A STUDY ON VISCERAL FAT IN RELATION TO ANTHROPOMETRY AND LIPID PROFILE USING DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA)

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

HUSM	Hospital Universiti Sains Malaysia
DEXA	Dual energy x-ray absorptiometry
VAT	Visceral adipose tissue
SAT	Subcutaneous adipose tissue
TG	Triglyceride
LDL	Low density lipoprotein
HDL	High density lipoprotein
BMI	Body Mass Index
WC	Waist circumference
ROI	Region of interest

ABSTRAK

Latar belakang: Lemak organ perut memainkan peranan yang penting dan sebagai penanda kepada pelbagai penyakit dan mobiditi. Lemak organ perut adalah lemak yang mengelilingi organ dalaman abdomen. Pembahagian lemak ini di dalam badan manusia bergantung kepada banyak faktor seperti umur, jantina dan etnik. Tujuan kajian ialah untuk mengkaji perkaitan antara lemak organ perut dengan jantina, antropometri dan profil lemak menggunakan *dual energy x-ray absorptiometry* (DEXA).

Metodologi: Subjek dikumpul dari Hospital Universiti Sains Malaysia, Kelantan. Kriteria kemasukan kajian adalah dewasa berumur 18 hingga 50 tahun. Subjek yang mengandung, mempunyai berat badan melebihi 150kg, mempunyai masalah kesihatan yang memerlukan ubat *anti-lipid* dan menggunakan ubat steroid dalam tempoh yang panjang (melebihi 2 minggu) tidak layak menyertai kajian ini. Ukuran antropometri seperti ukur lilit pinggang, tinggi, berat dan indeks jisim tubuh dikira. Subjek perlu berpuasa sekurang-kurangnya 8 jam untuk ujian darah lemak. Hanya keputusan ujian darah lemak dalam tempoh 3 bulan sebelum atau selepas imbasan DEXA diambil kira. Imbasan DEXA dilakukan untuk mendapatkan nilai lemak organ perut dalam unit gram (g).

Keputusan: Seramai 112 orang subjek yang memenuhi kriteria kajian. Terdapat perbezaan signifikan lemak organ perut antara subjek lelaki dan perempuan (p=0.024), yang mana subjek lelaki menunjukkan nilai lemak organ perut lebih tinggi berbanding subjek perempuan. Kajian juga menunjukan perkaitan yang kuat antara lemak organ perut dengan ukur lilit pinggang, indeks jisim tubuh dan berat dalam kedua-dua jantina. Terdapat perkaitan yang signifikan antara lemak organ perut dengan profil lemak TG (r=0.500, p=0.000), LDL (r=0.222, p=0.019) dan HDL (r= -0.440, p=0.000); yang mana HDL yang menunjukkan perkaitan negatif dengan tisu lemak perut.

Kesimpulan: Keputusan kajian menunjukkan perbezaan signifikan lemak organ perut antara jantina. Terdapat perkaitan signifikan antara lemak organ perut dengan ukur lilit pinggang, indeks jisim tubuh dan profil lemak. Oleh itu, ukur lilit pinggang, indeks jisim tubuh dan profil lemak boleh digunakan sebagai petunjuk untuk menjangka lemak organ perut.

Kata kunci: *Dual energy x-ray absorptiometry*, Profile lemak, Lemak organ perut, Indeks jisim tubuh, Ukur lilit pinggang

ABSTRACT

Background: Visceral fat plays important role and as an indicator in many health related disease and morbidity. It is defined as body fat that stored within the abdominal cavity around the important internal organs. The fat distribution in the human body is dependent on many factors such as gender, age and ethnicity. The purpose of this study is to determine the correlation between visceral fat and gender, anthropometry; and lipid profile using dual energy x-ray absorptiometry (DEXA).

