COMPARISON OF GLIMEPIRIDE-METFORMIN VERSUS DAPAGLIFLOZIN-METFORMIN IN PATIENTS WITH TYPE 2 DIABETES

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BAHRIA UNIVERSITY MEDICAL & DENTAL COLLEGE

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This Dissertation is dedicated to my parents

For their endless love, support, and encouragement

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ABSTRACT

The efficacy of common prescribed anti-hyperglycemic agents such as metformin, α-glycosidase inhibitors, dipeptidyl peptidase-4 inhibitors, sulfonylureas, glucagon-like peptide-1 analog, thiazolidinediones, and non- sulfonylurea secretagogues and are insulin-dependent. The efficacy of these drugs is diminished in dysfunction of pancreatic islet β-cells during type 2 diabetes mellitus (T2DM). Comparatively, dapagliflozin, a highly selective inhibitor of sodium glucose co-transporter 2 (SGLT-2), is insulin-independent, control glycemia by reducing reabsorption of glucose by the proximal tubule in the kidney by increasing glucosuria. The aim of the study was to evaluate and compare the efficacy, safety, and tolerability of dapagliflozin with the glimepiride in patients with type 2 diabetes inadequately controlled with metformin monotherapy. The study was conducted in National Medical Center, Karachi on 210 diagnosed patients of type 2 diabetes mellitus. Male and female patient's ≥ 45 years of age, fulfilling criteria were included in the study. These patients were systematically randomized into 2 groups each having 105 members. Group A was received tablet Dapagliflozin 10mg once daily plus tablet metformin 500 mg TDS and group B was received tablet glimepiride 4mg once daily plus tablet metformin 500 mg TDS, and for 12 weeks. They were undergoing investigations including HbA1c, FBS, LFTs, RFTs, Lipid Profile, ketonuria, glucosuria, puss cell count, and bacteria. For the analysis, descriptive statistics were calculated using SPSS version 25. The mean fasting blood sugar in group A at week 0, week 6 and week 12 was 184.05±14.82 mg/dL,137.02±12.30 mg/dL and 101.40±16.85 respectively while in group B was 178.19±9.04 mg/dL,146.23±12.54 mg/dL and 121.89±9.22 mg/dL respectively. While, mean Hb1AC in group A at week 0 and week 12 was 7.83±0.54 % and 6.91±0.74 % while mean Hb1AC in group B at week 0 and week 12 was 6.91±0.74 % and 7.91±0.49%. Further we have found significant mean difference for fasting blood sugar and HbA1c at different intervals between the study groups and within the groups.

Furthermore, the safety profiles of these in patients with type 2 diabetes were evaluated. The normal levels of lipid profile and liver function test were found within and between the groups during week 0, week 6 and week 12. The urine analysis has shown no pyuria, leukocyte esterase and bacteria at week 0, week 6 and week 12. The mean ketone in group A at week 0, week 6 and week 12 was 0.23±0.10 mg/dL,0.23±0.10 mg/dL and 0.22±0.10 mg/d respectively while in group B was 0.31±0.13 mg/dL,0.32±0.13 mg/dL and 0.32±0.13 mg/dL respectively. Furthermore, we found insignificant mean difference for WBC at week 0 (p=0.931), week 6 (p=0.864) and week 12 (p=0.921) with respect to study groups. The difference in glucosuria was identified in 6^{th} week (group A: 12% mild, 82% moderate and 6% severe ; group B: 98% mild and 2% moderate) and 12^{th} week (2% mild, 9% moderate and 89% severe ; group B: 99% mild and 1% moderate) in group A and B. Dapagliflozin-metformin is clinically more effective to treat glycemia in uncontrolled T2DM with metformin patients as compared to Glimepride-metformin combination. Furthermore, Dapagliflozin co-administered with metformin did not produce adverse effects.

Key words:

Diabetes Mellitus, Dapagliflozin, glimepiride, metformin, SGLT-2 inhibitor

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ALT	Alanine transaminase
AST	Aspartate transaminase
ATP	Adenosine triphosphate
DM	Diabetes mellitus
FBG	Fasting blood sugar
FFA	Free fatty acids
HDL	High density lipoprotein
IDF	International diabetes federation
IGT	Impaired glucose tolerance
IR	Insulin resistance
LDL	Low density lipoprotein
MODY	Maturity onset diabetes of the young
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
OGTT	Oral glucose tolerance test
PKC	Protein kinase C
PPBG	Post-prandial blood glucose
RAGE	Receptor of advanced glycation end products
T1DM	Type 1 Diabetes mellitus
T2DM	Type 2 Diabetes mellitus
TG	Triglyceride
VLDL	Very low-density lipoprotein
WHO	World health organisation

CHAPTER 1

INTRODUCTION

1.1 Diabetes Mellitus (DM)

Diabetes mellitus (DM) is defined as metabolic disorder, consisting of chronic hyperglycemia due to dysregulation of insulin secretion, action or both (Chaudhury *et al.*, 2017). Metabolic anomalies of macromolecules, including proteins, lipids and carbohydrate moiety represents role of insulin as anabolic pancreatic hormone. The key factors responsible for metabolic abnormalities include decreased secretion of insulin to get an adequate metabolic response and high-level insulin resistance (IR) in liver, skeletal muscles, adipose tissue. The severity of diabetic symptoms depends on type and duration of disorder as, pre-diabetic patients mostly asymptomatic, while with the extent of uncontrolled condition, marked symptoms appear. Children with type 1 diabetes mellitus (T1DM) commonly suffer from polydipsia, polyphagia, polyuria, blurred vision and weight loss. Sustained-uncontrolled diabetes mellitus responsible to suffer the patient with stupor, coma, and even death due to ketoacidosis or nonketotic hyperosmolar syndrome (Kharroubi & Darwish, 2015).

Diabetes mellitus (DM) is one of the oldest diseases known to mankind. About 3000 years ago it was first reported in Egyptian manuscript. In 1936 distinction was made between type 1 and type 2 DM. Type T2DM was first reported as a component of metabolic syndrome in 1988.

Diabetes is initially characterized as the disease involving the excessive urine production in the Egyptian manuscripts which were written in 1500 B.C. In the Indian

literature, it was referred as the ‘Madhu meha’ (Honey urine- the urine which attracted ants), also it was the Indian physicians who classified the disease into Type I and Type II. In first century A.D. Aretaeus the Cappadocian named the disease as ‘Diabetes’ which is a Greek word for ‘Siphon’, and the term ‘Mellitus- Latin word for sweet like honey’ was introduced by John Rollo in 1798, as this type is different from other types in which urine has no taste. In the book ‘The Canon of Medicine’, diabetic gangrene was not only observed, but also indicated by abnormal appetite, and the mixture of fenugreek, lupin, and zedoary seeds was mentioned as the remedy of it (Lakhtakia, 2013).

Diabetes is one of the major health care problem that has reached alarming levels: approximately 500 million people worldwide have diabetes. Globally 463 million diabetic adults were found in 2019, while the mortality rate was 4.2 million and 760 billion dollars were spent for treatment. There are significant chances to increase 51% diabetic population till 2045 and the statistics will be reached to 700 million as stated by International diabetes federation (IDF)-2019 (R. Williams *et al.*, 2020).

In 2019, the countries with the largest number of people with diabetes aged 20-79 are the India, United States of America and China, and the number is expected to rise till 2030. The number of diabetic people in Pakistan is expected to exceed till 2045 and will move to 3rd rank where the United States is present (R. Williams *et al.*, 2020).

The countries with the highest diabetes rate in the middle east and north Africa (MENA) region are Pakistan (19.9%) and Sudan (22.1%). The countries where maximum number of diabetic adults between 20 to 79 years are found in Pakistan (19.4 million), Iran (Islamic Republic of Iran) (5.4 million) and Egypt (8.9 million) (R. Williams *et al.*, 2020).

Pakistan is ranked in the top 10 among people with diabetes, impaired glucose tolerance, and undiagnosed diabetes (20-79 years) in 2019, 2030 and 2045. According to IDF-2019, Pakistan was listed at 4th rank with 19.4 million diabetic population in 2019, whereas at 2045, the rank and population both will be increased (3rd rank; 37.1 million diabetic population) (R. Williams *et al.*, 2020).

As per IDF 2019 report, 8.5 million undiagnosed diabetic population in Pakistan was reported in 2019. And the rank for impaired glucose tolerance in 2030 and 2045 will be reached to 7th (11.8 million), and 8th (16.5 million) respectively (R. Williams *et al.*, 2020).

Further IDF has reported that women's mortality rate for diabetes was higher than men in the MENA region, estimated at approximately 248,300 and 170,600 deaths in 2019, respectively. This may be because the number of women with diabetes was slightly higher than men. The total mortality rate due to diabetes in Pakistan was highest, reaching 159,000 by 2019 (R. Williams *et al.*, 2020).

In 2019, the cost to treat diabetes in MENA region was approximately EUR 24.9 billion. The total annual cost of diabetes-related health in 2045 is expected to reach EUR 38.6 billion (R. Williams *et al.*, 2020).

Overall, the proportion of healthcare expenses for diabetes was 15.2% of the total area. The largest proportion of expenditures is used due to diabetes in Lebanon (20.4%), Sudan (20.7%) and Pakistan (19.7%). In Oman, the lowest percentage of healthcare costs (6.8%) for DM is reported. The annual consumption of per diabetes patients from MENA region generally varies. Pakistan was estimated at $83 according to IDF (R. Williams *et al.*, 2020).

1.1.1 Overview of Glucose metabolism

Followed by taking the meal, the glucose upsurge stimulates the insulin to secrete from pancreatic β-cells, to enhance biotransformation and glucose uptake into muscles and adipose tissues. During starvation, the glycogenolysis in liver provides glucose to sustain brain function, regardless of dependent on insulin activity. Furthermore, insulin takes part in glucagon secretion from pancreatic alpha cells and decreases serum fatty acids, which ultimately decline hepatic gluconeogenesis (Baynes, 2015) .

1.1.2 Pathophysiology of Diabetes mellitus

Dysregulation in the feedback loop mechanism of insulin secretion from β-cells of pancreas or resistance of insulin-sensitive receptors that are found in muscle, liver, and adipose tissue, eventually disturbs glucose metabolism. This declines glucose uptake and reverse extracellular hypoglycemic and intracellular hyperglycemic balance. Thus, intracellular hypoglycemia stimulates glucose production through glucogenesis and gluconeogenesis in liver *via* lipolysis and declines protein anabolism. The pathological conditions lead to this including, diabetic ketoacidosis, polyphagia, reduced wound healing, and cachexia. On the other side, extracellular hyperglycemia cause osmotic dieresis and diabetic coma. (Asmat *et al.*, 2016; Zheng *et al.*, 2018)

1.1.3 Classification of Diabetes Mellitus (DM)

Type 1 diabetes mellitus (T1DM) is characterized as deficiency of insulin level due to T-cell induced autoimmune destruction of β cells (De Ferranti *et al.*, 2014; Kerner & Brückel, 2014). It predominantly found in childhood, and adolescence, and can be present in adulthood. The common symptoms include polyuria, polydipsia, and polyphagia. The pathophysiology involves in T1DM is declined insulin, hyperglycemia, and ketoacidosis. (De Ferranti *et al.*, 2014)

Immune-based diabetes mellitus (DM) is characterized by cell-induced autoimmune destruction of insulin-secreting β-cells of pancreas. The T lymphocytes abnormally activates to cause insulitis (inflammation of pancreas) and produce antibodies that show their activity against β-cells (antibody-mediated response). This might be constituted as a key predictor for immune destruction (Kakleas *et al.*, 2015).

Previous studies propose that immune system-induced T1DM development is probably because of i) inflammatory disease and ii) clinical characteristics that are raised from insulin-secreting β-cell loss. However, the proper evidence has not existed that shows the association of autoantibodies with immune system-induced T1DM

pathogenetic mechanism. Pancreatic islet autoantibodies in normoglycemia have been shown strong marker to detect immune system-induced T1DM in advance. Furthermore, pancreatic tyrosine phosphatase (IA2A) autoantibodies, and glucamic acid decarboxylase (GADA), are suggested markers for early validation to suspect immune system-induced T1DM diagnosis.

Genetic factors that lead to immune system-induced T1DM include HLA DR and DQ 120 genotypes and the gene for this T1DM is shown to be expressed in insulin-secreting pancreatic β-cells. Environmental factors include viruses (mumps, enterovirus, cytomegalovirus, and ljungan virus), nutrition and low Vitamin D concentration (Kakleas *et al.*, 2015).

Idiopathic T1DM have some miscellaneous categories that have unknown etiologies. The patients have been shown permanent insulinopenia (insufficient insulin level) and susceptible to develop ketoacidosis, however, there is no positive evidence for β-cell autoimmunity. Patients of Idiopathic T1DM is suffered from i) episodic ketoacidosis and ii) exhibited fluctuating levels of insulin deficiency between each episode. Previous studies supported the Idiopathic T1DM association with inheritance but no association with HLA. (Association, 2017)

Type 2 diabetes mellitus (T2DM) is characterized by decreased insulin secretion from pancreatic β-cells and peripheral insulin resistance (IR). (Al-Goblan *et al.*, 2014; Kaveeshwar & Cornwall, 2014). IR is caused the increase level of plasma fatty acid (FA), decrease glucose uptake to muscles and lipolysis. All these events eventually lead to hepatic gluconeogenesis. Insulin resistance and insulin-secreting β-cell dysfunction occur simultaneously for T2DM development. (Al-Goblan *et al.*, 2014)

Gestational diabetes is characterized as glucose intolerance during pregnancy. The development of insulin resistance and high insulin secretion may susceptible to cause gestational diabetes (Association, 2004; Kampmann *et al.*, 2015; Zhu & Zhang, 2016). Normally, 2nd trimester (mid-pregnancy) initiates IR and progress till the third trimester. The possible factors that leads to IR includes, placenta secreting hormones and adipokines, such as, growth hormone, tumor necrosis factor alpha- α (TNF-α) and

lactogen. Moreover, increase the level of steroid hormones, such as estrogen, progesterone, and cortisol causes an imbalance in glucose insulin ratio during pregnancy, thus, insulin secretion increases to compensate IR. Furthermore, enhanced adipose tissues, increased maternal adipose deposition, decreased physical activity and high caloric intakes play the role to produce glucose intolerance (Alfadhli, 2015).

Monogenic diabetes has been constituted by single-gene disorders. Proper diagnosis is assisted to explain the pathogenesis of disease, described the risk factors contributed to develop diabetes within the family and enabled to manage by modifying treatment. Optimal management is needed in diabetes to prevent the patient from chronic complications (Szopa *et al.*, 2015).

Monogenic diabetes includes maturity-onset diabetes (glucokinase gene, HNF1A gene, HNF4A gene, ABCCB gene), renal cysts and diabetes (HNF1B gene), maternity inherited diabetes and deafness (mtDNA 3243 gene), permanent neonatal diabetes (KCNJ11 gene, insulin gene), neonatal diabetes (KCNJ11 gene) and delay epilepsy, transient neonatal diabetes (6q24), wolfram syndrome, Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, and Wolcott-Rallison syndrome (Association, 2017).

The changes in exocrine pancreas and decline functional activity of pancreatic acinar cells and ductal cell has been observed in both T1DM and T2DM. Exocrine pancreatopathy is existed in diabetes mellitus but the mechanism and clinical manifestations still unclear. Detail studies are needed to understand exocrine-mediated diabetic pancreatopathy, produce optimum treatment strategy and differentiate with chronic pancreatitis (Mohapatra *et al.*, 2016). The disease of exocrine pancreas includes neoplasia, trauma, pancreatopathy, cystic fibrosis, hemochromatosis, fibrocalculous pancreatitis and others (Association, 2017).

Drugs mediated hyperglycemia and diabetes is found to be a global problem. The drugs alter the secretion of normal insulin levels, produce insulin resistance or toxicity in pancreatic cells and increase gluconeogenesis. Antihypertensive agents have not been observed to induce glucose production. Increase glycemic profiles have been

reported by using thiazide diuretics, β-blocking drugs, renin-angiotensin system inhibitors, and calcium channel blockers. Hyperglycaemia and diabetogenic effect may also increase by using lipid-modifying drugs, includes statins. Nicotinic acid tends to alter glycemic events. Fluoroquinolones, in high or even in moderate doses produce life-threatening effects. Glucose metabolism is altered by using enzyme inhibitors such as nucleoside reverse transcriptase inhibitors, and protease inhibitors. Pentamidine interacts with pancreatic cells and inhibits its function for normal glucose homeostasis. The anti-epileptic drugs, such as phenytoin and valproic acid has been reported to alter glycemic events. Antipsychotic drugs of second generation produces insulin resistance that leads to hyperglycemia, ketoacidosis and diabetes mellitus. A high dose of an antidepressant drug is found to be mediate diabetes in patients. Patients having ketoacidosis and receiving theophylline and β-adrenergic agonists are susceptible to produce hyperglycemia (Association, 2017).

Glucocorticoids are associated to produce steroid diabetes. Chemotherapeutic agents tend to induce hyperglycemia, such as calcineurin inhibitor decrease insulin secretion from pancreatic β cells. Oral contraceptives with an increasing concentration of estrogen have been associated to mediate hyperglycemia. Drugs of growth hormone and somatostatin have been found to induce glucose level (Fathallah *et al.*, 2015).

As per World health organisation report (2019), several hormones including, cortisol, glucagon, growth hormone, and epinephrine have been observed to antagonized insulin mechanisms of action. Increase secretion of these hormones due to endocrine disorder is associated to develop diabetes. The successful treatment for underlying causes of hormone excess is capable to resolve the hormones-mediated hyperglycemia. Somatostatinoma (tumor of pancreatic delta cell) is another factor that leads to diabetes, by inhibiting pancreatic insulin secretion. The normal glucose level is achieved followed by the successful treatment of the tumor. (Organization, 2019). The endocrine disorders that lead to diabetes include cushing syndrome, glucagonoma, hyperthyroidism, acromegaly, pheochromocytoma, and others.

1.1.4 Screening strategies

The screening strategies for pre, diabetes and diabetes are mentioned by American Diabetes Association.

Table 1.1: Clinical examination for diabetes (Association, 2019)

	Conditions	Diagnosis
Fasting plasma glucose	8 hours on fasting state before testing	≥126 mg/dL
Oral glucose tolerance test (OGTT)	Orally administered 75g anhydrous glucose load before 2 hours of examination	≥200 mg/dL
HbA1C	8 hours on fasting state before testing	≥6.5% (48 mmol/mol)

Table 1.2: Clinical examination for pre-diabetes (Association, 2019)

	Conditions	Diagnosis
Fasting plasma glucose	8 hours on fasting state before testing	100-125 mg/dL
Oral glucose tolerance test	Orally administered 75g anhydrous glucose load before 2 hours of examination	140-199 mg/dL
HbA1C	8 hours on fasting state before testing	5.7-6.4% (39-47 mmol/mol)

1.1.5 Recommendations for diagnosis

The recommendations to diagnose diabetes is based on the clinical examination of HbA1c and fasting plasma glucose (FPG) level, while OGTT is preferred in case of any doubt in diagnosis. Furthermore, repetition is suggested to assure the diagnosis. The diabetic report by Euro Heart Survey has demonstrated OGTT as main examination to diagnose diabetes in cardio vascular patients, other than FPG or HbA1c (Organization, 2019).

1.1.6 Mechanism of insulin in glucose regulation

Extracellular glucose is taken up by plasma-membrane glucose transporters 2 (GLUT2) and initiates intracellular glycolysis. The increase adenosine triphosphate (ATP) level alters the ATP/ADP balance, which subsequently close ATP-regulated potassium (K^{+}) channels. Followed by, increase intracellular calcium level opens voltage-gated calcium (Ca^{+2}) channels, which in turn, triggers insulin secretion (Röder *et al.*, 2016).

1.1.7 Insulin resistance

Insulin secretion and insulin resistance (IR) are the two primary contributors of developing diabetes. Insulin resistance is initiated before the onset of diabetes mellites (Gustafson *et al.*, 2015). The modern lifestyle, abdominal obesity, excessive adipose tissues, and sedentary lifestyle are the key factors to cause insulin resistance. Some of the normal individuals experience insulin resistance of similar levels as found in diabetes. IR continuously is enhanced or declined the insulin secretory and compensation feedback loop, which eventually leads to a pre-diabetic state (impaired

glucose tolerance). Hyperglycaemia, free fatty acid, and hyperinsulinemia cause reactive oxygen spices formation, enhance oxidative stress and triggers mechanisms that lead to stress. The combination of all these events inhibits insulin activity and its secretion that eventually progress the onset of T2DM. (Tangvarasittichai, 2015).

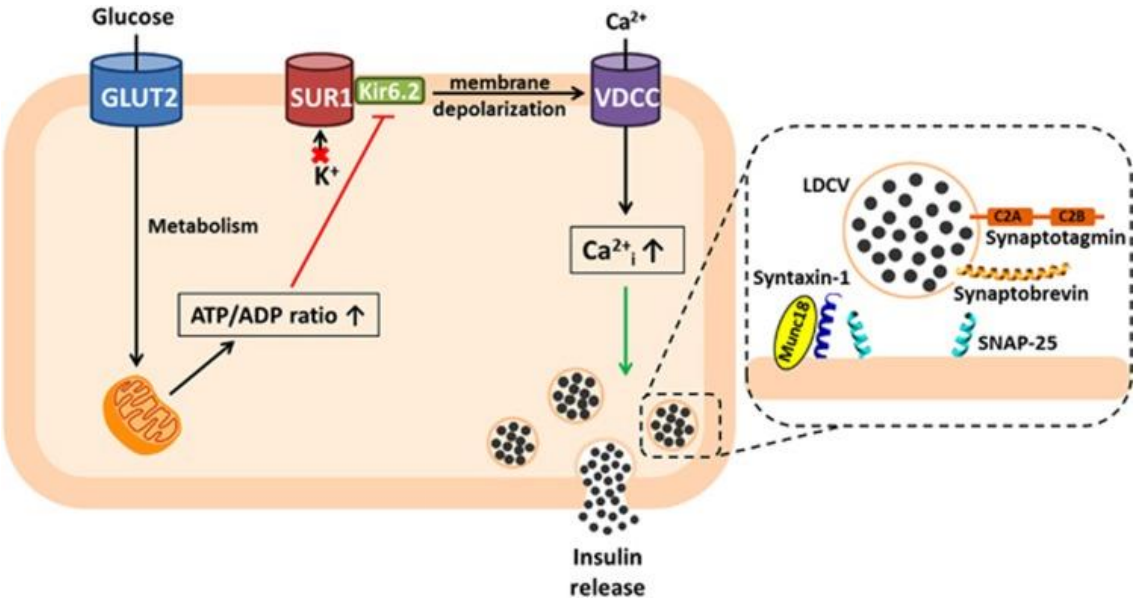


Figure 1.1: Glucose-induced insulin secretion (Röder et al., 2016).

1.1.8 Factors responsible for the pathogenesis of diabetes mellitus (DM)

The increasing prevalence of type II diabetes is associated with the epidemic of obesity. Correspondingly, type I diabetes is also caused by similar etiology. Whereas, the pathophysiology behind type 1 diabetes (autoimmune) remains to be studied and identified. The particular molecular mechanism leading to increase the prevalence of T1DM is still unclear, especially in the young age group individuals (Al-Goblan *et al.*, 2014).

Wilkin was proposed the "accelerator hypothesis", that is recognized as the highly accepted theory that defines the relation of body mass with T1DM. The researcher ofthis well-known theory has suggested that the rise in body mass has increased chances to develop T1DM in young age peoples. At diagnosis, the body mass index (BMI) is inversely associated with age. The increased body mass promotes insulin resistance that leads to the development of T1DM. Still, gaps are found to in the exact mechanism that underlying the association between the development of T1DM and obesity. Further investigations are needed (Al-Goblan *et al.*, 2014).

The previously reported data for T2D has suggested the impaired endothelial function is associated with obesity and IR in both diabetes and pre-diabetes. In the condition of increasing body mass, β-cells of pancreas is unable to regulate its normal functions and leads towards insulin resistance. Moreover, the adipose tissues secretes non-esterified fatty acids (NEFAs) during obesity is might be responsible to cause insulin resistance (IR) and impaired β-cell functions (Al-Goblan *et al.*, 2014).

Chromosome 6p21 contains HLA region has been responsible for progression of T1D (X. Hu *et al.*, 2015; Pociot & McDermott, 2002). Subsequently, insulin gene has also responsible for leading T1D. The polymorphisms in insulin gene have regulated

the insulin mRNA expression in thymus gland and are the possible cause to affect the development of immune tolerance towards insulin hormone (Kracht *et al.*, 2017; Pociot & McDermott, 2002).

Earlier findings have been suggested the contribution of epigenetics to leads T1DM. DNA methylation at CpG dinucleotides (cytosine residues) is associated with T1DM. The methylation at CpG sites is primary difference between diabetic (T1DM) and non-diabetic individuals (Rakyan *et al.*, 2011; Stefan *et al.*, 2014).

Environmental associations have been triggers genetic factors to increase autoimmunity that ultimately leads to progressed T1DM (Skyler *et al.*, 2017). Though, the severe exposure to environmental conditions is generated by the increased risk of the onset of islet autoantibodies actions that lead to childhood-onset of T1DM. However, the gap is still present to identify the variation in age to develop diabetes in children.

The importance to discuss food intake with diabetes is still needed to explore. Such as, the study has conducted at early age in which dairy products or high milk administered and found that the high IgA (antibodies) level in cow's formula milk enhanced the risk to develop T1D independently (Virtanen *et al.*, 1994).

Viral infections are another underlying pathophysiology to increasing prevalence of diabetes, however, still, no proper evidence is defined for a specific viral strain that linked to T1DM (Van Belle *et al.*, 2011).

The epidemiological data has been reported the association of coxsackievirus B (an enterovirus) to develop T1DM due to the occurrence of RNA and protein molecules of this virus in pancreas and surrounding tissues of T1DM. This virus produces infection in pancreatic β cells, stimulate inflammation and activate innate immunity during the onset of T1DM. Furthermore, coxsackievirus B induced interferon-alpha that activates autoimmunity that combat with β cells and this activity may be deleterious. (Hober & Sauter, 2010).

The study was taken to identify the association among viral infection, autoimmunity, β cell function, insulitis, and survival of patient with T1DM. Enterovirus has shown to produce infection in pancreatic β cells in T1DM patients, which leads to produce inflammation and impaired regular functions. (Dotta *et al.*, 2007)

Viruses may also exhibit autoimmune effect through molecular mimicry of mechanisms, molecular mimicry is the process that involves the sharing of antigenic characteristics between pancreatic β cells and environmental substances. This mimicry increases to originate immune responses directly against produced autoantigens of virus which looks like the actual antigens and leads to cellular impairment (Paschou *et al.,* 2018). Modifications in gut microbiomes levels has been further listed as another main cause of progress towards pathogenesis of T1D (C. Hu *et al.,* 2015).

Diabetes has shown hyperglycemia, increase triglycerides (TG), free fatty acids (FFA), and decrease low- and high- density lipoprotein elevated circulating levels of FFA acids, TG and LDL and decrease HDL-cholesterol. The diabetic- mediated dyslipidemia significantly plays a vital role to induce cardiovascular complications in T2DM outpatients. Furthermore, many epidemiological data have suggested that low HDL, high TG, and low LDL starts the development of T2DM and capable to self-regulate the pathogenesis of T2DM. The bystander effect has been reflected between insulin resistance and dyslipidaemia to the development of T2DM. The previously reported data on genetic models of mouse and isolated pancreatic islets of mice and humans suggested the pathophysiological factors for disrupt levels of lipoproteins is the development of insulin resistance, impaired β-cell and dysregulate insulin secretion (von Eckardstein & Sibler, 2011).

Stimulation of the immune system, especially innate immune activity in pancreatic islets of langerhans has been vital element to cause T1DM. Viral infections in pancreatic β-cells cause the increase secretion of type I interferon (IFN) which stimulate numerous IFN-upregulated genes, the expression of genes has been indicator of diabetes. But , some other studies have also suggested that IFN-upregulated genes overexpress during many other primary or secondary pathological conditions, and even in the absence of viral infection (Lundberg *et al.,* 2016).

1.1.9 Intracellular molecular mechanism leads diabetic complications

Glucose is utilized in euglycemic state to produce ATP by undergoing glycolysis and kerb cycle pathways. Glucose is further utilized to release NADPH and produce ribose *via* pentose phosphate pathway. Excess glucose enters into glucogenesis pathway to convert into glycogen and increases lipogenesis for fatty acid synthesis. While, during diabetes, 30% of glucose undergoes to polyol pathway to convert into fructose.

