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Dapagliflozin Effects on Hematological Parameters, and Renal Function in Type II Diabetic Patients

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صدق الله العظيم

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Supervision certification

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with sincere appreciation

Muthanna K. Zaki

Abstract

Type 2 diabetes mellitus (T2-DM) is a progressive disease that if not well-controlled leads to a variety of complications including hematological and renal problems which necessitate the importance of evaluating the potential of antidiabetics in reducing and/or preventing these complications. The aim of the study was to investigate the effects of dapagliflozin on hematological parameters, erythropoietin (EPO), and renal function in patients with T2-DM to evaluate the safety, efficacy and potential benefits of dapagliflozin on those patients. The study was designed as a retrospective cohort trial and was conducted between November 2023 and April 2024 at a private clinic in Mosul, Nineveh Province. It included three groups, the control group (group 1) involved 41 apparently healthy individuals, (group 2) involved 40 patients with T2-DM on metformin-only therapy, and (group 3) involved 30 patients with T2-DM on combination therapy of dapagliflozin and metformin. The baseline characteristics included age, sex, body mass index (BMI), and the duration of diabetes. Complete blood count (CBC), EPO level, serum uric acid (SUA), and renal function tests were measured and compared among the three groups. Serum creatinine was significantly higher in group 2 as compared to group 1 ($p < 0.013$), while the addition of dapagliflozin as an add-on therapy to metformin reduced the creatinine levels to be insignificant as compared to group 1. Serum urea levels were significantly higher in group 2 and group 3 as compared to group 1 ($p < 0.001$); however, there were no significant differences between group 2 and group 3. Furthermore, SUA was significantly lower in dapagliflozin as compared to group 2 ($p < 0.04$). EPO was significantly higher in patients who received dapagliflozin therapy ($p < 0.01$) as compared to group 1. Additionally, red blood cells (RBC), hemoglobin (Hb), and hematocrit (Hct) were also significantly higher in patients on dapagliflozin therapy. Moreover, white blood cells (WBC) were significantly higher in patients who received

dapagliflozin medication. Furthermore, there was a significant positive correlation between SUA, EPO, and hematological parameters in patients on dapagliflozin. On the other hand, there is a significant negative correlation between SUA, EPO, and hematological parameters in patients who were treated with metformin only. The study concluded that dapagliflozin therapy has demonstrated its beneficial effects on the kidney by reducing serum creatinine levels. SUA was also reduced with dapagliflozin medication, and this reduction was associated with a better impact on hematological parameters and EPO level.

Keywords: Dapagliflozin, Diabetes mellitus, Hematological disorders, SGLT2 inhibitors.

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List of Abbreviations

Abbreviation	Key
1,5AG6P	1,5-anhydroglucitol-6-phosphate
4PLC	4-parameter logistic curve
ACEIs	Angiotensin-converting enzyme inhibitors
ADA	American diabetes association
ADPS	N-ethyl-N-(3-sulfopropyl)-3-methylaniline
AGEs	Advanced glycation end products
AGIS	α-glucosidase Inhibitors
AMP	Adenosine monophosphate
AMPK	Adenosine monophosphate-activated protein kinase
ARBs	Angiotensin receptor blockers
ATP	Adenosine triphosphate
AVP	Arginine vasopressin
BMI	Body mass index
CBC	Complete blood count
CD	Cluster of differentiation
CHD	Coronary heart disease
CHF	Congestive heart failure
CKD	Chronic kidney disease
CLIA	chemiluminescent immunoassay
CRTase	Creatine amidinohydrolase
CrCl	Creatinine clearance
DKA	Diabetic ketoacidosis

Abbreviation	Key
DM	Diabetes mellitus
DNP	Diabetic nephropathy
DPG	Diphosphoglycerate
DPP-IV	Dipeptidyl peptidase IV
DSMES	Diabetes self-management education and support
DSPN	Distal symmetric polyneuropathy
eGFR	Estimated glomerular filtration rate
ELISA	Enzyme-linked immunosorbent assay
EPO	Erythropoietin
ESPMT	N-ethyl-N-sulfopropryl-m-toluidine
ESRD	End-stage renal disease
FBG	Fasting blood glucose
FSG	Fasting serum glucose
FDA	Food and Drug Administration
G-6-P	Glucose 6 phosphate
G-6-PDH	Glucose 6 phosphate dehydrogenase
GDM	Gestational diabetes
GLDH	Glutamate dehydrogenase
GLP-1	Glucagon-like peptide-1
Gluconate-6P	Gluconate 6 phosphate
GP	Glycoprotein
GSH	Glutathione
Hb	Hemoglobin

Abbreviation	Key
Hct	Hematocrit
HDL	High density lipoprotein
HEPES	N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid
HHS	Hyperosmolar hyperglycemic state
HI-1α	Hypoxia-induced factor-1α
HK	Hexokinase
HRP	Horseradish Peroxidase
LDL	Low density lipoprotein
MAPK	mitogen-activated protein kinase
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MES	2-morpholinoethane sulfonic acid
MI	Myocardial infarction
MNT	Medical nutrition therapy
NADH	Nicotinamide adenine dinucleotide
NLR	NOD-like receptors
NO	Nitric oxide
NPDR	Non-proliferative diabetic retinopathy
OD	Optical density
OGTT	Oral glucose tolerance test
OS	Oxidative stress

abbreviation	Key
PAD	Peripheral artery disease
PDR	proliferative diabetic retinopathy
PG	Plasma glucose
PI3K-Akt	Phosphatidylinositol-3 kinase
PKC	Protein kinase C
POD	Hydrogen peroxidase
PPAR-γ	peroxisome proliferator-activated receptor gamma
RBC	Red blood cell
RDW-SD	Red cell distribution width-standard deviation
RLUs	Relative light units
ROS	Reactive oxygen species
SGLT2	Sodium-glucose co-transporter 2
SOD	Superoxide dismutase
STD	Standard
T1-DM	Type 1 diabetes mellitus
T2-DM	Type 2 diabetes mellitus
TINIA	Turbidimetric inhibition immunoassay
TNFα	Tumor necrosis factorα
tPA	Tissue-type plasminogen activator
TRIS	Trisaminomethane
TTAB	Tetradecyltrimethylammonium bromide

Abbreviation	Key
Tx	Thromboxane
VEGF	Vascular endothelial growth factor
WBC	White blood cell
β-TG	β-thromboglobulin

Chapter one

Introduction, Aim and Objectives

1. Introduction

1.1. Diabetes mellitus

Diabetes mellitus (DM) has been known since antiquity. Its descriptions have been found in the Egyptian papyri, in ancient Indian and Chinese medical literature, as well as, in the work of ancient Greek and Arab physicians. It is characterized by weight loss and polyuria. The phrase DM was combined by the Greek physician Aertaeus. Diabetes means "to pass through" in Greek, and the Latin word mellitus means "honey" (relating to sweetness) (Deshmukh & Jain, 2015).

DM can be defined as a collection of metabolic disorders with varied causes involving total or relative insulin deficiency or insulin not working properly at the receptor site. This results in anomalies in the metabolism of carbohydrates, lipids, and proteins, which leads to persistently elevated levels of glucose in the blood. There are more than 400 million people with DM in the world, and that number is expected to rise to 592 million by 2035. About 7% of the population is thought to have type 2 diabetes mellitus (T2-DM), which is responsible for 90 to 95 % of all diabetes cases. The incidence of T2-DM has increased significantly, becoming a major global public health concern. This disorder is most common in low- and middle-income countries (Taderegew et al., 2020).

The disease presents with common symptoms including thirst, polyuria, unintentional weight loss, blurred vision, and tiredness. In severe cases, diabetic ketoacidosis (DKA) and hyperosmolar hyperglycemic state (HHS) may present, which are considered as acute complications of the disease and lead to unconsciousness, coma, and eventually death, if left untreated (Ramachandran, 2014). The chronic hyperglycemia of diabetes, especially

when poorly controlled causes long-term damage, dysfunction, and failure of different organs of the body. DM is associated with microvascular and macrovascular complications, nephropathy, retinopathy, and peripheral neuropathy, in addition to stroke, cardiovascular, and gastrointestinal disease which are examples of such complications (Cloete, 2022). The management of DM involves a combination of strategies including non-pharmacological and pharmacological interventions that aim to reduce blood glucose and control the symptoms in addition to reducing and preventing future complications (Guo et al., 2023).

1.2. Classification of diabetes mellitus

There are different types of diabetes that depend on the clinical situation of the patient at the time of diagnosis and include:

1.2.1. Type I diabetes mellitus

Type I DM (T1-DM) is caused by the destruction of beta cells in the pancreas and usually results in complete insulin deficiency. It can be presented as immune-mediated diabetes or idiopathic diabetes (Katsarou et al., 2017).

In immune-mediated T1-DM, which accounts for 5-10% of those with diabetes, the onset of the disease results from autoimmune destruction of the beta cells in the pancreas and was previously referred to as insulin-dependent diabetes or juvenile-onset diabetes. While many of those with T1-DM have permanent insulin deficiency and are at risk of DKA, they have no evidence of autoimmunity. These types of patients are considered to have idiopathic diabetes (Catarino et al., 2020).

1.2.2. Type II diabetes mellitus

This form of diabetes accounts for nearly 90-95% of diabetes patients and it was previously referred to as insulin-independent diabetes or adult-onset diabetes. It involves individuals who have relative rather than absolute insulin deficiency, insulin resistance, or both. This type of diabetes may be related to genetic or environmental factors (Kharroubi, 2015). In terms of pathogenesis, a defect in insulin action and insulin production leads to excessively elevated glucose levels in the bloodstream as shown in figure 1.1 (Nigussie et al., 2022). In instances of β-cell malfunction, there is a decrease in insulin release, which hinders the capacity of the body to regulate glucose levels within the normal range. On the other hand, insulin resistance causes the liver to produce more glucose while muscular, hepatic, and adipose tissue all reduce their glucose intake. Insulin resistance is typically less severe than β-cell dysfunction, even though both occur early in the pathophysiology and have a role in the development of the disease. when β-cell failure and insulin resistance exist together, hyperglycemia worsens and T2-DM progression occurs (Galicia-Garcia et al., 2020).

The majority of people who have this type of diabetes are obese, where obesity itself can create some degree of insulin resistance. Patients who are not obese generally have a higher percentage of body fat, mostly in the abdominal area (DeFronzo et al., 2015). Additionally, patients with T2-DM usually remain asymptomatic and undiagnosed for a long period because the hyperglycemia in these patients develops gradually and at the early stage of the disease is not enough to cause the classical symptoms of diabetes. Unfortunately, these patients are at increased risk of developing microvascular and macrovascular complications of diabetes (Ahmad et al., 2022). The risk factors for developing this type of diabetes include age, obesity, ethnicity, women with previous

gestational diabetes (GDM), and patients with hypertension or dyslipidemia (Y. Wu et al., 2014).

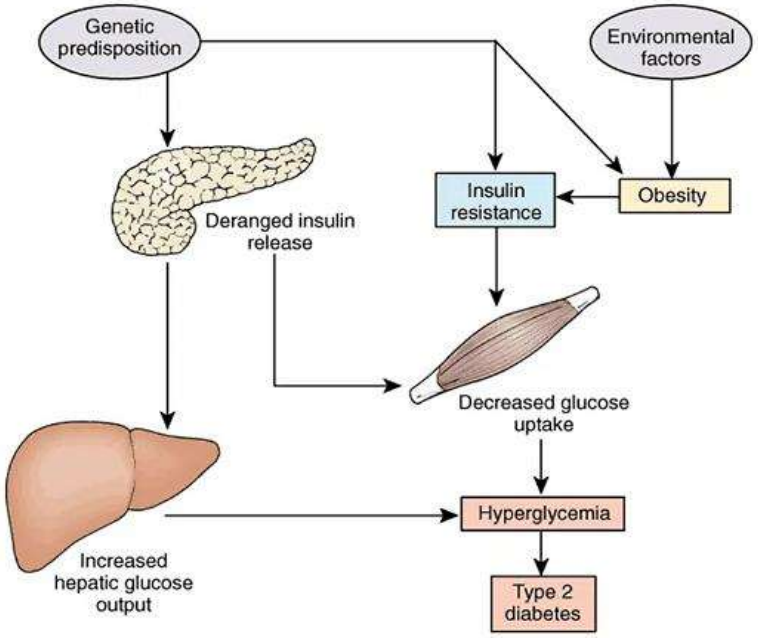

Figure 1.1: Pathogenesis of type 2 diabetes mellitus

Pathogenesis of T2-DM involves a reduction in the synthesis and release of insulin that may related to genetic predisposition in addition to insulin resistance that may related to obesity and environmental factors resulting in a reduction in glucose uptake by the tissues and hyperglycemia (Nigussie et al., 2022).

1.2.3. Gestational diabetes mellitus

This type of diabetes occurs during pregnancy and usually disappears after delivery. It can be presented at any stage of pregnancy but often develops in the second and third trimester. GDM is usually associated with insulin resistance that appears to result from the combined effect of increased maternal adiposity and hormones produced by the placenta. These hormones reduce the effect of insulin and include, human placental lactogen, human placental growth hormone, progesterone, and cortisol (McIntyre et al., 2019).

Other types of DM include monogenic diabetes which involves defects in the beta cell function or insulin action, disease of the exocrine pancreas,

endocrine disorders, drug or chemical-induced diabetes, infection-related diabetes, and diabetes associated with the specific syndrome (Wolfsdorf & Sperling, 2012).

1.3. Diagnostic criteria for diabetes mellitus

Diabetes can be diagnosed based on plasma glucose level by using the following tests:

A fasting blood glucose level (FBG) of 126 mg/dL (7 mmol/L) or higher after a period of fasting for at least 8 hours. Or 2-hour plasma glucose (PG) level during oral glucose tolerance test (OGTT) with 1.75 g/kg (maximum 75 g) of glucose equal to 200 mg/dL (11.1 mmol/L) or more. A hemoglobin A1c (HbA1c) level of 6.5% or higher, or a random plasma glucose level equals to or exceeds 200 mg/dL (11.1 mmol/L) with signs and symptoms of DM such as polyuria, polydipsia, and unintentional weight loss (Kumar et al., 2016).

If one lab result falls into the diabetes range and there are no signs of hyperglycemia, a repeat confirmatory laboratory test (such as FBG, HbA1C, 2hPG in OGTT) must be done on another day. The diagnosis of diabetes was confirmed when two separate tests were performed and the results were shown to be over the diagnostic limits (Imam, 2012). To avoid rapid metabolic deterioration and complications when the symptoms of hyperglycemia present, a confirmatory test is not required before the initiation of the treatment (Sacks, 2011).

1.4. Management of type 2 diabetes mellitus

1.4.1. Non-pharmacological therapy

The first and most important component of treating DM is educating patients about nutrition, physical activity, psychological and social support, and diabetic medications to treat hyperglycemia (Raveendran, 2018).

Diabetes self-management education and support (DSMES) is an approach that will assist patients with diabetes in learning more about how to take care of themselves. It also involves psychosocial and emotional support (Powers et al., 2017). Education topics that are important for optimal diabetes self-care include self-monitoring of blood glucose, insulin administration, guidelines for diabetes treatment during illnesses, prevention and management of hypoglycemia, skin and foot care, diabetes management before, during, and after exercise, and risk factor–modifying activities (Powers et al., 2020).

Medical nutrition therapy (MNT), on the other hand, is a term used by the American diabetes association (ADA) to define the adjusted coordination of caloric consumption with other aspects of diabetes treatment (insulin, exercise, and weight loss) (Barrea et al., 2023).

Physical activity is another essential non-pharmacological therapy for diabetes. Exercise is important for diabetic patients with many benefits including a reduction in cardiovascular risk, reduced hypertension, maintaining muscle mass, reduced body fat, and weight reduction. Lowering plasma glucose and enhancing insulin sensitivity are additional benefits of exercise. A minimum of 150 minutes of moderate aerobic physical exercise per week, spread out over three days, with no more than a two-day break, is recommended for individuals with diabetes by the ADA (Yang et al., 2019).

Psychosocial support for diabetic patients is an important part of their management. Anxiety, depression, or diabetes distress, should be identified and may need the care of a mental health specialist. Emotional disorders could result in a variation in behavior so that individuals may become less adherent to their medication regimen, diet, and exercise (Stoop et al., 2019).

1.4.2. Pharmacological therapy

The aims of pharmacological therapy for diabetic patients in most cases are to treat and prevent symptoms of hyperglycemia and to reduce the risks of microvascular, cardiovascular (CV), and other complications. Pharmacologic strategies available for the management of T2-DM involve oral glucose-lowering medications, insulin, and other agents that enhance glycemic control (Thrasher, 2017).

1.4.2.1. Glucose lowering agents

Improvement in the therapy of T2-DM led to the generation of oral glucose-lowering agents with various modes of action that act on different processes involved in the pathogenesis of T2-DM as shown in figure 1.2. According to their mechanisms of action, glucose-lowering medications are subdivided into agents that enhance insulin secretion, reduce glucose production, enhance glucagon-like peptide-1 (GLP-1) action, improve insulin sensitivity, or induce glucose excretion in urine. Insulin is sometimes the first-choice agent in the management of T2-DM (C. Bailey, 2015). Biguanides and sulfonylureas were the only two classes of oral glucose-lowering medicines available for almost 40 years. However, several new treatment alternatives have been introduced in the last 20 years, which involved different classes with different mechanisms of action (Preiser et al., 2020).