Methodology: Subjects were recruited from Hospital Universiti Sains Malaysia, Kelantan. The inclusion criteria was adult aged 18 to 50 years old. Participants who were pregnant, weighed more than 150kg, had any medical illness requiring anti-lipid medication and had prolonged usage of steroid (more than 2 weeks) were excluded from the study. Anthropometric measurements such as waist circumference, height, weight and BMI were performed. Participants needed to fast for at least for 8 hours for serum lipid profile test. Fasting serum lipid profile result obtained within 3 months before or after DEXA scan was considered valid. DEXA scan was performed to quantify the visceral fat in unit gram (g).

Results: Total of 112 participants fulfilled the study criteria. There was significant different of visceral fat between male and female (p=0.024), in which male subjects showed significantly higher VAT than female. It also showed strong correlation between VAT with waist circumference, BMI and weight in both genders. There was significant correlation between VAT with triglycerides (r=0.500, p=0.000), LDL (r=0.222, p=0.019) and HDL (r= -0.440, p=0.000); from which only HDL presented negative correlation with VAT.

Conclusion: The findings of the study showed significant different of visceral fat between gender. There is significant correlation between VAT and WC, BMI and serum lipid profile. Thus, WC, BMI and lipid profile can be used as indicator to predict VAT.

Keywords: Dual energy x ray absorptiometry, Lipids, Visceral fat, Body Mass Index, Waist Circumference

CHAPTER 1: INTRODUCTION

1.1 LITERATURE REVIEW

Adipose tissue is a connective tissue composed mainly of adipocytes that contain lipid droplets. It fills up space between organs and tissues and provides one of main source of stored energy, structural and metabolic support. Adipose tissue is distributed in different proportions in human body and the pattern of distribution is influenced by many factors such as age, gender, ethnicity, physical activity and medication (Wu *et al.*, 2001). It is distributed into two components, which are subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (Shuster *et al.*, 2012), and each has different metabolic characteristic.

Out of these two, visceral adipose tissue (VAT) gains particular attention as its association with various medical pathologies such as insulin resistance, cardiovascular disease, impaired lipid metabolism and predisposition to cancer (Kaess *et al.*, 2012; Ritchie and Connell, 2007). This may be explained by the fact that VAT has a higher lipolytic activity and it directly releases free fatty acids into the portal circulation (Kim *et al.*, 2011).VAT is defined as body fat stored within the abdominal cavity, around important internal organs such as liver, spleen, pancreas and intestines. VAT drains its blood into the portal vein and account for 6-20% of total body fat, with higher values in males than females. Adipose tissues in the retroperitoneal compartment, about 7% of total, do not drain into the portal vein and are therefore not considered VAT (Karastergiou *et al.*, 2012).

It is shown that there is difference in fat distribution between genders whereby women have more body fat than men. For the same body mass index (BMI), women typically present with about 10% higher body fat compared to men. However, the deleterious metabolic consequences of the central obesity are typical in men. Men store more fat in the visceral depot whereas women store more fat in the gluteal-femoral region (Blaak, 2001; Wu *et al.*, 2001). The pear-shaped body fat distribution of women is associated with lower cardio metabolic risk. It simply provides a safe lipid reservoir for excess energy, or may directly regulate systemic metabolism via release of metabolic products or adipokines (Karastergiou *et al.*, 2012). Ageing increases adiposity in both sexes, accumulation of visceral fat and ectopic fat deposition.

Visceral obesity can be estimated using several anthropometric measures, such as BMI, waist circumference (WC), waist-to-hip ratio, waist-to-height ratio, or sagittal abdominal diameter. BMI are the most commonly used diagnostic tool to characterise obesity. According to WHO, normal BMI ranges 18.5 to 24.9 kg/m², BMI greater than 25 kg/m² is defined as overweight and BMI greater than 30 kg/m² is defined as obese. International Diabetes Federation (IDF) has defined the WC cutoff values according to ethnicity and gender e.g., the WC values of 90 cm for men and 80 cm for women in South Asia and China (Alberti *et al.*, 2006). WC was measured at the midpoint between the inferior border of the subcostal margin and iliac crest in the mid-axillary line after normal expiration with the subject standing as stated in most literatures. Compared to the Caucasians, Asians typically exhibit a higher risk of obesity-related morbidity and mortality at a given BMI or WC. For example, Obesity in Asia Collaboration evaluated the optimal WC cutoff values for the identification of diabetes in 155,122 individuals from 10 countries in the Asia-Pacific region (86% Asian, 2 study populations were from Australia), and it was revealed that the cutoff values were 85 cm for men and 80 cm for women. This study showed that the prevalence of diabetes was consistently higher among Asians than that of Europeans at any given level of the BMI or WC. This clarifies that the ethnic differences play important role in the relationship between being overweight and having diabetes (Kim *et al.*, 2011).

