Pancreas Volume and Fat Deposition in Type II Diabetes Mellitus

Dr. Anusha A/P Ramanaidu

Department of Radiology, School of Medical Sciences,
Universiti Sains Malaysia Hospital (Hospital USM)

Dissertation Submitted In Partial Fulfilment Of The Requirement For Master Of Medicine (Radiology)

UNIVERSITI SAINS MALAYSIA

2022

Pancreas Volume and Fat Deposition in Type II Diabetes Mellitus

Dr. Anusha A/P Ramanaidu

Department of Radiology, School of Medical Sciences,
Universiti Sains Malaysia Hospital (Hospital USM)

Dissertation Submitted In Partial Fulfilment Of The Requirement For Master Of Medicine (Radiology)

UNIVERSITI SAINS MALAYSIA

2022

ACKNOWLEDGMENT

I would like to express my utmost gratitude for giving me the opportunity and endlessly guiding me throughout the completion of this thesis. The completion of this thesis would also not have been possible without the participation, cooperation, and assistance of so many people whose names may not all be enumerated. Their contributions are sincerely appreciated and gratefully acknowledged. However, I would like to place on record, my deepest and utmost appreciation, particularly to the following:

1. Assoc. Professor. Dr. Mohd Ezane Aziz my main supervisor, assisted and guided me in carrying out this study until its completion.
2. Assoc. Professor. Dr.Wan Mohd Izani Wan Mohamed, my co-supervisor, was supportive and provided invaluable insights during the progress of this study.
3. Associate Professor Dr. Mohd Shafie Abdullah, Associate Professor Dr. Juhara Haron, Dr. Khairil Amir Sayuti, Dr. Chandran Nadarajan, Dr. Ahmad Hadif Zaidin Samsudin, Dr. Wan Aireene Wan Ahmed, Dr. Nur Asma Sapiai, Dr. Nasibah Mohamad, Dr. Bazli Md Yusof, Dr. Fattah Rahiman Ghazali, Dr. Ahmad Aizuddin Mohamad Jamali, Dr. Lau Chiew Chea all of whom had directly or indirectly contributed their ideas and comments to the success of this study.
4. Radiographers, colleagues, and all the other staff in the Department of Radiology, Hospital Universiti Sains Malaysia (HUSM).
5. Finally, my family for their unconditional love, support, and motivation.

DISCLAIMER

I declare that this dissertation records the results of my study performed byme and that it is of my composition.

-- ----------------------------------

(ANUSHA A/P RAMANAIDU) (DATE)

TABLE OF CONTENT

LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

PV	Pancreatic volume
NPA	Normal Pancreatic Attenuation
BMI	Body Mass Index
HU	Hounsfield's unit
CT	Computed Tomography
FBS	Fasting Blood Sugar
HbA1c	Glycated haemoglobin
FLP	Fasting Lipid Profile
TG	Triglycerides
HDL-C	High-density lipoprotein-cholesterol
LDL-C	Low-density lipoprotein-cholesterol
NHMS	National Health and Morbidity Survey
PACs	Picture Achieve and Communication System

LIST OF FIGURES

ABSTRAK

Later Belakang

Diabetes mellitus adalah sekumpulan penyakit metabolik yang dikaitkan dengan kecacatan fungsi sel β atau rintangan insulin yang dicirikan oleh hiperglisemia akibat pengurangan isipadu pankreas dan peningkatan dalam lemak pankreas akibat kawalan gula yang lemah. Dalam pesakit dengan diabetes mellitus jenis II terdapat perkaitan yang kuat dengan isipadu pankreas dan pengecilan pankreas yang normal. Oleh itu, matlamat utama kajian ini adalah untuk mewujudkan pengurangan ketara dalam isipadu pankreas dan peningkatan dalam pengecilan pankreas normal dalam Diabetes Mellitus Jenis II, berbanding dengan populasi bukan diabetes mellitus, berdasarkan penilaian menggunakan tomografi berkomputer (CT) .Objektif lain adalah untuk melihat perkaitan faktor metabolik seperti indeks jisim badan (BMI), hemoglobin A1C (HbA1c), gula darah puasa, profil lipid puasa (jumlah kolesterol, lipoprotein berketumpatan tinggi (HDL), dan lipoprotein berketumpatan rendah (LDL,) dan trigliserida dalam pesakit diabetes mellitus jenis II.

Method:

Kajian keratan rentas prospektif telah dijalankan di Hospital Universiti Sains Malaysia (HUSM) ,Kota Bharu, Kelantan, Malaysia melibatkan 74 subjek yang menjalani CT scan abdomen / pelvis dimasukkan dalam kajian. Pesakit dikelaskan kepada diabetes mellitus type II dan non-diabetes mellitus. Di antara pesakit ini 39 adalah bukan diabetes dan 35 adalah diabetes. Pesakit ini termasuk lingkungan umur dari 35 tahun hingga 70 tahun dengan purata umur pesakit adalah 56 tahun. Terdapat 34 pesakit wanita dan 30 lelaki masing-masing. Isipadu pankreas dan pengecilan pankreas dibandingkan antara diabetes mellitus jenis II dengan non-diabetes mellitus menggunakan ujian t bebas. Penemuan imbasan CT abdomen dikumpul dan dianalisis menggunakan. Untuk isipadu pankreas, formula diameter berikut (AP*tail* + AP*body*)/2 x L(badan&ekor) X CCbody + $(APhead/2)^2$ x 3.14 x CChead. Penyusupan lemak diperoleh menggunakan ROI dan mengukur unit Hounsfield antara pankreas dan limpa HU(pankreas – limpa). Jumlah isipadu pankreas dan pengecilan pankreas kemudiannya dibandingkan antara diabetes mellitus jenis II dan bukan diabetes mellitus menggunakan ujian-t bebas. Kovariat dianalisis dengan menggunakan ANCOVA.

