EFFICACY OF MULTIGRAIN SUPPLEMENTATION IN TYPE 2 DIABETES MELLITUS: A RANDOMIZED HUMAN INTERVENTION TRIAL

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by

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LIST OF SYMBOLS

°C	Celsius
%	Percentage
µL	Micro liter
µmol	Micromole
cm	centimeter
g	Gram
g/dL	Gram per deciliter
g/L	Gram per liter
kg	Kilogram
mg	Milligram
min	Minute
ml	Milliliter
mmol	Millimole
mmol/L	Millimole per liter
mmHg	Milliliter per mercury
ng/ml	Nanogram per milliliter
nm	Nanometer
nmol/L	Nanomole per liter
pmol/mL	Picomole per milliliter
U/L	Unit per liter
U/ml	Unit per milliliter

LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
ADP	Adenosine diphosphate
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
DM	Diabetes mellitus
CAD	Coronary artery disease
GDM	Gestational diabetes mellitus
T2DM	Type 2 diabetes mellitus
HbA1c	Glycosylated hemoglobin
EFSA	European food safety authority
FDA	US food and drug Administration
NHMS	National health and morbidity surveys
RYR	Ryanodine receptor
OGTT	Two-hour oral glucose tolerance test
DKA	Diabetes ketoacidosis
HHS	Hyperosmolar hyperglycemic state
GSH	Glutathione
GPx	Glutathione peroxidase
SOD	Superoxide dismutase
LDL	Low density lipoprotein
HDL	High density lipoprotein
USM	Universiti Sains Malaysia

LIST OF APPENDICES

KEBERKESANAN SUPLEMEN BIJIRIN PELBAGAI DALAM KALANGAN PESAKIT DIABETES JENIS 2: SATU PERCUBAAN INTERVENSI MANUSIA SECARA RAWAK

ABSTRAK

Diabetes jenis 2 (T2DM) yang tidak terkawal boleh membawa kepada komplikasi mikrovaskular (nefropati, retinopati dan neuropati) dan penyakit kardiovaskular. Kandungan beta-glukan dalam bijirin mempunyai potensi untuk meningkatkan sensitiviti insulin, menurunkan tindak balas glukosa postprandial dan mengurangkan darjah keradangan. Gabungan bijirin yang betul bukan sahaja memenuhi keperluan tubuh manusia, tetapi juga menyediakan kandungan pemakanan yang penting dan munasabah. Walau bagaimanapun, tiada kajian di Malaysia yang telah dijalankan untuk menilai peranan bijirin pelbagai dalam T2DM. Matlamat kajian ini adalah untuk menentukan keberkesanan suplemen bijirin pelbagai pada (1) kawalan glisemik; (2) profil kardiometabolik; (3) tahap tekanan antioksidan dan oksidatif, status pemakanan and kualiti hidup (QoL); dan (4) keselamatan, pematuhan and toleransi dalam kalangan pesakit T2DM. Bermula Oktober 2020 hingga Jun 2021, seramai 50 orang dewasa yang menghidap T2DM, yang menerima rawatan diabetes standard di Klinik Rawatan Harian telah dibahagikan secara rawak kepada dua kumpulan. Kumpulan suplemen menerima suplemen bijirin pelbagai 30 g dua kali sehari (bersamaan dengan 3.4 g beta-glukan) dengan ubat standard selama 12 minggu, manakala kumpulan kawalan ditetapkan dengan ubat standard. Selepas 12 minggu intervensi, kawalan glisemik dalam kalangan kumpulan suplemen menunjukkan penambahbaikan yang ketara, seperti yang ditunjukkan dalam HbA1c (p <0.05), insulin serum (p < 0.0001). Kolesterol LDL (p<0.05), jumlah kolesterol (p<0.05),

nisbah pinggul pinggang (p<0.05), lemak badan (p<0.05) dan lemak visceral (p<0.05) telah berkurangan dengan ketara dalam kalangan kumpulan makanan tambahan. Peningkatan ketara dalam kepekatan TAC (p<0.0001), SOD (p<0.0001) dan GPx (p<0.0001) telah ditunjukkan, manakala tahap kepekatan MDA (p<0.01), Protein Carbonyl (p<0.0001) dan 8-OHG (p<0.01) menurun dalam kalangan kumpulan tambahan. Kumpulan suplemen bijirin pelbagai beta-glukan yang tinggi menunjukkan QoL yang lebih baik dalam keletihan tenaga (p<0.05). Pematuhan terhadap suplemen adalah memuaskan (82.9%) dengan aduan gastrousus yang minimum. Kesimpulannya, suplemen bijirin pelbagai dengan kandungan beta-glukan yang tinggi boleh menambah baik kawalan glisemik (HbA1c dan insulin serum), profil lipid (kolesterol LDL dan jumlah kolesterol), tahap antioksidan (TAC, SOD dan GPx) dan tekanan oksidatif (MDA, protein karbonil dan 8-OHG), status pemakanan (lemak badan, lemak visceral dan nisbah pinggang-pinggul) dan kualiti hidup (keletihan tenaga) dalam kalangan pesakit T2DM.

EFFICACY OF MULTIGRAIN SUPPLEMENTATION IN TYPE 2 DIABETES MELLITUS: A RANDOMIZED HUMAN INTERVENTION TRIAL

ABSTRACT

Uncontrolled type 2 diabetes mellitus (T2DM) may lead to microvascular complications (nephropathy, retinopathy and neuropathy) and cardiovascular diseases. The beta-glucan content in the grains has the potential to improve insulin sensitivity, lowering postprandial glucose response and reducing inflammation degrees. A proper combination of grain not only satisfy human body's need, but also provides essential and reasonable nutritional contents. However, no trial in Malaysia has been conducted to evaluate the roles of multigrain in T2DM. The current study is aimed to determine the effects of multigrain supplementation on (1) glycemic control; (2) cardiometabolic profile; (3) peripheral antioxidant and oxidative stress status, nutritional status and QoL; and (4) safety, compliancy and tolerability among the T2DM patients. From October 2020 to June 2021, a total of 50 adults living with T2DM, who were receiving standard diabetes care at Day Care Clinic were randomized into two groups. The supplementation group received twice daily 30 g multigrain supplement (equivalent to 3.4 g beta-glucan) with standard medication for 12 weeks, while the control group was prescribed with standard medication. After 12 weeks of intervention, the glycemic control among supplement group improved significantly, as shown in HbA1c ($p<0.05$) and serum insulin ($p<0.0001$). The LDL-cholesterol ($p<0.05$), total cholesterol ($p<0.05$), waist hip ratio ($p<0.05$), body fat ($p<0.05$) and visceral fat ($p<0.05$) were significantly reduced among supplement group. Significant elevations in TAC ($p<0.0001$), SOD ($p<0.0001$) and GPx ($p<0.0001$) concentrations were demonstrated,