Lipid is organic compound that is poorly soluble in water but miscible in organic solvents. There are 2 main sources of lipid in human bodies which are endogenous storage and exogenous dietary intake. Lipid plays critical role in biological life. It provides a structural component in cells and involved in metabolic and hormonal pathways. Increased level of blood lipid is eventually stored as adipose tissue. Vice versa the adipose tissue is also metabolized into circulating lipid in blood vessel which thus forms a vicious cycle of metabolism pathway.

Abnormal level of lipid in the blood is called hyperlipidaemia or dyslipidaemia. It is also called hyperlipoproteinemia due to its attachment to lipoproteins in the blood circulation. The clinically significant lipoproteins are low density lipoprotein (LDL) and high density lipoprotein (HDL). Excess LDL cholesterol contributes to blockage of the arteries, eventually leads to greater risk of cardiovascular disease, whereas in contrast the low HDL cholesterol contributes such risk.

Previous reports showed significant correlation between visceral fat and lipid profile, e.g. study conducted by Miazgowski *et al.* (2017) in healthy European men and women aged 20-30 years old, Mochizuki *et al.* (2011) in healthy and preclinical Japanese men and Vatanparast *et al.* (2009) in postmenopausal women in Canada.

Various methods are used to assess visceral fat in clinical setting, namely anthropometric measurement, bioelectrical impedance analysis and imaging. Imaging technologies including dual energy x-ray absorptiometry (DEXA), magnetic resonance imaging (MRI) and computed tomography (CT) have been identified as gold standards in body composition analysis. DEXA is the most advantageous choice due to its lower cost, easy access, rapid examination and little radiation.

The principle of DEXA is that it measures the attenuation of two energies emitted from the modality to distinguish fat, lean and bone mineral content. DEXA is able detect whole body fat mass accurately within 2% coefficient of variation and has the capacity for regional analysis (Hill *et al.*, 2007), including the visceral fat. This information is obtained through the whole body scan.

Whole body scan is able to give a full picture of individual body composition which includes total body and regional fat mass, total body and regional lean mass, total body and regional bone marrow content and density, as well as visceral fat mass. Visceral fat measurement is usually taken at L3 to L5 level, nearly similar region measured in CT and MRI. Shuster *et al.* (2012) mentioned that DXA region of interest (ROI) in the trunk extending 5 cm or 10 cm above the iliac crest and laterally to the edges of the abdominal soft tissue corresponds to the third to fifth lumbar vertebrae; and this is the region commonly used in CT imaging to assess VAT. Hill *et al.* (2007) also established similar landmarks and showed this region incorporates in CT slice at L3 to L4 level. Meanwhile, Vatanparast *et al.* (2009) put ROI for VAT manually by adjusting the lines between upper L1 and lower L4 and the inner costal margin of the whole body scan.

In view of the clinical importance of visceral adipose tissue and lipid profile to various diseases, plus the lack of study on association between them especially in Southeast Asia, this study was aimed to study on visceral fat in relation to gender, anthropometry and lipid profile in Malaysia population. This is in line with previous reports said that fat distribution is influenced by ethnicity, different population, age and gender as well as method of assessment.