Keputusan:

Pesakit diabetes mellitus jenis II mempunyai min isipadu pankreas yang jauh lebih kecil dengan perbezaan min 10.18cm^3 (95% CI = 2.16, 18.20). Purata isipadu pankreas dengan sisihan piawai (SD) 64.82cm^3 dalam diabetes mellitus jenis II dan 75.00cm^3 dalam subjek bukan diabetes mellitus. Isipadu pankreas dikategorikan ke dalam saiz normal atau berkurangan, pada pesakit diabetes dengan bahagian pankreas berkurangan yang lebih tinggi ($p = 0.003$). Tidak terdapat perbezaan yang signifikan dalam pengecilan pankreas normal antara pesakit diabetes mellitus jenis II dan bukan diabetes mellitus. Purata pengecilan pankreas HU(pankreas – limpa) mempunyai sisihan piawai (SD) sebanyak – 4.7 dan sisihan piawai (SD) bagi subjek diabetes mellitus jenis II dan bukan diabetes mellitus ialah– 5.74 dan – 3.86 masing-masing. Umur kovariat, HbA1c, dan paras gula darah puasa berkorelasi dengan ketara dengan isipadu pankreas dalam diabetes mellitus jenis II, di mana semua pembolehubah ini mempunyai korelasi songsang yang sederhana. Hanya indeks jisim badan (BMI) mempunyai korelasi yang ketara dengan peningkatan dalam pengecilan pankreas normal dengan korelasi songsang yang sederhana.

Kesimpulan:

Dalam kajian kami menggunakan pengukuran tomografi berkomputer (CT) multidetector bagi isipadu pankreas boleh digunakan sebagai alat untuk menyaring individu dengan diabetes mellitus jenis II, di mana terdapat perkaitan yang signifikan dengan pengurangan jumlah pankreas. Walau bagaimanapun, pengecilan pankreas biasa tidak menunjukkan korelasi yang signifikan antara diabetes mellitus jenis II dan bukan diabetes mellitus dalam kajian kami. Isipadu pankreas, umur faktor metabolik yang berubah-ubah, HbA1c, dan gula darah berpuasa mempunyai korelasi songsang yang sederhana. Dalam pengecilan pankreas normal, faktor metabolik berubah yang mempunyai korelasi hanyalah BMI.

ABSTRACT

Background:

Diabetes mellitus is a metabolic disease associated with a defect of β cell function that causes insulin resistance characterised by hyperglycemia. In the diabetes mellitus population, the reduction of pancreatic volume and an increase in the pancreas attenuation cause uncontrolled sugar in patients. In patients with type II diabetes mellitus, there is a strong association between pancreas volume and pancreatic attenuation. Many studies conducted in Europe and other Asian countries showed diabetes mellitus has an association with reduced pancreas volume and an increase in pancreas attenuation in diabetes mellitus population. The aim of this study is to establish a significant reduction in pancreatic volume and an increase in the pancreatic attenuation in type II diabetes mellitus, in comparison with the non-diabetes mellitus population, based on the assessment using computed tomography (CT) among the Malaysian population. In Malaysia, no study has been conducted to evaluate pancreas changes among type II diabetes mellitus patients. The various external factors such as lifestyle, diet, and body habitus influence these pancreatic changes. Another objective is to see metabolic factors' association with type II diabetes mellitus patients.

Methods:

A prospective cross-sectional study was conducted at Hospital University Sains Malaysia (HUSM), Kota Bharu, Kelantan, Malaysia involving 74 subjects who underwent elective CT scan abdomen/pelvis for various other indications were enrolled in this study. Patients were classified into type II diabetes mellitus and non-diabetes mellitus. Among these subjects 39 were non-diabetic and 35 were diabetic. The age of these subjects ranged from 35 to 70 years old with a mean age of 56 years old. There were 34 female and 30 male subjects respectively. A single observer was used to measure the pancreatic volume and pancreas attenuation. CT abdomen images were analysed and measured at the workstation using the Picture Archive and Communication system (PACS). Pancreas volume were measured by using formula (AP*tail* + AP*body*)/2 x L(*body&tail*) X CCb*ody* + (AP*head*/2) x 3.14 x CC *head*. The CC diameter is calculated by multiplication of the number of sections of a particular part of the pancreas according to the slice thickness. The pancreas attenuation was obtained using ROI and measuring Hounsfield units between the pancreas and spleen, then using the formula to measure pancreas attenuation (HU p–s). The volume of pancreas and pancreas attenuation were then compared between type II diabetes mellitus and non-diabetes mellitus using an independent t-test. The covariates were analysed by using ANCOVA.

Results

The type II diabetic mellitus patients had a significantly smaller mean pancreas volume with a mean difference of 10.18cm^3 (95% CI = 2.16, 18.20). The mean average of pancreatic volume with a standard deviation (SD) of 64.82cm^3 in Type II Diabetes Mellitus and 75.00cm^3 in Non-Diabetes Mellitus subjects. The pancreas volume is categorised into the normal or reduced size, in diabetic patients with a higher proportion of reduced pancreas ($p = 0.003$). There was no significant difference in normal pancreas attenuation between the type II diabetes mellitus and non-diabetes mellitus patients. The mean average of pancreatic attenuation HU (*pancreas – spleen*) has a standard deviation (SD) of – 4.7 and the standard deviation (SD) of type II diabetes mellitus and non-diabetes mellitus subjects was– 5.74 and – 3.86 respectively. The covariates age, HbA1c, and fasting blood sugar level significantly correlate with pancreatic volume in type II diabetes mellitus, in which all these variables had a moderate inverted correlation. Only body mass index (BMI) has a significant correlation with an increase in normal pancreatic attenuation with a moderate inverted correlation.