Hyperglycemia mediated polyol pathway decline the normal ratio of $NADPH/NADP^{+}$, reduce nitric oxide (NO) formation, increase sorbitol accumulation, enhance osmotic stress, increase fructose level (that in turns leads towards glycation), and cause non-alcoholic fatty liver disease (NFALD). Polyol pathway leads to diabetic complications, such as neuropathy, nephropathy, and retinopathy (Yan, 2018).

Chronic hyperglycemia leads to enhance hexosamine pathway. During this mechanism, glycolysis increases fructose 6-phosphate (F6P) formation to metabolized into glucosamine-6-phosphate (G6P) *via* glucosamine-fructose aminotransferase. G6P utilize UDP-*N*-Acetylglucosamine-1-phosphate uridyltransferase and convert into UDP-*N*-Acetyl glucosamine (UDP-GlcNAc). The UDP-GlcNAc stimulates O-glucosamine-*N*-Acetyl transferase hyperactivity. The increase in enzyme activity leads to modification in gene expression and increases tumor necrosis factor (TNF) -α and -ß levels, which prevent the mitogenesis in mesangial cells and increase collagen matrix to produce pro-oxidative species (Ighodaro, 2018).

Reactive dicarbonyl compounds, such as methylglyoxal (MGO), 3-deoxyglucosone (3-DG), and glyoxal (GO) are formed from various metabolic pathways (polyol pathway, Maillard reaction, and glycolysis) to produce irreversible advanced glycation end products (AGEs) (Khangholi *et al.*, 2016; Yamagishi *et al.*, 2015).

These non-enzymatic glycating products lead to cause IR (insulin resistance) as

it modifies the insulin structure which ultimately affects its activity and causes decrease glucose uptake. Furthermore, these products decrease insulin clearance and enhances insulin secretion. Moreover, AGEs may play its role to IR *via* enhanced expression of AGEs receptor (RAGE) and decline AGEs receptor-1 (AGER-1). Eventually, the low level of SIRT-1 disturbs insulin signaling and causes inflammation. AGEs are stimulated PKC-α (protein kinase C-α) and increase regulation of TNFα to affect insulin signaling mechanism and initiate inflammation.

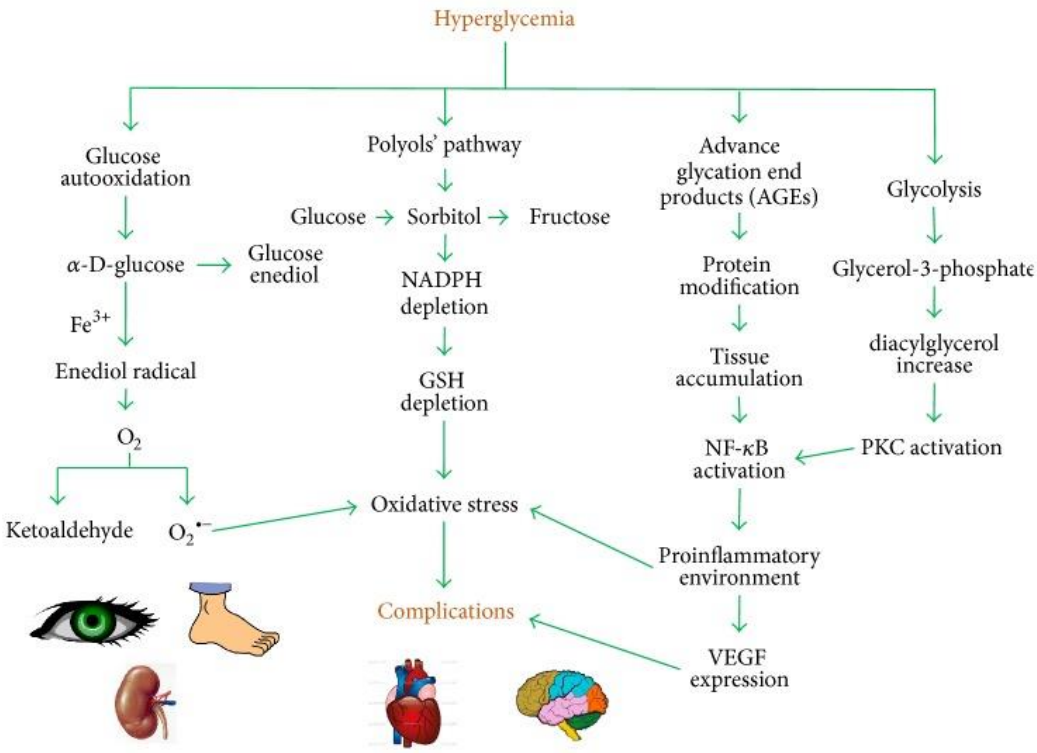


Figure 1.2: Intramolecular pathway of diabetic-hyperglycemia leads complications (Alemán-González-Duhart et al., 2016).

The reduce insulin synthesis and secretion are involved to cause pancreatic β-cell failure due to chronic hyperglycemia. AGEs are affected by insulin transcription and synthesis. Furthermore, AGEs showed its activity to inhibit insulin secretion by reduced iNOS activation that ultimately blocked cytochrome-c activity and depleted adenosine triphosphate molecule. AGEs are also reduced insulin release from β cells *via* exhibited activity in modification of citric acid cycle which leads to decreased adenosine triphosphate formation. The low adenosine triphosphate prevents closure of adenosine triphosphate-regulated potassium ion (K^+) channels which in turn reduces membrane to depolarize, decrease intracellular calcium ion (Ca^{+2}) level and cause reduced insulin secretion from pancreatic β-cells (Nowotny *et al.*, 2015).

1.1.10 Diabetic Complications

Chronic hyperglycemia and IR are potent element that takes part to develop atheroschlerosis and contributed to diabetic macrovascular complications. However, both hyperglycemia and IR are normally appears before the onset of diabetes (Liebow & Hellerstein, 1949).

Insulin resistance in liver cells, muscle, and adipose tissues are strongly shown correlation with weight gain, which ultimately enhanced free fatty acid production and reactive oxygen spices level. Both of these leads to progress atherosclerotic modifications and development of macrovascular complications. Chronic hyperglycemia also markedly induce reactive oxygen spices, that stimulate intracellular PKCs pathway and cause inflammatory modifications that leads to atherosclerosis

(Huang *et al.*, 2017).

The high mortality rate is observed in diabetes due to stroke, coronary- and, peripheral- artery disease. The excess free oxidative species level in diabetes produces negative effect on myocardial cell, by leading dysregulation in calcium signalling, dysfunction of contractile protein, and remodulation of myocardium. All these events leads to cardiac cell death (Lotfy *et al.*, 2017).

Diabetes with chronic microvascular complications leads to high morbidity along with mortality rate. About 30-45% of T2DM patients possess microvascular problems. The key elements to develop or progress the complications consist of uncontrolled glucose level, duration of diabetes, dyslipidemia, and increase blood pressure. Control glucose level decreases the chance to develop microvascular complications, such as nephropathy, retinopathy, and neuropathy. (Mehravar *et al.*, 2016)

Diabetes-mediated nephrology (DN) declines kidney physiology and leads to progress in diverse complications, such as hypertensive kidney disease (nephrosclerosis) and acute kidney failure (Alicic *et al.*, 2017). The clinical symptoms include, increase blood pressure, and proteinuria (Thomas *et al.*, 2015; Umanath & Lewis, 2018).

The pathophysiology leads to develop DN and end-stage renal disease (ESRD) initiates by chronic hyperglycemia. Diabetes-mediated AGEs (advanced glycation end products) increase growth factors and dysregulate hormone. AGEs release reactive oxygen spices (ROS) and inflammatory pathogens to cause glomerular modification, hyperfiltration hypertension and, renal hypertrophy. The clinical manifestations to diagnose DN include, albuminuria and, increase blood pressure. The structural modifications in kidney includes, extracellular matrix deposition, thick glomerular membrane, proliferation modification, and tubular atrophy (Umanath & Lewis, 2018).

Diabetic neuropathy is another common complication with 60% prevalence level in diabetic population. It develops after few years of onset of diabetes or uncontrol

glucose level, in some cases neuropathy is already present at the time of diabetic diagnosis (Timar *et al.*, 2016).

Diabetic neuropathy is affected quality of life and caused other diabetic complications. Patients sometimes are not able to distinguish temperature and burn themselves due to sensing fault temperature in their routine activities. Sensitivity loss and nociception loss able to cause ulcer formation and may lead to serious injuries, such as amputations. Previous research suggested that increased autonomic neuropathy of the heart is independently correlated with diabetic nephropathy. And cardiac autonomic neuropathy (CAN) is related to enhanced the frequency of morbidity and mortality in patients that having cardiovascular disease (Timar *et al.*, 2016).

Diabetic neuropathic patients have an increased risk of fall down that can lead to decreased mobility, increase sedentary lifestyle, and increase mortality level. The factors that are needed to consider to treat DN patients includes strength, proprioception, and fear of fall (Timar *et al.*, 2016).

Diabetic peripheral neuropathy (DPN) is characterized as sensorimotor polyneuropathy. Chronic hyperglycemia and cardiovascular complications lead to modify the metabolic process and alter microvessels to originate diabetic peripheral neuropathy. Sensory symptoms initiate from toes and increases towards upper limbs. Furthermore, pain sensation loss, tingling, hyperalgesia, allodynia, and burning are observed. Sensory symptoms are not able to indicate the severity behind the axonal loss. Patients with declaring severe pain have less or absence of sensory deficit under clinical exam or *via* electrodiagnostic examination. About 20-30% of DPN patients are suffered from neuropathic pain. (Juster-Switlyk & Smith, 2016)

Autonomic neuropathy (AN) is most common diabetes-mediated vascular complication. The clinical manifestations to diagnosis AN include, exercise intolerance, resting tachycardia, constipation, gastroparesis, orthostatic hypotension, erectile dysfunction, and sudomotor dysfunction. Autonomic neuropathy in cardiac vessels is leads to myocardial infarction, fatal arrhythmia, morbidity, and death. The cardiac autonomic neuropathy is assessed *via* heart rate variability (HRV) (Tiftikcioglu *et al.*,

2016).

Diabetes causes motor and sensory dysfunction in gastrointestinal tract that substantially leads to morbidity. Gastric emptying is relatively 30-50% delayed in chronic hyperglycemia, with appearing multiple symptoms, including impaired nutrition, uncontrol glycemia, and long-time is taken to absorb orally administered drugs. The extent to which delay emptying is variable and it is not well recognized. Diabetes-induced gastroparesis can be associated with clinical manifestations, including postprandial fullness, nausea, bloating, vomiting, while some patients are asymptomatic in gastroparesis and may produce relatively weak symptoms. Intestinal peristalsis is usually dysregulated in diabetes, it may lead to diarrhea or cause constipation (Rayner & Horowitz, 2006).

The diabetic foot is characterized by deep tissues lesions that are mediated by diabetic neurological complication and, lower limb vascular disorder. The prevalence of diabetic foot is gradually increased because of continuous increase in diabetes mellitus. The previous study reported that diabetes-induced diabetic foot is amputated in every 30 seconds (Zhang *et al.*, 2017).

Periodontal diseases, including, gingival and periodontal tissues are associated with chronic hyperglycemia-mediated diabetes mellitus. Periodontium is specialized tissues that perform its function by providing support to teeth and surround them. This is comprised of periodontal ligament, dentogingival junction, alveolar bone, and root cementum. The key function, other than mastication, is to provide support and protect and keep the tooth in position. However, periodontal disease is still not recognized as diabetes-mediated complication. The previous study has been suggested the poor glycemic level might be enhanced the risk to develop periodontitis and gingivitis. Diabetes mediated periodontal disorders have been associated to develop metabolic syndrome, cardiovascular complications, and obesity (Molina *et al.*, 2016).

Initially, diabetes was suggested to induced periodontal diseases, while sometime later, reverse association was observed that shown periodontitis as the risk factor to develop diabetes (Molina *et al.*, 2016).

Bone metabolism is affected by diabetes mellitus. The dysregulation in bone metabolism, including formation and resorption, affect bone, and both conditions have been found in diabetes. Chronic hyperglycemia has produced an effect on osteoblast cells. Diabetes is consistently reduced osteoblasts and osteocytes by increased intracellular apoptotic pathway, such as, advanced glycation end products is one the key component that initiates apoptosis by stimulating MAP (Mitogen-activated protein kinase) kinase pathway. Diabetes further interrupted bone formation by decline transcription factor expression that modulates osteoblast differentiation. The study has conducted in type 1 and 2 diabetes and observed the decreased intracellular alkaline phosphatase activity and formation of mineralized matrix. The high advanced glycation end products in diabetic patients are reduced the bone healing process. Furthermore, in diabetes, AGEs receptor (RAGE) is found in osteoblast that interrupted the sensitive intracellular mechanism (Wu *et al.*, 2015).

Diabetes mediated skin disorders are generally neglected and underdiagnosed. The complications include dry skin, cutaneous infection, and pruritus. Chronic hyperglycemia and advanced glycation end products can cause pruritus that can lead to major skin complications. (de Macedo *et al.*, 2016)

Several mechanisms to initiate skin disorders are recognized in which advanced glycation end products are well described. The uncontrolled glycaemic profile has produced strong influence on skin homeostasis by reducing keratin cells to proliferate and migrate, inhibit protein anabolism, increase intracellular apoptotic mechanism of endothelial cells, inhibit nitric oxide formation and impaired phagocytosis and chemotaxis in cells (de Macedo *et al.*, 2016).

Diabetes retinopathy has shown potential to cause blindness. The vision impairment or loss is due to diabetic mediated maculopathy and complications of proliferative diabetic retinopathy (vitreous hemorrhage, tractional retinal detachment and neovascular glaucoma) (Nentwich & Ulbig, 2015). The study has reported that type 1 diabetes leads retinopathy and nephropathy can increase risk of each other. This study has also reported diabetic retinopathy association with cardiovascular

impairments and high mortality rate in both types of diabetes (Nentwich & Ulbig, 2015).

1.1.11 Lifestyle interventions in Diabetes Mellitus

Healthy characteristics of routine life, including physical activity, healthy diet, and stress, are important factors affecting the development and progress of T2D (Chen *et al.*, 2015). Well-proper management in daily routine is a vital characteristic to treat diabetes, including provide diabetes self-management education, provide medical nutritional therapy to patients, increase physical activity, cessed smoking, and provide psychosocial care. Diabetic patients and practitioners should focus on how to have an optimal lifestyle from the time of the first diagnosis to improve the treatment of diabetes (Association, 2018).

Four critical periods have defined to assess the need for diabetes self-management education and support. These are assessed (i) at the time of diagnosis, (ii) every twelve months for assessment of nutrition, education, and emotional needs (iii) When new risk factors (health conditions, physical limitations, emotional factors, or basic living needs) starts that effect on self-management and (iv) When changes in care occur. (Association, 2018)

The practice to implement regular physical activity in daily routine is critically emphasized factor to maintain glucose level and provide impression of healthiness in diabetes. The health status of individual is defined as the proper recommendations and precautionary measures for intensity activity level. Physical activity is characterized by all body movements that initiated catabolism of biomolecules to utilized energy in the form of ATP. Exercise is defined as the properly planned, and well-structured level of physical activity. Exercise has been shown improvement in glucose tolerance in diabetes, decreased the risk to progress cardiovascular complications, and reduced body weight. Regular exercise might be reduced or delay the onset of T2DM. In type 1 diabetes, regular exercise has also been shown significant health benefits, such as decreased insulin resistance, improved muscle strength, cardiovascular fitness, and many others. The challenges to control hyperglycemia in diabetes is varied and based

on the type of diabetes, type of activity to recommend them, duration of persistent uncontrolled diabetes, individual age, and diabetes produced secondary complications. Therefore, the intensity of physical activity and exercise is suggested according to the specific requirement for metabolic health of individual (Colberg et al., 2016).

The encouragement should be given to diabetic and non-diabetic individuals to reduce the frequency of daily sedentary time and tried to adopt the frequent break up sitting time by activity bouts. Furthermore, changes in behavior have the potential to further promote practice to implement regular physical activity for a lifetime (Colberg et al., 2016).

1.1.12 Therapeutic approaches

Globally, metformin is used as alternative or adjunct approach to treat diabetes. Previous studies and clinical profiles of T2DM has been suggested metformin as effective and safe drug either use alone or adjunct with sulfonylurea. Metformin maintains glucose level by preventing gluconeogenesis and reduce the peripheral IR and stimulate insulin release from β cells. Furthermore, metformin has been shown its positive effects to reduce plasma lipid levels, which in turn weight loss (DeFronzo *et al.*, 1995).

Sulfonylureas are found to increase insulin concentration from pancreatic β-cells. The mechanism starts with binding of sulfonylureas with its receptor on insulin-secreting β- cells. The ATP-dependent channel blocked the inflow of K^+, depolarized the plasma membrane, and increased the flow of calcium ion into β cells. Subsequently, calcium ions bind with filaments of actomyosin and initiate insulin exocytosis that is used for glucose homeostasis (Rendell, 2004; Sola *et al.*, 2015).

Sulfonylureas have short term duration for its action, and this is usually prescribed in combination therapies to get optimal response. The common side effects associated with sulfonylureas including, headache, nausea, malaise, dizziness, and diarrhea. Two sulfonylureas are avoided to prescribe due to similar mode of action, while it can be used with other medication (incretin-based agents, metformin, alpha-

glucosidase inhibitors, and insulin) to get optimum response (Grant & Graven, 2016).

The reports have shown detrimental cardiovascular sequelae (Middleton *et al.*, 2017), prolonged hypoglycemia (Sola *et al.*, 2015), myocardial infarction, ischemic stroke, cardiovascular death (Douros *et al.*, 2017), and hip fracture (Rajpathak *et al.*, 2015).

The sulfonylureas drugs that are prescribed generally includes, Amaryl (glimepiride), Glucotrol (glipizide), Tolinase (tolazamide), Diabinese (chlorpropamide) and Tolbutamide.

Meglitinides normalize glucose levels *via* stimulating insulin secretion from pancreatic β cells. It forms loose interaction with their receptors and shows that this has short duration to produce its maximum effect. Meglitinides use as alone or in combination with other drugs. Meglitinides are restricted to use with sulfonylurea due to similar mechanisms of action. Primary, it is used to decrease post-prandial glucose. The dysfunction of β cells decreases the effectiveness of meglitinides. It is metabolized in liver, thus, it has been suggested to avoid in case of liver dysfunction and proper caution is taken to administered in patients with renal dysfunction (Grant & Graven, 2016). The meglitinides drugs that are prescribed generally includes Starlix (nateglinide), and Prandin (repaglinide).

Alpha-glucosidase inhibitors (AGIs) inhibits alpha-glucosidase such as maltase, subsequently the sugar absorption from gut delay. The studies have been reported that besides delay absorption, AGIs further show its metabolic activity to ferment starch in the colon. AGIs use as first-line glucose therapy to treat T2DM because it works on postprandial hyperglycemia. The overdose of AGIs has not been shown hypoglycemic events, life-threatening events or increase body weight (van de Laar, 2008).

The frequency of abdominal pain, flatulence, and constipation was compared among Asian and non-Asian patients. The incidence of diarrhea was found higher in non-Asian populations relatively other. Due to specific mode of action of AGIs, their side effects are most often associated with gastrointestinal (GIT). Furthermore, the

meta-analyses study was also reported the increased incidence of GIT discomforts related to AGI, such as constipation, flatulence, abdominal pain, and diarrhea. The study of Van de Laar *et al*. shown that acarbose treatment had increased GIT adverse effects and that was parallel with increasing dose. The frequency of increased side effects might be according to districts. Hanefeld *et al*. observed GIT side-effects by AGI treatment, and the increased incidence of adverse effects was varied from one country to another country (Gao *et al.*, 2018).

The alpha-glucosidase inhibitors that are prescribed generally include, glyset, acarbose, and miglitol, voglibose, precose and glucobay.

Thiazolidinediones (TZDs) are used to increase insulin sensitivity that leads to decline in glucose levels. They produce its effects by acting as agonists of peroxisome proliferator-activated receptors gamma (PPARγ). TZDs-interaction with PPARγinitiates the receptor to regulate gene transcription that mainly involved to maintain glucose and produce adipose tissues (Alemán-González-Duhart *et al.*, 2016).

The side effects of TZDs include weight gain, edema, plasma-volume expansion, congestive heart failure, hepatotoxicity, fluid retention, and hemodilution. The mechanism behind toxicity may be mediated by activation of PPARγ or by some other pathway, thus, further studies are needed to explain this phenomenon (Alemán-González-Duhart *et al.*, 2016). The thiazolidinediones drugs that are prescribed generally include rosiglitazone, and pioglitazone.

The incretin hormones include, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) are contributed to stimulate pancreatic insulin secretagogue activity, that eventually maintains normoglycemia (Zietek & Rath, 2016). In T2DM the effect of incretin hormone is observed as impaired. But, still, its activity is controversial as previous studies shown unaltered, upregulated and downregulated GLP-1 secretion in T2DM. The reduction in GLP-1 response is observed followed by oral glucose intake by IGT and IFG individuals, while enhanced GLP-1 level is also observed IFG patients (Færch *et al.*, 2015).

Monami *et al* reported nausea, vomiting, diarrhoea, angioedema, pancreatitis, angina pectoris, chronic heart failure, arteriopathy of lower limbs, coronary artery revascularization, myocardial infarction, and stroke as serious or severe adverse events initiated by GLP-1 inhibitors (Monami *et al.*, 2009).

The GLP-1 receptor agonist drugs that are prescribed generally includes, Dulaglutide (Trulicity), Lixisenatide (Adlyxin), Albiglutide (Tanzeum), Liraglutide (Victoza), Exenatide (Byetta), and Semaglutide (Ozempic, Rybelsus).

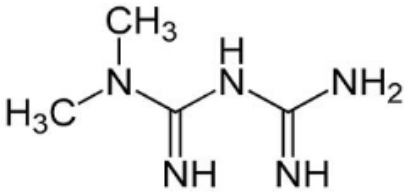


Figure 1.3: Structure of metformin (Rizvi et al.,

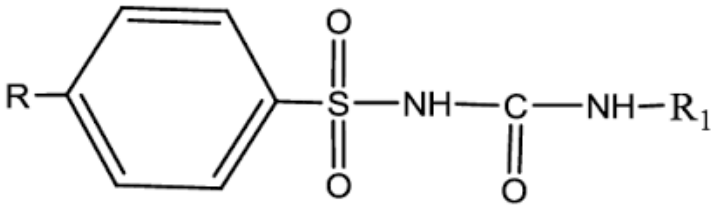


Figure 1.4: Structure of sulfonylurea (Yousef et al., 2018)

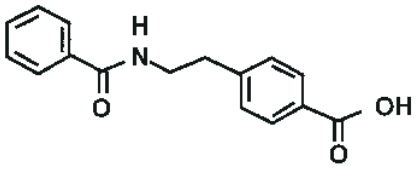


Figure 1.5: Structure of Meglitinides (Levien et al., 2001)

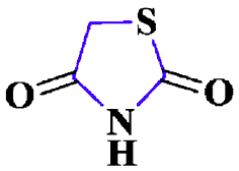


Figure 1.6: Structure of thiazolidinediones (Naim et al., 2017)

Dipeptidyl peptidase 4 (DPP-4) inhibitors are administered orally to decrease glucose-intolerance, prevent the degradation of active GLP-1lo, and glucose-dependent insulinotropic polypeptide, enhanced pancreatic glucose-dependent insulin secretion and suppressed glucagon secretion. Furthermore, DPP-4 inhibitors have shown their potential as an anti-atherosclerotic drug (Mita *et al.*, 2016)

Hypoglycemia is not seem in patients that was receiving DPP-4 inhibitors. While, it is recommended to down titrate the dose of these inhibitors in patients who was previously receiving treatment of insulin or a sulfonylurea. Furthermore, monitor the patients for hypersensitivity reactions and skin disorders during intervention. Especially, it is advised to cautiously monitor the renal function and liver function in patients that receive sitagliptin and vildagliptin, respectively. The previous evidences are not enough that clearly defines the association of DPP-4 inhibitors with pancreatitis, and the reported data of clinical trials is not suggested the risk for pancreatic cancer. However, both the FDA (food and drug administration) and EMA (European medicines agency) are investigating all the previously and ongoing research data through pharmacovigilance evaluations to elucidate the association of DPP-4 inhibitors with pancreas dysregulation or impairment and decide next regulatory action that should be needed (Karagiannis *et al.*, 2014).

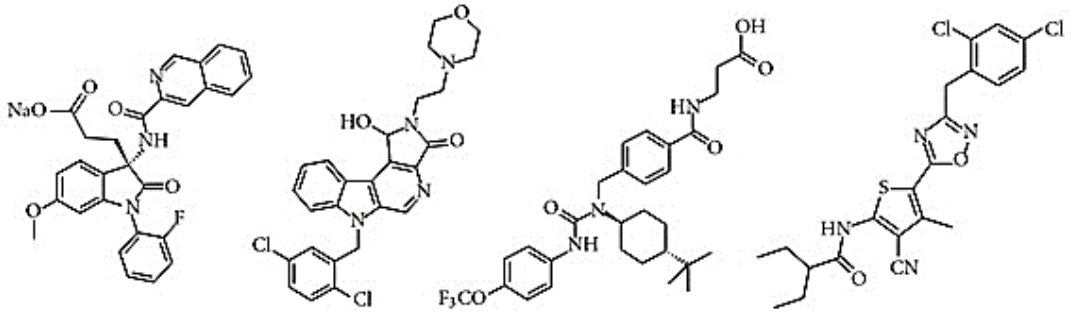
The Dipeptidyl peptidase 4 (DPP-4) inhibitors that are commonly prescribed includes, Galvus (Vildagliptin), Tradjenta (Linagliptin), Januvia (Sitagliptin), and Onglyza (Saxagliptin).

The glomerular filtration rate (GER) in normoglycemic individuals is

125 ml/min/1.73m^2, and each day kidney is filtered 180 grams of glucose. The glucose concentration in urine is absent and suggested effective reabsorption by kidney through sodium-glucose co-transporters (SGLT-1, and 2). In T2DM, the high glucose level is saturated with SGLT-2 transporters and resulted in glycosuria. SGLT-2 inhibitors prevent SGLT-2 activity in proximal tubule to reabsorb glucose, thus maintain glucose level (Dekkers *et al.*, 2018).

The mild infection in genital and urinary tract are seen frequently, but rarely resulting to discontinue the drug. These inhibitors are not recommend to patients with a previous history of genital or urinary tract infections. The long-term tolerability of drug for cardiovascular disease have been monitored in various ongoing trials. The increase LDL, short-term hypotension and volume depletion was seen in SGLT-2 inhibitors. As for malignancies, the only risk factor that found with dapagliflozin was urinary bladder cancer, which observed during follow-up, while it is found unlikely because of its early appearance after intervention of the inhibitor. In fact, SGLT-2 inhibitors were shown no significant association with adverse effects at all doses. So far, this seems to be a safe new class of OADs (oral antidiabetic drugs) for T2DM, especially because of considerable control in HbA1c level, body weight and blood pressure without resulting hypoglycaemia, SGLT-2 inhibitors contribute to the management of T2DM by offering new oral drug combinations (Halimi & Vergès, 2014).

The SGLT-2 inhibitors that are prescribed generally include, empagliflozin, dapagliflozin, and canagliflozin.