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CHAPTER 2: STUDY PROTOCOL

2.1 TITLE

A study on visceral fat in relation to anthropometry and lipid profile using Dual Energy X-ray Absorptiometry (DEXA)

2.2 OBJECTIVES

2.2.1 General objective

To study the correlation between visceral fat and gender, anthropometry; and lipid profile using Dual Energy X-ray Absorptiometry (DEXA)

2.2.2 Specific objective

1. To compare visceral fat between male and female subjects.
2. To study the correlation between visceral fat and BMI based on gender.
3. To study correlation between visceral fat and WC based on gender.
4. To determine the association between visceral fat and lipid profile (triglyceride, LDL and HDL).

2.3 METHODOLOGY

2.3.1 Study design

This was a cross sectional study.

2.3.2 Study location and duration

Study was conducted in Hospital Universiti Sains Malaysia (HUSM) from February 2016 until December 2017.

2.3.3 Study population and sample

2.3.3.1 Reference population

Adult subject aged 18 to 50 years old in Kelantan

2.3.3.2 Source population

Subjects in Hospital Universiti Sains Malaysia that fulfilled the study criteria

2.3.4 Sampling technique

Subjects were assigned by using convenience sampling method.

2.3.5 Sampling frame

2.3.5.1 Inclusion criteria

1. Adult aged 18 to 50 years old

2.3.5.2 Exclusion criteria

1. Any medical illness requiring anti-lipid medication
2. Body weight more than 150kg

3. Prolonged usage of steroid more than 2 weeks
4. Pregnancy

2.3.6 Study subject

Subjects were recruited via advertisement. The researcher selected the subjects who fulfilled the study criteria. The researcher explained to the subjects the purpose of the study, confidentiality of information, the nature of examination and the contraindications to DEXA. The risk of exposure to ionizing radiation was explained to the subjects, which was equivalent to one tenth of chest radiograph. Blood test was performed on the day of imaging if the subjects had no previous fasting serum lipid profile within last 3 months. Informed consent was obtained before subjects were enrolled into the study.

2.3.7 Honorarium

Breakfast was provided to the subjects after completion of the imaging such as hot drink and food.

2.3.8 Sample size calculation

For objective 1:

The sample size is calculated using two means comparison (independent t-test) with power of study 80%, confidence level 95%, the maximal standard deviation is 6.6

(Ranasinghe et al., 2013), effect size 0.5, drop out 10%. The corrected sample size was 70, so total sample was 70 x 2 = 140.

For objective 2:

The sample size calculation using correlation formula with significance level (alpha) 0.05, power of study 80%, drop out 10%, expected correlation coefficient 0.46 (Janssen et al., 2002) for male and expected correlation coefficient 0.60 (Janssen et al., 2002) for female. Total sample size calculated was 31 for male and 18 for female.

For objective 3:

The sample size calculation using correlation formula with significance level (alpha) 0.05, power of study 80%, drop out 10%, expected correlation coefficient 0.55 (Janssen et al., 2002) for male and expected correlation coefficient 0.76 (Janssen et al., 2002) for female. Total sample size calculated was 24 for male and 11 for female.

For objective 4:

The sample size calculation using G* Power tool 3.1, Family t-test with statistical test using linear bivariate regression: one group, size of slope. Significance level (alpha) 0.05, the power of study 80%, standard deviation-x 1048, standard deviation-y 1, slope H0 0, correlation 0.21(Vatanparast et al., 2009). The sample size calculated was 173.

Therefore, largest sample size calculated which is 173 subjects was used in this study.

2.3.9 Research tools

1. DEXA machine – Hologic Discovery A fan beam
2. Workstation – Hologic Discovery QDR software version 13.4.2
3. Physical examination
   a) Weight: digital weighing SECA scale
   b) Height: portable meter SECA scale
   c) WC: measuring tape

2.3.10 Operational definition

1. Lipid profile

Fasting lipid profile from venous blood that taken within 3 months or less, before or after the DEXA imaging. Subjects were fasted overnight at least for 8 hours before imaging. Results were according to UK universal unit, mmol/l.

2. Visceral fat

The value was taken from visceral adipose tissue mass, in unit gram. It was automatically calculated at the region provided by custom DXA Discovery software, which was equivalent to L3-L4 vertebra level and laterally to the edges of the abdominal soft tissue.