Conclusion:

Type II diabetes mellitus observed a significant reduction in pancreatic volume. For pancreatic volume, metabolic factors such as age, HbA1c, and fasting blood sugar have a moderate inverted correlation. However, the normal pancreatic attenuation doesn't show a significant correlation between type II diabetes mellitus and the non- diabetes Mellitus population. In type II diabetes mellitus only, BMI shows a significant correlation with an increase in pancreas attenuation.

CHAPTER 1: BACKGROUND

1.1 Introduction

Diabetes mellitus is a group of metabolic diseases that are characterised by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Most cases of diabetes fall into two broad etiopathogenetic categories: type 1 and type II diabetes mellitus. Type I diabetes or juvenile-onset diabetes (insulin-dependent diabetes) results from cellular-mediated autoimmune destruction of the β-cells by the body's own lymphocytes. Type II diabetes mellitus (non-insulin-dependent diabetes) is caused by improper action of insulin with a relative insulin deficiency. In the non-diabetes mellitus adult population, the blood glucose levels are within the normal range. There is no sign and symptoms of hyperglycemia or hypoglycemia (Of *et al.*, 2005).

Pancreas disorder has a significant cause of high sugar-causing diabetes mellitus. The pancreas has two main functions the exocrine function that helps digestion and the endocrine function that regulates blood sugar. The endocrine function is important because it consists of an Islet of Langerhans that creates and releases the hormone directly into the bloodstream. These main pancreatic hormones are insulin, which lowers the sugar level, and glucagon, which raises blood sugar. The pancreas helps regulate blood sugar levels by producing insulin. Insulin hormone helps to transport glucose from the blood into cells which are later converted into energy. In some situations, when the glucose level in the bloodstream becomes too high the insulin hormone is needed to be released to help bring the sugar back to normal. Without enough insulin, blood sugar levels can increase rapidly and cause damage to the kidneys, heart, and other organs in the body. Diabetes mellitus is a disease that requires close monitoring by a healthcare professional.

National Health and Morbidity Surveys (NHMS) in Malaysia, have reported an increasing trend of diabetes mellitus for the past two decades. The recent cross-sectional studies in Malaysia have shown the prevalence of diabetes mellitus ranging from as low as 5.2% up to a high of 25.3% in 2015. The diagnosed and undiagnosed populations increased more than double from 1996 to 2015. In another study done in the United States, between the years 1990 and 2010, the number of diabetic patients tripled and new cases annually double. Diabetes mellitus patients have a 50% higher risk of death than and healthy adults. The National Health and Morbidity Survey (NHMS) 2019 has reported that the overall diabetes prevalence in adults ≥18 years in NHMS 2015 and 2019 was 13.4% and 18.3% respectively. The prevalence of overall diabetes for adults aged 30 years and above was 24.1% in NHMS 2019 (Ministry of Health, 2020). Long-term diabetes mellitus is associated with dysfunction which eventually leads to multiple organ failures, especially eye, heart, kidney, nerves, and blood vessels. All these complications are the major causes of morbidity and mortality. If there is a delay in treating diabetes mellitus patients and its complication may cause a dramatic increase in costs.

Many studies were conducted to assess the correlation between the reduction of pancreas volume and an increase in normal pancreas attenuation among type II diabetes mellitus. An increase in normal pancreas attenuation, there is still unsure pathophysiology whether is due to obesity or type II diabetes mellitus. The imaging modalities used for the assessment of the pancreas in previous studies are computed tomography (CT) and magnetic resonance imaging (MRI). In Malaysia no study has been conducted in the changes of pancreatic volume and pancreas attenuation in diabetes mellitus. Once identifying the causes of pancreatic changes between the diabetes mellitus and non-diabetes mellitus population it can help us managing and treating these patients.

1.2 Objectives

1.2.1 General Objective

The purpose of this study is to compare the pancreas volume and fatty infiltration between type II diabetes mellitus and non-diabetes mellitus patients, its associated factors.

1.2.2 Specific Objectives

1.2.2.1 To compare the pancreas volume in type II diabetes mellitus and non-diabetes mellitus patients.

1.2.2.2 To compare normal pancreas attenuation in type II diabetes mellitus and non-diabetes mellitus patients.

1.2.2.3 To determine the association of metabolic factors such as body mass index (BMI), hemoglobin A1C (HbA1c), fasting blood sugar (FBS), fasting lipid profile (total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL), and triglycerides) with pancreatic volume and pancreatic attenuation in type II diabetes mellitus.

1.3 Research Question

Question 1: Is there differences in pancreatic volume between patients with type II diabetes mellites with non-diabetes mellitus patients?

Alternative Hypothesis 1: There is a significant difference between pancreatic volume in type II diabetes mellitus patients and non-diabetes mellitus populations**.**

Question 2: Is there difference between normal pancreas attenuation with type II diabetes mellitus?

Alternative Hypothesis 2: There is an association of normal pancreas attenuation between type II diabetes mellitus patients and non-diabetes mellitus populations.

Question 3: Are there correlations between these metabolic factors such as body mass index (BMI), glycated hemoglobin (HbA1c), fasting blood sugar, fasting lipid (total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) and triglycerides) with pancreas volume and pancreatic attenuation in type II diabetes mellitus?

Alternative Hypothesis 3: There are correlations between these metabolic factors such as body mass index (BMI), glycated hemoglobin (HbA1c), fasting blood sugar, fasting lipid profile (total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) and triglycerides) with pancreatic volume and pancreatic attenuation in type II diabetes mellitus.