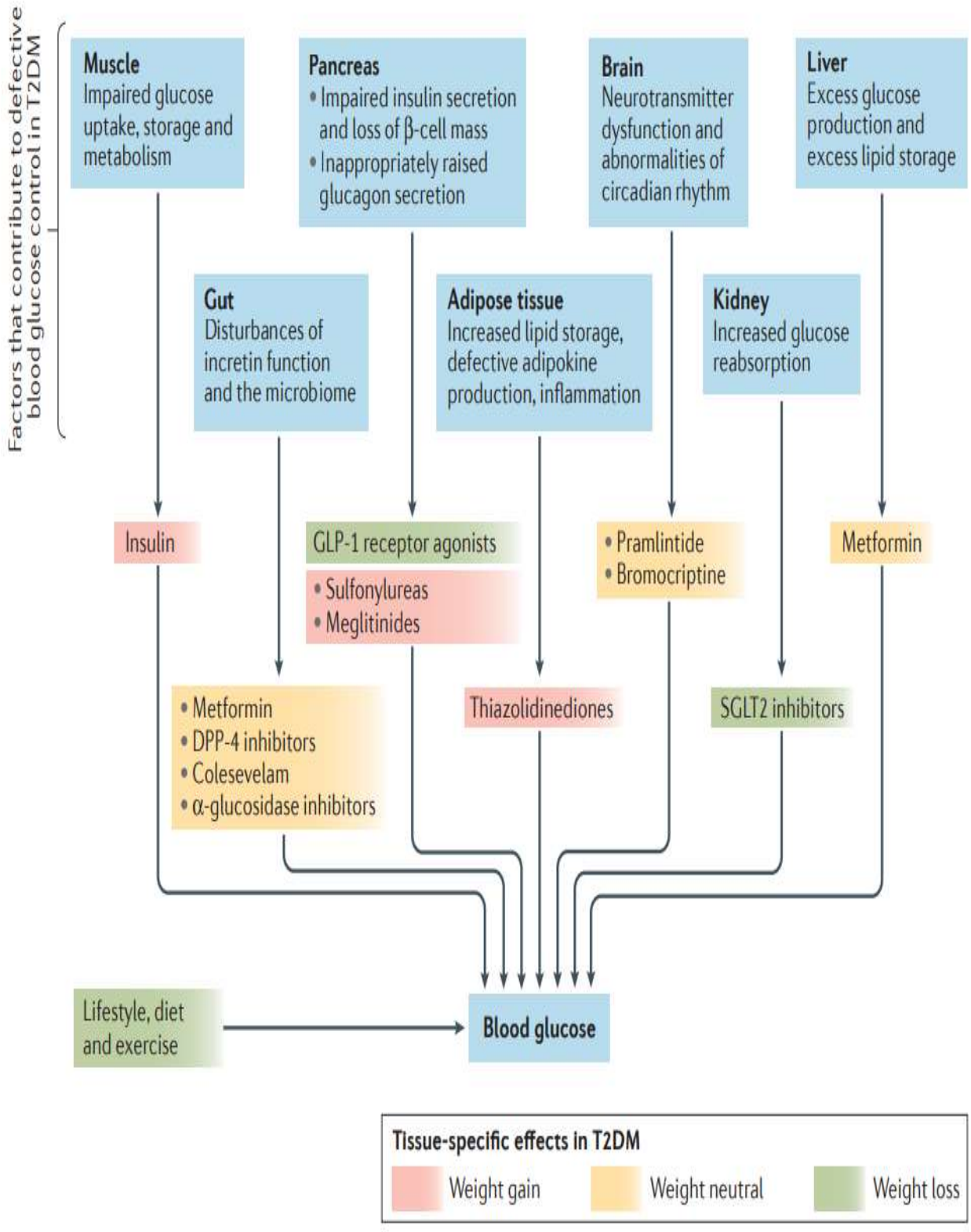

Figure 1.2: Sites of action of glucose-lowering drugs.

DDP-4: dipeptidyl peptidase IV inhibitors; GLP-1: glucagon like peptide-1; SGLT2: sodium-glucose cotransporter 2 (C. Bailey, 2015).

1.4.2.1.1. Biguanide

Metformin (dimethylbiguanide) is the only kind of biguanide that is available in clinical practice. The risk of lactic acidosis has led to the withdrawal of other biguanides, including buformin and phenformin. The most widely prescribed drug for T2-DM globally is metformin, which was first used in clinical practice in Europe in 1957 and in the United States in 1995 (C. J. Bailey, 2017). Metformin has become recognized as the primary treatment option for T2-DM, unless contraindicated or not tolerated, because of its efficacy, safety profile, and affordable price (Baker et al., 2021). This class of drugs acts by reducing hepatic glucose production and improves peripheral insulin sensitivity slightly. It enters cells through organic cation transporters and stimulates adenosine monophosphate (AMP)-dependent protein kinase. Mutations in these transporters may influence metformin responsiveness (Di Magno et al., 2022). Administering these medications lowers FBG and insulin levels, enhances lipid profiles, and promotes weight loss. There is an extended-release form of metformin that may alleviate some of the gastrointestinal adverse effects, such as nausea, vomiting, diarrhea, and metallic taste (Taylor et al., 2021).

Reduction in the micro- and macrovascular complications is accomplished with long-term use of metformin. The major adverse effects of metformin are lactic acidosis and vitamin B12 deficiency (Dyatlova et al., 2024). Metformin should not be given to people who have moderate renal impairment (estimated glomerular filtration rate [eGFR] is more than 30 mL/min), any type of acidosis, unstable congestive heart failure (CHF), severe hypoxemia, or liver disorder. Also, metformin is contraindicated for people who are in the hospital, who are unable to take medicine by mouth, or who are getting radiographic contrast agents (James G Boyle, Gerard A McKay, 2010).

1.4.2.1.2. Insulin secretagogues-agents that affect the adenosine triphosphate-sensitive potassium channel

This class of glucose-lowering agents is represented by sulfonylureas. They act by stimulating insulin secretion by interacting with the potassium channel (Adenosine triphosphate [ATP]-sensitive potassium channel) on the beta cells. These drugs have more efficacy in patients with relatively newly diagnosed T2-DM who have residual endogenous insulin secretion (Sola et al., 2015).

First-generation sulfonylureas: tolbutamide, chlorpropamide, and tolazamide have a longer half-life, higher risk of hypoglycemia, and more drug interaction so that they have not still utilized recently (Sola et al., 2015). Second-generation sulfonylureas such as (glibenclamide [glyburide], gliclazide, glimepiride, and glipizide) have a faster onset of action with better controlling on the postprandial glucose elevation, but the shorter half-life of some agents may require multiple daily dosing (Tirmizi, 2015). Long-term use of sulfonylureas is correlated with a reduction in the micro- and macrovascular complications. Particularly for older individuals, glimepiride and glipizide, which can be administered once daily, are preferred over glyburide (Kalra et al., 2015).

Nateglinide, repaglinide, and mitiglinide are not considered sulfonylureas but they also act through interacting with the ATP-sensitive potassium channel in the beta cells. These agents are given instantly before each meal because of their short duration of action to decrease glucose elevation after meals (Lv et al., 2020). Hypoglycemia may occur with insulin secretagogue therapy, especially in those with a longer duration of action, with greater incidence in elderly patients. The occurrence of hypoglycemia includes several causes such as delayed meals, alcohol consumption, prolonged physical

activity, or impaired renal function (Tourkmani et al., 2018). The metabolism of most sulfonylureas occurs in the liver to compounds (some of them are active, such as those of glibenclamide) that are cleared from the body by the kidney. So, their administration in patients with severe impairment in the function of the liver or kidney is not recommended (Thulé & Umpierrez, 2014). For patients with chronic kidney disease (CKD) requiring these agents, shorter half-life drugs, like glimepiride, glipizide, or repaglinide can be used with caution. An increase in weight is a common side effect of sulfonylurea treatment. Some sulfonylureas are highly interacting with alcohol and drugs such as warfarin, aspirin, ketoconazole, fluconazole, and α-glucosidase inhibitors (Gunaratne et al., 2018; Nam et al., 2019).

In the brain and the heart, there are similar kinds of ATP-sensitive potassium channels. All of these agents except glibenclamide have a low affinity for these channels. Although there are concerns that glibenclamide might affect heart function, studies have not demonstrated an increase in mortality rate from heart disease in patients treated with this drug or other drugs in this group (Scheen, 2021).

1.4.2.1.3. Insulin secretagogues-agents that enhance glucagon-like peptide-1 receptor signaling

Medications related to this group increase insulin production in response to glucose by enhancing the activity of incretins. It is divided into either agents that act as agonists for GLP-1 receptors or enhancers for the activity of endogenous GLP-1. Because insulin secretion is glucose-dependent, agents in this class do not cause hypoglycemia (unless other medicines that can cause hypoglycemia, like sulfonylureas, are used at the same time) (Meloni et al., 2013).

An increase in insulin production in response to glucose, a decrease in glucagon, and a slowing of the gastric emptying rate are the mechanisms by which GLP-1 receptor agonists work. In addition, these drugs decrease appetite and cause moderate weight reduction in the majority of patients (Nauck et al., 2021). Based on their duration of action, these drugs can be divided into short and long-acting agents. Short-acting GLP-1 receptor agonists include exenatide and liraglutide. Long-acting GLP-1 receptor agonists involve sustained-release exenatide, dulaglutide, and semaglutide (Hinnen, 2017).

GLP-1 receptor agonists can be used as concomitant therapy with other oral glucose-lowering agents such as metformin, sulfonylureas, and thiazolidinediones. The main side effects include nausea, vomiting, and diarrhea. Some formulations of these agents carry the Food and Drug Administration (FDA)-black box warning because of the increased risk of thyroid C-cell tumors in rodents and should not be used in patients with medullary carcinoma of the thyroid or multiple endocrine neoplasia. GLP-1 receptor agonists could affect the absorption of other drugs as they cause a slowing in the gastric emptying rate (Rajput et al., 2022).

Dipeptidyl peptidase IV (DPP-IV) inhibitors including sitagliptin, vildagliptin, and linagliptin act by inhibiting the degradation of endogenous GLP-1 and so improve the action of incretin. Patients may take DPP-IV inhibitors alone, as part of a dual or triple therapy, or even in conjunction with insulin. Reduced doses should be considered for patients with impaired renal function. Adverse effects related to DDP-IV inhibitors include rash, hypersensitivity reactions (involving anaphylaxis, angioedema, and Stevens-Johnson syndrome), and severe joint pain (Richter et al., 2008). Because there is evidence that GLP-1 receptor agonists and, to a lesser extent, DPP-IV inhibitors greatly raise the risk of acute pancreatitis, these drugs should not be given to people who already have pancreatic disease or who have other risk

factors for acute pancreatitis, like heavy alcohol use or very high serum triglycerides (Shihab, 2015).

1.4.2.1.4. Thiazolidinediones

Drugs involved in this class include pioglitazone, rosiglitazone, and troglitazone. Troglitazone is the prototype and it was presented in 1997 and then discontinued soon after because of its toxic effect on the liver. Rosiglitazone and pioglitazone were first made available in 1999. Rosiglitazone was withdrawn from the market in Europe in 2010, and its use was limited in the USA after reports of an association with an increased chance of heart disease. Because of a possible risk of bladder cancer, pioglitazone was withdrawn from the market in some European countries in 2011 (Consoli & Formoso, 2013).

Thiazolidinediones act by stimulating a nuclear receptor known as peroxisome proliferator-activated receptor gamma (PPAR-γ). This receptor presents in muscle, beta cells, liver, macrophages, and the vascular endothelium and to a lesser extent fat tissue. The stimulation of PPAR-γ regulates gene expression, leading to enhancement in fat storage, insulin sensitivity, and tissue glucose uptake. Additionally, it alters energy balance and decreases inflammation (Arnold et al., 2019). There is a little rise in triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) when using rosiglitazone. While pioglitazone reduces triglycerides, it elevates HDL to a greater degree and LDL to a lesser degree. It may be difficult to assess the clinical significance of the lipid alterations caused by these drugs (Madan, 2005).

Thiazolidinediones are contraindicated in patients with liver insufficiency or CHF (class III or IV). A worsening of diabetic macular edema rarely occurs in some patients according to the FDA warning. Postmenopausal

women who use these medications are at a higher risk of fractures. Premenopausal women with polycystic ovarian syndrome had ovulation-promoting effects of thiazolidinediones. However, the safety of thiazolidinediones during pregnancy has not yet been proven and thus women should be cautious when using these medications (Lebovitz, 2019).

1.4.2.1.5. Sodium-glucose co-transporter 2 inhibitors

Dapagliflozin, canagliflozin, and empagliflozin are medications that belong to this class. They are administered alone when lifestyle changes do not bring about the desired results or when metformin cannot be tolerated. In addition to insulin, various glucose-lowering medications can be utilized with sodium-glucose co-transporter 2 (SGLT2) inhibitors (Hsia et al., 2017). SGLTs are active membrane transporters that move sodium down its concentration gradient, generally into the cell, in association with moving certain hexose sugars or other molecules against their concentration gradient. The primary SGLTs are SGLT1 and SGLT2, glucose absorption in the gut is mostly controlled by SGLT1, while the renal reabsorption of most filtered glucose is mainly accomplished by SGLT2 (Pinto et al., 2022).

The reabsorption of a high concentration of filtered glucose entering the tubules is facilitated by SGLT2, a high-capacity glucose transporter that is found in segment 1 of the proximal tubules. While, reabsorption of a low concentration of filtered glucose is facilitated by SGLT1, a low-capacity glucose transporter that is found in segment 3 of the proximal tubules as shown in figure 1.3 (Perry & Shulman, 2020). The drugs cause an increase in glucose excretion in the urine by competitively blocking SGLT2, which in turn prevents glucose reabsorption and lowers the renal threshold for glucose. Therefore, insulin is not required for the glucose-lowering effect, and variations in insulin secretion or sensitivity do not affect its action (Ni et al., 2020). Inhibitors of

SGLT2 cause glycosuria and sodium loss in the urine, which in turn cause mild diuresis and calorie loss, which lead to a minor reduction in blood pressure (3-6 mmHg in systolic blood pressure) and weight loss (Wilding et al., 2018).

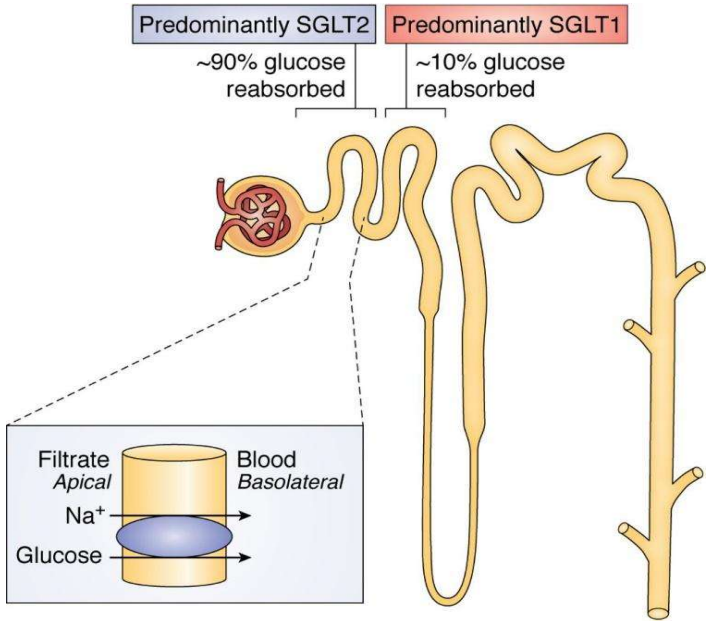

Figure 1.3: The location of sodium-glucose cotransporter 1 and sodium-glucose cotransporter 2 in the nephron

SGLT: Sodium-glucose cotransporter (Perry & Shulman, 2020).

Due to the increased glucose excretion in the urine, mycotic infections in the urinary tract and genitourinary tract commonly occur in both men and women, and the diuretic effect can lead to a reduction in the intravascular volume and acutely compromised renal function (Hazique et al., 2023). Inhibition of SGLT2 causes an increase in glucagon levels and accordingly the liver synthesis of glucose and ketones. Euglycemic DKA may occur during illness or when ongoing glucosuria masks stress-induced requirements for insulin. Therefore, individuals with T1-DM or a pancreatogenic form of DM associated with insulin insufficiency should not use these medications. Also, there is a chance that dapagliflozin could raise the risk of bladder cancer (Tentolouris et al., 2019).

These drugs reduce cardiovascular disease and all-cause cardiovascular mortality in patients with T2-DM who have established cardiovascular disease. The greatest potential benefit of SGLT2 inhibitors in terms of morbidity and mortality benefits could be reduced heart failure and cardiovascular death (Tuttle et al., 2021). Several concepts have evolved to explain the positive effect of SGLT2 inhibitors on cardiovascular outcomes as shown in figure 1.4. The reduced cardiovascular mortality may be attributed, in part, to the improved glycemic control that is seen in individuals on SGLT2 inhibitors (Perry & Shulman, 2020).