3. Age

Age was taken according to year the subject was born.

4. Body mass index

Body mass index was defined as the body mass in kilograms divided by the square of the body height in meters, in unit kg/m^2.

2.3.11 Data collection

Data were collected from Radiology Information System (RIS) and Picture Archive Communication System (PACS) in HUSM. Subject's information was collected in the data sheet and labeled with serial number in order to maintain privacy and confidentiality of subject.

2.3.11.1 Anthropometric measurement

1. Weight: Subject stand straight without shoes and accessory and measured to the nearest 0.1kg.
2. Height: Subject stand straight with chin up and measured to the nearest 0.1m without shoes.
3. Waist circumference: Subject stand with feet 25-30cm apart so that weight evenly distributed. Measurement was taken at midway between the inferior margin of the last rib and the iliac crest in a horizontal plane. The measurer fitted the tape snugly but not compressed soft tissues and measured to the nearest 0.1cm.

2.3.11.2 Image acquisition

All subjects wore a hospital gown and were asked to remove all materials that could attenuate the x-ray beam, e.g. jewelry, pin and metal. All subjects were in supine position and whole body scan using fan beam technology was performed. Through this whole body scan, a standard soft tissue examination included total body and regional measurement (head, trunk, arms and legs) of fat mass, lean tissue and bone mineral content. The sub region in the abdomen at the level of L3-L4 was interpreted as visceral adipose tissue (VAT). It was analysed into estimated VAT mass, estimated VAT volume and estimated VAT area. Estimated VAT mass was taken as visceral fat in unit gram. The duration of the imaging was about 10 minutes. The machine was calibrated according to standard procedures before each scanning session.

2.3.12 Image analysis

All image processing was performed using Hologic QDR system Version 13.4.2 software. Images were reviewed and analysed by researcher.

2.3.13 Statistical analysis

Data from data sheet were entered and analysed using Statistical Package for Social Sciences SPSS system (version 22). Subjects were grouped according to gender. Descriptive statistics for subject data were expressed as mean and standard deviation. The normality of visceral fat, lipid profile, BMI and WC were tested. The mean differences of visceral fat between male and female subjects based on age group were compared using independent student t-test. The correlation between visceral fat and BMI and WC based on gender were determined using Pearson correlation. The association between visceral fat and lipid profile were determined using Pearson correlation. The results of student t-test and Pearson correlation were presented using tables.

CHAPTER 3: MANUSCRIPT

3.1 TITLE PAGE

A STUDY ON VISCERAL FAT IN RELATION TO ANTHROPOMETRY AND LIPID PROFILE USING DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA)

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3. All radiographer in charge for Dual Energy X-ray Absorptiometry (DEXA).
4. Ethical approval from members of Human Research Ethics Committee USM (HREC).
5. Colleagues and all the staff in the Department of Radiology, HUSM who have supported me and lent me their helping hand in completing this study.
6. Loving husband, parents and sibling; millions of thanks and love for the sacrifices they have made in the whole year of study. Their prayer and patience have made me stay strong in this journey.

MAIN DOCUMENT

3.2. TITLE

A STUDY ON VISCERAL FAT IN RELATION TO ANTHROPOMETRY AND LIPID PROFILE USING DUAL ENERGY X-RAY ABSORPTIOMETRY (DEXA)

3.3 ABSTRACT

Background: Visceral fat plays important role and as an indicator in many health related disease and morbidity. It is defined as body fat that stored within the abdominal cavity around the important internal organs. The fat distribution in the human body is dependent on many factors such as gender, age and ethnicity. The purpose of this study is to determine the correlation between visceral fat and gender, anthropometry; and lipid profile using dual energy x-ray absorptiometry (DEXA)

Methodology: Subjects were recruited from Hospital Universiti Sains Malaysia, Kelantan. The inclusion criteria was adult aged 18 to 50 years old. Participants who were pregnant, weighed more than 150kg, had any medical illness requiring anti-lipid medication and had prolonged usage of steroid (more than 2 weeks) were excluded from the study. Anthropometric measurements such as waist circumference, height, weight and BMI were performed. Participants needed to fast for at least for 8 hours for serum lipid profile test. Fasting serum lipid profile result obtained within 3 months before or after DEXA scan was considered valid. DEXA scan was performed to quantify the visceral fat in unit gram (g).