CHAPTER 2: LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a group of metabolic diseases that are characterised by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Most cases of diabetes fall into two broad etiopathogenetic categories in type I diabetes or juvenile-onset diabetes (insulin-dependent diabetes) and type II diabetes (non-insulin-dependent diabetes) (Of *et al.*, 2005). Type II diabetes mellitus is the most common form of diabetes mellitus, which accounts for > 90% of all cases of adult-onset diabetes mellitus in Malaysia. In Type II diabetes mellitus there is a progressive decline in β cell function associated with insulin resistance in muscle and adipose tissue (Ministry of Health, 2020). The National Health and Morbidity Survey (NHMS) 2019 has reported that the overall diabetes prevalence in adults ≥18 years in NHMS 2015 and 2019 was 13.4% and 18.3% respectively. The prevalence of overall diabetes for adults aged 30 years and above was 24.1% in NHMS 2019 (Ministry of Health, 2020). It is very important to identify and treat diabetes mellitus patients as soon as possible. Long-term diabetes mellitus is associated with dysfunction which eventually leads to multiple organ failure.

.

2.2 Pancreas Anatomy and Imaging

The pancreas is a retroperitoneal organ transversely in the upper abdomen that overlays across the L1 to L3 vertebral bodies. The pancreas is usually in the anterior pararenal space. The head pancreatic is bounded by the C-loop of the duodenum. The tail of the pancreas is seen on the left side of the abdomen seen at the splenic hilum. The pancreas is approximately 12–15cm in length (Walker, 2018). The pancreas contains complex histology with a combination of both endocrine and exocrine cells. Endocrine cells clustered together and form an Islet of Langerhans found within exocrine pancreatic cells that account for approximately 1- 2 % of the entire pancreatic organ. Beta-cells of the pancreas act as glucose sensors which accordingly release insulin output to the prevailing blood glucose level. Insulin is important for the promotion of glucose storage and the prevention of glycogen breakdown (Walker, 2018). The pancreas is intricately lobulated and contained within a delicate fibrous membrane. The exocrine pancreas consists of acini, which release their secretions into the major channels through ductulus. The group of Islets of Langerhans found between the acini cells. These different kinds of secretory cells are distinguished by histochemical staining in the islets. Glucagon is secreted by the Islets Alpha cells, whereas pro-insulin is secreted by the beta cells (a congener of insulin). Somatostatin is secreted by a third cell type, the delta cell.

The pancreas can be visualised by various imaging modalities such as ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI) (Djuric-Stefanovic *et al.*, 2012) Out of all these modalities, CT is the best choice to measure the pancreatic volume and measure the fat infiltration within the pancreas. Computed tomography (CT) study is a non-invasive method that takes a short acquisition time. It is also available worldwide. In our current study, evaluating the pancreas volume and pancreas attenuation are adequate by measuring in a non-contracted phase (Djuric-Stefanovic *et al.*, 2012; Kim *et al.*, 2014). Ultrasound is not a choice of modality for the evaluation of the pancreas due to its anatomical location. There is a limitation in the visualisation of the pancreas due to gases in the stomach and colon obscuration. The tail of the pancreas is commonly not well visualised on ultrasound. Another disadvantage of ultrasound is the lack of standardised measurement techniques. Ultrasound imaging is also operator-dependent and influenced by imaging pre-set (gain, depth, and frequency). The patient's body habitus influences pancreas imaging by ultrasound (Kim *et al.*, 2014). CT imaging is less costly compared to MRI. MRI is another alternative to measure fat content, but it is challenging and requires a long time in image acquisition. In imaging MRI, the chemical shift artifact is usually common due to the pancreas being a small organ with an irregular margin and surrounded by visceral fat (Kim *et al.*, 2014). There are many patient factors such as the history of operation with the use of material that is not compatible with MRI, claustrophobia, and unable to cooperate (Kim *et al.*, 2007).

2.3 Pancreas Volume

Pancreatic volume increases linearly with age. It increases linearly from childhood to adolescence and reaches a plateau from age 40 to 60 years old. In the older age group above the age of 70 years old, there is a reduction in the volume of an older age group (Lu *et al.*, 2019). Many other factors that will affect the volume of the pancreas such as age, gender, obesity, physical inactivity, and different ethnic groups (Lim *et al.*, 2014). Several pathological conditions that can be associated with pancreatic atrophy such as chronic pancreatitis, pancreatic adenocarcinoma, post pancreas tumour resection, etc (Djuric-Stefanovic *et al.*, 2012).

In Type II diabetes mellitus there is a combination of both insulin resistance and decreased beta cell mass (Macauley *et al.*, 2015). Blood glucose levels usually do not rise unless there is a decline of approximately 50% of the pancreas insulin secretory function usually done by biopsy and obtaining the tissue for histological correlation of fatty infiltration (Macauley *et al.*, 2015). There is a strong correlation between the reduction of pancreatic volume and a hyperglycaemic state. This is mainly caused by the reduction or death of B cell islets of Langerhans leading to progressive loss of insulin secretion (Of *et al.*, 2005; Tee and Yap, 2017).

There are various methods can be used in measuring pancreas volume. The patients performed CT abdominal for routine clinical evaluation due to various other common indications. These groups of patients examined can be either in or out or in patients.

Firstly, need screening of all the patients who had done computerised tomography of the abdomen or pancreas as planned. The population included in this study is chosen depending on the inclusion and exclusion criteria. Once the patient has been scanned and the images are acceptable, the pancreas in its optimal view in the axial slice will be identified. Then the cranial-caudal (CC) dimensions of the entire pancreas are measured. The anterior-posterior diameter of the body (AP body) and tail (AP tail) was measured by using the splenic vein to the anterior margin of the pancreas (Figure 1a). Anterior-posterior diameter of the head (AP head) was measured at the level of the superior mesenteric vein and artery (Figure 1b). At the same level, the width/ length of the pancreatic head in lateral–lateral (LL) diameter was measured at the same level (LL head). The entire length of the body and tail (LL body & tail) was measured as the maximal linear distance from the neck to the tip of the pancreatic tail. After getting all these measurements, CC diameters were calculated by multiplication of the number of slices of a particular part of the pancreas identified. Finally, the pancreas volume is calculated by using this formula $(AP_{tail} + AP_{body})/2 \times L(_{body\&tail}) \times CCb_{ody} + (AP_{head}/2)^2 \times 3.14 \times CC_{head}$.

