An open-label, uncontrolled study to evaluate the effect of verapamil on glycaemic control in type 2 diabetes mellitus patients with hypertension in Saudi Arabia

Emtenan Al-Harbi, PhD candidate

Strathclyde Institute of Pharmacy and Biomedical Sciences

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Abstract:

Introduction: Type 2 diabetes is a common chronic disease that continues to globally increase in prevalence and is a major healthcare burden. Diabetes and hypertension are frequently coexistent conditions and the use of antihypertensive agents is common in diabetic patients. One antihypertensive agent, verapamil, had tentatively shown potentially positive effects on glycaemic control in assorted pre-clinical models.

Aim: To evaluate the effect of verapamil on glycaemic control in hypertensive type 2 diabetic patients.

Method: Type 2 diabetic hypertensive subjects were recruited from King Fahad Medical City, Riyadh, KSA to receive oral verapamil therapy. Blood pressure and glucometabolic parameters including fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), C-peptide, and Homeostatic Model Assessment Insulin Resistance (HOMA-IR) were monitored at baseline and after 6 months of verapamil therapy.

Results: 35 patients (16 male, 19 female) with a mean age of 57.2 years were recruited. The use of verapamil was associated with non-significant decreases in HbA1c (0.2 ± 1.0%, *P*=0.25%), FPG (0.5 ± 1.8 mmol/L, *P*=0.11), C-peptide (0.1 ± 0.3 nmol/L, P=0.06), and HOMA-IR (0.3 ± 0.9, *P*=0.05).. However a sub-group of 17 participants had a decrease in HbA1c that was ≥ 0.5%. Univariable logistic regression showed that baseline BMI, HOMA-IR, and C-peptide (*P*<0.05) were significantly associated with HbA1c reductions of ≥ 0.5%. HbA1c levels were affected by sitagliptin use, metformin dose, insulin use, duration of diabetes, neuropathy, and retinopathy (*P*<0.05). Additionally, insulin use was negatively associated with FPG levels but had no association with HOMA-IR and C-peptide levels (*P*<0.05).

Conclusion: Verapamil was metabolically neutral and allowed stabilization of glycometabolic parameters in type 2 diabetic individuals. Additional research exploring why there was variable response to verapamil therapy is warranted.

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Chapter 1

1.1 Introduction

Diabetes mellitus (DM) is a chronic metabolic disease where patients have an abnormally high level of glucose in their blood due to poor insulin sensitivity, insufficient insulin secretion, or a combination of the two.[3,4] It is one of the most common chronic diseases that continues to increase in prevalence as a global health problem due to fast-economic development, ageing populations, and unhealthy lifestyles.[5,6] Diabetes mellitus affects millions of people of all ages, gender, racial and ethnic groups.[3,5,7]

1.2 The global prevalence of diabetes

According to data from the recent International Diabetes Federation (IDF) atlas, United Kingdom (UK) diabetes prevalence in adult patients aged 20-79 years was estimated to be 5.6 %. While in the United States (US) the prevalence is higher, at 13.3%.[3] Globally, according to the recent IDF atlas, diabetes prevalence in adult patients aged 20-79 years was estimated to be 9.3% (527 million people) and predicted to increase to 10.2% (578 million) by 2030 and 10.9% (700 million) by 2045.[3,8]

1.2.1 Prevalence of diabetes in the Kingdom of Saudi Arabia (KSA)

Over the past three decades, KSA has realised significant economic growth and a notable improvement in life quality which has led to remarkable changes in lifestyle. This in turn has caused a significant increase in the incidence rate of diabetes.[9-11] This dramatic increase has reached an alarming rate.[12,13] According to IDF, KSA is one of

the top 10 countries for diabetes prevalence in 2011 and is projected to stay in the top 10 by 2030.[14] It is considered as having the seventh-highest rate of diabetes incidence worldwide and projected to have the sixth-highest rate in 2035.[15]

According to the recent IDF atlas, KSA has a diabetes incidence of 18.3%.[3] However, a higher prevalence was detected in Saudi studies. In 2011, a study reported that the prevalence of diabetes was 34.1% in males and 27.6% in females.[15] In 2014, another study reported a prevalence rate of diabetes of 25.4% for subjects aged ≥30 years and 40.2% for subjects aged ≥45 years.[16] In a more recent 2019 study, over 25% of the adult population has diabetes and this figure is projected to more than double by 2030.[11]

1.3 Global mortality burden of diabetes

In 2019, the IDF reported that around 4.2 million deaths (11.3% of global deaths) worldwide were attributable to diabetes in the age range 20 to 79 years. This is equivalent to a death every 8 seconds.

1.4 Global economic burden of diabetes

Globally diabetes imposes a large economic burden on individuals, national healthcare systems, and countries. Also, the IDF estimates that at least 760 billion United States Dollars (USD) were spent worldwide in diabetes-related healthcare costs in 2019. This represents 10% of total healthcare spending on adults. This represents a 4.5% increase on the 2017 estimate and is expected to continue in grow, reaching 825 billion USD by 2030 (an increase of 8.6%) and 845 billion USD by 2045 (an increase of 11.2%).[3,7] According to the American Diabetes Association (ADA), the costs of diabetes

increased by 26% from 2012 to 2017 as a result of the increase in both incidence of diabetes and treatment cost per diabetic patient.[17]

1.4.1 KSA economic burden of diabetes

In KSA, diabetes imposes a large economic burden on individuals, the national healthcare system, and the country. The national healthcare burden due to diabetes is estimated to exceed $870 million. This only represents a direct cost and does not included indirect costs associated with diabetes, such as absenteeism, loss of productivity from disease-related complications, unemployment due to disability, and early mortality due to the disease.[18] People diagnosed with diabetes, on average, have medical healthcare expenditures that are ten times higher than what expenditure would be in the absence of diabetes ($3,686 versus $380). Consequently, early diagnosis, optimal control of diabetes, and prevention of new cases are crucial to help minimize disease burden and decrease the healthcare costs associated with diabetes.[3,4,12]

1.5 Classification of diabetes

Diabetes can be classified into different categories including Type1 diabetes, Type 2 diabetes, secondary diabetes, and gestational diabetes mellitus (GDM). Typically GDM is diagnosed in the second or third trimester of pregnancy while secondary diabetes develops due to other causes e.g. drug-induced diabetes due to glucocorticoid drug therapy. Type 1 diabetes accounts for 5–10% of all diabetic cases and is also known as "immune-mediated diabetes", "juvenile-onset diabetes", and “insulin-dependent diabetes”, because it depends upon its management by exogenous insulin administration. The most common type of diabetes, Type 2 diabetes, accounts for 90–

95% of all diabetic cases. It is also known as "non-insulin-dependent diabetes" and "adult-onset diabetes".[3,4,19,20]

Type 1 and Type 2 diabetes are distinctive diseases in which the clinical presentation and disease progression varies considerably between patient cohorts.[3,4,19] Correct classification is critical to guiding optimal therapy, but a minority of patients cannot easily be classified as Type 1 or Type 2 diabetics at the time of diagnosis.[19] The difficulties in differentiating between the diabetic types at the onset of diagnosis in these patients typically disappear over time.[19,21] Future classification of diabetes will likely depend on the pathophysiology of the underlying beta cell (β-cell) dysfunction, which are the insulin-secreting cell in the pancreatic islets of Langerhans. In addition, classification may depend on the stage of the disease and can be classified according to glucose status as being normal, impaired, or diabetic.[19,22] Type 2 diabetes will be discussed thoroughly thereafter as it is the most common type of diabetes and directly relevant to this research.

1.5.1 Type 2 diabetes

Type 2 diabetes accounts for the vast majority (around 90%) of diabetic cases worldwide (463 million people). Its prevalence is increasing in most countries and has a high degree of variability worldwide.[3,22-24] Moreover, it was estimated that 50.1% of diabetic cases (231.9 million of the 463 million adults living with diabetes) are undiagnosed type 2 diabetics.[3,24] In 2011, a study[25] performed in KSA to determine the prevalence of type 2 diabetes, found that in people≥30 years old the overall prevalence was 31.6%. Prevalence was significantly higher in males (34.7%) than in females (28.6%) ($P < 0.001$).[25] A systematic review done to highlight the prevalence

and future projections of type 2 diabetes in KSA found that the prevalence of type 2 diabetes is 32.8% and the predicted prevalence will be 35.4% in 2020; 40.4% in 2025 and 45.4% in 2030.[12]

1.6 Diagnosis of type 2 diabetes

Glycated haemoglobin (HbA1c) is the major tool for assessing glycaemic control and many clinicians tend to use HbA1c as a diagnostic test where its measurement reflects both fasting and postprandial glucose concentrations over three months.[26,27] It has a strong predictive value for diabetic complications and reflects average glycaemia over a period of approximately 3 months.[27-35] HbA1c can be performed at any time of the day and does not require any special patient preparation such as fasting.[27,32-35]

Fasting plasma glucose (FPG) is defined as having no calorific intake for at least 8 h prior to a measurement. FPG can be used as screening test for diabetes but it is not recommended over an HbA1c test as it requires the patient to fast for at least 8 hours before the test and the sampling process of fasting glucose is more complex than for HbA1c which can lead to a greater risk of measurement errors.[36] Also the test reproducibility of FPG is lower than HbA1c[37] and it has a lower ability to predict longer-term clinical outcomes.[31]

However, the use of FPG and an oral glucose tolerance test (OGTT) as screening tests for diabetes are commonplace and may be promoted by physicians due to the relative unavailability and high cost of the HbA1c test.[38]

According to ADA recommendations[19,39], the criteria for diagnosis of diabetes should be one of the following measures:

- Random plasma glucose (RBG) level ≥ 200 mg/dL (11.1 mmol/L) in a patient with classic diabetic symptoms.
- FPG ≥ 126 mg/dL (7.0 mmol/L).
- Two hours postprandial glucose (2-hpg) ≥ 200 mg/dL (11.1 mmol/L) following an OGTT (performed as described by the World Health Organization (WHO), using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water). A normal blood glucose level is lower than 140 mg/dL (7.8 mmol/L). A blood glucose level between 140 and 199 mg/dL (7.8 and 11 mmol/L) is considered impaired glucose tolerance, or prediabetes.
- HbA1c value ≥6.5%.

1.7 Monitoring of type 2 diabetes

All people with type 2 diabetes should have regular check-ups to monitor their glycaemic control, risk level, and disease progression. HbA1c is the major tool for assessing glycaemic control and diabetes complications.[27-33,40]

Patient self-monitoring of blood glucose (SMBG) and continuous glucose monitoring (CGM) may also help in evaluating glycaemic control and is used in combination with HbA1c measurements, particularly in individuals on intensive insulin therapy.[27]

Epidemiological analysis of the UK Prospective Diabetes Study (UKPDS 2000) showed that for every 1% reduction in HbA1c, the relative risk for microvascular complications decreased by 37%, diabetes-related deaths 21%, and myocardial infarction 14%.[41]

Generally, in adult diabetic (non-pregnant) patients, 7% is considered an acceptable goal of HbA1c. For selected patients with specific characteristics (short duration of diabetes, type 2 diabetes treated with nonpharmacological therapy or on metformin only regime, or having no significant cardiovascular disease), the physician might suggest a lower HbA1c goal (6.5%) if this can be reached without causing significant hypoglycaemia or other drug-related side effects. In contrast, in some patients, the physician might accept a higher HbA1c goal (8%) if they have a history of severe hypoglycaemia, extensive comorbid conditions, severe microvascular or macrovascular complications, or long-standing diabetes with a history of severe high blood glucose or having difficulty in achieving their HbA1c goal despite an adequate therapy plan and medication adherence. [27]

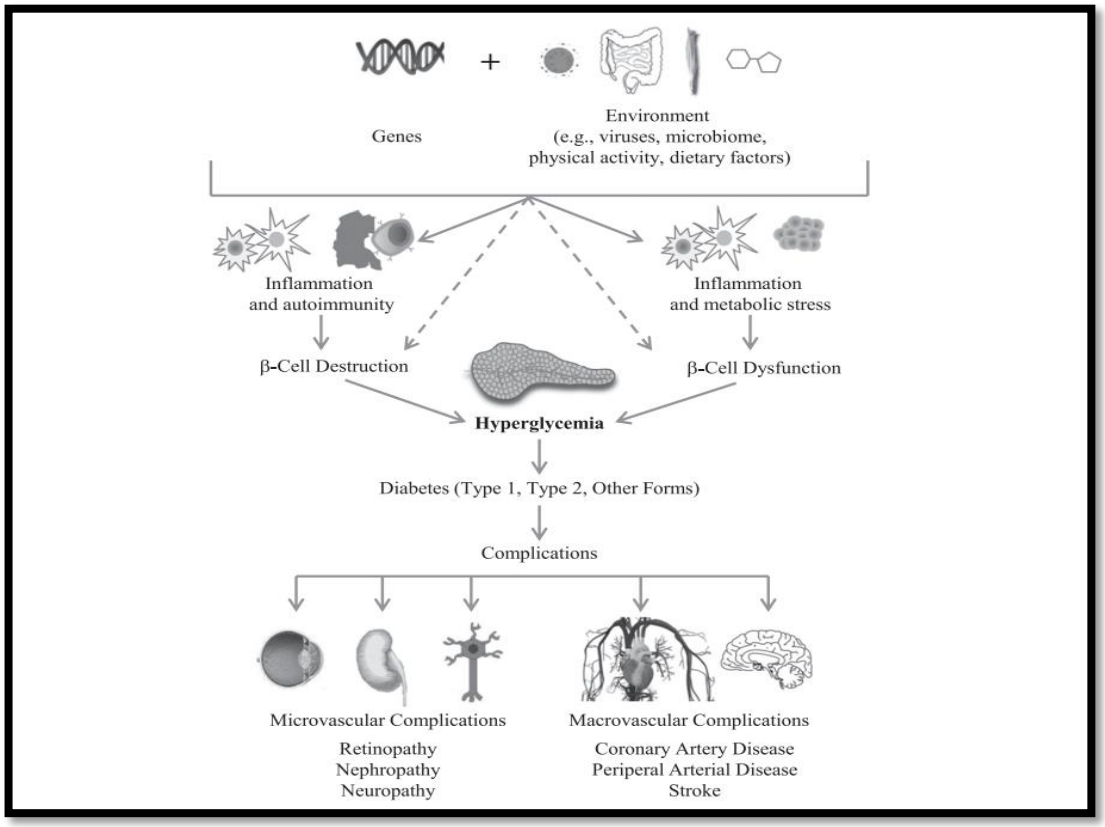
Generally, the accuracy of the HbA1c test is excellent for National Glycohemoglobin Standardization Program (NGSP) certified assays (www.ngsp.org). NGSP-certified assays are standardized HbA1c test results and help in significantly reducing inter-country variability among HbA1c results reported. Whilst individual countries may not report HbA1c using identical units, there are established equations to enable the conversion between different reporting units.[40] However, conditions that affect red blood cell turnover (anaemia, recent blood transfusion, end-stage kidney disease, and pregnancy) may result in discrepancies between the HbA1c measurement and the patient's true mean glycaemia. Also, some patients have haemoglobin variants such as sickle cell trait (Hbs), which interfere with and affect the accuracy of the HbA1c measurement. So, for patients with any haemoglobin trait, the selection of an HbA1c assay method not affected by patient factors should be carefully undertaken to ensure an accurate HbA1c result.[27,32,33,38,42]

HbA1c testing should be performed at least twice a year in patients who are meeting their individual HbA1c goal but should be undertaken quarterly in patients whose therapy has changed or who are not meeting their glycaemic goals. [27,32,33]

1.8 Risk factors for type 2 diabetes

Type 2 diabetes is characterized by both insulin resistance and β-cell dysfunction.[43] Insulin resistance is mainly caused by obesity [44] while β-cell function in individuals is determined by genetic factors.[45] Different genetic and environmental factors can increase the risk of diabetes. These factors impact inflammation, autoimmunity, and metabolic stress in diabetic patients as shown in Figure 1.1.[22] Diabetic risk factors can be classified into modifiable and unmodifiable risk factors. The unmodifiable risk factors for type 2 diabetes are advancing age, race/ethnicity, family history, maternal history of diabetes, and male sex. Modifiable risk factors include obesity, smoking, lack of physical activity, low socioeconomic status, low educational level (increases risk by 41%), low occupation level (increases risk by 31%), low-income level (increases risk by 40%)[6,22] and presence of conditions associated with insulin resistance such as hypertension and dyslipidemia.[27] Globally, obesity is considered a major modifiable risk factor for diabetes.[46,47] In 2015, it was reported that between 80% and 90% of all Saudi type 2 diabetes patients had obesity as a risk factor.[48] Diabetes prevention programs should aim to correct all modifiable risk factors.[11,49]

Figure 1. 1: Genetic and environmental factors for developing diabetes



Reproduced from Skyler et.al.[22]

1.9 Complications of type 2 diabetes

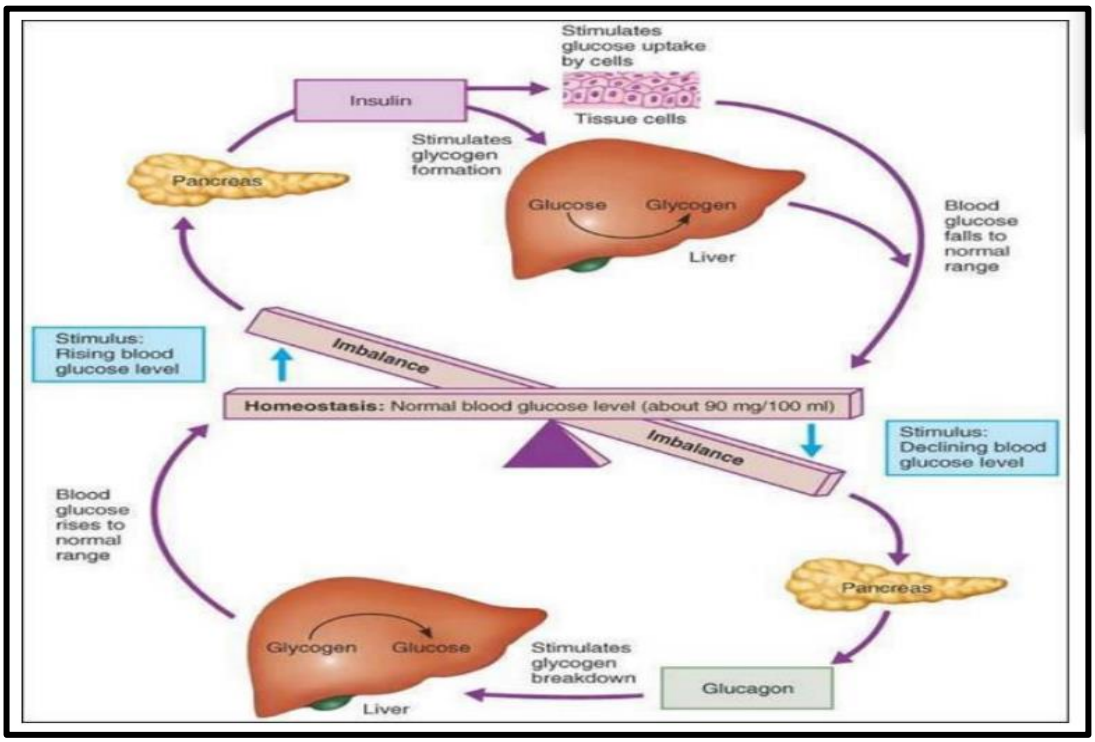
Type 2 diabetes is the leading cause of blindness, amputation, kidney disease, cardiomyopathy, cerebrovascular, and peripheral artery disease. Diabetes-related complications can be classified into microvascular complications (nephropathy, retinopathy, and neuropathy) and macrovascular complications (cardiovascular disease, stroke, and peripheral artery disease).[4,11,50,51] These complications increase the morbidity and mortality in diabetic patients.[22] Atherosclerotic cardiovascular disease (ASCVD) is one of the most common causes of morbidity and mortality in diabetic patients. A large proportion of people with type 2 diabetes also have hypertension and hyperlipidemia which must be considered collectively.[50] The prevalence of

hypertension in patients with diabetes is higher than in the general population and mainly associated with hyperlipidemia and central obesity.[22,52-57] On other hand, the prevalence of diabetes is around 2% of patients with hypertension per year.[54] In 2013, a systematic review found that among diabetic obese patients, hypertension rates were ≥70% in Asia and more than 80% in Europe with lower rates of over 30% reported in North and South America.[55] As diabetes and hypertension are frequent coexistent diseases, the use of antihypertensive agents is common in diabetic patients.[22,52]

1.10 Pathophysiology of type 2 diabetes

As shown in Figure 1.2 [58], normally when blood glucose levels are elevated, this will stimulate β-cells in the pancreatic islets of Langerhans to secrete insulin. The secreted insulin stimulates glucose uptake from the blood into the liver and other tissues such as skeletal muscles. This reduces blood glucose levels back to normal. Conversely, a reduction in blood glucose concentration is directly detected by alpha-cells in the pancreatic islets of Langerhans. These cells are responsible for releasing glucagon which stimulates the liver to release glucose until it returns to a normal range. The capacity to lower blood glucose is dependent on pancreatic β-cell responsiveness to glucose levels and the body's sensitivity to insulin.[58]

Figure 1. 2: Blood glucose level maintenance in the body.



Reproduced from Ogori A Friday.[58]

β-cell dysfunction, which results in insufficient insulin secretion, is the basis for understanding the pathophysiology of type 2 diabetes.[59-61] Type 2 diabetes, develops as a result of the progressive loss of pancreatic β-cell function when β-cells are unable to secrete sufficient insulin (relative insulin deficiency.[62]) to maintain and sustain metabolic requirements, frequently in the presence of insulin resistance within the peripheral tissues.[19,39,61-64] The deficiency of plasma insulin and low glucose tolerance causes insulin resistance, which is the fundamental symptom underlying the potential development of type 2 diabetes. Type 2 diabetes is primarily linked with insulin secretory defects related to inflammation and metabolic stress, with other factors such as genetic factors playing a role.[22] Insulin resistance develops with ectopic fat deposition in the liver and muscle. This fat may also accumulate in the pancreas and is associated with a decline in β-cell function, islet inflammation, and subsequent β-

cell death (β-cell apoptosis).[65] However, in the early phases of diabetes, significant weight loss can reduce intrapancreatic fat and restore pancreatic function.[66]

Many studies report that the loss of functional β-cell mass is a hallmark of diabetes.[67-71] A decrease in β-cell mass occurs when there is a rise in the frequency of β-cell apoptosis while the rate of new islet formation remains unaffected.[62] In patients with type 2 diabetes, β-cell mass is reduced by 20–65%, leading to impaired and delayed insulin secretion. These defects cause hyperglycaemia in patients with type 2 diabetes. Following diabetes diagnosis, hyperglycaemia tends to become more complicated, severe, and difficult to treat. The progressive nature of diabetes is usually due to the continuous decrease of β-cell function and mass.[22]

1.11 Assessment of β-cell dysfunction

Endogenous insulin secretion is most commonly measured by assessment of C-peptide levels. C-peptide is released exclusively by pancreatic β-cells as a by-product of the enzymatic cleavage of proinsulin to insulin and is produced in equimolar amounts to insulin.[72-75] It is considered as a direct measure of β-cell function and has a plasma half-life of 30 min.[73-75] According to the Diabetes Control and Complications Trial (DCCT), C-peptide range in healthy individuals is 0.3–0.6 nmol/L (fasting) or 1–3 nmol/L (postprandial).[76]

The measurement of C-peptide levels is clinically informative where fasting C-peptide level ≥ 0.2 nmol/L is an indicator of residual β-cell function which is linked with enhanced glucose control, diminished risk of certain diabetes complications such as eye and kidney disease, and a decreased incidence of severe hypoglycaemia (blood glucose level less than 70 mg/dL).[27,74,75] Also, it has been reported that Type 2 diabetic

patients who have fasting C-peptide ≤ 0.2 nmol/l have better control if they use insulin therapy rather than oral treatments.[75,77]

The measurement of C-peptide can be either by measuring stimulated or fasting C-peptide. Stimulated C-peptide is the most frequent measurement for endogenous insulin secretion.[73] Stimulation of C-peptide secretion by either glucose, mixed meal tolerance test (MMTT), glucagon or arginine administration will afford the most sensitive and valid method to assess β-cell function.[73-75,78,79] However, although all of these stimuli have been used in clinical trials, stimulation with MMTT is the most physiologically relevant method of insulin secretion.[74] Although both MMTT and glucagon stimulation tests are highly reproducible, MMTT shows significantly higher levels of C-peptide than the glucagon stimulation test.[78] Also, patients commonly prefer MMTT because of nausea that may occur with the arginine test.[73-75]

The measurement of stimulated C-peptide has limitations such as it is an expensive, time-consuming test, and unacceptable to some patients because it requires overnight fasting. There is also a need to allow 90 minutes to complete the test and postponement of their morning insulin dose. However, in the clinical trial centers measuring fasting C-peptide, it may be easier to obtain, correlates well with stimulated C-peptide, and is more convenient. However, with disease progression, there will be proportionally a greater decrease in stimulated versus basal C-peptide responses. Consequently, measurement of basal or fasting values alone lack the sensitivity of responses to dynamic testing.[73,74]

Generally, C-peptide levels are associated with diabetes type, age at diagnosis, and duration of disease.[75] Unfortunately, there have been relatively few prospective studies that have assessed alterations in C peptide level from prediabetes to the diagnosis of

diabetes. Studies done by Tsai et al. and Sosenko et al. found that there is a progressive deterioration in C-peptide levels that is generally moderate during the pre-diabetic period compared with changes after diagnosis with diabetes. In a study done in 2020, the research group found that in patients with insulin-treated type 2 diabetes, low levels of fasting C-peptide were linked with higher glycaemic variability and risk of hypoglycaemia. They suggested that C-peptide levels should be taken into account when treating type 2 diabetes with insulin treatment and assessing their hypoglycaemic risk.[80]

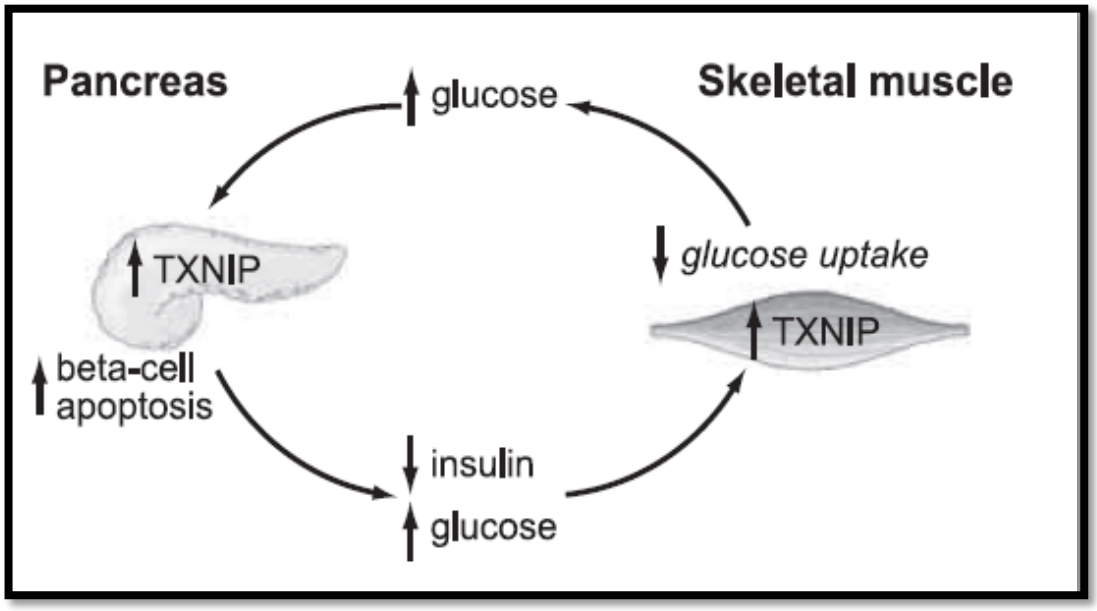
1.12 Reasons for β-cell dysfunction

In the University of Birmingham, Alabama, USA, a research group performed a study that identified thioredoxin-interacting protein (TXNIP) as a critical factor involved in β-cell biology and identified it as the most important controller for β-cell function and insulin production in vivo.[81-83] In diabetic patients, there was an upregulation of TXNIP which was critical for glucotoxicity-induced β-cell death.[82,84,85] Chronic exposure of β-cell to high glucose levels induces glucotoxicity and promotes β-cell apoptosis leading to further worsening of hyperglycaemia.[85,86] Genetic ablation of TXNIP promotes endogenous β-cell survival and avoids development of diabetes by preventing β-cell apoptosis and increasing pancreatic β-cell mass which leads to increased insulin production (anti-diabetic effects).[69,81-84,87-89] Moreover, elevated TXNIP levels also contribute to β-cell dysfunction which in turn leads to inhibition of insulin production.[83,90]

Furthermore, a study investigating the pathways by which TXNIP induces apoptosis found that TXNIP shuttles within the β-cell and translocates from the nucleus to the

mitochondria and initiates an apoptotic cascade.[50] In addition, other studies have consistently shown that TXNIP deficiency protects pancreatic β-cells against oxidative stress, glucose toxicity, and apoptosis [68,82,91] and experimentally has rescued mice from diabetes by preserving β-cell mass and function.[83] A study[70] done to examine the extra-pancreatic effect of TXNIP downregulation found that its downregulation likely increased muscle and adipose glucose uptake and decreased hepatic glucose production. Figure 1.3 show the effects of TXNIP upregulation and glucose toxicity on pancreas and skeletal muscles.[70]

Figure 1. 3: Role of TXNIP in glucose toxicity in β cell and impaired glucose uptake in the periphery.



Reproduced from Parikh et al [70]

1.13 Assessment of insulin resistance

For the assessment of insulin resistance, DeFronzo et al. have considered the euglycaemic clamp method as the gold standard method [92] and the best technique for assessing insulin resistance as it provides a direct measurement of the general body sensitivity to insulin, specifically in skeletal muscle. This technique is an accurate and direct measurement of insulin resistance and can differentiate between peripheral and hepatic insulin resistance.[93] Despite, its usefulness, it is costly and time-consuming.

The first simple method for evaluation of insulin sensitivity is the Homeostatic Model Assessment Insulin Resistance (HOMA-IR). It was first developed by David Matthews et al. in 1985 and has been widely used for the estimation of insulin resistance in clinical settings and research. This method quantifies insulin resistance by calculating insulin sensitivity (%S) and β-cell function (%B) of the pancreas from fasting plasma glucose (FPG) and either fasting insulin or C-peptide concentrations.[94,95]

The HOMA-IR test assesses hepatic rather than peripheral insulin resistance.[93] Since hepatic glucose production (HGP) is the main determinant of FPG concentration, and fasting plasma insulin (FPI) concentration is the main regulator of HGP, the HOMA-IR index is practically a measure of hepatic insulin resistance. A value of less than 2.5 is considered a normal level for HOMA-IR.[94] For easy interpretation, lower HOMA-IR values indicate greater insulin sensitivity, whereas higher HOMA-IR values indicate lower insulin sensitivity.[94,96] There are two main methods of calculating HOMA-IR, the first one is HOMA1 which is the original model.[95-97]

HOMA1-IR = FPI x FPG / 22.5

Where FPG is expressed in mmol/L and FPI is expressed in mmol/L. In this equation, the constant 22.5 should be replaced by 405 if glucose is reported in mg/dL.[97] The problem with the original formula is that it underestimated %S and overestimated % B.[95] A second variant of the equation, called HOMA2 (a computer model) is available as an online calculator.[96,97]

The HOMA2 calculator was released by the University of Oxford, Centre for Diabetes, Endocrinology, and Metabolism in the UK and is considered to be more accurate.[96] The second equation is referred to as the quantitative insulin sensitivity check index (QICKI) which inverse of the sum of the logarithms to base 10 of the FPG and FPI values: QUICKI = 1/ [(log FPG + (log FPI)] where FPI is expressed in mmol/L and FPG is expressed in mg/dL.[94]

The reported values of QUICKI for various populations are; non-diabetic obese (0.331 ± 0.010); non-obese (0.382 ± 0.7) and diabetics (0.304 ± 0.7).[94] Both methods use fasting plasma glucose and insulin levels to quantify insulin resistance and correlate well with the results of the euglycaemic clamp method.[94] A study done in 2000 by Bonora and colleagues showed a strong correlation between HOMA-IR and euglycaemic clamp over different levels of glucose tolerance and insulin sensitivity.[98] Moreover, Shoji et al. showed that HOMA-IR can be an alternative technique to assess resistance to insulin.[99] Quantification of insulin resistance using HOMA-IR is more convenient[100] and considered as the most popular, commonly accepted method of measurement.[97]

1.14 Assessment of Medication Adherence

Adherence to medications has been identified as a key issue for positive health outcomes. According to WHO, adherence is defined as "the extent to which a person's behaviour corresponds with agreed recommendations from a health care provider".[101] The proper evaluation of drug efficacy and assessment of patient adherence is essential to ensure successful diabetes treatment.[102] Primary medication adherence occurs when a patient fills the first prescription for a new medication.[103] Poor medication adherence is very common and leads to diminished effectiveness of treatment, and this can lead to unwanted complications, deterioration in their condition, and increase the risk of death.[101,104-106]

It has been determined that up to 50% of patients with a chronic disease are making medication-related decisions without first seeking medical advice and becoming "non-adherent" with their prescribed medication, compromising potential treatment benefits. Therefore, health providers should routinely assess patient medication adherence.[107]

Although there is no accepted gold standard methodology for assessing medication adherence, numerous methods have been described in the literature.[101,105] Measurement of medication adherence can be done by several methods including self-reporting adherence questionnaires such as the Morisky Medication Adherence Scale, drug level monitoring, and monitoring prescription refill rates.[108] Also, self-reporting is considered the simplest, accurate, and least expensive method, with high sensitivity and specificity of more than 70 %.[108] George et al. reported that the Morisky questionnaire was a valid scale for detecting non–adherence.[109]

Non-adherence to lifelong treatments in chronic diseases such as diabetes and hypertension are a global phenomenon.[75,77,78,82,110] According to WHO, the average adherence rate in developed countries is 50%.[101] This prevalence is consistent with a recent study reported that 48.6% of Saudi patients with type 2 diabetes had good adherence.[111]

There is a positive association between increased adherence rate and a decrease in HbA1c.[112,113] A study found that for each 10% increase in adherence to glucose-lowering drugs, HbA1c significantly decreased by 0.14%.[85] Similarly, another study also reported an average reduction of 0.16% in HbA1c values for each 10% increase in adherence.[86] Also, numerous studies have observed an association between low adherence to antihypertensive medications and uncontrolled hypertension.[109,114-116]

Obstacles that lead to non-adherence may include factors such as complexity of treatment regimens, intolerance to drug-related side effects, long-term multidrug therapy, and insufficient information or directions given to the patient.[117,118] Medication side effects often negatively impact medication adherence. Grant et al. reported that side effects of diabetic and antihypertensive agents were a major reason for medication non-adherence in diabetic patients.[119]

A systematic review of randomized controlled trials showed that pharmacists have a significant role to play in type 2 diabetic patient care by helping patients to increase their adherence level and realise the maximum effectiveness of their medication.[120]

1.15 Management of type 2 diabetes

Screening offers an opportunity to identify people who are required to initiate the treatment as soon as possible to prevent or delay disease progression and minimize potential complications.[121,122] In addition, maintaining blood glucose levels as close as possible to a normal range, without hypoglycaemia, is the optimal goal in the management of type 2 diabetes.[121]

1.15.1 Self-management in diabetes

Type 2 diabetic patients generally have a good understanding of the need for medications but commonly adjust their dosage and administration time according to their daily schedules which leads to a high level of self-management.[101,120,123] In 2016, a systematic review suggested that health care providers should appreciate individual patient preferences and how this impacts the self-management by generating an agreed care plan.[123] In 2017, a review study done in Saudi Arabia, recommended that urgent attention is required to develop, support, and implement health interventions, guidelines, and policies that will help in assisting the prevention, diagnosis, management, and promotion of self-management of diabetes.[13]

1.15.2 Non-pharmacological management

Overcoming the modifiable risk factors for diabetes include undertaking good physical activity, avoiding smoking and following a healthy diet. Adopting these healthy lifestyle approaches provides an opportunity to prevent or delay the onset of diabetes in people with a high risk of diabetes.[27,124] It is recommended that overweight adults with type 2 diabetes should do moderate-to-strong intensity physical activity for at

least 150 min spread over at least 3 days/week (no more than 2 consecutive days without activity) and maintained at least 5% weight loss. Shorter durations (minimum 75 min/week) of intensive physical activity may be sufficient for younger and more physically fit patients.[4]

Many studies consistently identify the benefit of controlling obesity in type 2 diabetes management.[125-129] Weight loss enhances insulin sensitivity in the liver and skeletal muscle tissues[130] and may also decrease pancreatic fat accumulation.[126] Deficits in insulin secretion can sometimes be partially reversible with a healthy diet and weight loss in recently-diagnosed type 2 diabetes.[66,131] Weight management is very important in the management of all type 2 diabetic patients regardless of their disease onset or body mass index (BMI) and can be achieved by appropriate dietary measures supplemented by physical activity, in addition to initiating glucose-lowering agents such as metformin that facilitate weight loss.[125-129,132] In advanced cases of obesity, the use of anti-obesity medications may be required to help patients adhere to a low-calorie diet by minimising hunger and lack of fullness signals that can appear when trying to lose weight. In very advanced obesity cases (BMI $\geq$40 kg/m^2), bariatric surgery can be performed in cases that do not achieve sufficient weight loss and improvement in blood glucose level with nonsurgical methods.[4,22,132,133] Obese type 2 diabetic patients who realise modest and sustained weight loss show an enhanced glycaemic control and reduce their need for anti-diabetic medications.[4,125,129] However, a small number of studies have also shown that extreme dietary energy restriction using very-low-calorie diets (< 800 calories/day) are capable of reducing HbA1c in obese type 2 diabetic patients to less than 6.5% and fasting glucose levels to less than 126 mg/dL without the use of anti-diabetic medications.[127,134,135] Unfortunately, weight loss induced

improvements in blood glucose control are most likely to be realized in newly diagnosed type 2 diabetics where insulin secretory capacity is relatively well preserved and insulin resistance is the primary issue.[125,128,134,136,137]

As part of an overall healthy lifestyle, all type 2 diabetic patients should be advised not to use tobacco products or electronic-cigarettes. In those diabetic patients who do smoke, a routine component of diabetes care should include appropriate counseling, and offering a form of smoking cessation treatment.[4]

1.15.3 Pharmacological management

There are many classes of anti-diabetic medications available to reverse the hyperglycaemia observed in type 2 diabetes. These have different mechanisms of action and target several pathophysiological components of the disease. Many very important considerations should be considered before prescribing these agents including comorbidities, hypoglycaemic risk, possible drug side effects, the potential of a drug to impact on patient's weight, patient preferences, and finally drug cost.[4,129] The currently available anti-diabetic drugs for type 2 diabetes are shown in Table 1.1.[2] In 2011, a study showed favourable combined changes in β-cell function and insulin sensitivity over time with rosiglitazone when used as initial monotherapy in type 2 diabetes.[138] However, according to 2022 ADA recommendations,[1] metformin is the primary drug of choice in the management of type 2 diabetes. Once initiated, metformin should be continued so as long as it is tolerated and not contraindicated. Metformin is contraindicated in cases such as severe renal disease (eGFR < 30ml/min) and acute metabolic acidosis (rare).[1,4] Metformin is generally well tolerated by patients, although upon initiation of metformin gastrointestinal side effects can be

experienced and its long-term use may be linked with vitamin B12 deficiency so periodic measurement of vitamin B12 levels is recommended, especially in patients who also have peripheral neuropathy or anaemia.[1,4]

After initiation of metformin and non-pharmacological therapy, treatment should be considered without delay for type 2 diabetic patients who are not meeting their treatment goals. According to 2022 ADA recommendations the treatment schedules are shown in Figure 1.4.[1]

Table 1. 1: Drug-specific and patient factors to consider when selecting antidiabetic drugs in the adult with type 2 diabetes according to ADA recommendations.