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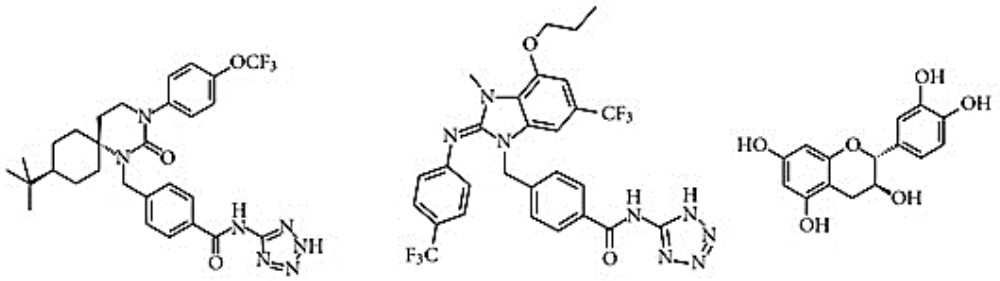


5 6 7 (catechin)

Figure 1.7: Structures of GLP-1 receptor agonist (Willard et al., 2012)

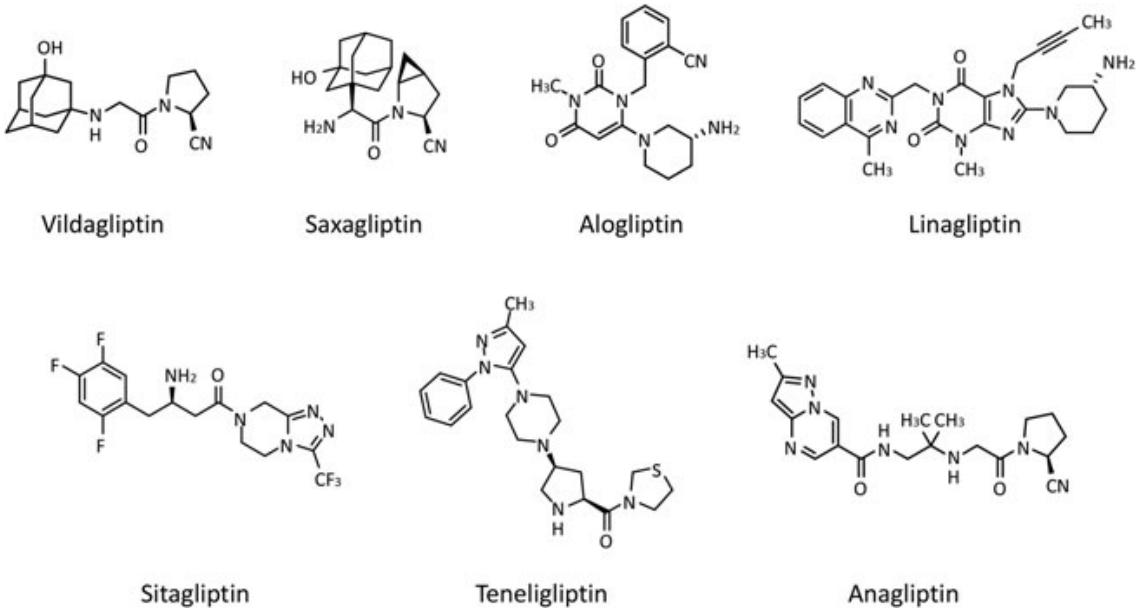
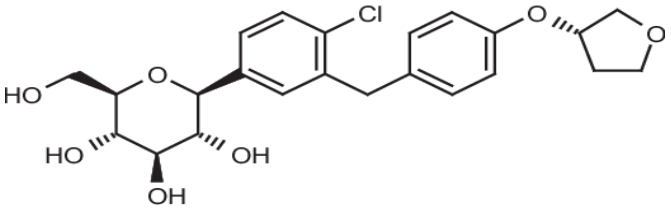
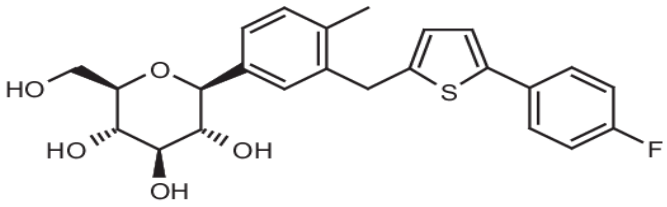


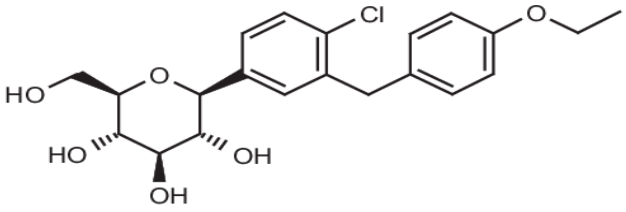
Figure 1.8: Structures of dipeptidyl peptidase-4 inhibitors (Nakamaru et al., 2016)



Empagliflozin



Canagliflozin



Dapagliflozin

Figure 1.9: Structures of SGLT-2 inhibitors (Chrysant, 2017)

1.2 Hypothesis

A) Null hypothesis

There is no difference in effect of glimepiride- metformin versus dapagliflozin-metformin for treatment of T2DM.

B) Alternate hypothesis

There is a difference in effect of glimepiride- metformin versus dapagliflozin-metformin for treatment of T2DM.

1.3 Objectives of study

This study will be conducted in Pakistani population having uncontrolled T2DM with metformin with the objectives:

1) To observe the efficacy of dapagliflozin-metformin in patients with type 2 diabetes.
2) To observe the efficacy glimepiride-metformin in patients with type 2 diabetes.
3) To compare the efficacy of dapagliflozin and glimepiride in patients with type 2 diabetes.
4) To compare the safety of dapagliflozin vs glimepiride-metformin in patients with type 2 diabetes.

1.4 Statement of the problem

Lifelong treatment is required for a T2 DM. No cure is available for the disease, moreover available first and second-line pharmacological treatment options have adverse effects that add to the agony of the patient.

1.5 Significance of study

The pathophysiology to progress T2DM is complexed and multifaceted. The key elements that leads to T2DM includes pancreatic β-cell dysfunction, peripheral IR, enhance gluconeogenesis in the liver, and increased lipolysis during obesity. Furthermore, the other known intracellular mechanisms include, high glucagon secretion, reduce incretin response, reabsorb high glucose in the kidneys and few neurotransmitter dysregulations, are also involved in the pathophysiology of T2DM.

Currently prescribed antidiabetic drugs have been under developing stages to act on the underlying cause that progress or develop T2DM. Mostly, glycemic control in T2DM patient is uncontrolled, only 53 % of diabetic patients are achieved glycemic goals with their currently therapeutic regimen.

However, even in diabetic patients having control glycemia requires multiple antidiabetic agents to manage their disease. Therefore, new drug development is required in the field.

In the current study, the inhibitor of the sodium-glucose cotransporter-2 (SGLT-2) in proximal convoluted tubule was used for treatment of diabetes. The SGLT-2 inhibitors that are currently approved or under investigation include canagliflozin, ipragliflozin, dapagliflozin, and empagliflozin. This dissertation was focused on the efficacy and safety profile of dapagliflozin-metformin combination in T2DM patients.

1.6 Operational definitions

Diabetes mellitus (DM)

Diabetes mellitus (DM) is defined as metabolic disorder, consisting of chronic hyperglycemia leads by dysregulation of insulin secretion, or insulin sensitivity or both. The diagnostic criteria for T2DM are FPG $\geq$ 7.0 mmol/L or HbA1C $\geq$ 6.5% with no caloric intake for at least 8 hours ; or 2-hour followed by plasma glucose load or 75-gm

oral glucose tolerance test $\geq$ 11.1 mmol/L; and random plasma glucose $\geq$ 11.1 mmol/L at any time of day (Goldenberg & Punthakee, 2013).

HbA1c

It is glycosylated haemoglobin and is glycaemic index in diabetes mellitus. The cutoff point to label the patient as diabetic is 6.5% (Sherwani *et al.*, 2016).

BMI

WHO has recommended the cutoff criteria for BMI is: <18 kg/m^2 is underweight, between 18-24.9 kg/m^2 is normal weight, between 25-29.9 kg/m^2 is Overweight, and $\geq$ 30 kg/m^2 is considered as obese. While between 30-34.9 is class I obese, between 35.0-39.9 is class II obese, and $\geq$ 40.0 is class III obese (Novosad *et al.*, 2013; Younas *et al.*, 2013)

CHAPTER 2

LITERATURE REVIEW

Kidney, bean-shaped organ, is the key mediator to regulate blood sugar homeostasis by stimulating gluconeogenesis, glycolysis and glucose reabsorption from glomerular filtrate (Alsahli & Gerich, 2017). At post-absorptive state, 20-25% glucose is secreted into blood circulation that is produced by gluconeogenesis in kidney. In this fasting level, kidney use approximately 10% glucose as compare to total utilization of glucose by the body. Followed by postprandial period, the formation of glucose from kidney enhance and produce 60% glucose. Kidneys are filtered about 180 g of glucose regularly and reabsorbed in the blood circulation. Hormones (specifically insulin and catecholamines), glucose transporters, and enzymes are prominent elements that contribute kidney functions (Alsahli & Gerich, 2017).

The kidney has been performed crucial part in glucose filtration, reabsorption, fluid regulation and electrolyte balance (Zhao *et al.*, 2016). Normoglycemia is maintained in non-diabetic individuals through proper glucose regulation by kidney. Healthy and non-diabetic individual filtered 180 g of glucose regularly through renal glomeruli and then reabsorbed the glucose through proximal convoluted tubules that contain GLUTs (passive facilitated glucose transporters), and the active SGLTs (sodium-glucose cotransporters). These transporters are utilized especial pump (Na+ ATPase, K+ ATPase) due to glucose transportation towards the cells against concentration gradient (Ahmadieh *et al.*, 2016).

Chronic hyperglycemia increases the renal filtration of glucose from glomeruli and enhances tubular glucose reabsorption. While under normal conditions of GFR

(glomerular filtrate rate) and blood glucose, the proximal tubule of kidney reabsorbs all the previously filtered glucose. The increase of glucose load in proximal tubule under diabetic condition is the source of glucosuria. Additionally, more glucose is reabsorbed from kidney that ultimately increases the blood glucose concentration (Vallon & Thomson, 2017).

Renal tubules provide transport to glucose molecules, sodium-glucose transporters -1 and 2 expression are upregulated in chronic hyperglycaemia, thus enhancing the threshold to produce glucosuria (Song *et al.*, 2016). The intracellular mechanism for increase upregulation of both SGLT-1 and SGLT-2 under diabetic condition is still unexplained completely, but few data suggested the uncontrolled glycemia may have more contribution other than insulin level, as the increase glucose load in proximal tubular cells of nephron increases and cause upregulation of transporters. SGLT-2 receptors are downregulated under normoglycemia, SGLT-2 inhibitors are produced glycosuria under high glucose load in tubules, and reduced glucose reabsorption according to the need of body. Previously study suggested that the preserved SGLT-1 function by using specifically SGLT-2 inhibitors decrease the development of hypoglycemia and maintain glucose level in diabetes (Verbrugge *et al.*, 2015)

The sodium-glucose co-transporter-2 in proximal convoluted tubules are inhibited to reduced glucose reabsorption and facilitates glycosuria production which eventually leads to maintain plasma glucose level in diabetes (Chao, 2014; Kalra, 2014). This complete action of these inhibitors are fully dependent on blood concentration of glucose and, inconsistent with the mechanism of action of GLUTs drug (thiazolidinediones), SGLT-2 inhibitors are independent with the insulin level.

Consequently, SLGT-2 inhibitors have weak potential to progress hypoglycemia, and not able to overly stimulate pancreatic β cells. These inhibitors perform its action in renal tubules, therefore its efficacy decreases in kidney impairment (Kalra, 2014; Rosenwasser *et al.*, 2013).

The structure of dapagliflozin drug contains C-linked glucoside, the aglycone moiety (non-sugar group) is attached to glucose molecule through carbon-carbon (C-C) interaction, which provides stability of drug against glucosidase enzymes. The chemical name of 408.87g dapagliflozin is (2S,3R,4R,5S,6R)-2-[4-chloro 3-(4-ethoxybenzyl) phenyl]-6 (hydroxymethyl)tetrahydro2H-pyran-3,4,5-triol), while its molecular formula is $C_{21}H_{25}ClO_6$. (Kasichayanula *et al.*, 2014).

Dapagliflozin is inhibited the function of sodium-glucose cotransporter 2 (SGLT2) from proximal convoluted tubule (Plosker, 2012). This drug is considered as new class that administered orally against hyperglycemia, possessed innovative mode of action, and accepted as safe medication by the food and drug administration (FDA), United States of America. The effective and tolerable dapagliflozin at orally administration have potential to decline the uncontrolled T2D-mediated complications and eventually improve quality of life (Al AdAwi *et al.*, 2019).

The studies have reported the novel agent of 1990s has shown potential to act against T2DM with glucosuria, but unfortunately, this was found as poor bioavailability because of limited absorption and high degradation. But dapagliflozin drug has opened the door for the discovery of other promising drugs to treat T2DM, such as SGLT2 inhibitors. Canagliflozin (SGLT2 inhibitor) is the first FDA approved anti-hyperglycaemic agent that inhibits reabsorption of glucose form renal proximal convoluted tubule and leads to inhibit blood glucose in diabetic patients. Followed by, dapagliflozin is selected as second FDA approved drug that act as SGLT-2 inhibitors (Al AdAwi *et al.*, 2019).

Dapagliflozin is specifically acted on SGLT-2 and inhibits its activity to reabsorb glucose (Vallon & Thomson, 2017), and ultimately increase glucosuria that eventually maintains glucose homeostasis. This drug mechanism is initiated with high glucose load in proximal tubule and independent with both insulin secretion or action (Plosker, 2012; Schwartz & Katz, 2016).

The efficacy of dapagliflozin was studied at phase III level for drug development and shown the decrease in HbA1c level from baseline. Similarly, another

earlier study was shown decrease fasting plasma glucose along with body weight in diabetic patients as compared with control (Filippatos *et al.*, 2015).

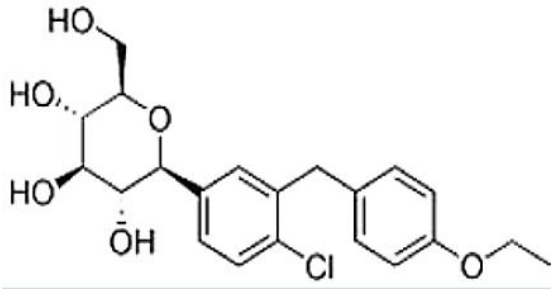


Figure 2.1: Structure of dapagliflozin (Mante et al., 2017)

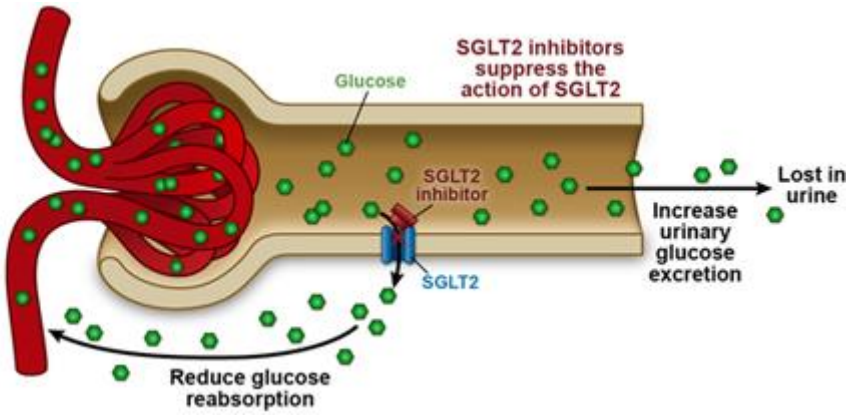


Figure 2.2: Mode of action of SGLT-2 inhibitors (Zaccardi et al., 2016)

The double-blind trial study was recruited in randomized 485 dapagliflozin treated-T2DM patients and control. The drug (2.5, 5 or 10 mg) was given in morning (main cohort) or evening (exploratory cohort). The analysis of main cohort study (−0.58%, −0.77%, −0.89%, at 2.5, 5 and 10mg dose) and exploratory cohort (−2.88% with 5 mg and −2.66% with 10 mg) at week 24 was shown decrease HbA1c level relative to baseline (Filippatos *et al.*, 2015).

The randomized, double-blind, placebo-controlled study on 261 individuals for 12 and 24 weeks was performed to identify the efficacy of dapagliflozin. The drug dose of dapagliflozin from 1 to 50 mg was shown effective when compared with metformin, group as compared to placebo (Fioretto *et al.*, 2015).

Another study with T2D patients treated with dapagliflozin monotherapy was shown dose-dependent decline of glycosylated haemoglobin compared with their baseline level. Similarly, dapagliflozin was observed significantly reduced FPG and body weight as compared with control and metformin-treated patients. The mild genital and urinary tract infections were found in dapagliflozin-treated patients as compared to control, while hypoglycaemia effect was not found in dapagliflozin groups (Fioretto *et al.*, 2015).

The considerable adverse effects of dapagliflozin treatment has not found to impair liver or kidney or fluctuate baseline serum electrolytes. While, the first week of dapagliflozin administration was caused decline eGFR level, followed by baseline levels was achieved. Over all, dapagliflozin-treatment up to 2 years in diabetic patients has not shown renal impairment, diabetic nephropathy and acute nephrotoxicity (Fioretto *et al.*, 2015).

Another study was reported that hypovolemia, hypotension, and dehydration were uncommon, but rarely found in none serious level in the dapagliflozin treated group as compared with control. The increased risk to progress volume depletion events was found in dapagliflozin- and loop-diuretics- treated patients. Statistically

increased levels of dyslipidemia and week to moderate increase of low-and high-density-lipoprotein cholesterol, total cholesterol and reduce triglycerides were reported. There are some pieces of evidence that shown ketoacidosis in diabetic patients that were treated with SGLT-2 inhibitors. However, dapagliflozin effect on ketoacidosis is inconsistent with previously reported findings. The investigation of this issue is going on by the European Agency of Medicine and the US FDA by review all pooled analyses and will study the modifications that are required in these medicines or not (Fioretto *et al.*, 2015).

Metformin hydrochloride is white crystal drug of 129.16 g/mol. This is chemically termed as N, N-dimethylimidodicarbonicarbonidediamide hydrochloride. The molecular formula is C4H11N5.HCl and freely soluble in water (H_2O), slight/weak solubility in alcohol, and insolubility against methylene chloride and acetone (da Trindade *et al.*, 2018).

Several pieces of evidence have reported the favorable effective/risk ratio of metformin by analysed through pharmacodynamics, clinical efficacy, pharmacokinetics, and intracellular mechanistic study. Metformin is first-line choice for T2DM patients, and it is also cost-effective. Globally, it is considered as most known prescribed diabetic therapy, and it is considered as essential medicine included in the World Health Organization list (C. J. Bailey, 2017).

Metformin, a non-medical complex medicine possessing multiple sites of action and intracellular molecular pathways. Metformin is acted in two ways; directly or indirectly on gut to enhanced glucose utilization, intensify glucagon-like peptide (GLP-1), and alter microbiome and, acts on liver to decrease gluconeogenesis. At intracellular downstream, metformin acts on liver mitochondrial respiratory chain, causing to stimulate 5' AMP-activated protein kinase (AMPK), decrease insulin resistance (by initiating fat metabolism) and reducing cyclic adenosine monophosphate (cAMP), which in turn, declining the expression of the gluconeogenic enzyme. Metformin also inhibited fructose-1,6-bisphosphatase action in liver *via* adenosine monophosphate (AMP). Further, hepatic, renal, and intestinal, effects of metformin in

humans is required to identify further strong benefits mechanism followed by long-term medication in diabetic humans (Rena *et al.*, 2017).

Metformin is anti-diabetic treatment having good safety profile. The gastrointestinal side effects are commonly observed with transient symptoms that spontaneously resolved or avoided by change in dosage. Metformin monotherapy is not produced hypoglycaemia until it is not prescribed as conjunction with other anti-diabetic drugs, such as sulfonylureas or insulin. About 4% of diabetic participants were discontinued metformin because of its increased adverse events. Chronic adverse effects are rare and commonly observed due to lactic acidosis in renal or hepatic impairment patients or may be associated with other contraindications. Metformin treatment has been reduced weight in diabetic patients, and therefore creates difference with other antidiabetic therapies that are linked to maintain or gain weight. To date, metformin most commonly prescribed to manage diabetes rather than weight loss in diabetic or healthy individuals (Group, 2012).

Metformin is generally prescribed to treat diabetes, but it can target aging-mediated intracellular mechanisms (Barzilai *et al.*, 2016). Metformin stimulates anti-aging mechanisms by inhibiting inflammation, DNA damage, inflammatory-cytokine induced DNA protein expression and it can initiate adenosine monophosphate-induced protein kinase (AMPK) signalling mechanism.

Furthermore, metformin averts DNA impairment from superoxide through preventing reactive oxygen (ROS) formation by reverse electron flux, and by reducing mTOR- (mammalian target of rapamycin) mediated superoxide synthesis mechanisms (Valencia *et al.*, 2017).

A weight-loss potential of metformin in diabetic and non-diabetic individuals was studied. The 6- month clinical study recruited on 199 volunteers administered 2500 mg metformin and compared with placebo, thus, weight loss of 5.8 kg was observed in treated group, while placebo volunteers shown 0.8 kg mean weight gain (Igel *et al.*, 2016).

In the Biguanides and Prevention of Risks in Obesity (BIGPRO1) study, 457 non-diabetic obese individuals were treated for 1 year with metformin 850 mg BID therapy or placebo. The decreased body weight of 1.2 kg was observed in the metformin arm with placebo. In another 15-days study of 30 non-diabetic obese volunteers with treated metformin 500 mg BID or placebo; 2.6 kg decrease body weight was found in the metformin arm (Igel *et al.*, 2016).

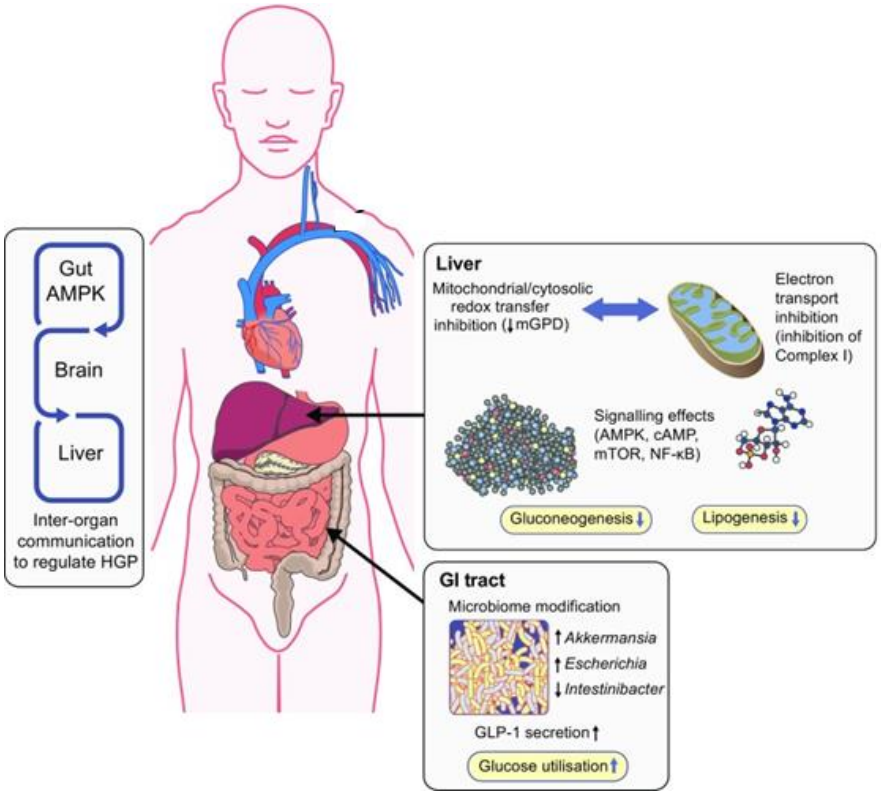


Figure 2.3: Mode of action of metformin (Rena et al., 2017)

Malin et al. study also consistent with previous studies, they observed approximately 3 kg weight loss by metformin treatment (2000 mg/day) in 32 prediabetic individuals for 12-weeks. The meta-analyses on non-diabetic obese subjects that had received metformin monotherapy was shown 1.92 kg decrease body weight compared to baseline, along with a decreased of 38% risk to progress type 2 diabetes (Igel *et al.*, 2016).

Another meta-analysis study observed decrease mean 1.1 kg weight followed by treated with variable dosing and duration of metformin therapy. Furthermore, liraglutide monotherapy (anti-obesity) also used to compare and was observed that it reduced 1.7 kg weight (Igel *et al.*, 2016).

The previous evidence is suggested that metformin is effective for cardiovascular treatment (CVD) rather than diabetes mellitus. However, prospective randomized trials that used the drug in non-diabetic patients have shown neutral potential on cardiovascular problems. The further study on randomized controlled trials are needed, that explains better understanding of targeting mechanism and using metformin. Moreover, novel preclinical nondiabetes-inflammatory markers were identified that uses metformin as therapeutic strategy and thus benefit to utilized metformin as repurposing in CVD complications (Rena & Lang, 2018).

The previous study data has suggested that metformin has to be prompted attempts to use as probe in prevention of cancer activity in clinical trials (Chae *et al.*, 2016). Metformin has inhibitory effects on tumor proliferation markers; however, the gap is still present to identify the association of metformin with survival rate. It is better to identify the tumor histology and stage at which an optimum effect of metformin as an anti-cancer therapy is observed. The effective administration of

metformin as immunotherapy requires to be further verified and valid authentic pieces of evidence to establish likely future benefits (Rena & Lang, 2018).

Glimepiride belongs to first in sulfonylurea group of the third generation with possessing organic properties and having $C_{24}H_{34}N_4O_5S$ molecular formula (Ahmed *et al.*, 2016). The comparation of glimepiride with other two generations has shown high potential and duration of action (Reges *et al.*, 2018).

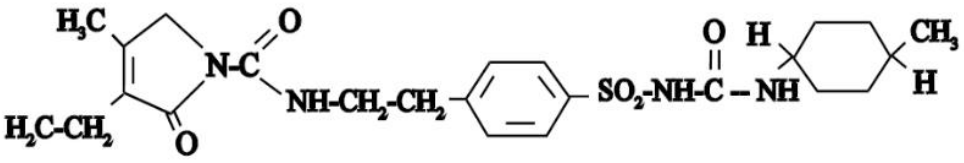


Figure 2.4: Structure of glimepiride (Jacob, 2014)

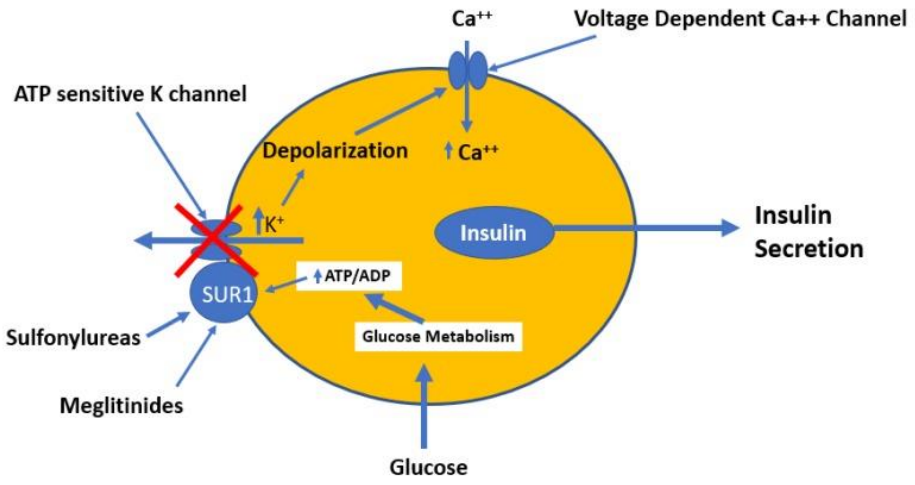


Figure 2.5: Mode of action of glimepiride (Feingold, 2019)

Glimepiride is glucose-lowering drug from the group of sulfonylurea (Kabadi, 2015) of either the second or third-generation. The drug is increased the insulin from pancreas and enhanced the sensitivity of the intracellular insulin receptor to the action of insulin. This drug is chemically water-insoluble and half-life of this is approximately 5-hours. Following oral administration, glimepiride is absorbed within 1-hour and shows a peak of drug plasma at 2-3 hrs (Ahmed *et al.*, 2016).

Sulfonylureas drugs are increased glucose tolerance by stimulated glucose-induced insulin secretion from pancreatic β-cells, and probably the inverse association would be found among insulin release and blood glucose level. Although, the meta-analysis in glimepiride-treated diabetic individuals is observed the decrease glucose level and retain similar insulin concentration in their circulation. This evidence has assumed that glimepiride treatment exerted extrapancreatic, and insulin-like activity on muscle and adipose tissues.