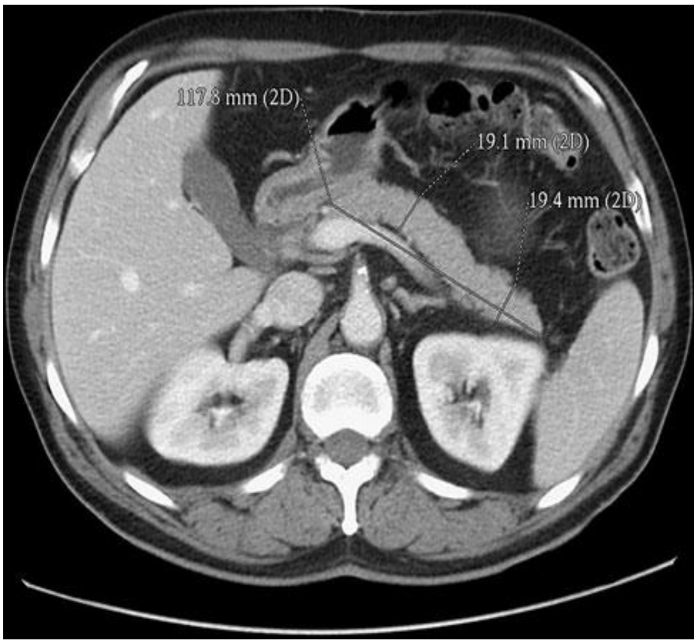

Figure 1a. AP measurement method of the body and tail of pancreas.

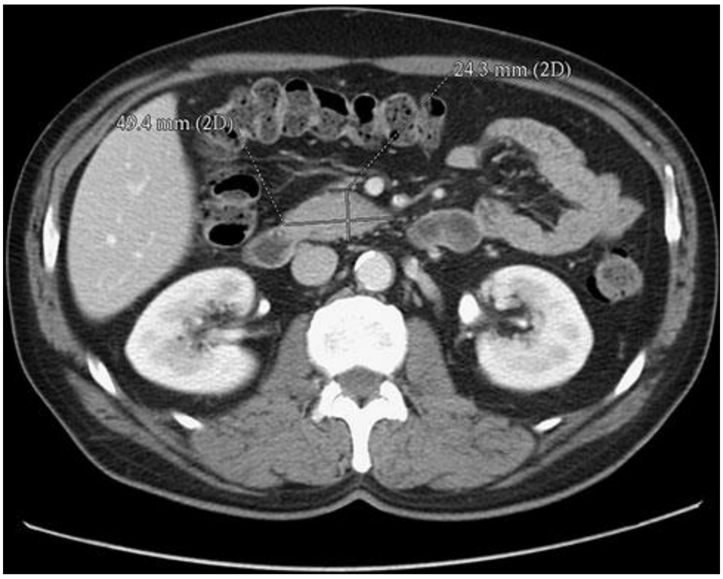

Figure 1b. Measurement method of the head of pancreas

The second method is the measurement of the pancreas volume by the summation of area technique (Figure 1c). The pancreas parenchyma is manually outlined in each slice by using the region of interest (ROI). Subsequently, the outline areas are calculated automatically. The product of the pancreas area and section thickness represents the pancreas volume in every single section. Total pancreas volume will compute by summation of all slices of the pancreas manually or automatically. The software was used to automatically calculate the area within the defined traces (Lim *et al.*, 2014).

.

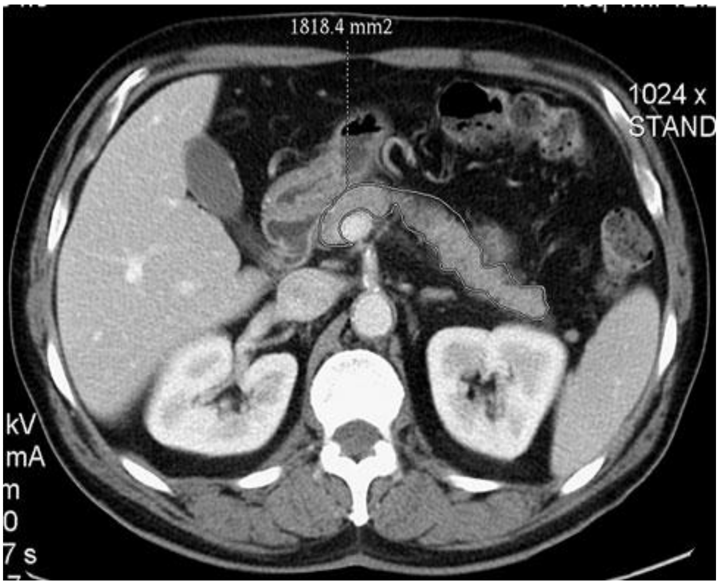

Figure 2. Summation of area technique.

A study by Jeon et al. (2017) found a significant reduction in pancreas volume in patients with diabetes mellitus compared non diabetes mellitus population. The authors hypothesised that this difference may be caused by pancreatic inflammation, as evidenced by elevated levels of C-reactive protein and interleukin 6 in diabetes mellitus patients. This research was conducted on the Pancreatic Cancer Translational Research Consortium (PanCarc), which is an international consortium made up of investigators from more than thirty institutions worldwide who are working together to identify new targets for cancer therapy. Epidemiological studies that have looked at the relationship between type 2 diabetes and pancreatic volume or size have consistently shown a reduction in pancreatic volume in diabetic patients relative to non-diabetic controls. In one study, diabetic patients had an average decrease of 23% in pancreatic volume compared to non- diabetes mellitus population. These findings suggest poor glycaemic control may lead to reduced pancreatic function (Eigl et al., 2020).