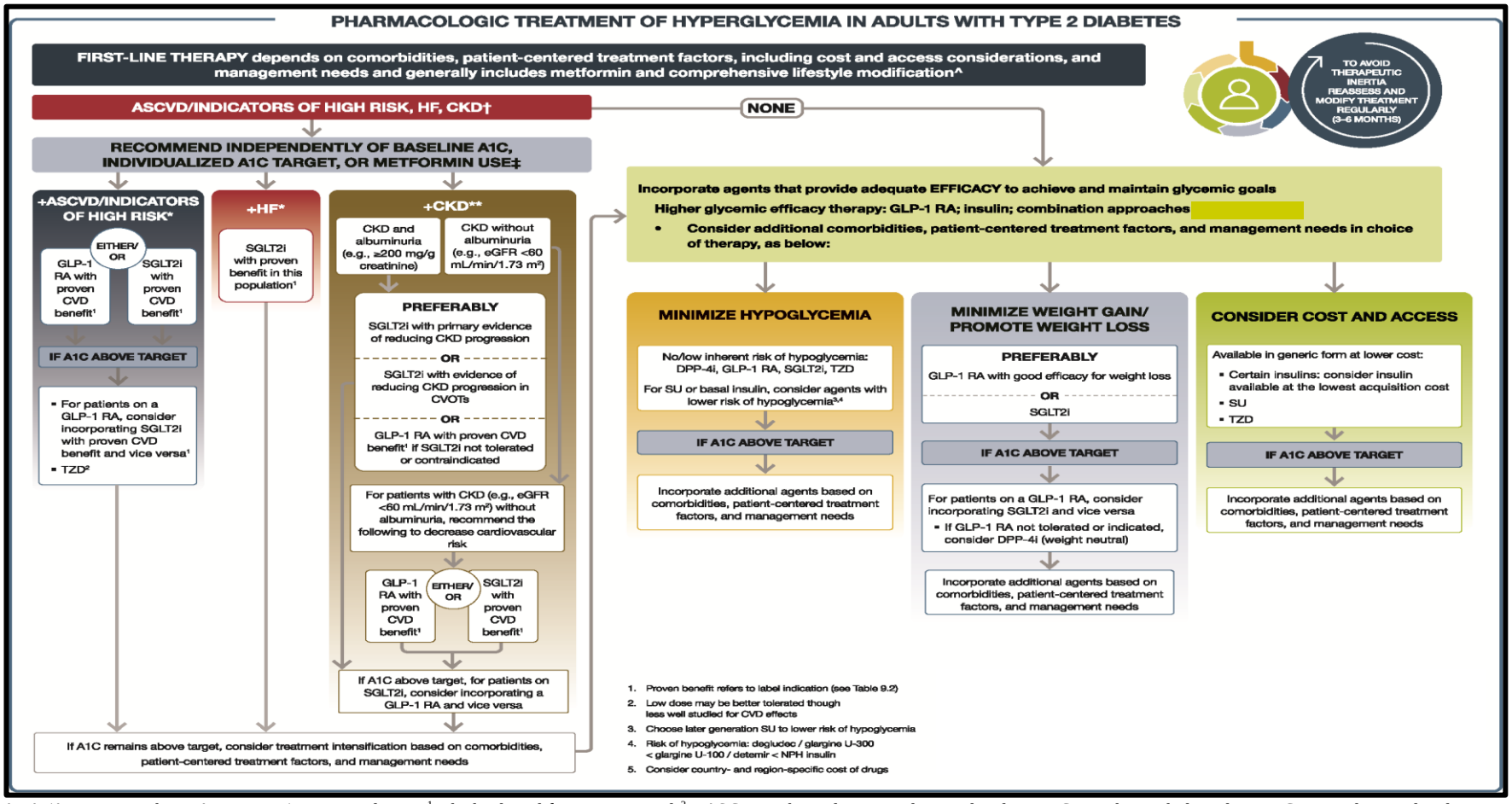
	Efficacy	Hypoglycemia	Weight change	CV effects		Cost	Oral/SQ	Renal effects		Additional considerations
				ASCVD	HF			Progression of DKD	Dosing/use considerations*	
Metformin	High	No	Neutral (potential for modest loss)	Potential benefit	Neutral	Low	Oral	Neutral	▪ Contraindicated with eGFR <30 mL/min/1.73 m^2	▪ Gastrointestinal side effects common (diarrhea, nausea) ▪ Potential for B12 deficiency
SGLT2 inhibitors	Intermediate	No	Loss	Benefit: empagliflozin†, canagliflozin†	Benefit: empagliflozin‡, canagliflozin, dapagliflozin‡, ertugliflozin	High	Oral	Benefit: canagliflozin§, empagliflozin, dapagliflozin§	▪ See labels for renal dose considerations of individual agents ▪ Glucose-lowering effect is lower for SGLT2 inhibitors at lower eGFR	▪ Should be discontinued before any scheduled surgery to avoid potential risk for DKA ▪ DKA risk (all agents, rare in T2D) ▪ Risk of bone fractures (canagliflozin) ▪ Genitourinary infections ▪ Risk of volume depletion, hypotension ▪ ↑LDL cholesterol ▪ Risk of Fournier's gangrene
GLP-1 RAs	High	No	Loss	Benefit: dulaglutide†, liraglutide†, semaglutide (SQ)† Neutral: exenatide once weekly, lixisenatide	Neutral	High	SQ; oral (semaglutide)	Benefit on renal end points in CVOTs, driven by albuminuria outcomes: liraglutide, semaglutide (SQ), dulaglutide	▪ See labels for renal dose considerations of individual agents ▪ No dose adjustment for dulaglutide, liraglutide, semaglutide ▪ Caution when initiating or increasing dose due to potential risk of nausea, vomiting, diarrhea, or dehydration. Monitor renal function in patients reporting severe adverse GI reactions when initiating or increasing dose of therapy.	▪ **FDA Black Box:** Risk of thyroid C-cell tumors in rodents; human relevance not determined **(liraglutide, dulaglutide, exenatide extended release, semaglutide)** ▪ GI side effects common (nausea, vomiting, diarrhea) ▪ Injection site reactions ▪ Pancreatitis has been reported in clinical trials but causality has not been established. Discontinue if pancreatitis is suspected.
DPP-4 inhibitors	Intermediate	No	Neutral	Neutral	Potential risk: saxagliptin	High	Oral	Neutral	▪ Renal dose adjustment required (sitagliptin, saxagliptin, alogliptin); can be used in renal impairment ▪ No dose adjustment required for linagliptin	▪ Pancreatitis has been reported in clinical trials but causality has not been established. Discontinue if pancreatitis is suspected. ▪ Joint pain
Thiazolidinediones	High	No	Gain	Potential benefit: pioglitazone	Increased risk	Low	Oral	Neutral	▪ No dose adjustment required ▪ Generally not recommended in renal impairment due to potential for fluid retention	▪ **FDA Black Box:** Congestive heart failure **(pioglitazone, rosiglitazone)** ▪ Fluid retention (edema; heart failure) ▪ Benefit in NASH ▪ Risk of bone fractures ▪ Bladder cancer (pioglitazone) ▪ ↑LDL cholesterol (rosiglitazone)
Sulfonylureas (2nd generation)	High	Yes	Gain	Neutral	Neutral	Low	Oral	Neutral	▪ Glyburide: generally not recommended in chronic kidney disease ▪ Glipizide and glimepiride: initiate conservatively to avoid hypoglycemia	▪ FDA Special Warning on increased risk of cardiovascular mortality based on studies of an older sulfonylurea (tolbutamide)
Insulin – **Human insulin**	High	Yes	Gain	Neutral	Neutral	Low (SQ)	SQ; inhaled	Neutral	▪ Lower insulin doses required with a decrease in eGFR; titrate per clinical response	▪ Injection site reactions ▪ Higher risk of hypoglycemia with human insulin (NPH or premixed formulations) vs. analogs
Insulin – **Analogs**						High	SQ			

Reproduced from ADA (American Diabetes Association)[1] which adapted from from Davies et al.[2] * For agent-specific dosing recommendations, please refer to the manufacturers' prescribing information. † FDA-approved for CVD benefit. ‡ FDA-approved for HF indication. § FDA-approved for CKD indication. CVOT, cardiovascular outcomes trial; DPP-4, dipeptidyl peptidase 4; GLP-1 RA, glucagon-like peptide 1 receptor agonist; NASH, nonalcoholic steatohepatitis; SQ, subcutaneous; T2D, type 2 diabetes.

In patients who are unresponsive to oral antidiabetic drugs, injectable medications may be used (Figure 1.5).[1] Glucagon-like peptide-1 receptor agonists (GLP-1 RA) are generally recommended over insulin as the first-line agent.[4,132] However, early initiation of insulin should be adopted if there are signs of ongoing catabolism (unexplained patient weight loss), symptoms of hyperglycaemia are present with HbA1c levels greater than 10 %, or blood glucose levels more than 300 mg/dL. Two studies have reported that Type 2 diabetic patients who have no residual C-peptide level (≤ 0.2 nmol/L) will have better control if they use insulin therapy rather than oral antidiabetic drugs.[75,77]

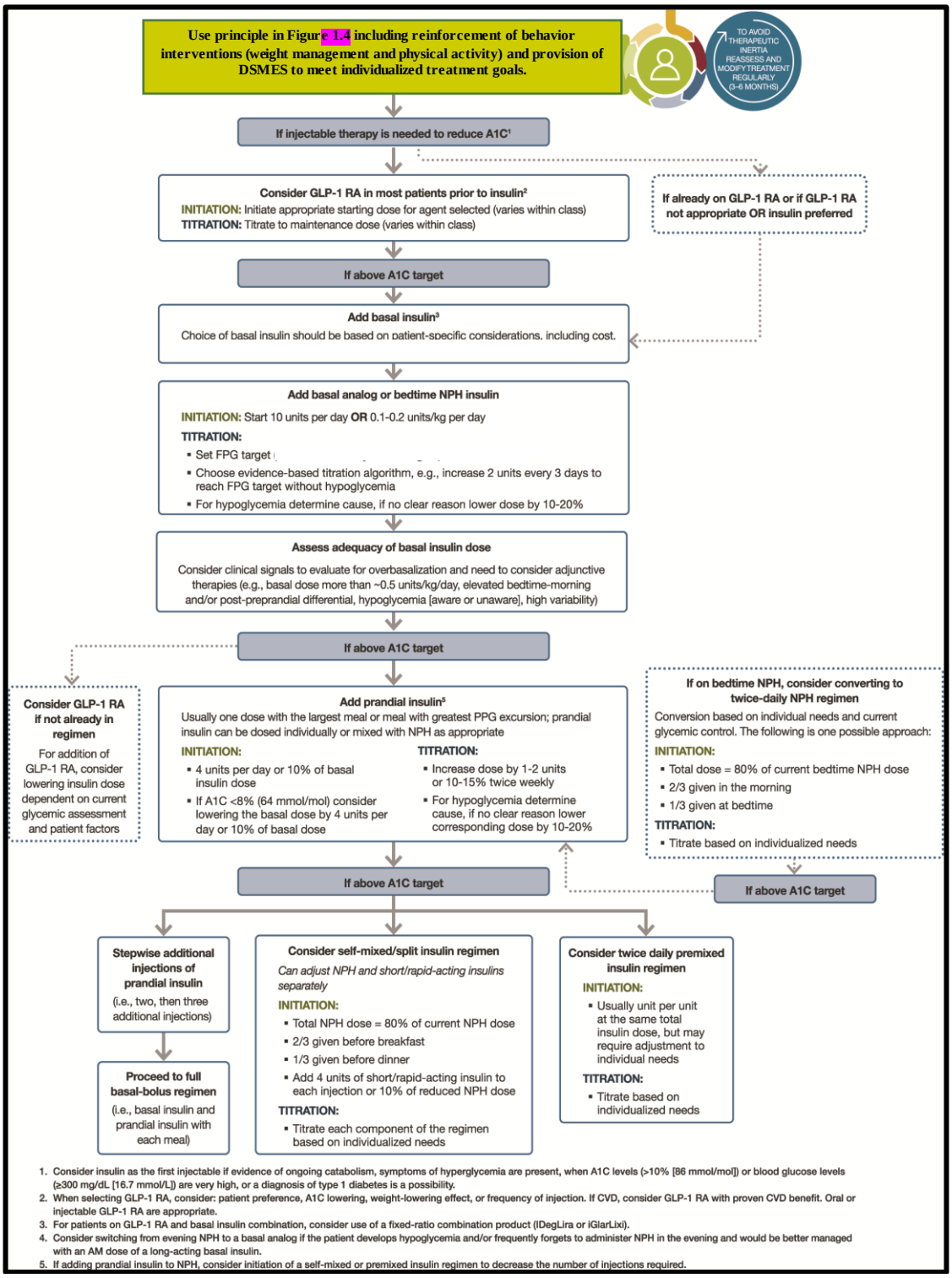
Reevaluation of the medication regimen should be performed at regular intervals, generally, every 3–6 months depending on patient needs, and therapy adjusted according to response.[1,4] Special consideration should be made to type 2 diabetic patients with any renal impairment, cardiovascular disease, or those who are obese.[1,4]

Figure 1. 4: Glucose-lowering medication in type 2 diabetes according to ADA recommendations.



ADA (American Diabetes Association)recommendation [1] which adapted from Davies et al.[2] *ASCVD, atherosclerotic cardiovascular disease; CKD, chronic kidney disease; CVD, cardiovascular disease; CVOTs, cardiovascular outcomes trials; DPP-4i, dipeptidyl peptidase 4 inhibitor; eGFR, estimated glomerular filtration rate; GLP-1 RA, glucagon-like peptide 1 receptor agonist; HF, heart failure; HFrEF, heart failure with reduced ejection fraction; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; SGLT2i, sodium–glucose cotransporter 2 inhibitor; SU, sulfonylurea; T2D, type 2 diabetes; TZD, thiazolidinedione.

Figure 1. 5: Intensifying to injectable therapies in type 2 diabetes according to ADA recommendations.



ADA (American Diabetes Association)[1] which adapted from Davies et al.[2] DSMES, diabetes self-management education and support; FPG, fasting plasma glucose; GLP-1 RA, glucagon-like peptide 1 receptor agonist; max, maximum; PPG, postprandial glucose.

1.15.4 Special considerations in the management of type 2 diabetes

1.15.4.1 Obese patients with type 2 diabetes

A recent meta-analysis of 227 randomized controlled trials of anti-diabetic in type 2 diabetics found that HbA1c changes were not associated with baseline BMI, indicating that obese patients can benefit from the same types of treatments as normal-weight diabetic patients.[139] However, when considering pharmacological treatments for obese patients, whenever possible, glucose-lowering medications that promote weight loss or are weight neutral should be chosen.[1,129] Agents associated with weight loss include metformin, α-glucosidase inhibitors, GLP-1 RA, amylin mimetics, and SGLT2 inhibitors. DPP-4 inhibitors have a neutral effect on body weight but insulin, thiazolidinediones, and sulfonylureas are associated with weight gain.

1.15.4.2 Diabetes patient with kidney impairment

Glucose control must be adapted in these patients since kidney impairment predisposes to hypoglycaemia. Besides, caution should be adopted in monitoring disease progression using HbA1c values in diabetes patients with kidney impairment because the reduction of red blood cell production and survival and the increased destruction of red blood cells may occur which can cause falsely lowered HbA1c results especially in patients who require to undergo renal dialysis.[140]

In 2020, a review regarding Type 2 diabetes management in patients with chronic kidney disease (CKD) conclude that metformin should be the first pharmacological treatment applied.[141] This is consistent with 2022 ADA recommendation [1] which state that metformin should be considered the first-line treatment for all patients with type

2 diabetes, including those with CKD. Estimated glomerular filtration rate (eGFR) should be monitored while taking metformin and the benefits and risks of continuing treatment.[142] Metformin should not be initiated for patients with an eGFR <45 mL/min/1.73m^2.[142] According to the recent recommendation of the national institute for health and care excellence (NICE) [143], in adults with type 2 diabetes that are using metformin, reviewing the dose should be done if the estimated eGFR is below 45 ml/minute/1.73m^2. In addition, metformin drug should be stopped if the eGFR is below 30 ml/minute/1.73m^2.[143]

If HbA1c is above target in patients with established CKD, SGLT2i or GLP-1 RA are the preferred second-line agents.[141,144] SGLT2 inhibitors and GLP-1 RAs should be considered for those who require another drug added to metformin to attain target HbA1c or cannot use or tolerate metformin.[141] In patients with an eGFR ≥30 mL/min/1.73m^2 and urinary albumin >30 mg/g creatinine, the use of SGLT2 inhibitors should be considered to reduce the risk of hypoglycaemia, CKD progression or cardiovascular events.[141,142] In addition, the use of SGLT2 inhibitors e.g. dapagliflozin has also reduced hospitalization due to CKD progression.[1,4,21,144]

Also, in patients with CKD who are at increased risk for cardiovascular events, the use of GLP-1 RA may reduce the risk of progression of albuminuria, cardiovascular events, and hypoglycaemia and appear to possibly slow CKD progression.[141,142]

1.15.4.3 Diabetic patient with cardiovascular diseases

Patients with type 2 diabetes who have established atherosclerotic cardiovascular disease (ASCVD), heart failure, or at high risk of ASCVD should use anti-diabetic medications that have demonstrated benefits in cardiovascular diseases which are

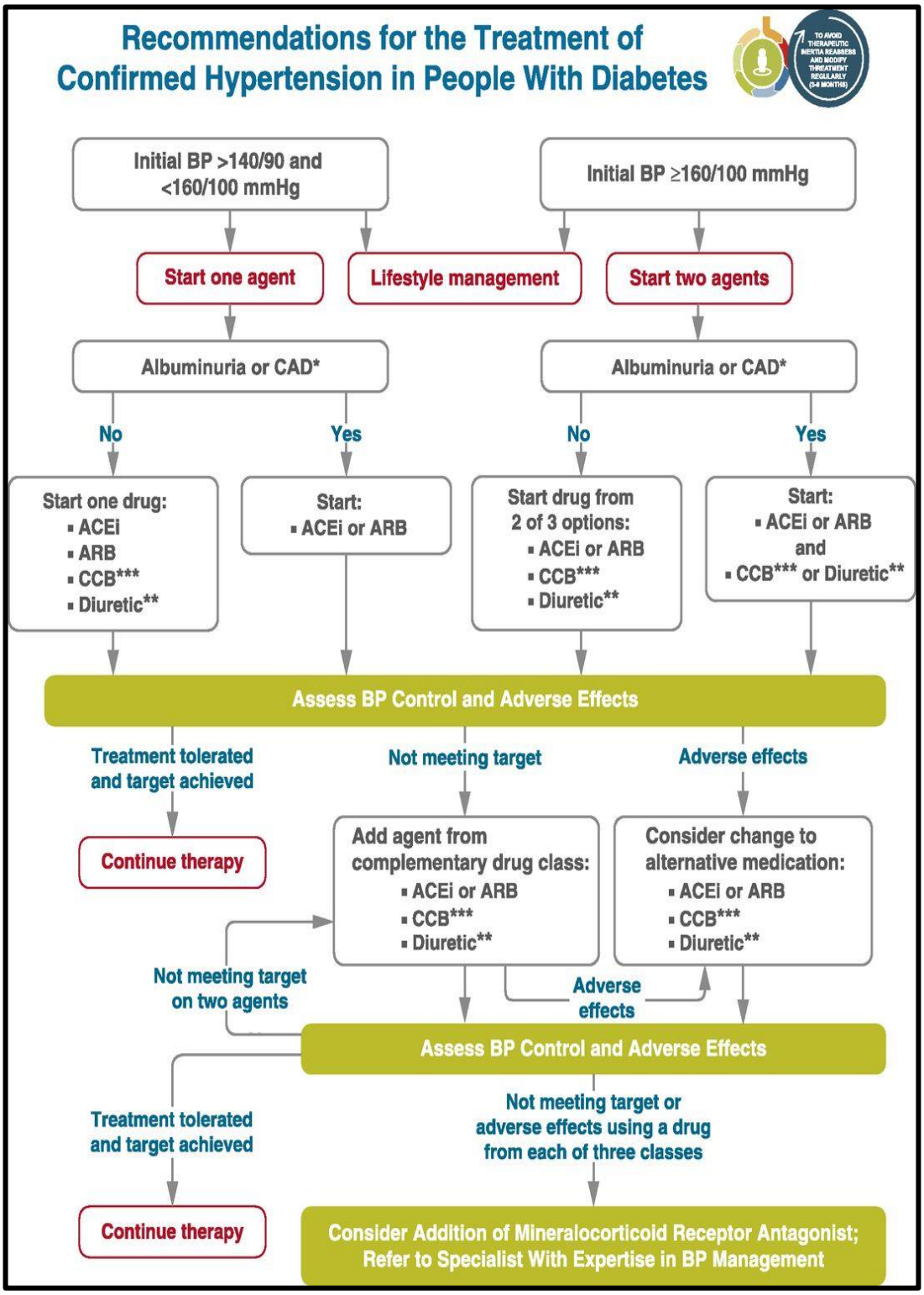
SGLT2 inhibitors and /or GLP-1 RA.[1,4,132,145,146] Among patients with ASCVD at high risk of heart failure or in whom heart failure coexists, an SGLT2 inhibitor is recommended.[132,144-146] The use of SGLT2 inhibitors e.g. dapagliflozin has also reduced hospitalization due to heart failure.[1,4,21,144] Metformin is also considered cardioprotective drugs.[21,141]

1.15.4.4 Diabetes patient with hypertension

All hypertensive patients with diabetes should monitor their blood pressure at home. For patients with diabetes and hypertension, blood pressure targets should be individualized through a shared decision-making process that addresses CVD risk, potential adverse effects of antihypertensive medications (e.g. hypotension, syncope, falls, acute kidney injury, and electrolyte abnormalities), and patient preferences.[4,50] Generally, for patients with diabetes and hypertension who are at higher CVD risk, a blood pressure target of 130/80 mmHg is considered appropriate. While in patients with diabetes and hypertension at lower risk for CVD, a blood pressure target of 140/90 mmHg is considered appropriate. Figure 1.6 provides an algorithm for the treatment of confirmed hypertension in people with diabetes.[49]

Both ADA recommendations[39] and Joint National Committee 8 (JNC 8) [147] are agreed on the target of blood pressure in diabetic patients and the choices of the antihypertensive drugs that recommended for diabetic hypertensive patients. Table 1.2 shows the different lowering effect range of different classes of antihypertensive.[148]

Figure 1. 6: Recommendations for the treatment of confirmed hypertension in people with diabetes.



ADA (American Diabetes Association)[1] which Adapted from de Boer et al[53]. *An angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) is suggested to treat hypertension for patients with coronary artery disease (CAD) or urine albumin-to-creatinine ratio 30–299 mg/g creatinine and strongly recommended for patients with urine albumin-to-creatinine ratio ≥300 mg/g creatinine. **Thiazide-like diuretic; long-acting agents shown to reduce cardiovascular events, such as chlorthalidone and indapamide, are preferred. ***Dihydropyridine calcium channel blocker (CCB). BP, blood pressure.

Table 1. 2: The lowering blood pressure effect of different antihypertensive drug classes

Anti hypertensive drug classes	Lowering blood pressure effect
ACE inhibitors e.g., benazepril, captopril, enalapril, lisinopril, perindopril, ramipril, and trandolapril	The average of BP lowering efficacy is -8 mm Hg for SBP and -5 mm Hg for DBP. [149]
ARBs e.g. candesartan and irbesartan	The average of BP lowering efficacy is -8 mm Hg for SBP and -5 mm Hg for DBP. [150]
α1-blockers e.g., doxazosin, prazosin, and terazosin.	The average of BP lowering is -8 mm Hg for SBP and -5 mm Hg for DBP. [151]
β1-blockers e.g., atenolol, bisoprolol, acebutolol, and metoprolol.	The average of BP lowering efficacy is -8 mm Hg for SBP and -5 mm Hg for DBP. [152]
Calcium channel blockers e.g., verapamil, nifedipine, diltiazem, amlodipine.	The average of BP lowering efficacy is -10 mm Hg for SBP and -7 mm Hg for DBP for dihydropyridines.[153] The average of BP lowering efficacy is -8 mm Hg for SBP and -6 mm Hg for DBP for non-dihydropyridines.[153]
Thiazide diuretics e. g hydrochlorothiazide	The average of BP lowering is -9 mm Hg for SBP and -4 mm Hg for DBP.[154]
Loop diuretics e.g., furosemide	The average of BP lowering is -7.9 mm Hg for SBP and -4.4 mm Hg for DBP.[155]

*BP, blood pressure. SBP, systolic blood pressure. DBP, diastolic blood pressure.

In September 2021, a study[156] done to investigate the effects of five major classes of antihypertensive drugs on the risk of new-onset type 2 diabetes, they found that in comparison to placebo, angiotensin-converting enzyme inhibitors (RR 0·84 [95% CI 0·76–0·93]) and angiotensin II receptor blockers (RR 0·84 [95% CI 0·76–0·92]) reduced the risk of new-onset type 2 diabetes; however, the use of β blockers (RR 1·48 [95% CI 1·27–1·72]) and thiazide diuretics (RR 1·20 [95% CI 1·07–1·35]) increased this risk, and neutral effect was found for calcium channel blockers (RR 1·02 [95% CI 0·92–1·13]).

1.16 Why is there still a need for new management options for type 2 diabetes?

Unfortunately, globally half of diabetic cases (231.9 million of the 463 million adults living with diabetes) are undiagnosed type 2 diabetics.[3,24] In US, one-third of type 2 diabetes cases remain undiagnosed.[157] So once diagnosed with type 2 diabetes, patients have already experienced an aggressive rise in HbA_{1c} and lost almost 80% of β-cell function.[158] So antidiabetic drugs are actually initiated after β-cell mass or function has significantly declined to a critical level when patients already have elevated HbA1c values and experiencing complications, with the potential coexistence of diseases such as hypertension presenting.

Early screening for all patients with risk factors for type 2 diabetes may help in earlier diagnosis and treatment of those patients before complications appear. However, early screening is difficult to perform for all patients at risk of diabetes due to financial and resource limitations within health care systems.[159] Also, many of the current therapeutic drugs available for type 2 diabetic patients come with an increased risk of

hypoglycaemic events, potential weight gain, and significant side effects, highlighted by the fact that some of them have an FDA black box. In addition, combinations of anti-diabetic agents with distinct mechanisms of action are usually required to achieve adequate drug therapy as monotherapy is often unsatisfactory. However, combination therapy can lead to additive side effects and/or may be associated with unwanted drug-drug interactions, inappropriate pharmacokinetics, toxicity, and reduce patient compliance.[160,161] Also, renal and cardiovascular disease commonly coexist with type 2 diabetes and some anti-diabetic medications are completely contraindicated or must be used with caution.[4] According to a systematic review and meta-analysis that incorporated 61 trials, the clinical effects of glucose-lowering agents need time to be visible (reduction of HbA1c levels by between 0.5 to 1.25%, unlikely to fall more than 1.5% on average, details are in Table 1.3) and that is mostly needed between 4 to 6 months after initiation of therapy to reach maximal reduction in HbA1c levels.[26] All of this leads to poor glycaemic control, especially in high risk patients.[22,92,162] Globally, between 40% and 60% of patients have poorly controlled diabetes.[163-166,22,92,162] In 2018, a study found a higher prevalence of poorly controlled diabetes (74.9%) in Saudi patients and the most important risk factors for poor glycaemic control in type 2 diabetics were a family history of diabetes, a longer duration of diabetes, inadequate physical activity, and obesity.[167] Some previous reports have suggested that a high body mass index (BMI) during diabetes treatment is associated with a high rate of decline in insulin secretion capacity in the patient.[168,169] Also, it has been reported that the insulin secretion capacity of patients with type 2 diabetes declines progressively with the duration of diabetes.[170]

Table 1. 3: Percentage of HbA1c reduction anticipated by different oral anti-diabetic classes

Oral anti-diabetic classes		Percentage of HbA1c reduction*
Alpha-glucosidase inhibitors		1%
Biguanides		1%
Dipeptidyl peptidase-4 inhibitors		0.75%
Meglitinides		0.75%
Sulfonylureas		1.25%
Thiazolidinedione	**Rosiglitazone**	1.25%
	Pioglitazone	1%

Adapted from Sherifali D .et.al. [26]

Finally, despite the availability of the current therapeutic agents, morbidity and mortality continue to rise with the associated healthcare costs.[5] So, the need for improved management of diabetes and to help overcome the problem of poor diabetic control is ongoing.[171-180]

1.17 Trials on new type 2 antidiabetic agents

In 2013, a synthetic copy of fibroblast growth factor 21 (FGF21) was suggested to offer protection against obesity and potentially boost the actions of insulin. However, a month-long trial in type 2 diabetic patients found that FGF21 had no statistical effect on body weight, insulin, and blood sugar levels.[173] In 2018, an animal study found that FGF21 is still being considered for potential use in type 2 diabetes as its overproduction in healthy animals fed a standard diet prevented the increase in weight and insulin resistance associated with ageing. However, further studies to examine its safety and efficacy in humans are still required.[181]

In 2015, Buse and his colleagues completed two studies.[171] The first study, a phase 1 study, found that a delayed-release metformin tablet that delivered the drug to the colon had lower systemic bioavailability in comparison to either an immediate-release or extended-release metformin tablet that delivered the drug to the upper small intestine. The second phase 2 study found that the delayed-release was at least as effective as similar doses of metformin extended-release in decreasing blood glucose levels over a 3-month administration period. They attributed the glucose-lowering effect of metformin on a lower bowel-mediated mechanism of metformin action with a resultant decreased risk of lactic acidosis as less risk of systemic metformin accumulation, making it safer in patients with renal impairment. [171]

A 2017 study[172] examining the effects of a daily dose of a low molecular weight protein tyrosine phosphate (LMPTP) inhibitor in obese mice with insulin resistance was performed. The insulin receptor is a protein tyrosine kinase, which after the engagement with insulin, auto-phosphorylates and phosphorylates several downstream targets.[176]

Tyrosine phosphatases that dephosphorylate the insulin receptor are potential therapeutic targets as LMPTP promotes insulin resistance and diabetes through an action on the liver. So, the chemical inhibition of LMPTP activity increases insulin-induced activation of the liver insulin receptors, increased fasting insulin levels, and improves glucose tolerance in obese mice. However, further studies on LMPTP inhibitor to establish safety and efficacy in humans are still required.[172]

In a 2017 review article[178] exploring the effect of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in the management and prevention of Type 2 diabetes found no

clinical data to support their use in type 2 diabetes other than salicylates. Salicylates may be associated with a decline in HbA1c and FPG and can act as a promotor of anti-inflammatory effects and higher levels of insulin. However, the lack of high quality and well-powered comparative clinical trials that are adequately designed prevent solid conclusions to be drawn. Ultimately salicylate use may have limited clinical application in a diabetic population who are at greater risk of cardiac and renal impairment.[178]

Likewise, a 2017 meta-analysis of randomized clinical trials found that there are no significant effects of vitamin D3 supplementation on glycaemic control in type 2 diabetics.[179] However, a more recent 2019 suggested that the vitamin D3 analog, alfacalcidol, could significantly ($P < 0.001$) improve glucose and lipid metabolism in a type 2 diabetic rat model, particularly when combined with metformin.[180] However, further studies to establish alfacalcidol's efficacy in humans as a glucometabolic agent are still required. A 2019 phase 2 study[174] to examine an oral hepato-selective glucokinase activator (TTP399) was associated with a clinically significant and sustained reduction in HbA1c (0.9%) compared to placebo in type 2 diabetes. TTP399 did not cause hypoglycaemia and had no detrimental effect on plasma lipids, liver enzymes, and blood pressure.[174] However, additional studies are still needed to determine the long-term efficacy and safety profile of TTP399.