The recent study has suggested the decrease in endogenous glucose formation by glimepiride other than insulin secretagogues actions of the drug. The decline of therapeutically enhanced increase insulin level may be imperative to discuss, as previous findings hypothesized the association of fasting plasma insulin (FPI) and consequent heart disease progression. While, A clearer explanation is needed to explore the insulin effects on cardiovascular system (Massi-Benedetti, 2003).

Del Guerra et al. were identified the glimepiride direct effects on pancreatic islets. They isolated pancreatic islet cells from 7 human donors and cultured them to assess glucose-induced insulin secretion after glimepiride treatment. They observed that increased glimepiride concentrations caused enhanced insulin secretion. Consequently, glimepiride monotherapy leads to stimulate physiologic insulin level,

revealing that glimepiride has biphasic insulin secretion that was dependent on the ambient blood glucose concentration (Massi-Benedetti, 2003).

Goldberg et al assessed the efficacy of glimepiride (1, 4, or 8 mg) in randomized 304 T2DM patients up to 14-week. At the end of the study period, all glimepiride doses decreased FPG, PPG, and HbA1c levels than placebo. Median FPG levels were lowered by 43, 70, and 74 mg/dL at 1, 4, and 8 mg glimepiride doses, respectively. HbA1c levels (1mg: 1.2%, 4mg: 1.8%, and 8 mg: 1.9%) and postprandial plasma glucose (1mg: 63, 4mg: 92, and 8 mg: 94 mg/dL) were declined. Comparative with 1-mg glimepiride, 4- and 8-mg doses was shown greater potential, however, 4-mg glimepiride exhibited optimum antihyperglycemic activity (Basit *et al.*, 2012),

Schade et al was conducted placebo-controlled, randomized clinical trial by administered 1–8 mg dosage of glimepiride up to 10 weeks in T2DM. In this study, decreased fasting plasma glucose (FPG) by 46 mg/dL, postprandial plasma glucose (PPG) by 72 mg/dL, and HbA1c by 1.4% was observed as compared with placebo group. Optimum glycemic level (HbA1c < 7.2%) was identified in 69% of glimepiride-treated subjects relatively 32% of controls group. While, non-fasting insulin and C-peptide levels were also enhanced in the study volunteers (Basit *et al.*, 2012).

Glimepiride is decreased both the levels of FPG and PPG as compare to placebo and once-daily dose is equivalent to administered the twice-daily doses. Studies are also suggested that glimepiride is able to controls the blood glucose levels by initiating its effect on insulin release, which is appeared to be greater approximately 2-hours after taking the meals than under fasting conditions. These outcomes of Basit *et al.*, 201 is suggested that glimepiride monotherapy is enhanced the insulin and C-peptide secretion under physiologic conditions (Basit *et al.*, 2012).

The glimepiride safety and efficacy were identified in multiple controlled studies, the decreased risk of hypoglycemia and increased body weight relative to other sulfonylureas drugs were observed. Glimepiride was effective to reduce blood

FPG, PPG, and HbA1c levels, moreover, it was suggested as useful and cost-effective therapeutic choice to control T2DM (Basit *et al.*, 2012).

The findings of reported study for this combination suggested the effective profile of the combination and considered it as safe therapeutic choice for poorly controlled T2DM patients that was before on metformin monotherapy. Rosenstock et al. suggested the association of this combination with improvement in 2-hour PPG, FPG, and HbA1c. The maximum patients were found with HbA1c < 7.0% compared to dapagliflozin plus metformin or saxagliptin plus metformin. The weight loss was another additional benefit that was associated with dapagliflozin. Patients were tolerated with add-on medication with comparable rates of adverse effects to monotherapies. Consistently, genital infections were appeared in T2DM patients that receiving saxagliptin and dapagliflozin combination as compared to patients with dapagliflozin monotherapy. The risk to progress hypoglycemia was < 1% in combination therapy (D. M. Williams & Stephens, 2015).

The randomized, parallel-group, placebo-controlled trial, double-blind study was performed in T2DM patients. All the subjects were receiving dapagliflozin added to glimepiride treatment up to 48-weeks. The combination was improved glucose tolerance and weight loss (Strojek *et al.*, 2014).

The 16-week randomized, clinical phase III trial, double-blind placebo-controlled study was performed by Schumm-Draeger et al. The inclusion criteria of patients were based on previously taken metformin (≥ 1500 mg/day of twice for 24-hours) and had poorly glycaemic control randomized to receive dapagliflozin (2.5 or 5 mg of twice/day), placebo or 10 mg of dapagliflozin once daily. Patients (n= 400) randomized to 2.5 mg, 5 mg (twice daily), 10 mg (once daily) or placebo co-administered with metformin (twice daily). The significantly reduced adjusted mean change of HbA1c in the dapagliflozin groups were found as compared to placebo [(2.5 mg dapagliflozin: −0.52% vs. −0.30%, $p = 0.0106$) (and 5 mg dapagliflozin: −0.65% vs. −0.30%, $p < 0.0001$)] (Schumm-Draeger *et al.*, 2015).

Sjöström et al were pooled the data from seven studies to evaluate associations of dapagliflozin-induced weight loss and decrease in % of HbA1c in T2DM patients. The therapy was based on 10 mg of dapagliflozin alone or in combination for 24-weeks. They suggested that 2 kg of reduction in body weight with dapagliflozin was contributed to 6% decrease of total baseline HbA1c level (Sjöström *et al.*, 2015).

A 52-week, open-labeled, study was conducted in T2DM patients having inadequately controlled glycemia with quadruple oral hyperglycemia drugs (metformin, and dipeptidyl peptide 4 inhibitors, and glimepiride) to identify the safety 10 mg of dapagliflozin. After 52 weeks of intervention, patients were showed clinically normal cholesterol (163.2 ± 32.5 mg/dL), triglyceride (160.4 ± 83.6 mg/dL), HDL (42.9 ± 8.7 mg/dL), and LDL (95.2 ± 27.6 mg/dL) levels (Ku *et al.*, 2019).

Stephan Matthaei et al were recruited patients with HbA1c of 7.0-10.5% (53-91 mmol/mol) and treated with dapagliflozin with metformin plus sulfonylurea (group A) once in 24-houre or placebo (group B) till 24-weeks. Group A was showed 184.5mg/ dL of total cholesterol, 101 mg/dL of LDL, 49.5 mg/dL of HDL cholesterol, 2.1 LDL/HDL cholesterol ratio and 173.8 mg/dL of triglycerides (Stephan Matthaei *et al.*, 2015).

The clinical phase 3, double-blind, multicentre trial was conducted on 546 T2DM patients who previously received metformin (≥1500 mg) once daily and had poorly control glycaemic profile. They were randomly divided into 4 groups according of dapagliflozin dose; 2·5 mg (group 1), 5 mg (group 2), or 10 mg (group 3), or placebo (n=137). After 24-weeks of once daily dose intervention, the frequency of UTI were 7 (5%) in placebo, 4(3%) in 2.5mg dose, 7(5%) in 5mg dose and 9(7%) in 10mg dose (C. J. Bailey *et al.*, 2010).

Häring *et al* was observed improve hyperglycemia, systolic blood pressure, and weight

after 24 weeks of treatment with 10 mg or 25 mg empagliflozin add-on to metformin, this effective action was sustained up to 76th week, while empagliflozin administration over 76 weeks was found to be well tolerated. Empagliflozin as add-on

to metformin might be beneficial therapeutic selection for T2DM people that incapable to prevent hyperglycemia with only metformin (Häring *et al.*, 2014).

Canagliflozin (100mg or 300mg) plus metformin produced considerable higher response to control HbA1c and weight loss relative with metformin monotherapy, both concentrations of drug in combination has shown tolerability effect as similar as shown in monotherapy. Besides, canagliflozin at 100mg or 300mg used as monotherapy produced relatively more decreased of HbA1c level, and body weight than metformin monotherapy. Overall, the findings were supported the efficacy profile and safety level in add-on therapy with canagliflozin (100mg or 300mg) and metformin in drug-naive T2DM patients, especially for patients that possess 8.5% (69 mmol/mol) HbA1c level. The report was suggested that canagliflozin might be best therapeutic alternative to metformin in diabetic population (Rosenstock *et al.*, 2016).

Metformin and glyburide are orally administered glucose-lowering drugs used in both T2DM and gestational diabetes. The add-on of these drugs was increased the efficacy and decrease insulin requirement in patients. Furthermore, the effective results were not found in patients who failed to response to these medications or experienced adverse effects by using both of these (Nachum *et al.*, 2017).

The diabetic patient received 50 mg vildagliptin in combination with glimepiride, achieved 6.5% endpoint HbA1c in 45% treated subject's comparative to 3% placebo volunteers. Moreover, consistent with the American Diabetes Association (ADA) recommendation regarding HbA1c, 65.9% of patients were observed <7% HbA1c level at the endpoint compared with 11.6% of placebo individuals. Relatively, the use of sitagliptin (dipeptidyl peptidase-4 inhibitor) with glimepiride specifically in the Caucasian population was observed, ADA goal that is <7% in HbA1c were insignificant among sitagliptin- glimepiride treated and non-treated individuals by showing 10.8 and 8.7% HbA1c, respectively, even though subjects had shown slightly lesser HbA1c control at 8.4% baseline (Kikuchi *et al.*, 2010).

Sitagliptin-added glimepiride resulted considerably improved glucose homeostasis. Sitagliptin addition for 52 weeks improved insulin-secreting pancreatic

β-cell physiology comparative with placebo group and baseline levels. The 1-year use of this combination was considered as well tolerated (Tajima *et al.*, 2011).

The prospective study was conducted to estimate the metabolic effect of glimepiride (5 mg) with metformin (500mg) on inadequately controlled 125 T2DM patients of Moradabad, North India. The significant reduced level of aspartate aminotransferase (12^{th} week: 34.8±6.4 mg/dL; 24^{th} week: 34.8±6.99 mg/dL; Baseline: 37.2±6.99 mg/dL), and alanine transaminase (12^{th} week: 37.6±9.2 mg/dL; 24^{th} week: 37.6±9.2 mg/dL; Baseline: 40.9±14.5 mg/dL) was found in 12^{th} and 24^{th} week as compared to baseline (Singh *et al.*, 2016).

The controlled, open-label study over 24-weeks, was conducted by Kesavadev et al in 440 randomized patients of T2DM. The patients were received metformin and insulin combination therapy plus 1–3 mg of glimepiride. The reduce level of HbA1c and total daily dose of insulin were found by addition of glimepiride. Furthermore, hypoglycemic events were occurred in patients by glimepiride treatment at the end of the study (Kesavadev *et al.*, 2017).

Randomized, double-blind, multicenter, controlled trial was performed in Italy. Total 47 patients were received glimepiride 2 mg once per day for 12-months. Followed by intervention, plasma transaminase were evaluated at 3, 6, 9, and12 months of treatment and found both AST (U/L) (3^{rd} month: 22±7, 6^{th} month: 21±8, 9^{th} month 21±3, 12^{th} month 20±6; Baseline 21±5) and ALT (U/L) (3^{rd} month: 23±6, 6^{th} month: 22±9, 9^{th} month 22±3, 12^{th} month 21±5; Baseline 22±4) were clinically normal levels (Nishihama *et al.*, 2017)

CHAPTER 3

METHODOLOGY

3.1 Study design

Randomized clinical trial.

3.2 Subjects / Animals

Males and females diabetic patients of age ≥ 45 years fulfilling the inclusion criteria were recruited in the current study after informed consent.

3.3 Place of sample collection / Setting

Medical OPD of National Medical Centre

3.4 Inclusion criteria

Patients having following features were included in the study:

1. Adult (aged ≥45years) patients (both male or female) with T2DM and insufficient glycemic control with exercise, diet, and metformin monotherapy IR* (≥1500 mg/day or maximum tolerated dose with dose that was unchanged for 12-weeks before randomization)

2. ≥7% and ≤10% HbA1c

3. BMI ≤45 kg/m^2 at screening

4. Normal base line investigations (CBC, LFTs, RFTs, Lipid profile)

3.5 Exclusion criteria

1. Patients who was taken glucose-lowering medicines other than metformin IR ≤12 weeks before to randomization

2. Patients with eGFR<60 mL/min/1.73 m2 (MDRD) at the time of screening

3. Patients suffering with liver disease (ALT, AST or alkaline phosphatase >3 x ULN) at the time of screening

4. Patients suffering from terminal illness or cancer.

5. Lactating or pregnant women.

3.6 Duration of study

(a) Individual study period

12 Weeks

(b) Total period of study

6 months

3.7 Sample size estimation

Sample Size for Frequency in a Population

Population size (for finite population correction factor or fpc)(*N*):	720
Hypothesized % frequency of outcome factor in the population (*p*):	26.3%+/-5
Confidence limits as % of 100(absolute +/- %)(*d*):	5%
Design effect (for cluster surveys-*DEFF*):	1

Sample Size(*n*) for Various Confidence Levels

Confidence Level (%)	Sample Size
95%	211
80%	109
90%	163
97%	243
99%	301
99.9%	388
99.99%	447

Equation

Sample size $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$

Results from OpenEpi, Version 3, open source calculator--SSPropor
Print from the browser with ctrl-P
or select text to copy and paste to other programs.

3.8 Sampling technique

Systemic random sampling (randomized sampling technique)

3.9 Human subjects

210 individuals

3.10 Materials used (Drugs/ Chemicals/ Proforma /Questionnaire/any other)

Drugs

1) Tab Dapagliflozin 10mg
2) Tab Glimepiride 4mg.
3) Tab Metformin 500mg

Instruments:

1) Spectrophotometer
2) ELISA Reader
3) Immunoassay analyser
4) Automated chemistry analyzer
5) Hematology analyzer
6) Refrigerator
7) Freezer

Kits:

1) HbA1c Kit
2) Glucose Kit
3) LFTs Kit

4) Lipid profile kit

Other;

1) Subject evaluation form

3.11 Protocol of study

After the ethical approval of clinical trial from ethical committee of Bahria university

Patients fulfilling the inclusion criteria were enrolled for the study after taking informed written consent (attached).

All required information such as name, age, sex, occupation, address, phone contact, associated illness including trauma, hepatic, renal, cardiovascular etc., were recorded on prescribed performa specially designed for the study.

A wash out period of at least 72 hours for any medication to the patient were given before the start of the study. 210 diagnosed Diabetes patients were selected from Medical OPD of National Medical Center Karachi. The patients were divided randomly in two groups, each consisting of 105 patients.

Group A:

This group were included 105 patients, who will receive Tab Glimepiride 4mg once daily plusTab Metformin 500 mg orally thrice daily for 12 weeks.

Group B:

This group were included 105 patients, who were given with Tab Dapagliflozin 10mg once daily plusTab Metformin 500 mg orally thrice daily for 12 weeks.

Base line investigations

Before enrolment for the study necessary investigations was done:

1. HBA1C and FPG
2. Lipid profile
3. Liver function test
4. Urine DR

All the parameters mentioned above will be recorded on 0, 6^{th} and 12^{th} weeks.

3.11.1 HbA1c

This method to detect HBA1c is used Tetradecyltrimethylammonium bromide (TTAB) eliminate interference from leukocytes by working as the detergent in the hemolyzing reagent. The patient sample pre-treatment for the removal of labile HbA1c is not necessary step. All the variants of glycated hemoglobin and have identical HbA1c antibody-recognizable regions are determined by this assay.

The patients HbA1c identification is according to turbidimetric inhibition immunoassay to hemolyze the patient blood sample.

Fasting sample of patient and addition of R1 reagent (buffer/antibody). Glycohemoglobin (HbA1c) in the sample is produced soluble antigen-antibody complexes by reacted with anti-HbA1c antibody. Because only one specific HbA1c antibody site is present in HBA1c molecule, insoluble complexes formation does not take place.

The polyhaptens in reagent 2 (R2) is produced an insoluble antibody-polyhapten complex by reacted with excess anti-HbA1c antibodies. This complex can be easily determined turbidimetrically.

Sample: Fasting blood

Preparation of working solution: The reagents found in the kit was ready to use.

Storage and stability: Reagents were stored at +2 to +8 ˚C

Procedure

The patients whole blood samples were lysed by using hemolyzing reagent to release the hemoglobin from erythrocytes. Next, reagent 1 was added and read the absorbance of Hb at 570 nm. The absorbance is directly proportional to the Hb concentration in the test sample. After that, reagent 2 was added and the absorbance was read 660 nm. The increase absorbance was indicated the proportionality of HbA1c concentration in sample.

Reference value: 4-6 %

3.11.2 Fasting plasma glucose (FPG)

Principle

Hexokinase by ATP is catalysed the glucose phosphorylation to convert it into glucose-6-phosphate.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

The Glucose-6-phosphate dehydrogenase enzyme is oxidized glucose-6-phosphate (G-6-P) to gluconate-6-phosphate by NADP. The increase blood glucose concentration is exhibited the increase rate of formation of NADPH during the enzymatic reaction and is measured photometrically.

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H}^{+}$$

Sample: Fasting plasma

Storage and stability: Reagents was stored at +2 to +8 ˚C

Procedure

To get the optimum performance of the enzymatic assay for glucose detection, the given directions of document for the analyser concerned were followed. According to protocol, instructions of analyzer-specific assay manual were referred.

Calculations:

The automatic calculation of the analyte concentration of each patient sample was done by Roche/Hitachi cobas c systems.

The Conversion factors are : mmol/L x 18.02 = mg/dL
mmol/L x 0.1802 = g/L
mg/dL x 0.0555 = mmol/L

Reference value:70-100 mg/dL

3.11.3 Lipid profile

3.11.3.1 Cholesterol

Principle

Hydrogen peroxide (H_2O_2) is oxidized 4-aminoantipyrine and phenol to produce quinoemine (red color) that can be quantify spectrophotometrically. The increase the formation of color is directly proportional to the level of cholesterol in the sample of the patient.

Sample: Fasting serum

Storage and stability: Reagents were stored at +2 to +8 ˚C

Procedure notes

Analytic Measurement Range (AMR): 4 - 800 mg/dL

Extended Range:

4 – 8000 mg/dL with automatic rerun (1:10).

Cholesterol values above 8000 mg/dL as >8000 mg/dL

Cholesterol values below 4 mg/dL as <4 mg/dL.

Reference value:

≥ 18 years: <200 mg/dL

<18 years: <170 mg/dL

3.11.3.2 High density lipoprotein (HDL)

Summary of test principle

Magnesium or dextran sulfate reagent adds to the sample to produce water-soluble complexes and non-high-density lipoprotein-cholesterol. Followed by reagent 2 is added, HDL-cholesterol esters convert to HDL- cholesterol through PEG-cholesterol esterase (enzyme). The HDL-cholesterol acts upon by PEG-cholesterol oxidase, and previously produced hydrogen peroxide is combined with 4-amino-antipyrine and HSDA by peroxidase to produce a blue or purple pigment that measures photometrically at 600 nm.

Storage and stability: Reagents were stored at +2 to +8 ˚C

Procedure

Serum was used for this assay. And the assay was run according to the proper guidelines of the manufacture protocol.

The ranges of Roche are:

	No risk	Moderate risk	High risk
Female:	> 65 mg/dL	45-65 mg/dL	< 45 mg/dL
Males :	> 55 mg/dL	35-55 mg/dL	< 35 mg/dL

Reference value: 40-60 mg/dL

3.11.3.3 Triglyceride (TG)

Principle

The test sample incubate with lipoproteinlipase (LPL) is liberated glycerol ($C_3H_8O_3$) and free fatty acid (FFA). The produce glycerol in the presence of ATP (adenosine triphosphate) and glycerol kinase produce G-3-P (glycerol 3 phosphate) and liberate ADP (adenosine-5-diphosphate). This G-3-P forms DAP (dihydroxyacetone phosphate) and H_2O_2 (hydrogen peroxide) in the presence of GPO (glycerol phosphate dehydrogenase). At last, the red color compound, quinoneimine dye, is formed by H_2O_2, 4-aminophenazone and p-chlorophenol reaction that is catalyses by peroxidase.

Sample: Fasting serum
Preparation of working solution: The reagents found in the kit were ready to use.

Storage and stability: Reagents were stored at +2 to +8˚C

Procedure

Three test tube marked as blank, standard and sample. Working solution 1ml and 10µl of triglycerides standard was taken in test tube marked as standard. 1ml of working solution and 10µl of serum was added in test tube marked as sample. 1ml of working solution and 10µl of distilled water was taken in blank test tube. The test tubes were left for 5 min for incubation at 37˚C. Then tubes were placed in spectrophotometer and the absorbance of standard and sample was read against blank at 500nm.

Calculation: Absorbance of sample/absorbance of standard x triglycerides 200 mg/dl

Reference value: 44-148 mg/dL

3.11.3.4 Low density lipoprotein (LDL)

LDL-cholesterol was calculated by formula given below
Formula: LDL cholesterol = Total cholesterol - Triglycerides/5 -HDL cholesterol
Reference value: 130-160 mg/dl

3.11.4 Liver Function Test

3.11.4.1 Aspartate transaminase (AST)

Principle

γ-glutamyltransferase (GGT) is transferred the γ-glutamyl molecule to glycylglycine moiety of L-γ-glutamyl -3-carboxy-4-nitroanilide. The 5-amino-2-nitrobenzoate is liberated and its amount is proportional to the biological activity done by GGT in the patient sample. It is quantified by taking the increase in absorbance.

Sample: Plasma

Storage and stability: Reagents were stored at +2 to +8˚C
Procedure notes:

The results were reported to the nearest whole number in U/L.

Analytical Measurement Range (AMR): 3 U/L - 1200 U/L. In the decrease mode, the extended measuring range with the automatic rerun function (1:11) of the analyzer was 3-13200 U/L. The results from the diluted sample, utilizing the rerun function, were automatically multiplied by a factor of 11.

Values less than 3 were reported as <3 U/L.

Values more than 13200 were reported as >13,200 U/L.

Calculations:
The COBAS 6000/8000 system was automatically calculated the GGT activity of each patient sample.

Reference value:

Male: 8-61 U/L

Female: 5-36 U/L

3.11.4.2 Serum glutamic pyruvic transaminase (SGPT)

Principal

Alanine aminotransferase (ALT) is catalysed the formation of pyruvic acid and glutamic acid at pH 7.4 and 37°C by alanine and α-ketoglutaric acid. After that, phenylhydrazine is added to produce phenylhydrazone with pyruvic acid. This phenylhydrazone, under alkaline conditions is produce reddish brown colour. The ALT enzymatic activity can be quantifying by taking optical density at 505 nm.

Sample: Plasma

Preparation of working solution: The reagents found in the kit were ready to use.

Storage and stability: Reagents were stored at +2 to +8˚C

Procedure

The pipetting parameters are as follows:

		Dilutent (H_2O)
R1	59 µL	10 µL
Sample	11 µL	26 µL

SR	17 µL	9 µL
Total volume	132 µL	

Reference range: 2-700 U/L (0.03-11.7 µkat/L)

3.11.4.3 Alkaline phosphatase (AP)

Principle

Magnesium ions and zinc ions is cleaved the p-nitrophenyl phosphate from phosphatases to produce p-nitrophenol and phosphate. The formed p-nitrophenol is released directly proportional to the alkaline phosphatase (ALP) activity. It is quantified by measuring the increased in sample absorbance.

Sample: Plasma

Preparation of working solution: The reagents found in the kit were ready to use.

Storage and stability: Reagents were stored at +2 to +8˚C

- Procedure Notes:

AMR: (Analytical Measurement Range): 3 – 1200 U/L
Report values of greater than AMR as >1200 U/L

Calculations:

The cobas c 111 analyzer is calculated automatically the analyte activity of patient sample. The U/L x 0.0167 = µkat/L conversion factor was used.

Reference range:

Males: 40-129 U/L

Females: 35-104 U/L

3.11.4.4 Bilirubin

Principle

Conjugated and unconjugated bilirubin is coupled with a diazo reagent in surfactant to produce azobilirubin (dye). This reaction (diazo reaction) is accelerated by adding the solubilizing agent, such as surfactant. The greater the absorbance , the greater will be tthe total (conjugated and unconjugated) bilirubin level in the sample.

Sample: Blood

Preparation of working solution: The reagents found in the kit were ready to use.

Storage and stability: Reagents were stored at +2 to +8˚C

Procedure

To achieve the optimum performance of the HbA1C assay, the directions given by manufacture protocol were followed.

Reference

Direct bilirubin ≤ 3.4 μmol/L (≤ 0.20 mg/dL)

3.11.5 Urine DR

3.11.5.1 Glucosuria

The 5 ml of benedict's reagent was added into 8 drops (0.5 mL) of urine sample. Boil the content for 2 minutes over the flame. The results were analysed according to appear of the colour ; blue to cloudy green colour represents negative

result, yellow green represents + (<0.5% glucose), greenish yellow represents ++ (<0.5-1 % glucose), yellow represents +++ (1-2 % glucose), orange to brick red represents ++++ (2 % glucose).

3.11.5.2 Ketonuria

All the reagents were prepared according to company's protocol. 50 µL of β-Hydroxybutyrate added into standard wells, while 50 µL test sample added into test wells. Incubate all the test ad sample wells followed by adding 50 µL of Reaction Reagent to each well for 30-minutes. The absorbance was measured in spectrophotometric microplate reader at 450 nm.

3.11.5.3 White blood cell count

The the uncentrifuged urine specimens was used to count WBC by hemocytometer microscopically. 1millimeter of urine sample was trapped by KOVA Petter. Next, transferred to notch on a KOVA slide 10 chamber hemocytometer. The 6.6 ml of the urine sample were drawn into the KOVA Slide 10 chamber by capillary action. The cells were counted that found in per small grid, took average and to obtain total cells in 1 mL, multiplied the cells count with 90.

3.11.5.4 Pyuria

Block and Nyan technique were used to count pyuria, in which the specimen has been well shaken initially, and then few drops of sample were transferred into a Neubauer counting bar chamber and the count were made of the number of pus cells in a cm^3 (cubic millimetre).

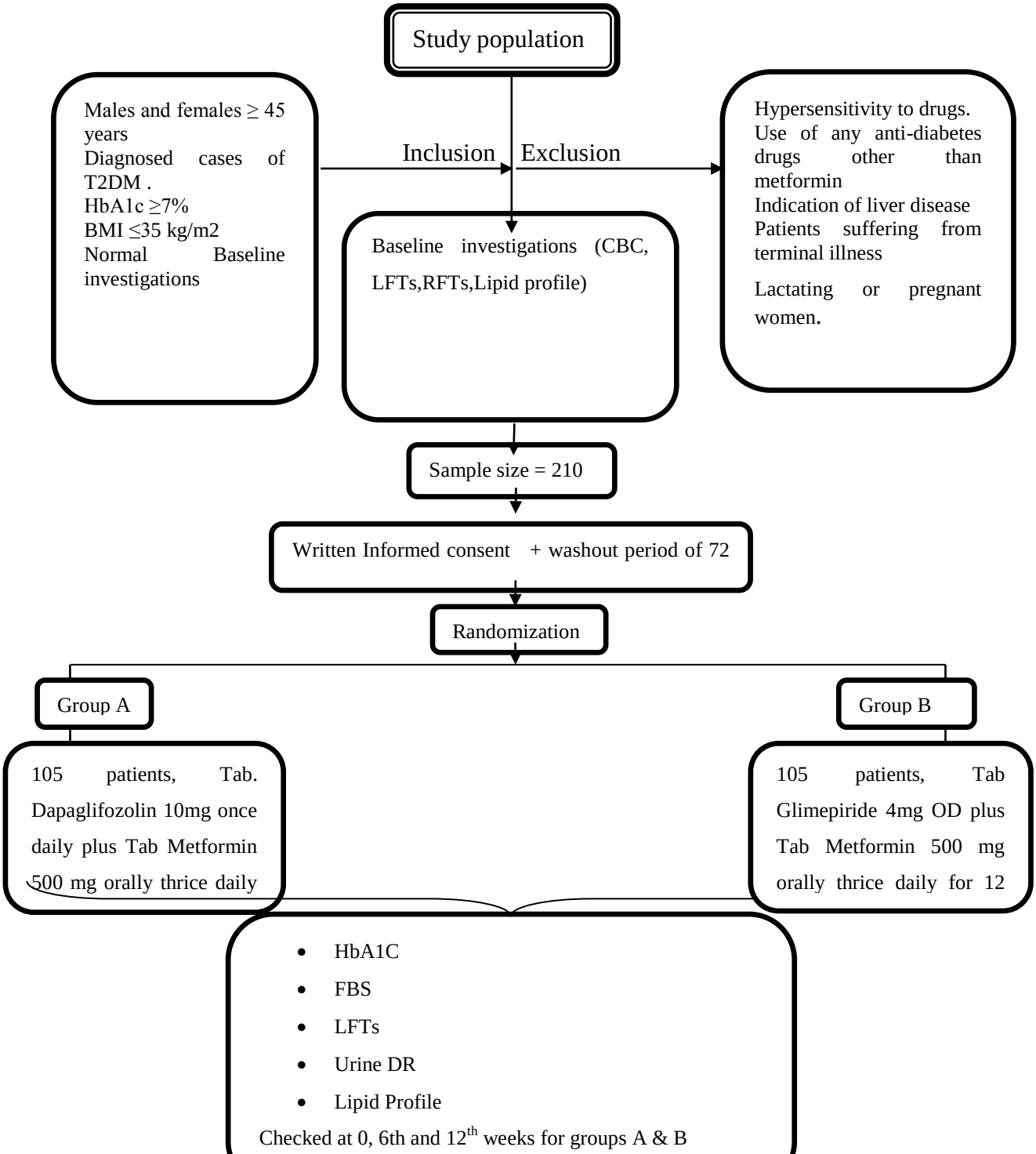
3.11.5.5 Bacteriuria

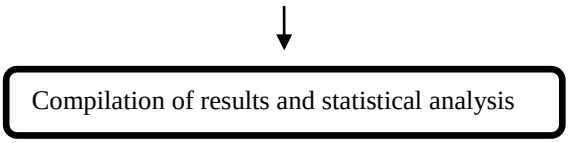
The dry film was made by spreading the drops of uncentrifuged urine specimen in the glass slide. Gram stain was added to fixed and stained. The presence of bacteria was visualized under oil immersion lens.