while the level of MDA (p<0.01), protein carbonyl (p<0.0001) and 8-OHG (p<0.01) were reduced among supplement group. High beta-glucan multigrain supplementation group showed better QoL in energy fatigue (p<0.05). The compliance towards the supplement was satisfactory (82.9%), with minimal gastrointestinal complaints. Conclusively, high beta-glucan multigrain supplementation could improve glycemic control (HbA1c and serum insulin), lipid profile (LDL cholesterol and total cholesterol), peripheral antioxidant (TAC, SOD and GPx) and oxidative stress status (MDA, protein carbonyl and 8-OHG), nutritional status (body fat, visceral fat and waist-hip ratio) and QoL (energy fatigue) among T2DM patient.

CHAPTER 1

INTRODUCTION

1.1 Background

Diabetes mellitus (DM) is a highly prevalent and heterogeneous metabolic disorder characterized by the presence of hyperglycemia due to impairment of insulin secretion, defective insulin action, or both (Punthakee et al., 2018). The chronic hyperglycemia of DM is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2014). According to the National Health and Morbidity Survey (2019), one in every five adults in Malaysia has diabetes, proportionate to 3.9 million people aged 18 years and above. The prevalence of diabetes among adults in Malaysia has increased from 11.2% in 2011, to 13.4% in 2015, and recorded 18.3% in 2019.

DM is classified into four types, the type 1 diabetes, type 2 diabetes, other types (genetic defects of beta-cell function, insulin action and etc) and gestational diabetes mellitus (GDM) (ADA, 2014). Type 2 Diabetes Mellitus (T2DM) is the foremost common frame of DM, which accounting for more than 90% of all cases of adult-onset DM in Malaysia (Sami et al., 2017). T2DM has attained epidemic proportions worldwide, with 415 million cases estimated globally in 2015, and the number is expected to increase dramatically in the next decades, reaching 642 million by 2040 (Ogurtsova et al., 2017).

The main aim of diabetes management is targeted at reducing the acute and chronic diabetes complications, via the effective control of plasma glucose, blood pressure, lipid profile and body weight, concurrently (ADA, 2014). The distinction between effective treatment and cure is obscured within the case of diabetes, but few individuals can remission it through diet changes, and be able to reach and maintain

normal blood sugar levels without, or with minimum medication. In particular, nutrition or dietary therapy is one of the trending complementary medicine, with the ultimate goal to control and remission (by averting resulting complications after its onset) (Koithan & Devika, 2010).

Achieving optimal glycemic control in T2DM is essential to prevent long-term micro- and macrovascular complications. A number of factors other than glycated hemoglobin (HbA1c) level will influence treatment regimens, and practice guidelines emphasize the need for concomitant treatment of other cardiovascular risk factors (Gracia-perez, et al., 2013). Majority of patients with T2DM failed to control glycemia via healthy dietary intake and lifestyle modifications, and pharmacotherapy. Within this context, a tight glycemic control with a HbA1c level ≤7% is recommended for T2DM patients in general, while a more stringent glycemic control with a HbA1c level ≤ 6.5%, is desirable for those who are younger, newly diagnosed and without complications. Yet, maintaining the glycemic control, optimizing the treatment, and ensuring the patient adherence consistently prove to be the major challenges in T2DM management globally. In a large- scale cross-sectional study, only 31.4% of patients who had any insulin use were shown to have a HbA1c level ≤7% (Selvin et al., 2016).

Wholegrain is defined as consisting of the entire grain (bran, endosperm and germ), and most fiber from wholegrain is in insoluble type, including cellulose, hemicellulose and lignin, with exception of barley and oat (relevant sources of soluble fiber such as beta-glucan, pentoses, and arabinoxylan) (Fardet, 2010; Kyrø et al., 2018). Wholegrain is a good source of dietary fiber, resistant starch, antioxidants and other important micronutrients, such as folic acid and vitamins (Pepa et al., 2018) . The fiber content in wholegrain has been reported to improve the insulin sensitivity, lowering postprandial glucose response and lowering inflammation (Wirström et al., 2013; Li et

al., 2016). In addition, laboratory and epidemiological investigations have reported that wholegrain, especially barley and oat, contain a high amount of beta-glucan, which has been proven to lower blood glucose levels, improving glucose tolerance, ameliorating hyperlipidemia, improving immunity and decreasing infections (Dedeepiya et al., 2012). According to the Malaysian Dietary Guidelines (2020), a high dietary fiber diet of 20-30g/day is recommended for diabetes prevention, with wholegrain accounting for 50% of total grain intake. Therefore, the proper consumption of wholegrain can act as a complementary treatment for T2DM patients. Consumption of 1 cup of cooked oat bran, or ½ cup of cooked barley, or 1 slice of multi-wholegrain fiber meal bread provided more than 5g of dietary fiber (MNT, 2013). In parallel, the demand of the multigrain in the food market is increasing tremendously, due to an increased awareness of managing chronic diseases by ingesting health promoting foods (Niu et al., 2017). Multigrain, a proper combination of few types of grains, could satisfy human body's need with essential nutritional content (Jing et al., 2018).