A small number of human studies have examined the inhibitory effects of calcium channel blockers (CCB) on pancreatic β-cells.[182-187] All first-generation CCB inhibit L-type calcium channels, which are expressed in heart and pancreatic β-cells.[188]

However, a study revealed the unique finding that verapamil in a dose-dependent manner has an inhibitory effect on both the basal activity and the stress-activated

transport activity of GLUT1. GLUT1 transporter is expressed in a wide variety of tissues such as muscle tissue and is largely responsible for a basal level of glucose uptake. Interestingly, verapamil had to be present during the activation phase (glucose deprivation) to be inhibitory. This suggests a direct interaction of verapamil with a component of the activation process. This suggested that verapamil interferes primarily with the activation process rather than with the transporter itself. The inhibition of GLUT1 may be a contributing factor to the hyperglycaemia observed in verapamil toxic dose.[189] However, in verapamil nontoxic dose, animal studies show promising results of verapamil enhancing β-cell survival and function and improving glucose profiles.[67,70,83,188] Limited human studies have hinted at a possible effect of verapamil improving overall glucose profile.[182-187]

1.18 Why Verapamil?

Verapamil, one of the first-generation L-type calcium channel blockers, has been used widely in clinical practice to treat hypertension, cardiac arrhythmias[190], cluster headaches[191], and migraines[192] However, verapamil has not been formally evaluated as a potential blood glucose-lowering agent to treat diabetes. However, animal studies show promising results of verapamil enhancing β-cell survival and function and improving glucose profiles.[67,70,83,188] Limited human studies have hinted at a possible effect of verapamil improving overall glucose profile.[182-187] Therefore, a well design prospective study that aim to study the dual therapeutic potential of verapamil drug in is very important and critical to assess and clarify the ability of verapamil to improve glycometabolic response in addition to its blood pressure control in diabetic hypertensive patients'.

1.19 Reasons for limited information on the effect of verapamil on serum glucose levels

The likely explanation for this is that previous large clinical studies on verapamil primarily focus on cardiovascular outcomes. Also, these studies were performed on patients who may or may not have been diabetic. Additionally, as CCB are not first-line antihypertensive drugs in diabetic patients, the potential signals resulting from effects on glycaemic control may have been overlooked or potentially attributed to a traditional antidiabetic agent and/or lifestyle interventions.[188]

1.20 Diabetic animal models evaluating the potential role of verapamil

A previous animal study that found that TXNIP-deficient mice were protected against diabetes, strongly supporting the role of TXNIP inhibition in the observed glucose-lowering effects of verapamil.[83] In 2012, a study was done to explore the ability of verapamil to downregulate the pro-apoptotic TXNIP marker in a mouse model.[188] The researchers found that verapamil was able to significantly decrease TXNIP mRNA expression in the type 2 diabetic model by more than 70% and improve insulin sensitivity, significantly increase serum insulin levels, and reduced β-cell apoptosis. Interestingly, these effects were dose-dependent and were realised at elevated glucose levels of 11.1 mmol/L but not at normoglycaemic values of 5 mmol/L. Verapamil was well tolerated and no significant side effects were detected.[188] Also, animal studies showed a significant reduction in the risk of cardiac complications in verapamil users

and it was found to be beneficial especially in diabetic cardiomyopathy because of its ability to decrease TXNIP expression and apoptosis in heart tissue.[83,84,193]

The results from the animal studies[83,84,188,193] were promising in terms of the possibilities of translating these findings into human studies to examine the pancreatic and extra-pancreatic effect of verapamil's TXNIP downregulation.

1.21 Human studies for verapamil role in diabetes

In 1991, a single-blinded placebo-controlled crossover study was conducted on 10 patients with type 2 diabetes only receiving a diabetic diet without any antidiabetic drug intervention.[182] Half of the patients received verapamil-SR 240 mg twice daily and half received placebo. After a three-week washout period, patients were crossed over to the alternative treatment and the metabolic study was repeated. After 7 days of treatment, C-peptide and FPG were measured. C-peptide was not significantly different during placebo and verapamil administration but verapamil lowered FPG from a mean value of 209.0 to 185.6 mg/dL; the difference between the two groups was statistically significant (the mean difference was 1.3 mmol/L, P <0.05).

Khodneva and colleagues performed an observational cross-sectional study on middle-aged and older (above 45 years) diabetic adults to examine the association between the use of CCB in general, and verapamil specifically, on FPG levels.[183] They used data from the REasons for Geographic and Racial Differences in Stroke (REGARD) study. The study sample was 4987 adults with diabetes who were enrolled between 2003 and 2007 from the continental United States. The study sample (4987 patients) was included 1484 (29.6% of the study sample) CCB users, of which 174 (3.4% of the study sample) were verapamil users. They found that verapamil users (174 patients)

had an average FPG level that was 9.6 mg/dL (P= 0.03) lower compared with CCB non-users (3494 patients). This, however, was not statistically significant different to controls.[183] They found no statistically significant differences in FPG between verapamil users (15 patients) and non-users (646 patients) among those not concurrently receiving glucose-lowering medications (the mean difference was -1.1 mg/dL, P =0.91). Also, they found no statistically significant differences in FPG between verapamil users (116) and non-users (2049) among those receiving only oral antidiabetic agents (the mean difference was -6.0 mg/dL, P = 0.19).[183] In addition, they found that the difference in FPG between verapamil users (15 patients) and non-users (319 patients) was more pronounced for those only on insulin (mean difference -37.4 mg/dL), but this did not reach statistical significance (P = 0.06). The last subgroup was for verapamil treated participants who received a combination of insulin and oral antidiabetic agents, they found that verapamil significantly affects the FPG in this subgroup, with verapamil users (43 patients) having on average -24.1 mg/dL lower FPG compared to non-users (799 patients) (P = 0.04).

A randomized, double-blind trial was undertaken to compare the effect of combination therapy of non-dihydropyridine calcium channel blocker (verapamil) plus an angiotensin-converting enzyme inhibitor (trandopril) versus β-blocker (atenolol) plus a diuretic (chlorthalidone) on HbA1c in patients with type 2 diabetes and mild-to-moderate hypertension.[186] The study population was a total of 463 hypertensive outpatients with non–insulin-treated type 2 diabetes with age ranging from 40-80 years on stable anti-diabetic therapy for at least 3 months before enrolment. Patients were randomly treated with fixed combinations of either 180 mg verapamil SR plus 1 mg Trandolapril or 50 mg atenolol plus 12.5 mg chlorthalidone once daily each following

a two-week washout period. The main outcome measures were HbA1c, FPG, and systolic and diastolic blood pressure. After 20 weeks, they found that HbA1c (primary outcome measurements) was stable at 7.9% after administration of verapamil SR plus trandolapril and increased from 7.8% to 8.6% with atenolol plus chlorthalidone. Actually, increasing in HbA1c values in the atenolol plus chlorthalidone was unsurprising as this follows known pharmacological effect on glucose profile. The difference of HbA1c became significant after 4 weeks and remained significant after 20 weeks of treatment where it reached 0.8% (P= 0.0001).[186] Also, the secondary outcomes measurements were assessed after 20 weeks. They found a significant mean difference in blood pressure in systolic blood pressure (4.85 mmHg, P=0.001), diastolic blood pressure (1.79 mmHg, P=0.0222), and FPG (-1.17mmol/L, P= 0.0001). A prospective, randomised, double-blind, parallel controlled trial was carried out in 11 Spanish hospitals.[194] A total of 103 type 2 diabetic patients with a mean age 54.9±9.3 years (23.5 % patients on insulin) with uncontrolled blood pressure on monotherapy antihypertensive drugs were recruited. Patients were randomised to treatment groups: verapamil SR/trandolapril 180/2 mg (VT) or to enalapril/hydrochlorothiazide 20/12.5 mg (EH) for 6 months. All patients were counselled to maintain the same antidiabetic therapy throughout the study. HbA1C was not modified on VT: baseline, 5.91±1.43%; end of treatment, 5.94 ±1.62%, but increased on EH: baseline, 5.96 ±1.25%; final, 6.41 ±1.51%, (ANOVA interaction P=0.040). The mean blood glucose changed from 143±55 mg/dL to 119 ±53 mg/dL in the VT group and from 133± 34 mg/dL to 132± 42 mg/dL in the EH group (ANOVA, P=0.018). Overall BP was significantly reduced from 157.3 ±12.0/98.3±6.4 mm Hg to 140.5 ±14.5/86.1 ± 8.2 mm Hg (P< 0.1) and albuminuria significantly decreased from 508.6±693.8 mg/24 hours to 253.4± 517.2

mg/24 hours ($P < 0.1$), both without significant differences between treatments. They conclude that the combination verapamil/trandolapril seems to permit a better metabolic control than enalapril/hydrochlorothiazide.

A study done by Rubio et.al was performed to examine the effect of using a fixed-dose trandolapril (2mg)-verapamil (180mg) versus trandolapril (2mg) alone on type 2 diabetes normotensive patients with proteinuria.[195] Sixty patients were randomly assigned to each group. Patients in both groups were treated for 6 months with monthly evaluation. Baseline FPG levels were comparable between groups but final FPG assessments were observed to be significantly lower in the fixed dose trandolapril-verapamil group (139 ±19 mg/dL) compared with the trandolapril group (154 ± 22 mg/dL; $P < 0.001$).

In 2019, the first systemic review critically examining all relevant human studies to assess whether verapamil-based treatment was associated with improved glycometabolic control in patients with type 2 diabetes. The review indicated that plasma glucose levels were lowered significantly by verapamil-based treatment in patients with type 2 diabetes (mean change -13 ± 5.29 mg/dL; $P = 0.049$); HbA1c values were instead not affected by the verapamil use (mean change - 0.10 ± 0.12%; $P = 0.453$).[196]

A recent randomized, double blind, placebo-controlled study [197] done by Malayeri et. al. and includes non-insulin type 2 diabetic patients who were only on two oral antidiabetic medications (sitaglibtin and metformin). Malayeri et. al study aimed to evaluate the efficacy and safety of oral verapamil administration in 44 patients between 40 and 67 years and were diagnosed with diabetes for at least 5 years. In their study, patients were randomized to either 120 mg verapamil -SR (120mg) or placebo and the

result showed a significant reduction in HbA1c mean level in non-insulin user patients receiving 120 mg verapamil of 0.5% after 3 months (P=0.047).[197]

To the best of our knowledge, Malayeri.et.al.[197] are the first group to prospectively investigate the gene expression of TXNIP and GLP1R mRNA in type 2 diabetes patients. They found that by the end of the study (after 3 months), TXNIP gene expression and GLP1R mRNA had no significant difference from baseline (P>0.05).

In 2018, a Phase 2 clinical trial [198] in adult patients with recent-onset type 1 diabetes, explored the addition of oral verapamil (titrated over the first three months from 120 mg to 360 mg once daily dose) or placebo over a total of 12 months to their insulin therapy on glycaemic control. They found that verapamil treatment, compared with the placebo, significantly improved mixed-meal-stimulated C-peptide values (P = 0.0186). They also found that both verapamil and control groups were maintained excellent glycaemic control throughout the trial, as demonstrated by an average HbA1c measurement between 6 and 7%, there was a nonsignificant trend toward a lower HbA1c in the verapamil group (P = 0.083). Also, verapamil treatment was well tolerated and not associated with any clinically significant adverse events. They concluded that verapamil may be a safe and effective novel approach to improve endogenous β-cell function and diminish insulin dose requirements and hypoglycaemic occurrences in adult individuals with recent-onset type 1 diabetes.[198]

Other studies have had different endpoints where they aimed to assess the incidence of type 2 diabetes in patients using verapamil versus non-user. The international verapamil SR-trandolapril (INVEST) study was performed to examine predictors of type 2 diabetes development.[185] A total of 16,176 patients aged 50 years and older without diabetes at entry were investigated for newly diagnosed diabetes during

follow-up. They compared verapamil SR and trandolapril combination therapy versus atenolol and hydrochlorothiazide combination therapy on controlling blood pressure in patients with clinically stable coronary artery disease. At the 24-month follow-up, participants treated with verapamil and trandolapril combination therapy were less likely to develop diabetes than those treated with the atenolol and hydrochlorothiazide combination therapy (7.0% vs 8.2%, hazard ratio 0.85, p <0.01).

Also, Yin et al undertook a retrospective study[187] to compared the incidence of type 2 diabetes in adults prescribed oral verapamil using a population-based cohort study utilising Taiwan's National Health Insurance Research Database from 2000 to 2011. The study enrolled 4930 patients in the verapamil cohort and the same number in matched cohort (using other CCBs). During follow-up periods 340 of 4930 patients in the matched cohort and 260 patients in the verapamil cohort developed type 2 diabetes. The incidence rates were 6.41 and 8.07 per 1000 population per year among verapamil and other CCB users, respectively. The adjusted hazard ratio (HR) for type 2 diabetes associated with the use of verapamil versus other CCBs was significant (0.80 [95% confidence interval, 0.68 to 0.94; P = 0.006]).

1.22 Conclusion of literature

Diabetes is one of the most common chronic diseases and carries a significant disease burden for the individual, for society and is projected to increase at an alarming rate.[4,5,23,199,200] Current glucose-lowering drugs provide inadequate blood glucose control[22,92,162-166] The common comorbidity of hypertension can be readily treated using verapamil.[190,201,202] Limited animal studies show promising results of verapamil in enhancing β-cell survival and function and improving glucose profiles.[67,70,83,188]

The available human studies have hinted at a possible effect of verapamil improving overall glucose profile.[182-187] However, the role of verapamil remains unclear due to variability in sample size and study design. The proposed Ph.D. research aims to further explore the role of verapamil in type 2 diabetes and to address some of the remaining unanswered.

1.23 Study Rational

Globally, type 2 diabetes is one of the most common chronic diseases and at present life style changes and antidiabetic medications are the only method to minimize and control disease progression. The available human studies are very limited. These studies have shown or suggested a possible effect of the antihypertensive verapamil on improving patient glycaemic control. The opportunity to examine the effects of verapamil in type 2 diabetic mellitus patients by assessing endogenous insulin secretion and/or insulin sensitivity underpinned the rationale of the study.

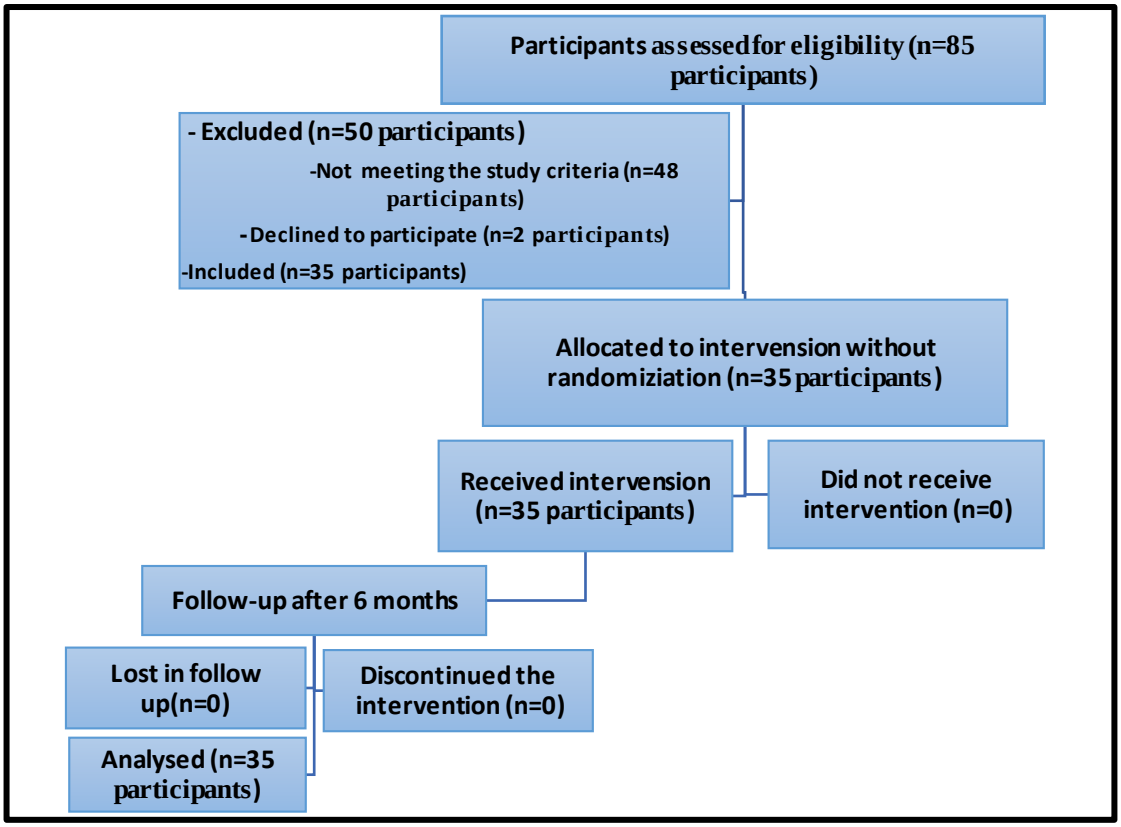
Chapter 2

2. Research Methods

2.1 Summary of clinical trial design

The clinical trial is an open, uncontrolled study conducted at a single center trial site. It recruited thirty-five hypertensive type 2 diabetic patients (16 males, 19 females), mean age 57.2 ± 7.7 years, to receive oral verapamil therapy in the form of a sustained-release (SR) tablet (ISOPTIN-SR™). All patients were initiated on 120 mg verapamil SR (half a tablet) once daily and dose was adjusted to the desired therapeutic response and maintained at that dose for the remainder of the 6-month trial period. An overview of the trial process is shown in Figure 2.1.

Figure 2. 1: Flow chart of trial process



2.2Research Objectives

The research objective was to evaluate the effect of verapamil on glycaemic control in a hypertensive type 2 diabetic population.

2.3 Hypothesis

2.3.1 Null Hypothesis

Using verapamil in type 2 diabetic patients with hypertension will not affect the enhancement of endogenous insulin secretion and/or insulin sensitivity .

2.3.2 Alternative hypotheses

Using verapamil in a hypertensive type 2 diabetic patients will affect the enhancement of endogenous insulin secretion and/or insulin sensitivity.

2.4 Research methodology relating to clinical trial

This study was conducted at the King Fahad Medical City, Riyadh, KSA and recruited 35 type 2 diabetic subjects with hypertension. Subjects were recruited to receive oral verapamil therapy in the form of a slow-release (SR) 240 mg tablet (ISOPTIN SR®). Patients were initiated on 120 mg verapamil SR (half a tablet) once daily and dose was adjusted to the desired therapeutic response and maintained at that dose for the remainder of the 24-week trial period. The trial patients recruitment starting from (December 2016) with the trial officially ending on (December 2019). The recruited patients were followed for 24 weeks after verapamil intervention. A descriptive outline of the study can be found in Table 2.1 below.

Table 2. 1: Descriptive outline of the study

Descriptive information	
Study Type	Interventional Quasi Experimental study
Study Phase	Phase 2
Study Design	Open, uncontrolled
Study Setting	Cardiology clinics at King Fahd Medical City
Condition	Adult Type 2 diabetic patients with uncontrolled hypertension
Study Duration	24 weeks
Intervention	Verapamil 120,240, or 360 mg SR tablet (ISOPTIN SR) depending upon individual patient need.

2.4.1 Eligibility criteria

2.4.1.1 Inclusion criteria

1. Patients with Type 2 diabetes based on 2016 ADA Criteria as discussed in chapter 1. [39]
2. Uncontrolled hypertensive in diabetic patients > 140/90 mm Hg based on 2016 ADA Criteria as discussed in chapter 1. [39]
3. Male or female gender.
4. ≥ 18 years of age.
5. Body mass index (BMI) > 18.
6. Females and males of reproductive potential, willing to use medically acceptable birth control until 3 months after completion of treatment period.
7. Signing the written informed consent.

2.4.1.2 Exclusion criteria

1. Patients involved in any other experimental study.
2. Patients on less than 12 months of insulin therapy initiation.
3. Pregnant females or lactating females who intend to provide their own breast milk to the baby during the study.
4. Cardiac medical conditions that, in the opinion of the investigator, would interfere with safe completion of the trial:
    - Uncompensated heart failure, fluid overload, myocardial infarction or evidence of ischemic heart disease or left ventricular dysfunction; hypotension (systolic pressure <90 mm Hg); PR interval prolongation on electrocardiogram (ECG) or bradyarrhythmia (e.g., sick sinus syndrome, AV block); and atrial flutter or fibrillation within the 12 weeks before intervention.
5. Non-cardiac medical condition that, in the opinion of the investigator, would interfere with safe completion of the trial such as secondary hypertension, resistant hypertension, history of epilepsy, cancer, cystic fibrosis, sickle cell anaemia, diabetes secondary to pancreatic disease, untreated hypothyroidism or active Graves' disease with hyperthyroidism, evidence of active infection, advanced or end stage organ failure, and a psychiatric or medical disorder that would prevent giving informed consent.
6. Pre-exciting medications that the patient on which interacting adversely with verapamil or contraindicated to be taken together.

2.4.2 Data collection procedure

The effect of verapamil on blood glucose profile will be tested prospectively.

2.4.2.1 Baseline visit

- Type 2 diabetic patients who are having uncontrolled hypertension will be included in the baseline examinations.
- Their demographical data will be collected during the baseline visit.
- Patients' habits will be recorded (smoking, exercise and diet)
- General baseline examination including temperature, heart rate, blood pressure.
- Patients have to be fasted and able to comply with special instructions before the baseline lab tests. Patients will be asked to return on the second day for a fasted blood sample before starting verapamil (details in Figure 2.2).

2.4.2.1.1 Data collection sheets for base line data

Part I: Medical record number, patient code, age, sex, nationality, education, employment, marital status, smoking, exercise, and diet habits.

Part II: Vital signs, BMI, weight, height, blood pressure, history of dyslipidemia, information about diabetes (type of diabetes, duration of diabetes, antidiabetic medication, history of ketoacidosis, and history of any diabetic complication), and laboratory tests clinical assessment (details about part 1 and 2 are in Figure 2.3).

2.4.2.2 Intervention plan

- After providing informed consent and completion of screening baseline assessments, 35 subjects fitted the inclusion criteria and received a starting oral dose of 120 mg verapamil daily. This dose was adjusted to the desired therapeutic response and maintained at that dose for the remainder of the 24-week trial.
- The medical physician ordered the following baseline laboratory tests (FPG, HbA1c, fasting C-peptide, lipid profile, renal profile (including eGFR) and liver profile) before verapamil was commenced.
    - Estimation for glomerular filtration rate (eGFR) was done using widely-used equations for estimating GFR from serum creatinine which is the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation[203].
        - $eGFR = 141 \times \min(Scr/\kappa, 1)\ \alpha \times \max(Scr/\kappa, 1)\text{-}1.209 \times 0.993^{Age} \times 1.018$ [if female] × 1.159 [if African American]
            - where:
                - Scr is serum creatinine in mg/dL,
                - κ is 0.7 for females and 0.9 for males,
                - α is -0.329 for females and -0.411 for males,
                - min indicates the minimum of Scr/κ or 1
                - max indicates the maximum of Scr/κ or 1
- During the treatment period, subjects were contacted weekly via telephone for an assessment of adverse events, concomitant medications, diabetes management habits/lifestyle and study product compliance. Patients were

also allowed to contact the investigator as needed during the study duration.

2.4.2.3 Follow up visits

- At follow up visit 1 at week 12 (monitoring visit) subjects returned to the study site to have their medication compliance monitored, blood pressure and pulse assessment, clinical and adverse events assessment, concomitant medications assessment and repeat of some laboratory tests (lipid profile, renal profile (including eGFR) and liver profile).

- At follow up visit 2 at week 24 (end of study visit) subjects returned to the study site for repeat all of the baseline laboratory tests, assessment blood pressure and pulse assessments, medication compliance assessment, clinical and adverse events assessment, and concomitant medications assessment.

2.4.2.3.1 Data collection sheets for the follow up visits

1. General follow up data sheets included the following (details in Figure 2.4):

Part I: Patient's code, age, education, employment, marital status, smoking, exercise, diet habits.

Part II: Vital sign, BMI, weight, height, blood pressure, history of dyslipidemia, information about diabetes (type of diabetes, duration of diabetes, anti-diabetic medication, history of ketoacidosis, and history of any diabetic complication), adherence assessment score, current verapamil dose (if the patients were on 120mg, 240mg, or 360 mg), and laboratory tests clinical assessment.

2. Adherence sheet (Figure 2. 5): Assessing the adherence using the translated Arabic version of the Morisky Medication Adherence Scale (MMAS-8) after taking the permission.[204]

    - It is an 8-item scale, items 1–7 were recorded as 'yes/no' dichotomous responses (scored 0/1) and item 8 was recorded using a 5-point Likert scale (never/rarely scored 1, other responses scored 0). Thus, the total score of the 8-item scale ranges from 0 to 8 and a total score of 8 was considered to represent high adherence and scores 6 or 7 were considered as average adherence where less than 6 is considered poor adherence.

3. Data sheet for monitoring verapamil safety/side effect (Figure 2. 6).

Figure 2. 2: Special instruction before measuring C-peptide level

تحضير المريض اختبار البيتيد سي (C-peptide test)

على المريض ايقاف بعض الادويه الادويه الحافضة للسكر التي قد تتدخل في نتيجة الفحص وعليه اتباع الاتي:

الدواء	الاحتياطات الواجب اتخاذها للقيام بالتحليل
☐ الانسولين قصير المدى	لا ياخذ الجرعة الصباحيه و يجب ان يكون العشاء سلطة ولا يحتوى على نشويات . ويصوم المريض بعد العشاء 14 ساعة الى وقت التحليل في الصباح و يستطيع ان يشرب الماء فقط خلال فترة صومه. وبعد التحليل يستطيع المريض ان ياخذ فطوره وجرعة الإنسولين كالمعتاد.
☐ الانسولين متوسط المدى	
☐ الانسولين طويل المدى	**1) اذا كان المريض ياخذ جرعة الانسولين طويل المدى في المساء :** لا ياخذ الجرعة المسائيه و يجب ان يكون العشاء سلطة ولا يحتوى على نشويات . ويصوم المريض بعد العشاء 14 ساعة الى وقت التحليل في الصباح و يستطيع ان يشرب الماء فقط خلال فترة صومه. وبعد التحليل يستطيع المريض ان ياخذ جرعة الإنسولين طويل المدى. **2) اذا كان المريض ياخذ جرعة الانسولين طويل المدى في الصباح :** لا ياخذ الجرعة الصباحيه و يجب ان يكون العشاء سلطة ولا يحتوى على نشويات . ويصوم المريض بعد العشاء 14 ساعة الى وقت التحليل في الصباح و يستطيع ان يشرب الماء فقط خلال فترة صومه. وبعد التحليل يستطيع المريض ان ياخذ جرعة الإنسولين طويل المدى.
ملاحظة هامه جدا: للمرضى الذين يستخدمون الانسولين بجميع انواعه: 1) اذا شعر المريض باعراض هبوط فعليه ان يقطع صيامه ويتعامل مع هبوط معدل السكر كالمعتاد . وعليه فقط اعلامنا بذلك وسيتم التواصل معه لمساعدته في تنظيم معدل السكر لديه و لترتيب موعد اخر. 2) ان يراقب المريض قراءة السكر في الدم خصوصا قبل النوم فإذا تجاوزت 250 mg/dl فعليه ان ياخذ دواءه كالمعتاد ولا يحضر للفحص في الصباح فقط عليه اعلامنا بذلك وسيتم التواصل معه لمساعدته في تنظيم معدل السكر لديه و لترتيب موعد اخر.	
الادويه الفمويه الحافضة للسكر	
• Metformin (Glucophage)	لا تاخذ الجرعة المخصصة لوجبة العشاء و يجب ان يكون العشاء سلطة ولا يحتوى على نشويات . ويصوم بعد العشاء 14 ساعة الى وقت التحليل و يستطيع ان يشرب الماء خلال فترة صومه. وبعد التحليل يستطيع ان يستخدم علاجه كالمعتاد.
• Gliclazide (Diamicron)	يجب على المريض يجب ان يكون العشاء سلطة ولا يحتوى على نشويات . ويصوم بعد العشاء 14 ساعة الى وقت التحليل و يستطيع ان يشرب الماء خلال فترة صومه وبعد التحليل يتسطيع المريض ان ياخذ فطوره وجرعة الدواءالصباحيه كالمعتاد.وعلى المريض مراعاة الاتي: 1) اذا كان المريض ياخذ جرعته في المساء فعليه ان لاياخذ جرعة الليل في الليلة التي تسبق يوم التحليل. 2) واذا كان المريض ياخذ جرعته في الصباح فعليه ان لاياخذ جرعة صباح في يوم التحليل.
• Glimepiride (Amaryl)	

اسم المريض:

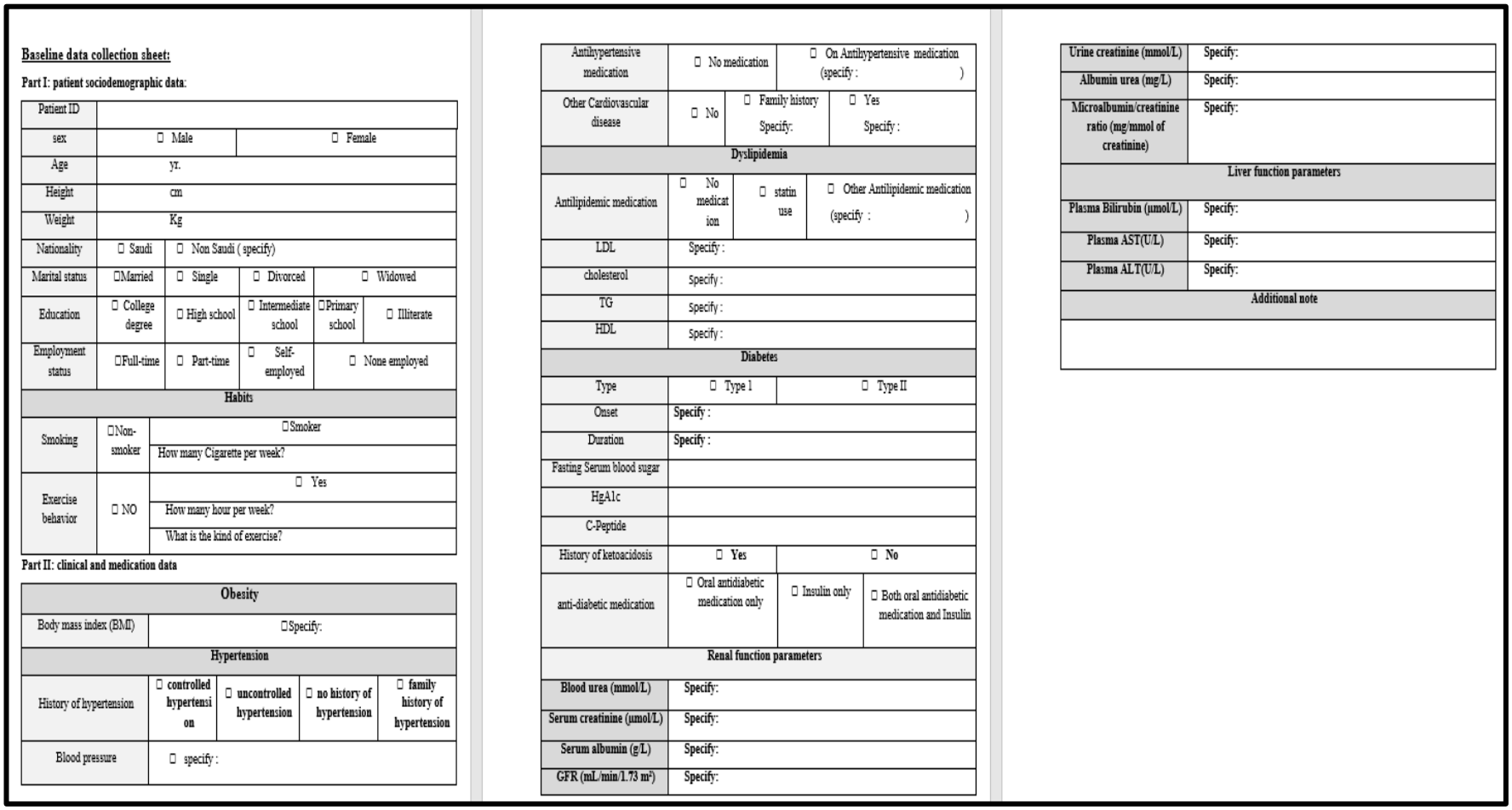
توقيع المريض:

التاريخ:

ختم العيادة

`

Figure 2. 3: Base line data collection sheet



Baseline data collection sheet:

Part I: patient sociodemographic data:

Patient ID					
sex	☐ Male	☐ Female			
Age	yr.				
Height	cm				
Weight	Kg				
Nationality	☐ Saudi	☐ Non Saudi (specify)			
Marital status	☐Married	☐ Single	☐ Divorced	☐ Widowed	
Education	☐ College degree	☐ High school	☐ Intermediate school	☐Primary school	☐ Illiterate
Employment status	☐Full-time	☐ Part-time	☐ Self-employed	☐ None employed	
Habits					
Smoking	☐Non-smoker	☐Smoker			
		How many Cigarette per week?			
Exercise behavior	☐ NO	☐ Yes			
		How many hour per week?			
		What is the kind of exercise?			