3.12 Statistical analysis

The data of the patients was analyzed by using the statistical package of social sciences (SPSS) version 25.0. All continuous variables were presented in mean and standard deviation. The qualitative variables were shown in frequency and percentages. To see the difference between Pre and Post findings, parametric t-test and Paired t-test were performed. P-value < 0.05 was considered as statistically significant.

3.13 Algorithm of study





CHAPTER 4

RESULTS

Total 200 patients of either gender with age more than 45 years meeting inclusion criteria of study were included in the study to evaluate and compare the efficacy, safety, and tolerability of dapagliflozin-metformin with the glimepiride-metformin in patients with type 2 diabetes inadequately controlled with metformin monotherapy.

In both study groups, Group A (dapagliflozin + metformin) and Group B (glimeperide+metformin) 100 patients were Included. Descriptive statistics were calculated using SPSS version 25. Qualitative variables were presented in terms of frequency and percentage. Quantitative variables were presented in term of mean and standard deviations. Mean comparison was done by independent t-test and dependent test as appropriate considering $p \leq 0.05$ as significant.

In group A, 38 were males and 62 were females out of 100 patients, while in group B, 42 patients were male and 58 were females. The age and BMI were found insignificant between both A (age: 55 years; BMI: 31.5 kg/m^2) and B (age: 57 years; BMI: 30.5 kg/m^2) groups.

Mean fasting blood sugar in group A at week 0, week 6 and week 12 was 184.05±14.82 mg/dL,137.02±12.30 mg/dL and 101.40±16.85 respectively while mean fasting blood sugar in group B at week 0, week 6 and week 12 was 178.19±9.04 mg/dL,146.23±12.54 mg/dL and 121.89±9.22 mg/dL respectively. We found significant mean difference for fasting blood sugar at week 0(p=0.001), week 6(p=0.000) and week 12(p=0.000) with respect to study groups. We found significant mean difference in group A for week 0-week 6(p=0.000), week 6-week 12(p=0.000) and week 0-week 12(p=0.000). We also found significant mean difference in group B for week 0-week 6(p=0.000), week 6-week 12(p=0.000) and week 0-week 12(p=0.000) (Figure-4.1, Table-4.1 and Table-4.2).

Mean Hb1AC in group A at week 0 and week 12 was 7.83±0.54 % and 6.91±0.74 % while mean Hb1AC in group B at week 0 and week 12 was 6.91±0.74 % and 7.91±0.49%. We found significant mean difference for Hb1AC at week 0(p=0.00) and week 12 (p=0.000) with respect to study groups. We found significant mean difference in group A for week 0-week 12(p=0.000) while we also found significant mean difference in group B for week 0-week 12(p=0.000) (Figure-4.2, Table-4.3 and Table-4,4).

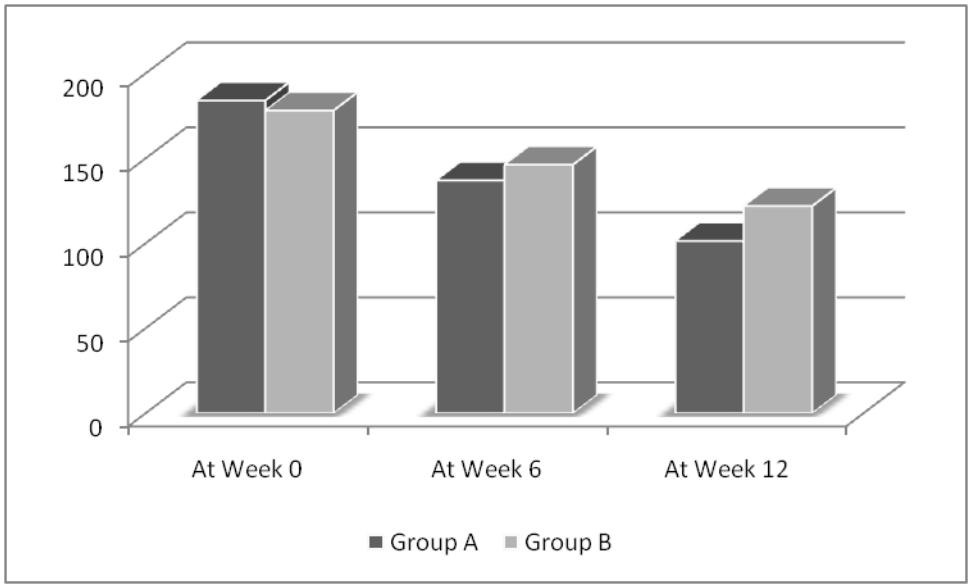


Figure 4.1 FPG (mg/dL) at week 0, week 6 and week 12, (N=200) .

Table 4.1: Fasting blood glucose levels between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	184.05±14.82	178.19±9.04	5.86	0.001[S]
At Week 6	137.02±12.30	146.23±12.54	-9.210	0.000[S]
At Week 12	101.40±16.85	121.89±9.22	-20.49	0.000 [S]

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.2: Fasting blood glucose levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6	47.030	0.001[S]	14.542	0.001[S]
Week 0-Week 12	82.650	0.000[S]	10.443	0.000[S]
Week 6-Week 12	35.620	0.000 [S]	11.894	0.000 [S]

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

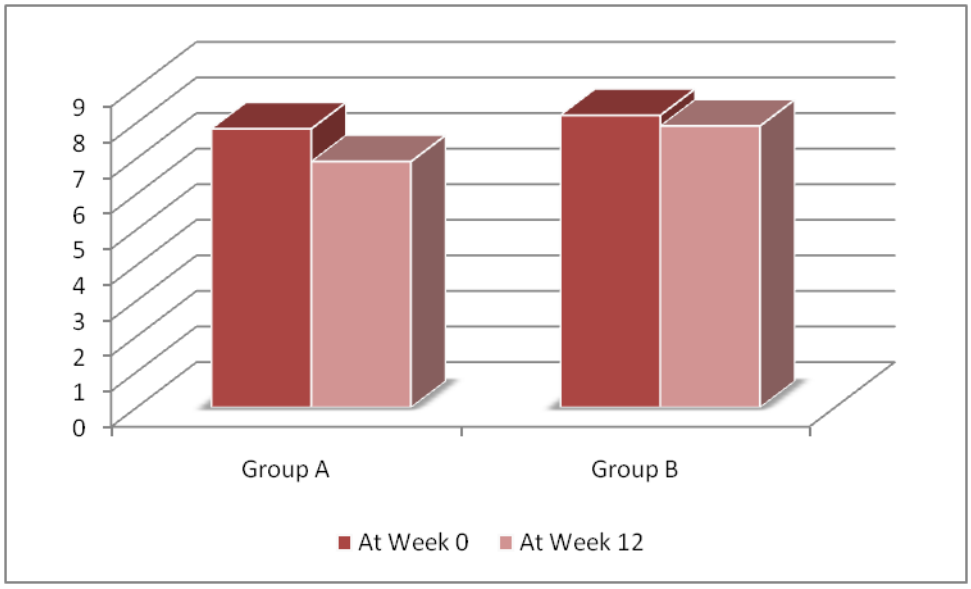


Figure 4.2 HbA1c (%) at week 0 and week 12 in group A and B

Table 4.3: HbA1c (glycated hemoglobin) between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	7.83±0.54	8.21±0.45	-0.377	0.000^{S}
At Week 12	6.91±0.74	7.91±0.49	-1.000	0.000^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.4: HbA1c (glycated hemoglobin) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 12	-0.920	0.001^{S}	0.296	0.001^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Mean SGPT in group A at week 0, week 6 and week 12 was 27.77±4.28 IU/L, 27.34±4.32 IU/L and 27.86±4.43 IU/L respectively while in group B at week 0, week 6 and week 12 was 27.77±4.28 IU/L, 27.70±4.47 IU/L and 27.82±4.47 IU/L respectively (Figure-4.3, Table-4.5 and Table-4.6)

Mean alkaline phosphatase in group A at week 0, week 6 and week 12 was 77.32±6.09 IU/L, 81.37±5.48 IU/L and 79.87±5.55 IU/L respectively while mean alkaline phosphatase in group B at week 0, week 6 and week 12 was 78.98±9.35 IU/L, 80.13±10.28 IU/L and 82.05±10.42 IU/L respectively. We found insignificant mean difference for alkaline phosphatase at week 0 (p=0.139), at week 6 (p=-0.289) and week 12 (p=0.067) with respect to study groups (Table-4.9 and Table-4.10)

Mean total bilirubin in group A at week 0, week 6 and week 12 was 0.56±0.17 mg/dl , 0.55±0.17 mg/dl and 0.55±0.16 mg/dl respectively while mean total bilirubin in group B at week 0, week 6 and week 12 was 0.50±0.15 mg/dl, 0.56±0.17 mg/dl and 0.58±0.17 mg/dl respectively (Table-4.11 and Table-4.12).

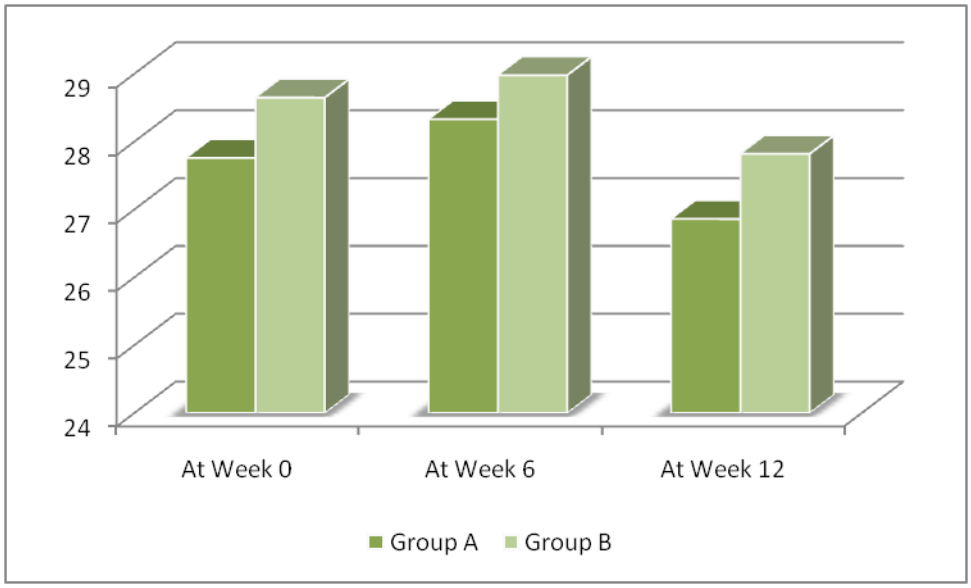


Figure 4.3 SGPT (IU/l) at week 0, week 6, and week 12, (N=200)

Table 4.5: SGPT (IU/L) levels between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	27.76±4.29	28.65±4.07	-0..890	0.134^{NS}
At Week 6	28.33±4.24	28.98±3.91	-0.650	0.261^{NS}
At Week 12	26.86±3.92	27.82±4.47	-0.960	0.108^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.6: SGPT (IU/L) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	-0.570	0.372 ^{NS}	-0.330	0.530 ^{NS}
Week 0-Week 12º	0.9000	0.091 ^{NS}	0.830	0.160^{NS}
Week 6-Week 12º	1.470	0.012^{S}	1.160	0.022^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

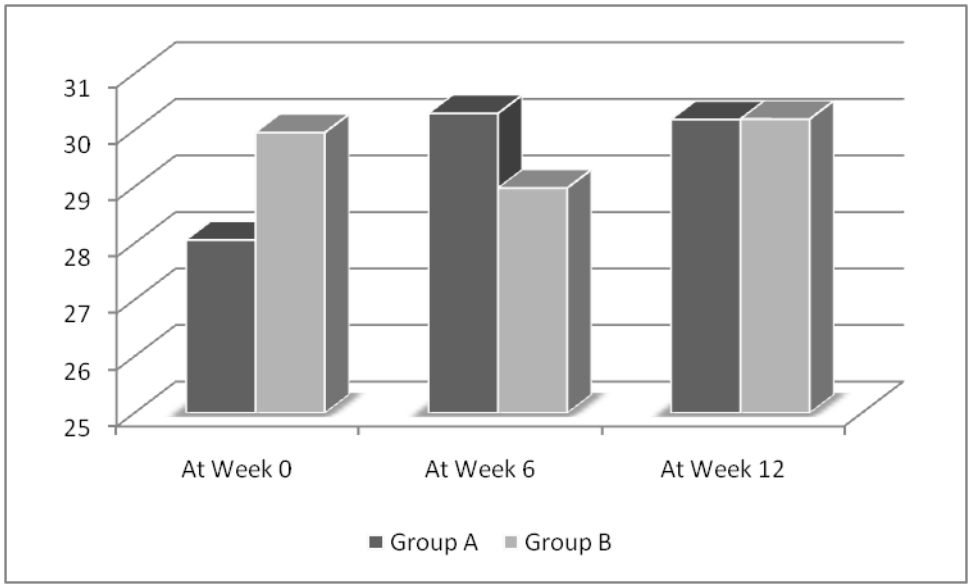


Figure 4.4 SGOT (IU/dL) at week 0, week 6 and week 12

Table 4.7: SGOT (IU/dL) levels between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	28.06±4.23	29.96±3.94	1.100	0.059^{NS}
At Week 6	30.30±5.33	28.98±4.58	1.320	0.062^{NS}
At Week 12	30.19±6.56	30.20±4.75	-0.010	0.990^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.8 SGOT (IU/dL) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	-2.24	0.001^{S}	-2.02	0.001^{S}
Week 0-Week 12º	-2.13	0.007^{S}	-3.240	0.000^{S}
Week 6-Week 12º	0.11	0.884^{NS}	-1.220	0.059^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.9: Alkaline phosphatase (IU/l) levels between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	77.32±6.09	78.98±9.35	-1.660	0.139^{NS}
At Week 6	81.37±5.48	80.13±10.28	1.240	0.289^{NS}
At Week 12	79.87±5.55	82.05±10.42	-2.180	0.067^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.10 Alkaline phosphatase (IU/l) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	-4.050	0.000^{S}	-1.150	0.417^{NS}
Week 0-Week 12º	-2.550	0.002^{S}	-3.070	0.023^{S}
Week 6-Week 12º	1.500	0.058^{NS}	-1.920	0.134^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.11: Bilirubin total (mg/dL) levels between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	0.56±0.17	0.50±0.15	0.059	0.013^{S}
At Week 6	0.55±0.17	0.56±0.17	-0.01	0.679^{NS}
At Week 12	0.55±0.16	0.58±0.17	-0.035	0.156^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.12: Bilirubin total (mg/dL) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	0.004	0.874 NS	-0.065	0.006S
Week 0-Week 12º	0.007	0.752 NS	-0.087	0.000S
Week 6-Week 12º	0.003	0.905 NS	-0.022	0.406 NS

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

To achieve the objective, we were identified the risk of urinary tract infections (UTIs) followed by combination therapy. The parameters involved to study UTIs through urine sample include, ketone and glucose level, and bacteria, WBCs, and pus-cells count. Mean puss cell in group A at week 0, week 6 and week 12 was 0.00±0.00 ,2.68±1.51 and 4.49±1.61 respectively while Mean puss cell in group B at week 0, week 6 and week 12 was 0.00±0.00 ,0.00±0.00 and 0.00±0.00 respectively. (Table-4.13)

In group A, 97% were found mild and 3% moderate level of sugar at week 0 while 12% were found mild,82% moderate and 6% with severe sugar level at week 6. At week 12, 2% were found with mild,9% with moderate and 89% with severe sugar level. In group B, 96% were found mild, 3% with moderate and 1% with severe level of sugar at week 0 while 98% were found mild and 2% with moderate sugar level at week 6. At week 12, 99% were found with mild and 1% with moderate sugar level. (Table-4.14 and Table-4.15,, Figure-4.5, Figure-4.6).

Mean ketone in group A at week 0, week 6 and week 12 was 0.23±0.10 mg/dL,0.23±0.10 mg/dL and 0.22±0.10 mg/d respectively while mean ketone in group B at week 0, week 6 and week 12 was 0.31±0.13 mg/dL,0.32±0.13 mg/dL and 0.32±0.13 mg/dL respectively (Table-4.16).'

Mean white blood cell count in group A at week 0, week 6 and week 12 was 2.01±0.82x10^9 cells/liter, 1.99±0.82 82x10^9 cells/liter and 2.03±0.59 x10^9 cells/liter respectively while mean white blood cell count in group B at week 0, week 6 and week 12 was 2.00±0.81 x10^9 cells/liter, 2.01±0.82 82x10^9 cells/liter and 2.02±0.81 x10^9 cells/liter respectively. We found insignificant mean difference for WBC at week 0 (p=0.931), with week 6 (p=0.864) and with week 12 (p=0.921) with respect to study

groups. Furthermore, all patients were found with null leukocyte esterase at week 0, week 6, and week 12 as well as null bacteria at week 0, week 6 and week 12 (Table-4.17 and Table-4.18, Graph-3).

The microscopic analysis of urine specimen has not shown bacteria in dapagliflozin-metformin treated group (A) and glimepiride-metformin treated group (B). (Table4.20)

Table 4.13: Pus cell (unit) at week 0, week 6 and week 12, (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)
At Week 0	0.00±0.000	0.00±0.00
At Week 6	2.68±1.51	0.00±0.00
At week 12	4.49±1.61	0.00±0.00

Group A treated with dapagliflozin+ metformin and group B treated with glimepiride + metformin.

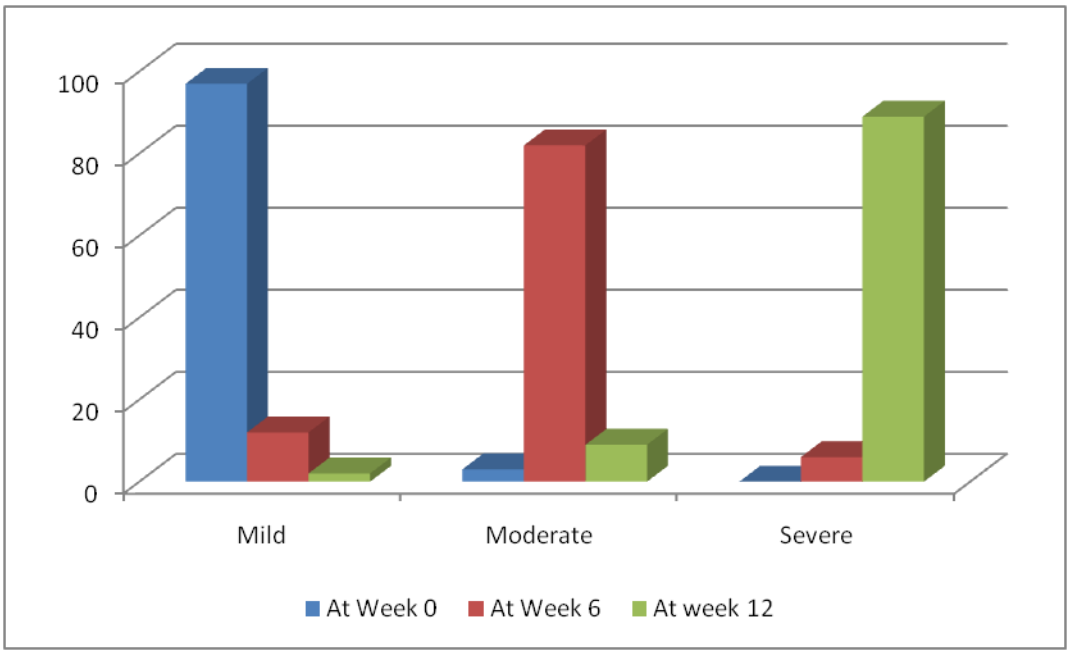


Figure 4.5 Glucosuria in group A

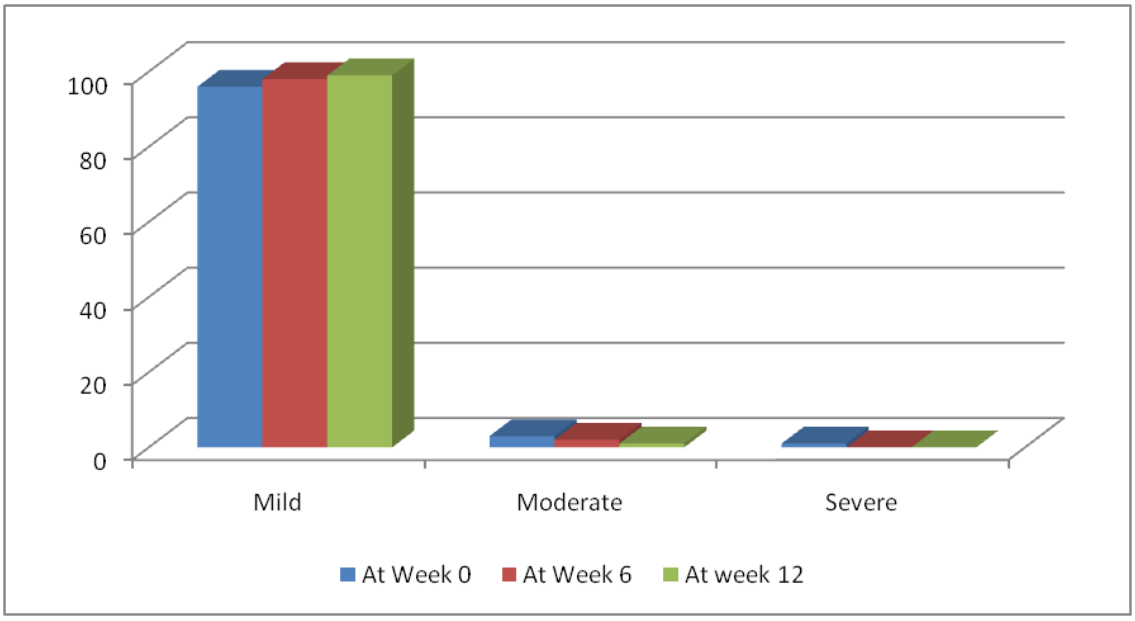


Figure 4.6 Glucosuria in group B

Table 4.14: Glucosuria in group A at week 0, week 6 and week 12, (N=200)

		Mild (N = %)	Moderate (N = %)	Severe (N = %)
Group A	At Week 0	97 (97)	3 (3)	0 (0)
	At Week 6	12 (12)	82 (82)	6 (6)
	At week 12	2 (2)	9 (9)	89 (89)

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin.

Table 4.15: Glucosuria in group B at week 0, week 6 and week 12, (N=200)

		Mild (N = %)	Moderate (N = %)	Severe (N = %)
Group B	At Week 0	96 (96)	3 (3)	1 (1)
	At Week 6	98 (98)	2 (2)	0 (0)
	At week 12	99 (99)	1 (1)	0 (0)

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin.

Table 4.16: Ketonuria (mg/dL) at week 0, week 6 and week 12, (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)
At Week 0	0.23±0.100	0.31±0.13
At Week 6	0.23±0.10	0.32±0.13
At Week 12	0.22±0.10	0.32±0.13

Group A treated with dapagliflozin+ metformin and group B treated with glimeperide+ metformin.

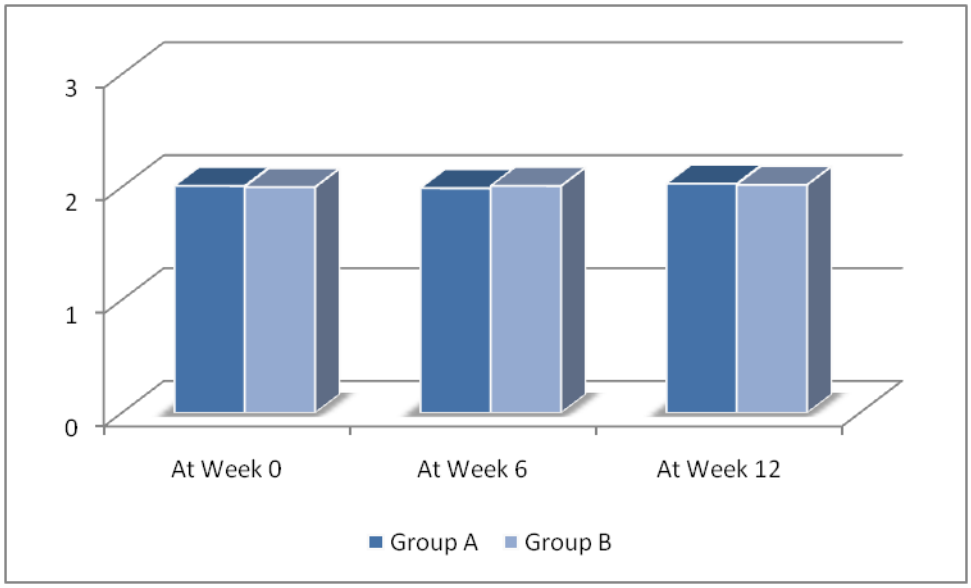


Figure 4.7 White blood cell count (x 109 cells/liter)

Table 4.17: WBC (x 109 cells/liter) levels between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	2.01±0.82	2.00±0.81	0.0100	0.931^{NS}
At Week 6	1.99±0.82	2.01±0.82	-0.02	0.864^{NS}
At Week 12	2.03±0.59	2.02±0.81	0.01	0.921^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.18: WBC (x 109 cells/liter) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	0.0200	0.320^{NS}	-0.010	0.657^{NS}
Week 0-Week 12º	-0.200	0.838^{S}	-0.020	0.817^{NS}
Week 6-Week 12º	-0.04	0.682^{NS}	-0.010	0.910^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.19: Leukocyte esterase (Unit) at week 0, week 6 and week 12, (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)
At Week 0	0.00±0.00	0.00±0.00
At Week 6	0.00±0.00	0.00±0.00
At Week 12	0.00±0.00	0.00±0.00

Group A treated with dapagliflozin+ metformin; and group B treated with glimeperide+ metformin.

Table 4.20: Bacteria at week 0, week 6 and week 12 (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)
At Week 0	0.00±0.00	0.00±0.00
At Week 6	0.00±0.00	0.00±0.00
At Week 12	0.00±0.00	0.00±0.00

Group A treated with dapagliflozin+ metformin; and group B treated with glimeperide+ metformin.

Mean cholesterol in group A at week 0 and week 12 was 189.74±23.24 mg/dL and 188.95±23.45 mg/dL while mean cholesterol in group B at week 0 and week 12 was 185.21±23.65 mg/dL and 189.29±23.47 mg/dL (Table-4.21 and Table-4.22, Figure-4.8).

Mean triglyceride in group A at week 0 and week 12 was 145.07±19.95 mg/dL and 144.56±19.42 mg/dL while mean triglyceride in group B at week 0 and week 12 was 143.39±17.52 mg/dL and 148.38±19.23 mg/dL (Table-4.23 and Table-4.24, Figure-4.9).