However, SGLT2 inhibitors alter glucose consumption in the body, promoting fat utilization and increasing ketone metabolism in the heart. This may provide cardiovascular benefits by modulating the energy metabolism of the heart (Yokono et al., 2014). Additionally, SGLT2 inhibitors have been demonstrated to reduce the activity of the NLR family, pyrin domain-containing 3 (NLRP3) inflammasome, and this anti-inflammatory effect may be attributable to increased ketones that result in improvements in the cardiac function (Butts et al., 2015). Furthermore, clinical evidence suggests that reductions in blood pressure can partially—but not entirely—explain improved cardiovascular outcomes in people using an SGLT2 inhibitor (Shen et al., 2020).

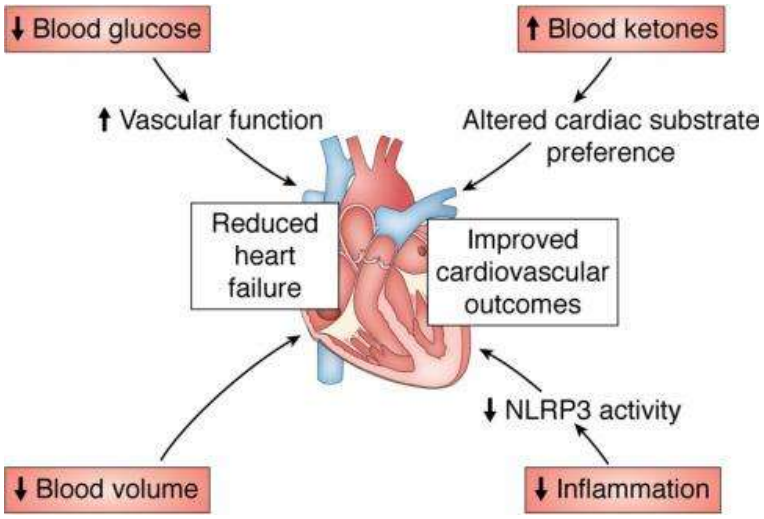

Figure 1.4: Proposed pathways for sodium-glucose cotransporter 2 inhibitors to minimize heart failure and enhance cardiovascular outcomes.

NLRP3: NLR pyrin domain-containing 3 inflammasome (Perry & Shulman, 2020).

SGLT2 inhibitors have been demonstrated to have renoprotection effects in diabetic patients via multiple mechanisms involving enhancement in glycemic control, improvement in tubuloglomerular feedback, lowering intraglomerular hypertension, and reducing proteinuria as shown in figure 1.5 (Heerspink et al., 2018). Although SGLT2 inhibitors demonstrate efficacy in slowing the progression of diabetic kidney disease, they should not be initiated in patients with eGFR less than 60 mL/min/ 1.73 m^2. Nonetheless, they can be continued in patients with eGFR as low as 45 mL/min/1.73 m^2. None of these agents should be used in patients with eGFR less than 30 mL/min/1.73 m^2 (Garcia-Ropero et al., 2018).

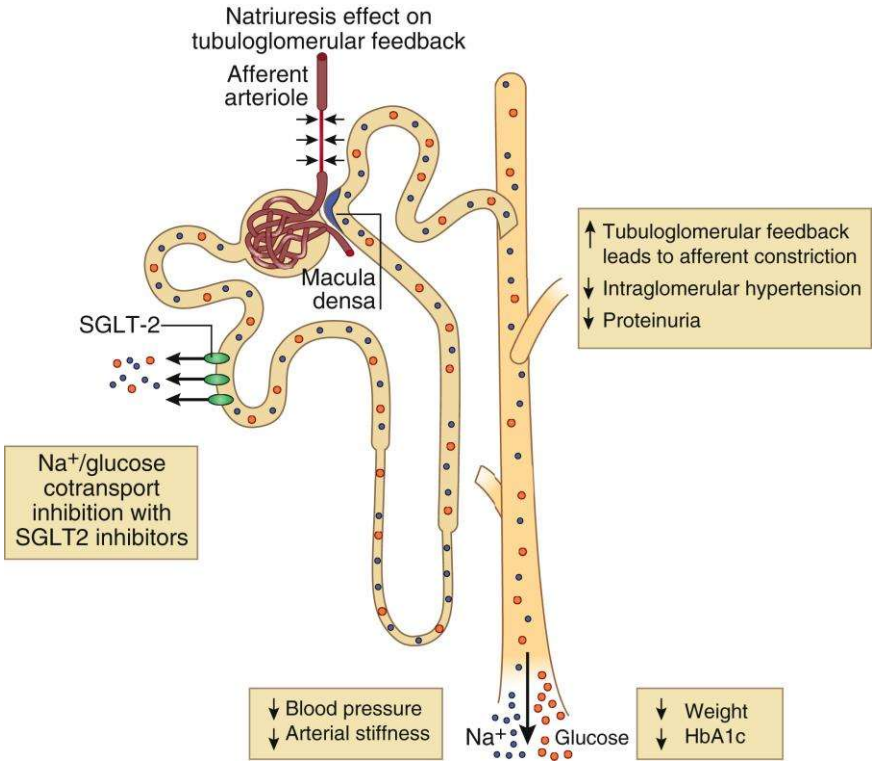

Figure 1.5: Renoprotection effects of sodium-glucose cotransporter 2 inhibitors.

HbA1c: hemoglobin A1c (Heerspink et al., 2018).

1.4.2.1.6. α-glucosidase inhibitors

In the early 1990s, acarbose was the first α-glucosidase inhibitor (AGI) to be introduced. Later, voglibose and miglitol were made available in some countries. AGIs inhibit α-glucosidase enzymes competitively on the brush border of enterocytes that line the intestinal villi. This prevents the enzymes from breaking down disaccharides and oligosaccharides into monosaccharides. AGIs have different binding strengths for various α-glucosidase enzymes, which leads to unique activity profiles. For example, acarbose binds to glycoamylase better than other glucosidases, while miglitol is a stronger inhibitor of sucrase (Z. Liu & Ma, 2017).

AGIs reduce post-prandial hyperglycemia by decreasing glucose absorption, and they do not affect glucose utilization or insulin secretion. The

major side effects of these agents include flatulence, abdominal distention, and diarrhea which may lead to treatment discontinuation (Dirir et al., 2022). These side effects are related to the enhanced carriage of oligosaccharides to the large intestine. Hypoglycemia is not common. AGIs do not cause an increase in weight, and they do not exhibit clinically significant drug interactions (Dirir et al., 2022; Hedrington & Davis, 2019).

1.4.2.2. Insulin therapy in type 2 diabetes mellitus

The commencement of insulin treatment is suggested when intensive hyperglycemia (more than 9% HbA1c) is seen at diagnosis, especially if hyperglycemic symptoms (polyuria or polydipsia) or any catabolic signs (weight loss or ketosis) are observed (B.-W. Lee et al., 2017). Additionally, patients with compromised hepatic or renal functioning should be considered for insulin when adequate glycemic control is not obtained or in individuals who have experienced myocardial infarction (MI), stroke, or underwent major surgery. However, it should be considered for patients with T2-DM who cannot control glucose levels despite the use of appropriate oral hypoglycemic agents (Wallia & Molitch, 2014).

Generally, insulin alone is not perfect management for poorly controlled T2-DM, as it is conjugated with both an increase in weight and insufficient blood glucose control. Deferent combinations of insulin with oral hypoglycemic agents now exist and are broadly utilized (Pagkalos, 2011).

1.5. Complications of type 2 diabetes mellitus

The majority of the mortality and morbidity associated with diabetes is caused by its complications, which impact numerous organ systems. There are two main types of complications: vascular and nonvascular. Vascular complications were either microvascular or macrovascular. Retinopathy,

neuropathy, and nephropathy are examples of microvascular complications, while coronary heart disease (CHD), peripheral artery disease (PAD), and cerebrovascular disease are examples of macrovascular complications (Rask-Madsen & King, 2013). Microvascular complications are diabetes-specific and usually do not occur until the second decade of hyperglycemia, while additional pathophysiologic features shared with the entire population (such as dyslipidemia and hypertension) are associated with macrovascular complications and may develop before hyperglycemia is established. Nonvascular complications include infections, skin changes, loss of hearing, gastrointestinal abnormalities, genitourinary abnormalities, increased risk of dementia, and impaired cognitive function as shown in table 1.1 (Okuducu Teran, 2021). Since T2-DM is frequently asymptomatic for a long time before diagnosis, many people with the disease may already have complications related to hyperglycemia and insulin resistance when they are diagnosed (L. Huang et al., 2022).

Table 1.1: Complications of type 2 diabetes mellitus

<table>
<tr><th colspan="3">Complications of type 2 diabetes mellitus</th></tr>
<tr><th colspan="2">Vascular</th><th rowspan="2">Nonvascular</th></tr>
<tr><th>Microvascular</th><th>Macrovascular</th></tr>
<tr><td rowspan="2">Retinopathy</td><td rowspan="2">Coronary heart disease</td><td>Gastrointestinal</td></tr>
<tr><td>Genitourinary</td></tr>
<tr><td rowspan="3">Neuropathy</td><td rowspan="3">Peripheral arterial disease</td><td>Dermatology</td></tr>
<tr><td>Infections</td></tr>
<tr><td rowspan="2">Dementia and impaired cognitive function</td></tr>
<tr><td rowspan="2">Nephropathy</td><td rowspan="2">Cerebrovascular disease</td></tr>
<tr><td>Hearing loss</td></tr>
</table>

1.5.1. Mechanisms of Complications

The main etiologic factor contributing to DM complications is chronic hyperglycemia, but it is unclear what mechanism(s) exactly causes such diverse cellular and organ damage. The complications are likely multifactorial with an emerging hypothesis that hyperglycemia results in epigenetic alterations that affect the expression of genes in influenced cells (Stefano et al., 2016).

Furthermore, chronic hyperglycemia leads to the creation of advanced glycation end products (AGEs), which join to specific cell surface receptors. On the other hand, the nonenzymatic glycation of intra- and extracellular proteins leads to cross-linking of proteins. These changes result in glomerular and endothelial dysfunction, altered extracellular matrix composition, and hastened atherosclerosis (V. P. Singh et al., 2014). Growth factors may play an important role in some diabetes-related microvascular complications; for example, vascular endothelial growth factor A (VEGF-A) is raised locally in diabetic proliferative retinopathy (Gupta et al., 2013). Moreover, hyperglycemia may have a unifying mechanism in that it increases the production of superoxide anions or reactive oxygen species (ROS) in the mitochondria, which may activate several pathways (González et al., 2023).

The mechanisms of macrovascular complications (including MI and stroke) that occur in DM are glucose-related mechanisms but also involve insulin resistance, and traditional cardiovascular risk factors (dyslipidemia, hypertension) (Zakir et al., 2023). Furthermore, insulin cannot effectively prevent adipose tissue lipolysis, which increases the amount of fatty acids delivered to the liver, muscles, endothelial cells, and cardiac tissues. This causes an accumulation of triglycerides, diacylglycerol, and ceramides in the tissues (Ormazabal et al., 2018).

1.5.2. Ophthalmological Complications in type 2 diabetes mellitus

Progression of diabetic retinopathy, which leads to significant macular edema and the development of new blood vessels, is the primary cause of visual loss. There are two stages of diabetic retinopathy proliferative and non-proliferative. Retinal vascular microaneurysms, blot hemorrhages, and cotton-wool spots are hallmarks of non-proliferative diabetic retinopathy (NPDR), which often manifests later in the first decade or early in the second decade of hyperglycemia. The pathophysiologic process involved in NPDR includes loss of retinal pericytes, increased retinal vascular permeability, alterations in retinal blood flow, and abnormal retinal microvasculature, all of which can lead to retinal ischemia (Nentwich, 2015; W. Wang & Lo, 2018)

The hallmark of proliferative diabetic retinopathy (PDR) is the appearance of neovascularization in response to retinal hypoxemia. These recently developed blood vessels manifest close to the macula and optic nerve, they ruptured easily, causing vitreous hemorrhage, fibrosis, and ultimately retinal detachment. However, although not all patients with NPDR go on to develop PDR, their chance of progressing to PDR within 5 years increases with the severity of the non-proliferative disease (Lechner et al., 2017).

1.5.3. Neurological complications in type 2 diabetes mellitus

Diabetic neuropathy occurring in nearly 50% of individuals with long-standing T2-DM expresses as a diffuse neuropathy (distal symmetrical polyneuropathy (DSPN) and/or autonomic neuropathy), a mononeuropathy, and/or a radiculopathy/polyradiculopathy. Progression of neuropathy, like other DM complications, is correlated with the duration of diabetes and glycemic management. Smoking, cardiovascular disease, increased triglycerides, hypertension, and body mass index (BMI) (the greater the BMI, the greater the risk of neuropathy) are additional risk factors (Tesfaye

et al., 2010). Nerve fibers, both myelinated and unmyelinated, are lost. Diabetic neuropathy should only be diagnosed after ruling out other potential causes, as its clinical manifestations are similar to those of other neuropathies (Chang & Yang, 2023).

DSPN is the most prevalent form of diabetic neuropathy. It usually manifests as pain and loss of distal sensory function, but up to half of those who have it do not experience any symptoms (Kasznicki, 2014). Symptoms may include a sensation of numbness, tingling, burning, or sharpness that begins in the feet and spreads proximally. Pain which typically involves the lower extremities is frequently present at rest and worsens at night. Both an acute (lasting less than 12 months) and chronic form of painful diabetic neuropathy can occur. Due to anomalies in both large and small nerve fibers, DSPN greatly increases the risk of foot ulcers, falls, and the need for lower limb amputations (C. Lin et al., 2020).

Individuals with long-standing T2-DM may develop signs of autonomic dysfunction involving the parasympathetic (cholinergic) and sympathetic (adrenergic) systems (Lamotte & Sandroni, 2022). DM-related autonomic neuropathy can disturb multiple organ systems, including the cardiovascular (decreased heart rate variability, resting tachycardia, and orthostatic hypotension), gastrointestinal (delayed gastric emptying [gastroparesis] and altered small- and large-bowel motility) genitourinary (cystopathy and sexual dysfunction), sudomotor, and metabolic systems (Sharma et al., 2020). Additionally, Sympathetic nerve system dysfunction causes hyperhidrosis in the upper limbs and anhidrosis in the lower limbs. A greater chance of foot ulcers can result from anhidrosis, which causes the skin on the feet to become more dry and cracked (Volmer-Thole & Lobmann, 2016). Mononeuropathy (dysfunction of specific cranial or peripheral nerves) is less frequent than polyneuropathy and manifests as pain and motor impairment in the distribution

of a single nerve (Tracy & Dyck, 2008). Diabetic radiculopathy or polyradiculopathy, is a condition characterized by intense pain that affects one or more nerve origins and is potentially associated with motor impairment (Rinaldo et al., 2017).

Lower extremity complications are common in T2-DM and they are also a major cause of morbidity in those patients (Naidoo et al., 2015). The increased prevalence of these disorders in diabetic patients is due to the interaction of several pathogenic factors, including neuropathy, abnormal foot biomechanics, PAD, and inadequate wound repair (Riandini et al., 2021). Peripheral sensory neuropathy hinders the normal protective systems and allows the patient to endure major or recurrent small trauma to the foot, usually without knowing about the wound (Nativel et al., 2018). Anhidrosis and abnormalities in the superficial blood flow of the foot are caused by autonomic neuropathy, which can lead to skin drying up and the development of fissures. Foot alteration (hammer toe, claw toe deformity, prominent metatarsal heads, Charcot's joint) and abnormalities in the muscle mechanics of the foot are the outcomes of motor and sensory neuropathy (Jain & HC, 2020).

PAD and poor wound healing impede recovery of small breaks in the skin, allowing them to expand and become infected. Factors that increase the chance of foot ulcers or amputations include male sex, diabetes for more than ten years, peripheral neuropathy, an abnormal foot structure (such as bony abnormalities, calluses, or thickened nails), PAD, smoking, a history of foot ulcers or amputations, impaired vision, inadequate glucose control, and diabetic nephropathy, especially dialysis (Alex et al., 2010).

1.5.4. Renal complications in type 2 diabetes mellitus

Diabetic nephropathy (DNP) is the common cause of CKD and end-stage renal disease (ESRD) requiring renal replacement therapy (Lim, 2014). CKD

in individuals with DM is associated with a higher risk of cardiovascular disease. Unfortunately, Individuals with DNP usually have diabetic retinopathy. The presence of CKD in the absence of retinopathy in T2-DM should prompt investigation for different reasons for kidney disease (Jeng et al., 2016). Similar to other microvascular complications, chronic hyperglycemia plays an essential role in the pathogenesis of DNP. However, the mechanisms by which chronic hyperglycemia leads to DNP are incompletely understood. Hemodynamic changes in the renal microcirculation (glomerular hyperfiltration or hyperperfusion and elevated glomerular capillary pressure), structural alterations in the glomerulus (increased extracellular matrix, thickening of basement membrane, loss of podocyte and foot processes, mesangial expansion, fibrosis) as shown in figure 1.6, and the effects of solubilized factors (growth factors, endothelin, angiotensin II, AGEs) are all involved in the pathogenesis of DNP (Alicic et al., 2017; Amorim et al., 2019; Vallon & Komers, 2011).