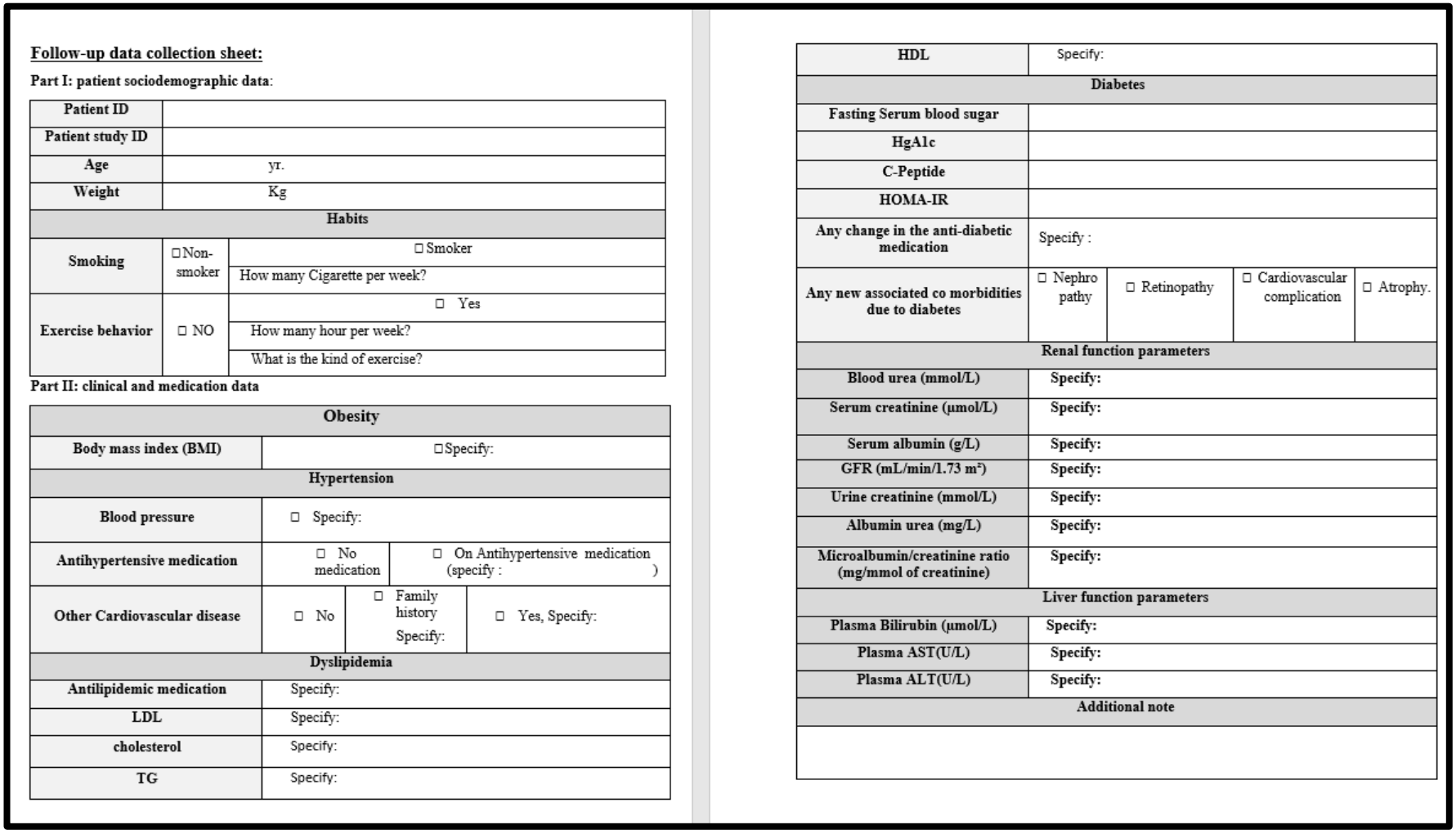
Part II: clinical and medication data

Obesity				
Body mass index (BMI)	☐Specify:			
Hypertension				
History of hypertension	☐ **controlled hypertension**	☐ **uncontrolled hypertension**	☐ **no history of hypertension**	☐ **family history of hypertension**
Blood pressure	☐ specify :			

Antihypertensive medication	☐ No medication	☐ On Antihypertensive medication (specify :)	
Other Cardiovascular disease	☐ No	☐ Family history Specify:	☐ Yes Specify :
Dyslipidemia			
Antilipidemic medication	☐ No medication	☐ statin use	☐ Other Antilipidemic medication (specify :)
LDL	Specify :		
cholesterol	Specify :		
TG	Specify :		
HDL	Specify :		
Diabetes			
Type	☐ Type 1	☐ Type II	
Onset	**Specify :**		
Duration	**Specify :**		
Fasting Serum blood sugar			
HgA1c			
C-Peptide			
History of ketoacidosis	☐ **Yes**	☐ **No**	
anti-diabetic medication	☐ Oral antidiabetic medication only	☐ Insulin only	☐ Both oral antidiabetic medication and Insulin
Renal function parameters			
Blood urea (mmol/L)	**Specify:**		
Serum creatinine (µmol/L)	**Specify:**		
Serum albumin (g/L)	**Specify:**		
GFR (mL/min/1.73 m²)	**Specify:**		

Urine creatinine (mmol/L)	**Specify:**
Albumin urea (mg/L)	**Specify:**
Microalbumin/creatinine ratio (mg/mmol of creatinine)	**Specify:**
Liver function parameters	
Plasma Bilirubin (µmol/L)	**Specify:**
Plasma AST(U/L)	**Specify:**
Plasma ALT(U/L)	**Specify:**
Additional note	

Figure 2. 4: Follow-up data collection sheet



Follow-up data collection sheet:

Part I: patient sociodemographic data:

Patient ID		
Patient study ID		
Age	yr.	
Weight	Kg	
Habits		
Smoking	☐Non-smoker	☐ Smoker
		How many Cigarette per week?
Exercise behavior	☐ NO	☐ Yes
		How many hour per week?
		What is the kind of exercise?

Part II: clinical and medication data

Obesity			
Body mass index (BMI)	☐Specify:		
Hypertension			
Blood pressure	☐ Specify:		
Antihypertensive medication	☐ No medication	☐ On Antihypertensive medication (specify :)	
Other Cardiovascular disease	☐ No	☐ Family history Specify:	☐ Yes, Specify:
Dyslipidemia			
Antilipidemic medication	Specify:		
LDL	Specify:		
cholesterol	Specify:		
TG	Specify:		

HDL	Specify:			
Diabetes				
Fasting Serum blood sugar				
HgA1c				
C-Peptide				
HOMA-IR				
Any change in the anti-diabetic medication	Specify :			
Any new associated co morbidities due to diabetes	☐ Nephro pathy	☐ Retinopathy	☐ Cardiovascular complication	☐ Atrophy.
Renal function parameters				
Blood urea (mmol/L)	**Specify:**			
Serum creatinine (µmol/L)	**Specify:**			
Serum albumin (g/L)	**Specify:**			
GFR (mL/min/1.73 m²)	**Specify:**			
Urine creatinine (mmol/L)	**Specify:**			
Albumin urea (mg/L)	**Specify:**			
Microalbumin/creatinine ratio (mg/mmol of creatinine)	**Specify:**			
Liver function parameters				
Plasma Bilirubin (µmol/L)	**Specify:**			
Plasma AST(U/L)	**Specify:**			
Plasma ALT(U/L)	**Specify:**			
Additional note				

Figure 2. 5: The translated Arabic version of the Morisky Medication Adherence Scale (MMAS-8)

Arabic version of the Morisky Medication Adherence Scale (MMAS-8)

		نعم	لا
1	هل تنسى احيانا تناول الدواء؟		
2	ينسى الناس احيانا تناول ادويتهم لاسباب اخرى غير النسيان. هل تتذكر خلال الاسبوعين الاخيرانك نسيت الدواء في اي يوم؟		
3	هل انقطعت وتوقفت عن تناول الدواء دون ان تخبر طبيبك لانك شعرت ان حالتك تسوء اكثر اذا تناولت الدواء؟		
4	حين تسافر اوتغادر المنزل هل تنسى احيانا اخذ الدواء؟		
5	هل تناولت الدواء بالامس؟		
6	حين تشعر ان اعراض المرض عندك قد اصبحت تحت السيطرة هل تتوقف احيانا عن تناول الدواء		
7	تناول الدواء كل يوم مشكلة مزعجة لبعض الناس هل شعرت يوما بالضيق نتيجة التزامك بخطة الدواء الخاص بك		
8	ماهي درجة الصعوبة في التذكر تناول الدواء؟		

	نعم	لا
ابدا او نادرا		
مرة واحدة		
احيانا		
عاليا		
دائما		

ختم العيادة

اسم المريض:

توقيع المريض:

التاريخ:

Figure 2. 6: Safety Sheet for Monitoring of verapamil side effect.

Patient's study ID:	**Date:**

Side effects monitoring data sheet

Common symptoms	**No**	**Rarely**	**Some time**	**Commonly**
☐ Constipation	☐	☐	☐	☐
☐ Headache	☐	☐	☐	☐
☐ Dizziness or lightheadedness	☐	☐	☐	☐
Less common symptoms	☐	☐	☐	☐
☐ Heartburn	☐	☐	☐	☐
☐ Swelling of the hands, feet, ankles, or lower legs	☐	☐	☐	☐
☐ Difficulty breathing or swallowing	☐	☐	☐	☐
☐ Slow heartbeat	☐	☐	☐	☐
☐ Fainting	☐	☐	☐	☐
☐ Blurred vision	☐	☐	☐	☐
☐ Rash	☐	☐	☐	☐
☐ Nausea	☐	☐	☐	☐
☐ Extreme tiredness or joint pain	☐	☐	☐	☐
☐ Unusual bleeding or bruising	☐	☐	☐	☐
☐ Fatigue (unusual tiredness)	☐	☐	☐	☐
☐ Loss of appetite	☐	☐	☐	☐
☐ Pain in the upper right part of the stomach	☐	☐	☐	☐
☐ Yellowing of the skin or eyes	☐	☐	☐	☐
☐ flu-like symptoms	☐	☐	☐	☐
☐ Sleep disturbance	☐	☐	☐	☐

2.4.3 Objective measurements

1. HbA1c level will be determined by the change from baseline and after 6 months of daily verapamil use.
2. FPG level will be measured after at least 8 hours of fasting. The level change from baseline and after 6 months of daily verapamil use will be determined.
3. Parameters of endogenous insulin secretion:
    - Fasting C-peptide level as determined by the change from baseline and after 6 months of verapamil use.
        - A greater improvement in insulin production for our participants, would provide an indication of the efficacy of this intervention.
4. Parameter of insulin sensitivity:
    - HOMA2 (computer model): HOMA-IR level as determined by the change from baseline to 6 months after daily verapamil use.
        - A greater improvement in insulin sensitivity for our participants, would provide an indication of the efficacy of this intervention.

2.5 Sample Size

A sample size of 35 achieves 80% power to detect a mean of paired differences of 0.5 % in the HbA1c values with an estimated standard deviation of differences of 1 and with a significance level (alpha) of 0.05 using a two-sided paired t-test using Stata 16.1 (Stata Corp- College Station- TX- USA)

2.6 Statistical analysis

The data was manually entered in Excel 2016 and then imported into Stata 16.1 for analysis. The categorical variables are presented as N (%). Normality test (Shapiro-Wilk) was performed to check the distribution of data and appropriate parametric/non-parametric statistical tests were applied accordingly. Non-normal data were compared with Wilcoxon signed-rank test for matched pairs and normal data were compared with paired t-test. Factors affecting the response to verapamil were assessed using univariable logistic regression analysis. The changes in the repeated measures and factors affecting them were assessed with random effect regression.

Chapter 3

3.1 Results

Between December 2016 to December 2019, 35 participants were enrolled into the study. The mean age was 57.2 ± 7.7 years and 54.3% of participants were female (n= 19). All participants had type 2 diabetes with a duration of diabetes ranging from 4 to 20 years. Around half of the participants (n=17, 48.6%) were receiving insulin as part of their treatment. All participants had a normal ECG (normal sinus rhythm) and normal ejection fraction (EF) (65.51± 5.75%). Demographic characteristics are presented in Table 3.1. Most of the participants (n= 30) had an education level lower than high school (Figure 3.1), and 60% were not performing any exercise (Figure 3.2). The concurrent medications are listed in Table 3.2. The majority of patients were on metformin (100%), sitagliptin (40%), aspirin (80.0%), calcium carbonate (74.4%), or cholecalciferol (vitamin D_3) (74.4%). All participants had normal renal and liver function tests at baseline assessment, as shown in Table 3.3.

Medication adherence was assessed using the Arabic version of the Morisky Medication Adherence Scale (MMAS-8). The median baseline adherence level was 8, ranging from 6 to 8. The majority of participants (32 participants, 91.4%) had high adherence levels with a maximum score of 8 in MMAS-8. Later, after 3 and 6 months, the median adherence was 8, ranging from 7 to 8

Table 3 .1:Demographic characteristics of the participants

Baseline characteristics	(n= 35)
Age (Mean ± SD)	57.2 ± 7.7 years
Females (n, %)	19, 54.3%
Insulin user (n, %)	17, 48.6%
Smoking (n, %)	3, 8.6%
Body mass index (BMI) (Mean ± SD)	34 ± 5.8 kg/m^2
Ejection fraction (EF) (Mean ± SD)	65.5 ± 5.7%
Pulse (Mean ± SD)	108.9 ± 7.7 beats per minute (bpm)
Systolic blood pressure (Mean ± SD)	156.2 ± 7.5 mmHg
Diastolic blood pressure (Mean ± SD)	91.4 ± 4.1 mmHg
Mean arterial blood pressure (Mean± SD)	113 ± 4.5 mmHg
Duration of diabetes (Mean ± SD)	13 ± 5.6 years
Glycated haemoglobin (HbA1c) (Mean ± SD)	8.4 ± 1.3%
C-peptide (Mean ± SD)	0.9 ± 0.3 nmol/L
Homeostatic Model Assessment Insulin Resistance (HOMA-IR) (Mean ± SD)	2.3 ± 0.8
Fasting plasma glucose (FPG) (Mean ± SD)	8.7 ± 2.1 mmol/L
Diabetic neuropathy (n, %)	8, 22.9%
Diabetic retinopathy (n, %)	4, 11.4%

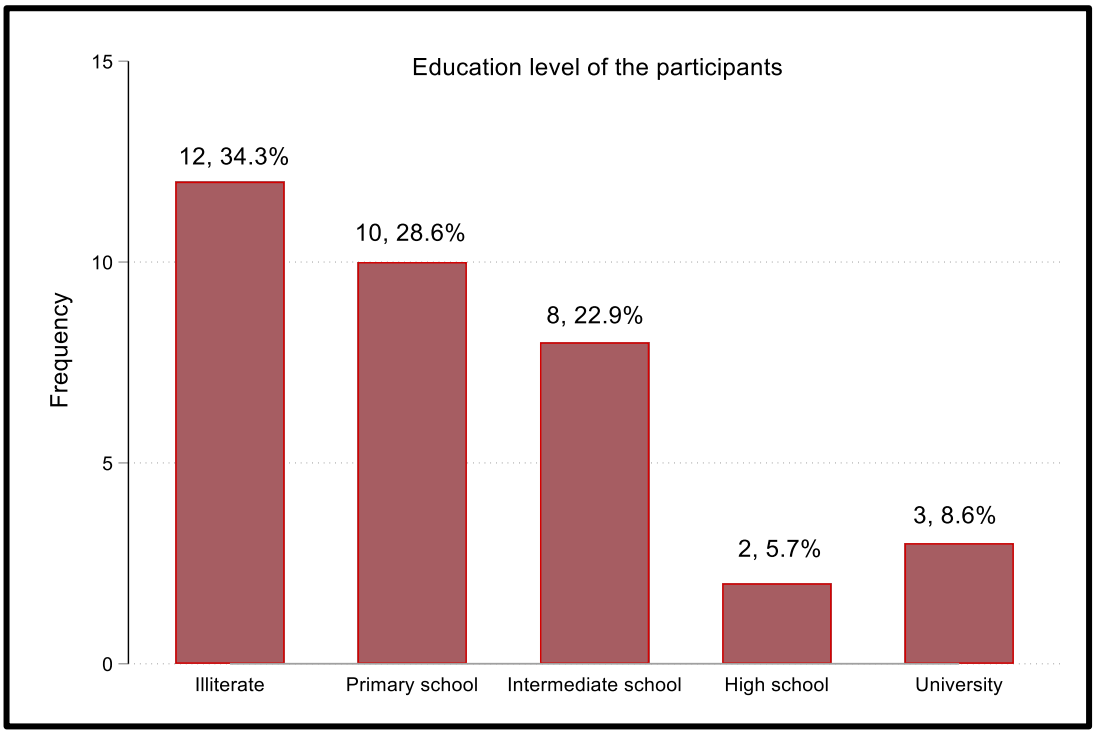


Figure 3. 1: Education level of the participants.

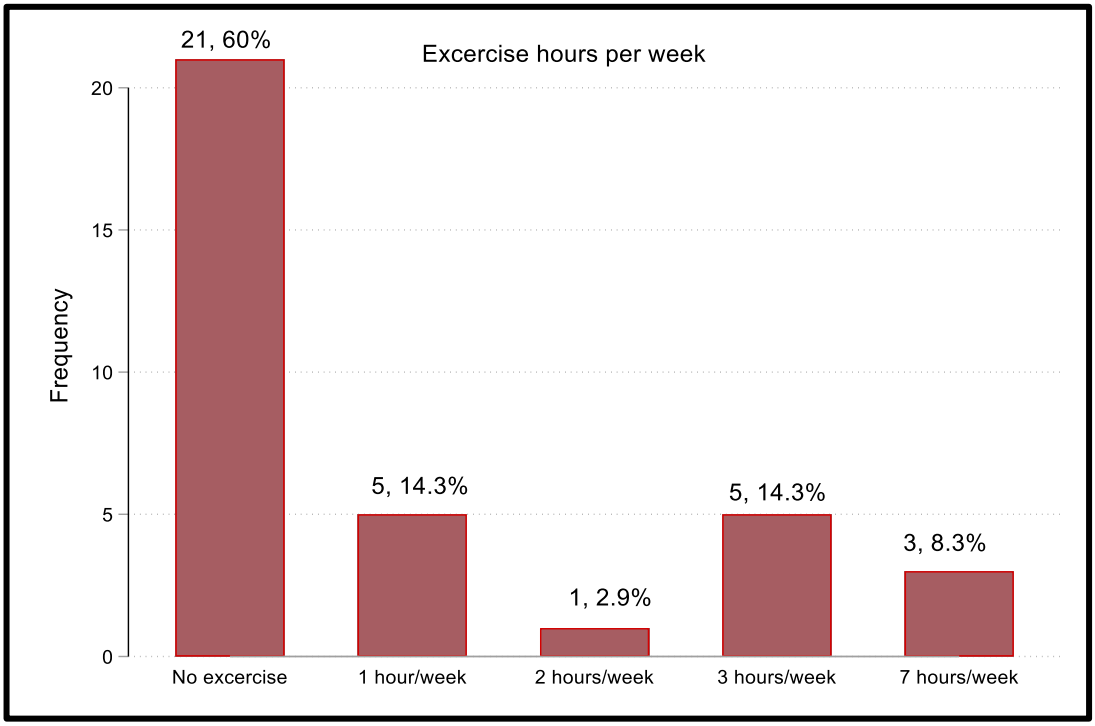


Figure 3. 2: Frequency of exercise performance by study participants.

Table 3. 2: Medication history at the baseline.

Medications	Frequency*	Number of patients at baseline (%)
Anti-hypertensive medications		
Lisinopril 20 mg	OD	15 (42.9)
Irbesartan 150 mg	OD	4 (11.4)
Irbesartan 300 mg	OD	11 (31.4)
Perindopril arginine 20 mg	OD	2 (5.7)
Candesartan 8 mg	OD	3 (8.6)
Verapamil 240 mg	OD	0
Verapamil 120 mg	OD	0
Antidiabetic medications		
Metformin 1 gm	BID	20 (57.1)
Metformin 500 mg	BID	5 (14.3)
Metformin 500 mg	TID	10 (28.6)
Insulin aspart 100 units/ml, 3 ml flexpen	OD	10 (28.6)
Insulin glargine 100 units/ml, 3 ml pen	OD	17 (48.6)
Liraglutide 18 mg/3ml (3ml) pen injector	OD	8 (22.9)
Sitagliptin 100 mg	OD	14 (40.0)
Glibenclamide 2.5 mg	OD	1 (2.9)
Glibenclamide 5 mg	OD	3 (8.6)
Gliclazide MR 60 mg	OD	7 (20.0)
Glimepiride 2 mg	OD	1 (2.9)
Anti-lipidemic medications		
Atorvastatin 10 mg	OD	22(62.9)
Atorvastatin 20 mg	OD	3(8.6)
Other medications		
Vitamin B complex	OD	10 (28.6)
Calcium carbonate 600 mg	OD	26 (74.4)
Choleciferol (vitamin D_3) 50000 IU	OD	26 (74.4)
Aspirin 81 mg EC	OD	25 (71.4)
Multivitamin and minerals	OD	13 (37.1)
Alendronate 70 mg	OD	1 (2.9)
Carbimazole 5 mg	OD	1 (2.9)
Omeprazole 20 mg	OD	4 (11.4)
Esomeprazole 20 mg	OD	8 (22.9)
Natural tear eye drops	QID	26 (74.4)

*****OD, once a day; BID, two times per day; TID, three times per day; QID, four times per day

Table 3. 3: Baseline renal, liver, and lipid profile of participants.

Organ function	Mean ± SD
Renal profile tests (normal values)	
• **Serum creatinine (Female: 0.6–1.1 mg/dL; male: 0.70–1.30 mg/dL) ****	Female: 0.8 ± 0.1 Male: 0.9 ± 0.2
• **GFR (≥ 90 mL/min/1.73 m²)***	85.5 ± 14.2
Liver profile tests**	
• **Plasma Total Bilirubin (0.3-1.1 mg/dl)**	0.6 ± 0.2
• **Plasma AST (11-47 U/L)**	31.4 ± 10.7
• **Plasma ALT (7-53 U/L)**	19.3 ± 11.8
• **Serum albumin (3.5-5 g/dl)**	4.2 ± 0.2
Lipid profile tests**	
• **Cholesterol (<200 mg/dl)**	75.1 ± 21.5
• **HDL (>35 mg/dl)**	37.10 ± 5.23
• **LDL (<100 mg/dl)**	52.9 ± 31.0
• **TG (<160 mg/dl)**	34.5 ± 28.3

*Normal range for Glomerular filtration rate; GFR[205] , ** Normal ranges according to Transitions of Care in Pharmacy Casebook.[206] ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; TG, triglyceride,.

The main objective of the study was to examine the effect of verapamil after 6 months of intervention on glucometabolic parameters (HbA1c, FPG, C-peptide, and HOMA-IR) in type 2 diabetic hypertensive patients.

Regarding HbA1c, the skewness of data at baseline was -0.193 and after 6 months of intervention was 0.29. As both skewness values were between -0.5 and 0.5, the data distribution is considered symmetrical. Box plot of the baseline and 6-months of HbA1c is presented in Figure 3.3. HbA1c was normally distributed and the measures were compared using paired t-test. After six months of verapamil intervention, there

was a mean reduction of 0.2 (± 1.0) % in HbA1c (Figures 3.4). However, this was not statistically significant from the baseline (p = 0.25, Table 3.4).

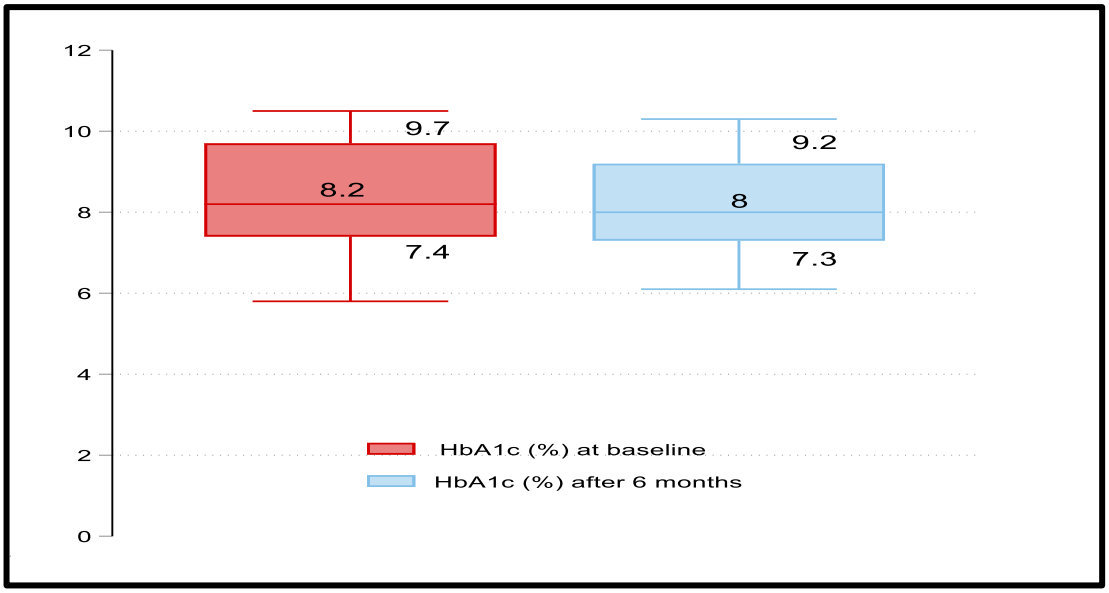


Figure 3. 3: Box and whisker plot of HbA1c values at baseline and after six months of intervention with verapamil.

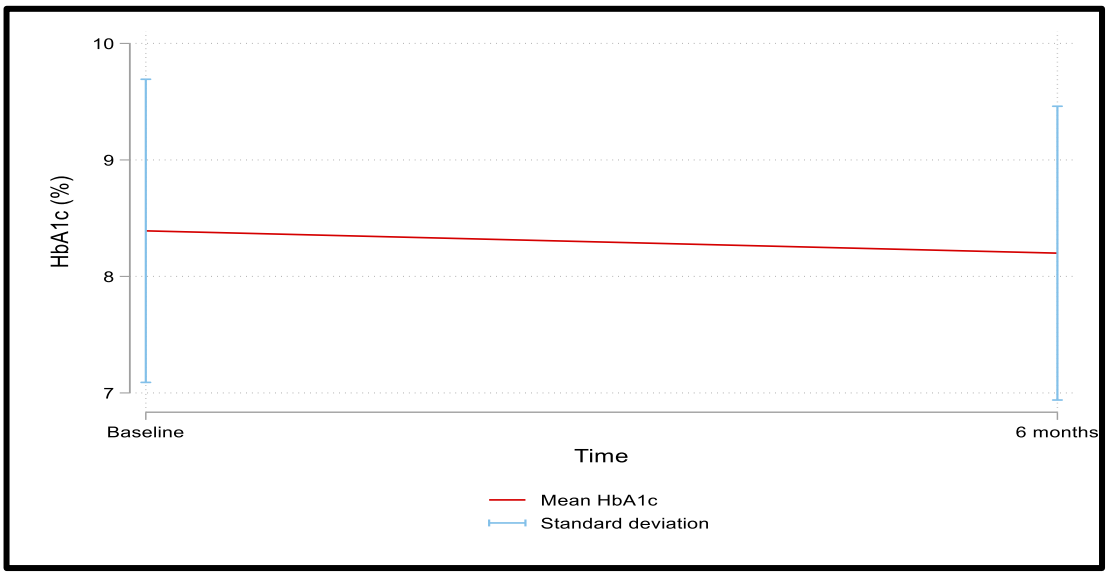


Figure 3. 4: Change of mean HbA1c values at baseline and after six months of intervention with verapamil.

Regarding FPG, skewness of data at baseline was 0.381 and after six months of intervention was 0.328. As both skewness values were between -0.5 and 0.5, the data distribution is considered symmetrical. The box plot of FPG at the baseline and after six months is presented in Figure 3.5. After 6 months of verapamil intervention, there was a reduction of 0.5 (± 1.8) mmol/L in FPG (Figures 3.6). A paired t-test showed no significant effect of verapamil on FPG (*P* =0.11, Table 3.4).

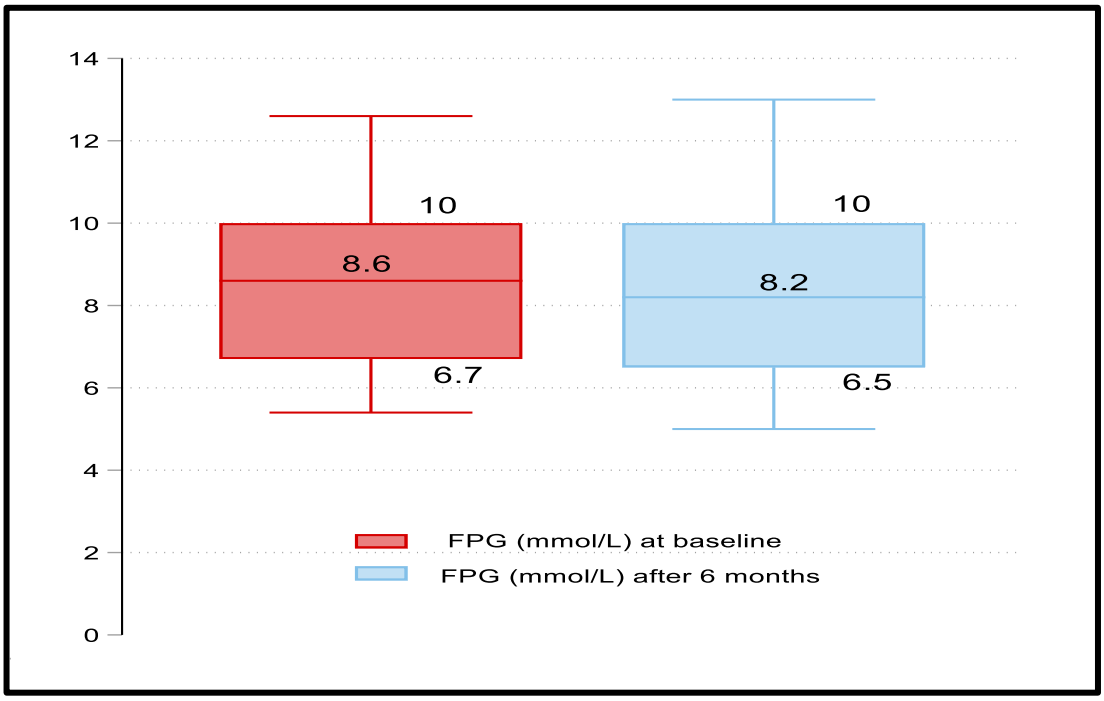


Figure 3. 5: Box and whisker plot of FPG values at baseline and after 6 months of intervention with verapamil.

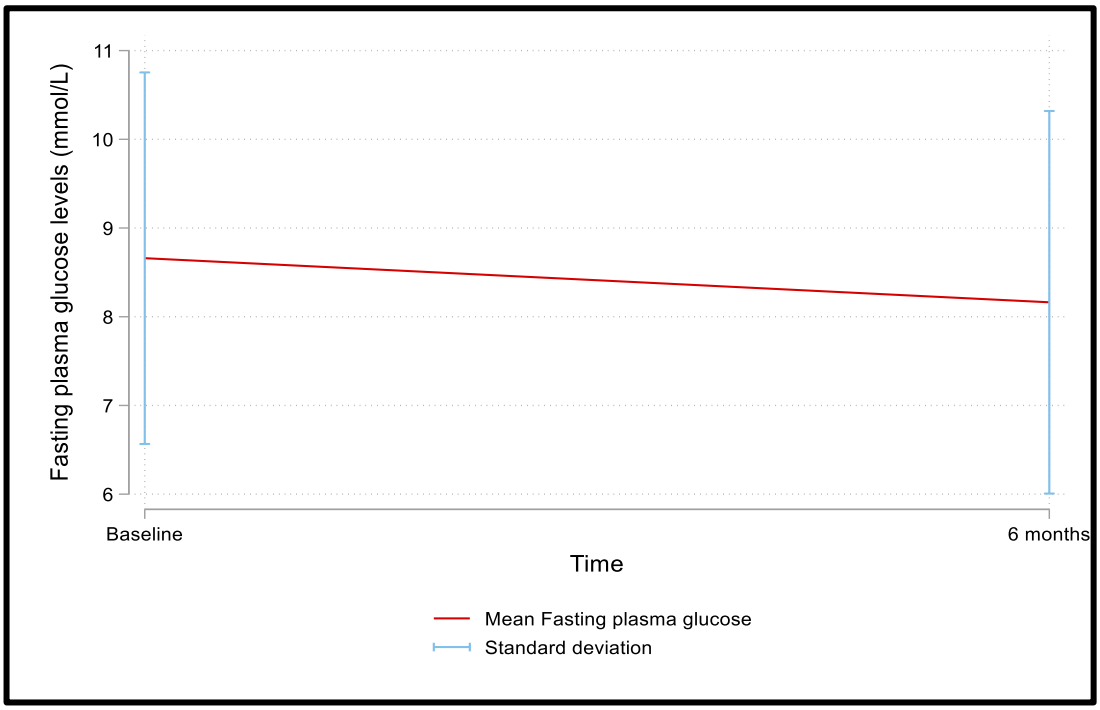


Figure 3. 6: Change in the mean FPG level from the baseline and after six months of verapamil use.

Regarding C-peptide, the skewness of data at baseline was 0.182 and after 6 months of intervention was 0.22. As both skewness values were between -0.5 and 0.5, the data distribution is considered asymmetric. The box plot of C-peptide at baseline and after 6 months is presented in Figure 3.7. After 6 months of verapamil intervention, C-peptide was reduced by 0.1 (±0.3) nmol/L (Figures 3.8). A paired t-test showed no significant effect of verapamil on C-peptide (P= 0.06, Table 3.4).

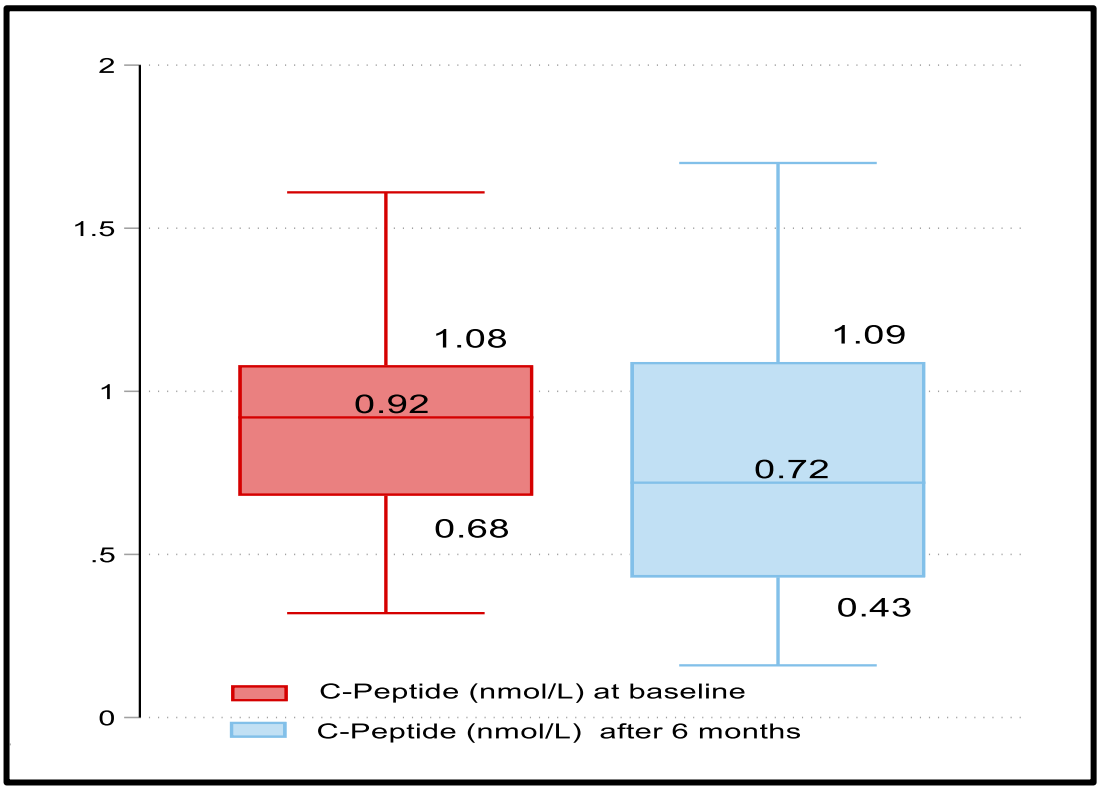


Figure 3. 7: Box and whisker plot of C-peptide values at baseline and after 6 months of intervention with verapamil.