Mean high-density lipoproteins (HDL) in group A at week 0 and week 12 was 53.96±3.27 mg/dL and 57.45±5.14mg/dL while mean HDL in group B at week 0 and week 12 was 53.33±2.67mg/dL and 56.48±3.70 mg/dL. There was significant mean difference for HDL at week 0 with week 6 for group A (p=0.000 and group B (p=0.000) (Table-25 and Table-4.26, Figure-4.10).

Mean low-density lipoproteins (LDL) in group A at week 0 and week 12 was 142.02±12.52mg/dL and 140.57±10.82mg/dL while mean LDL in group B at week 0 and week 12 was 144.98±14.34 mg/dL and 140.98±10.36 mg/dL (Table-4.27 and Table-4.28, Figure-11).

Table 4.21: Cholesterol (mg/dL) levels between group A and group B (N=200)

	Group A	Group B	Mean Difference	P-Value
At Week 0	189.74±23.24	186.21±23.65	3.530	0.288^{NS}
At Week 12	188.95±23.45	195.03±19.50	-6.080	0.048^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.22: Cholesterol (mg/dL) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	0.7900	0.810^{NS}	-8.820	0.003^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

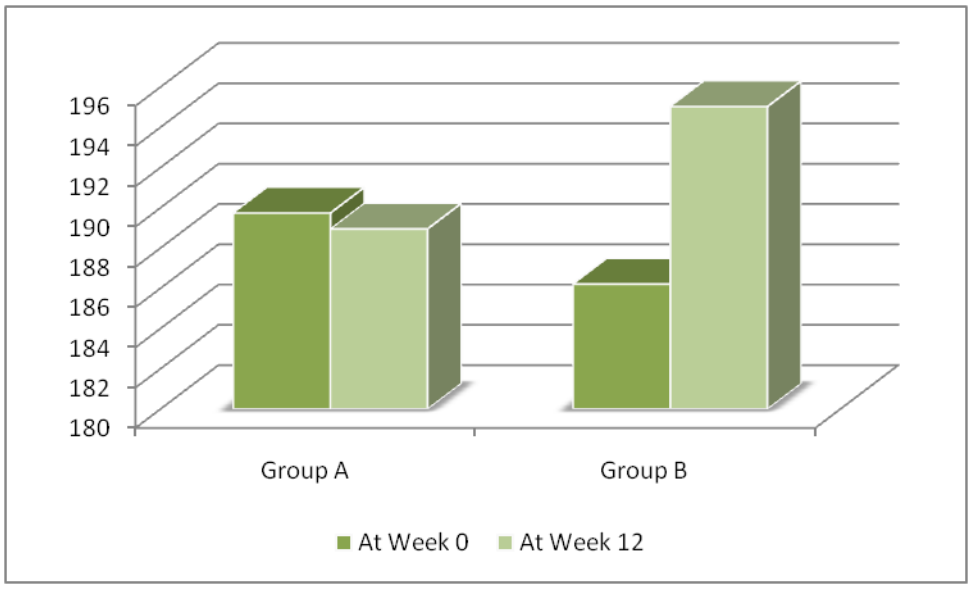


Figure 4.8 Cholesterol (mg/dL) at week 0 and week 12

Table: 4.23: Triglyceride (mg/dL) levels between group A and group B (N=200)

	Group A	Group B	Mean Difference	P-Value
At Week 0	145.07±19.95	143.39±17.52	1.680	0.528 ^{NS}
At Week 12	144.56±19.42	148.38±19.23	-3.820	0.164 ^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.24: Triglyceride (mg/dL) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6^{o}	0.510	0.854 ^{NS}	-4.990	0.058 ^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

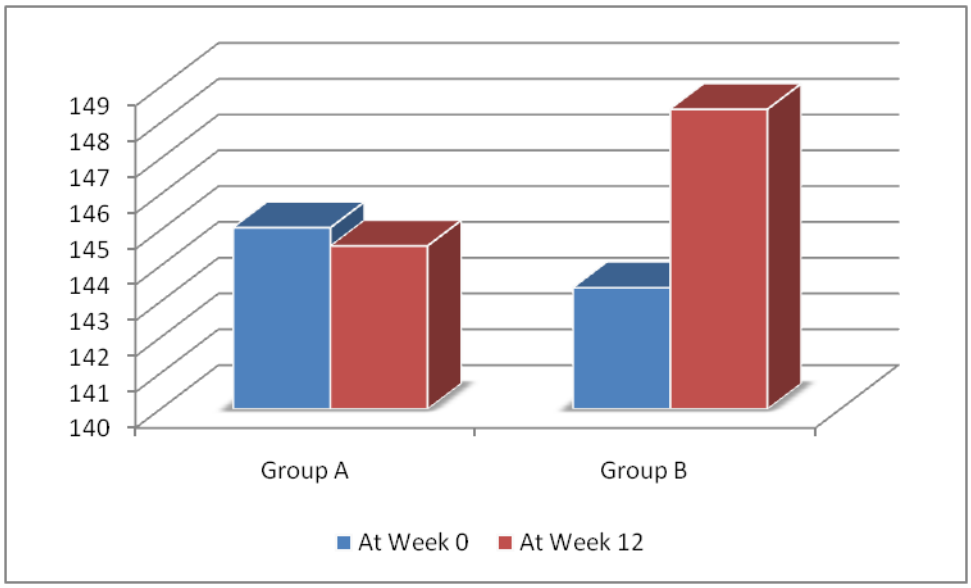


Figure 4.9 Triglyceride (mg/dL) at week 0 and week 12.

Table 4.25: HDL (mg/dl) levels between group A and group B (N=200)

	Group A (Mean±SD)	Group B (Mean±SD)	Mean Difference	P-Value
At Week 0	53.96±3.27	53.33±2.67	0.630	0.138^{NS}
At Week 12	57.45±5.14	56.48±3.70	0.970	0.128^{NS}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table: 4.26: HDL (mg/dl) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	-3.490	0.000^{S}	-3.150	0.000^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimeperide+ metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

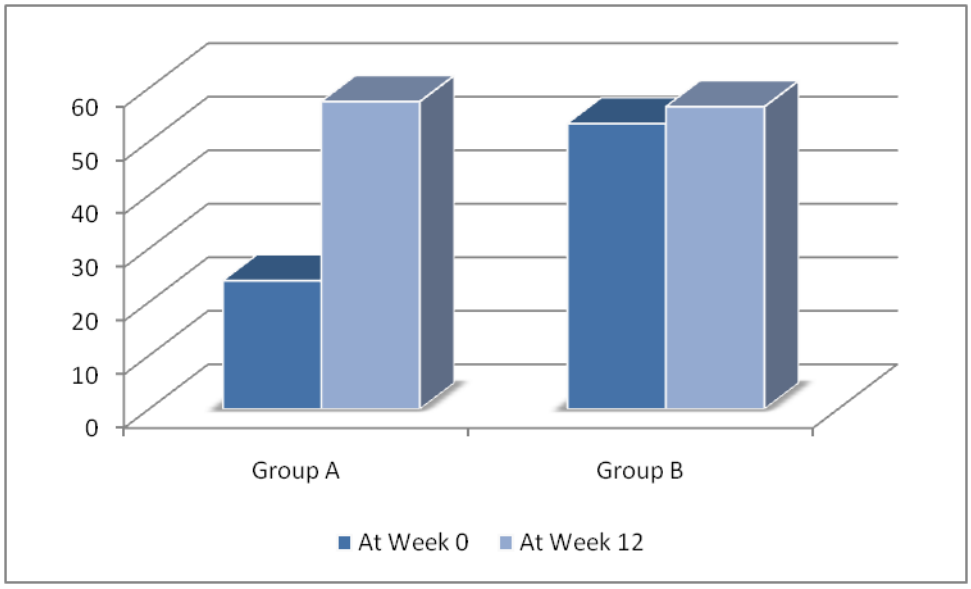


Figure 4.10 High density lipoprotein (mg/dL)

Table 4.27: LDL (mg/dl) levels between group A and group B (N=200)

	Group A	Group B	Mean Difference	P-Value
At Week 0	142.02±12.52	144.98±14.34	-2.960	0.122^{NS}
At Week 12	140.57±10.82	140.98±10.36	-0.410	0.785^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Independent t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

Table 4.28: LDL (mg/dl) levels at week 0-week 6, week 0-week 12 and week 6-week 12 (N=200)

	Group A (Mean difference)	P-Value	Group B (Mean difference)	P-Value
Week 0-Week 6º	1.450	0.353^{NS}	4.000	0.014^{S}

Group A: treated with dapagliflozin+ metformin; Group B: treated with glimepiride + metformin; Paired t-test was applied: S=Significant at 0.05, NS=Not Significant at 0.05.

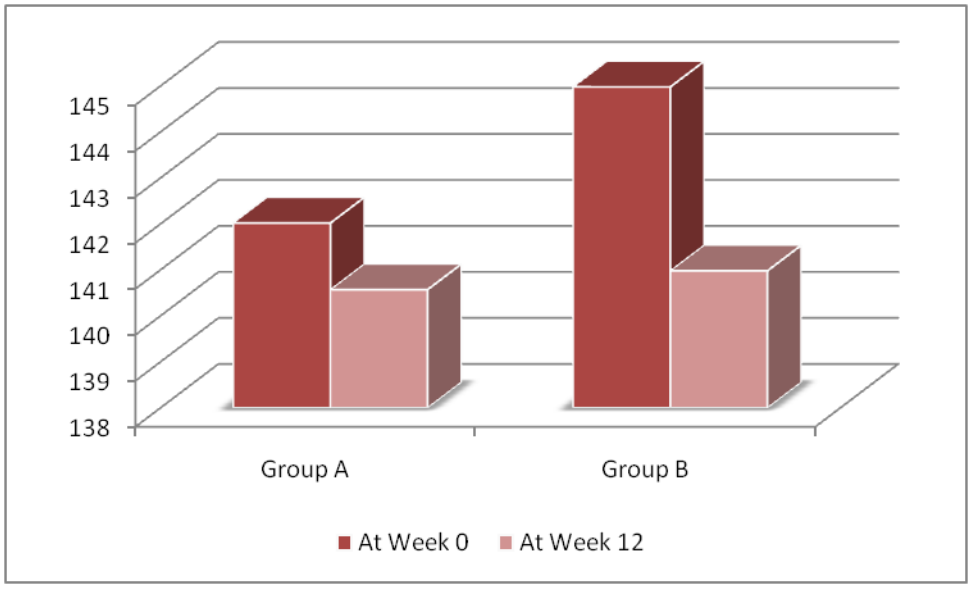


Figure 4.11 Low density lipoprotein (mg/dL)

CHAPTER 5

DISCUSSION

The uncontrolled chronic hyperglycemia in diabetes over time can greatly damage tissues throughout the body and produce serious life-threatening effects or even death. The most common complications include cardiovascular disorders (heart attack, stroke), eye disorders (blindness), renal failure, neuropathy (cause tingling and pain), certain infections (leads to amputation), and dental problems. The control blood sugar level greatly prevents, manage, or delay to progress uncontrolled hyperglycemia-induced secondary complications. Therefore, the present study was aimed to identify the efficacy and safety of dapagliflozin and glimepiride as add-on to metformin in the Pakistani population having uncontrolled T2DM with metformin.

Our primary objective was to assess the efficacy of dapagliflozin as add-on to metformin in T2DM patients. Therefore, group A was recruited 100 patients, who received tab dapagliflozin of 10 mg once daily plus tab metformin of 500 mg orally thrice daily for 12 weeks. The quantification of finding plasma glucose (FPG) has been one of the parameters to identify the onset of diabetes or uncontrolled diabetes. The increase level of FPG is initiated due to insufficient insulin production from pancreatic β cells that subsequently leads to prevent glucose uptake to muscle or liver. Regarding this, we have found an increased blood glucose level (203.56±20.15 mg/dL) in patients that were previously treated with metformin alone. Followed by 6^{th}-week of treatment in group A patients, we have found drastically decrease FPG i.e 137±12. mg/dL ($p<0.000$) as compare to metformin monotherapy (0-week). And, at 12^{th} week of treatment, the significantly normal blood sugar level i.e 101±16 mg/dL has identified as compared to metformin monotherapy (week 0) and week-6^{th} of

dapagliflozin add-on with metformin administration.

The gradually decrease in FPS from 1st-12 weeks is the prominent action of dapagliflozin-metformin combination. Dapagliflozin (SGLT-2) acts on SGLT-2 of proximal convoluted tubule and inhibits its activity to reabsorb glucose and that eventually maintains glucose homeostasis. The mechanism of the drug is initiated with high glucose load in proximal tubule (Plosker, 2012; Schwartz & Katz, 2016).

The efficacy of dapagliflozin-metformin combination was further identified by assessed HbA1c level. As, this is another indicative parameter of diabetes. The paired t-test analysis was found to significantly decrease baseline glycated hemoglobin (HbA1c) level at 12th -week as compare to 0-week. This finding was consistent with our previously dapagliflozin-metformin combination decreased FBS level, as the decrease formation of the glucose-Hb linkage indicates the decrease presence of excessive sugar in the bloodstream. At 0-week (only metformin treatment) of our study, the blood glucose level has found increased which enhanced the non-enzymatic Hb-glucose glycation reaction, whilst the dapagliflozin-metformin combination markedly decrease glucose that ultimately reduces hemoglobin glycation. Our findings are supported that addition of dapagliflozin to metformin is effective to produce glycemic control.

Similar to our findings, the long-term (4 years) glycaemic response of dapagliflozin-metformin combination in inadequately controlled T2D was assessed previously in randomized, double-blind, phase III clinical study. Followed by 208 weeks of intervention, sustained reduction in glycated haemoglogin (HbA1c) level i.e −0.30% [95% CI (confidence interval)] was identified. Fewer patients reported hypoglycaemia in the dapagliflozin group (5.4 %). Our study was conducted over the period of 12th-week, and we have not found hypoglycaemia in group A (Del Prato *et al.*, 2015)

Consistently, the randomized double-blind study on T2D (poorly controlled by metformin alone) was conducted by Rosenstock *et al*. The patients before received the dapagliflozin plus metformin therapy were shown mean baseline HbA1c level of 8.9%

(i.e: 74 mmol/mol). At week-24 of therapy, the adjusted mean change from the baseline HbA1c level was found –1.2% (i.e: –13.1 mmol/mol). The proportion of enrolled patients to achieve HbA1c less than 7% (53 mmol/mol) was 22% (Rosenstock *et al.*, 2015).

Sjöström et al were pooled the data from seven studies to evaluate associations of dapagliflozin-induced weight loss and decrease in % of HbA1c in T2DM patients. The therapy was based on 10 mg of dapagliflozin alone or in combination for 24-weeks. They suggested that 2 kg of reduction in body weight with dapagliflozin was contributed to 6% decrease of total baseline HbA1c level (Sjöström *et al.*, 2015). Inconsistent with body weight-HbA1c relation, we have found that the decrease HbA1c was due to increase glucosuria by dapagliflozin.

The 16-week randomized, clinical phase III trial was performed by Schumm-Draeger et al. The inclusion criteria of patients were based on previously taken ≥1500 mg/day of metformin twice for 24-hours and had poorly glycaemic control randomized to receive 2.5 or 5 mg of dapagliflozin twice/day, placebo or 10 mg of dapagliflozin once daily. Patients (n= 400) randomized to 2.5 mg, 5 mg (twice daily), 10 mg (once daily) or placebo co-administered with metformin (twice daily). The significantly reduced adjusted mean change of HbA1c in the dapagliflozin groups were found as compared to placebo [(2.5 mg dapagliflozin: −0.52% vs. −0.30%, $p = 0.0106$) (and 5 mg dapagliflozin: −0.65% vs. −0.30%, $p < 0.0001$)] (Schumm-Draeger *et al.*, 2015).

The hypothesis was tested the association of lowered bloodstream glucose concentration by dapagliflozin with improved pancreatic β-cell function and IR in T2DM patients. The analysis of homeostasis model assessment of β-cell function (HOMA β-cell) were shown that dapagliflozin improves pancreatic β‑cell function and this was possibly due to reduction in glucotoxic. Prominently, the authors were suggested that the glucose toxicity effect of chronic hyperglycemia on pancreatic β-cell function in T2DM patients is reversible within some extent (Fioretto *et al.*, 2015). The positive response to lower the glycemia in dapagliflozin-metformin treated patients of our study was may be due to its effect on β-cell function.

In the previously reported study, two randomized controlled trials were conducted in diabetic patients that were taken dapagliflozin- or metformin- alone, or in combination. They were shown significantly greater decreased glycated protein (HbA1c) levels in combination therapy as compared to both other monotherapies. In trial 1, the reduction of HbA1c was found -1.35% for metformin, -1.19% for dapagliflozin, and -2.05% for dapagliflozin-metformin as compare to before drug administration levels. In another trial, the reduction of HbA1c was found -1.44% for metformin, -1.45% for dapagliflozin, and -1.98% for dapagliflozin-metformin as compare to before drug administration levels (Henry *et al.*, 2012).

A 52-week, open-labeled, prospective observational study was conducted in T2DM patients having inadequately controlled glycemia with quadruple oral hyperglycemia drugs (metformin, and dipeptidyl peptide 4 inhibitors, and glimepiride) to identify the effectiveness of 10 mg of dapagliflozin. After 52 weeks of intervention, patients was showed reductions in HbA1c level from $9.0 \pm 1.3\%$ to $7.8 \pm 1.2\%$ and FPG level from 191.2 ± 67.8 to 138.1 ± 68.2 mg/dL (Ku *et al.*, 2019). Inconsistently, we have found the effectiveness of dapagliflozin-metformin combination and also compare this with glimepiride-metformin treatment for 12^{th} week. Furthermore, FBG was reduced from 203.56±20.15 to 101.40±16.85 mg/dL and HBA1C from 8.56±7.33 to 6.71±0.56%.

S Matthaei et al were identified the durability and tolerability of 10 mg/day of dapaglifozin over 52 weeks (i.e 24-week randomized, double-blind period plus 28-week double-blind extension) as add-on to metformin and sulphonylurea in T2D. At 52-weeks, HbA1c (−0.8% *vs* −0.1%) and FBG (−1.5 mmol/l *vs* 0.6 mmol/l) levels showed greater improvement from baseline with dapaglifozin as compared to placebo. The frequency of patients to achieve HbA1c <7.0% with dapaglifozin was 27.3% and placebo was 11.3% (S Matthaei *et al.*, 2015). The dapaglifozin as add-on to metformin and sulphonylurea combination was inconsistent add on with respect to our study to decrease levels of FBS and HBA1C, our findings has shown significant results only metformin add on with dapaglifozin.

To identify the efficacy and safety of dapagliflozin in T2DM, randomized double-blind placebo-controlled trial was conducted over 102-weeks on 274 participants. At 102-weeks, significant differences *vs* metformin (low-dose) with 5 mg of dapagliflozin (5.8 mmol/mol [0.53%], P = 0.018) and 10 mg of dapagliflozin (4.8 mmol/mol [0.44%], P = 0.048) were found. The FPG for 5 and 10 mg of dapagliflozin were 0.69 mmol/L (P = 0.044), and 1.12 mmol/ L, respectively. The 0–4.6% and 5.3% of hypoglycemic events were observed in metformin and dapagliflozin groups, respectively (C. Bailey *et al.*, 2015). These findings were found after 102-weeks which is inconsistent with our study. The dapagliflozin-metformin group in our study has shown the decrease levels in 12^{th} week.

Our next objective was to identify the best effective which was to compare the efficacy of glimepiride as add-on to metformin in uncontrolled diabetic patients, we included 100 patients, who have received 4 mg of glimepiride once daily plus with 500 mg of metformin orally thrice daily for 12 weeks (group B). We have found that the FPG was uncontrolled with metformin monotherapy, while the treatment with glimepiride-metformin combination has shown statistically significant decrease FPG level at 6^{th} week and this further this therapy produces prediabetic state at 12^{th} week. The decrease of glucose was due to addition of glimepiride with metformin, as, glimepiride (sulfonylureas) is acted as an insulin secretagogue. It reduces glucose level by stimulating the release of insulin from β cells of pancreas and by increasing activity of intracellular receptors of insulin.

The significantly marked reduction in baseline HbA1c level has found from previously treated metformin monotherapy patients after glimepiride add-on metformin therapy for 12^{th} week. These findings have provided strength to our previous baseline levels of FBS that have shown decrease glucose by addition of glimepiride plus metformin.

Similarly, with our findings, Shimpi et al were identified the effect of metformin-glimepiride on glycaemic control in T2DM. The inclusion criteria of diabetic patients in their study was based on glycosylated hemoglobin (HbA1c) level that was > 7%. Total 31 diabetic volunteers were taken treatment of 1000 mg of

metformin with 2 mg of glimepiride for 12 weeks. The decrease in HbA1c level was observed (-1.4%). Furthermore, the FPG, cholesterol, triglyceride and LDL reduction were found in patient (Shimpi *et al.*, 2009).

Consistent with our findings, randomized, double-dummy, double-blind, parallel-group, multicenter trial was carried out in France by Charpentier et al over 5-months. The T2D patients of 35-70 years aged which had inadequately controlled diabetes by taken 2550 mg of metformin monotherapy daily for at least 4 weeks was selected. They were randomly divided into three groups; i) metformin alone, ii) glimepiride-metformin add-on and, iii) glimepiride alone. The results were revealed that glimepiride co-treated with metformin in T2D patients were prominent glycaemic control (HBA1C: -0.74±0.96% ; FBS: -1.8 ± 2.2 mmol/l change ; PPBG: -2.6 ± 3.9 mmol/l change) as compared with other monotherapy treated groups, such as glimepiride (HBA1C: 0.27±1.10% ; 0.7±3.1 mmol/l change ; PPBG: 0.1±5.1 mmol/l change) and metformin (HBA1C: + 0.07±1.20%; + 0.8±0.4 mmol/l change ; PPBG: + 1.1±5.9) (Charpentier *et al.*, 2001).

Another randomized, double-blind, multicentre, active-controlled trial was conducted over 52-week to identified the efficacy of glimepiride-metformin combination therapy in inadequately controlled T2DM. Baseline patient age was 56.1 ± 9.7 years, duration of diabetes was 7.8 ± 6.4 years and HbA1c level was 8.5% ± 0.8% (69 ± 9.0 mmol/mol). The adjusted mean change from baseline in HbA1c level after the intervention was found −0.98% (−10.7 mmol/mol) (Frias *et al.*, 2020).

Similarly, randomized, double-blind, parallel-group, 52-week trial was assessed the efficacy and safety of 1-6 mg/d of glimepiride-metformin dual add-on therapy in patients with T2D. The patients recruited in the study were had HbA1c level between 7.5-10.5% and received ≥ 1500 mg/d of metformin previously. The glimepiride-metformin combination was resulted in reduction in HbA1c and greater frequency of patients was achieved 7.3% of A1C. The combination was observed overall well-tolerated, while 44.0% of hypoglycemia incidence was observed with glimepiride-metformin (Frias *et al.*, 2018).

The prospective study was conducted to estimate the metabolic effect of

glimepiride (5 mg) with metformin (500mg) on inadequately controlled 125 T2DM patients of Moradabad, North India. The significant reduced level of FPG at 12, and 16-week was observed as compared to baseline (116±14.5 mg/dL, 107±18.7 mg/dL, 185.0± 49.04 mg/dL; $p<0.05$). The PPBG was reduced from 275.9±63.5 to 167.0±27.0 mg/dL at 12-weeks and 141.3±31.0 mg/dL at 24-weeks of intervention. Furthermore, the reduced HbA1c level (12^{th} week: 7.6±0.81%; 24^{th} week: 6.7±0.7%; Baseline: 8.99±1.36%) was seen as compared to baseline (Singh *et al.*, 2016). The drug concentration in their study was inconsistent with our study design to achieve the reduction of FPG and HbA1c level, we have given 4mg glimepiride.

The open-label, randomized study was carried out to study the effect of metformin-glimepiride (1000/2mg) on glycaemic control in T2DM. Patients with HbA1c more than 7% were included in the study. At week 12 of treatment, the HbA1c was 9.5±0.4% and FPG was 205.7±27.4 mg/dL in patient (Shimpi *et al.*, 2009). The concentration of metformin and glimepiride i.e 1000mg and 2 mg, respectively, for 12^{th} week of the study was inconsistent with our study design. We have found the optimal level of reduction at 5oomg of metformin and 4mg of glimepiride.

Randomized, open labelled study were performed in 80 T2DM patients. Patients were received Metformin(500mg)-Glimepiride(1mg) over 30-weeks. They were shown the reduced FBS (mg/dL) (week-15^{th}: 120.60±14.01, week-30^{th}: 107.80±12.41 vs baseline: 176.06±32.56) PPBS (mg/dL) (week-15^{th}: 166.48±17.97, week-30^{th}: 156.22±14.59 vs baseline: 272.34±39.89), and HbA1c (%) (week-15^{th}: 7.66±0.51, week-30^{th}: 6.78±0.27 vs baseline: 8.89±0.53) levels at 15^{th} and 30^{th} week as compare to baseline (Prakash *et al.*). Our study was inconsistent with their findings of 15^{th} week FBS levels, as we have found the similar results at 12^{th} week rather than 15^{th} week but the % of HbA1c was similar.

A prospective comparative study was conducted for a period of 6 months on T2DM patients, subjects were received metformin 500mg + glimepiride 2mg. Baseline levels of FBS, PBS, and RBS were 123, 188.9 and 154.3 mg/dL and after follow-up, 104.6, 144.2 and 127 mg/dL, respectively, were observed (Sridevi *et al.*). The 6^{th} month duration of their study was inconsistent with our study design, this long time-

period of their study was back the FBS that was very close to normal range, but in our study, we have found the 121.82±9.24 mg/dL, which is close to diabetic level.

Similar to our findings, total of 30 patients that were received the treatment plan of glimepiride-metformin shown 4.44 ±1.3 % of HbA1c, 132.63±4.97 mg/dL of FPG and 191.07±8.64 mg/dL of PPBS (Nishanth *et al.*, 2018).

The prospective study was carried by T et al for a period of 6 months in T2DM patients with > 35 years of age, HbA1c >7% and FPG >140 mg/dL. Patients were treated with combination therapy of Glimepiride + Metformin (2/500 mg, 1-0-1) and clinically analysed at 3^{rd} and 6^{th} week. The mean glycosylated haemoglobin (at 3-months: 8.25±1.1%, at 6^{th}-months: 7.51±1.77 % *vs* base line: 8.73±1.66 %), FPG (at 3-months: 228.35± 61.05 , at 6^{th}-months: 200.47±75.17 *vs* base line: 257.38±59.25 mg/dL), were found decrease as compare to baseline level (T *et al.*, 2017). The drug concentration in and duration of their study were inconsistent with our study to achieve the reduction of FPG and HbA1c level, we have given the treatment with 4mg glimepiride for 12^{th} week.

The longitudinal interventional study was performed in 60 patients who were initially taken metformin (500 mg bid) with poor glycemic control. In their study, patients were received glimepiride-metformin (1 mg / 500 mg bid) for total 3-months period. Average FBS level before initiation of therapy was 204.13 mg/dL. At the end of 3rd month of therapy, an average of FBS was reduced to 132.5 mg/dL. Mean PPBS was 288.57 mg/dL and at the end of 3rd month of therapy, this reduced to 203.47 mg/dL. Baseline mean value of HbA1c before with combination therapy was 8.49% and at the end of 12 weeks was 8.53% (Gullapalli & Desai, 2018). The HbA1c levels in their study was not found much difference after the treatment, while in our 12^{th} week study, this level has reduced from 8.21±0.45 to 7.76±0.45%.

Previously, the prospective observational study was conducted among T2DM patients who did not achieve adequate glycaemic control with metformin monotherapy. Patients were received 2 mg of glimepiride once daily and as add-on therapy to metformin 1.5 to 2 gm in single or divided doses for 6 months. There was

reduction in mean FBS (122.4±31.9 *vs* 175.6±47.1 mg/dL), PPBS (193.0±70.7 *vs* 277.3±68.3 mg/dL) and HbA1c (6.9±1.1 *vs* 8.3±1.1 mmol/mol) as compare to baseline levels. The glimepiride was caused hypoglycaemia in patients (Sarkar *et al.*, 2019). Inconsistent with their study, we have not found hypoglycaemia during the complete time-period.