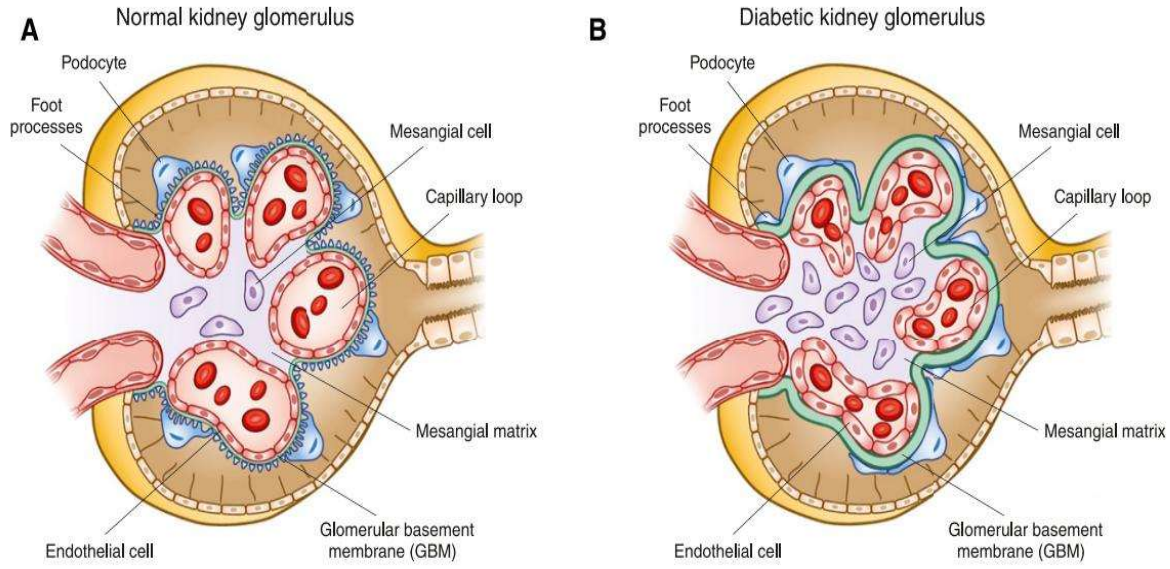

Figure 1.6: Normal kidney morphology and structural alterations in diabetes mellitus.

Diabetic kidney disease results in structural changes, involving thickening of the glomerular basement membrane, fusion of foot processes, loss of podocytes with mesangial matrix expansion (Alicic et al., 2017).

The history of DNP is associated with a sequence of events. The development of glomerular hyperperfusion and renal hypertrophy, marked by an elevation of eGFR, happens in the initial years of DM. Over the first five years of diabetes, the glomerular basement membrane thickens, the glomeruli get hypertrophy, and the mesangial volume expands, while the eGFR returns to a normal range (Bakris, 2011). These events lead to the development of albuminuria which is described by the ADA as a chronically elevated urinary albumin-to-creatinine ratio of more than 30 mg/g on a spot specimen (Christofides & Desai, 2021). The albuminuria can regress with improvement in glycemic control or with improvement in blood pressure control using angiotensin-aldosterone system blockade and/or SGLT-2 inhibitor therapy in some patients with DM and albuminuria of short period. However, when a significant decrease in eGFR and albuminuria develops, the resulting pathologic alterations are typically permanent (Selby & Taal, 2020).

The DNP that develops in T2-DM differs from that of T1-DM in that albuminuria may be present at the time of diagnosis, reflecting its extended asymptomatic period and hypertension more often contributes to albuminuria and reduced eGFR. Finally, it is important to keep in mind that albuminuria in T2-DM can have secondary causes other than DM, including hypertension, congestive heart failure, prostate disease, or infection (Reutens, 2013). On the other hand, DM can lead to type IV renal tubular acidosis, also known as hyporeninemic hypoaldosteronism. Individuals with this condition are more likely to have hyperkalemia and acidemia, and medicines (especially angiotensin-converting enzyme inhibitors [ACEIs], angiotensin receptor blockers [ARBs], and mineralocorticoid receptor antagonists) may make these problems worse (Sousa et al., 2016). Furthermore, Radiocontrast-induced nephrotoxicity can occur in people with DM. Proven nephropathy and volume loss are risk factors for this poisoning. All patients with DM who undergo

radiographic procedures involving contrast dye should drink plenty of water before and after the procedure to prevent dehydration, and their serum creatinine levels should be monitored for at least two days after the procedure (Heyman et al., 2013).

1.5.5. Cardiovascular complications in type 2 diabetes mellitus

Cardiovascular diseases increased in both individuals with T1-DM and T2-DM. Additionally, diabetes worsens the prognosis for CHD and MI compared to non-diabetics. In people with diabetes, CHD is more likely to include several vessels. DM increases the risk of cerebrovascular illness in addition to CHD. Also, CHF is common in long-standing DM. Thus, type 1 and type 2 DM both double the risk of cardiovascular death in men and quadruple it in women after adjusting for all known cardiovascular risk factors (Fan, 2017; Oe et al., 2021; Rosano et al., 2017).

Furthermore, the risk for coronary artery-related events is comparable in T2-DM patients without a history of MI compared to non-diabetic individuals with a history of MI. (Budoff et al., 2016). Diabetes is associated with an increased risk of cardiovascular disease and death due to the interplay between hyperglycemia and other risk factors including dyslipidemia (high triglycerides, cholesterol, and LDL with low HDL), hypertension, sedentary lifestyle, obesity, and smoking (Martín-Timón, 2014).

The mechanisms beyond cardiovascular diseases in diabetic patients involve endothelial dysfunction, heightened platelet activation and thrombosis, and alterations in plaque properties as shown in figure 1.7 (Desai et al., 2015). Diabetic patients without CHD exhibit endothelial dysfunction that correlates with the duration of diabetes. Additionally, diabetes has been demonstrated to enhance platelet activation and increase both primary and secondary platelet aggregation. Furthermore, diabetes is associated with elevated plasma

fibrinogen levels and reduced fibrinolytic activity caused by impaired tissue-type plasminogen activator (tPA) activity (Li, Weber, et al., 2021). Moreover, it has been reported that coronary tissue from diabetic patients exhibits higher levels of lipid-rich atheroma and macrophage infiltration on histologic examination, in comparison to those without diabetes. These features are believed to indicate susceptible plaque that is at a greater risk of rupturing (Kovarnik et al., 2017).

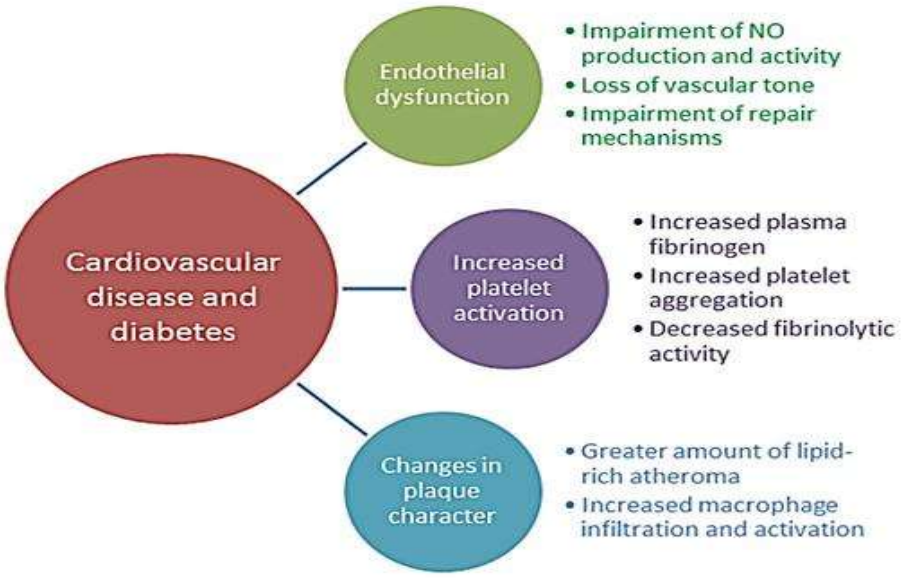

Figure 1.7: Mechanisms of cardiovascular complications in diabetic patients.

(Desai et al., 2015)

Compared to older individuals with long-standing T2-DM, the cardiovascular risk is less in younger individuals with a shorter duration of the disease. In populations where diabetes has not yet been diagnosed, elevated HbA1c predicts not just diabetes risk but also CHD, stroke, and overall mortality (Raghavan et al., 2019). However, patients with diabetes often experience "silent ischemia," or the lack of chest discomfort, and a comprehensive evaluation of the heart is required before any major surgical procedure (Manistamara et al., 2021).

1.5.6. Hematological complications in type 2 diabetes mellitus

Hematologic complications are commonly development in T2-DM. They include abnormalities in the activity, morphology, and metabolism of red blood cells (RBCs), white blood cells (WBCs), and platelets, as well as abnormalities in hemostasis and plasma proteins (Biadgo et al., 2016).

Unlike diabetic complications in other organ systems, which may require years to occur and are irreversible, hematologic abnormalities may be identified early in the course of the disease and usually can be reduced with appropriate metabolic control (Farooqui et al., 2019). Although hematologic abnormalities usually do not present as primary signs of diabetes, they may contribute to more severe issues, such as anemia, infection, and hypercoagulability (Kachekouche et al., 2018). Nonetheless, some hematologic alterations, such as glycosylated Hb, are useful indicators for monitoring blood glucose control. There are several mechanisms involved in hematological complications of DM including protein glycosylation, oxidative damage, ketoacidosis, and other mechanisms (Cho et al., 2008).

1.5.6.1. Mechanism of hematological complications in type 2 diabetes mellitus

1.5.6.1.1. Protein glycation

Hyperglycemia results in the nonenzymatic binding of glucose to cellular proteins, that cause chemical alterations with subsequent modifications in protein structure and/ or function. This process is described as nonenzymatic protein glycation. Cells with insulin-independent glucose uptake (in which intracellular glucose concentrations reflect blood glucose levels) are most affected by hyperglycemia and protein glycosylation. Erythrocytes, platelets, and endothelial cells have insulin-independent glucose uptake, in addition to

cells in neural, ocular, and renal tissue (Y. Wang et al., 2021). Over weeks to months, glucose-modified proteins undergo additional rearrangement processes in addition to complex reactions resulting in the formation of AGEs. These products with early glycation products, are accountable for numerous pathologic effects. However, both glycosylated proteins and AGEs can serve as indicators of long-term glucose control (Shimizu et al., 2019).

1.5.6.1.2. Oxidative damage

Oxidative damage is another crucial mechanism in the progress of diabetic complications. It is caused by the interaction of oxygen radicals (i.e., hydrogen peroxide, hydroxyl radical, and superoxide anion) with cellular proteins, lipids, and nucleic acids. The deleterious effects of oxidation involve protein denaturation, membrane lipid peroxidation, and disruption of cellular synthetic functions (Chaudhary et al., 2023). In diabetes, oxidative damage is caused by both excessive oxygen radical formation and weakened antioxidant defense ability. Furthermore, oxidative stress is accountable for changed iron metabolism, micronutrient abnormalities (e.g., zinc or ascorbate deficiency), and activation of neutrophils and monocytes (Iacobini et al., 2021).

1.5.6.1.3. Ketoacidosis, and other mechanisms

Ketoacidosis can result in severe metabolic abnormalities that can be responsible for hematologic complications. Some alterations are secondary to electrolyte depletion, dehydration, renal failure, infection, and acidosis that happen in ketoacidosis (Dhatariya et al., 2020). Excessive ketones also have direct adverse effects on the immune functions of WBCs and contribute to oxidative damage in RBCs, even though the mechanism of these alterations is not defined. Furthermore, acidemia resulting from excessive ketoacids (13-hydroxybutyrate and acetoacetate) can significantly reduce the oxygen-carrying capacity of Hb (Moraes & Surani, 2019).

Other metabolic abnormalities that happen in diabetes, involving hyperlipidemia, decreased insulin levels, and reduced glycosaminoglycans, also have potential hematologic effects. None of the mechanisms involved in diabetic complications are completely separate from one another, instead, they are highly correlated (Forbes & Cooper, 2013).

1.5.6.2. Types of hematological complications in type 2 diabetes mellitus

1.5.6.2.1. Red blood cell abnormalities in type 2 diabetes mellitus

The RBCs have been studied broadly as a model system in DM for biochemical abnormalities occurring in cell types exhibiting insulin-independent glucose uptake. Diabetic complications that affect RBCs include Hb glycosylation, altered oxygen affinity, decreased deformability, membrane protein and lipid abnormalities, Heinz body formation (Heinz bodies are clumps of precipitated Hb that result from oxidative damage to Hb), volume and morphological changes, aggregation, reduced membrane fluidity, oxidative stress, and metabolic alterations as shown in figure 1.8. Many of these abnormalities result in a reduced RBC lifespan, which may lead to anemia in diabetic patients (Y. Wang et al., 2021).

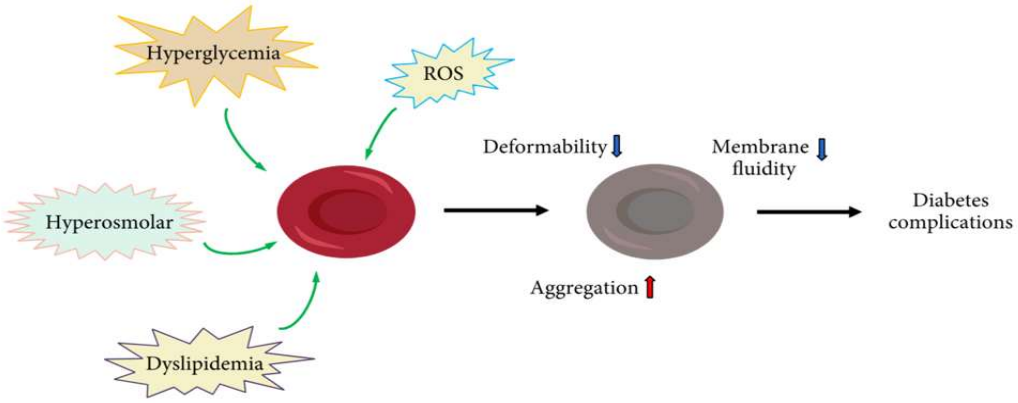

Figure 1.8: Red blood cell abnormalities in type 2 diabetes mellitus.

Erythrocytes in diabetic individuals are at greater risk of aggregation, decreased cell deformability, and impaired membrane fluidity due to numerous factors such as hyperglycemia, hyperosmolarity, oxidative stress, and lipid metabolism disturbance. These alterations in RBC ultimately give rise to microcirculation disorder and diabetic complications. ROS: reactive oxygen species (Y. Wang et al., 2021).

Glycation affects Hb function by meddling in the binding site of 2,3-diphosphoglycerate (2,3-DPG). As a result, glycohemoglobin loses its ability to properly bind and deliver oxygen. Although the definite concentration of 2,3-DPG may be different in diabetes, glycohemoglobin binds oxygen more tightly, reducing oxygen supply to the tissues and resulting in tissue hypoxia. The clinical significance of this phenomenon has not been determined, because diabetic patients can compensate for reduced tissue oxygen tension by enhancing cardiac output and blood perfusion. However, in diabetic patients with microvascular defects, such a compensatory mechanism may not be possible (Castilho et al., 2003).

Deformability is an important RBC function that relies on normal membrane structure and function as well as normal cytosolic fluidity. Because RBCs should pass within capillaries significantly smaller than their diameter, reduced deformability may critically impair rapid and homogeneous perfusion within the microcirculation. Hyperglycemia can reduce RBC deformability by

its effect on membranes (glycation and oxidation), as well as by raising intracellular viscosity through the production of sorbitol via the polyol pathway. Furthermore, less deformability of erythrocytes also shortened their life span. When broken erythrocytes gathered in micro-vessels, they blocked blood flow, which finally caused tissues to lack oxygen (Barshtein et al., 2023; Buys et al., 2013).

Glycation of spectrin, the main structural protein in the membrane, leads to protein crosslinking and conformational alterations, with possible rigidification of the RBC membrane. In addition, the glycation of calcium ATPase, which is another membrane protein, results in the excessive accumulation of intracellular calcium, which can result in echinocytosis, oxidative damage, and hemolysis. Furthermore, there are increases in Membrane-bound Hb in RBCs from diabetics, which further hardens RBC membranes and makes them more susceptible to oxidative damage (Saleh, 2015).

The capacity of RBC to clump together is called aggregation. A decrease in total protein content (especially glycoproteins) of the erythrocyte membrane and an increase in sialidase activity leads to a decrease in sialic acid on the surfaces of erythrocytes in diabetic patients. Consequently, the superficial negative charge of the cells decreases, and erythrocyte clumping increases (Babu, 2021).

The relative lateral fluidity of the lipids and proteins in RBC membranes is referred to as RBC membrane fluidity. For cells to continue functioning normally, biomembrane functions such as signal transduction and cellular metabolism rely on membrane fluidity. The deformability and aggregation of RBCs are worsened by a decrease in their membrane fluidity, which is caused by a rise in nonenzymatic glycosylation, ROS, and lipid peroxidation

(Bianchetti et al., 2021). High blood viscosity and coagulation, caused by increased aggregation and decreased erythrocyte fluidity and deformability due to hyperglycemia, lead to microcirculation disorders and are a major contributor to macrovascular and microvascular complications in diabetes (Cho et al., 2008).

Even though RBCs cannot synthesize or modify fatty acids, there is an exchange of fatty acids between the plasma and the membrane that can result in the alteration of RBC membrane fatty acid composition in individuals with hyperlipidemia (Maturu & Varadacharyulu, 2012). In diabetes, enhanced lipolysis leads to the mobilization of adipose tissue, which contains high levels of saturated fatty acids and low levels of polyunsaturated fatty acids. RBC membrane fatty acid profiles changed consequently. With reduced polyunsaturated fatty acids, the RBC membrane becomes less fluid and deformable (Dilworth et al., 2021).