1.2 Problem statement

The Global Burden of Disease Study (2019) for Iranian indicated that 20.1% of deaths and 11.5% of disability-adjusted life-years due to non-communicable diseases result from DM. Similarly, T2DM is the most prevalent non-communicable disease in Malaysia, with prevalence of 18.3% among adult 18 years of age in 2019 (NHMS, 2019). T2DM is one of the most common metabolic diseases in 2015, with 415 million cases, and this number is expected to increase dramatically in the next decades reaching 642 million by 2040 (Ogurtsova et al., 2017). This causes a major challenge to the Malaysian public health system, incurring an additional healthcare cost of USD 1.07 to 1.83 (MYR 4.49 to 7.67) million annually (Zhang et al., 2010). According to

International Diabetes Federation (IDF) 2019, the global economic burden of DM is also massive, with an estimated annual expenditure of USD 760 billion in 2019, projected to grow by 11.2% to USD 845 billion by 2045 (Saeedi et al., 2019). Above that, up to 92.3% of the practitioners believed that patients would not comply with insulin treatments, and a multinational survey reported that 72.5% of their patients did not comply with insulin regimens (Lee et al., 2013).

Current diabetes medication is aimed to control the prognosis of diabetes complications. However, it is possible for some people to remission the diabetes condition through diet changes, and be able to reach the normal blood glucose levels without medication (ADA, 2012). Furthermore, lower therapeutic efficacy owing improper or ineffective dosage regimen of the medication, low potency and altered side effects due to drug metabolism and lack of target specificity, solubility and permeability problems are the major drawbacks associated with the use of the conventional drugs (Feingold, 2022). Despite the advent of promising anti-hyperglycemic agents, the major challenges in efficient diabetes treatment include optimizing the existing therapies to guarantee optimum and balanced glucose concentrations, as well as reducing long-term diabetes-related complications (Tan et al., 2019). In such a scenario, nutritional regimen is important part of the foundation for the treatment of diabetes that can combat the diabetes complications (Pandey et al., 2011; Yeung et al., 2018; Gray & Threlkeld, 2019). The European Food Safety Authority (EFSA), (2011) claims that oats and barley beta-glucans are effective to decrease the LDL-cholesterol, and increasing satiety, that leads to a reduction in energy intake, reducing post-prandial glycemic responses, and improve the digestive function which is beneficial for T2DM. Furthermore, The United States Food and Drug Administration (FDA) has approved the health claims for dietary fibers, beta-glucan (at least 3g/day) from oats for reducing plasma cholesterol levels

and cardiovascular disease risk (FDA, 2019). Therefore, there is a need to investigate the effectiveness of the enrichment of the high beta-glucan grain supplementation using clinical trials among T2DM patients.

1.3 Justification of the study

Diabetes is one of the most common form of glandular disease in the world, and is responsible for about four million deaths per year (Shaw et al., 2010). T2DM is happened when the beta-cells in the pancreas malfunction and/or insulin resistance develops in the liver, skeletal muscle, or adipose tissue, hyperglycemia arises and resulting in an excess level of glucose circulating in the blood (Zheng et al., 2018). Metabolic abnormalities in carbohydrates, lipids, and proteins result from the importance of insulin as an anabolic hormone. Low levels of insulin to achieve adequate response, and/or insulin resistance of target tissues, mainly skeletal muscles, adipose tissue, and to a lesser extent, liver, at the level of insulin receptors, signal transduction system, and/or effector enzymes or genes are responsible for these metabolic abnormalities (Kharroubi & Darwish, 2015). Dietary interventions have been considered as foundation for the treatment of diabetes (Gray et al., 2019). A diet rich in wholegrain fiber, independent of energy, fat, and other dietary factors, can reduce mortality rates among T2DM (Rezvani et al., 2011). The comorbidities and complications of T2DM were associated with cost and economic burden. This happened when the length of inpatient stay and frequency visits of outpatients were significantly associated with costs (Ganasegeran et al., 2020). Therefore, by controlling the diabetes complications, it might reduce the economic burden.

Studies have looked into the advantages of single grain-based wheat bread for diabetes management in recent years (Rezvani et al., 2011; Montazerifar et al., 2016),

particularly in lowering the incidence of T2DM complications. However, the effects of high beta-glucan multigrain on T2DM is less understood, and is currently uncertain. This study aimed to investigate the effects of 12-week multigrain supplementation in T2DM patients. The changes of glycemic control, cardiometabolic health metrics, peripheral oxidative stress, nutritional status, QoL, safety and compliancy were assessed, concurrently.

1.4 Research question

i. What are the effect of high beta-glucan multigrain supplementation among the T2DM patients?
ii. What is the effect of multigrain supplementation on glycemic control among the T2DM patient?
iii. What is the effect of multigrain supplementation on cardiometabolic profile among the T2DM patient?
iv. What is the effect of multigrain supplementation on peripheral oxidative stress status, nutritional status and QoL among the T2DM patient?
v. What is the effect of multigrain supplementation on safety, compliancy and tolerability among the T2DM patient?

1.5 Research objectives

1.5.1 General objective:

To determine the effect of high beta-glucan multigrain supplementation among the T2DM patients.

1.5.2 Specific objectives

i. To assess the effects of multigrain supplementation on glycemic control.

ii. To evaluate the effects of multigrain supplementation on cardiometabolic profile.

iii. To explore the effects of multigrain supplementation on peripheral oxidative stress status, nutritional status and QoL.

iv. To determine the safety, compliancy and tolerability towards the multigrain supplementation program.

1.6 Research hypotheses

1.6.1 Hypotheses 1

Null Hypothesis:

H_0 = The multigrain supplementation is not effective to improve glycemic control.

Alternative Hypothesis:

H_a = The multigrain supplementation is effective to improve glycemic control.

1.6.2 Hypotheses 2

Null Hypothesis:

H_0 = The multigrain supplementation is not effective to improve cardiometabolic profile.

Alternative Hypothesis:

H_a = The multigrain supplementation is effective to improve cardiometabolic profile.

1.6.3 Hypotheses 3

Null Hypothesis:

H_0 = The multigrain supplementation is not effective to improve peripheral oxidative stress, nutritional status and QoL.

