grade 2 to grade 1 and 2 patients from grade 1 to grade 0. This might be due to the number of patients in grade 2 is greater to patient in grade 1 in pre intervention phase. We expect that the liver steatosis grading in patients who improved from grade 2 to grade 1 will further improve to grade 0 if we prolonged the study duration. From the same table, we assumed that one patient improved from grade 3 to either grade 2 or grade 1.

On the other hand, no significant changes were observed in the median liver steatosis grade and fibrosis level of the nine participants in the control group.