The average volume of an erythrocyte is called mean corpuscular volume (MCV), and the coefficient of variation of MCV is called RBC volume distribution width (RDW). Higher RDW numbers mean that MCV is less consistent, which is usually due to changes in erythrocyte maturity or breakdown. MCV and RDW are both positively related to the occurrence of diabetic complications and are reported to be a potential risk factor for diabetic-related complications therefore; they can be used as a predictor of these complications (Blaslov et al., 2019).

The capacity of erythrocytes to survive and transport oxygen depends on their normal morphology (H. Wang et al., 2020). Using a light microscope, the shape of erythrocytes from healthy people to people with T2-DM was compared. "Bowl-shaped" erythrocytes, which are thought to be the most deformable cells, make up 55% of the cells in healthy people. They were

followed by discocytes, which are thought to be more rigid cells, at 44%. In diabetes individuals without vascular problems, there is no significant difference as compared to the healthy control group. On the other hand, patients with vasculopathy had a statistically significant decrease in bowl-shaped cells and an increase in discocytes (60%) (MORTAŞ et al., 2021).

An imbalance between the antioxidants of the body and oxidative activities, with an oxidative tendency, is referred to as oxidative stress ((Pizzino et al., 2017). Autoxidation of glucose occurs under hyperglycemic conditions and is thought to be the primary mechanism for erythrocyte free radical generation. Hyperglycemia also reduces antioxidant capacity by lowering antioxidant levels in tissues, such as vitamin E, catalase, glutathione (GSH), and superoxide dismutase (SOD) (Papachristoforou et al., 2020).

Diabetes is combined with an alteration in cellular metabolism (Jiang *etal.*, 2020). Based on the concentration gradient in erythrocytes, glucose transporter 1 promotes insulin-independent glucose transmembrane transfer. In the RBC of T2-DM patients, there are changes in the rate of glucose uptake, enzyme activity, and the synthesis and use of intermediate metabolites and ATP (Y. Wang et al., 2021).

1.5.6.2.2. Anemia and type 2 diabetes mellitus

One of the most prevalent blood diseases among people with T2-DM is anemia. Numerous investigations have shown that individuals with diabetes who also have renal insufficiency usually develop anemia (Taderegew et al., 2020). Some additional research has also shown that diabetics can experience anemia before any signs of kidney damage become apparent. However, Patients presenting with DNP are more likely to experience anemia and a more severe form of the condition compared to patients presenting with other causes of renal failure (Forte et al., 2011). Several mechanisms are suggested to cause anemia

in diabetic patients including nephropathy and reduced erythropoiesis, iron deficiency, inflammation state, and low testosterone level as shown in figure 1.9 (D. K. Singh et al., 2009).

Sympathetic denervation of the kidneys resulting from autonomic neuropathy and the renal microvascular complication that occurs in T2-DM will lead to reduced erythropoietin (EPO) production. In DNP, anemia due to an EPO deficit might manifest before the appearance of severe renal failure (Tsai & Tarng, 2019). On the other hand, another study found that 70% of anemic patients with normal renal function also had low EPO levels, indicating that functional EPO insufficiency may not be related to the severity of renal failure (Thomas, 2006). Chronic hyperglycemia and elevated ROS levels may lead to lower stability and accelerated degradation of hypoxia-inducible factor-1α (HIF-1α), which could be another mechanism contributing to diminished EPO levels in diabetic patients (Xiao et al., 2013).

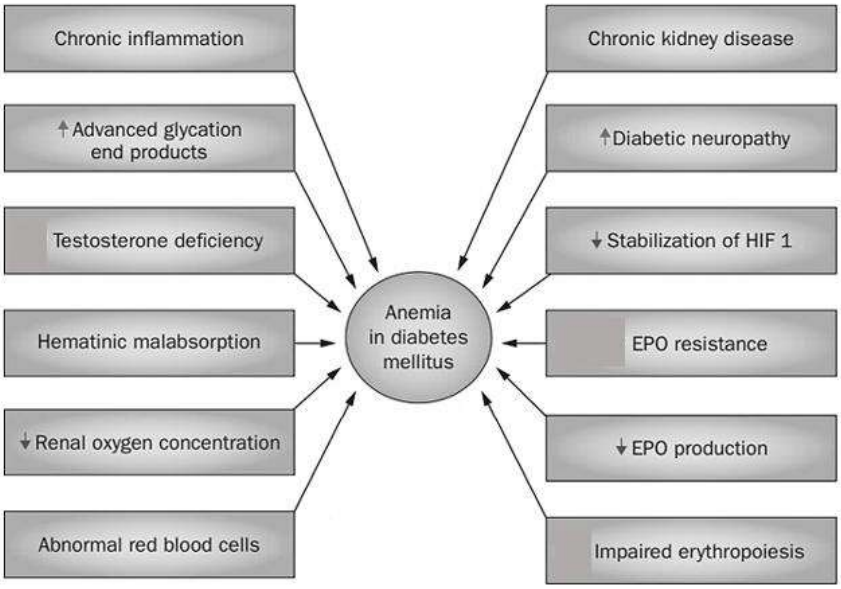

Figure 1.9: Mechanisms of anemia in type 2 diabetes mellitus.

HIF 1: hypoxia-inducible factor-1, EPO: erythropoietin; ↑: increase: ↓: decrease (D. K. Singh et al., 2009).

Decreased erythropoiesis in T2-DM may be potentially caused by a diminished bone marrow response to EPO stimulation as a result of microvascular damage, which may impact bone marrow perfusion and abnormal levels of cytokines related to systemic inflammation that occurs in DM (Fadini, 2011). Notably, some research indicates that AGEs accumulation in diabetes mellitus can also impact bone marrow hematopoiesis (B. Wang et al., 2018).

Iron deficiency was shown to be relatively common in patients with ESRD and T2-DM. This finding is regarded as reasonable when considering that glycation caused by persistent hyperglycemia affects transferrin receptors and could lower the binding rates of the receptor to iron, lowering the amount of iron available (Ma et al., 2021). Anemia related to iron insufficiency is more frequent in individuals with T2-DM and chronic kidney disease because of a reduction in the intestinal absorption of iron due to inflammation caused by kidney disease (Soliman et al., 2017).

Chronic inflammation is a characteristic of DM, and thus it is reasonable to be considered a key factor in the development of RBC dysfunction and anemia in diabetic patients (Tsalamandris et al., 2019). The elevation of specific proinflammatory cytokines, such as interleukin-1, tumor-necrosis factor α (TNFα), and interferon-γ, is associated with anemia in numerous chronic medical diseases including DM. Typical hematological abnormalities seen in inflammatory anemia include normochromic and normocytic anemia, decreased erythropoiesis, and a shortened lifespan for RBC (Nemeth & Ganz, 2014).

Low testosterone levels in men have been thought to be a cause or contributor to anemia for a long time (J. H. Lee et al., 2022). RBC production rises when testosterone levels are high, which is probably partly because

testosterone increases the levels of EPO (Bachman et al., 2010). Anemia was prevalent in men with T2-DM and low testosterone levels, according to a cross-sectional study. This suggests that anemia and low testosterone levels have mechanistic overlap in patients with T2-DM (Grossmann et al., 2009).

1.5.6.2.3. White blood cell abnormalities in type 2 diabetes mellitus

Neutrophil, monocyte, and lymphocyte functions may be influenced by metabolic alterations that occur in diabetes. These alterations affect the ability of leukocytes to respond to infectious agents and immune stimuli and may increase the susceptibility of diabetic patients to infection (Thimmappa et al., 2023). Neutrophil chemotaxis, phagocytosis, and intracellular bactericidal activity may also be reduced. Lymphocytes from poorly controlled diabetics show reduced proliferative responses to mitogen stimulation and decreased T- and B-cell surface membrane markers. However, Leukocyte dysfunction is generally reversed by efficacious blood glucose control (Kolluru et al., 2012).

Because infection is a common triggering factor of diabetic ketoacidosis, these alterations in immune function are important in predisposing patients to ketoacidosis (M. V. Lin et al., 2010). Like RBCs and platelets, leukocytes are less deformable in diabetic patients, as tested by measuring their filterability through small pores. Reduced deformability can reduce the ability of leukocytes to migrate through endothelial pores and result in capillary closure, one of the initial pathologic changes leading to microangiopathy. Decreased leukocyte deformability may also result in increased blood viscosity and hypercoagulability (Szablewski & Sulima, 2017).

1.5.6.2.4. Platelet abnormalities in type 2 diabetes mellitus

There are several mechanisms by which diabetes affects the platelets function including changes in platelet aggregability, thromboxane production,

membrane glycation, platelet size, platelet glycoprotein (GP) receptors, platelet secretion products, oxidative stress, and intracellular mechanisms as shown in figure 1.10 (Ferroni et al., 2004). Improvement in platelet aggregation in response to various agonists (such as thrombin, collagen, arachidonic acid, and epinephrine) due to impaired calcium homeostasis, activation of protein kinase C (PKC), decreased production of platelet-derived nitric oxide (NO), and increased formation of superoxide has been shown in patients with T2-DM compared to non-diabetic individuals (Kaur et al., 2018).

Platelets show enhanced thromboxane (Tx) production as evidenced by increased urinary excretion of 11-dehydro-TxB2. This increase in Tx production has been associated with both micro- and macroangiopathy that occur in diabetic patients. Also, Membrane glycation together with alteration in lipid composition results in impaired fluidity of the platelet membrane (Piechota et al., 2022; Santilli et al., 2015). In addition, alterations in platelet size have been reported in patients with T2-DM, predominantly larger platelets are found in those patients, and larger and younger platelets are considered to be more reactive resulting in increased platelet aggregation (Yilmaz & Yilmaz, 2016). Furthermore, platelets of diabetic patients have increased numbers and activity of multiple GP receptors on the platelet membrane. Accordingly, increases in GP IIb/IIa (fibrinogen receptor), GPIb-IX (von Willebrand factor receptor), GPIa/IIa (collagen receptor), and the cluster of differentiation (CD) 62 (P-selectin receptor) have been detected in some diseases including DM. In addition, an increase in the CD40-CD40 ligand system has been demonstrated in DM patients. All these changes are expected causative factors for increased platelet aggregability (Kakouros et al., 2011; Seijkens et al., 2013)

Upon platelet activation, cytokine-like proteins are released from the α-granules including β-thromboglobulin (β-TG) and platelet factor-4. Increased levels of these proteins have been observed in DM patients and this elevation

is found to be associated with diabetic angiopathy such as proliferative retinopathy (Jindal et al., 2011). Functional platelet defects in DM have been associated with several intracellular alterations, including a reduction in Na^{+}/K^{+} ATPase activity and increased Ca^{+2} ATPase activity resulting in increased intracellular Ca2+ concentrations and platelet hyperactivity. Also, reduced intracellular Mg^{+2} in diabetic platelets may enhance platelet activity in addition to decreasing NO production (Takaya et al., 2007). Furthermore, most of the factors of both intrinsic and extrinsic pathways of coagulation have been reported to be altered in DM. Coagulation activation markers such as prothrombin fragments and thrombin-antithrombin complexes have been reported to be increased in DM. Hyperglycemia is associated with thrombin activation (based on increased plasma markers), hyperfibrinogenemia, and increased levels of factors VII, VIII, X, XI, XII, and von Willebrand's factor. Additionally, patients with DM may have lower activities of anticoagulants such as antithrombin III and protein C (Addai-Mensah et al., 2019).

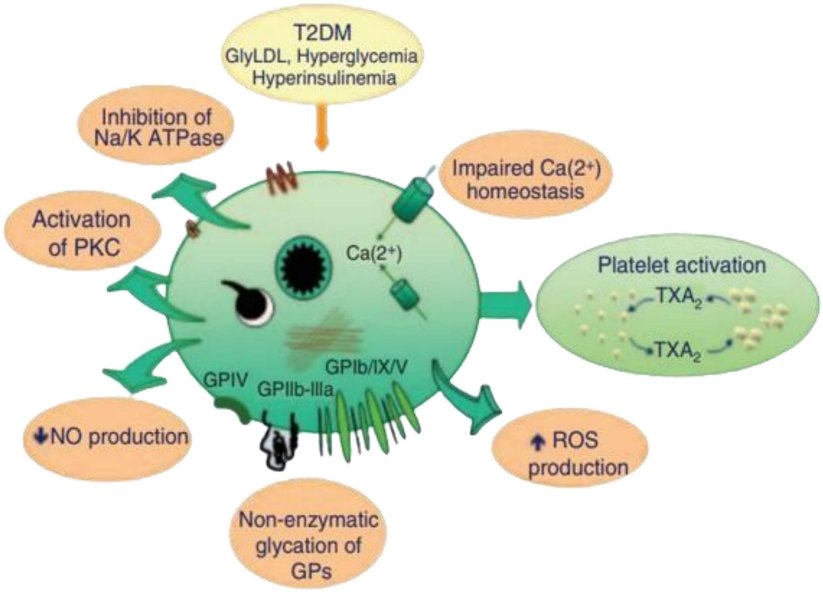

Figure 1.10: Platelet abnormalities in type 2 diabetes mellitus.

As shown, high blood sugar levels, glycated low-density lipoproteins (GlyLDL), and hyperinsulinemia can cause several alterations, including decreased nitric oxide (NO) production, an increase in reactive oxygen species (ROS), inhibition of platelet membrane Na+/K+-adenosine triphosphatase (Na+/K+-ATPase) activity, activation of protein kinase C (PKC), and nonenzymatic glycation of glycoproteins (GPs) on the membrane. All of these changes result in increased platelet aggregation in diabetic patients (Ferroni et al., 2004).

1.6. Aim and objectives of the study

1.6.1. Aim

1. Investigation of the association between dapagliflozin as an add-on therapy to metformin and certain hematological changes in type 2 diabetic patients
2. Comparison of the hematological parameters of diabetic patients using a combination of dapagliflozin and metformin to those using metformin-only therapy.
3. Evaluation of the impact of dapagliflozin as an add-on therapy to metformin on renal function in patients with T2-DM.

1.6.2. Objectives

1. Identifying a suitable sample of diabetic patients who are eligible for inclusion in the study.
2. collecting data on hematological parameters including EPO, RBC count, WBC count, Hb levels, platelet count, and other relevant parameters from the selected patients.
3. Analyzing and comparing the hematological parameters of diabetic patients taking a combination of dapagliflozin and metformin to metformin-only users.
4. Measuring of uric acid, urea, creatinine levels, and calculative creatinine clearance to assess renal function, and compare the result between diabetic patients who take a combination of dapagliflozin and metformin to those using metformin-only therapy.
5. Finding a potential correlation between the obtained results in the studied groups.

6. Drawing conclusions and providing recommendations to health care professionals based on the findings, highlighting the implications for clinical practice and further research.

Chapter two

Subjects Materials and Methods

2. Subjects, Materials, and Methods

The present study is a retrospective cohort study conducted between November 2023 and April 2024 in the private clinic of Dr. Mohammed Harith Al-Saaty, an internal medicine specialist, and a private medical laboratory, in Mosul, Nineveh province. The Collegiate Committee for Medical Research Ethics approved the project of trial under the following code: CCMRE-phA-23-17, on 8/11/2023 (appendix III). The questionnaire (appendix IV) was distributed to the patients with the consent to participate (appendix II) and the clinicians examined the participating patients to rule out any abnormalities.

2.1. Subjects

2.1.1. Controls

Forty-one persons participated in the study as a control group (group 1). They all appeared clinically healthy, had no chronic diseases, and were not receiving any chronic medication. There were 21 males and 20 females, and their mean age was 53.56 ± 9.82 years.

2.1.2. Patients

Two patient groups were enrolled in the present study:

- **T2-DM on metformin therapy (T2-DM+MET group; group 2):** 40 participants were enrolled in this group, and were equally divided between males and females. The participants have been diagnosed with T2-DM with a duration of 5.58 ± 3.41 years and were using metformin (1000 mg twice daily) for at least one year with a mean age of 53.53 ± 8.64 years.

- **T2-DM on metformin and dapagliflozin therapy (T2-DM+MET+DAPA group; group 3)**: 30 participants were enrolled in group 3, and they were 16 males and 14 females. The participants have been diagnosed with T2-DM with a duration of 4.96 ± 2.92 years and were using metformin and dapagliflozin (1000 /5 mg twice daily) for at least one year with a mean age of 56.81 ± 7.54 years.

2.1.3. Inclusion criteria

- Male and female.
- Type 2 diabetic patients on metformin (1000 mg twice daily) therapy for a duration not less than 1 year.
- Type 2 diabetic patients on metformin and dapagliflozin (1000 /5 mg twice daily) therapy with a duration of not less than 1 year.

2.1.4. Exclusion criteria

- Patients with T1-DM.
- T2-DM patients on insulin or any other hypoglycemic drugs.
- Smoking.
- Patients with cardiovascular disease (including MI, coronary artery diseases, PAD, and stroke).
- Patients with renal diseases (including nephropathy).
- Patients with hematological disease.
- Other diabetic complications (including retinopathy and neuropathy).
- Pregnant women and lactating mothers.