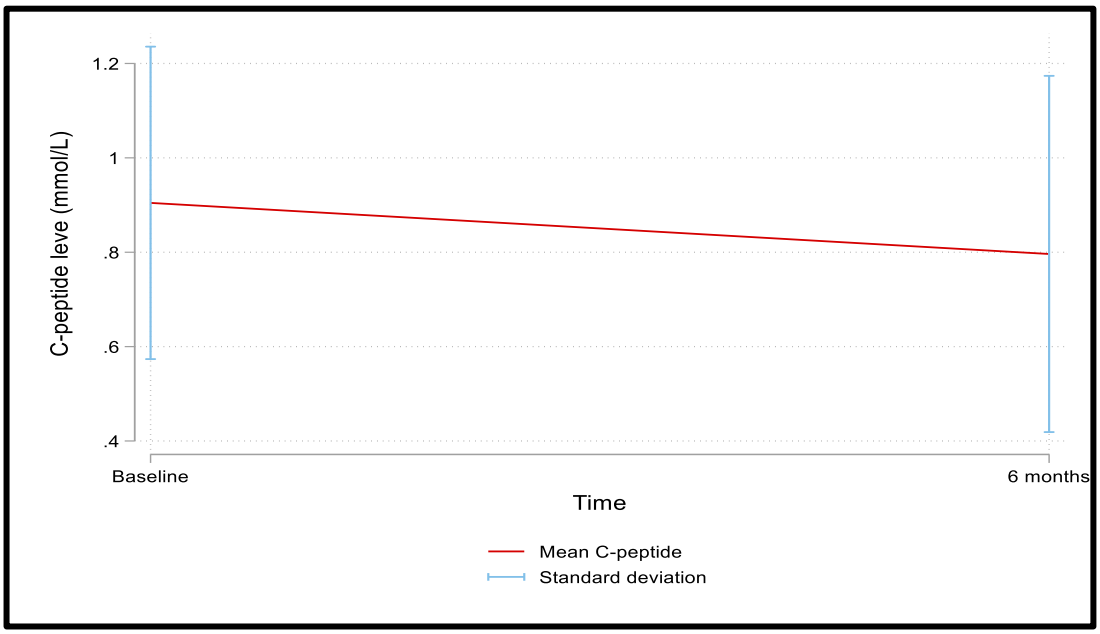


Figure 3. 8: Change in the mean C-peptide level from the baseline and after six months of verapamil use.

Regarding the last parameter HOMA-IR, the skewness of data at baseline was 0.06 and after six months of intervention was 0.35, as both skewness values were between -0.5 and 0.5, the data distribution is considered asymmetrical. The box plot of HOMA-IR at baseline and after six months is presented in Figure 3.9. After 6 months of verapamil intervention, there was a reduction in HOMA-IR by 0.3(±0.9) nmol/L (Figures 3.10). A paired t-test showed no significant effect of verapamil on HOMA-IR after 6 months of intervention (P= 0.05, Table 3.4).

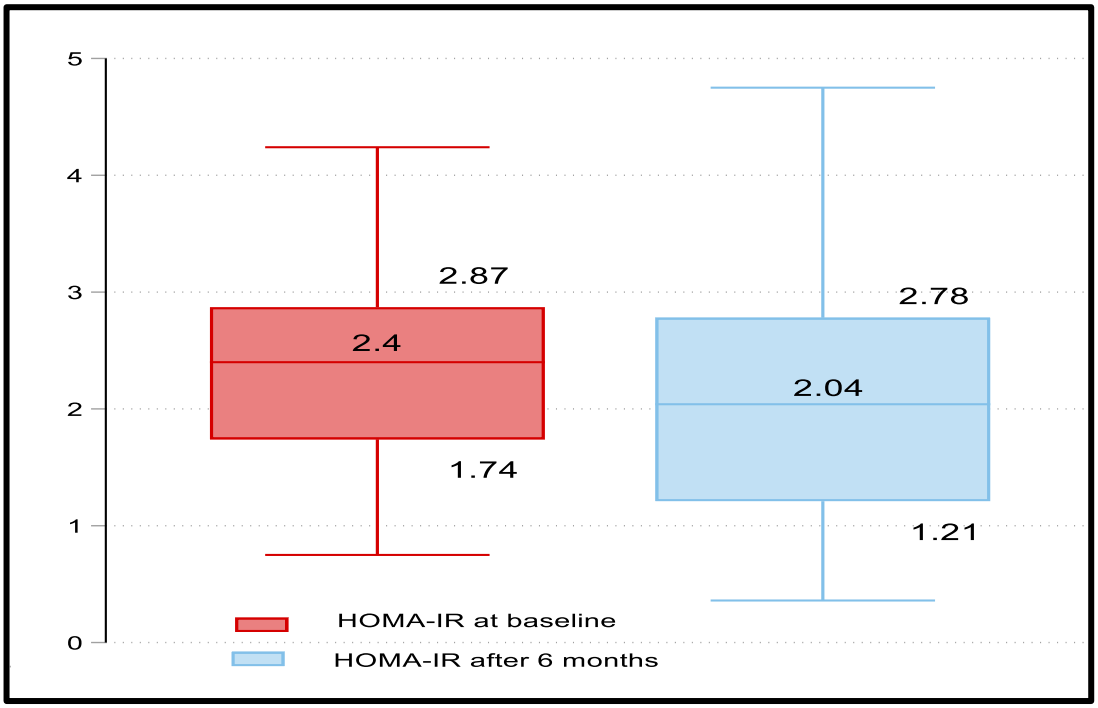


Figure 3. 9: Box and whisker plot of HOMA-IR values at baseline and after six months of intervention with verapamil.

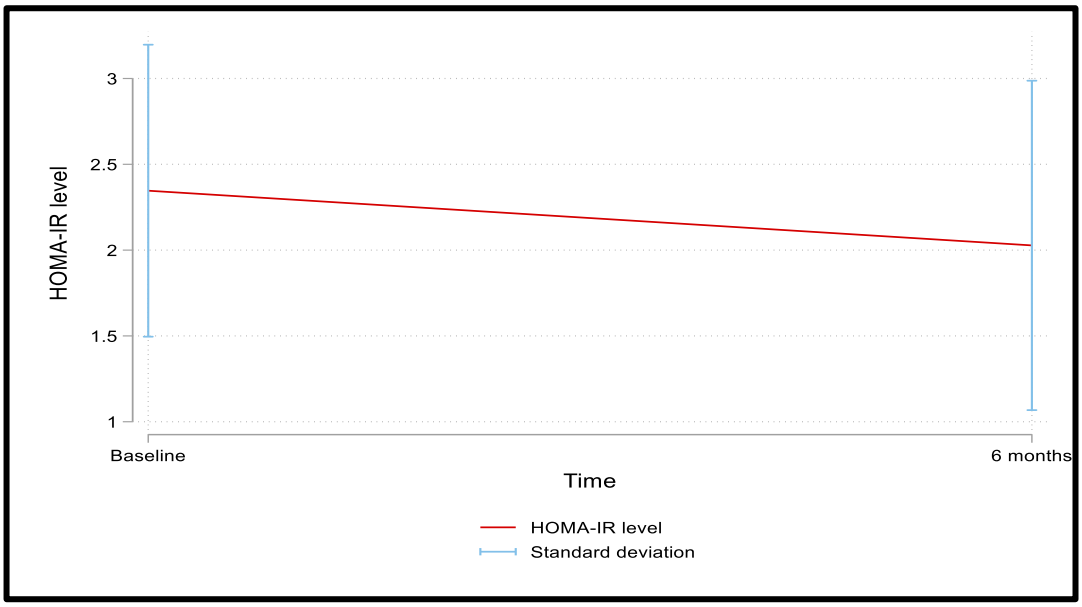


Figure 3. 10: Change in the mean HOMA-IR level from the baseline and after six months of verapamil use.

Table 3. 4: Mean difference of the clinical parameters at baseline and six months of intervention with verapamil.

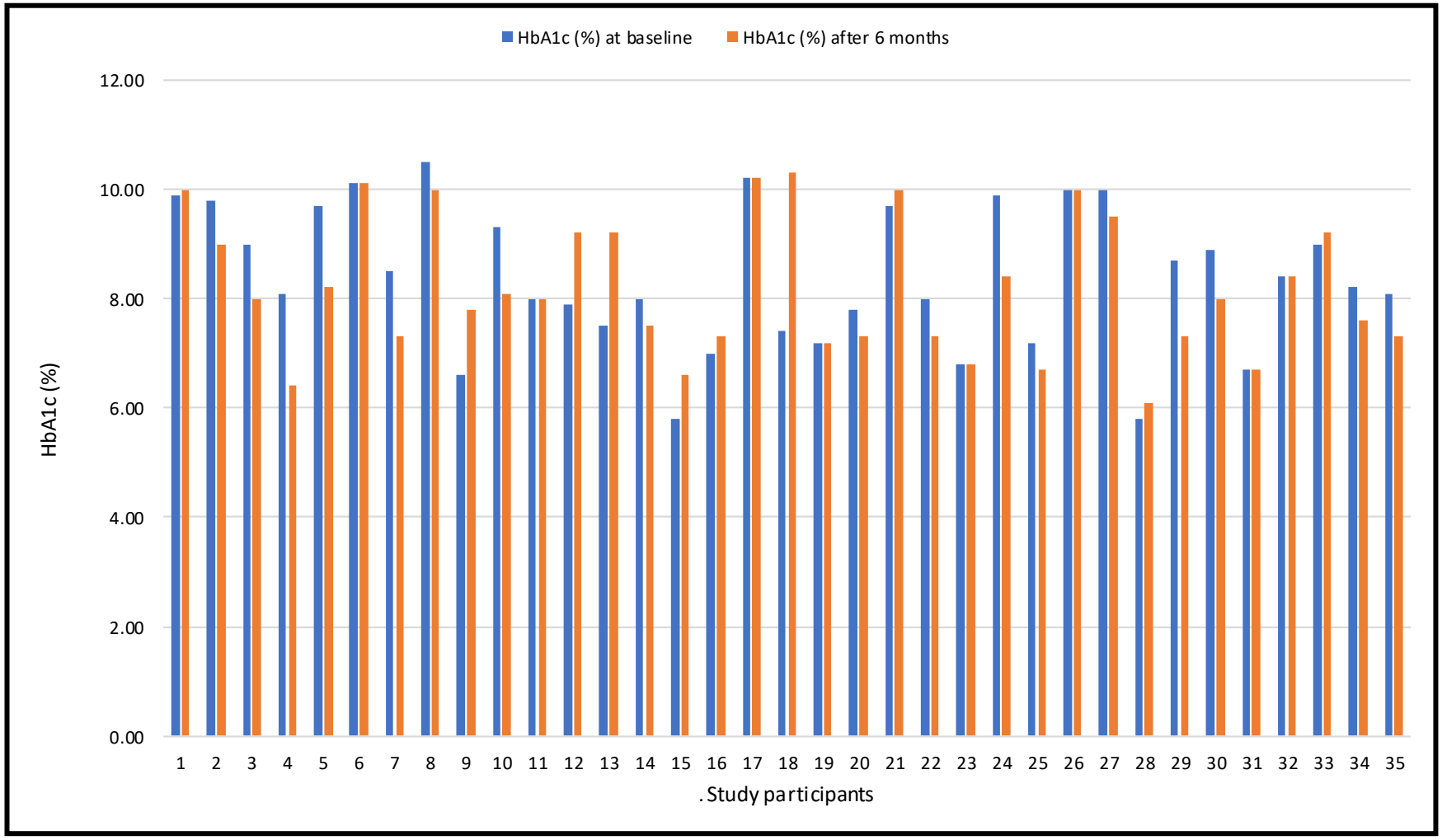
Parameter	0 – 6 months (Mean difference ± SD)	*P*
HbA1c (%)	0.2 ± 1.0	0.25
Fasting plasma glucose (mmol/L)	0.5 ± 1.8	0.11
C-Peptide (nmol/L)	0.1 ± 0.3	0.06
HOMA-IR	0.3 ± 0.9	0.05

3.1.1 Sub-analysis

All glucometabolic parameters decreased after using verapamil; however, the decrease was not statistically significant (Table 3.4). The current study undertook a sub-analysis that examined the effect of verapamil use on each participant. HbA1c values were exclusively chosen for this sub-analysis as it is the major clinical, biochemical

parameter for assessing glycaemic control. HbA1c values at baseline and after 6 months of intervention were shown for each participant in Figure 3.11, accordingly the study group was divided into responders or non-responders. The box and whisker plot of the sub-group analysis for HbA1c (responders versus non-responders) is presented in Figure 3.12. A responder was defined as a participant who achieved a reduction of $\geq 0.5\%$ in HbA1c value following 6 months of verapamil therapy. Around half of the participants (n=17, 48.6%) were responders based on this definition. They had a statistically significant response to therapy following six months of verapamil with a mean reduction of 0.9±0.4% in HbA1c from their baseline values ($P<0.001$, Figure 3.13). On the other hand, participants classified as non-responders exhibited a significant increase in HbA1c values relative to their baseline values (mean difference of -0.5±0.80% HbA1c). ($P<0.001$, Figure 3.13).

Figure 3. 11: The values of HbA1c at baseline and after six months of intervention were detected for each participant.



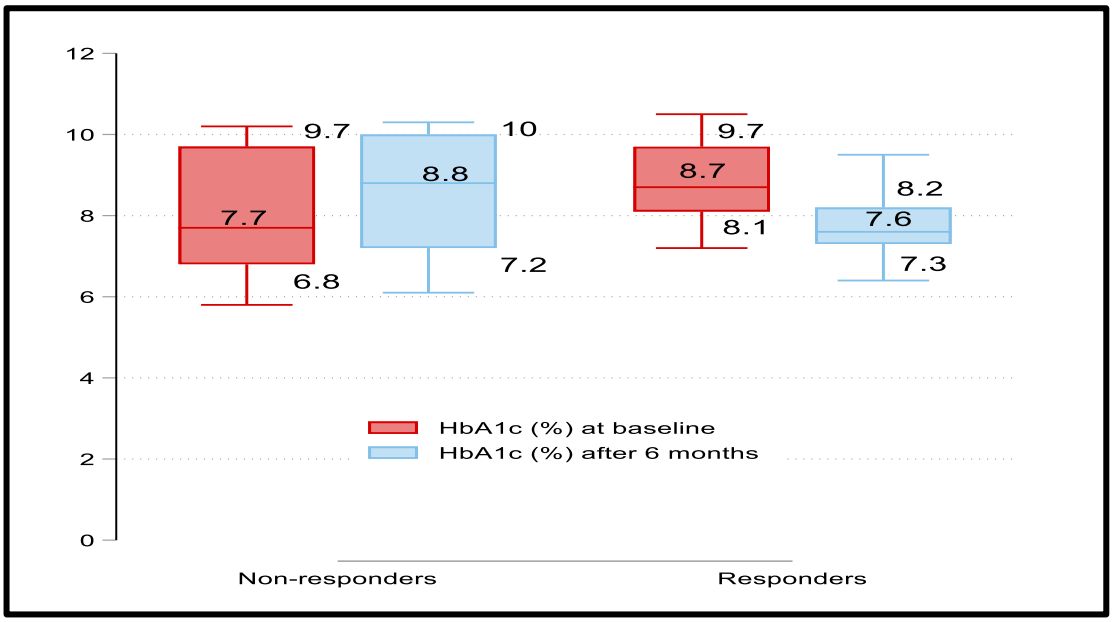


Figure 3. 12: Box and whisker plot of the sub-group analysis for HbA1c (responders versus non-responders).

The interaction between time and response was evaluated using random effect regression and revealed a significant decrease in HbA1c in participants who achieved the desired response at 6 months (coefficient: -1.43 (95% confidence interval: -1.83 to -1.01); p<0.001) (Figure 3.13).

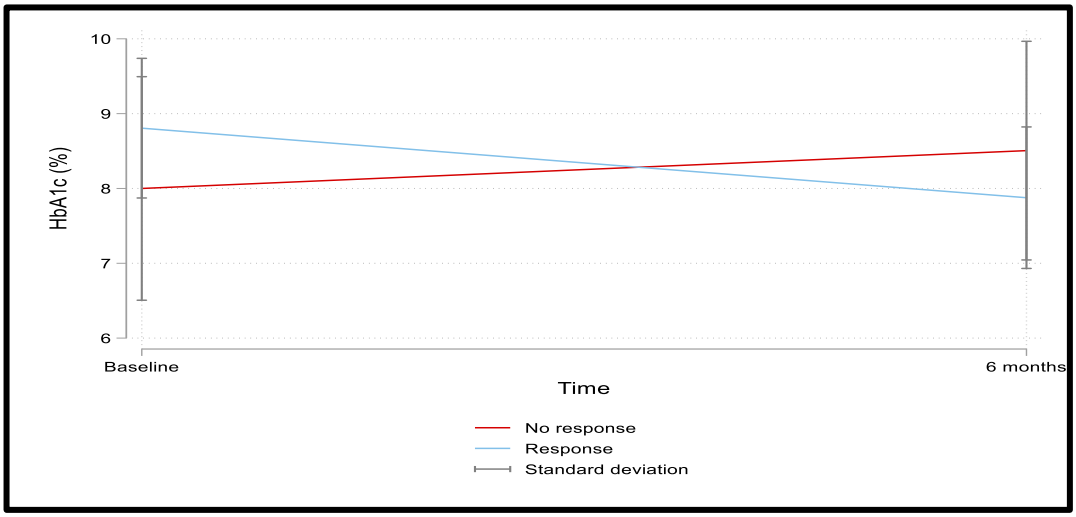


Figure 3. 13: Change in mean HbA1c in responders and non-responders after six months of treatment with verapamil.

Univariable logistic regression analysis was done for factors that affecting response to verapamil. The result of the univariable logistic regression analysis confirmed that baseline BMI, HOMA-IR, and C-peptide were significantly higher in responders (Table 3.5).

Table 3. 5: Logistic regression for factors affecting response to verapamil therapy

	Univariable	
	OR (95% CI)	**P**
Gender	0.7 (0.18- 2.66)	0.60
Age	0.98 (0.90- 1.07)	0.66
Baseline HbA1c	1.7 (0.95- 3.02)	0.07
Baseline FPG	1.04 (0.75- 1.42)	0.83
Baseline C-peptide level	75.89 (3.27- 17.6)	0.01
Baseline HOMA-IR	6.16 (1.62- 23.49)	0.01
Sitagliptin	1.78 (0.45- 6.97)	0.41
Insulin	0.35 (0.09- 1.37)	0.13
Baseline BMI	1.24 (1.06- 1.45)	0.01
Metformin dose	0.48 (0.18- 1.27)	0.14
Duration of diabetes	1.04 (0.92- 1.17)	0.56
Education	1.44 (0.81- 2.56)	0.21
Exercise	1.12 (0.80- 1.56)	0.50
Smoking	2.27 (0.19- 27.58)	0.52
Neuropathy	1.08 (0.22- 5.22)	0.93
Retinopathy	1.07 (0.13- 8.56)	0.95

3.1.2 Factors affecting the change in HbA1c level

Univariable random effect regression analysis was used to evaluate the effect of different baseline variables on the change of HbA1c level after 6 months of verapamil therapy. Sitagliptins were associated with a significant decrease in HbA1c. However, insulin, higher metformin dose, longer duration of diabetes, higher baseline FPG levels, retinopathy and neuropathy were associated with higher HbA1c level after 6 months. (Table 3.6) (Figures 3.14- 3.18)

Table 3. 6: Factors affecting the HbA1c levels from the baseline to the 6-months follow-up

	Coefficient (95% CI)	P-value
Gender	-0.45 (-1.24- 0.33)	0.26
Age	0.01 (-0.04- 0.07)	0.59
BMI	0.04 (-0.03- 0.10)	0.30
Sitagliptin	-1.40 (-2.07- -0.75)	<0.001
Insulin	1.39 (0.74- 2.03)	<0.001
Metformin dose	0.62 (0.11- 1.13)	0.02
Duration of diabetes	0.12 (0.06- 0.18)	<0.001
Education	-0.07 (-0.39- 0.25)	0.67
Exercise	0.01 (-0.18- 0.20)	0.92
Smoking	-1.29 (-2.64- 0.07)	0.06
Neuropathy	1.13 (0.26- 2.0)	0.01
Retinopathy	1.35 (0.18- 2.51)	0.02
Ejection fraction	-0.02 (-0.09- 0.05)	0.61
Baseline HOMA-IR	0.1 (-0.4- 0.6)	0.69

Baseline C-peptide	-0.23 (-1.45- 0.99)	0.71
Baseline FPG	0.34 (0.19- 0.5)	<0.001

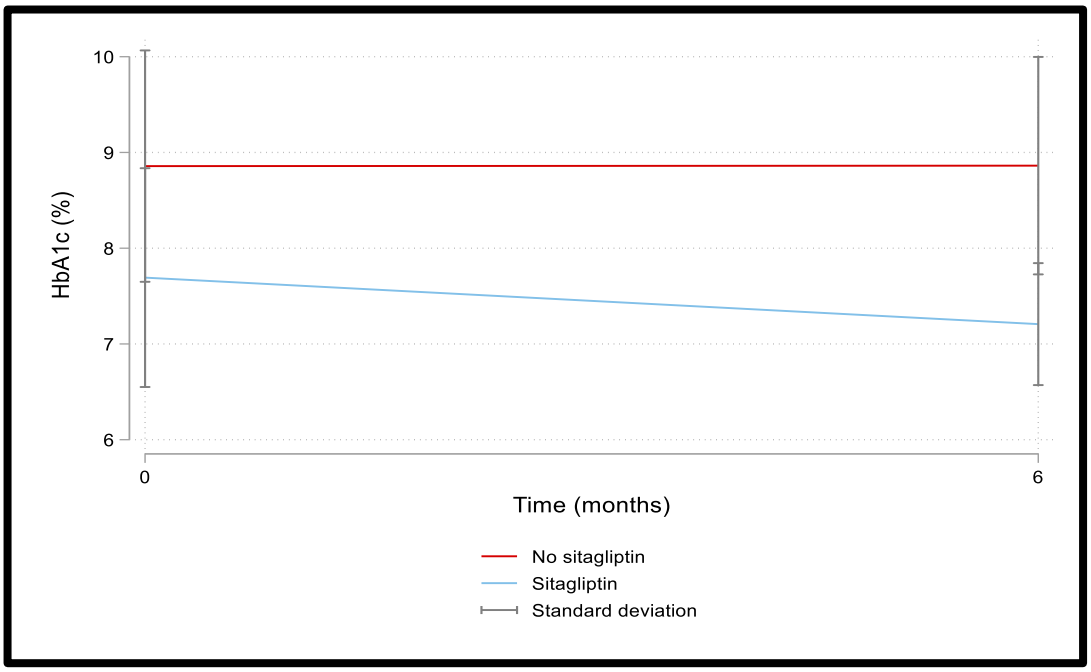


Figure 3. 14: The change in mean HbA1c levels with and without sitagliptin. **sitagliptin.**

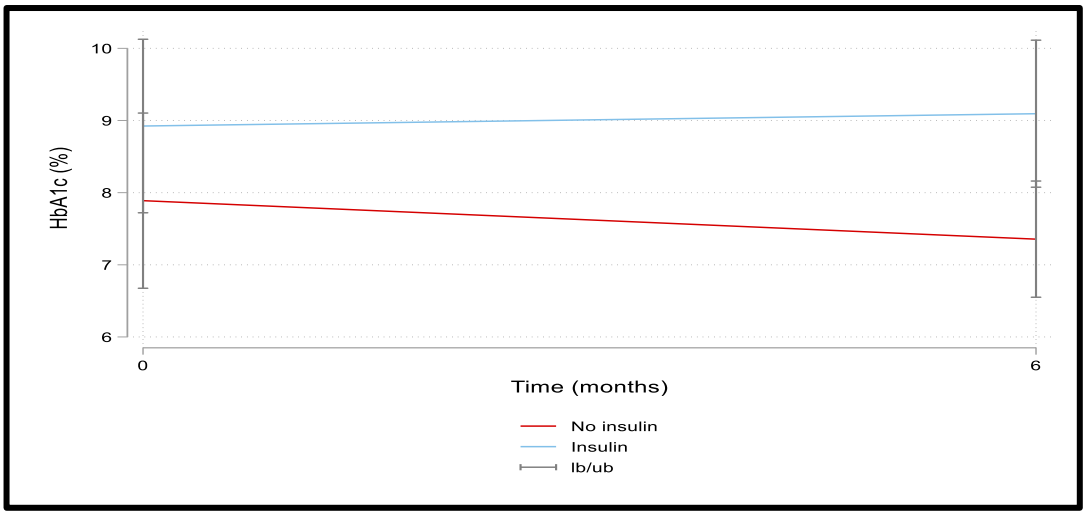


Figure 3. 15: The mean change in HbA1c levels with and without insulin.

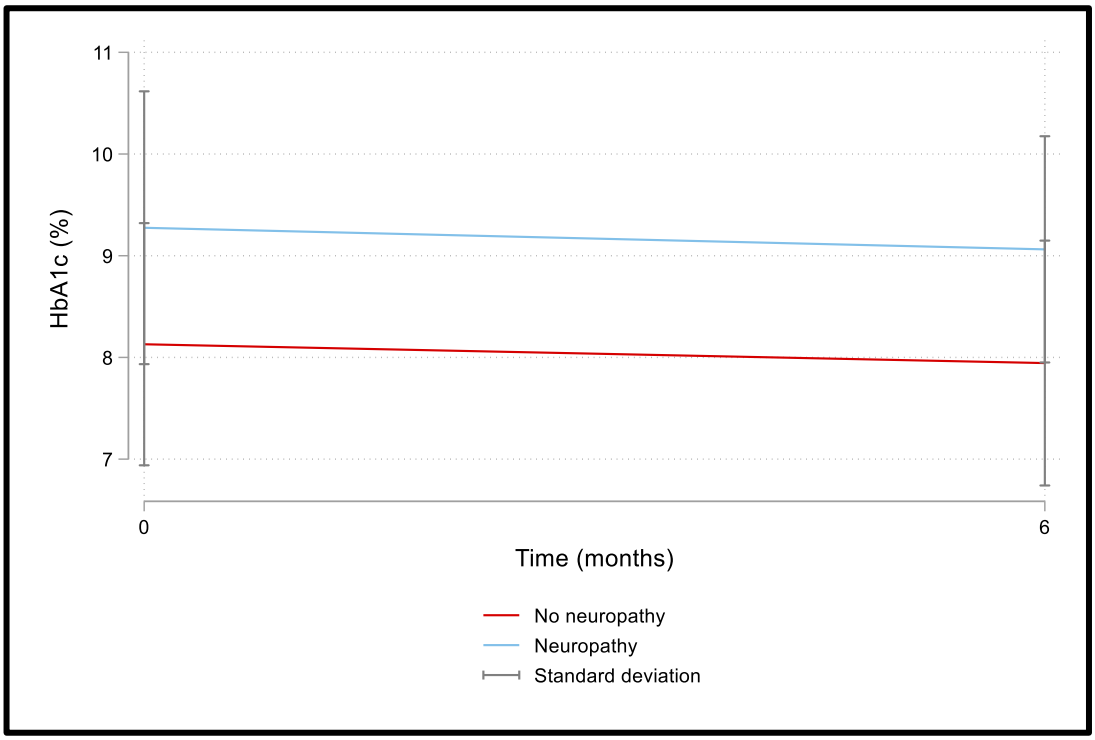


Figure 3. 16: The change of mean HbA1c levels in patients with and without neuropathy.

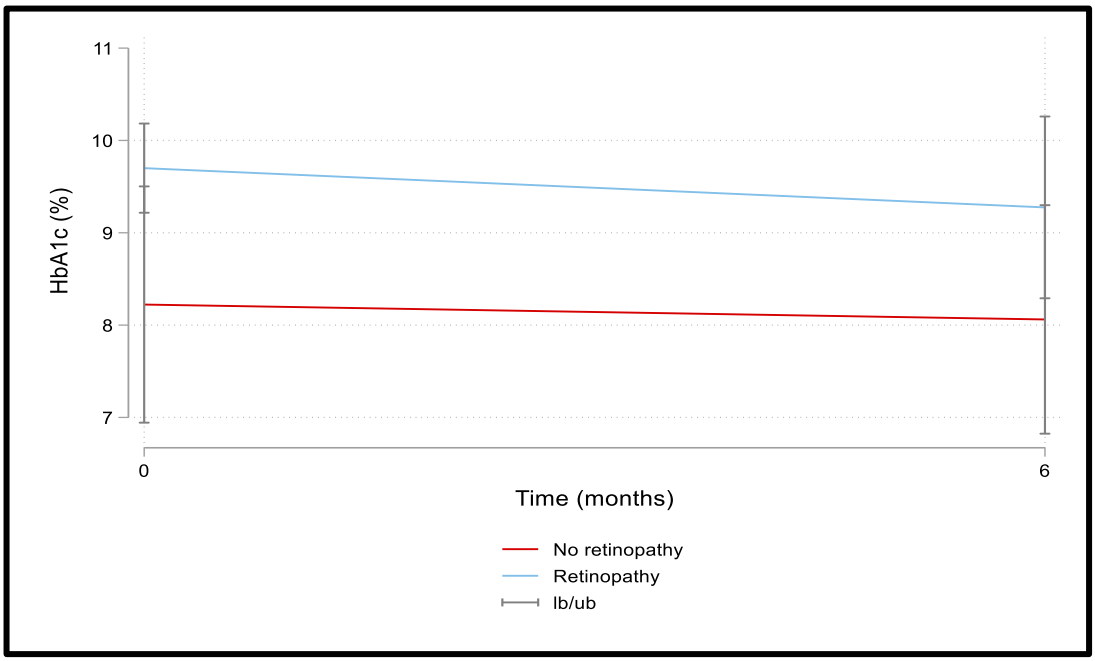


Figure 3. 17: The mean change of HbA1c levels in patients with and without retinopathy.

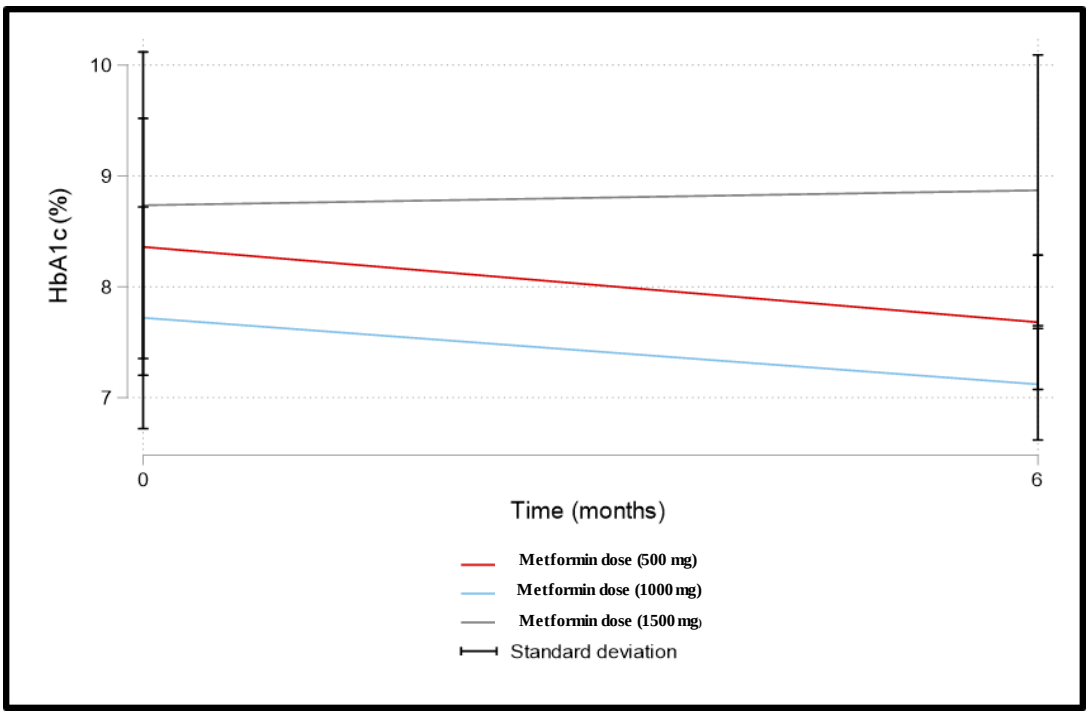


Figure 3. 18: The change of mean HbA1c levels in patients with different dose of metformin.

3.1.3 Factors affecting the change in FPG levels

As shown in Table 3.7, sitagliptin and smoking were associated with lower FPG levels; however, insulin use, baseline HbA1c levels, high metformin dose, and longer duration of diabetes were associated with higher FPG (Figure 3.19-3.22). However, higher baseline FPG levels, retinopathy and neuropathy were not associated with FPG level. (Table 3.7)

Table 3. 7: Factors affecting the FPG levels from the baseline to the 6-months follow-up.

	Coefficient (95% CI)	P-value
Gender	-0.44 (-1.73- 0.85)	0.50
Age	-0.003 (-0.09- 0.08)	0.94
BMI	0.07 (-0.04- 0.18)	0.20

Sitagliptin	-1.98 (-3.11- -0.84)	0.001
Insulin	2.39 (1.18- 3.28)	<0.001
Metformin dose	1.16 (0.37- 1.96)	0.004
Duration of diabetes	0.17 (0.07- 0.28)	0.001
Education	-0.01 (-0.54- 0.51)	0.98
Exercise	-0.05 (-0.36- 0.26)	0.76
Smoking	-2.49 (-4.64- -0.34)	0.02
Neuropathy	0.95 (-0.56- 2.46)	0.22
Retinopathy	0.74 (-1.28- 2.75)	0.48
Ejection fraction	0.02(-0.10- 0.13)	0.77
Baseline HOMA-IR	0.23 (-0.54- 0.99)	0.56
Baseline C-peptide	-0.38 (-2.36- 1.59)	0.70
Baseline HbA1c	0.69 (0.25- 1.14)	0.002

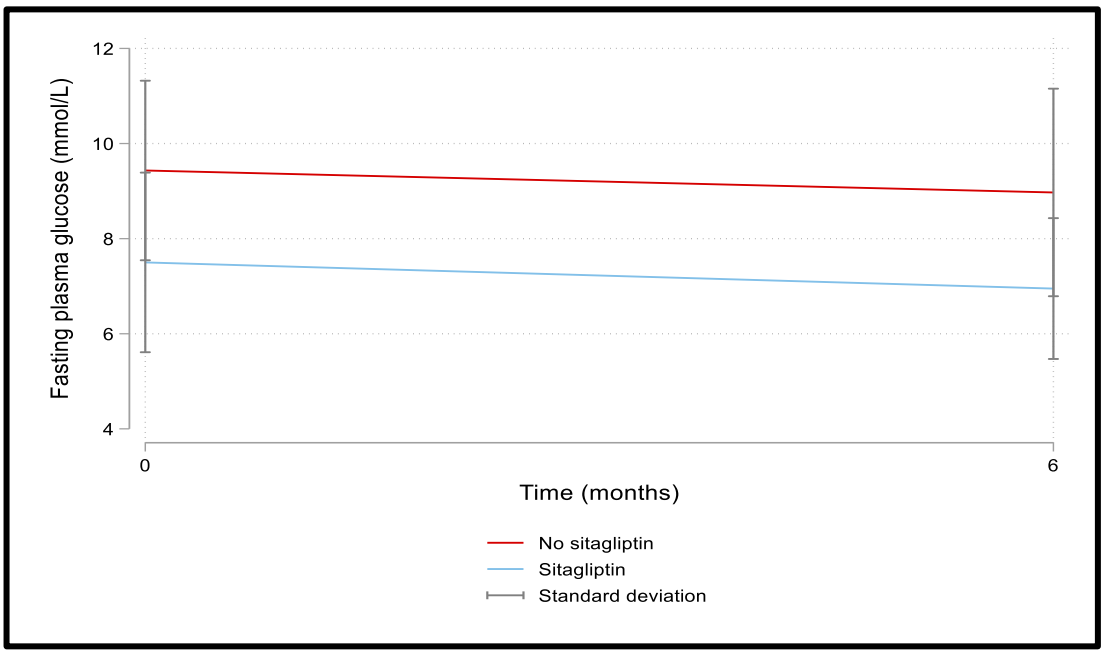


Figure 3. 19: Change in mean FPG level in patients with and without sitagliptin.