2.4 Pancreatic steatosis

Pancreas steatosis refers to the accumulation or replacement of pancreatic parenchyma by fat cells. Fat replacement within the pancreas has various synonyms such as pancreatic lipomatosis, fatty replacement, fatty infiltration, non-alcoholic fatty pancreatic disease, pancreatic steatosis, and lipomatous pseudohypertrophy (Kim *et al.*, 2007; Hori *et al.*, 2016; Report, 2017). Generally in elderly and obese populations pattern of fat infiltration is usually diffuse (Ibrahim *et al.*, 2017). As fat accumulation increases in the pancreas, it usually leads to pancreas dysfunction and death of nonadipocytes through lipoapoptosis, eventually leading to diabetes mellitus (Ibrahim *et al.*, 2017; Miyake *et al.*, 2018). Fat accumulation in the pancreas classically shows reduced attenuation of the pancreas in CT imaging. The attenuation of the pancreas is compared with surrounding structures such as the spleen and liver to suggest infiltration of fat. Usually, the pancreas with fat infiltration will show a lower attenuation with the adjacent spleen (Ibrahim *et al.*, 2017).

The population is included in this study only after both inclusion and exclusion criteria are fulfilled. A pancreatic image from the non-contrast phase of CT abdomen or pancreas identifies the best view of the pancreas in the axial view is selected. Two methods that can be used to identify fatty infiltration within the pancreas parenchyma are by measuring attenuation using Hounsfield's units (HU) of the pancreas in selected CT images (Kim *et al.*, 2014; Hori *et al.*, 2016). Fat within the pancreas was usually unevenly distributed. Therefore, measuring the mean attenuation of the pancreas gives average attenuation. Attenuation of the pancreas was measured within the circular region of interest (ROI) at three different areas of the pancreas (head, body, and tail) (Kim *et al.*, 2014). The mean attenuation of the three measurements of ROI represents the attenuation pancreas of each subject individually (Kim *et al.*, 2014; Miyake *et al.*, 2018). Attenuation of the spleen were also measured at three different areas, and mean attenuation is calculated as seen in Figure 2a, 2b, and 2c. The ROI of the pancreas and spleen are measured simultaneously at the same slice thickness. Mean attenuation pancreas and spleen are identified as P and S respectively. After calculating the mean attenuation of the pancreas and spleen respectively, a pancreatic fat index can be calculated. The first method in measuring pancreatic fat index is by the difference between P- S. It shows to be a good indicator of a fatty pancreas. Many studies have shown the amount of pancreatic fat component correlates histologically demonstrated with P- S (Kim *et al.*, 2014; Jeong *et al.*, 2018; Çoruh *et al.*, 2020). The first method is to measure the difference between the pancreas-splenic attenuation, HU (*pancreas - spleen*). value measures ≤ - 5 Hounsfield Unit (HU) are categorised to be a fatty pancreas. Patients with a pancreas-splenic attenuation difference > -5 HU have categorized the non-fatty pancreas (Ibrahim *et al.*, 2017; Çoruh *et al.*, 2020).

The second method of quantifying pancreatic fat index is by calculating the P/S ratio. The ratio of P/S, with a value of < 0.7 shows the presence of significant infiltration of fat in the pancreas (Koç and Taydaş, 2020).

Figure 3a. Measurement of the head of pancreas HU.

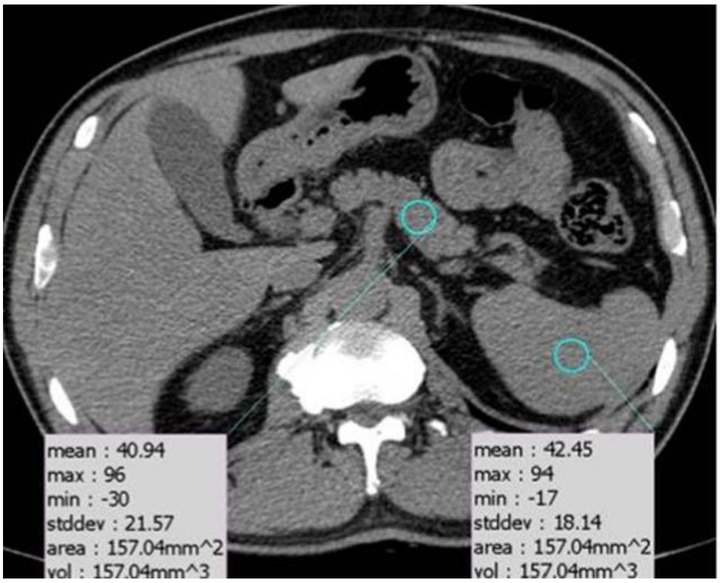

Figure 3b. Measurement of the body of pancreas HU.

Figure 3c. Measurement of the tail of pancreas HU

An increase in normal pancreas attenuation is a common problem in diabetes, and it causes impaired of the pancreas function (Cha & Hollander, 2021). There is strong evidence that pancreatic fat infiltration is commonly seen in diabetes mellitus and is not simply a result of obesity. It also suggests that diabetics may have different levels of susceptibility to developing pancreatic fat accumulation based on their genetic makeup. Given the prevalence of pancreatic fat infiltration in diabetes, the pathophysiology needs to be understood. Then adequate measures can be taken to prevent or reverse this condition.

2.5 Pitfalls and solutions occurred during the measurement of the pancreas volume and normal pancreatic attenuation using CT abdomen.