Alternative Hypothesis:

H_a = The multigrain supplementation is effective to improve peripheral oxidative stress, nutritional status and QoL.

1.6.4 Hypotheses 4

Null Hypothesis:

H_0 = The multigrain supplementation is not safe and tolerated.

Alternative Hypothesis:

H_a = The multigrain supplementation is safe and well tolerated.

CHAPTER 2

LITERATURE REVIEW

2.1 Type 2 Diabetes Melitus

2.1.1 Disease characteristics

Type 2 Diabetes Mellitus (T2DM), which is most typically identified in middle or later adulthood, affects patients who have a range of health conditions, including high blood pressure, obesity, and inactivity. Over 21 million Americans have diabetes, with T2DM accounting for over 90% of cases (Bullard et al., 2018). It is not surprising that T2DM has recently grown increasingly prevalent in teens and younger individuals given the global obesity pandemic. This disease typically manifests itself gradually as muscle and liver cells slowly lose their sensitivity to the effects that endogenous insulin has on lowering glucose levels (Petersen & Shulman, 2018). The insulin resistance that develops as a consequence is accompanied by higher levels of glucose in the blood and, ultimately, contributes to the progressive impairment of beta cells (Cerf et al., 2013). Treatments for diabetes can range from dietary management and increased exercise regimens to the use of one of the many anti-diabetic medications, and even insulin injections, depending on the severity of the disease as well as other patient characteristics (Ministry of Health Malaysia, 2020).

Studying the relationship between the development and manifestations of T2DM can be difficult for a number of reasons. One of these reason is that the majority of people who have this disorder also have a number of comorbid disorders and lifestyle characteristics that may also affect cognition to some degree. These conditions include hypertension, abnormal lipid profiles, cardiovascular disease, obesity, inactivity, depression, and iatrogenic hypoglycemia (Feinkohl et al., 2015). Additionally, since T2DM typically develops over the course of several years, it is not possible to accurately

estimate the duration or extent of glycemic excursions prior to a formal diagnosis. This is due to the fact that T2DM typically develops slowly (Feinkohl et al., 2015).

2.1.2 T2DM in Malaysia

The findings of the National Health and Morbidity Surveys (NHMS) in Malaysia, which are the most comprehensive and nationally representative available health data of adults for the years 1996, 2006, 2011, 2015, and 2019, indicated that the prevalence of diabetes has been on an upward trend for more than two decades. From 1996 to 2019, the overall prevalence of DM, including cases that were known but not diagnosed, increased by more than double (Zanariah et al., 2008; National Institutes of Health Ministry of Health Malaysia, 2019). It is extremely concerning that approximately half of the total diabetes cases that have been reported come from people who have diabetes that has not yet been diagnosed. At its most recent reported value of 18.3%, the prevalence of DM in Malaysia is comparable to that of other countries. More than 60% of the world's diabetic population lives in the Asia-Pacific region, specifically in Japan, Brunei Darussalam, Singapore, and the Republic of Korea; however, China and India recorded the highest in prevalence overall, accounting for almost half of all cases combined (Nanditha et al., 2016). According to the International Diabetes Federation (IDF) (2021), the number of people who have diabetes in the South-East Asia (SEA) region, including Malaysia, will increase by 68%, reaching 152 million by the year of 2045.

The NHMS data revealed that the majority of the patients in 2015 (79.1%) and 2019 (85.6%) were taking oral anti-diabetic medications within the past two weeks as opposed to insulin therapy (25.1% in 2015 vs 25.7% in 2019). The same report also mentioned that 88.0% of the T2DM patients had received specific diabetes dietary advice from healthcare personnel, while 75.4% claimed to have been advised by

healthcare personnel to lose weight, and up to 23.0% opted for traditional and complementary medication (NHMS, 2019).

In terms of gender and ethnicity, the prevalence of females (18.4%) was highest compared to males (18.2%), and Indians (31.4%) still hold the throne of the highest prevalence among other ethnic groups, followed by Malays (21.6%), Chinese (15.1%) and Bumiputera Sarawak (12.3%). Overall raised blood glucose was more common among the widow(er) / divorcee (33.2%), those with no formal education (28.7%), retirees (45.8%) and originated from the B40 group (18.5%). However, in term of geographical area, the prevalence of overall DM was similar in both urban and rural areas, where Negeri Sembilan (33.2%) has recorded the highest prevalence, followed by Perlis (32.6%) and Pahang (25.7%) (NHMS, 2019).

2.1.3 Economic burden of T2DM

The rising incidence of diabetes in the world's population poses huge economic problems to most nations in the region and has the potential to slow the pace of both national and international progress. Malaysia has the highest rate of diabetes in Western Pacific region, costing around 600 million US dollars per year (Ganasegeran et al., 2020; Ganasegeran et al., 2021). In 2010 alone, an estimated USD 600 million was spent on diabetes-related healthcare in the country, accounting for approximately 16% of the overall national healthcare budget (Zhang et al., 2010). The estimate was within the range of the total annual costs, between USD 141.6 million and USD 174 billion in their global systematic review (Ng et al., 2014).

In neighbouring countries such as Singapore, the total economic cost of T2DM for the country's working-age population, nearly 140,000 persons, was estimated to be USD 787 million in 2010, accounting for 0.35% of the country's gross domestic product (Png et al., 2016). The direct medical costs and complications in China were estimated

to be USD 26 billion in 2007 (Wang et al., 2009), while the total estimated cost of diabetes was USD 174 billion in the United States in 2007 (Dall et al., 2008), and USD 31.9 billion in India (Tharkar et al., 2010). As a result, the rising prevalence of diabetes causes an economic burden and a significant concern for the society as a whole in the form of the cost of medical care, treatment, medications, and complications.