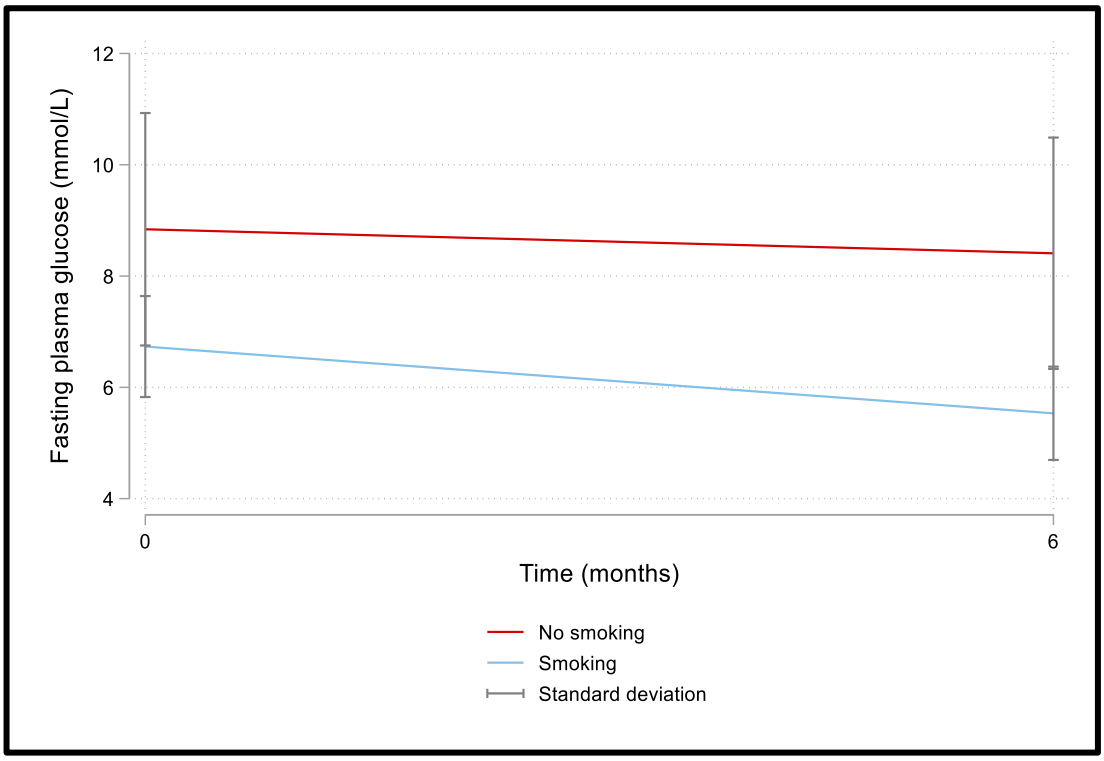


Figure 3. 20: Change in mean FPG levels in smoker versus nonsmoker patients.

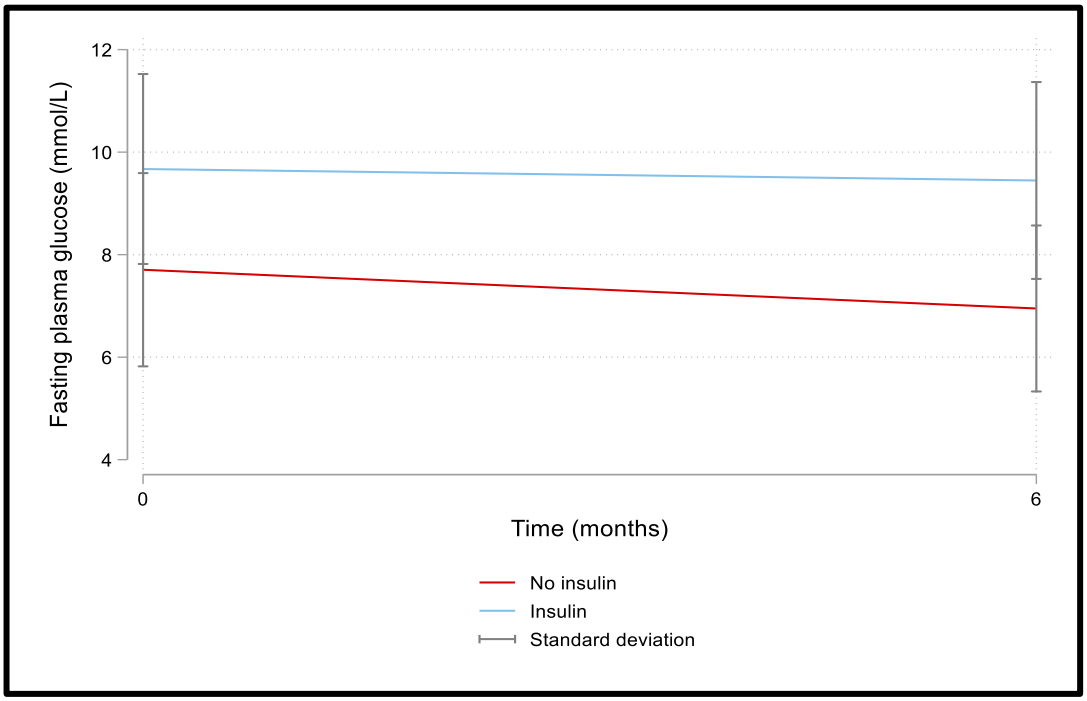


Figure 3. 21: The mean change in FPG levels with and without insulin use

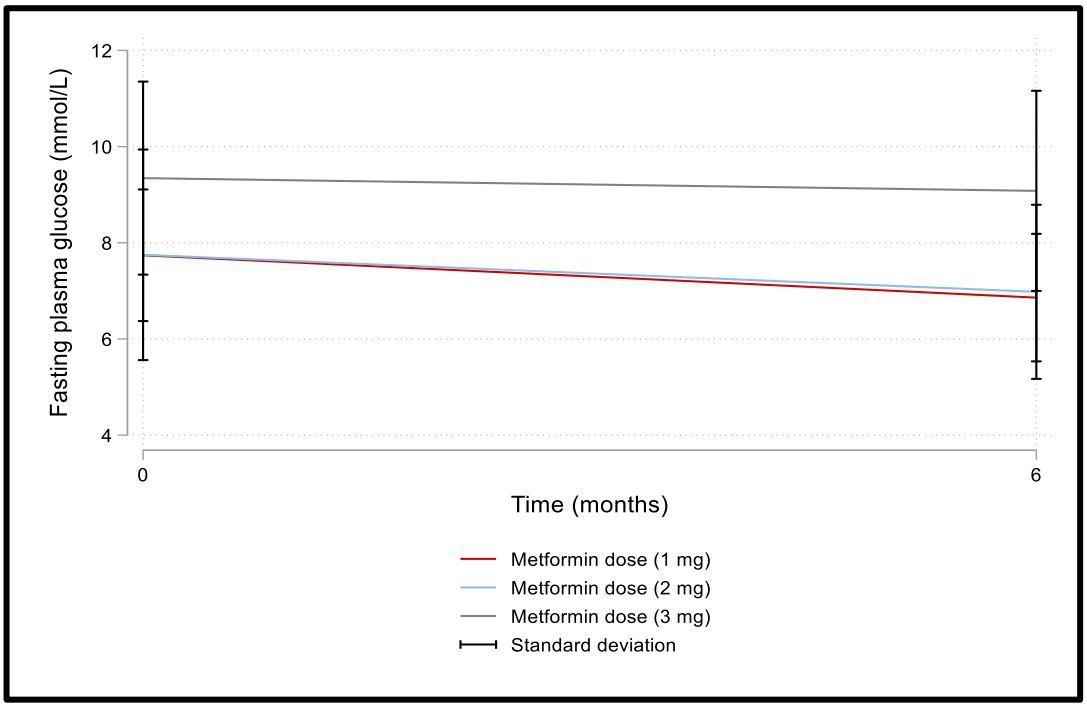


Figure 3. 22: The change of mean FPG levels in patients with different dose of metformin

3.1.4 Factors affecting HOMA-IR levels

As shown in Table 3.8, the increase in HOMA-IR level was associated with higher BMI and C-peptide levels at baseline. However, insulin, higher metformin dose, longer duration of diabetes, higher baseline FPG levels, retinopathy and neuropathy were not associated with higher HOMA-IR level after 6 months of verapamil intervention. (Table 3.8)

Table 3. 8: Factors affecting the HOMA-IR levels from the baseline to the 6-months follow-up

	Coefficient (95% CI)	P-value
Gender	0.05 (-0.47- 0.57)	0.85
Age	-0.03 (-0.06- 0.001)	0.06
BMI	0.06 (0.02- 0.10)	0.004
Sitagliptin	0.34 (-0.18- -0.86)	0.20
Insulin	-0.26 (-0.78- 0.25)	0.32
Metformin dose	-0.11 (-0.47- 0.25)	0.54
Duration of diabetes	-0.01 (-0.06- -0.04)	0.75
Education	0.13 (-0.07- 0.34)	0.19
Exercise	0.07 (-0.05- 0.20)	0.23
Smoking	0.32 (-0.60- 1.25)	0.49
Neuropathy	-0.36 (-0.97- 0.24)	0.24
Retinopathy	-0.43 (-1.24- 0.37)	0.29
Ejection fraction	-0.01 (-0.05- 0.04)	0.74
Baseline C-peptide	2.01 (1.55- 2.47)	<0.001
Baseline FPG	0.06 (-0.64- 0.19)	0.35
Baseline HbA1c	-0,01 (-0.21- 0.20)	0.93

3.1.5 Factors affecting C-peptide levels

As shown in Table 3.9, the increase in C-peptide was associated with higher BMI and HOMA-IR levels at baseline However, insulin, higher metformin dose, longer duration of diabetes, higher baseline FPG levels, C-peptide levels at baseline, retinopathy and neuropathy were not associated with higher C-peptide level after 6 months. (Table 3.9)

Table 3. 91: Factors affecting the C-peptide levels from the baseline to the 6-months follow-up.

	Coefficient (95% CI)	P-value
Gender	0.03 (-0.17- 0.25)	0.73
Age	-0.01 (-0.03- 0.0004)	0.06
BMI	0.02 (0.01- 0.04)	0.01
Sitagliptin	0.20 (-0.002- 0.41)	0.05
Insulin	-0.17 (-0.38- 0.02)	0.08
Metformin dose	-0.08 (-0.22- 0.06)	0.25
Duration of diabetes	-0.01 (-0.03- -0.01)	0.38
Education	0.06 (-0.02- 0.14)	0.17
Exercise	0.03 (-0.02- 0.08)	0.20
Smoking	0.22 (-0.15- 0.59)	0.24
Neuropathy	-0.18 (-0.42- 0.06)	0.15
Retinopathy	-0.21 (-0.53- 0.11)	0.21
Ejection fraction	-0.003 (-0.02- 0.01)	0.69
Baseline HOMA-IR	0.30 (0.23- 0.37)	<0.001
Baseline FPG	-0.002 (-0.05- -0.05)	0.94
Baseline HbA1c	-0.02 (-0.11- 0.5)	0.55

3.1.6 The long-term glucometabolic effect of verapamil

As mentioned this study is 6 months trial (24 weeks) but as a part of analysis we found that it is interesting if we follow the respond patients to see if the verapamil effect on the glycometabolic parameter will continue to be observed in the respond patients. Therefore, the long-term effects of verapamil therapy were examined in the responder group after 12 months of verapamil therapy initiation. The change in HbA1c, FPG, C-

peptide, and HOMA-IR after 6 and 12 months of intervention with verapamil are shown in Figures 3.23-3.26, respectively. The effect of verapamil on the glucometabolic parameters was evaluated after 12 months of intervention in the responders using random effect regression. The results showed a significant decrease in all parameters after 6 and 12 months compared to the baseline value. The main decrease occurred in the first 6 months of therapy (Table 3.10).

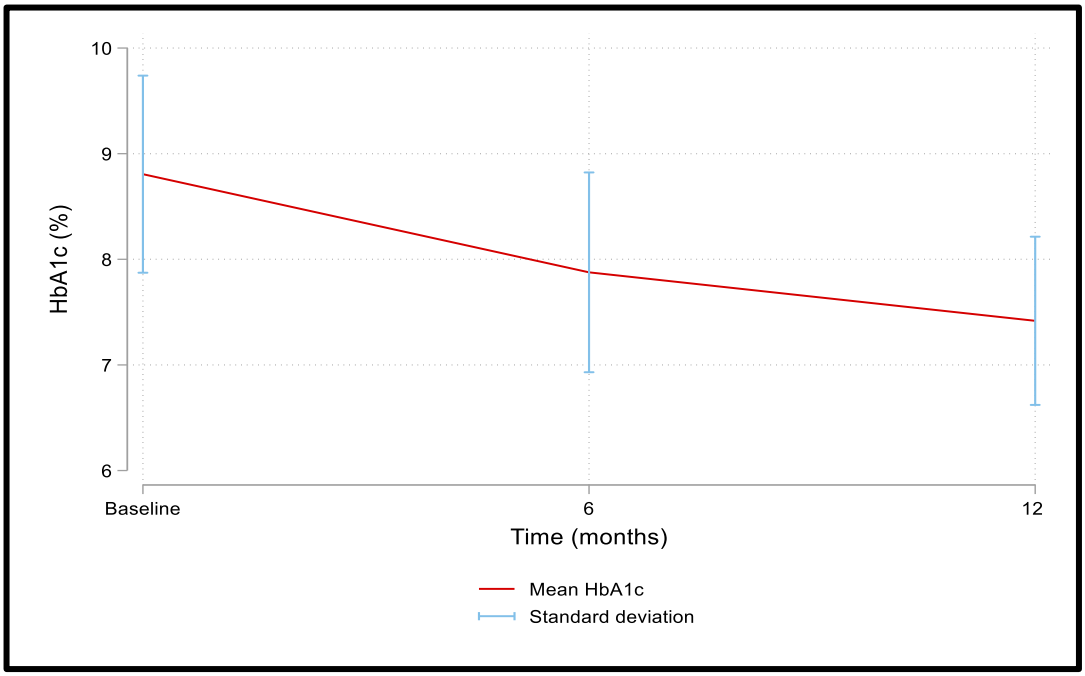


Figure 3. 23: The change of mean HbA1c values at 6 and 12 months of intervention with verapamil.

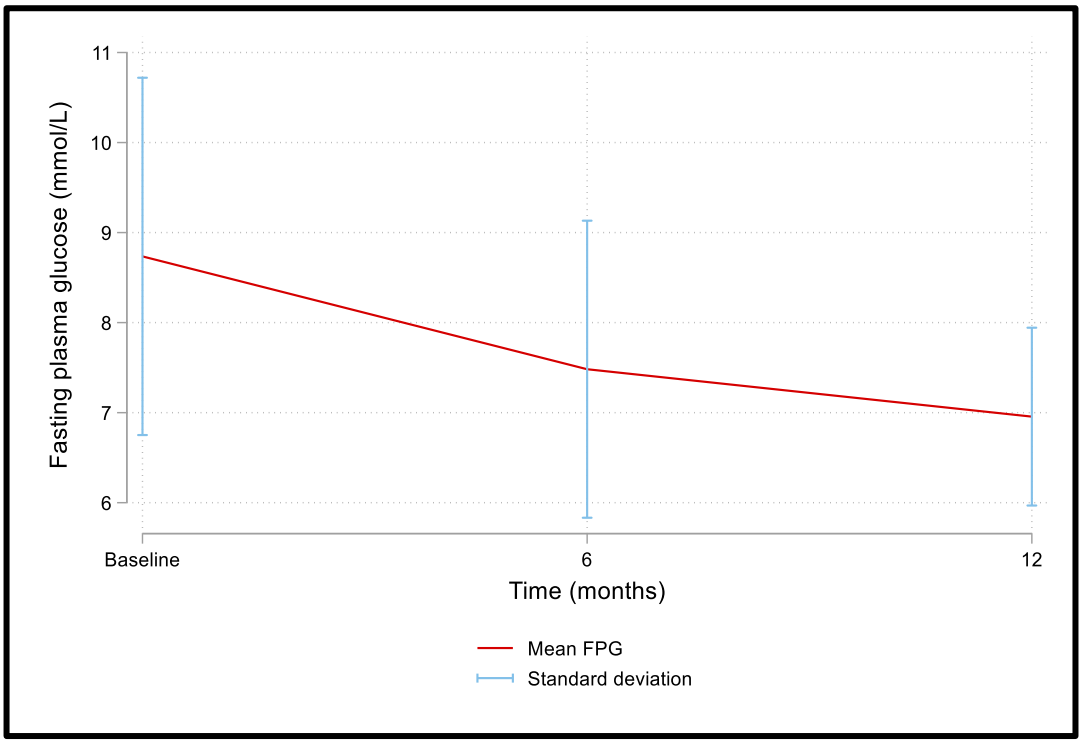


Figure 3. 24: The change of mean FBG values at 6 and 12 months of intervention with verapamil.

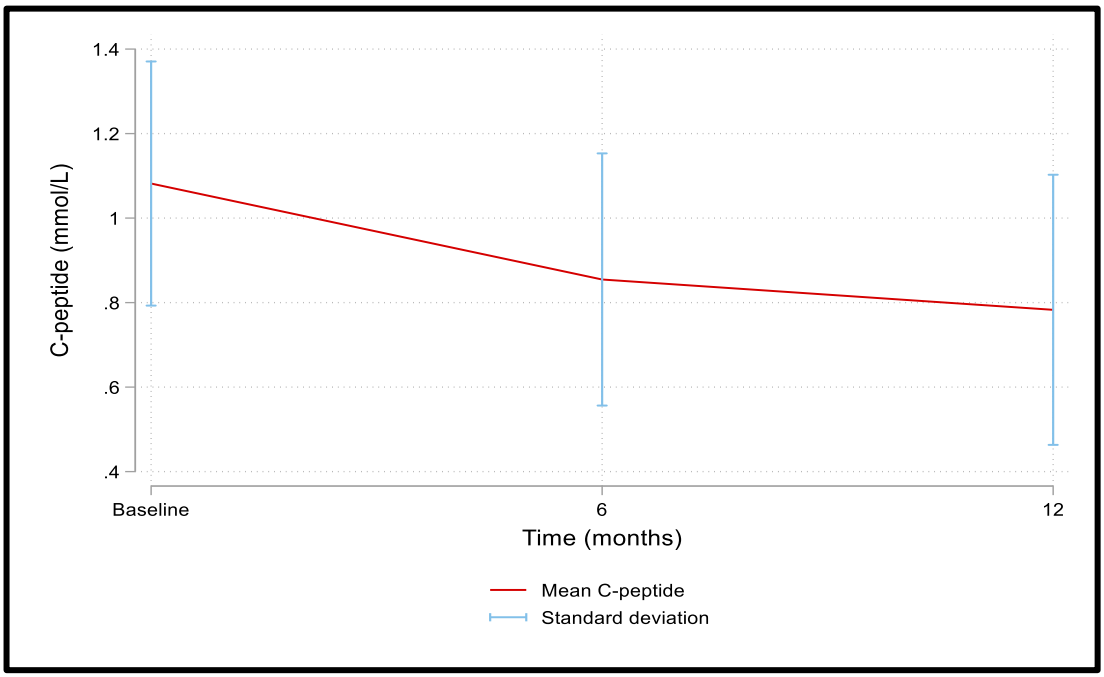


Figure 3. 25: The change of mean C-Peptide values at 6 and 12 months of intervention with verapamil.

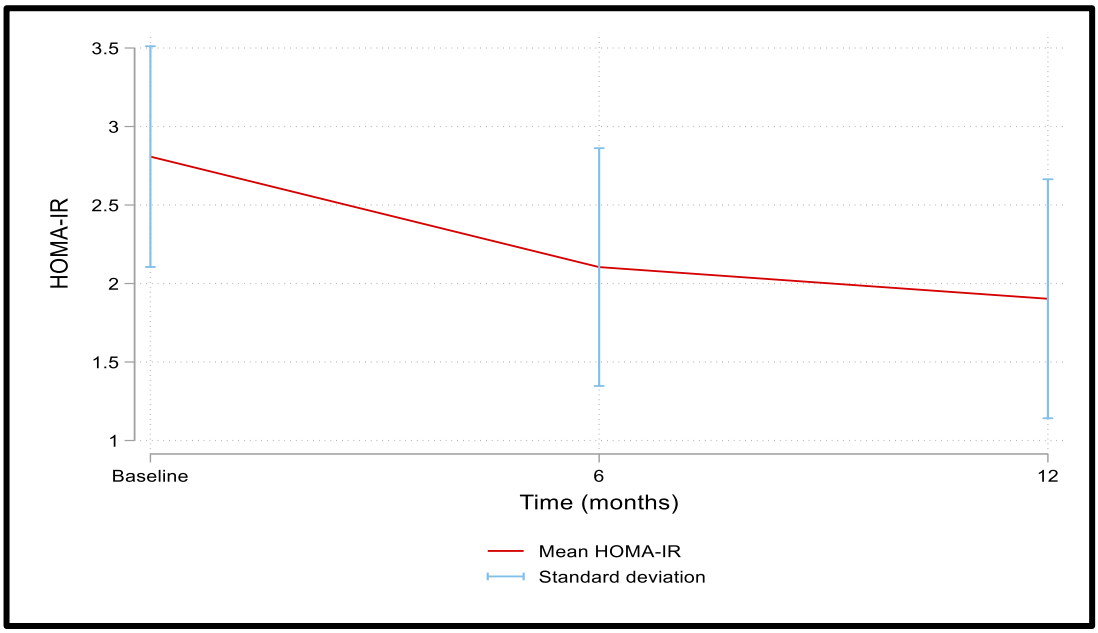


Figure 3. 26: The change of mean HOMA-IR values at 6 and 12 months of intervention with verapamil.

Table 3. 2 : Comparison of clinical parameters at baseline, 6 and 12 months of intervention with verapamil for responder group (17 participants)

	Mean± SD	Coefficient (95% CI)	P
HbA1c (%) baseline	8.8 ± 0.9		
HbA1c after 6-months	7.9 ± 0.9	-0.93 (-1.29- -0.57)	<0.001
HbA1c after 12-months	7.4 ± 0.8	-1.39 (-1.75- -0.57)	<0.001
HbA1c between 6-12 moths		-0.46 (-0.82- -0.10)	0.012
FPG (mg/dL) at baseline	8.7 ± 2		
FPG after 6-months	7.5 ± 1.6	-1.25 (-2.08- -0.43)	<0.001
FPG after 12-months	7.0 ± 0.99	-1.78 (-2.60- -0.96)	<0.001
FPG between 6-12 moths		-0.52 (-1.35- 0.30)	0.21
C-peptide(nmol/L) at baseline	1.1 ± 0.3		
C-peptide after 6-months	0.9 ± 0.3	-0.23 (-0.35- -0.10)	<0.001

C-peptide after 12-months	0.8 ± 0.3	-0.30 (-0.42- -0.18)	<0.001
C-peptide between 6-12 moths		-0.07 (-0.19- 0.05)	0.25
HOMA-IR at baseline	2.8 ± 0.7		
HOMA-IR after 6-months	2.1 ± 0.8	-0.70 (-1.02- -0.39)	<0.001
HOMA-IR after 12-months	1.9 ± 0.8	-0.91 (-1.22- -0.59)	<0.001
HOMA-IR between 6-12 moths		-0.20 (-0.52- 0.11)	0.21

3.2 Monitoring

3.2.1 Monitoring blood pressure response to verapamil

All participants were initiated on verapamil 120 mg sustained released (SR) once daily (half tablet of verapamil-SR 240 mg). Blood pressure and pulse were assessed to monitor the verapamil efficacy as an anti-hypertensive. Patients were followed up by phone on a weekly basis, and according to patients' self-reporting, no cases of hypertension or hypotension were recorded. In the hospital, blood pressure and pulse were measured after 3 and 6 months of verapamil intervention.

The mean arterial pressure (MAP) was calculated using the following formula, MAP = diastolic blood pressure + 1/3 (systolic blood pressure – diastolic blood pressure).[207] Changes in the mean arterial pressure in responders and non-responders after 3 and 6 months was assessed using random effect regression. The MPA at baseline (113±4.5), 3 months (101.2± 7.9) and 6 months (94.4±6.2). The change in the mean arterial pressure was significant after 6 month of verapamil intervention (coefficient: -3.11 (-3.45 to -2.76); p<0.001) with no difference between responders and non-responders (coefficient: 2.8 (-1.33 to 6.93); *P*=0.18; Figure 3. 27).

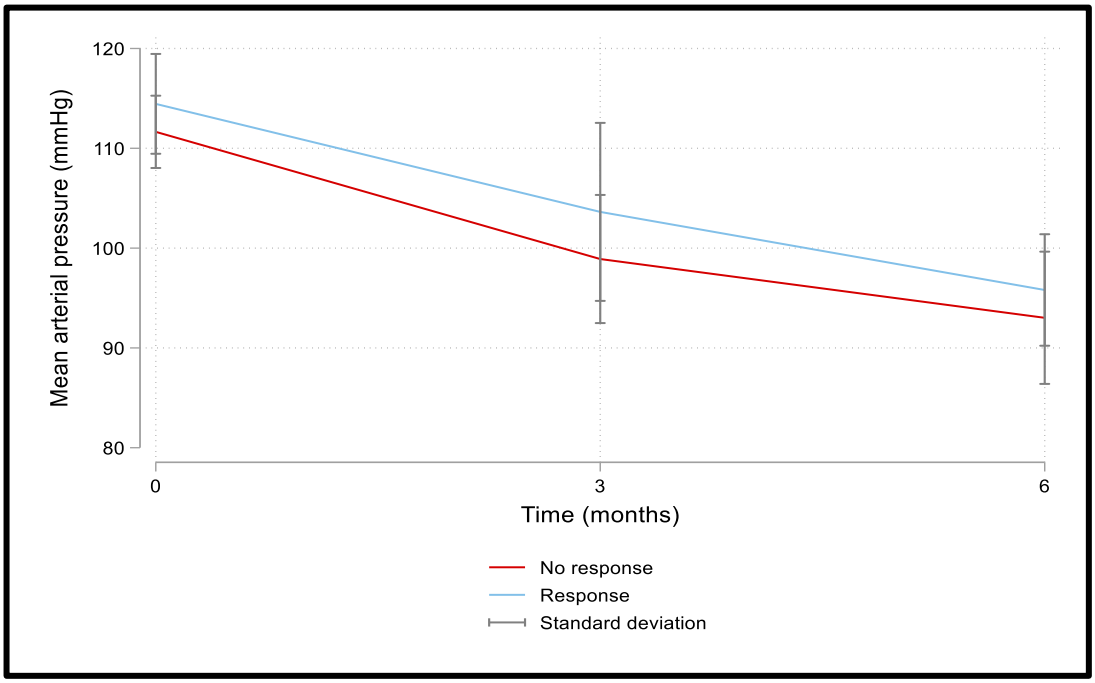


Figure 3. 27: The mean arterial pressure at baseline, after 3 months, and after 6 months of intervention with verapamil for responders and non-responders.

Also, changes in pulse in responders and non-responders after 3 and 6 months was assessed using random effect regression. Mean pulse at baseline (108.9± 7.7), 3 months (89.1± 6.2) and 6 months (73.4± 7.6). There was a significant decrease in pulse (-5.9 (-6.5- -5.4), $P< 0.001$) after 6 month of verapamil intervention with no difference between responders and non-responders (0.6 (-2.5- 3.6), $P= 0.72$; Figure 3.28).

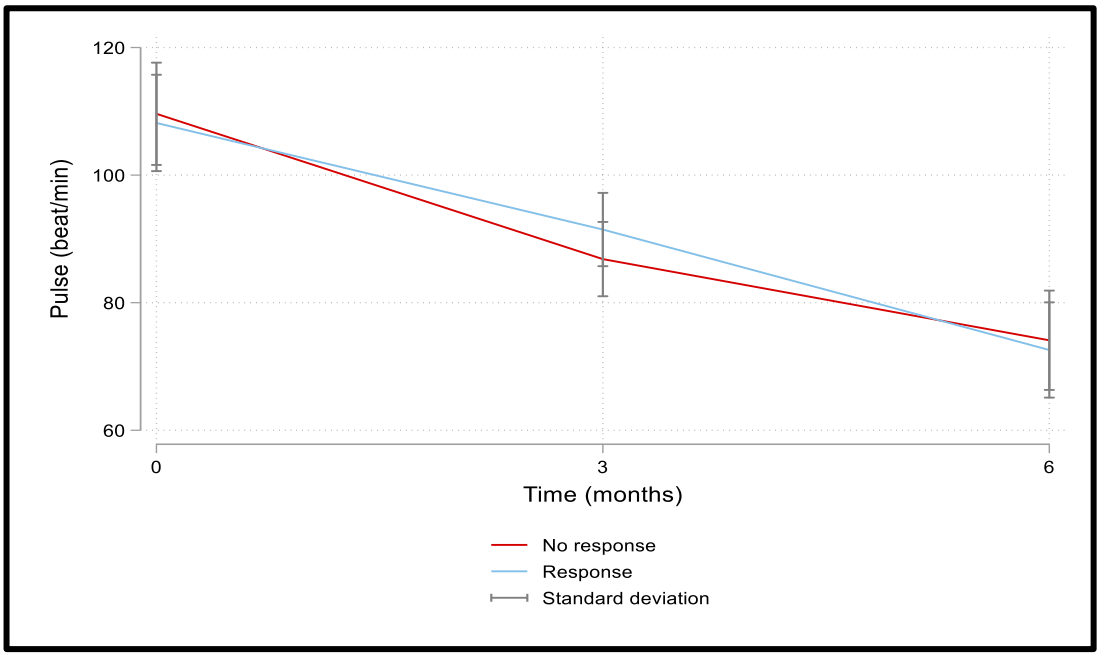


Figure 3. 28: Pulse at baseline, after 3 months, and after 6 months of intervention with verapamil for responders and non-responders.

To assess the patients' response to verapamil and the need for increasing the verapamil dose, the values of systolic and diastolic blood pressure and pulse for each participant were evaluated after 3 months and 6 months of the intervention. According to the systolic and diastolic blood pressure and the calculated mean arterial pressure and pulse rate of the participants after 3 months of intervention 8 participants (22.9%) had their dose increased to 240 mg (one tablet) once daily to control their hypertension. After 6 months, all of the participants reached the target blood pressure level (systolic blood pressure <140, diastolic blood pressure <90, and pulse rate <100 bpm) without the need for any further increase in their verapamil dose.

Blood pressure parameters were also assessed for responders after 12 months of verapamil intervention. The data of blood pressure and pulse parameters between 6 and 12 months after verapamil intervention were analysed using the random effect

regression with no significant difference (*P*= 0.62 and 0.8, respectively), with all participants achieving target levels (systolic blood pressure <140, diastolic blood pressure <90, and pulse rate <100 bpm) without need for further increase in verapamil dose or causing unwanted side effects such as hypotension (Figures 3.29 and 3.30).

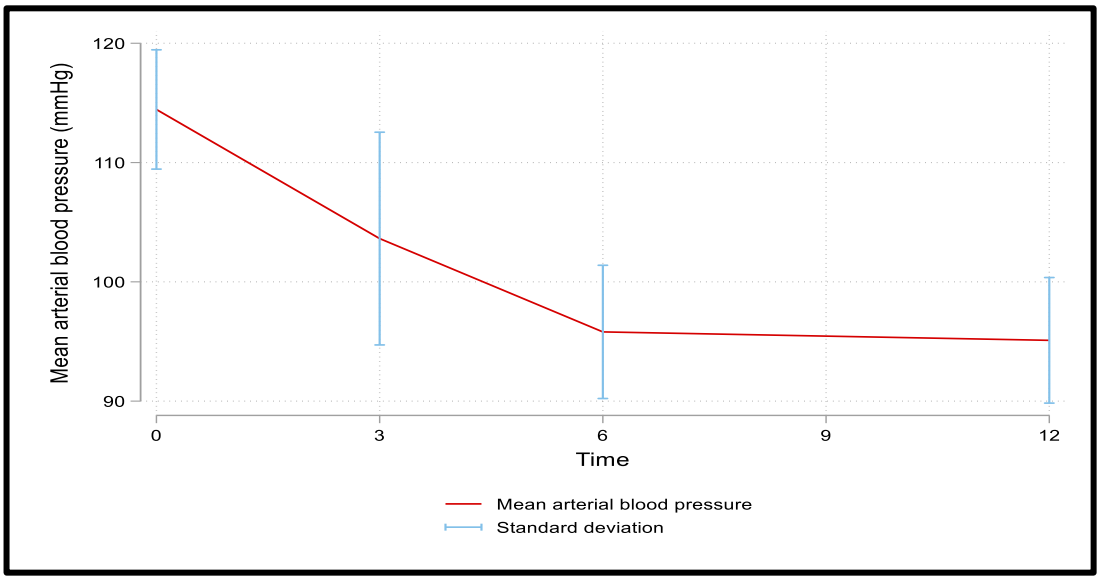


Figure 3. 29: The change in the mean arterial blood pressure in responders.

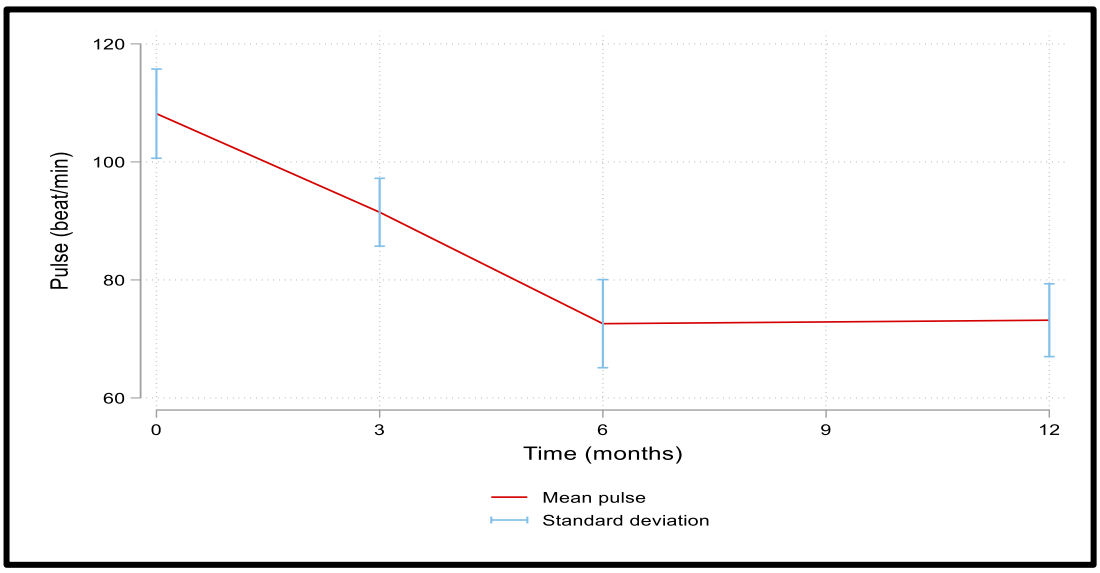


Figure 3. 30: The change in mean pulse rate in responders.

3.2.2 Verapamil side effects

Verapamil's side effects were assessed after 3 months of therapy using the side-effect monitoring data sheet (Figure 2.6). Verapamil was well tolerated by most patients, with one patient reported nausea (2.85%). Another reported fatigue (2.85%) and one experienced a headache (2.85%). All of the adverse events were transient and spontaneously resolved, with no treatment interruption. There were no serious side effects that required medical intervention or discontinuing verapamil (Table 3.11). Another assessment was done after 6 months of verapamil intervention; the side effects of verapamil were assessed using the side-effect monitoring data sheet (Figure 2.6). Verapamil was well tolerated by most patients, with only two patients (5.71%) reported constipation and one reported dizziness (2.85%). One patient reported fatigue (2.85%) and another experiened a headache (2.85%). All of the adverse events were transient and spontaneously resolved, with no treatment interruption. There were no serious side effects that required medical intervention or discontinuing verapamil as shown in Table 3.11.