The patient is usually in a supine position and asked to take a deep breath and hold it during a CT abdomen/pancreas image acquisition. The motion artifact of breathing can cause misregistration of CT images causing suboptimal imaging. The solution to this problem was to ask the patient to follow instructions and hold his/ her breath during image acquisition (Gilbeau *et al.*, 1992).

Oral contrast medium has its own advantages and disadvantages. Oral contrast medium is usually given 30 to 45 minutes prior to performing the CT abdomen images. The most common disadvantages are when the given contrast might poo along the 1st and 2nd part of the duodenum or the adjacent surrounding small bowel loops which may causes streak artifact. This artifact can cause difficulty in the delineation of the pancreas. Therefore, measuring the pancreas can be challenging (Gilbeau *et al.*, 1992).

Administration of intravenous injection contrast helps in better visualisation of vessels. It gives a more accurate determination in measuring pancreas size. However, intravenous injection contrast can cause a risk of worsening renal function in patients with diabetes mellitus (Gilbeau *et al.*, 1992; Report, 2017). Metformin is the choice of oral hypoglycemia treatment used in type II diabetes mellitus. For patients on metformin who requires iodinated contrast material during CT scan study, the estimated glomerulus rate (eGFR) needs to be calculated. In reference to The European Society of Urogenital

Radiology, if the estimated glomerular filtration rate (eGFR) of less than 60 mL/min, then metformin should be withheld at the time of contrast administration. The theory behind, this is patients on metformin will have a decrease in renal clearance leading to an increase in lactic acidosis, causing a mortality rate of up to 50% (Baerlocher, Asch and Myers, 2013). A non-contrast study is adequate in measuring the pancreatic volume and normal pancreatic attenuation.

In measuring fat attenuation of pancreas and spleen using ROI, a standard measurement of a circular area of 1.0 cm2 using a three-dimensional workstation (Kim *et al.*, 2014; Miyake *et al.*, 2018). ROI of 1.0 cm^2 size is an appropriate choice in an atrophied pancreas. Using a larger area of ROI will be difficult to locate and measure the attenuation. If a larger ROI is used, it could cause an inadequate attenuation value of the pancreas. In the region of circular (ROI) the adjacent vessels, calcifications, cysts, and biliary and pancreatic ducts should be avoided (Kim *et al.*, 2014; Miyake *et al.*, 2018). Few of the sample population, peripheral margins of the pancreas are poorly defined due to its adjacent retroperitoneal fat. Therefore, pancreatic attenuation is measured as close as possible to the splenic vein to obtain more pancreatic parenchyma (Miyake *et al.*, 2018).

2.6 The changes in the pancreas volume and pancreatic attenuation in correlation with metabolic factors.

Metabolic factors included in our study is body mass index (BMI), glycated hemoglobin (HbA1c), fasting blood sugar, fasting lipid (total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) and triglycerides). Few studies has shown that factors also have an influence on the pancreatic volume and normal pancreas attenuation (Gilbeau *et al.*, 1992; Miyake *et al.*, 2018; Koç and Taydaş, 2020). All these bloods, glycated hemoglobin (HbA1c), fasting blood sugar, fasting lipid (total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) and triglycerides) should be within 6 – 12 months from the date of CT scan performed. Most of these patients has a clinic appointment 6 monthly where all this blood investigations are routinely taken and monitored. Patients with diabetes mellitus with good control of sugar level can show less reduction in pancreatic volume and pancreatic attenuation compared to diabetes mellitus patients with poorly controlled sugar (Macauley *et al.*, 2015). A short duration of blood investigation with the CT scan images can give us clue who well the sugar level is controlled.

The World Health Organization (WHO) criteria for body mass index (BMI) classifies a BMI of 25-29.9 kg/m^2 as overweight and >30 kg/m^2 as obese. As recent studies have proven body weight plays a major role in metabolic disease. Recently population in Malaysia with an increasing trend of body weight shows a very high incidence rate of diabetes mellitus. In obesity, ectopic fat accumulation can be seen infiltrating the pancreas which eventually causes dysfunction of the pancreas. Obesity increases the risk of developing type II diabetes by almost 30%, and people with type II diabetes are two-and-a-half times more likely to be overweight or obese than those who do not have the disease. Additionally, people with type II diabetes are more likely to suffer from other conditions such as hypertension, strokes, heart failure, fatty liver disease, and gallstones (Padhi *et al.*, 2020).

Patients with dyslipidaemia (FSL, HLD, LDL and triglyceride) usually gets the fat deposition in the subcutaneous adipose. Once the subcutaneous adipose tissue is not able to appropriately store excessive, fat it will be redirected to visceral and ectopic tissues like the liver and pancreas. These will lead to fat accumulation in the pancreas eventually causing pancreatic dysfunction (Tee and Yap, 2017). In the Malaysian population fat distribution, the reason for the increasing trend of body weight is attributed to economic growth, urbanisation, and a sedentary lifestyle. However, in western countries, the consumption of fast food over the past decade plays an important role.

The sugar control HbA1c and fasting blood sugar are usually high in diabetes mellitus patients as the pancreas loses its capacity to release adequate insulin causing high blood sugar levels. Few studies showed that pancreatic volume is strongly associated with β cell function. Therefore, measuring the pancreatic volume indirectly helps us to determine the functioning pancreatic parenchyma. The smaller the pancreas volume it affects the sugar control.