2.2 Pathophysiology of T2DM

A dysfunction in the feedback loops between insulin action and insulin secretion leads to abnormally high glucose levels in the blood (Stumvoll et al., 2005). When beta cells are dysfunctional, insulin secretion is decreased, which makes it more difficult for the body to maintain physiological glucose levels. The organs involved in T2DM development include the pancreas (beta-cells and alpha-cells), liver, skeletal muscle, kidneys, brain, small intestine, and adipose tissue (Defronzo, 2009). Evolving data suggest the role for adipokine dysregulation, inflammation, and abnormalities in gut microbiota, immune dysregulation, and inflammation, have emerged as important pathophysiological factors (Schwartz et al., 2016). On the other hand, insulin resistance is linked to an increase in glucose production in the liver as well as a reduction in glucose uptake in adipose tissue, muscle, and the liver. Beta-cell dysfunction is typically more severe than insulin resistance, despite the fact that both processes take place early in the pathogenesis and contribute to the development of the disease. As a result, hyperglycemia is amplified when both beta-cell dysfunction and insulin resistance are present, which leads to the progression of T2DM (Cerf et al., 2013; Zheng et al., 2018).

2.2.1 (A) Beta-cell physiology

It is necessary to ensure the proper function of healthy beta-cells by maintain the cellular integrity and to strictly control the mechanisms and pathways that are

involved in the physiology of beta-cells (Cerf et al., 2013). In the biological study, one of the beta-cells' responsibilities is to synthesize an adequate amount of insulin, which is referred to as pre-proinsulin. During the maturation process, with the help of several protein in the endoplasmic reticulum, the pre-proinsulin undergoes a conformational modification to produce proinsulin (Bunney et al., 2017). Following that, pro-insulin is moved from the endoplasmic reticulum to golgi apparatus, where it enters into immature secretory vesicles and is subsequently cleaved into C-peptide and insulin (Fu et al., 2012; Halban, 1994). The maturation process involves the storage of insulin in granules, where it remains until the release of insulin is triggered. A response to high concentrations of glucose is primary stimulus that sets off the release of insulin. A few other factors such as the amino acids, fatty acids and hormones, that induced the release of insulin into the bloodstream (Boland et al., 2017).

A solute carrier protein known as glucose transporter 2 (GLUT2), which also serves as a glucose sensor for beta cells, is the primary mechanism where beta cells take in glucose in response to an increase in the concentration of glucose in the bloodstream. When glucose first enters the cell, glucose catabolism is triggered, leading to an increase in the ratio of ATP to ADP found inside the cell. This, in turn, causes ATP-dependent potassium channels in the plasma membrane to close. When ATP is used as an energy source, it can break down into ADP and become less effective. This happens because ADP is a weaker form of ATP. ATP is an energy that enables proteins in the plasma membrane to alter the shape of glucose molecules. Ca^{2+} is able to enter the cell as a result of this, which leads to the depolarization of the membrane and the opening of the voltage-dependent Ca^{2+} channels. The increase in the Ca^{2+} concentration within the cell causes the priming and fusion of the secretory insulin-containing granules to the plasma membrane, which ultimately results in the release of insulin from the cell, the process

called exocytosis (Fu et al., 2012; Boland et al., 2017; Rorsman & Ashcroft, 2018; Seino et al., 2011) (Figure 2.1).

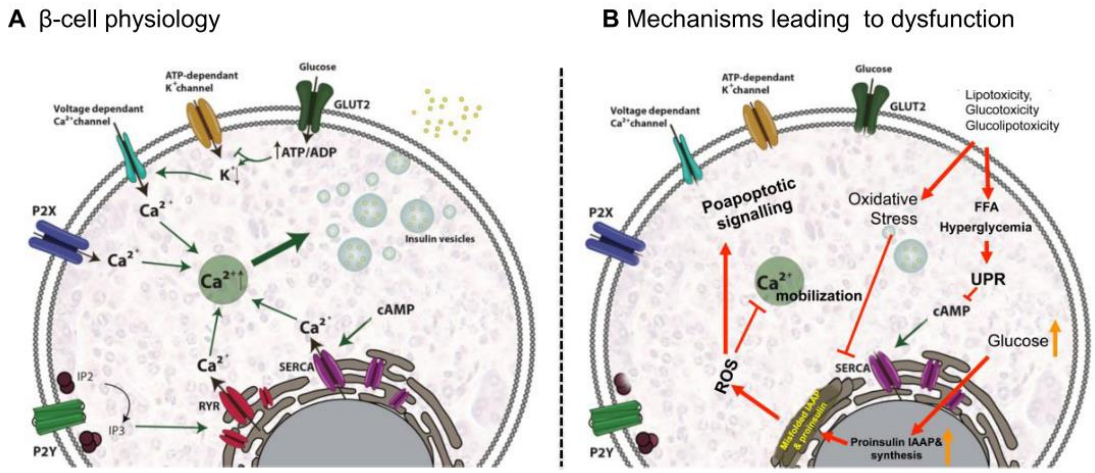

Figure 2.1 Mechanisms of beta cell physiology (A) and leading to dysfunction of beta cell (B).

(Source: Garcia et al., 2020)

Additionally, RY receptors (RYR) have the capacity to amplify Ca^{2+} signals and, as a result of their strategic locations within the cell and their capacity to mediate Ca^{2+} induced Ca^{2+} release (CICR), may play significant roles in stimulus-insulin secretion coupling. It is involved in the stimulation of insulin secretion, RYR amplifies Ca^{2+} signals when the channel is sensitized by messenger molecules produced by nutrient metabolism or ligand-binding (Islam, 2002), as shown in Figure 2.1. However, additional cell signals can also help or improve the way that beta-cells release insulin. Among them, cyclic adenosine monophosphate (cAMP) may be the most significant messenger enhancing the release of insulin. According to growing evidence, cAMP mobilizes secretory vesicles that contain insulin by reducing intracellular Ca^{2+} reservoirs and raising intracellular Ca^{2+} concentrations (Cuíñas et al., 2016).