2.2. Materials

2.2.1. Blood sampling

Seven milliliters of blood were withdrawn from all participants after fasting state for 8-12 hours in the morning, including patients and control, using disposable syringes and then divided into two parts: 2 ml were transferred into an anticoagulant EDTA tube, and were used to measure HbA1c and complete blood picture. The remaining blood was transferred into a plain tube and allowed to clot at room temperature for 10 minutes, then the serum was separated by centrifugation for 10 minutes at 3,000 RPM. Then the separated serum was carefully dispensed using micro-pipetting and used to measure fasting serum glucose (FSG), ferritin level, and renal function tests including creatinine, urea, and uric acid. The remaining serum was stored in Eppendorf tubes at -80 °C, to be then used for testing EPO.

2.2.2. Instruments

The following instruments in table 2.1, were utilized during the study:

Table 2.1: The devices used in the research

Instrument	Company	Manufacturing country
Incubator	FAITHFUL	China
ACCENT 200 chemistry auto analyzer	CORMAY	Poland
Microplate reader	CHROMATE	China
Centrifuge	HIGHTOP LY8A	Poland
Mindray BS-240 chemistry auto analyzer	Mindray	China
Hematology auto analyzer	Sysmex	China

2.3. Methods

2.3.1. Renal function tests

2.3.1.1. Determination of serum creatinine

Creatinine was determined in serum by kinetic colorimetric assay (at wavelength 546 nm) using a BS-240 auto analyzer and a kit supplied by Shenzhen Mindray Bio-medical Electronic company (China), with catalog number PDIBS20050.

2.3.1.1.1. Test principle

Creatininease converts the creatinine in the sample to creatine. Creatine amidinohydrolase (CRTase) hydrolyzes the creatine to sarcosine and urea. The oxidative demethylation of sarcosine is then catalyzed by the enzyme sarcosine oxidase, resulting in the formation of glycine, formaldehyde, and hydrogen peroxide. A quinonimine with a maximum absorbance at 546 nm is produced when hydrogen peroxide reacts with N-ethyl-N-sulfopropryl-m-toluidine (ESPMT) and 4-aminoantipyrine in the presence of peroxidase enzyme. The color intensity of the reaction product is directly proportional to the creatinine concentration in the sample.

$$\text{Creatinine} + H_2O \xrightleftharpoons{\text{Creatininase}} \text{Creatine}$$

$$\text{Creatine} + H_2O \xrightleftharpoons{\text{CRTase}} \text{Sarcosine} + \text{Urea}$$

$$\text{Sarcosine} + H_2O \xrightleftharpoons{\text{Sarcosine oxidase}} \text{Glycin} + HCHO + H_2O_2$$

$$2H_2O_2 + \text{4-aminoantipyrine} + \text{ESPMT} \xrightleftharpoons{\text{peroxidase}} \text{Quinonimine} + 4H_2O$$

2.3.1.1.2. Reagents

Table 2.2: Serum creatinine test reagents

Reagents	Contents	
R1	CRTase	> 40 KU/L
	Sarcosine oxidase	> 7 KU/L
	Ascorbic acid oxidase	2KU/L
	Catalase	> 100 KU/L
	ESPMT	0.47 mM
R2	Creatininase	> 400 KU/L
	Peroxidase	> 50 KU/L
	4-aminoantipyrine	2.95 mm/L

2.3.1.2. Determination of serum urea

Urea was determined in serum photometrically (at wavelength 340 nm) and based on an enzymatic kinetic test with urease and glutamate dehydrogenase (GLDH), using a BS-240 auto analyzer and a kit supplied by Shenzhen Mindray Bio-medical Electronic company (China), with catalog number PDIBS200010

2.3.1.2.1. Test principle

Urease hydrolyzes urea to form ammonium and carbonate as shown in the following equation:

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + CO_3^{2-}$$

In the presence of GLDH and the coenzyme nicotinamide adenine dinucleotide (NADH), α-oxoglutarate reacts with ammonium to yield L-glutamate. In the second step of this reaction, the oxidation of two moles of NADH to NAD+ occurs for every mole of urea hydrolyzed.

$$NH_4^+ + \alpha - \text{Oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + \text{NAD}^+ + H_2O$$

There is a direct proportion between the rate of reduction in NADH and urea concentrations in the specimen measured photometrically.

2.3.1.2.2. Reagents

Table 2.3: Serum urea test reagents

Reagents	Contents	
R1	Trisaminomethane (TRIS) buffer	120 mmol/L
	ADP	750 mmol/L
	Urease	≥ 40 KU/L
	GLDH	≥ 0.4 KU/L
R2	NADH	1.2 mmol/L
	α-Oxoglutarate	25 mmol/L

2.3.1.3. Determination of serum uric acid

Uric acid was determined in serum by enzymatic colorimetric test at a wavelength (primary/second: 546/670 nm), using an ACCENT-200 auto analyzer and a kit supplied by CORMAY company (Poland), with catalog number 7-208.

2.3.1.3.1. Test principle

The cleavage of uric acid into allantoin and hydrogen peroxide is facilitated by the enzyme uricase.

$$\text{Uric acid} + 2\ H_2O \xrightarrow{\text{Uricase}} \text{allantoin} + CO_2 + H_2O_2$$

Hydrogen peroxide reacts with 4-aminoantipyrine in the presence of hydrogen peroxidase (POD) to yield a quinoneimine (colored compound).

$$2\ H_2O_2 + H^+ + \text{4-aminoantipyrine} \xrightarrow{\text{POD}} \text{quinoneimine dye} + 4\ H_2O_2$$

The intensity of the color produced by the formed quinoneimine is directly proportional to the concentration of uric acid, and this relationship is determined by measuring the increase in absorbance.

2.3.1.3.2. Reagents

Table 2.4: Serum uric acid test reagents

Reagents	Contents
R1	Ascorbate oxidase ≤ 104 µkat/L Peroxidase (POD) ≤22.4 µkat/L 4-aminoantipyrine ≤1.2 mmol/L Sodium hydroxide ≤0.8 % Buffer PIPES (PH 7.0) ≤ 120 mmol/L Stabilizers, preservative, detergent
R2	Buffer PIPES (PH 7.0) ≤ 60 mmol/L N-ethyl-N-(3-sulfopropyl)-3-methylaniline (ADPS) ≤ 2 mmol/L Uricase ≤ 9.9 µkat/L Ferricyanide potassium ≤ 22.8µmol/L Sodium hydroxide ≤ 0.4 % Stabilizers, preservative, detergent

2.3.1.4. Determination of estimated creatinine clearance

The estimated creatinine clearance (CrCl) was calculated from serum creatinine using the Cockcroft-Gault equation as below:

$$\mathbf{CrCl} = \frac{\mathbf{[140 - age\ (years)\ ] \times weight\ (kg)}}{\mathbf{serum\ creatinine\ (mg/dl) \times 72}}\ \mathbf{(\times 0.85,\ for\ female)}$$

2.3.2. Hematological profile

2.3.2.1. Determination of complete blood picture

Hb, RBC, WBC, Platelets, hematocrit (Hct), MCV, mean corpuscular Hb (MCH), mean corpuscular Hb concentration (MCHC), and RDW- standard deviation (RDW-SD) were all estimated in whole blood using Sysmex hematology auto analyzer (Japan).

2.3.2.1.1. Reagents

The following solutions were used: Isotonic Diluent (composed of inorganic salts, buffer, preservative, and purified water), a lysis solution, and an enzymatic cleaner.

2.3.2.1.2. Test principle

Sysmex hematology auto analyzer is a fully automated 18-parameter hematology analyzer. The device is equipped with a three-part impedance-based hematology analyzer. The mechanism of this device is based on the modification of the impedance of the aperture that is calibrated and travels through an electrolyte along a constant path, defined by two electrodes placed on either side of the aperture. The cells pass through the aperture by applying a vacuum to one side, effectively resisting their physical volume to the path of passage. A voltage impulse is recorded at the terminal of the electrodes, and its magnitude is directly proportional to the volume of the cells. The resulting impedance is used to determine the count of RBCs, WBCs, and platelets.

2.3.2.2. Determination of serum ferritin

The CL-series free assay, an automated chemiluminescent immunoassay (CLIA), and a kit from Shenzhen Mindray Bio-medical Electronic company (China) were used to measure the serum ferritin level, with catalog number 105-004220-00 and carried out using a BS-240 auto analyzer device.

2.3.2.2.1. Test principle

To find out how much free ferritin is in a sample, the CL-series free test uses a two-site sandwich procedure. Placing the sample, paramagnetic microparticles

covered with a monoclonal anti-ferritin antibody (mouse), and the anti-ferritin antibody alkaline phosphatase conjugate into a reaction vessel is the initial step. Following incubation, free ferritin in the sample binds to an anti-ferritin antibody-coated microparticle and an anti-ferritin antibody conjugate labeled with alkaline phosphatase to create a sandwich complex. While other unbound materials are washed away, microparticles are magnetically trapped. The addition of the substrate solution to the reaction vessel is the second step. The process is catalyzed by anti-ferritin antibody (mouse)-alkaline phosphatase conjugate in the immunocomplex retained on the microparticle. A photomultiplier integrated inside the apparatus measures the chemiluminescent reaction as relative light units (RLUs). RLUs produced during the reaction are directly correlated with the amount of free ferritin in the sample. A calibration curve can be used to find the free concentration.

2.3.2.2.2. Reagents

Table 2.5: Serum ferritin test reagents

Reagents	Contents
R1	Paramagnetic microparticles coated with monoclonal anti-Ferritin antibody (mouse) in TRIS buffer with preservatives.
R2	Monoclonal anti-Ferritin antibody (mouse)-alkaline phosphatase conjugate in TRIS buffer with preservatives.
R3	TRIS buffer with preservatives.

2.3.2.3. Determination of erythropoietin

Serum EPO was estimated by Sandwich enzyme-linked immunosorbent assay (ELISA) test using a universal microplate reader from CHROMATE and a kit supplied by SUNLONG company (China), with catalog number SL0679Hu.

2.3.2.3.1. Test principle

This ELISA kit uses the Sandwich-ELISA technique as its approach. The Microelisa stripplate included in this kit has been pre-coated with an antibody that specifically targets EPO. The relevant microelisa stripplate wells are supplemented with standards or samples and mixed with the specific antibody. Next, a horseradish Peroxidase (HRP)-conjugated antibody that specifically targets EPO is introduced into each well of the microelisa stripplate and allowed to incubate. Free components are removed by washing. The TMB (3,3',5,5'-Tetramethylbenzidine) substrate solution is introduced into every well. Only the wells that include both EPO and HRP-conjugated EPO antibodies will exhibit a blue color, which will subsequently change to yellow upon the injection of the stop solution. The optical density (OD) is determined using spectrophotometry at a wavelength of 450 nm. The OD value is directly related to the concentration of EPO. The concentration of EPO in the samples can be determined by comparing the OD of the samples to the standard curve.

2.3.2.3.2. Reagents

Pre-coated Microplate 96 well plate (12 strips x 8 wells).

0.5mL Standard.

6mL chromogen solution A.

6mL chromogen solution B.

6mL HRP-conjugate reagent.

6mL sample diluent.

5mL standard Diluent.

20mL Wash solution.

6mL Stop solution.

2.3.2.3.3. Assy procedure

Dilute the standard (STD) by small tubes first as shown in figure 2.7, and then pipette the volume of 50 ul from each tube to the microplate well, each tube uses two wells, a total of 12 wells. Series dilution of the standard is illustrated in table 2.6.

Table 2.6: Series dilution of the standard for erythropoietin test

180 pg/ml	STD No.1	300 µl Original STD + 150 µl STD diluents
120 pg/ml	STD No.2	300 µl STD No.1 + 150 µl STD diluents
60 pg/ml	STD No.3	150 µl STD No.2 + 150 µl STD diluents
30 pg/ml	STD No.4	150 µl STD No.3 + 150 µl STD diluents
15 pg/ml	STD No.5	150 µl STD No.4 + 150 µl STD diluents
7.5 pg/ml	STD No.6	150 µl STD No.5 + 150 µl STD diluents

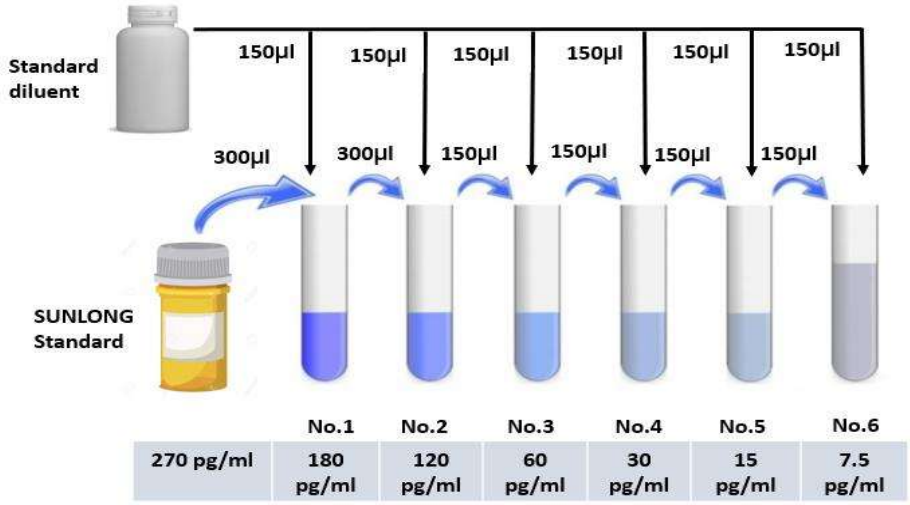

Figure 2.1: Series dilution of erythropoietin test

In the microelisa stripplate, leave a well empty as a blank control. In sample wells, 40 µl sample dilution buffer and 10 µl sample are added. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking. After that incubate for 30 min at 37 °C after sealing with closure plate membrane.

Dilute the concentrated washing buffer with distilled water (30 times for 96T and 20 times for 48T). Carefully peel off the Closure plate membrane, aspirate, and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.

After that Add 50 µl HRP-conjugate reagent to each well except the blank control well, incubation for 30 min at 37 °C and then washing. Afterward add 50 µl chromogen solution A and 50 µl chromogen solution B (coloring agents) to each well, mix with gentle shaking, and incubate at 37°C for 15 minutes. Please avoid light during coloring. Then add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow. Read absorbance O.D. at 450 nm using a microtiter plate reader. The OD value of the blank control

well is set as zero. The assay should be carried out within 15 minutes after adding the stop solution.

2.3.2.3.4. Calculations and results

Known concentrations of human EPO standard and its corresponding reading OD are plotted on the log scale (x-axis) and the log scale (y-axis) respectively. The concentration of human EPO in the sample is determined by plotting the OD of the sample on the Y-axis. The original concentration is calculated by multiplying the dilution factor.

2.3.3. Glycemic status

2.3.3.1. Determination of fasting serum glucose

Glucose levels in the serum were measured using a photometric approach at wavelengths of 340/409 nm. The enzymatic reference method with hexokinase was employed, utilizing an ACCENT-200 auto analyzer and a kit provided by Cormay company (Poland), with catalog number 7-252.

2.3.3.1.1. Test principle

Hexokinase (HK) enzyme facilitates the conversion of glucose to glucose 6 phosphate (G-6-P) by utilizing ATP.

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

In the presence of NADP, the resultant G-6-P is oxidized to gluconate 6 phosphate (gluconate-6-P) by glucose 6 phosphate dehydrogenase (G-6-PDH), NADPH is also generated in this phase, and the rate of formation is related to the glucose concentration, which is determined photometrically.

$$\text{G-6-P} + \text{NDP}^+ \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-p} + \text{H}^+$$

2.3.3.1.2. Reagents

Table 2.7 : Fasting serum glucose test reagents

Reagents	Contents	
R1	TRIS buffer	100mmol/L
	pH 7.8	
	Mg2+	4 mmol/L
	ATP	≥ 1.7 mmol/L
	NADP	≥ 1.0 mmol/L
	Preservative	
SR	HEPES buffer (N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid)	30 mmol/L
	pH 7.0	
	Mg2+	4 mmol/L
	HK (yeast)	≥ 130 µkat/L
	G-6-PDH (E. coli)	≥ 250 µkat/L
	Preservative	

2.3.3.2. Determination of glycated hemoglobin

HbA1c in hemolyzed whole blood is measured using turbidimetric inhibition immunoassay (TINIA) with an ACCENT-200 auto analyzer and a kit from Cormay company (Poland), with catalog number 7-111.

2.3.3.2.1. Test principle

Tetradecyltrimethylammonium bromide (TTAB) was employed as a buffer in the hemolyzing reagent to remove disruption caused by white blood cells. TTAB is used as a detergent to selectively lyse erythrocytes while preserving the integrity of leukocytes. This assay can detect all glycated Hb variants at the N terminus of the β chain that shares antibody-recognizable areas with HbA1c.

2.3.3.2.2. Reagents

Table 2.8: Hemoglobin A1c test reagents

Reagents	Contents
R1: Antibody reagent	MES buffer (2-morpholinoethane sulfonic acid) 0.025 mol/L TRIS buffer 0.015 mol/L, pH 6.2 HbA1c antibody (ovine serum) ≥ 0.5 mg/mL Detergents; stabilizers; preservative.
SR: Polyhapten reagent	MES buffer: 0.025 mol/L TRIS buffer: 0.015 mol/L, pH 6.2 HbA1c polyhapten: ≥ 8 µg/mL Detergents; stabilizers; preservative.