2.7 Conceptual framework

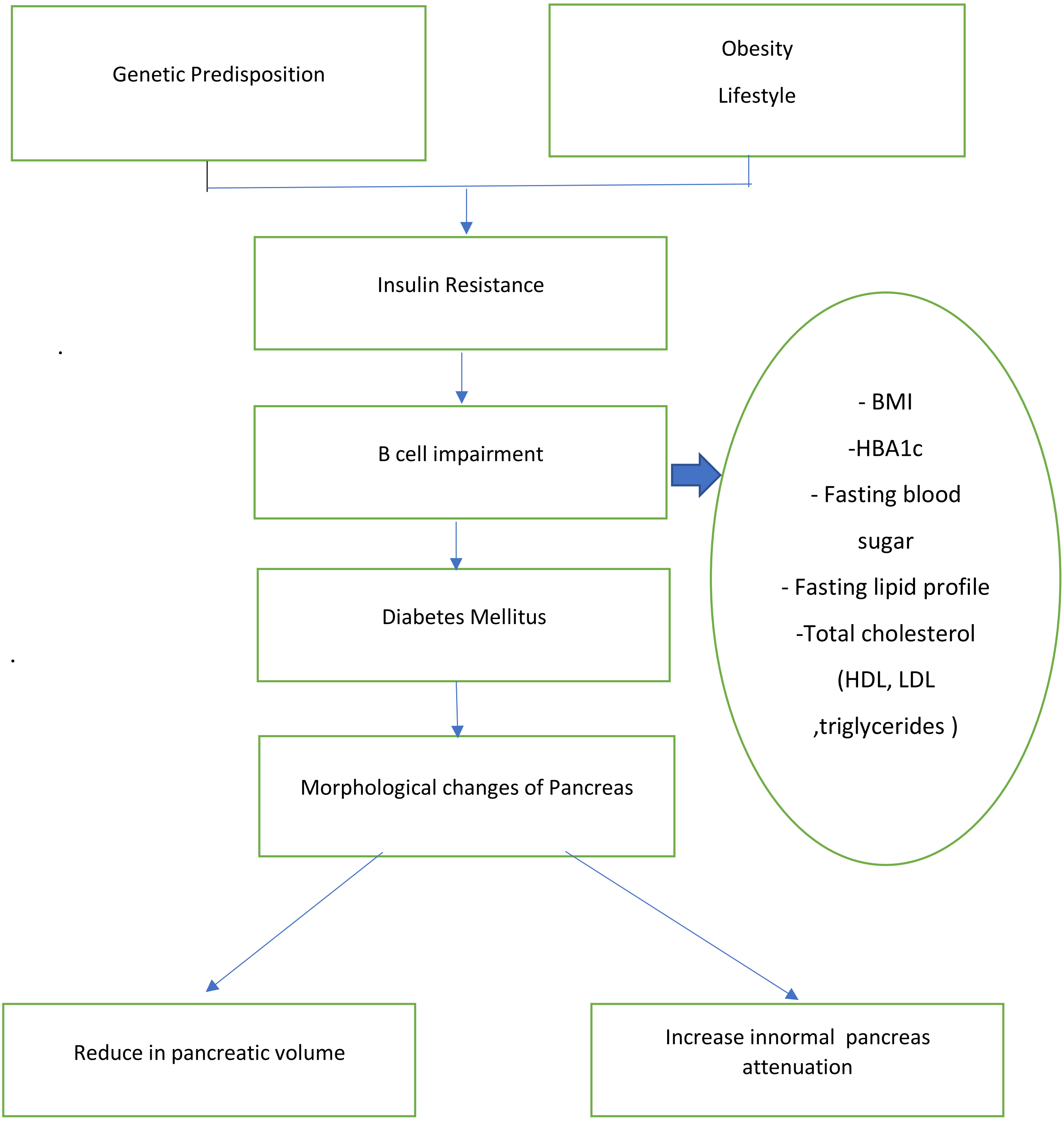

Figure 4: Conceptual framework

2.8 Rationale of Study

Research and studies have shown that there is a significant reduction in pancreatic volume and an increase in normal pancreatic attenuation in type II diabetes mellitus patients in comparison with the non-diabetes mellitus population.

As a result of the mentioned above, the study aims to establish there is a strong association between type II diabetes mellitus patients by using the non-contrasted CT abdomen findings. As we know type II diabetes mellitus is characterised by insulin resistance which has a strong association with a reduction in the pancreatic volume and fatty pancreas. Therefore, the outcome of this study might help the endocrinologist or clinicians in giving the adequate treatment and care needed. Non-contrasted CT abdomen is used as an ancillary tool to diagnose to identify high-risk populations and subsequently the optimum care given to them. This finally can improve the life quality of patients.

CHAPTER 3: METHODOLOGY

3.1 Study design

Cross sectional study (prospective) was conducted to compare pancreatic volume and increase in pancreas attenuation in type II diabetes mellites and non-diabetes mellitus**.**

3.2 Study Population

3.2.1 Reference Population - type II diabetes mellitus patients.

3.2.2 Source population - type II diabetes mellitus treated in HUSM.

3.2.3 Target / Study Population - type II diabetes mellitus came for elective CT abdomen for various indication in HUSM between 1st December 2020 until 22nd June 2021.

3.2.4 Sampling Frame - Elective patients scheduled for CT abdomen/ pancreas for various indications. These patients need to meet the inclusion and exclusion criteria.

3.3 Sample Size Calculation.

The sample size estimation for objectives 1 and 2, the sample size was calculated using single mean formula using Sample Size Calculator for Two Independent Mean, Kubang Kerian Kelantan Malaysia Unit of Biostatistics and Research Methodology, School of Medical Sciences, University Sains Malaysia, which is a computer software created by Najib, MY (2015), Unit of Biostatistics and Research Methodology, School of Medical Sciences, Health Campus, Universiti Sains Malaysia.

Objective 1

To compare the pancreas volume in type II diabetes mellitus and non-diabetes mellitus patients.

Objective 2

To compare the fat infiltration of pancreas in type II diabetes mellitus and non-diabetes mellitus patients.