Additionally, there is strong evidence that extracellular ATP is a key regulator of beta-cell function. It is well known that when glucose is stimulated, beta-cells release ATP by exocytosing insulin granules. Independent of glucose, purinergic signaling

through P2Y and P2X purinergic receptors promotes Ca^{2+} mobilization and controls insulin exocytosis. P2X-type receptors are ATP-activated ligand-gated ion channels that are non-selective for cations, whereas P2Y purinoreceptors have been reported to be coupled to G-proteins (Lustig et al., 1993; Simon et al., 1995). It has been suggested that the release of insulin from P2Y receptors may be mediated by intracellular Ca^{2+} mobilization in response to inositol-1,4,5-trisphosphate (IP3) formation, which causes the release of Ca^{2+} from ER stores and amplifies the Ca^{2+} signal that initiates exocytosis (Blachier & Malaisse, 1988; Garcia et al., 2020).

2.2.2 (B) Mechanisms leading to beta-cell dysfunction

The mechanisms leading to beta-cell dysfunction has been linking to the major factors to the clinical manifestations of T2DM, and its complications that are traditionally associated with beta-cell death or dysfunction, which is may affect the production of insulin. Hyperglycemia and hyperlipidemia are frequently present in an excessive nutritional state and physical inactivity, which is similar and contributing to obesity, favoring insulin resistance and chronic inflammation. Due to variations in their genetic susceptibility, beta-cells are exposed to toxic pressures in these conditions, including inflammation, inflammatory stress, endoplasmic reticulum stress, metabolic/oxidative stress, and amyloid stress, all have the potential to eventually result in the loss of islet integrity (Christensen & Gannon, 2019). Beta-cell dysfunction is caused by an excess of free fatty acids (FFAs) as well as hyperglycemia. This leads to the increased expression of endoplasmic reticulum stress, which in turn activates the apoptotic unfolded protein response (UPR) pathways (Yamamoto et al., 2019).

In fact, metabolic and oxidative stress caused by obesity, including the formation of lipotoxicity, glucotoxicity, and glucolipotoxicity, can cause damage to beta-cells (Halban et al., 2014). Stress caused by high levels of saturated fatty acids can

activate the unfolded protein response pathway via multiple mechanisms. These mechanisms include inhibition of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), which is responsible for ER Ca^{2+} mobilization; activation of IP3 receptors; or direct impairment of ER homeostasis. Additionally, sustained high glucose levels increase proinsulin biosynthesis and islet amyloid polypeptides (IAAP) in beta cells, which leads to the accumulation of misfolded insulin and IAAP, as well as an increase in the production of oxidative protein folding-mediated reactive oxygen species (ROS) (Yamamoto et al., 2019). These effects change the physiological mobilization of endoplasmic reticulum Ca^{2+}, favour proapoptotic signals, promote the degradation of proinsulin mRNA, and cause the release of interleukin (IL)-1, which attracts macrophages and intensifies local islet inflammation (Halban et al., 2014) (Figure 2.1).

In order to precisely satisfy the requirements of the metabolic process, insulin secretion needs to be subjected to stringent regulation. Because of this, maintaining the proper integrity of the islets of langerhans from pancreas which is necessary in order to enable beta cells to respond appropriately to the requirements of metabolism (Do & Thorn, 2015). Under pathogenic conditions, the mechanism described above can ultimately lead to the disruption of islet integrity and organization, impairing optimal cell-to-cell communication within pancreatic islets, contributing to poor regulation of insulin and glucagon release, and ultimately exacerbating the hyperglycemia (Garcia et al., 2020). Insulin secretory dysfunction is the primary cause of beta-cell death and a fundamental component of T2DM. Insulin secretory dysfunction can be caused by errors in the synthesis of insulin precursors or insulin itself, as well as disruptions in the mechanism that controls insulin secretion. A failure in the folding of proinsulin is an additional finding that is commonly linked to insufficient insulin production and diabetes (Liu et al., 2018). For instance, a reduction in the expression of the GLUT2

glucose transporter would have an effect on the downstream signalling pathway (Do & Thorn, 2015).

Figure 2.2 explains the T2DM progression. Beta-cells initially compensate for increasing insulin resistance by increasing insulin production and synthesis as well as beta-cell proliferation. This occurs when beta cells divide more frequently. However, prolonged insulin resistance and inflammation can lead to stress and decompensation in beta-cells, which can result in impaired glucose tolerance (or prediabetes). The presence of both hyperglycemia and hyperlipidemia over an extended period of time can cause beta cell glucolipotoxicity and the death of beta-cells. T2DM is caused when the functional beta cell mass decreases as a result of dedifferentiation and/or the death of beta-cells (Gracia et al., 2020).

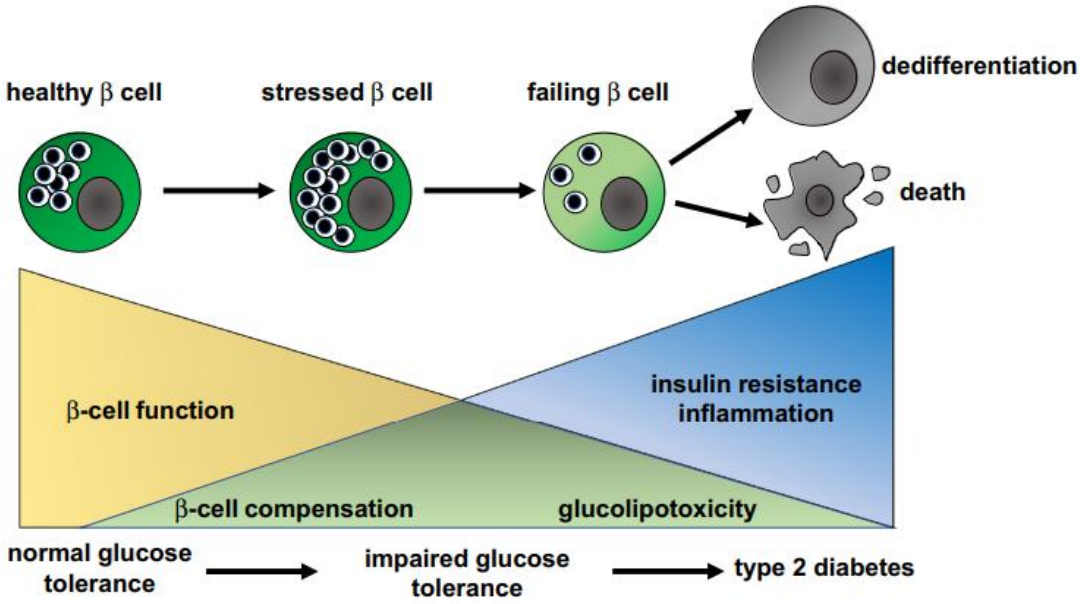

Figure 2.2 Schematic of T2DM progression

(Source: Christensen & Gannon, 2019)