Most previously, the study was conducted on 180 patients of T2DM. Patients were received combination of glimepiride plus metformin. Mean blood glucose level (mg/dL) before treatment was 182.4, after 3-months was 132.9, at 6^{th}-month was 108.1. Mean PPBG level (mg/dL) before treatment was 230.4, after 3-months was 170.2, and at 6^{th}-months was 148 (Lavania, 2019). The results at 3-months of mean fasting blood glucose level was high as compare to our study. We have found 121.82±9.24 mg/dL at 12^{th} week.

The study was conducted on T2DM patients for a period of 6 months (December 2011 – June 2012) at Andhra Pradesh. The decreased FBS (112±22.5 vs 131±.45 mg/dL), and HbA1c levels (7.216±0.485 vs 7.616±0.625 mg/dL) were found after glimepiride-metformin intervention as compare to pre-treatment (Krishna *et al.*, 2015). We also have found decrease levels but the difference of reduction with before treatment and after treatment has increased (FPG: 202.94±20.33 vs 121.82±9.24 mg/dL; HbA1c 8.21±0.45 vs 7.76±0.45%) as compare to their study.

Stephan Matthaei et al were recruited patients with HbA1c of 7.0-10.5% (53-91 mmol/mol) and treated with dapagliflozin with metformin plus sulfonylurea (group A) once in 24-hours or placebo (group B) till 24-weeks. Baseline HbA1c level in group A was 8.08% [65 mmol/mol] and placebo group was 8.24% [67 mmol/mol]). The FPG level in group A was 167.4 mg/dL [9.29 mmol/L] and placebo group was 180.5 mg/dL [10.02 mmol/L]). The proportion of patients who was able to achieve a therapeutic glycemic response (HbA1c <7.0% [53 mmol/mol]) with combination therapy was 31.8% as compared to placebo group, that was 11.1% ($P < 0.0001$) (Stephan Matthaei *et al.*, 2015).

Dapagliflozin and glimepiride have been frequently used as anti-diabetic drugs (Hussain Syed *et al.*, 2015; Rizvi *et al.*, 2016), dapagliflozin is inhibited the function of sodium-glucose cotransporter 2 (SGLT2) from proximal convoluted tubule (Plosker, 2012; Schwartz & Katz, 2016), while glimepiride increases the production of insulin from the β cells of pancreas and enhances the sensitivity of the intracellular insulin receptor to the action of insulin. They both are clinically used to treat T2D patients. Therefore, in the present study, our next objective was to identify the best effective drug to treat uncontrolled T2D by comparing the efficacy of dapagliflozin and glimepiride as add on to metformin in patients with type 2 diabetes.

The patients who previously treated with metformin monotherapy having inadequately controlled glycemia with metformin has shown higher HbA1c (8.56±7.33). The T2D patients who were received 10 mg of dapagliflozin once and 500 mg of metformin through oral route for three times in 24-hours for a year (group A) has exhibited significantly greater reduction (6.71±0.56) as compared to patients who have received 4 mg of glimepiride once daily in combination with 500 mg of metformin orally three times till 12 weeks (7.76±0.45).

With the same treatment, the FPG was identified at week 6 and 7 and has shown significantly decreased level followed by 6-weeks of treatment with dapagliflozin-metformin combination (137.02±12.30) as compared to glimepiride-metformin add on therapy (146.23±12.54). Furthermore, at 12^{th} week, dapagliflozin-metformin adjunct therapy has significantly produced normal blood glucose levels (101.40±16.85 mg/dL) as compare to glimepiride-metformin, that was shown pre-diabetic state (121.82±9.24 mg/dL). To the best of our knowledge, this is the first study that compares the treatment of dapagliflozin and glimepiride as add-on to metformin in uncontrolled diabetes. Furthermore, we have identified that taken 10 mg of dapagliflozin once in 24 hours with 500 mg of metformin three times was the optimum dose that controls diabetes by preventing HbA1c level and FPG.

Based on positive findings of dapagliflozin- and glimepiride-metformin add-on therapy to maintain glycemia, our next objective was to identify the safety of these combinations in patients with type 2 diabetes.

The increase range of pharmaceutical drugs available to treat diseases are continued to expand gradually, one of the unavoidable adverse effects of drug-mediated disease, include cardiovascular complications. Therefore, the lipid profile of the patients was assessed followed by combination therapy.

Followed by taken dapagliflozin-, or glimepiride-metformin combination by patients, the normal cholesterol level in patients is suggested no risk to develop atherosclerosis, coronary heart disease or cardiovascular disease in them. The normal high-density lipoprotein (HDL) in our study has suggested the sustained role of it, i.e the cholesterol is transported from the periphery tissues (arterial wall cells) to the liver to excrete into bile or metabolized into bile salts before excretion. Furthermore, the normal low density of lipoprotein (LDL) and triglyceride (TG) is not initiated the atherosclerosis or blocked artery or produced coronary artery diseases.

In the most previous study, patients who had myocardial infection was treated with dapagliflozin and found reduced risk to develop major adverse cardiovascular events, whereas no effect was found in patients who did not have previous myocardial infection, or who had atherosclerosis (Furtado *et al.*, 2019).

Similar to our findings of dapagliflozin, A 52-week, open-labeled, prospective observational study was conducted in T2DM patients having inadequately controlled glycemia with quadruple oral hyperglycemia drugs (metformin, and dipeptidyl peptide 4 inhibitors, and glimepiride) to identify the safety 10 mg of dapagliflozin. After 52 weeks of intervention, patients were showed clinically normal cholesterol (163.2 ± 32.5 mg/dL), triglyceride (160.4 ± 83.6 mg/dL), HDL (42.9 ± 8.7 mg/dL), and LDL (95.2 ± 27.6 mg/dL) levels (Ku *et al.*, 2019).

The review report was pointed out that glimepiride (second-generation drug), has a decreased affinity for cardiovascular tissue and may produce least negative effects, however not all previous analysis observations have been similar. As, one of the retrospective cohort study was involved T2DM and observed insignificant variation in mortality rate with the use of glimepiride alone. Another study was found that cardiovascular mortality, nonfatal myocardial infection, and risk of mortality was

enhanced by glimepiride monotherapy, in diabetic patients who were already at high risk for cardiovascular events (Azimova *et al.*, 2014). Our study was inconsistent to show cardiovascular effects by glimepiride-metformin add on therapy.

Stephan Matthaei et al were recruited patients with HbA1c of 7.0-10.5% (53-91 mmol/mol) and treated with dapagliflozin with metformin plus sulfonylurea (group A) once in 24-houre or placebo (group B) till 24-weeks. Group A was showed 184.5mg/ dL of total cholesterol, 101 mg/dL of LDL, 49.5 mg/dL of HDL cholesterol, 2.1 LDL/HDL cholesterol ratio and 173.8 mg/dL of triglycerides (Stephan Matthaei *et al.*, 2015). In present study, the patients were treated with dapagliflozin with metformin only and shown positive results to decrease glycemia with no cardiovascular effects without using of sulfonylurea, as mentioned by the study of Stephan Matthaei et al.

In another previous study, the lipid profile levels with dapagliflozin plus metformin in T2DM patients were detected and shown statistical insignificant changes in lipid parameters as compared to baseline level (total cholesterol: 189.5±33.9 *vs* 184.1±40.1; LDL: 111.1±32.2 *vs* 104.9±36.4 ; HDL: 47.7±11.9 *vs* 52.2±13.0 ; TG: 145.8±62.9 *vs* 135.1±54.2) during the complete study period of Scorsone et al (Scorsone *et al.*, 2018). These normal values are consistent with our findings.

The prospective study was conducted to estimate the metabolic effect of glimepiride (5 mg) with metformin (500mg) on inadequately controlled 125 T2DM patients of Moradabad North India. The significant reduced level of TC (12^{th} week: 168.5±31.1 mg/dL; 24^{th} week: 204±40.2 mg/dL; Baseline: 148±30.6 mg/dL), TAG (12^{th} week: 139.9±38.2 mg/dL; 24^{th} week: 121.1±29.7 mg/dL; Baseline: 167±58.4 mg/dL), LDL (12^{th} week: 98.0±26.5 mg/dL; 24^{th} week: 80.9±29.3 mg/dL; Baseline: 124±33.7 mg/dL) and VLDL (12^{th} week: 21.9±9.07 mg/dL; 24^{th} week: 17.7.9±5.7 mg/dL; Baseline: 28.1±14.4 mg/dL) were found as compared to baseline level but no significant rise in HDL (12^{th} week: 48.5±9.51 mg/dL; 24^{th} week: 49.5±12.9 mg/dL; Baseline: 48.2±9.6 mg/dL) was seen as compared to baseline level (Singh *et al.*, 2016). Our study has not observed the changes till 12^{th} week at 4 mg glimepiride, not 5 mg.

The another prospective study was carried for a period of 6 months in T2DM patients with > 35 years of age, HbA1c >7% and FPG >140 mg/dL. Patients were treated with combination therapy of Glimepiride + Metformin (2/500 mg, 1-0-1) and clinically analyzed at 3rd and 6th week. The mean total cholesterol (at 3-months: 144.62 ± 9.21, at 6th-months: 118.4 ± 5.59 *vs* base line: 150.68 ± 3.50 mg/dL), serum TG (at 3-months: 161.14 ± 8.90, at 6th-months: 141.63 ± 6.04 *vs* base line: 180.25 ± 6.7 mg/dL), and LDL (at 3-months: 46.67 ± 9.39, at 6th-months: 49.14 ±8.9 *vs* base line: 60.80 ± 11.25 mg/dL) were found decrease as compare to baseline level. Whereas, HDL level (at 3-months: 34.89 ± 5.78, at 6th-months: 37.81 ± 7.50 *vs* base line: 33.26 ± 8.01 mg/dL) was observed increase at 3rd and 6th month (T *et al.*, 2017). Our finding with respect to HDL was inconsistent with this reported study, as we have not found the increase or decrease the level of HDL.

Similar with our findings, the open-label, randomized study was carried out to study the effect of metformin-glimepiride (1000/2mg) on glycaemic control in T2DM. Patients with HbA1c more than 7% were included in the study. At week 12 of treatment, the total cholesterol was 118.5±6.4 mg/dL, serum triglyceride was 114±17.5 mg/dL, HDL was 36.43±1.58 mg/dL, and LDL cholesterol was 67.21±11.2 mg/dL in patient (Shimpi *et al.*, 2009).

A study of glimepiride-metformin in 30 patients were shown 157.03 ± 6.80 mg/dL of TG, 41.27 ± 1.44 mg/dL of HDL, 87.0 ± 3.10 mg/dL of LDL, and 36.40 ± 1.94 mg/dL of HDL (Nishanth *et al.*, 2018). The number of the patients in our study was 100 patients who received the glimepiride-metformin, i.e inconsistent with the recruited patients of Nishanth et al study but the findings were similar.

Inconsistent with our findings, the study on T2DM patients for a period of 6 months (December 2011 – June 2012) at Andhra Pradesh was shown decreased TC (201.6±33.19 vs 224.0±35.8 mg/dL), TG (192.3±69.0 vs 207.7±74.7 mg/dL), LDL (144.07±25.1 vs 160.0±30.8 mg/dL) and VLDL (38.2±13.9 vs 41.3±15.8 mg/dL) level after glimepiride-metformin intervention as compare to pre-treatment. The increase ejection systolic Fraction (60.0±15.4 vs 58.9±16.4) and HDL cholesterol levels (38.0±3.35 vs 37.3±3.03 mg/dL) were observed for the 12-weeks study period as

compared to baseline (Krishna *et al.*, 2015). The current study has found insignificant changes in lipid profile.

A study in Iraqis was performed and identify the effects of metformin monotherapy, and its combination with glimepiride on lipid panel in 50 newly on-set T2DM patients. They were designed open-label, randomized study. Patients were treated with either metformin (group 1; n=20), glimepiride (group 2, n=10) or both (group 3, n=20). After 12-weeks of interventions they were observed significantly reduced levels of TC and LDL in all groups, while HDL was increased prominently in only metformin treated patients (group 1) as monotherapy and its add-on with glimepiride patients (group 3) (Najim *et al.*, 2013). These are the inconsistent findings with respect to glimepiride-metformin combination in our study, as we have observed the insignificant changes after the intervention.

Similar with our findings, the study performed in diabetic with obese patients were showed the safe HDL (mg/dL) (32.94±5.08, 35.83±4.69, 38.94±4.76), LDL (mg/dL) (137.26±50.01, 124.53±51.8, 109.12±39.58), VLDL (mg/dL) (43.19±6.69, 38.19±5.67, 31.27±5.23), and TG (mg/dL) (215.94±33.47, 190.94±28.34, 156.33±26.17) level, and CHO/HDL (6.28±1.80, 5.39 ±1.62, 4.49±1.15) and LDL/HDL (4.19±1.53, 3.48±1.43, 2.81±1.01) ratio in 1^{st}, 2^{nd}, and 3^{rd} visit after the invention of metformin and Glimepiride combination therapy (Subrahmanyam *et al.*, 2013).

Next, we were identified the safety profile of these combinations by liver function test (LFT), as, drug induced-liver injury has been found in many previously reported studies. The possible mechanism involves in mediating liver inflammation includes, inhibition of intracellular metabolic cascade, covalent interaction of the drug to intra- or extra-cellular proteins, dysregulation of mitochondrial physiology, induction of apoptosis and blockage of cell membrane transport pumps.

The identification of safety of dapagliflozin- and glimepiride-metformin add-on therapy in hepatocellular hepatotoxicity was manifested by serum glutamic-oxaloacetic transaminase or alanine aminotransferase (ALT or SGOT), serum

glutamic-pyruvic transaminase or aspartate aminotransferase (AST or SGPT), and bilirubin quantification. We have found clinically insignificant results in both combinations from 0-12 weeks.

The normal ALT in both groups has suggested that the amino group transfer from L-alanine to α-ketoglutarate by ALT to produce pyruvate and L-glutamate (i.e the primary function of ALT) was normal in all patients of both groups. Next, the mean values of AST in group A and B was according to normal physiology has suggested that activity of aspartate transaminase to bio-catalyse the conversion of aspartate and α-ketoglutarate to oxaloacetate and glutamate remain sustained normally to maintain liver physiological regulations. Furthermore, the mean values of bilirubin in group A and B were same at 0-week, 6^{th}- week and 12^{th}- week. Thus, the bilirubin in blood samples of patients has not indicated liver disease.

All these above findings were represented that both add-on therapies was unable to produce hepatotoxicity.

Furthermore, these combination therapy effect on cholestatic hepatotoxicity was identified by estimating serum alkaline phosphatase levels, and these combinations at 0-, 6- and 12-week has again shown insignificant results to progress medical condition.

SGLT-2 inhibitors were recovered the liver regulation of NAFLD patients with T2DM. The study was conducted to identify the effect of dapagliflozin combined with metformin on the liver function of T2DM with NAFLD. Patients with alanine aminotransferase level > 40 IU/L were included. Dapagliflozin treatment found ALT decline (−21.1 U/L, respectively) and the proportion of patients with normal alanine aminotransferase level followed by therapy was found 80.0% (Choi *et al.*, 2018)

The prospective study was conducted to estimate the metabolic effect of glimepiride (5 mg) with metformin (500mg) on inadequately controlled 125 T2DM patients of Moradabad, North India. The significant reduced level of aspartate aminotransferase (12^{th} week: 34.8±6.4 mg/dL; 24^{th} week: 34.8±6.99 mg/dL; Baseline: 37.2±6.99 mg/dL), and alanine transaminase (12^{th} week: 37.6±9.2 mg/dL; 24^{th} week: 37.6±9.2 mg/dL; Baseline: 40.9±14.5 mg/dL) was found in 12^{th} and 24^{th} week as

compared to baseline (Singh *et al.*, 2016). While, in current study, we have found insignificant changes in liver function test followed by 12^{th} week of treatment with the same combination.

Similar to our findings of dapagliflozin-metformin combination, A 52-week, open-labeled, prospective observational study was conducted in T2DM patients who previously had inadequately controlled glycemia with quadruple oral hyperglycemia drugs (metformin, and dipeptidyl peptide 4 inhibitors, and glimepiride) to identify the safety 10 mg of dapagliflozin. After 52 weeks of intervention, patients were showed clinically normal aspartate aminotransferase (26.8 ± 13.0 IU/L), and alanine aminotransferase (30.7 ± 16.4 IU/L) levels (Ku *et al.*, 2019).

Position statement of the ADA (American diabetic association) and EASD (European Association for the Study of Diabetes) suggests avoiding use of insulin secretagogues in severe hepatic disease, due to the risk of hypoglycemia. CDA (Canadian Diabetes Association) is suggested to use alternate drugs to treat T2DM in hepatic failure patients. Furthermore, BNF (British National Formulary) recommend, "Insulin secretagogues agents, such as glimepiride, should be avoided to give or prescribed with proper caution at low doses to T2DM with chronic liver disease patients." But glimepiride must be avoided in patients with severe hepatic failure. Guidelines from Indian Council of Medical Research (ICMR) also mentioned avoidance of sulfonylureas in hepatic insufficiency and acute hepatitis. Consensus guidelines by EASLGD (Egyptian Association for the Study of Liver and Gastrointestinal Disease Egyptian Association for the Study of Liver and Gastrointestinal Disease) restricted the usage of sulfonylureas in severe hepatic disease due to increased risk of hypoglycaemia (Gangopadhyay & Singh, 2017).

The previously reported non-randomized, single-arm, open-label trial by Tobita et al was conducted in 16 patients having nonalcoholic steatohepatitis (NASH) with T2DM. The enrolled participants were taken 5 mg of dapagliflozin once in a day upto 24-weeks study period. Followed by, the liver function tests including, alanine aminotransferase, ferritin, serum concentrations of aspartate aminotransferase, and type IV collagen 7S was done and found significantly improved levels (Tobita *et al.*,

2017). This shows that the combination is not produced adverse effect to liver.

Dapagliflozin (SGLT2 inhibitor) eliminates primarily by glucuronidation. The pharmacokinetic studies have been observed that patients having moderate to severe liver dysfunction had greater systemic exposure to the drug as compared to healthy volunteers (normal liver physiology). The exposure of drug in body was highly associated with creatinine clearance by kidney. But the above conclusions were not found statistically significant and suggested that the decision to prescribed dapagliflozin to liver cirrhotic patients with T2D should be individually assessed, as the long-term safety and efficacy profile had not been specifically studied in patients with liver cirrhosis. Furthermore, proper caution is greatly needed when both hepatic dysfunction and renal impairment is found (García-Compeán *et al.*, 2016).

Similar to our findings of glimepiride-metformin combination, randomized, double-blind, multicenter, controlled trial was performed in Italy. Total 47 patients were received glimepiride 2 mg once per day for 12-months. Followed by intervention, plasma transaminase were evaluated at 3, 6, 9, and12 months of treatment and found both AST (U/L) (3^{rd} month: 22±7, 6^{th} month: 21±8, 9^{th} month 21±3, 12^{th} month 20±6; Baseline 21±5) and ALT (U/L) (3^{rd} month: 23±6, 6^{th} month: 22±9, 9^{th} month 22±3, 12^{th} month 21±5; Baseline 22±4) were clinically normal levels (Nishihama *et al.*, 2017).

One of the predisposition to progress or develop urinary tract infections in T2DM is glucosuria. Previous studies show that pharmacologically mediated glucosuria with SGLT2 inhibitors increases the risk to develop genital infections and, comparatively lesser extent to produce UTIs. Therefore, safety of our combinations was identified in the risk of urinary tract infections (UTIs) followed by add-on therapy. The parameters involved to study UTIs through urine sample include, ketone and glucose level, bacteria, WBCs, and pus-cells count.

Ketones are biochemical molecules that are formed during oxidation of fat for energy production. During diabetics, their formation is increased due to insulin deficiency that raised glucose level in the bloodstream and prevented its uptake to liver

and muscle cells. This ultimately is led to increase the fat oxidation by cells for energy rather than glucose catabolism and resulted in ketonuria. During the complete 12th week study period, we have not found ketonuria in both groups.

The previous study was reported that glimepiride (sulfonylureas drug) increased glucose tolerance by stimulated glucose-induced insulin secretion from pancreatic β-cells. Although, the meta-analysis in glimepiride-treated diabetic individuals was observed the decrease glucose level and retain similar insulin concentration in their circulation. Their evidence was assumed that glimepiride treatment exerted extrapancreatic, and insulin-like activity on muscle and adipose tissues. Similarly, in our study, group B has not shown ketone bodies, as glimepiride-metformin combination are able to produce insulin secretion, that ultimately inhibit ketone body formation.

SGLT2 inhibitors, such as dapagliflozin, can directly and indirectly stimulate the secretion of glucagon, which stimulates the fatty acids oxidation and ketone bodies formation (Rosenstock *et al.*, 2019).

The patients recruited in this study were initially average HbA1c and FBS level were 8.21±0.45% and 203.56±20.15 mg/dL, respectively. And according to this range, 500 mg of metformin is efficient to maintain insulin action for uptake of glucose into liver and muscle cells and prevent fat oxidation that ultimately inhibits ketone formation.

Furthermore, ketoacidosis is medical emergency condition arising in above 8.21±0.45 % of HbA1c and 203.56±20.15 mg/dL of FBS and may lead to cerebral edema, hypokalemia and pulmonary edema. In our study, the patients more than this average range of glycemic index was drop out from the study and treated under special observations.

Storgaard et al was presented a case study of diabetic ketoacidosis in obese, inadequately controlled T2D male patient who treated with dapagliflozin (SGLT-2 inhibitor). The patient was admitted in the hospital with diabetic ketoacidosis 5 days

after taking dapagliflozin. The initial observed signs and symptoms were nausea and dizziness, blood pressure was 170-systolic 103-diastolic, shown tachycardic (119 bpm), 15.3 mmol/l blood glucose level (mild hyperglycaemia), severe ketonuria and metabolic acidosis (pH 7.08). The patient was responded better with infusions of insulin, glucose and saline, and after 72-hours, he was discharged with prescribed only insulin. After 1-month, dapagliflozin-insulin was given to him and no recurrent signs of ketoacidosis was developed. Dapagliflozin may influence the ketone bodies formation and acidosis during acute illness or any other pathological conditions that increased the demand of insulin (Storgaard *et al.*, 2016).

Three patients were reported ketonuria, one patient was in the dapagliflozin-saxagliptin-metformin treatment and two patients was dapagliflozin-metformin treatment. But, according to clinical review, these patients had not a diagnosed for diabetic ketoacidosis (Rosenstock *et al.*, 2019).

Inconsistent with our findings, the pooled analysis of safety profile of dapagliflozin in phase IIb and phase III clinical trials was done by S. Jabbour et al. They found in the 21-study pool that 1 patient was suffered with serious adverse effect of diabetic ketoacidosis and 3 were adverse effect of ketonuria/metabolic acidosis due to dapagliflozin (S. Jabbour *et al.*, 2018).

Renal proximal convoluted tubules transport glucose molecules by sodium-glucose transporters (SGLT) -1 and 2. Their expression is increased during uncontrolled diabetes, therefore enhancing the threshold to produce glucosuria. In the present study, we identify the effect of dapagliflozin- and glimepiride-metformin adjunct on glucosuria. From the 0 week we have found the mild glucose in urine, which is one of the prominent manifestations of diabetes. At week-6, group A patients (dapagliflozin-metformin) have shown moderate level of glucosuria, whereas group B was still shown mild level. At week 12, severe increased was observed in group A, while group B was again similar as previous observations.

The differences in the findings probable due to taken of SLGT-2 inhibitor, which was dapagliflozin. This antidiabetic agent binds with SGLT-2 to reduce the glucose reabsorption and allow it to increase glucose level in urine. Therefore, till 6^{th}-

week consistent use of dapagliflozin produce mild effect while this markedly increased in 12^{th} week. On the other hand, glimepiride, another anti-diabetic agent, increases the production of insulin from the β cells of pancreas and enhances the sensitivity of the intracellular insulin receptor to the action of insulin to uptake glucose into the cell and metabolized it. This agent produces no role to increase glucosuria, as consistent with previous findings.

Komoroski et al were suggested that dapagliflozin demonstrated pharmacokinetic property and dose-proportional amount of glucosuria that was sustained over 24-hours, and indicated that it was suitable for clinical use once-daily doses (Komoroski *et al.*, 2009).

The study on 14 HNF1A-MODY (hepatocyte nuclear factor 1α -maturity-onset diabetes of the young), 19 GCK-MODY (Glucokinase-maturity-onset diabetes of the young), and 12 T2D patients was performed to identify the glucosuria by SGLT-2 inhibitors. All studied patients were received 10 mg of dapagliflozin once in the morning and added with their previously using therapy of diabetes. To identify the response of dapagliflozin on glucosuria, they were analyzed the changes in urinary glucose/creatinine ratio and serum 1,5-Anhydroglucitol level. They were concluded that one HNF1A-MODY, two GCK-MODY, and one T2DM patient were positive glucosuria before the therapy (dapagliflozin administration), while followed by using SGLT-2 inhibitor, the glucose was found in urine samples of all studied volunteers. The significant changes in average glucose/creatinine ratio followed by dapagliflozin treatment were found in groups (20.51 ± 12.08 mmol/mmol for HNF1A-MODY, 23.19 ± 8.10 mmol/mmol for GCK-MODY, 9.84 ± 6.68 mmol/mmol for T2DM) (Hohendorff *et al.*, 2017). Similar with their findings, we have found glucosuria, but we had the population of uncontrolled T2D patients.

The safety profile from 12 randomized, placebo-controlled trials were study to identify the relationship of glycosuria and UTI in diabetic patients with inadequately controlled glycemia by showing HbA1c level was between 6.5%-12%. The patients were treated with 2.5, 5, or 10mg of dapagliflozin or placebo once for 24-hours, either as alone or co-treated with sulfonylurea, metformin, thiazolidinedione or insulin, upto

5-6 months. Followed by, UTI infections were quantified and concluded that treatment of T2D with 5 or 10 mg of dapagliflozin once-daily was slightly increased the risk of UTI. But this mild to moderate rise was clinically controllable. Most importantly, the data was unable to demonstrate a considerable dose-UTI relationship (Johnsson *et al.*, 2013). Inconsistently, all the patients that was received 10 mg dapagliflozin has not shown mild or moderate UTI infection during the complete study period, but we have found glucosuria at different extent.

Diabetes-mediated complications, e.g nerve impairment, and decreased blood flow to the extremities increase vulnerability to get infection and stimulate immune system. White blood cells (WBCs), also termed as leukocytes, are one of the vital parts of the immune system defense, that is increased to prevent hyperglycemia- or drug-mediated infections (such as drugs increases glucosuria-induced infections). To achieve our objective, the WBCs were counted in the urine sample to detect the effect of these combinations on urinary tract infection (that may increase dye to glucosuria or drug combination) in both groups for 12-weeks. Followed by the intervention, we have found the normal count of leukocytes at 0-, 6- and 12- week of group A (i.e 2.01±0.82, 1.99±0.82, and 4.07±0.62, respectively) and B (2.00±0.81, 2.01±0.82, and 2.02±0.81, respectively).

To increase the credibility and validity of our previous WBCs finding, pus cells were counted, and leukocyte esterase level was quantified. The pus cell (liquor puris), is dead, white blood cells, that is accumulated in infection due to immune system response, has found insignificant in both groups. Next, leukocyte esterase, which is produced by WBCs, has also not found in urinary samples in both groups' male and female patients.

Furthermore, urinary tract infection was assessed by growth of bacteria in urinary sample. From 0- to 6-week and 6- to 12-week of dapagliflozin- and glimepiride-metformin adjunct therapy, the bacterial growth has not observed.

These results provide strength to the findings that given combination therapies are not able to produce abnormalities associated with urinary tract infection in patients throughout the 12-week study period. The levels of mild- or severe glucosuria in 6^{th}-

and 12th-week in patients taking dapagliflozin-metformin combination was not enough to progress urinary tract infections.

The meta-analysis study recruited 52-randomized controlled trials and showed a dose-dependent drug reaction between dapagliflozin monotherapy with increase vulnerability of UTIs and genital infections. But these were highly common in diabetic females' patients. The analysis was shown that UTIs and genital infections are mostly found in the first 24–26 weeks (beginning of treatment) and afterward incidence was decreased. These infections are usually mild and can be treated with conventional treatment (standard oral drugs and topical antifungal agents). Most importantly, these infections were observed specifically in females suffering previously form genital infection or in men suffering previously from previous balanitis (Papakitsou *et al.*, 2019).