2.3.3.2.3. Hemoglobin A1c estimation

The HbA1c in the sample interacts with the anti-HbA1c antibody from R1 (buffer/antibody) to create soluble antigen-antibody complexes. This reaction is exclusive to HbA1c and will only take place if HbA1c is detected in the sample. The following step involves creating an insoluble antibody-polyhapten complex by reacting the polyhaptens from SR (buffer/polyhapten) with an excess of anti-HbA1c antibodies. The presence of an insoluble complex can be detected using turbidimetric

analysis, where the turbidity intensity is directly related to the concentration of HbA1c in the sample.

2.3.3.2.4. Determination of hemoglobin and calculation of hemoglobin A1c

Hb produced from the hemolyzed sample is converted into a derivative with a unique absorption spectrum and bi-chromatically assessed during the preincubation phase (sample + R1) of the immunological reaction. The ultimate result is expressed as the percentage of HbA1c in the sample and is determined by finding the HbA1c/Hb ratio, as shown below:

$$\text{HbA1c (\%)} = (\text{HbA1c/Hb}) \times 91.5 + 2.15$$

The ratio between HbA1c and Hb of each sample was automatically calculated by the Mindray auto analyzer.

2.4. Statistical Analysis

Data are shown as mean values with standard deviation (mean±SD). A paired t-test was used to compare individual tested data. To investigate the statistical differences between the analyzed groups, a one-way analysis of variance (ANOVA) was used, followed by Tukey's post hoc test to identify any significant variability in the group means. Pearson's correlation coefficients, linear regression, and 95% confidence intervals were used to investigate the correlations between the analyzed variables. Before any statistical analysis, the enrolled groups were validated for normal distribution of data using normality tests (Kolmogorov-Smirnov, Shapiro-

Wilk). GraphPad Prism 8.0.1 was utilized for statistics and considered data to be statistically significant when p-values were less than 0.05.

Chapter three

Results

3. Result

3.1. Baseline characteristics in patients and control groups

Out of the 111 participants enrolled in the study, 30 patients were part of group 3 (received a combination of dapagliflozin and metformin), 40 patients were in group 2 (received metformin as a monotherapy), and 41 individuals belonged to group 1 (the control group). Demographic and baseline characteristics including age, sex, BMI, and duration of diabetes are shown in table 3.1. BMI was significantly higher in group 2 and group 3 as compared to the control group, ($p < 0.001$). On the other hand, Groups 2 and 3 were comparable regarding these parameters.

Table 3.1: Baseline characteristics of the studied groups

Parameter	Group 1 (n=41)	Group 2 (n=40)	Group 3 (n=30)
Age (year)	53.56 ± 9.82	53.53 ± 8.64	56.81 ± 7.54
N (M/F)	41 (21/20)	40 (20/20)	30 (16/14)
BMI (kg/m^2)	24.08 ± 0.87	29.70 ± 4.58 ***	30.48 ± 3.77 ***
Duration of diabetes (year)	-	5.58 ± 3.41	4.96 ± 2.92

Results are shown as mean±SD and are different significantly where indicated (***$p < 0.001$ in comparison to group 1). using one-way ANOVA followed by Tukey's post hoc test. BMI; body mass index, N; number, M; male, F; female.

3.2. Renal function tests and glycemic status

Renal function tests in T2-DM following administration of metformin or a combination of metformin and dapagliflozin showed a significantly high level of urea in comparison to the control group, ($p < 0.001$). Additionally, patients involved in group 2 expressed a significantly higher creatinine level than in group 1 ($p < 0.013$). On the other hand, there were no significant changes in creatinine clearance among the studied groups. However, there was a significantly lower serum uric acid (SUA) in group 3 as compared to group 2 ($p < 0.04$).

Regarding glycemic status, FSG was significantly higher in group 2 and group 3 than in group 1 ($p < 0.001$). HbA1c was also significantly greater in group 2 and group 3 versus group 1 ($p < 0.001$). On the other hand, HbA1c was higher in group 3 as compared to group 2 ($p < 0.01$) as shown in table 3.2.

Table 3.2: Assessment of renal function tests and glycemic status in the studied groups

Parameter	Group 1 (n=41)	Group 2 (n=40)	Group 3 (n=30)
Urea (mg/dL)	23.38 ± 4.75	27.93 ± 4.22***	27.45 ± 3.95***
Creatinine (mg/dL)	0.72 ± 0.13	0.85 ± 0.22*	0.81 ± 0.11
Creatinine clearance	100.07 ± 22.20	111.90 ± 29.57	113.40 ± 25.56
SUA (mg/dL)	4.34 ± 1.06	4.75 ± 0.86	4.21 ± 0.63#
FSG (mg/dL)	85.25 ± 12.18	128.1 ± 39.13***	121.3 ± 31.79***
HbA1c (%)	5.42 ± 0.37	6.68 ± 1.39***	7.66 ± 1.06***##

Data are presented as mean ± SD and are significantly different where indicated (*p <0.05, ***p <0.001; in comparison to group 1 and #p <0.05, ##p <0.01; in comparison to group 2). SUA: Serum uric acid; FSG: fasting serum glucose; HbA1c: hemoglobin A1c.

3.3. Erythropoietin and Hematological parameters

In patients involved in group 3, there was a significantly higher level of EPO as compared to group 1 ($p < 0.01$). Furthermore, the concentration of RBCs was significantly higher in group 3 versus group 2 ($p < 0.05$). Similarly, Hb showed a significantly higher level in group 3 than in group 2 ($p < 0.01$). Moreover, the concentration of Hct was significantly higher in group 3 when compared to group 1 ($p < 0.05$).

The level of WBCs in group 3 was significantly higher in comparison to group 1 and group 2 ($p < 0.05$ and 0.001 respectively). Other differences in the hematological parameters were insignificant among the three studied groups as shown in table 3.3.

Table 3.3: Erythropoietin and Hematological parameters of the studied groups

Parameter	Group 1 (n=41)	Group 2 (n=40)	Group 3 (n=30)
EPO (pg/mL)	17.28 ± 3.11	18.43 ± 1.48	19.72 ± 2.35**
Ferritin (ng/mL)	86.17 ± 53.18	97.39 ± 55.95	87.15 ± 46.36
Hb (g/dL)	13.94 ± 1.37	13.61 ± 1.71	14.82 ± 1.86##
RBCs (×10^6 / µL)	4.87 ± 0.39	4.76 ± 0.36	5.09 ± 0.70#
WBCs (×10^3/ µL)	7.14 ± 1.42	6.80 ± 1.37	8.30 ± 1.70*###
Plts (×10^3/ µL)	245.3± 59.23	236.4 ± 52.55	239.5 ± 70.99
Hct (%)	42.70 ± 3.81	43.60 ± 3.95	46.01 ± 5.58*
MCV (fL)	88.64 ± 7.44	91.58 ± 4.94	90.91 ± 4.12
MCH (pg)	29.00 ±1.78	29.83 ± 1.89	29.23 ± 1.95
MCHC (g/dL)	32.70 ± 1.37	31.86 ± 3.75	32.13 ± 1.31
RDW-SD (%)	48.88 ± 8.00	49.40 ± 9.14	46.23 ± 2.99

Data are presented as mean ± SD and are significantly different where indicated (*p <0.05, **p <0.01; in comparison to group 1 and #p <0.05, ##p <0.01, ###p <0.001; in comparison to group 2). Hb: hemoglobin; EPO: erythropoietin; RBCs: red blood cells; WBCs: white blood cells; Plts: platelets; Hct: hematocrit; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW-SD: red cell distribution width- standard deviation.

3.4. Relationships between uric acid, erythropoietin, and hematological parameters

In group 3, patients demonstrated a statistically significant positive correlation between SUA and EPO, as shown in figure 3.1. Furthermore, figure 3.2 revealed a direct relationship between SUA and Hb, indicating a positive link. Moreover, a positive association was also obtained between SUA and RBCs as illustrated in figure 3.3. Similarly, there was a direct relationship between SUA and Hct as seen in figure 3.4. SUA and WBCs exhibited a positive association (figure 3.5). Additionally, figure 3.6 clearly showed a positive link between SUA and RDW-SD.

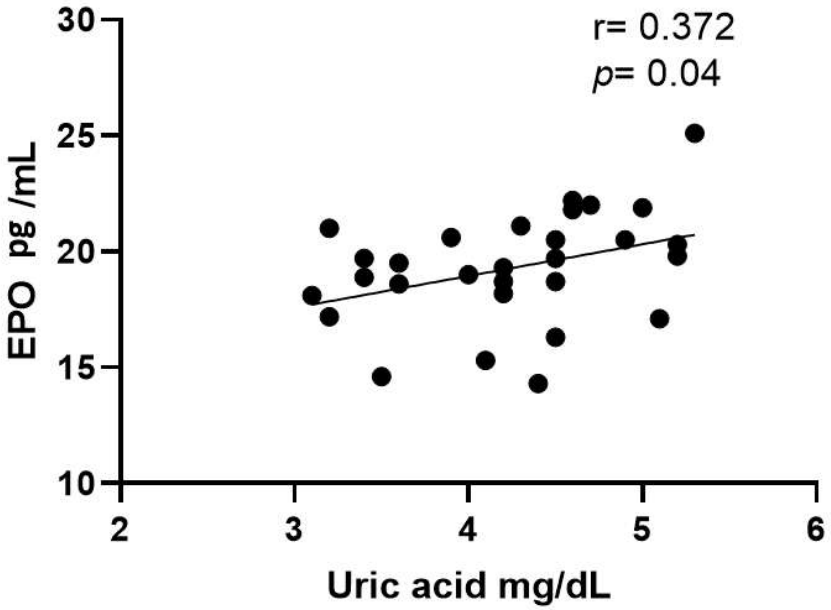

Figure 3.1: Correlation of serum uric acid and erythropoietin in group 3

EPO: Erythropoietin

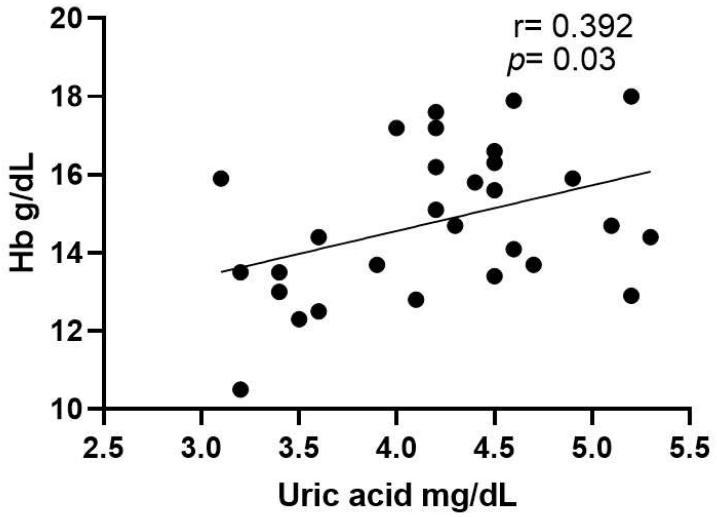

Figure 3.2: Correlation between serum uric acid and hemoglobin in group 3

Hb: Hemoglobin

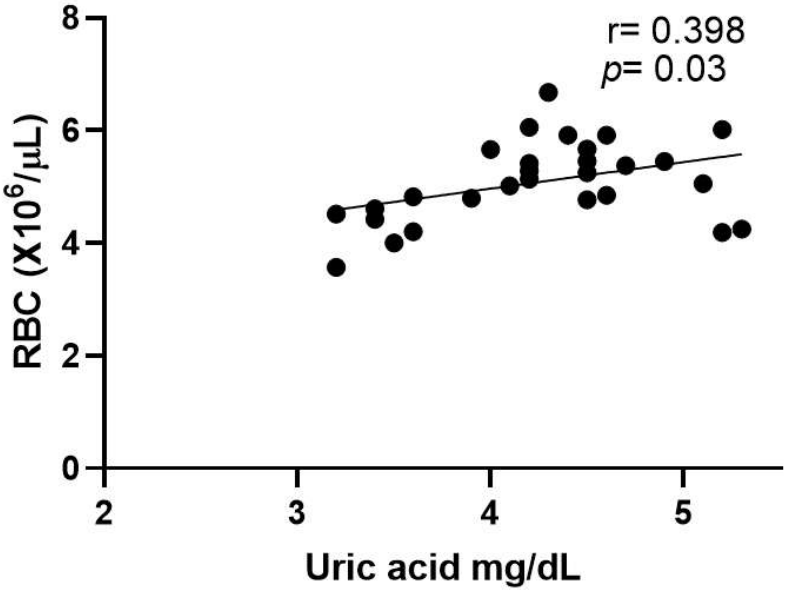

Figure 3.3: Correlation between serum uric acid and red blood cells in group 3

RBC: Red blood cell

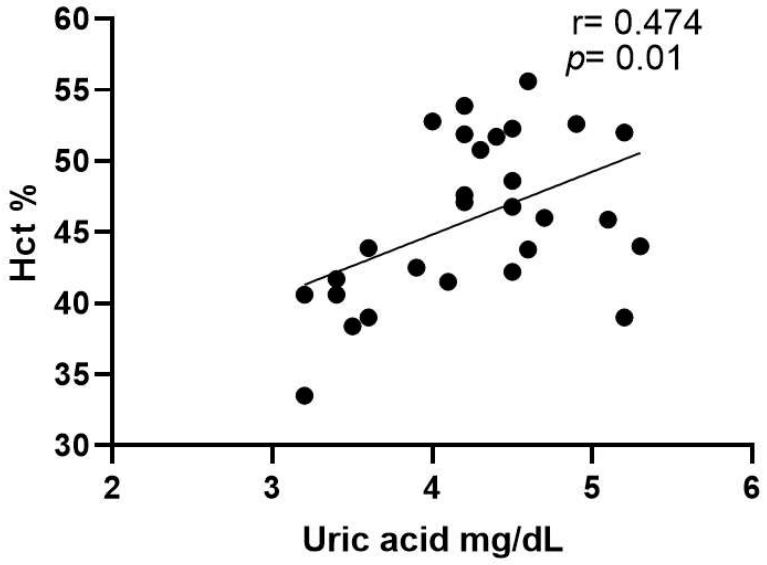

Figure 3.4: Correlation between serum uric acid and hematocrit in group 3

Hct: hematocrit

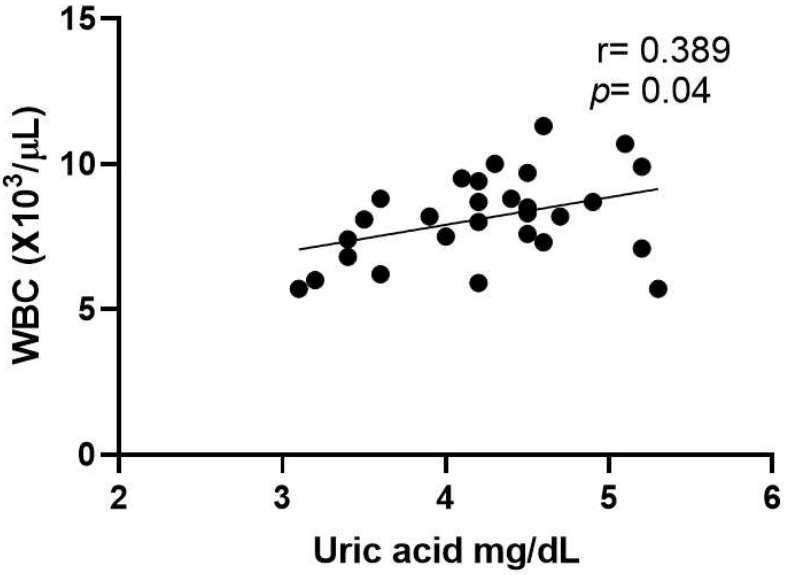

Figure 3.5: Correlation between serum uric acid and white blood cells in group 3

WBC: White blood cell

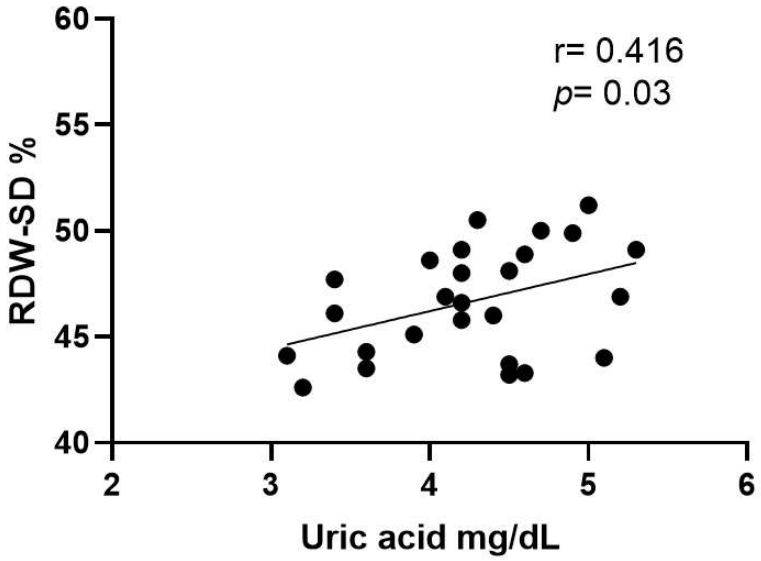

Figure 3.6: Correlation between serum uric acid and red cell distribution width-standard deviation in group 3

RDW-SD: Red cell distribution width-standard deviation

Conversely, findings of the present study revealed that T2-DM patients on metformin-only therapy have a statistically significant negative correlation between SUA and EPO as seen in figure 3.7. Similarly, the negative correlation between SUA and other parameters in this group extended to include Hb (figure 3.8), RBCs (figure 3.9), and Hct (figure 3.10). On the other hand, there are no statistically significant correlation between the other measured parameters that were tested in the statistical analysis.