2.3 Symptom and signs of diabetes

The signs and symptoms of T2DM are listed below:

- Polyuria: urinating often and passes excessive or abnormally large amounts of urine each time
- Polydipsia: feeling very thirsty or excessive thirst
- Nocturia: urinary frequency and having to urinate more often at night
- Malaise fatigue: extreme fatigue usually occurs along with malaise
- Altered vision: Blurry vision
- Frequent infections: Cuts/bruises that are slow to heal
- Tingling, pain, or numbness in the hands/feet

(Source: Clinical Practice Guidelines (CPG): Management of T2DM , 2020)

2.4 Diagnosis of T2DM

The diagnosis of T2DM is measured using random plasma test, fasting plasma glucose (FPG) test, two-hour oral glucose tolerance test (OGTT) and HbA1c. The random plasma is the simplest test and doesn't require fasting before taking the test and the indicator for this test is 200 or more than 200mg/dl (11.1 mmol/L) of blood glucose it probably indicates diabetes but has to be reconfirmed. Furthermore, fasting plasma glucose is a sample that taken after eight hours overnight fast. According to CPG (2020), FPG level of more than 126 mg/dL (7.0 mmol/L) is consistent with the diagnosis. OGTT is a test that using plasma glucose level and tested before and again two hours after the subject consumes 75 grams of glucose for the purpose of this test. If the plasma glucose (PG) level in the 2-hour sample is higher than 200 mg/dL (11.1 mmol/L), then diabetes mellitus can be diagnosed. Additionally, it is a common test; but, compared to FPG, it is less convenient, more expensive, and plagued with substantial difficulties of variability. Patients have to abstain from taking any medications that may have an effect on their glucose tolerance for a period of three to

five days, including steroids and thiazide diuretics. They also have to consume a diet that contains at least 150 grams of carbs per day. Finally, the HbA1c is the test that provides an estimate of the blood glucose levels as an average over the past two to three months. Patients are considered to have prediabetes mellitus if their HbA1c level between 5.7% and 6.2%. The HbA1c test is one that is simple, quick, and standardized, and it exhibits less variation than other tests due to the variables that come before the actual analysis. It is not significantly impacted by short-term illnesses or by stress. However, HbA1c is expensive and has a number of problems, including a decreased sensitivity, as the numerous diseases, such as sickle cell disease, pregnancy, anemia, hemodialysis, blood loss or transfusion, or erythropoietin therapy, can have an impact on it. It has not been adequately validated in groups that are not white (Goyal & Jialal, 2022).

2.5 Complications of Diabetes Mellitus

Generally, the injurious effects of hyperglycemia are separated into macrovascular complications (coronary artery disease, peripheral arterial disease, and stroke) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy) (Fowler et al, 2008).

2.5.1 Acute complications

Acute complications are major, life-threatening consequences that can occur quickly and fast as a result of uncontrolled high blood glucose (hyperglycemia) or low blood sugars (hypoglycemia) (hypoglycemia). Hypoglycemia is characterized by fluctuating blood sugar levels throughout the day that fall below 70 mg/dL. This condition is often referred to as having low blood sugar. Extreme hypoglycemia, which occurs when a person's blood sugar level falls below 54 mg/dL, can make the patient's

existing health condition worse, causing them to have trouble walking or seeing clearly, to feel weak, to behave strangely or to feel confused, and to have seizures. As a result, it is essential to have an understanding of the factors that might lead to hypoglycemia, or low blood sugar. Some of these factors include taking an excessive amount of insulin or medicine, failing to consume an adequate amount of carbohydrates, and other factors (Centers for Disease Control and Prevention, n.d.).

Hyperglycemia involves two acute metabolic consequences of diabetes that are the most serious are diabetic ketoacidosis (DKA) and the hyperosmolar hyperglycemic state (HHS). Despite the existence of well-developed diagnostic criteria and treatment regimens, DKA and HHS continue to be major contributors to morbidity and mortality among diabetes patients (Kitabchi et al., 2009). DKA manifests itself when there is an insufficiency of insulin in the body, which prevents glucose from entering cells to be used as a source of energy. Instead, the liver oxidizes fat to make fuel, a process that results in the production of chemicals known as ketones. Ketones can reach potentially harmful levels in the body if there is an excessive amount of production of them in a short period of time (Centers for Disease Control and Prevention, 2021). The fundamentals of managing diabetic ketoacidosis include treating dehydration and electrolyte imbalance, administering insulin, addressing precipitating factors, and preventing complications (Ministry of Health Malaysia, 2020). HHS occurs in people with T2DM who experience very high blood glucose levels (often over 40mmol/l). It can develop over a course of weeks through a combination of illness (e.g infection) and dehydration (Diabetes UK, 2015). As a consequence of obesity and high body mass index (BMI), there is the resistance of the peripheral tissue to the action of insulin. The beta-cell in the pancreas continues to produce insulin, but the amount is not enough to

counter the effect of the resistance of the end organ to its effect (Adeyinka & Kondamudi, 2022)

2.5.2 Chronic complications

Chronic complication is a long-term complication that plays a major role in the initiation of diabetic vascular complications through many metabolic process which are divided into microvascular and macrovascular complications. Diabetes microvascular complications are those long-term complications that affect or involving small blood vessels such as capillaries. Too much sugar for a long-term can harm the eyes, kidneys and nerves. This microvascular complication typically include retinopathy, nephropathy, and neuropathy (Zimmerman, 2016; Cade, 2008). Diabetic retinopathy (DR) is a microvascular condition that can impair the peripheral retina, the macula, or both, and is a major cause of visual loss and blindness among diabetics due to elevated of glucose level (Cade, 2008). Diabetic nephropathy is defined as persistent proteinuria and it can progress to overt nephropathy, which is characterized by progressive decline in renal function resulting in end-stage renal disease (Fowler, 2008). Neuropathy is a diverse disorder caused by nerve damage. The disorder is defined based on the damaged nerves, which include focal, diffuse, sensory, motor, and autonomic neuropathy (ADA, 2009; Fowler, 2008).