Results: Total of 112 participants fulfilled the study criteria. There was significant different of visceral fat between male and female (p=0.024), in which male subjects showed significantly higher VAT than female. It also showed strong correlation between VAT with waist circumference, BMI and weight in both genders. There was significant correlation between VAT with triglycerides (r=0.500, p=0.000), LDL (r=0.222, p=0.019) and HDL (r= -0.440, p=0.000); from which only HDL presented negative correlation with VAT.

Conclusion: The findings of the study showed significant different of visceral fat between gender. There is significant correlation between VAT and WC, BMI and serum lipid profile. Thus, WC, BMI and lipid profile can be used as indicator to predict VAT.

Keywords: Dual energy x ray absorptiometry, Lipids, Visceral fat, Body Mass Index, Waist Circumference

3.4 INTRODUCTION

Adipose tissue is a connective tissue composed mainly of adipocytes that contain lipid droplets. It fills up space between organs and tissues and provides one of main source of stored energy, structural and metabolic support. Adipose tissue is distributed in different proportions in human body and the pattern of distribution is influenced by many factors such as age, gender, ethnicity, physical activity and medication (1). It is distributed into two components, which are subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) (2), and each has different metabolic characteristic.

Visceral adipose tissue (VAT) gains particular attention as its association with various medical pathologies such as insulin resistance, cardiovascular disease, impaired lipid metabolism and predisposition to cancer (3, 4). This may be explained by the fact that VAT has a higher lipolytic activity and it directly releases free fatty acids into the portal circulation (5).VAT is defined as body fat stored within the abdominal cavity, around important internal organs such as liver, spleen, pancreas and intestines. VAT drains its blood into the portal vein and account for 6-20% of total body fat, with higher values in males than females.

Increased VAT causes increased proportion of small and dense low density lipoprotein (LDL) and high density lipoprotein (HDL), as well as triglyceride. This is due to remodeling of lipoprotein by various enzymes such as cholesteryl ester protein and hepatic triglyceride lipase. Smaller HDL cholesterol is prone to degradation and clearance from blood circulation, subsequently causing low HDL level in visceral obesity individuals. In contrast, small and dense LDL cholesterol is highly atherogenic as it becomes less bind to the LDL receptor result in increased its residence time and number in blood, thus next causing less its clearance from circulation. With longer LDL cholesterol residence time in circulation, it is more likely to interacts with endothelial surface of the artery and prone to LDL oxidation inside the artery wall. Increased triglyceride is also atherogenic as much as LDL.

It is shown that there is difference in fat distribution between genders whereby women have more body fat than men. For the same body mass index (BMI), women typically present with about 10% higher body fat compared to men. However, deleterious metabolic consequences of the central obesity are typical in men. Men store more fat in the visceral depot whereas women store more fat in the gluteal-femoral region (1, 6). As we grow older, adiposity is increased in almost equal distribution in both sexes with accumulation of visceral fat and ectopic fat deposition. Ageing causes redistribution of adipose tissue towards central or android phenotype in peri-menopausal women likely due to fall in estrogen levels.

Visceral obesity can be estimated using several anthropometric measures, such as BMI, waist circumference (WC), waist-to-hip ratio, waist-to-height ratio, or sagittal abdominal diameter. BMI are the most commonly used diagnostic tool to characterise obesity. According to WHO, normal BMI ranges 18.5 to 24.9 kg/m^2, BMI greater than 25 kg/m^2 is defined as overweight and BMI greater than 30 kg/m^2 is defined as obese. International Diabetes Federation (IDF) has defined the WC cutoff values according to ethnicity and gender e.g., the WC values of 90 cm for men and 80 cm for women in South Asia and China (7). WC was measured at the midpoint between the inferior