Stephan Matthaei et al was recruited patients with HbA1c of 7.0-10.5% (53-91 mmol/mol) and treated with dapagliflozin with metformin plus sulfonylurea (group A) once in 24-houre or placebo (group B) till 24-weeks. Adverse events of genital infections were occurred in 48.6% of group A patients as compared to group B (5.5% of group A, and 0% of group B; $P = 0.029$) was found. Events of UTI were reported by 6.4% of both A and B group patients (Stephan Matthaei *et al.*, 2015). We have found the insignificant UTI infections by use of dapagliflozin only with metformin in T2DM.

The durability and tolerability of 10 mg/day of dapaglifozin over 52 weeks (i.e 24-week randomized, double-blind period plus 28-week double-blind extension) as add-on to metformin and sulphonylurea in T2D was performed. Adverse events (dapaglifozin: 69.7; placebo: 73.4 %) and serious adverse events (dapaglifozin: 6.4%; placebo: 7.3%) were insignificant among both groups. The 15.6% and 8.3% hypoglycaemic were found with dapaglifozin and placebo, respectively. The genital infections were found 10.1% of dapaglifozin patients than 0.9% of placebo group and UTI proportion was similar in the two groups i.e 10.1% and 11.0% (S Matthaei *et al.*, 2015). Similarly, the present study has shown insignificant UTI infection but has

shown no hypoglycaemia due to the dapaglifozin-metformin treatment over the period of 12th month study.

Randomized double-blind placebo-controlled trial was conducted over 102-weeks on 274 participants to identify the efficacy and safety of dapagliflozin in T2DM. At 102-weeks, events of genital infections and of UTIs was observed more frequently in the dapagliflozin than metformin (low dose) group. But, all these adverse events were more commonly found in women, and most of them were only single episodes (C. Bailey *et al.*, 2015).

To identify the glucosuria and UTI relationship in T2DM patients having HbA1c > 6.5% to 12%, Johnsson et al were pooled the safety data analysis from randomized, placebo-controlled trials. Patients were taken 2.5, 5, or 10 mg of dapagliflozin or placebo once daily, either alone or combination with metformin for 12 to 24 weeks. Followed by intervention, increase glucosuria was found, but no incidence of UTI was reported even in gradually increased dapagliflozin dosage in patients. The discontinuation frequency because of UTI with dapagliflozin-treated T2DM patients were 8 (0.3%) and with placebo-treated T2DM patient was 1 (0.1%). Most of the UTI diagnosed infections were seem mild-moderate level and can be treated with standard antimicrobial agent (Johnsson *et al.*, 2013). The duration of the study may lead to produce mild to moderate level of infection, as our 12th week study design has not shown this mild-moderate level of UTIs.

The clinical phase 3, double-blind, multicentre, placebo-controlled, parallel-group trial was conducted on 546 T2DM patients who previously received metformin (≥1500 mg) once daily and had poorly control glycaemic profile. They were randomly divided into 4 groups according of dapagliflozin dose; 2·5 mg (group 1), 5 mg (group 2), or 10 mg (group 3), or placebo (n=137). After 24-weeks of once daily dose intervention, the frequency of UTI were 7 (5%) in placebo, 4 (3%) in 2.5mg dose, 7 (5%) in 5mg dose and 9 (7%) in 10mg dose (C. J. Bailey *et al.*, 2010). These results have not shown the drug-UTI relationship, same as our findings.

The genital infections and UTIs can found in patients that received treatment of dapagliflozin alone or in combination. Most often these infection found in women, and

in many studies, few number of cases were reported that had severe adverse effects and attempt was taken to discontinue them during study (C. J. Bailey *et al.*, 2010; S. A. Jabbour *et al.*, 2014; Nauck *et al.*, 2011; Parikh *et al.*, 2015).

Similar to our insignificant UTI findings, T2DM patients who had uncontrolled glycemia (baseline HbA1c 7.5–12%) were recruited in the study of Henry et al. They divided patients in to three groups i) dapagliflozin-metformin, dapagliflozin and metformin monotherapy. The 5 mg dosage of dapagliflozin was used in combination or in monotherapy during study 1, whereas 10 mg was used in study 2. In study 1 (5gm dapagliflozin) the frequency of UTI were 7.7% in combination, 7.9% in dapagliflozin alone and 7.5% in metformin alone. Whereas in study 2, the frequency were 7.6% in combination, 11.0% in dapagliflozin alone and 4.3% in metformin alone (Henry *et al.*, 2012).

The study was performed in T2DM patients (inadequately controlled on metformin) who had mean age was 60.7 years, HbA1c was 7.2%, and body mass was index 31.9 kg/m^2. Patients were received 10 mg/day of dapagliflozin, or placebo added to metformin. After 24-week of intervention, the frequency of UTI in Placebo + metformin group were 4% and Dapagliflozin10 mg plus metformin group were 3% (Bolinder *et al.*, 2014). The present study was designed for the period of 12^{th} week only but this short duration has still shown insignificant findings.

Glimepiride is produced hypoglycemia *via* activated the insulin secretion from β cells of pancreas and by increased the insulin sensitivity in receptors of peripheral tissue (Mohd *et al.*, 2015). Our findings have not shown hypoglycemia in Glimepiride-metformin combination, this may due to observation of patients for short duration i.e 12 week.

Inconsistent with our findings, the previous study was recruited patients with HbA1c of 7.0-10.5% (53-91 mmol/mol) and treated with dapagliflozin with metformin plus sulfonylurea (group A) once in 24-houre or placebo (group B) till 24-weeks. Adverse events were occurred in 48.6% of group A patients and 51.4% of group B

patients. Significantly 12.8% of group A patients as compared with 3.7% of group B experienced hypoglycemia (P = 0.024). (Stephan Matthaei *et al.*, 2015).

The study of Chirila et al was observed decrease hyperglycemia and increase frequency of hypoglycemia with use of glimepiride-metformin by 780 diabetic patients at all visits. (Chirila *et al.*, 2016)

The controlled, open-label study over 24-weeks, was conducted by Kesavadev et al in 440 randomized patients of T2DM. The patients were received metformin and insulin combination therapy plus 1–3 mg of glimepiride. The reduce level of HbA1c and total daily dose of insulin were found by addition of glimepiride. Furthermore, hypoglycemic events were occurred in patients by glimepiride treatment at the end of the study (Kesavadev *et al.*, 2017). The long-term use of this combination was may produce the hypoglycemia in patients. Our study has observed the patients till 12 weeks of intervention.

The systematic review and meta-analysis of 214 article of T2DM patients was concluded that the patients who had received glimepiride-metformin combination shown reduced HbA1c level and lower hypoglycemia adverse events (Sukmawan, 2019).

Another study was mentioned that reduced 3.72±4.17 mmol/L of 2h-PPG and 2.16±2.51 mmol/L of FPG. The proportion of patients who achieved an HbA1c less than 7% at week 32 was 56.0% in the glimepiride group. An average body weight gain of 2.53±1.21 kg in the glimepiride group was observed.

The percentage of patients experiencing any hypoglycemia event (ie, symptomatic event or event of plasma glucose concentration <54 mg/dL regardless of symptoms) was 5.8% vs 34.8% in glimepiride (5.8% vs 34.8%). In patients receiving glimepiride, the hypoglycemia event rate was higher in patients with baseline HOMA-2%β ≤median versus >median (2.29 vs 1.60 events/patient-year; adjusted IRR = 1.737; 95% CI, 1.439–2.097). The association between lower β-cell function at baseline and increased prevalence of hypoglycemia was particularly strong in patients aged ≥75

years (adjusted IRR = 2.409; 95% CI, 1.686–3.442; P < 0.001), although it was also significant in patients aged 65 to <75 years old (adjusted IRR, 1.654; 95% CI, 1.339–2.043; P < 0.001).

CHAPTER 6

CONCLUSION

6.1 Conclusion of the study

It is concluded that:

i. Dapagliflozin add-on to metformin is clinically effective to treat hyperglycemia in uncontrolled T2DM (A).

ii. Glimepride-metformin combination is effective to prevent hyperglycemia in inadequately control T2DM (B).

iii. Dapagliflozin-metformin (A) has better outcome in management of T2DM as compared to glimepride-metformin (B).

6.2 Recommendations

It is recommended that

i. Dapagliflozin should be co-administered with metformin to treat inadequately control glycemia in patients of T2DM.

ii. Futher studies are required to address the dapagliflozin-metformin as therapeutic option in other types of diabetes and diabetes with other pathological complications

iii. Future studies should be conducted to observe its cost-effectiveness on patients of T2DM with other commonly prescribed commercially available drugs.

iv. Future studies can be conducted in T2DM with kidney failure patients.

v. Long term intra molecular studies should be conducted to closely observe the effect of dapagliflozin-metformin on upregulation of pancreatic β cell and SLGT-2 receptors.

6.3 Strengths of the study

i. Dapagliflozin co-administered with metformin to treatment of glycemia in patients of poorly control T2DM.

ii. To the best of our knowledge this is the first study regarding the comparative effect of dapagliflozin-metformin with glimepiride-metformin, on inadequately control diabetes.

iii. Efficacy of present pharmacological treatment with respect to FBS and HBA1C were performed.

iv. Safety profile with respect to hepatic, urinary tract, and cardiovascular diseases of the drug groups were closely observed at intervals of 6^{th} and 12^{th}- week.

6.4 Limitations of the study

i. Single centric study.

ii. Patients only with uncontrolled diabetes were included in the study.

iii. Dapagliflozin, glimepride and metformin monotherapy group were not included.

iv. The designed time period to study the effect was short.

v. Number of patients were less.

CHAPTER 7

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Appendix A

FRC Approval letter

FACULTY RESEARCH COMMITTEE
BAHRIA UNIVERSITY MEDICAL & DENTAL COLLEGE

Ref No: FRC-BUMDC -13/ 2019/Phar-008

Date: 9th October, 2019

To,
Dr. M. Kamran Yousuf
M.Phil. Student
Department of Pharmacology
BUMDC-Karachi

Subject: **APPROVAL OF SYNOPSIS**

The Faculty Research Committee has approved the synopsis of below mentioned Student.

Name of Student: Dr. M. Kamran Yousuf

Title: **Comparison of Glimepiride-Metformin Versus Dapagliflozin-Metformin in Patients with Type 2 Diabetes**

Further this letter is recommended and referred to ERC for approval on ethical grounds.

Regards
Assist Prof. Dr. Mehreen Lateef,
CO- CHAIRPERSON FRC-BUMDC

Cc:
Director General
Principal
FRC Record
PG Secretariat

Appendix B

ERC Approval letter

Ref no: FRC/BUMDC -13/2019-Phar-008 **MS11**

Approval of Research Proposal

Mr/Miss/Ms/Mrs/ **Dr. M. Kamran Yousuf**

Registration No. **06-115182-003**

Dear MS/MPhil Student,

I am pleased to inform you that your research proposal on : **Comparison of Glimepiride-Metformin Versus Dapagliflozin-Metformin in Patients with Type 2 Diabetes** has been approved. You may, therefore, continue you research on this theme and produce a quality thesis, as per the HEC requirements.

I take this opportunity to remind you that you must complete your thesis, and defend it successfully, by **SPRING 2021**; this is the date which marks the end of the Extended Duration of your programme. However, to remain eligible for honours and awards, you must complete the thesis, and successfully defend it, by the end of 10 week into the next semester after the final semester.

I wish you every success.

Dated: 09/10/19

(CHAIRPERSON FRC)

Distribution:

- DG
- Principal
- Student's File (with the HOD/PGP Coordinator)
- Student

Appendix C

Consent form

CONSENT FORM

DIRECTOR / HEAD OF INSTITUTE / HOSPITAL

I am giving my consent as Director / Head of institute/ Hospital for granting permission for facilitating MPhil project of Dr Muhammad Kamran Yousuf, student of Batch-2 (2018-2020) MPhil Program – Pharmacology, at Bahria University Medical & Dental College Karachi.

The facilitation will include enrollment of patients and access to data from the clinic /ward, as per directions of Prof Dr Sajid Abbas Jaffery

Name: Dr OMER JUNG MD
Designation: ADMINISTRATOR.
Name of Institute: National Medical Center
Name of Hospital: National Medical Centre
Name of Clinic: Medical OPD
Date: 2-9-2019
Signature with stamp:

DR. OMER JUNG - MD
Medical Administrator
N.M.C

INFORMED CONSENT FORM FOR PATIENT

You are giving your consent to participate voluntarily and at your own will in this research clinical trial project that aims to treat your disease of Osteoarthritis by giving drug:

A. Tab. Dapaglifozolin 10mg once daily per orally for 12 weeks.

OR

B. Tab Glimepiride 4mg once daily per orally for 12 weeks.

PLUS

C. Tab Metformin 500 mg thrice daily per orally for 12weeks.

You have been told the possible side effects of the drugs (A). These can cause Genital tract infections (vulvo vaginal candidiasis), urinary tract infections, and hypoglycemia.

You have been told the possible side effects of the drugs (B). These can cause Hypoglycemia, hyperinsulinemia (intense hunger, fatigue) and renal dysfunction.

You have been told the possible side effects of the drugs (C). It can cause nausea, vomiting, diarrhea, lactic acidosis (fast shallow breathing, muscle pain & cramps), vitamin B12 deficiency (anemia, peripheral neuropathy).

You have been explained in detail the nature and significance of participating in the project and you understand the provided explanation.

You have been told that findings of your disease and your data will be kept strictly confidential and will be used only for the benefit of community, publications and paper presentations.

You have been explained that laboratory investigations will be conducted for the diagnosis and start of drug therapy and to monitor the therapy and side effects of the given drugs. For this purpose you fully agree to give your blood samples at the beginning and end of study and when required in between.

You also agree to give all relevant information needed, in full and to the best of your knowledge to the researcher. It is clarified to you that no incentive will be provided to you for participating in the studyexcept the cost oflab investigations and drugs, whereas you do have the right to withdraw from the study at any time.

You are advised to contact Dr. Kamran Yousuf on mobile number: 0333-9800961 or visit National Medical Centre in case of any query/ emergency related to your disease.

Name of Patient:_____ S/D/W/0_____

Treatment Assigned to Patient: _____

Signature / Thumb impression of Patient: _____

Name of Researcher: _____

Signature of Researcher:_____

Date: _____

مریض کا اجازت نامہ

میں رضا کارانہ طور پر حصہ لینے اور اپنی مرضی کے مطابق اس تحقیقاتی عمل میں اپنی رضامندی دے رہی ہوں/ رہا ہوں اس مقصد ادویات کے ذریعے میرے شوگر کے مرض کا علاج کرنا ہے۔

یہ علاج درج ذیل مراحل پر مشتمل ہے:

اے۔ ٹیبلیٹ: ڈیاگلائی فوزولن(Dapglifozolin)10mg روزانہ ایک مرتبہ 12 ہفتے تک۔

یا

بی۔ ٹیبلیٹ: گلمپر ائیڈ(Glimepiride)4mg روزانہ ایک دفعہ 12 ہفتے تک۔

+

سی۔ ٹیبلیٹ میٹفورمن(matformin)500mg گرام دن میں تین دفعہ 12 ہفتے تک۔

آپ کو دی گئی دوا 'الف' لینے_، سے شوگر کم ہوسکتی، پیشاب کا انفیکشن ہوسکتا ہے اور اندام نہانی میں فنگس انفیکشن ہوسکتا ہے۔
آپ کو بتائی گی دوسری B دوا سے شوگر کم ہوسکتی ہے، شدید بھوک لگ سکتی، تھکاوٹ ہوسکتی ہے اور گردوں پر اثر ہوسکتا ہے۔
آپ کو بتائی گی C دوا سے متلی، الٹی، اسہال ہوسکتے ہیں اور لیکٹک ایسیڈوسس (جس میں تیز سانس اور عضلات میں درد ہوسکتا ہے) اور وٹیمن B 12 کی کمی ہوسکتی ہے۔

مجھے اس تحقیقاتی منصوبے میں حصہ لینے اس کی فطرت اور اہمیت کے بارے میں تفصیل سے بیان کیا گیا ہے اور میں نے فراہم شدہ وضاحت سمجھ لی ہے۔ مجھے بتایا گیا ہے کہ میری بیماری کے نتائج اور میری معلومات کو ہر چند خفیہ رکھا جائے گا اور صرف معاشرے میں شوگر کی بیماری کے فائدے کے لیے تحقیقی مقالہ لکھنے اور اشاعت کے لیے استعمال کیا جائے گا۔

مجھے وضاحت کی گئی ہے کہ تحقیقی علاج کے آغاز سے پہلے اور تحقیق کے اختتام پر لیبارٹری سے جانچ کی جائیں گی۔ اس مقصد کے لیے میرے خون کے نمونے تحقیقی علاج شروع کرنے سے پہلے اور تحقیق کے اختتام اور جب ضرورت ہوئی تو درمیان میں بھی لیے جائیں گے۔ میں پوری طرح سے تمام ضروری معلومات محقق کو دینے پر رضا مند ہیوں۔ مجھے یہ واضح کیا گیا ہے کہ اس تحقیق میں حصہ لینے کے لیے مجھے کوئی معاوضہ نہیں دیا جائے گا۔ لیبارٹری تحقیقات، کی فراہمی اور ادویات کی لاگت محقق کی جانب سے ادا کی جائیں گی۔ جب کہ میرے پاس کسی بھی وقت تحقیق سے دستبردار ہونے کا حق ہے۔

مجھے مشورہ دیا گیا ہے کہ ڈاکٹر محمد کامران یوسف سے موبائل نمبر 0333-9800961 پر یا میری بیماری سے متعلق کسی بھی سوال/ایمرجنسی کی صورت میں نیشنل میڈیکل سینٹر ہسپتال سے رابطہ کروں۔ ادویات کے نتیجے میں کسی بھی غیر معمولی صورتِ حال میں یہ محقق کی ذمہ داری ہوگی کہ وہ مریض کا اپنے اخراجات سے علاج کرائیں گی۔

☐ ☐ ☐

مریض کا نام _____

مریض کو دی گئی دوا اے بی سی

مریض کے دستخط/ انگوٹھے کا نشان _____

محقق کا نام _____

محقق کے دستخط _____

تاریخ _____

Ethical Review Committee
Bahria University Medical and Dental College
Karachi, Pakistan

PROFORMA

PRINCIPAL INVESTIGATOR'S (PI)

NAME: Muhammad Kamran Yousuf

QUALIFICATION(S): MBBS

DESIGNATION: Lecturer

DEPARTMENT: Pharmacology

EMAIL: kmran2010@gmail.com

CONTACT NUMBER: Cell phone: 0333-9800961 Office no: Ext: 1070

Guidelines and instructions for researchers:

1. It is the obligation of researcher to fill the application form and to ensure that all details provided are true.

2. Incompletely filled form will not be entertained

3. No urgent basis cases will be accepted.

4. The process may take up to 8 weeks

5. PI /Supervisor (in student's research) must sign the application where ever it is required.

6. Consent form must be attached.

Appendix D

Subject Evaluation Performa

Serial No: _____ Reg. No: _____ Date: _____

Patient's Name: _____ S/D/W/O _____

Age: _____ Sex: _____ Weight(kg): _____ Height(cm): _____

Address: _____

Phone No.: _____ Occupation: _____

Presenting Complaints: _____

Medical History: _____

Family History: _____

PHYSICAL EXAMINATION AT THE TIME OF REGISTRATION:

Vitals: BP: _____ Pulse: _____ Resp. Rate: _____

CVS: Heart Sounds: _____ Other: _____

Respiratory System: _____

Abdomen: _____

CNS: _____

BASELINE LAB ASSESSMENT:

(Dav-0)

1. **CBC:**
   Hb: _____ WBC: _____ Platelets: _____
2. FBS: _____
3. HbA1C: _____
4. LFTs: _____
5. Lipid Profile: _____
6. RFTs:
   Urea: _____ Creatinine: _____

Treatment assigned: _____

Group: _____

PATIENT'S FOLLOW-UP RECORD
First Follow-up Visit (6th week)

Date: _____

Compliance: _____

Adverse Effects: _____

Parameters Evaluation:

1. FBS: _____
2. HbA1C: _____
3. LFTs: _____
4. Lipid Profile: _____
5. RFTs:

   Urea: _____ Creatinine: _____

Final Visit (12th week)

Date: _____

Compliance: _____

Adverse Effects: _____

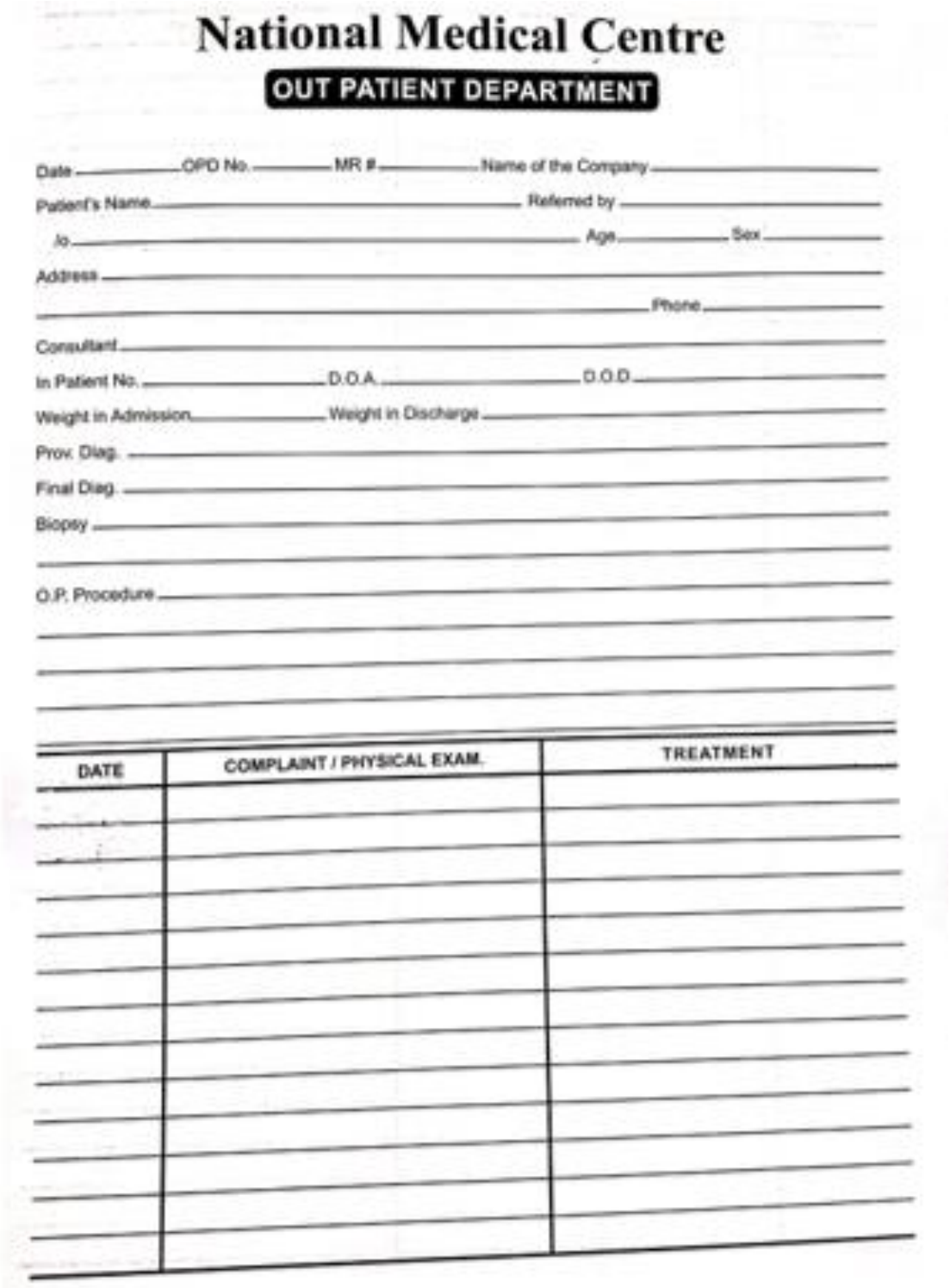
Parameters Evaluation:

1. FBS: _____
2. HbA1C: _____
3. LFTs: _____
4. Lipid Profile: _____
5. RFTs:

   Urea: _____ Creatinine: _____

Appendix E

Hospital Card



National Medical Centre

OUT PATIENT DEPARTMENT

Date _____ OPD No. _____ MR # _____ Name of the Company _____

Patient's Name _____ Referred by _____

S/o _____ Age _____ Sex _____

Address _____

_____ Phone _____

Consultant _____

In Patient No. _____ D.O.A. _____ D.O.D _____

Weight in Admission _____ Weight in Discharge _____

Prov. Diag. _____

Final Diag. _____

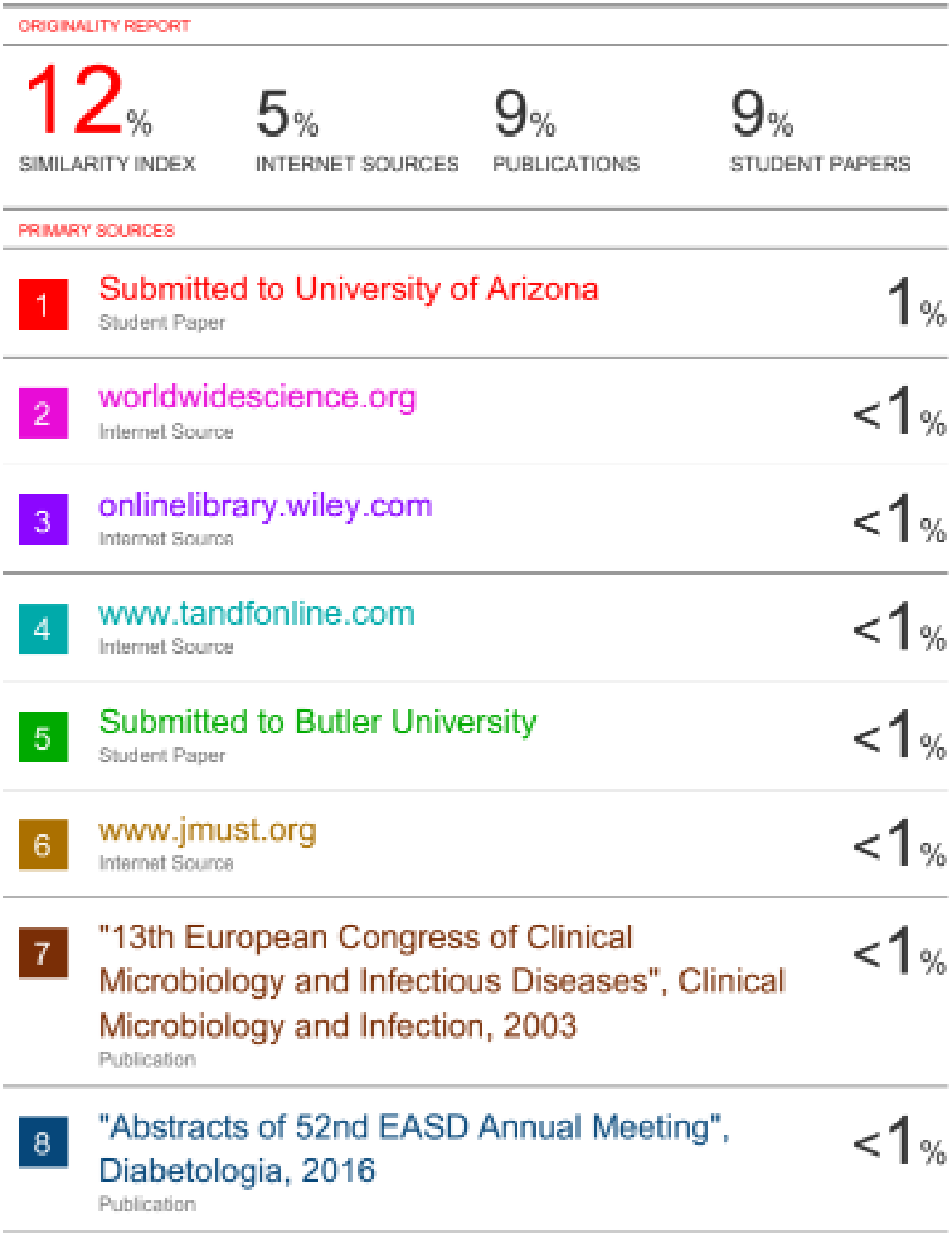
Biopsy _____

O.P. Procedure _____

DATE	COMPLAINT / PHYSICAL EXAM.	TREATMENT

Appendix F

Turnitin Plagirisim Check Report



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