Macrovascular complications are problems with arteries, veins, and other large blood vessels that last for a long time. Most diseases of the coronary arteries, peripheral arteries, and brain blood vessels are macrovascular complications of diabetes. The main cause of macrovascular disease is the process of atherosclerosis, which causes the walls of arteries all over the body to narrow. Atherosclerosis is thought to be caused by long-term inflammation and damage to the arterial walls in the peripheral or coronary vascular system (Fowler, 2008). Early macrovascular disease is also linked to

atherosclerotic plaque in the blood vessels that bring blood to the heart, brain, limbs, and other organs. While late-stage macrovascular disease causes these blood vessels to close off completely. This makes the risk of myocardial infarction (MI), stroke, claudication, and gangrene higher. Cardiovascular disease (CVD) is the main reason why diabetics lead to morbidity and mortality (Zimmerman, 2016).

2.6 Risk factor of T2DM

2.6.1 Sociodemographic and Psychosocial Risk Factor

2.6.1(a) Age

Age is one of the biggest risk factors for getting prediabetes and T2DM (A. M. Chang & Halter, 2003). Both T2DM and pre-diabetes are more common as people get older. Hyperglycemia is caused by a lack of insulin secretion, which gets worse with age, and increasing insulin resistance, which is caused by changes in body composition and sarcopenia. This is because as a person gets older, energy homeostasis gets worse and carbohydrate metabolism gets messed up (Barbieri et al., 2003; Mordarska & Godziejewska-Zawada, 2017).

The Baltimore Longitudinal Study of Aging demonstrated that insulin secretion after glucose load decreases with age, even when obesity and distribution of adipose tissue are accounted for. Tests on the kinetics of insulin excretion in elderly individuals revealed that, compared to younger individuals, postprandial insulin excretion is irregular and the amplitude of successive insulin pulses is lower (Meneilly et al., 1997). In people with T2DM, the impairment of cell function and dysfunction of insulin secretion are even more severe and are associated with a nearly total loss of the first phase of insulin secretion. Moreover, aging diminishes the sensitivity of pancreatic cells

to incretins. Incretins are less effective, leading to lower postprandial insulin levels and weaker suppression of glucagon secretion (A. M. Chang & Halter, 2003).

Insulin resistance, which increases with age, is the second most important cause of elevated blood glucose levels (Krentz et al., 2013). The distribution of adipose tissue in the elderly is changing (increased visceral adipose tissue), and the amount of fat tissue increases in contrast to the decrease in muscle mass that occurs with age. A consequence of the aging process is dysregulation of the hypothalamic-pituitary-adrenal axis (HPA axis), which results in a relative increase in cortisol levels. Additionally, cortisol causes insulin resistance in the liver (Mordarska & Godziejewska-Zawada, 2017).

When compared to younger patients, elderly patients are more likely to be hospitalized due to hypoglycemia, have a higher risk of myocardial infarction and end-stage renal disease, and are more likely to be affected by diabetes-related complications. In order to achieve the specific goals of therapy, older people who have had diabetes for a long time and who have a number of chronic complications need a more liberal approach. In addition, the therapy goals should make it a priority to prevent hypoglycemia, ensure the therapy is safe, and convince the patient to participate (Mordarska & Godziejewska-Zawada, 2017).

2.6.1(b) Ethnicity

Since ethnicity is defined as a complex multidimensional construct reflecting the confluence of biological factors and geographic origins, culture, economic, political, and legal factors, as well as racism, the concepts of race and ethnicity play important roles in understanding disparities in health and health care (Spanakis & Golden, 2013). The current literature describing disparities in diabetes varies in the terms used to define specific race/ethnic groups. As done previously and for consistency, the study used the term “non-Hispanic black” (NHB) to refer to individuals of African descent, “non-

Hispanic white" (NHW) for non-minority individuals, Hispanic American for those of Mexican, South American, Cuban, or Puerto Rican descent born and/or residing in the U.S., Asian American for individuals of South Asian (e.g. Indian), East Asian (e.g. Japanese, Chinese, Korean), Southeast Asian (e.g. Cambodian, Vietnamese, Laotian, Thai), and Pacific Island (e.g. Filipino) descent born and/or residing in the U.S., and Native American to refer to American Indians and Alaska Natives (Golden et al., 2012).

NHBs, Hispanic Americans, and Asian Americans were found to have a lower risk for developing cardiovascular complications of diabetes compared to NHWs in a systematic review of the risk between ethnicity and macrovascular complications (Lanting et al., 2005). Retinopathy is more common in minority groups compared to NHWs, regardless of race or ethnicity, as suggested by previous study (Golden et al., 2012; Lanting et al., 2005). Nephropathy follows this pattern, where if compared to non-Hispanic whites (NHWs), individuals who are black have a higher prevalence rate of ESRD but a lower mortality rate while on dialysis. A systematic review of the literature on neuropathy showed no significant differences between studies. However, these findings need to be interpreted with caution due to differences in the definition of diabetic neuropathy, the methods used in the individual studies, and the impact of cultural and linguistic barriers (Spanakis & Golden, 2013).

According to the biological factors, genetic factors have been considered as a potential explanation for race/ethnic differences in diabetes; however, the majority of studies suggest that the genetic architecture conferring an increased risk of T2DM is similar across race/ethnic groups, at least as far as common variants are concerned. Obesity represents one of the strongest contributors for the development of T2DM. NHBs had the highest prevalence rate of age-adjusted obesity of 49.5% whereas Mexican Americans and NHWs had rates of 40.4% and 34.3%, respectively (Golden et