3.2.3 Monitoring renal and liver function

Mean liver function tests (AST, ALT, bilirubin, and albumin) and renal function tests (serum creatinine and GFR) at baseline, 3 months, and after 6 months of intervention are shown in Figures 3.31-3.36, respectively and all of them were within the normal level. Random effect regression was done and showed that there was a significant increase in GFR and decrease in serum creatinine after 6 months of verapamil intervention (Table 3.12). In addition, Serum bilirubin and albumin were significantly decreased after 6 months of intervention (Table 3.12).

Table 3. 3: Incidence of verapamil side effects

Adverse drug reactions	n (%) at 3 months	n (%) at 6 months
Constipation	0	2 (5.71)
Dyspnea	0	0
Dizziness or light headedness	0	1 (2.85)
Bradycardia	0	0
Hypotension	0	0
Nausea	1 (2.85)	0
AV block	0	0
Headache	1 (2.85)	1 (2.85)
Oedema	0	0
Rash	0	0
Flushing	0	0
Fatigue	1 (2.85)	1 (2.85)
Elevated liver enzyme	0	0
Abnormal ECG (PR interval prolongation)	0	0
Heart burn	0	0
Swelling of hand, feet, ankle, or lower legs	0	0
Difficulty in breathing or swallowing	0	0
Fainting	0	0
Blurred vision	0	0
Rash	0	0
Extreme tendreness or joint pain	0	0
Unusual bleeding or bruising	0	0
Loss of appetite	0	0
Pain in the upper part of stomach	0	0
Yellowish of skin or eyes	0	0
Flu-like symptoms	0	0
Sleep disturbance	0	0

*Reproduce medilineplus,2021 [208]

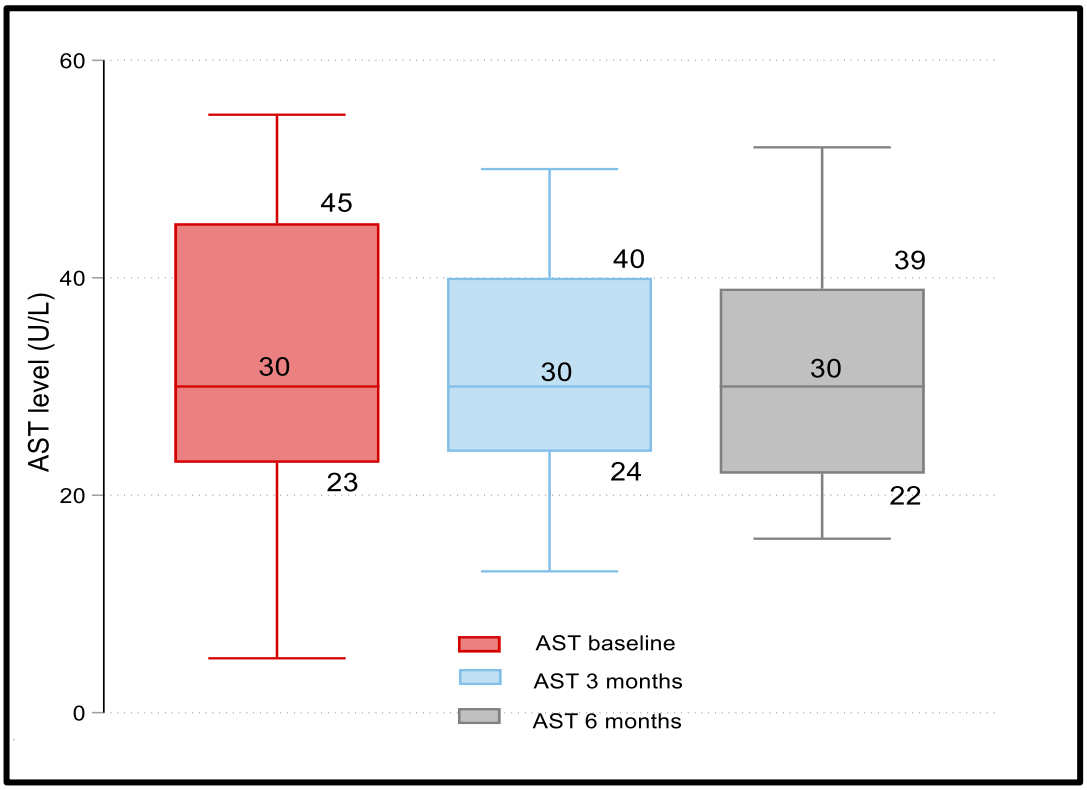


Figure 3. 31: Box plot of AST at baseline, 3 months, and 6 months of intervention with verapamil.

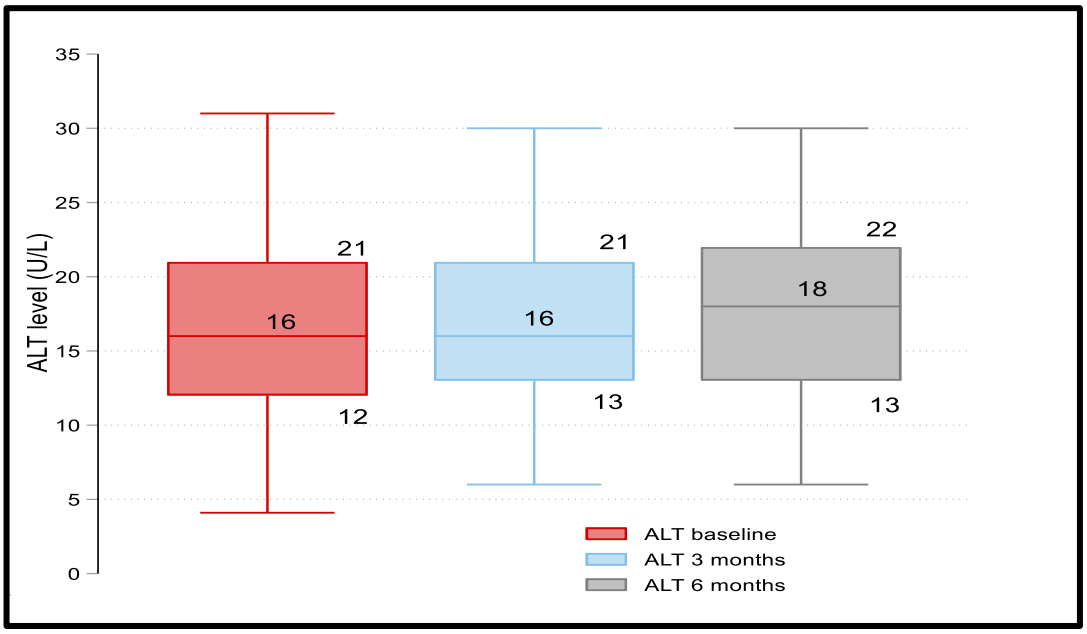


Figure 3. 32: Box plot of ALT at baseline, 3 months, and 6 months of intervention with verapamil.

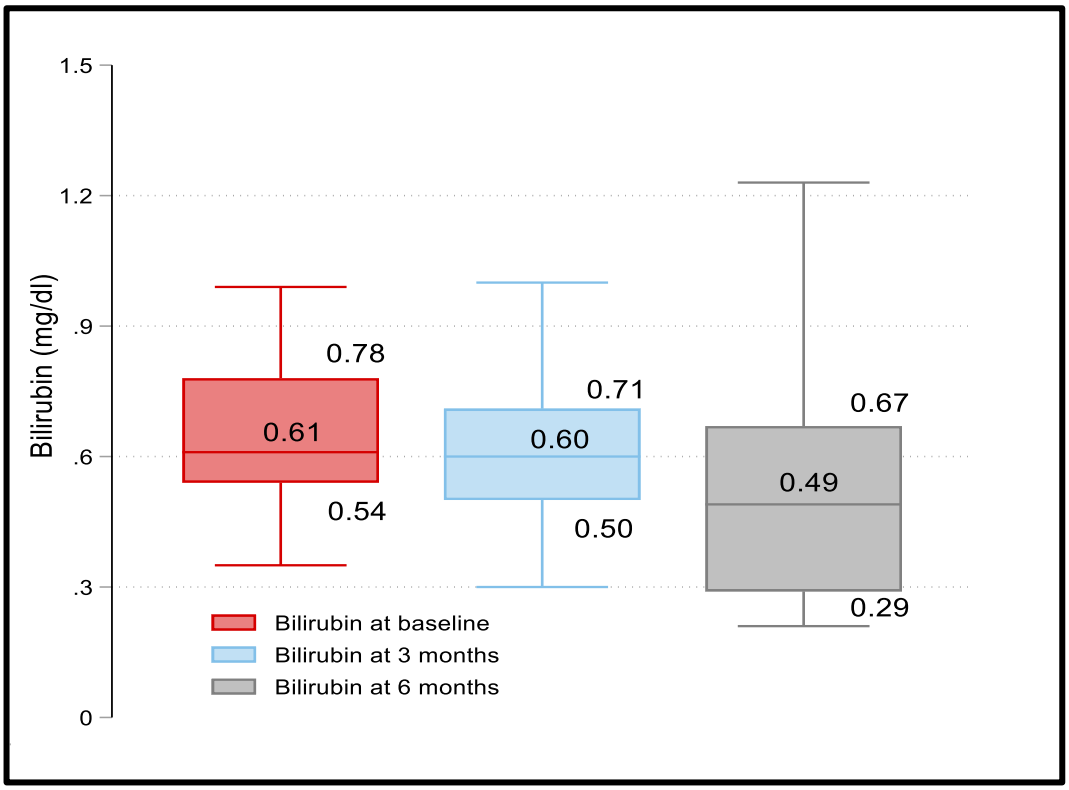


Figure 3. 33: Box plot of bilirubin at baseline, 3 months, and 6 months of intervention with verapamil.

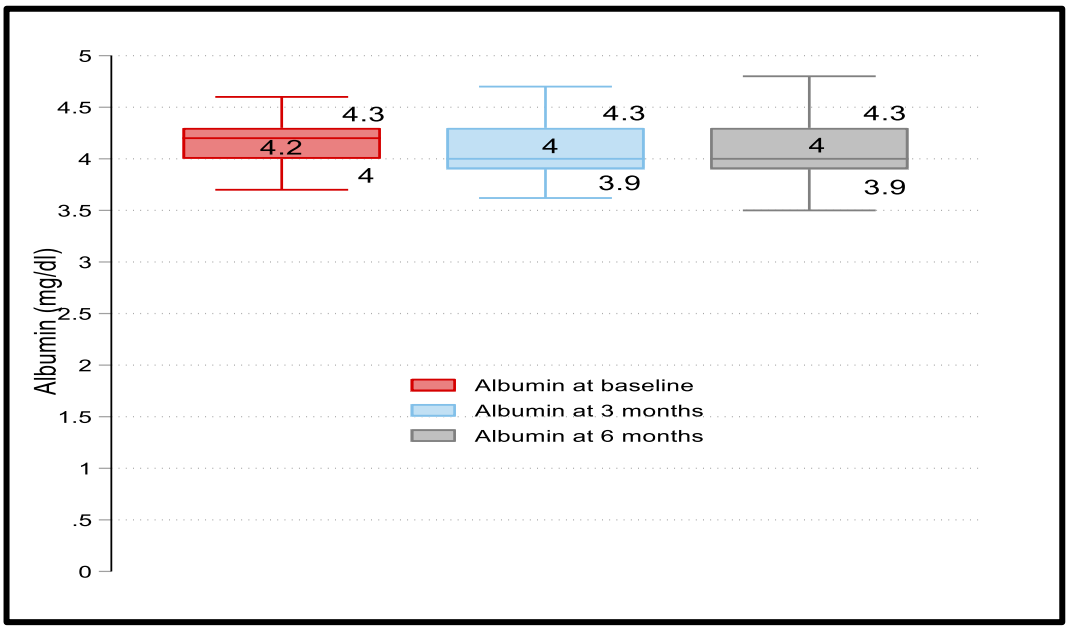


Figure 3. 34: Box plot of albumin at baseline, 3 months, and 6 months of intervention with verapamil.

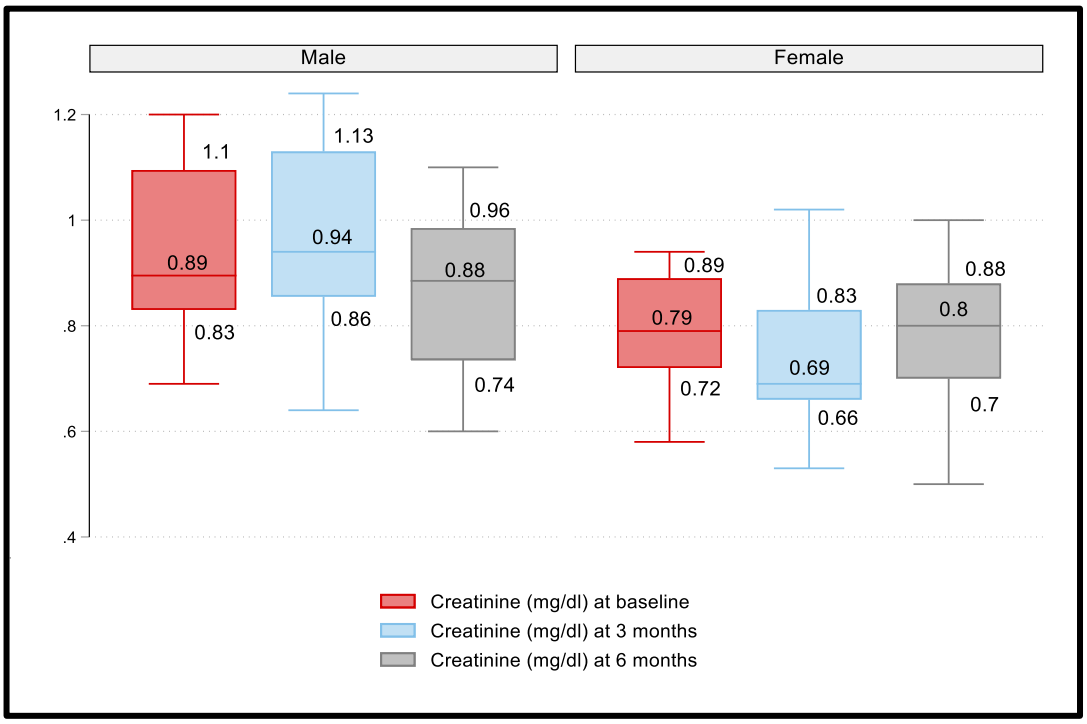


Figure 3. 35: Box plot of serum creatinine at baseline, 3 months, and 6 months of intervention with verapamil.

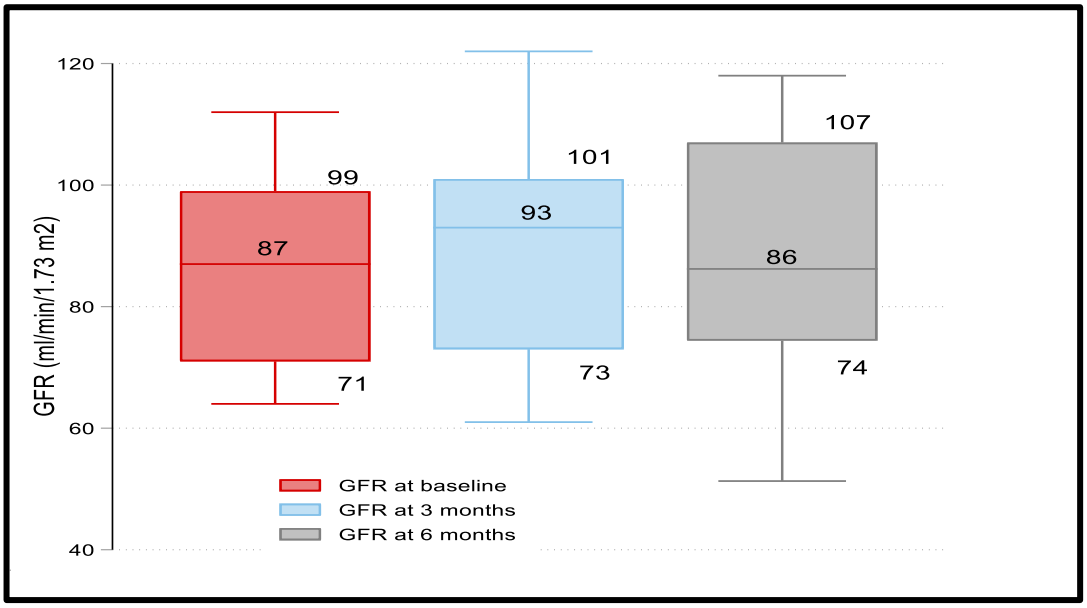


Figure 3. 36: Box plot of GFR at baseline, 3 months, and 6 months of intervention with verapamil.

Table 3. 4: Renal and liver function tests of participants after 6 months of intervention

Organ function	Coefficient (95% CI)	P value
Renal profile tests		
• **Serum creatinine** o **Female (0.6–1.1 mg/dL)** o **Male (0.70–1.30 mg/Dl)**	 -0.01 (-0.02- 0.001) -0.14 (-0.22- -0.06)	 0.04 <0.001
• **Glomerular filtration rate (GFR) (≥ 90 mL/min/1.73 m²)**	0.99 (0.004- 1.98)	0.049
Liver profile tests		
• **Plasma Total Bilirubin (0.3-1.1 mg/dl)**	-0.02 (-0.04- -0.01)	0.001
• **Plasma AST (11-47 U/L)**	-0.26 (-0.66- 0.13)	0.19
• **Plasma ALT (7-53 U/L)**	0.13 (-0.63- 0.38)	0.62
• **Serum albumin (3.5-5 g/dl)**	-0.02 (-0.04- -0.003)	0.02

*Normal range for Glomerular filtration rate; GFR[205] , ** Normal ranges according to Transitions of Care in Pharmacy Casebook.[206] ALT, alanine aminotransferase; AST, aspartate aminotransferase.

3.2.4 Monitoring changes in patient lipid profile whilst on study

Mean lipid profile (cholesterol, LDL, HDL, TG) at baseline, after 3 and 6 months of intervention are shown in Table 3.13 and Figure 3.37-3.40 and all of them were within the normal level. Random effect regression showed no significant differences in any of the lipid profile tests over the study period ($p>0.05$; Table 3.14).

Table 3. 5: The mean of cholesterol, HDL, LDL and triglycerides at baseline, 3 months and 6 months.

Lipid profile	Baseline (mean±SD)	After 3 months (mean±SD)	After 6months (mean±SD)	*P*-value
Cholesterol (mg/dL)	75.13± 21.49	73.65± 19.96	74.05± 17.37	0.75
HDL (mg/dL)	37.10± 5.23	37.47± 4.72	37.56± 4.26	0.56
LDL (mg/dL)	52.9± 30.98	49.55± 27.01	51.45± 22.79	0.64
TG (mg/dL)	34.49± 28.25	33.86± 36.4	34.62± 25.36	0.96

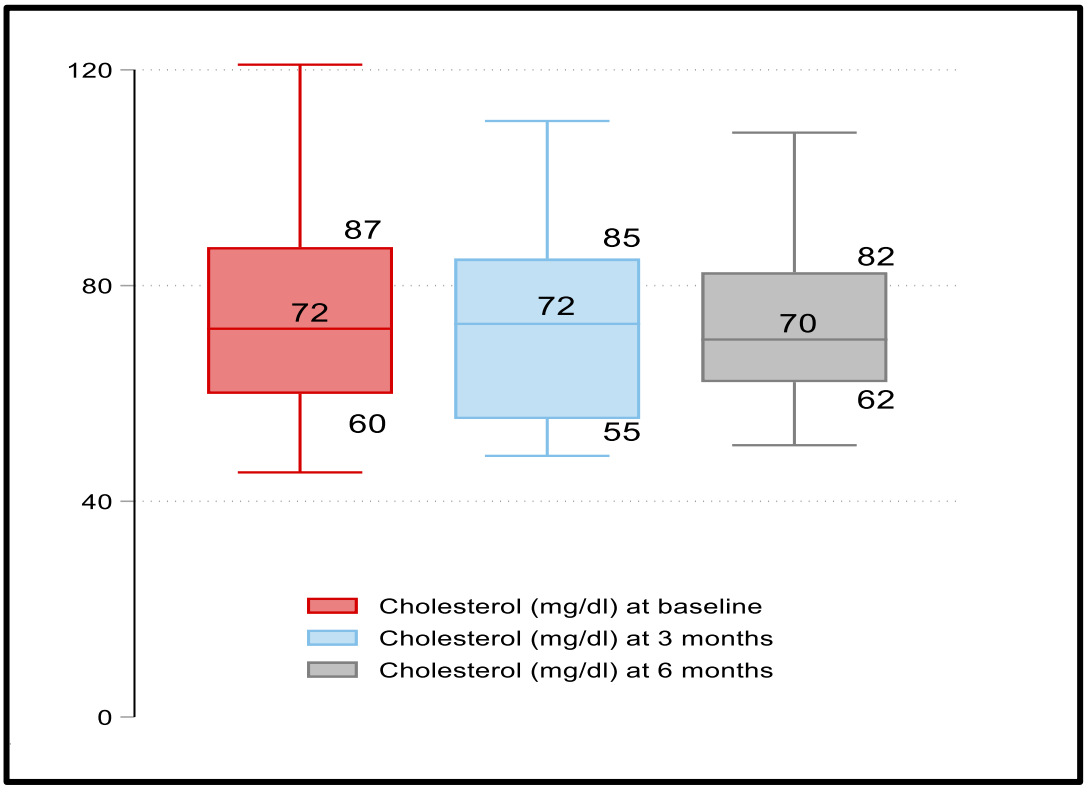


Figure 3. 37: Mean cholesterol level at baseline, after 3 and 6 months of intervention verapamil.

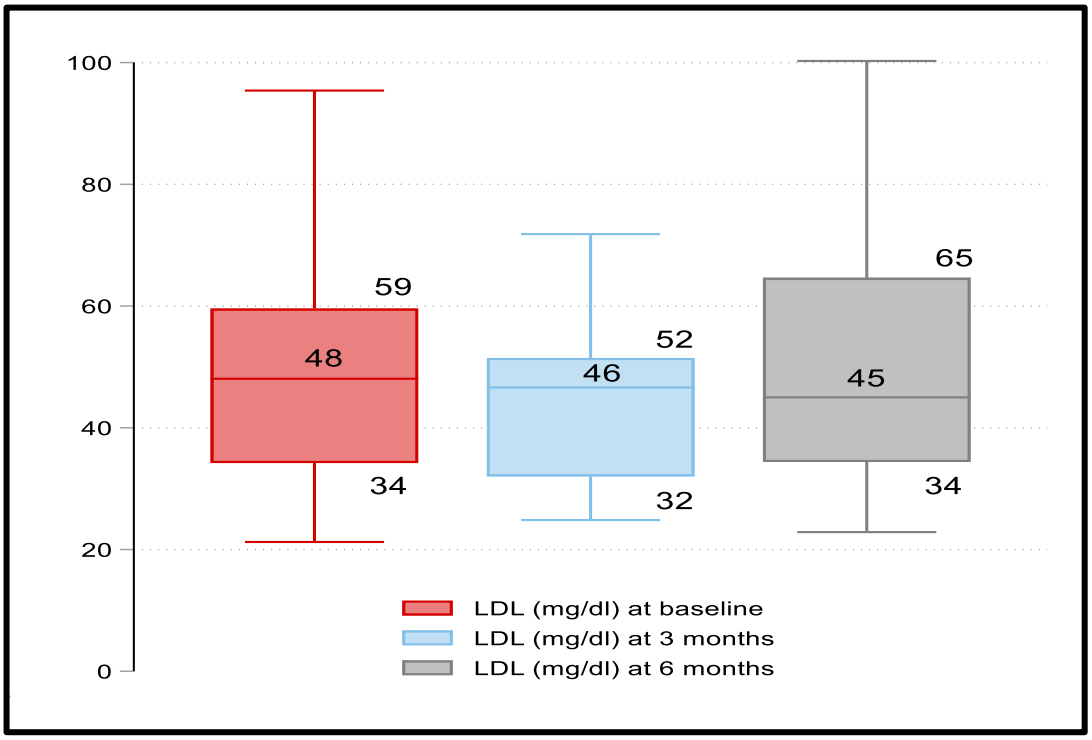


Figure 3. 38: Mean LDL level at baseline, after 3 and 6 months of intervention verapamil.

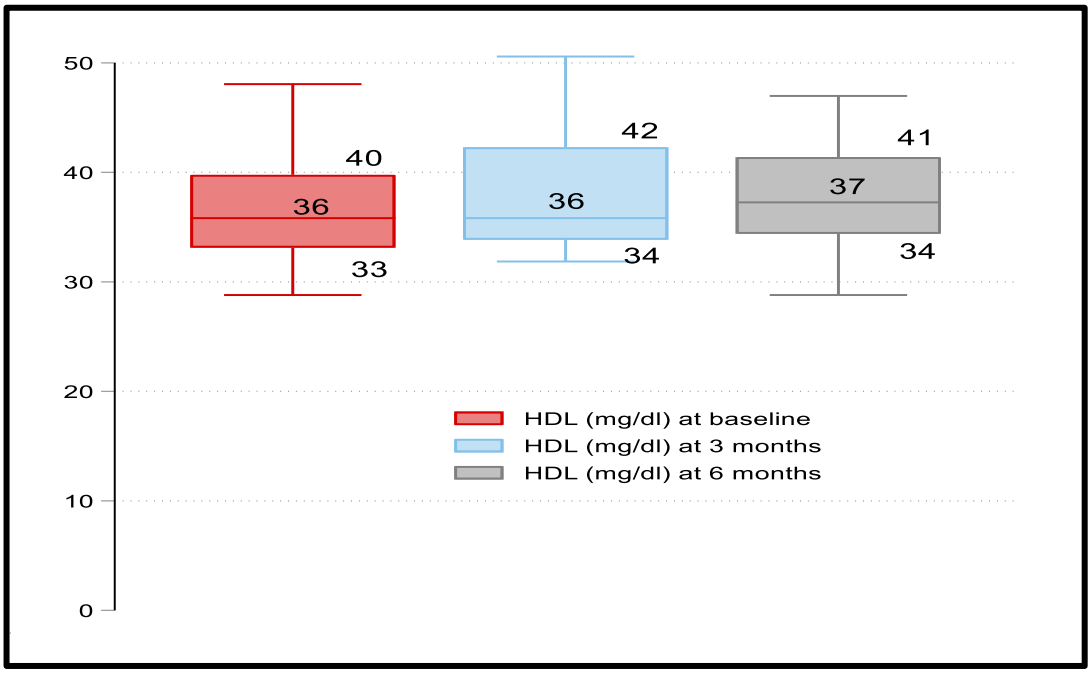


Figure 3. 39: Mean HDL level at baseline, after 3 and 6 months of intervention verapamil.

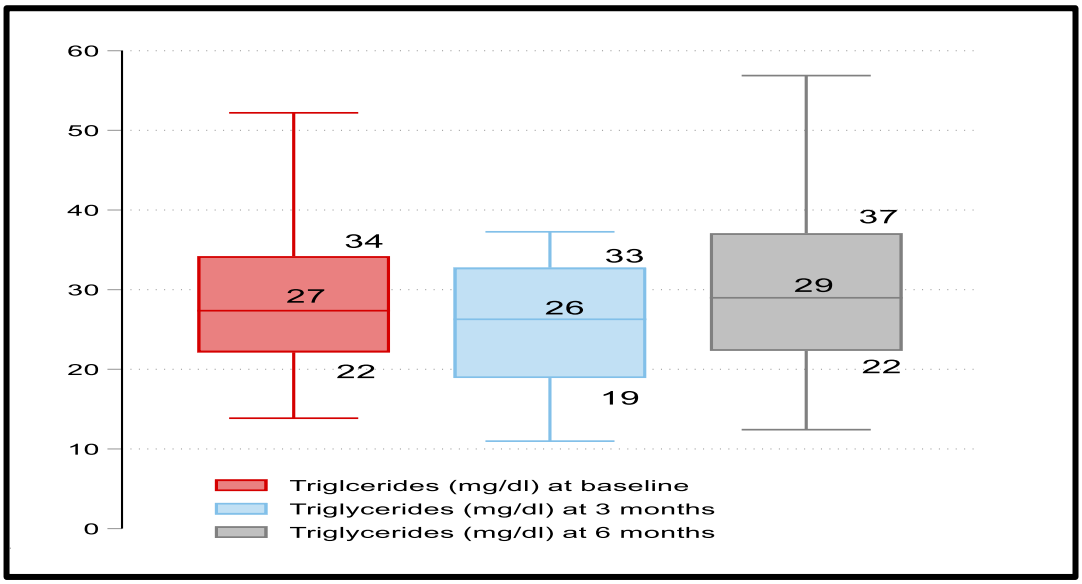


Figure 3. 40: Mean triglyceride level at baseline, after 3 and 6 months of intervention verapamil.

Table 3. 6: Mean difference of lipid profile tests at baseline and 6 months of intervention with verapamil.

Lipid profile	Coefficient (95% CI)	*P
Cholesterol mg/dl	-0.18 (-1.28- 0.92)	0.75
HDL mg/dl	0.08 (-0.18- 0.34)	0.56
LDL mg/dl	-0.24 (-1.25- 0.76)	0.64
TG mg/dl	0.02 (-0.85- 0.89)	0.96

**P*-value is for random effect regression

3.2.5 Monitoring changes in patient medication whilst on the study

There were no changes in anti-diabetic medication throughout the study period. Also, there were no changes in anti-hypertensive medication except for verapamil. The dose of verapamil was changed from 120 mg to 240 mg in eight patients as they required a higher dose to control their blood pressure. Some changes were undertaken to the other medications after 3 months of intervention as shown in Table 3.15.

Table 3. 7: Medication taken by participants throughout the study period.

Medications	Frequency*	N (baseline) (%)	N (3 months) (%)	N (6 months) (%)	% Of change from baseline
Anti-hypertensive medications					
Lisinopril 20 mg	OD	15(42.9)	15(42.9)	15(42.9)	0.
Irbesartan 150 mg	OD	4(11.4)	4(11.4)	4(11.4)	0.
Irbesartan 300 mg	OD	11(31.4)	11(31.4)	11(31.4)	0.
Perindopril arginine 20 mg	OD	2(5.7)	2(5.7)	2(5.7)	0.
Candesartan 8 mg	OD	3(8.6)	3(8.6)	3(8.6)	0.
Verapamil 240 mg	OD	0	0	8	0
Verapamil 120 mg	OD	0	35	27	0
Anti-diabetic medications					
Metformin 1 gm	BID	20 (57.1)	20 (57.1)	20 (57.1)	0.
Metformin 500 mg	BID	5 (14.3)	5 (14.3)	5 (14.3)	0.
Metformin 500 mg	TID	10(28.6)	10(28.6)	10(28.6)	0.
Insulin aspart 100 units/ml, 3 ml flexpen	OD	10(28.6)	3(8.6)	3(8.6)	0.
Insulin glargine 100 units/ml, 3 ml pen	OD	17(48.6)	15(42.9)	15(42.9)	0.
Liraglutide 18 mg/3ml (3ml) pen injector	OD	8 (22.9)	8 (22.9)	8 (22.9)	0.
Sitagliptin 100 mg	OD	14(40.0)	14(40.0)	14(40.0)	0.
Glibenclamide 2.5 mg	OD	1 (2.9)	1 (2.9)	1 (2.9)	0.
Glibenclamide 5 mg	OD	3(8.6)	3(8.6)	3(8.6)	0.
Gliclazide MR 60 mg	OD	7 (20.0)	7 (20.0)	7 (20.0)	0.
Glimepiride 2 mg	OD	1(2.9)	1(2.9)	1(2.9)	0.
Anti-lipidemic medications					
Atorvastatin 10 mg	OD	22(62.9)	22(62.9)	22(62.9)	0.
Atorvastatin 20 mg	OD	3(8.6)	3(8.6)	3(8.6)	0.
Other medications					
Vitamin B complex	OD	10(28.6)	11(31.4)	11(31.4)	-2.8
Choleciferol (vitamin D_3) 50000 IU	OD	26(74.4)	25(71.4)	25(71.4)	0.
Calcium carbonate 600 mg	OD	26(74.4)	27(77.1)	27(77.1)	-2.8
Aspirin 81 mg EC	OD	28(80.0)	28(80.0)	28(80.0)	0.
Multivitamin and minerals	OD	13(37.1)	14(40)	14(40)	-.2.9
Alendronate 70 mg	OD	1(2.9)	1(2.9)	1(2.9)	0.
Carbimazole 5 mg	OD	1(2.9)	1(2.9)	1(2.9)	0.
Omeprazole 20 mg	OD	4(11.4)	5(14.3)	5(14.3)	-.2.9
Esomeprazole 20 mg	OD	8(22.9)	7(20)	7(20)	2.9
Natural tear eye drop	QID	26(74.4)	27(74.4)	27(74.4)	-2.8

*OD, Once a day; BID, Two time per day; TID, Three time per day, N, number of participants

3.2.6 Monitoring adherence to treatments

Adherence level at baseline was detected to assess the participant adherence to their anti-hypertensive medication. Thirty-two participants (91.4%) had a high level of adherence, with an MMAS-8 score of 8.

Three participants (8.6%) scored either 6 (n=1) or 7 (n=2) which is considered an average level. Adherence to verapamil after 3 months, was high for 33 participants (94.3%), with an MMAS-8 score of 8. However, two participants (5.7%) had scored 7 (n=2) which considered an average level of adherence. Adherence to verapamil after 6 months, was high for 34 participants (97.1%), with an MMAS-8 score of 8. Only one participant (2.9%) had scored an average level of adherence (score of 7) (Table 3.16). There was a non-significant change in the adherence score (0.01 (-0.003- 0.3). *P*= 0.1) with no difference in adherence between responders and non-responders 0.05 (-0.1- 0.2), *P*=0.49)

Table 3. 8: The number and percentage of participants in each adherence level.

	High level (Score of 8)	Average level (Score of 7)	Average level (Score of 6)	Poor level (score <6)
At baseline (N, %)	**32 (91.4%)**	**2 (5.7%)**	**1 (2.9%)**	**0**
After 3 months (N, %)	**33 (94.3%)**	**2 (5.7%)**	**0**	**0**
After 6 months (N, %)	**34 (97.1%)**	**1 (2.9%)**	**0**	**0**

3.2.7: Monitoring BMI

As shown in Figure 3.41, the mean of BMI at baseline, after 3 months, and 6 months of intervention were very similar. After 6 months of intervention, participants' BMI

did not significantly change from the baseline with a mean difference of 0.1±0.5 Kg/m^2 (P=0.163). A box and whisker plot of BMI values at baseline and after 6 months of intervention with verapamil is shown in Figure 3.42. The change in BMI was not significant (Coefficient: -0.02 (-0.05 to -0.01); P= 0.14)

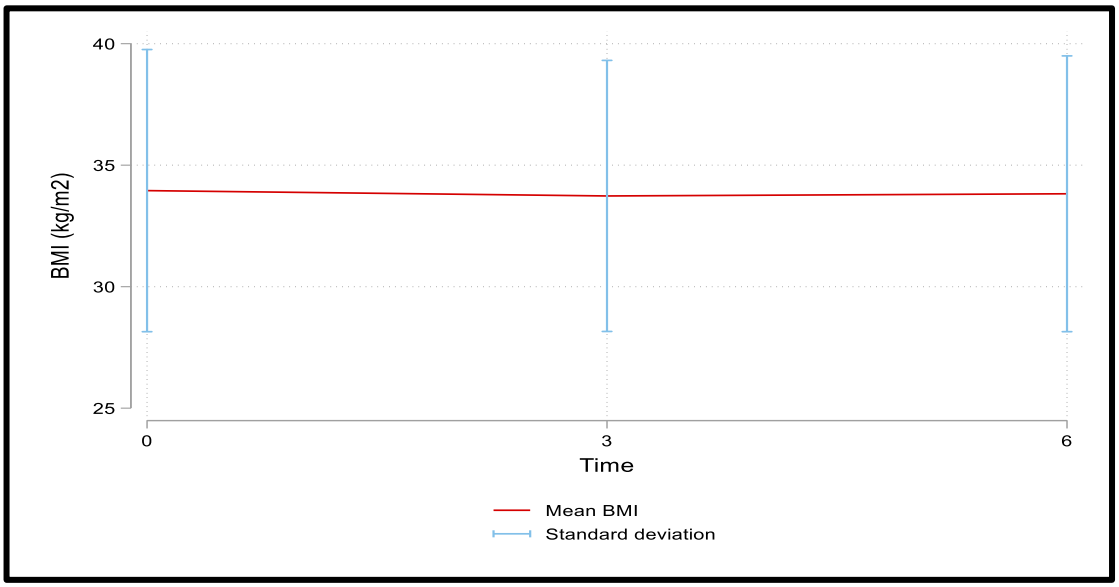


Figure 3. 41: Mean change in BMI in 3 and 6 months of therapy

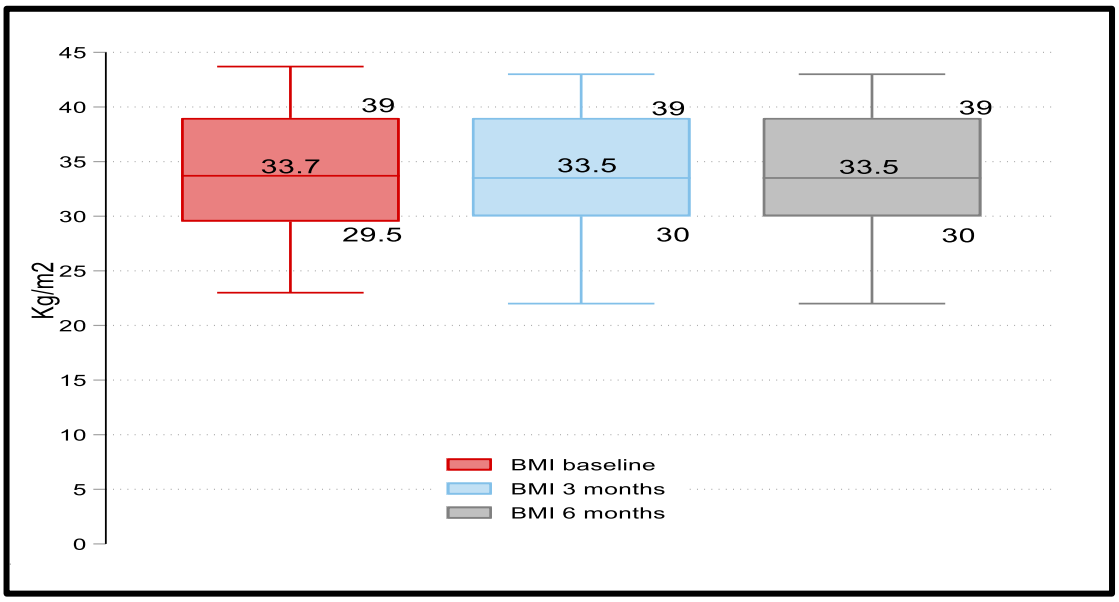


Figure 3. 42: Box and whisker plot of BMI values at baseline and after 3 and 6 months of intervention with verapamil.

Chapter 4

4.1 Discussion

In this study, the effect of verapamil on glucometabolic parameters in type 2 diabetic patients was evaluated after 6 months of verapamil use. We found that the use of verapamil in 35 type 2 diabetic patients was associated with a decrease in HbA1c, FPG, C-peptide, and HOMA-IR; however, the decrease did not reach a statistically significant level (P>0.05). Seventeen patients significantly achieved the required response to verapamil defined as a 0.5% decrease in HbA1c level. Univariable logistic regression showed that there were three factors significantly associated with the response; namely baseline BMI, HOMA-IR, and C-peptide (P<0.05). The change in HbA1c levels was affected by sitagliptin use, metformin dose, insulin use, duration of diabetes, neuropathy, and retinopathy. Additionally, insulin use was negatively associated with FPG levels but had no association with HOMA-IR and C-peptide levels. Verapamil was associated with improved renal function. However, it was associated with a decrease in albumin level.

Globally diabetes is a widespread disease and imposes a large economic burden.[3,7] Type 2 diabetes is the most common type and accounts for almost 90% of all diabetes cases.[3,22-24,209] The prevalence of type 2 diabetes in Saudi Arabia is 32.8%, and the predicted prevalence is 45.4% in 2030.[12] The coexistence of hypertension and diabetes is very common in KSA and globally.[53-56,210] Additionally, blood pressure control is an established strategy for preventing microvascular and macrovascular events in people with type 2 diabetes.[211-213] Therefore, identifying the dual therapeutic potential of an antihypertensive drug that may improve glycometabolic response in diabetic

patients' therapy is an appealing strategy. In addition, using combination therapy is very common in treating hypertension. Therefore, adding an antihypertensive drug that has a positive effect on blood glucose may help control the blood glucose level in diabetic patients.

Prior to this trial, the current evidence suggested that verapamil -one of the first-generation L-type non-dihydropyridine calcium channel blockers that have been widely used in clinical practice to treat hypertension disease- may have positive glycometabolic effect on type 2 diabetes mellitus.[67,70,83,84,182-188,193] Animal studies [83,84,188,193] were promising and offered the possibility of translating outcomes into humans to examine the pancreatic and extra-pancreatic effect of verapamil on TXNIP downregulation. Significant reduction in TXNIP mRNA expression in type 2 diabetic animal model by more than 70% improved insulin sensitivity, increased serum insulin levels, and reduced β-cell apoptosis. Verapamil was well tolerated, with no significant side effects recorded in animal studies.[188] At the initiation of this research, data about glycometabolic effects of verapamil in humans were limited.[183,185,187] Those studies had been explored in various diabetic populations with different trial designs and primary outcomes Table 4.1.

Table 4. 1: Summary of the previous studies regarding the glycometabolic effect of verapamil drug

study	Aim	Design	Participants	Age	intervention	Main outcomes results
Holzgreve.et al. 2003 [186]	Compared the effect of antihypertensive combination therapy with verapamil plus trandolapril versus atenolol plus chlorthalidone on HbA1c.	Randomized, double-blind trial.	463 subjects, type 2 diabetes and mild-to-moderate hypertension.	40-80 years	Combination of verapamil SR 180 mg plus 1 mg trandolapril versus atenolol l50 mg plus chlorthalidone 12.5 mg for 20 weeks.	• The mean difference between the two combinations in HbA1c was -0.79 % (-1.04 to -0.54) *P* = .0001), in FPG was -1.17 mmol/L (95% CI 1.64 to _0.70) *P*= .0001, in systolic blood pressure was 4.85 mmHg (CI 1.94-7.76) *P*=0.11, and diastolic blood pressure was 1.79 mmHg (CI 0.26-3.32) *P*=0.022.
Fernandez et al 2001 [194]	Compare, at equal blood pressure (BP) reduction, the effect of two different antihypertensive combinations on metabolic control and albuminuria.	Prospective, randomized, double-blind, Controlled trial.	103 type 2 diabetic patients with stable albuminuria with uncontrolled blood pressure on monotherapy antihypertensive drugs.	54.9±9.3 years	Combination of verapamil SR/trandolapril 180/2 mg (VT) versus combination of Enalapril/hydrochlorothiazide 20/12.5mg (EH) for 6 months.	• HbA1c, ANOVA interaction between the two combinations. *P*=0.040). • FPG (ANOVA, *P* 0.018). • BP and albuminuria were both significantly decreased (*P* < 0.1) without significant differences between treatments.

Khodneva et.al [183]	Examined associations between the use of CCBs in general and verapamil specifically on FPG.	Observational cross-sectional study	4987 adults with diabetes, included 1484 (29.6%) CCBs users, of which 174 (3.4%) were verapamil users.	45 years and older	Observational study comparing verapamil to CCBs non-users	• Verapamil users group had significantly decreased FPG -9.6 mg/dL lower compared to CCBs non-users group.
Bush et al 1991 [182]	To investigate the effect of verapamil on FPG	Prospective single-blind placebo-controlled cross-over study	10 participants normotensive type 2 diabetic patients	29-65 years	Five patients received verapamil-SR 240 mg twice a day versus 5 patients who received a placebo for 7 days	• C-peptide was not significantly different during placebo and verapamil groups. • FPG, the mean difference between verapamil and placebo groups was 1.3mmol/L, P = <0.05
Rubio et al 2004[195]	To compare the effect of fixed-dose trandolapril-verapamil (FDTV) with that of trandolapril on proteinuria.	Prospective open-label randomized study	60 normotensive, type 2 diabetic patients with proteinuria	The mean age in FDTV and trandolapril groups were 52.5 and 55 years respectively	2 mg trandolapril/180 mg verapamil FDTV once daily versus 2 mg trandolapril once daily	• FPG was significantly lower in the FDTV group (139 ±19) compared with the trandolapril group (154±22; $P < 0.001$). • No significant differences were observed between the two groups in mean baseline or final measurements of diastolic and systolic blood pressure, mean heart rate, or frequency of adverse events.

Based on what research had been undertaken it was uncertain whether verapamil had any useful antidiabetic activity and/or whether any antidiabetic activity was a consequence of a direct effect on pancreatic production of insulin. Therefore, the current clinical trial was designed to measure the effects of verapamil on C-peptide, a biochemical marker for endogenous insulin secretion, and biochemical parameters that reflect glycaemic control, namely – HbA1c, FPG, and HOMA-IR in type 2 diabetic hypertensive patients.

4.1.1 Effect of verapmil on HbA1c

HbA1c is the major biochemical parameter for assessing glycaemic control and has a strong predictive value for diabetic complications and reflects average glycaemia over a period of approximately 3 months.[27-33] HbA1c measurement can be performed at any time of the day and does not require any special patient preparation such as fasting.[27,32,33,36] In addition, the test reproducibility and ability to predict longer-term clinical outcomes of HbA1c are high.[31,37]

The results of the current study indicate that after six months of intervention, verapamil had no significant effect on HbA1c, with the mean difference before and after treatment being 0.2±1.0% (P =0.25). Two previous human studies that evaluated the effect of verapamil on HbA1c values reported results comparable to the result of the current study in respect to verapamil being metabolically neutral on HbA1c levels in type 2 diabetic hypertensive patients.[186,194] It is hard to make a direct comparison between their results and the current study result since the previous studies[186,194] investigated the effect of different antihypertensive combinations on glycaemic control and showed that antihypertensive combination that included verapamil was able to

stabilize the HbA1c level compared to other antihypertensive combinations that did not include verapamil.

The first study [186] included 463 hypertensive diabetics (type 2) patients that were randomly assigned to use once daily combinations of either 180 mg verapamil SR plus 1 mg trandolapril or 50 mg atenolol plus 12.5 mg chlorthalidone. At the end of the study, the verapamil and trandolapril combination group had remained glycaemically stable and there were no significant changes in HbA1c values (mean±SD) before and after treatment (7.9±1.17%, and 7.9±1.42% respectively) compared to the group that used the combination of atenolol and chlorthalidone (HbA1c increased from 7.8±1.26% baseline to 8.6±1.77%; P = 0.0001). The increase of HbA1c mean levels after using a combination of β-blockers and thiazide-like diuretic is considered clinically significant amongst most physicians in a diabetes care setting and such a combination is known to deteriorate glucose homeostasis.[156,214-216] This can happen due to different mechanisms such as decreasing the sensitivity to insulin and/or the release of insulin from pancreatic β cells in response to glucose [215,216] as well as weight gain [217] which leads to insulin resistance.[216,218] Also, increasing mean HbA1c after using chlorthalidone can affect glucose homeostasis by decreasing the blood flow within muscles leading to insulin resistance in skeletal muscles.[219]

The second study was performed by Fernandez et.al.[194] and evaluated the effects of the combination of verapamil SR/trandolapril 180/2 mg versus the combination of enalapril/hydrochlorothiazide 20/12.5 mg on 103 type 2 diabetic patients with a mean age 54.9±9.3 years with uncontrolled blood pressure for 6 months and each combination was used once daily. All patients were counselled to maintain the same antidiabetic therapy throughout the study. The HbA1c mean level did not change in

verapamil SR/trandolapril combination therapy group (5.91±1.43% pre-treatment and 5.94 ±1.62% post-treatment; P>0.05) but increased in enalapril/hydrochlorothiazide therapy group (5.96±1.25% pre-treatment and 6.41±1.51 post-treatment; *P*=0.04). The increase in the HbA1c mean level after using diuretic (hydrochlorothiazide) was anticipated since hydrochlorothiazide can affect glucose homeostasis. [156,219-225]

The results of the current study showed a reduction in the mean levels of HbA1c after 6 months of verapamil therapy but did not reach a significant level. However, data observation revealed that approximately half of the study sample had a significant improvement (> 0.5% reduction) in their HbA1c value. Therefore, based on improvement in HbA1c, study group were divided into responder and non-responder group. The responder group (n=17, 48.6%) had a statistically significant response to therapy following 6 months of verapamil with a mean difference (mean ± SD) of 0.9±0.4%, *P*<0.001 while the non-responder, showed a negatively significant difference with a mean difference of -0.5±0.80, *P*<0.001). The current study is not well powered to perform subgroup and multivariable analysis. Therefore, all factors and independent variables affecting the response were not well explored. To examine the association between participants' characteristics and response, univariable regression was conducted and showed that good response was associated with higher BMI, HOMA-IR, and C-peptide at the baseline.

Moreover, the factors affecting the change in HbA1c levels in all patients were evaluated. Sitagliptin use was associated with lower HbA1c level; however, insulin, higher metformin dose, longer duration of diabetes, higher baseline FPG levels, retinopathy, and neuropathy were associated with higher HbA1c.

A recent randomized, double-blind, placebo-controlled study [197] done by Malayeri et. al. and includes non-insulin type 2 diabetic patients who were only on two oral antidiabetic medications (sitagliptin and metformin). Malayeri et. al study aimed to evaluate the efficacy and safety of oral verapamil administration in 44 patients between 40 and 67 years who were diagnosed with diabetes for at least 5 years. There was no disclosure regarding the blood pressure condition of participants, verapamil tolerance, and relevant side effects such as hypotension. In their study, patients were randomized to either 120 mg verapamil -SR (120mg) or placebo. Malayeri et. al. study[197] showed a significant reduction in HbA1c mean level in non-insulin user patients receiving 120 mg verapamil of 0.5% after 3 months (P=0.047). (Table 4.2)

Malayeri et al. study[197] is a randomized trial performed in non-insulin user type 2 diabetic patients with no disclosure if participants were hypertensive or normotensive; however, the current study is a single arm research that included diabetic hypertensive insulin and non-insulin users. The current study found that insulin use was associated with higher HbA1c level but we performed univariable analysis. Therefore, the change in the HbA1c level could have affected by other confounders other than insulin such as duration of diabetes. The current study gives an insight that the response to verapamil may be affected by insulin use; a factor that should be considered in designing future studies. Also, in the Malayeri et al. study, BMI was 27, however, in the current study the participants were obese on average, their BMI was 34 ± 5.8 kg/m^2. This difference may reflects that as the current study participants had higher BMI so they had more insulin resistance and consequently less residual pancreatic function than participants in the Malayeri et al. study[197].

Table 4. 2: Comparision between the current study and the Malayeri et al study.

Study	Current study	Malayeri et al study[197]
Aim	To evaluate the effect of verapamil on glycaemic control in a hypertensive type 2 diabetic patients.	To evaluate the efficacy and safety of oral verapamil administration in type 2 diabetic patients.
Design	Open, uncontrolled Interventional Quasi Experimental study	Randomized, double blind, placebo-controlled study
Participants	35 Type 2 diabetes patients with uncontrolled hypertension	A total of 44 patients with type 2 diabetes
Age	Between 40 and 71 years	Between 40 and 67 years.
Duration since diagnosis	Range was 4-20 years	At least 5 years
Duration of study	6 months	3 months
Intervention	All participants received verapamil 120 mg SR tablet (ISOPTIN SR) depending upon individual patient need the dose was increased.	Participients randomlly allocated to recieve 120 mg verapamil -SR or placebo..
Main outcomes results	No significant decrease in HbA1c mean level and FPG after verapamil intervention (P>0.05). ○ The sub-analysis showed that non-insulin user was associated with significant reduction in HbA1c and FPG after 6 months (P<0.05).	1. No significant decrease in FPG mean level after verapamil intervention (P>0.05). 2. Significant dcrease in HbA1c in the verapamil group (P<0.05).

4.1.2 Effect of verapmil on FPG

The current study also investigated the effect of verapamil on FPG. Using FPG as a measurement for glucose control will not reflect long-term glycaemic control and the accuracy of the FPG value is not always guaranteed as it may be corrupted if people do not fast appropriately before measurement.[27,32,33,38] However, in some studies FPG was used as it is less expensive and more available than HbA1c. Therefore, the current study investigated the effect of verapamil on FPG to allow for the comparison between the current study results and the previous results of the studies that use the FPG as a measurement for glucose control. However, clinical information about the effect of verapamil on FPG is limited and heterogeneous with a different study design and sample size as shown in Table 4.1 which makes the comparison between the current study and the other available studies difficult.

The current study found that there were no significant effects of verapamil on FPG after 6 months of intervention compared to the baseline value, mean difference (mean±SD) was -0.5±1.8 mmol/L, P =0.11). Also, the current study found that non-insulin use was associated with a significant reduction in FPG levels after 6 months. Other factors that were associated with a significant changes in FPG levels were sitagliptin use and smoking status (decrease FPG), longer diabetes duration, insulin use, and higher metformin dose (increase FPG). Malayeri et al [197], reported a non-significant reduction in mean FPG in non-insulin user patients (44 participants) receiving 120 mg verapamil after 3 months (P=0.493) which is consistent with the current study. However, Fernandez et.al. [194] found that the difference in mean FPG level between both groups, verapamil SR/trandolapril 180/2 mg group versus enalapril/hydrochlorothiazide 20/12.5mg group, was statically significant (P =

0.018).[194] This difference is excepted as the increase in the FPG mean level after using diuretic (hydrochlorothiazide) was anticipated since diuretics may affect glucose homeostasis.

Also, a cross-sectional study done by Khodneva et al [183], found a statistically significant difference in FPG values between verapamil users (174 patients) and non-verapamil users (3494 patients) among hypertensive diabetic patients (mean difference was -9.6 mg/dL, *P* =0.03). However, when Khodneva and his colleagues[183] divided the participants into 4 sub-groups (not on medication, on oral antidiabetic drugs only, on oral antidiabetic drugs plus insulin therapy, and insulin therapy only), they found no statistically significant differences in FPG between verapamil users and non-users among all subgroups except for those on the combination of oral antidiabetic drugs and insulin therapy. In this group, verapamil users (43 out of 174) had on average -24.1 mg/dL lower FPG compared to non-verapamil users (799 patients) (*P* = 0.04). However, when they considered the participants who were only on oral antidiabetic medications, the reduction was not significant (mean difference 6 mg/dL (*P*= 0.19). Direct comparison between Khodneva et al. and the current research is difficult because of differences between study populations where the current research population comprising only type 2 diabetics while in Khodneva et al study[183], the study population was ill-defined but has been suggested to have an enriched type 2 diabetic population with no information on diabetes duration, disease severity and glycaemic control. Meaningful comparison is further compounded by imbalances in sample size between groups/subgroups and drug data e.g., verapamil dose and all drug(s) duration. Criteria for each sub-group were not determined e.g., what criteria the participants receiving insulin plus oral antidiabetic agents' combinations have

other than participants receiving only oral antidiabetic agents' combinations to understand the differences in their results. Also, information related to doses and how many oral antidiabetic drugs were used were not recorded for each subgroup. Also, FPG was the only measure undertaken to assess the glycometabolic effect of the verapamil making meaningful interpretation with the current clinical trial difficult.

Also, in another study[182] by Busch and his colleagues, undertaken on 10 normotensive type 2 diabetes patients who were only on diet to control their diabetes. They found that verapamil (240 mg twice a day for 7 days) significantly lowered FPG from a mean value of 11.6 mmol/L to 10.3 mmol/L (P <0.05). However, this result cannot be compared to the current study result because Busch et.al study[182] had a smaller sample size (10 patients), a higher verapamil dose (240 mg twice a day), and a shorter duration of treatment (7 days) than the current study. Furthermore, diabetic patients recruited to the Busch et.al. study were newly diagnosed, normotensive, and initiated diet control with no oral diabetes medication which different than the population of the current study. Also, they did not mention variability of the means nor present data on blood pressure before and after using verapamil. There was no disclosure regarding verapamil tolerance and relevant side effects such as hypotension, especially with such a high dose (240 mg) in normotensive individuals.

4.1.3 Effect of verapmil on C-peptide and HOMA-IR

As part of the current trial, C-peptide and HOMA-IR were measured. The current study found that C-peptide and HOMA-IR not significantly changed after 6 months of intervention with the mean difference (mean±SD) pre-and post-intervention being 0.1±0.3 nmol/L (P=0.06), and 0.3±0.9 (P =0.05) respectively. Univariable logistic regression showed that baseline BMI, HOMA-IR and C-peptide were significantly

associated with the response (P<0.05). High C-peptide levels and BMI at baseline were associated with increased HOMA-IR and, additionally, higher BMI was associated with higher C-peptide levels. Insulin use did not affect HOMA-IR and C-peptide levels and baseline HOMA-IR and C-peptide levels did not affect the change in HbA1c.

Upon to our knowledge, no previous study has measured the impact of verapamil on HOMA-IR values. In addition, the insulin level or C-peptide level should be available to be able to calculate the HOMA-IR theoretically and compare it with the current study result, Unfortunately, insulin levels has not been measured previously as a part of assessing the glycometabolic effect of verapamil on Type 2 diabetic patients. Bush et al study reported that C-peptide levels were not significantly different between placebo and verapamil treatment groups in noninsulin dependent diabetes participants however, the exact C-peptide values was not reported in the participants.[182]

Insulin resistance is postulated to begin in muscle tissue and muscle accounts for up to 70% of glucose disposal.[226] Therefore muscles are the main site for insulin resistance and muscle mass varies according to gender with men having higher skeletal muscle mass than women.[227]

Therefore, theoretically we could hypothesise that male patients, having generally higher muscles mass compared to females, should have had a better response to verapamil and higher insulin sensitivity level. However, in the current study, univariable logistic regression showed no association between verapamil response and gender (p>0.05).

Insulin resistance can affects the metabolism of lipids that promote an increase in free fatty acids level through different mechanisms.[226] One of these mechanisms is that insulin resistance leads to hyperinsulinemia and cause the decrease in ability of

lipoprotein lipase to metabolize triglycerides (TG) which lead to increases in serum triglycerides levels. Also, insulin resistance can lead to increased lipolysis which will result in increased flux of free fatty acid to the liver, leading to increased liver very low-density lipoprotein (VLDL) production. In addition, the insulin resistance can lead to increase fatty acids (FA) due to increased FA trapping by the high ability of the insulin-resistant adipocytes.

Verapamil is a known as inhibitor of CYP3A4 and if used with drugs that are metabolised by the cytochrome P-450 isoenzyme CYP3A4, drug interactions will occur. In the current study 25 participants were using simvastatin an anti-hyperlipidaemic drug and known to be metabolised primarily by CYP3A4. As known the concurrent use of verapamil with simvastatin lead to a known drug interactions which result in increasing the simvastatin level in the body and lead to increase incidence of simvastatin side effect, especially myopathy and more rarely rhabdomyolysis. However, this rarely happens if National Health Service (NHS) recommendation followed where the dose not exceed 20 mg daily.[228-230] In the current study, 22 patients were on simvastatin 10 mg and 3 patients were on simvastatin 20 mg. Patients' hyperlipidaemia was adequately controlled by using baseline simvastatin doses and there was no significant differences between baseline and end lipid profiles.

Muscle pain and tiredness were symptoms that were monitored throughout the study and no noticeable side effects attributable to statins were observed.

4.1.4 Long-term glycometabolic effects of verapamil

To examine the long-term glycometabolic effects of verapamil, 17 patients were followed up -who had a significant response to verapamil- for 12 months of verapamil

intervention. The results showed continuous reduction of HbA1c, FPG, C-peptide, and HOM-IR. However, the reduction was not significant between 6 and 12 months except for HbA1c values.

4.1.5 The effect of duration of diabetes on verapamil effect as glycometabolic agent

The longer the disease goes on the less residual pancreatic function and lose of glycaemic control as this shown in major clinical trials provide evidence of the increasing loss of glycaemic control over time in type 2 diabetes.[231-234] Unfortunately, no previous study had investigated the effect of duration of diabetes on verapamil glycometabolic effect for type 2 diabetes. Although, the U.K. Prospective Diabetes Study (UKPDS) showed that therapy with metformin, sulfonylurea, or insulin substantially lowered HbA1c but this effect reduced as duration of diabetes increased over 11 years.[232,233] Therefore, longer duration of diabetes may be a barrier for verapamil to give an appropriate glycometabolic effect. The result of the current study was consistent with the UKPDS as it showed that longer duration of diabetes was associated with higher HbA1c and FPG, with no effect on C-peptide and HOMA-IR. The mean duration of diabetes in the current study was 13 years and verapamil had neutral effects on glycometabolic parameters. However, further studies comparing newly diagnosed versus long duration type 2 diabetic patients are required to assess the effect of the duration of diabetes on the glycometabolic parameters.

4.1.6 Blood pressure response to verapamil therapy

Regarding the antihypertensive effect of verapamil, a review done on all antihypertensive classes found that the lowering BP effect of CCBs -including verapamil- are very comparable to other antihypertensive agents.[148] Also, according to JNC 8 [147] in case of diabetic hypertensive patients that are on monotherapy of ACEI or ARBs but are uncontrolled, adding a CCB is recommended. In the current study our participants were uncontrolled with ACEI or ARBs alone as some patients cannot tolerate the side effect of ACEI such as a dry cough therefore, the addition of a CCB was warranted. Also, in the current study, after initiating verapamil, there was a significant favorable decreases in blood pressure. The changes in the mean arterial pressure (MAP) in responders and non-responders after 3 and 6 months was assessed using random effect regression. MAP at baseline (113±4.5 mmHg), 3 months (101.2± 7.9 mmHg) and 6 months (94.4±6.2 mmHg). The change in the mean arterial pressure was significant after 6 month of verapamil intervention (coefficient: -3.11 (-3.45 to 2.76); P<0.001) with no difference between responders and non-responders (coefficient: 2.8 (-1.33 to 6.93); P=0.18). In addition, at the end of the study (6 months of intervention) all participants reached the target values without clinical hypotension being observed.

The chronotropic effects of verapamil on pulse rate where also assessed also, changes in pulse in responders and non-responders after 3 and 6 months was assessed using random effect regression. Mean pulse at baseline (108.9± 7.7 bpm), 3 months (89.1± 6.2 bpm) and 6 months (73.4± 7.6 bpm). There was a significant decrease in pulse (-5.9 (-6.5 to 5.4), P< 0.001) after 6 month of verapamil intervention with no difference between responders and non-responders (0.6 (-2.5 to 3.6), P= 0.72). In addition, at the

end of the study (6 months of intervention) all participants reached the target values without clinical bradycardia being observed.

4.1.7 Verapamil medication adherence and side effects

Medication adherence has been identified as a key issue in healthcare outcomes.[101] The current study measured compliance with treatment and it was assessed as being 94.3% overall using the Morisky Medication Adherence Scale. In addition, verapamil was well tolerated by most patients, with only two patients (5.71%) reported constipation and one reported dizziness (2.85%). One patient reported fatigue (2.85%) and another experiened a headache (2.85%). All of the adverse events were transient, resolved spontaneously and treatment was not interrupted. A study done by Khodneva et al [183] to measure the medication adherence to verapamil using a four-item Morisky scale reported lower adherence (67.3%) than the current study (94.3%). However, comparison between Khodneva et al.[183] and the current study is difficult as in Khodneva et al. study the medication adherence was self-reported and could not be confirmed by pharmacy refill data or medical records as was the case in the current study.

Rubio et al[235] found that compliance with verapamil treatment was 90% that is comparable to the current study result. Also, their result regarding the tolerability profile were a comparable with the current study result where they found that in each group (the group on trandolapril alone and the group on a combination of trandolapril with verapamil), there were three patients (10%) reported dizziness, and two patients (6.7%) had headache. In addition, all adverse events were transient, resolved spontaneously and treatment was not interrupted. Yin et al[187] found comparable

results, with verapamil compliance 97% on average but the tolerability profile between verapamil and control group was inconsistent with the result of the current study and Rubio et al.[235] where it was 61% and 62% experienced at least one adverse event during active treatment in verapamil group and control group, respectively. Also, Fernandez et al[194] found a higher percentage of adverse events than the current study with a percentage of adverse events recorded in verapamil and control groups of 29.4% and 30.8% respectively.

Recently Malayeri et al[197] found that verapamil was well-tolerated in patients who received the drug. Although some mild side effects were observed in a few patients, no significant side effects were reported. Also, no discontinuation of treatment or dose reductions were necessary but the study did not mention any details regarding the side effects that detected.

The overall results from all of the previous studies,[187,194,197,235] regarding verapamil tolerability were similar to the current result, where all adverse events were transient and resolved spontaneously, and treatment was not interrupted even in the studies that detected higher percentage of adverse effects, verapamil tolerability was similar to the control group which may reflect that the occurrence of side effects were not related to verapamil use and also the detected adverse effects did not significantly interrupt the study.

4.1.8 The effect of Verapamil on organ function

Verapamil is widely distributed throughout body tissues and the drug is eliminated by hepatic metabolism, with excretion of inactive products in the urine and/or faeces.[236] Therefore, renal function and liver function test were monitored throughout the current

study. Verapamil use was associated with reduction of serum creatinine and increase in GFR. On the other hand, verapamil was associated with reduction of bilirubin, and albumin. The effect of verapamil on renal function found in this study is similar to what reported in the literature in several pre-clinical and clinical studies.[237-239]

4.2 Study limitations

Recruitment criteria limited the overall sample size in the study period. However, the size was appropriate for the level of power/treatment effect. Larger sample size would have strengthened subgroup analysis. Univariable analysis was performed for factors affecting the change in glycometabolic parameters and the outcome could have affected by other confounders. The duration of follow-up could be a limitation and the effect of verapamil could have occurred beyond the duration of follow-up. Also, follow-up visits were a challenge as participants had chronic diseases and some of the study requirements were considered difficult by some patients because it required overnight fasting, postponement of their evening dose of insulin, and eating only a green salad as dinner. However, this was not an obstacle that may affect the accuracy of lab measurements, but it required contacting participants during the night before the appointment should be done to ensure that patients follow the study instructions properly. Also, rescheduling for another appointment for patients who cannot attend the appointment was not always easy to attend, especially for participants who lived outside the study setting (Riyadh).

4.3. Future work:

Data remains very limited on the glycometabolic effect of verapamil-based treatments so further experimental and prospective, longitudinal clinical trials, preferably multicentre studies, are needed to investigate glycometabolic effect of verapamil on type 2 diabetes. In addition, future studies including verapamil versus controlled group will be very important in giving clear definite conclusion even with small sample size. It is also going to be beneficial if a well-designed comparative study is initiated to compare the metabolic effect of verapamil versus other calcium channel blockers or other new strategies that may affect glycaemic parameters which will give additional information regarding the role of verapamil on the glycaemic parameters. In addition, future studies are recommended to detect if there is a difference in verapamil effect on glycometabolic parameters in type 2 diabetes in respect to duration of diabetes. Additionally, it will be interesting to evaluate in future studies whether early initiation of verapamil is going to be beneficial in preserving endogenous insulin secretion and preventing disease progression in diabetic hypertensive patients. It is recommended to include homogeneous patient groups in the future studies to be able to detect if verapamil has an effect on glycaemic parameters. The current study suggests that the effect can be different in diabetic patients receiving insulin versus non-insulin antidiabetic drugs. The response to verapamil varied greatly in our cohort, this suggests that withdrawal clinical trials could be a suitable design to study the effect of verapamil. In this study design, only responders will be included and followed over time.

Other factors affecting the glycaemic parameters should be considered such as the diet, exercise (including type and intensity of the exercise), BMI, the duration of diabetes,

the type and dose of antidiabetic drugs. Larger sample sizes are required to do subgroup analysis. Moreover, the duration of therapy should be considered as we observed a further reduction of HbA1c and FPG after 6 months of therapy. In addition, future studies are recommended to detect if there is a difference in verapamil effect on glycometabolic parameters in type 1 versus type 2 diabetes.

Finally, further studies with appropriate sample size to test the dose-dependent effect of verapamil on HbA1c and effect of verapamil on the progression of diabetic complications as the current study found verapamil was associated with improved renal function. If this confirmed by additional studies that means verapamil is not just a promising agent in short term managements to control diabetes (decreasing HbA1c), but it will also help in long term management as it helps in preventing the progression of diabetes by stopping nephropathy microvascular complications.

4.4. Summary

- As the coexistence of hypertensive in type 2 diabetic patients is very common, finding an anti-hypertensive agent that can be used in diabetic hypertensive patients that control both conditions is extremely beneficial.
- The current study found that:
    - Verapamil is metabolically neutral which at least allows stabilization of glycometabolic parameters with no glycaemic adverse effects on type 2 diabetes.
    - The change in HbA1c levels was affected by some of the participants' characteristics.

        - Sitagliptin use was associated with lower HbA1c; however, insulin use, higher metformin dose, longer duration of diabetes, higher baseline FPG levels, retinopathy and neuropathy were associated with higher HbA1c.
    - Additionally, insulin use was negatively associated with FPG levels but not associated with HOMA-IR and C-peptide levels.
    - Verapamil could have an enhanced effect in noninsulin user type 2 diabetes patients as non-insulin use was associated with a significant reduction in HbA1c and FPG levels after 6 months of verapamil therapy.
    - Extending the verapamil use was able to maintain the significant reduction in HbA1c level in the responder participants.
    - Verapamil was associated with improved renal function; however, it was associated with a decrease in albumin level. The effect of verapamil on albumin and bilirubin could be attributed to haemodilution effect of verapamil

4.5 Conclusion

The results of the current study found that verapamil is metabolically neutral which at least stabilize the glycometabolic parameters with no adverse glycaemic effects in type 2 diabetes. Response to verapamil may vary among type 2 diabetes patients and several factors could have affected the change in HbA1c levels, such as insulin use and duration of diabetes. These factors should be considered in designing future studies.

4.6 Innovation of the current study

- To the best of our knowledge,
    - The current study is the first prospective study that measures the C-peptide and HOMA-IR effect of verapamil on type 2 diabetic hypertensive patients.
    - The current study is the first prospective study investigating the association between different predictor factors and verapamil glycometabolic effect.
    - The current study is the first study in the Kingdom of Saudi Arabia that investigates the effect of verapamil on hypertensive patients with type 2 diabetes.
    - The current study is the first study that investigates the long-term effect of verapamil on hypertensive patients with type 2 diabetes.

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