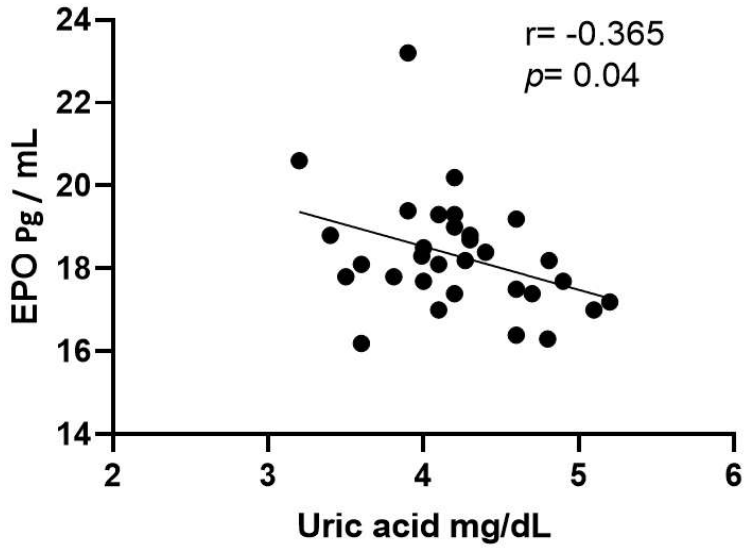

Figure 3.7: Correlation between serum uric acid and erythropoietin in group 2

EPO: Erythropoietin

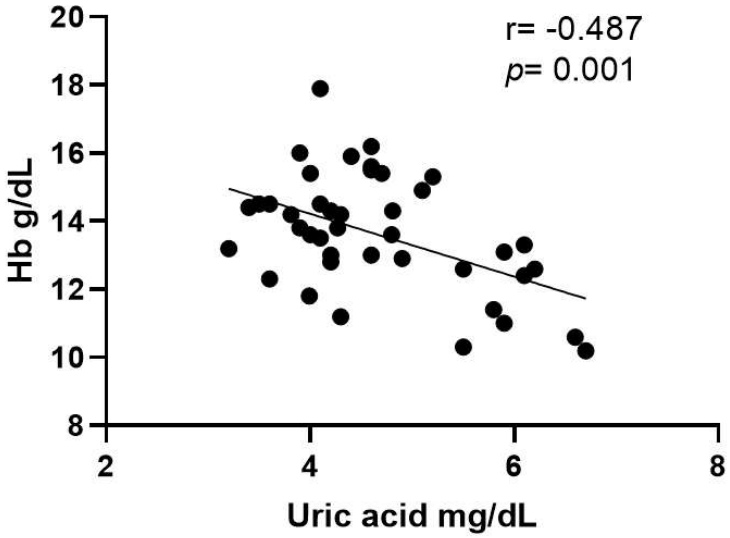

Figure 3.8: Correlation between serum uric acid and hemoglobin in group 2

Hb: Hemoglobin

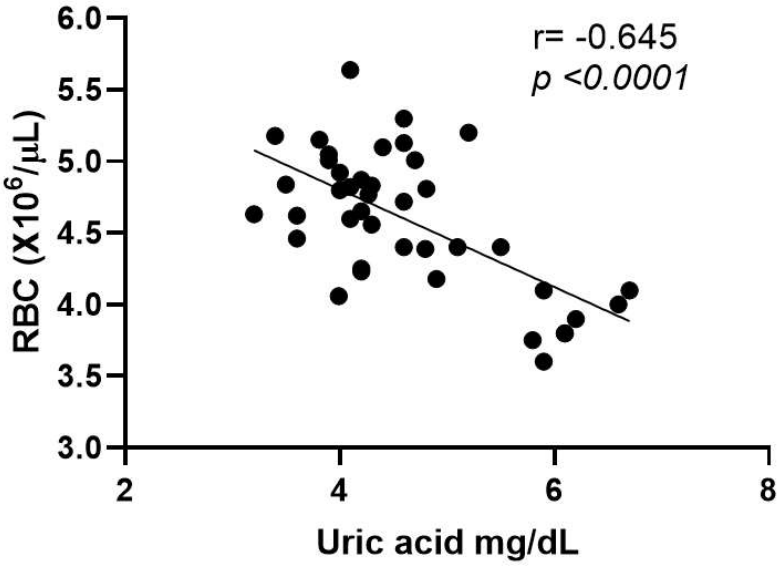

Figure 3.9: Correlation between serum uric acid and red blood cells in group 2

RBC: Red blood cell

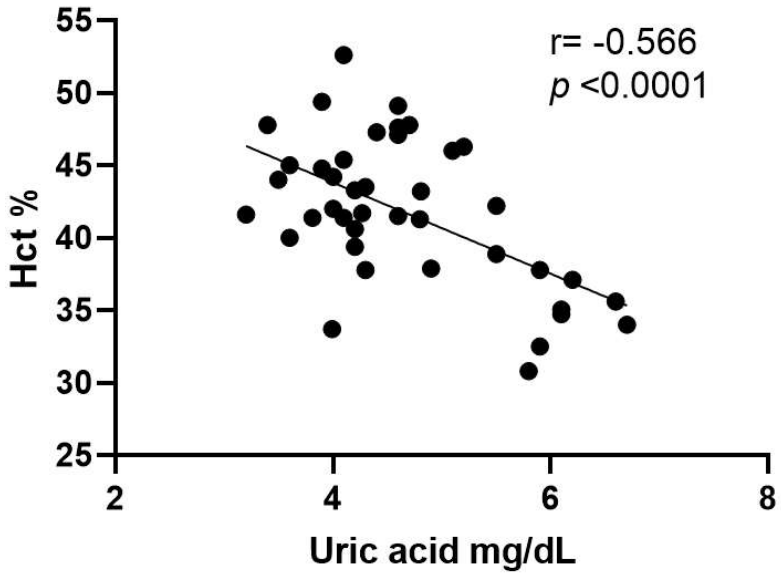

Figure 3.10: Correlation between serum uric acid and hematocrit in group 2

Hct: hematocrit

Chapter four

Discussion

4. Discussion

T2-DM is the most prevalent endocrine disorder, accounting for approximately 90% of all diabetes cases. It is characterized by elevated blood sugar values both when fasting and postprandial, along with relative insulin deficiency (Galicia-Garcia et al., 2020). If left untreated, hyperglycemia can lead to long-term microvascular and macrovascular complications such as nephropathy, neuropathy, retinopathy, and atherosclerosis, in addition to hematological issues (Chawla et al., 2016). The incidence of T2-DM is increasing worldwide with greater rates in Africa, Asia, and South America than in Europe or the United States. Thus T2-DM is regarded as a global epidemic condition (Khan et al., 2019).

Hematologic alterations are frequently reported in T2-DM patients. They include deviations in the function, morphology, and biochemical process of RBCs, WBCs, and platelets, as well as abnormalities in hemostasis and plasma proteins (Biadgo et al., 2016). Conversely, chronic hyperglycemia contributes to the pathogenesis of DNP which significantly progresses to CKD and ESRD necessitating renal replacement therapy (Lim, 2014).

Monitoring of hematological parameters is a potentially useful strategy for reducing diabetic-related complications in patients with T2-DM. RBC features are a useful tool for assessing diabetic patients. Erythrocyte-related indicators could offer more clinical data and be utilized to monitor the development of diabetes and associated consequences since RBCs could sense blood glucose variations earlier and consistently (Esraa R et al., 2020; Y. Wang et al., 2021). Additionally, Platelet count and mean platelet volume are markers of thrombotic potential and risk factors for diabetic microvascular complications. Likewise, impaired glucose tolerance is indicative of increased WBCs count, and T2-DM macroangiopathic and

microangiopathic outcomes are also associated with elevated WBCs count (Rafaqat & Rafaqat, 2023).

On the other hand, while a reduction in EPO level leads to the development of anemia in diabetic patients that requires medical intervention, a higher concentration of EPO contributes to diabetic retinopathy. Studies have shown that erythropoietin affects endothelial cells in the same manner as VEGF, which necessitated the monitoring of this parameter in diabetic patients (Bretz et al., 2020; Davidović et al., 2019).

In line with this, for people with T2-DM, renal function should be monitored at least annually to enable early treatment to preserve renal function (Christofides & Desai, 2021). Hence, it is important to assess the hematological parameters and renal function tests in these patients and to evaluate the impact of medications used in their treatment on these parameters.

Dapagliflozin is an oral, selective, and reversible inhibitor of SGLT2, which provides glycemic benefits without causing substantial hypoglycemia. There are also improvements in terms of blood pressure, weight, and lipids that could potentially lead to cardiovascular benefits (Narendran & Saeed, 2014). Additionally, it was demonstrated to have a positive impact on hematological parameters, EPO, and renal function in patients with T2-DM (Ahmed et al., 2023). However, in most clinical settings, dapagliflozin is used in combination with metformin in order to provide better glycemic status. Limited studies are available to assess the impact of this combination on hematological parameters and kidneys. Accordingly, this study was designed with a view to investigate the effects of dapagliflozin, as an add-on therapy to metformin, on hematological parameters, EPO levels, and renal function, to provide potential clinical insight into this combination to healthcare professionals.

4.1. The impact of dapagliflozin on glycemic status

In terms of glycemic status, the study revealed that FSG in patients who received a combination of dapagliflozin and metformin was not significantly reduced in comparison to metformin monotherapy. Conversely, Oldgren J et al. (2021) have shown a significant reduction in fasting glucose levels in diabetic patients who received dapagliflozin as an add-on therapy to metformin compared to metformin-only users. However, their study was different as the patients received 10 mg of dapagliflozin instead of 5 mg, and the study was limited by the short duration of treatment of 6 weeks (Oldgren et al., 2021). Furthermore, Cheng et al. (2021) revealed that there was a significant reduction in fasting glucose levels after using dapagliflozin as an additional therapy to metformin in patients with T2-DM. However, this study was different by the inclusion of only diabetic patients with metabolic syndrome (Cheng et al., 2021).

HbA1c, on the other hand, was significantly higher when dapagliflozin was added to metformin compared to metformin-only users. Contrary to our result, Bell et al. (2016) have shown a greater reduction in HbA1c in patients who received a combination of dapagliflozin and metformin therapy compared to patients who received metformin alone; however, this study is different as the overall participants were recently diagnosed with T2-DM and had a short-duration of diabetes (Bell et al., 2016). On the other hand, Cuatrecasas et al. (2024) have revealed that there are no significant changes in HbA1c after adding dapagliflozin to patients with T2-DM on metformin therapy. Again, their study involved only obese diabetic patients, which could be the source of variation in the obtained results (Cuatrecasas et al., 2024).

The observed increase in HbA1c levels in group 3 could be related to the effects of dapagliflozin on insulin resistance. A plausible mechanism involves the role of dapagliflozin in increasing fatty acid oxidation (Daniele et al., 2016). This process has been linked to increased oxidative stress in the body (David & Holwerda, 2023), which in turn, has been proven to increase insulin resistance and, hence, shows higher HbA1c readings (Tangvarasittichai, 2015). However, other causes beyond this result may related to difference in the disease severity and the life style of the patients (including diet, exercise, and adherence to medications). Additionally, patients may differ in their response to medications according to their genetic variations.

4.2. The impact of dapagliflozin on renal function tests

regarding renal function parameters, the present study showed a higher urea level in group 2 compared to the control group. However, adding dapagliflozin to metformin did not produce a significant change in urea level compared to metformin-only users. This result is in parallel with Bletsa et al. (2021) and Bailey et al. (2010), who also demonstrated the insignificant or neutral effects of dapagliflozin therapy on serum urea levels in T2-DM. (C. J. Bailey et al., 2010; Bletsa et al., 2021).

The current study has shown no significant differences in serum creatinine levels between the control and dapagliflozin plus metformin medication recipients. Conversely, individuals who were using metformin alone had noticeably elevated serum creatinine levels compared to the control group, indicating the positive role of dapagliflozin in reducing creatinine levels. In line with these results, Liu et al. (2023) also demonstrated the lowering effects of dapagliflozin on serum creatinine in patients with T2-DM (T. Liu et al., 2023). On the other hand, Henry and

colleagues (2012) have found that dapagliflozin does not affect serum creatinine in T2-DM patients when used as an add-on therapy to metformin. Nevertheless, their study is different in that the patients received different doses of metformin and the study was limited by the short duration of treatment of 6 months (Henry et al., 2012).

The lowering impacts of dapagliflozin medicine on blood creatinine in addition to its neutral effects on urea levels, demonstrated in the current study, may contribute to its beneficial impacts on the kidney, as revealed by multiple earlier investigations (BASKOY et al., 2023; Iijima et al., 2023; Provenzano et al., 2022). The suggested mechanisms for these beneficial effects could include increases in eGFR and urine sodium excretion. Furthermore, it reduces kidney damage by reducing oxidative stress, inflammatory responses, fibrosis, and endothelial activation. Additionally, dapagliflozin has been demonstrated to reduce tubular injury in diabetic patients (B. Huang et al., 2022; Urbanek et al., 2023).

The level of SUA was significantly lower in patients administering a combination of dapagliflozin and metformin therapy compared to patients on metformin only, highlighting the lowering impact of dapagliflozin medication on SUA, which is confirmed by several previous studies (Khalil et al., 2022; Mori et al., 2024; S. Wang et al., 2022).

The exact underlying mechanism for the reduction in SUA after using dapagliflozin remains unclear. The most accepted explanation could be attributed to the role of renal sodium-independent glucose transporter (GLUT9). The proximal tubules contain GLUT9 isoform 2, a transporter that excretes UA in exchange with glucose. Dapagliflozin by increased glucose excretion and raised tubular glucose concentration may enhance UA excretion via this transporter resulting in more exchange between uric acid in the blood and glucose in the filtrate. Additionally, the

collecting ducts contain GLUT9 isoform 2 which is responsible for the reabsorption of UA, high tubular glucose concentration hinders this transporter and leads to faster UA excretion (Yuan et al., 2020).

Changes in the expression of GLUTs are observed in diabetic kidney tissue. Long-term hyperglycemia in diabetic individuals raises the expression of GLUTs in the proximal tubule, and thus increasing glucose reabsorption. Patients with diabetes demonstrate three-fold greater reabsorption of glucose in comparison with healthy subjects (Sędzikowska & Szablewski, 2021). A possible suggestion is that the probable increase in the expression of GLUT9 in diabetic patients may be accountable for decreasing the efficacy of dapagliflozin in those patients and may be responsible for the insignificant reduction in FSG and the higher levels of HbA1c after the addition of dapagliflozin to metformin that are seen in the current study. However, more investigations are required about the impact of diabetes on these transporters and the effects of these transporters on the action of dapagliflozin therapy.

Furthermore, taking into account the lowering effects of dapagliflozin on SUA, it may be considered a promising antidiabetic therapy for diabetic patients with gout. Many studies have revealed the beneficial effects of dapagliflozin in diabetic patients with hyperuricemia and dapagliflozin was demonstrated to reduce the risk of gout in those groups of patients (Banerjee et al., 2023; Kochanowska et al., 2023).

4.3. The impact of dapagliflozin on erythropoietin and hematological parameters

Regarding the effects of dapagliflozin on hematological parameters in the current study, there are significantly high levels of RBCs, Hb, and Hct for patients who received dapagliflozin. Osonoi et al. (2023) also demonstrated the increased levels of RBCs, Hb, and Hct in patients with T2-DM and renal impairment on dapagliflozin (Osonoi et al., 2023). Additionally, Aberle et al. (2020) found that the administration of dapagliflozin raised RBCs, Hct, and reticulocyte count in T2-DM patients. However, unlike the current study, these patients received insulin in addition to dapagliflozin therapy (Aberle et al., 2020). Furthermore, Nomoto et al. (2017) also demonstrated the increasing effects of dapagliflozin on Hb and Hct in patients with T2-DM (Nomoto et al., 2017).

There are several proposed mechanisms beyond the elevation of RBCs, Hb, and Hct levels. Initially, it was thought that the increment in these parameters was due to the natriuretic action of dapagliflozin which led to a reduction in blood volume and hemoconcentration (Ekanayake & Mudaliar, 2023). However, a more potential explanation was related to the activation of arginine vasopressin (AVP) that resulted from the diuretic effects of dapagliflozin. In the experimental anemia model, AVP was found to rapidly increase RBC count and improve erythropoiesis independently on EPO (Eickhoff et al., 2019; Mayer et al., 2017). Furthermore, several previous studies have shown the increased levels of EPO with dapagliflozin therapy and reaching a peak within 2-4 weeks from the start of treatment (Lambers Heerspink et al., 2013; Sano et al., 2016)

EPO levels were significantly higher in this study in patients with dapagliflozin therapy compared to the control group which could be accountable for

increased RBC-related parameters in those patients. This finding is confirmed by a study that revealed a significant increase in EPO levels in patients with T2-DM on dapagliflozin therapy (Ghanim et al., 2020). Moreover, another study reported that patients who received dapagliflozin had significantly raised levels of EPO compared to placebo and hydrochlorothiazide groups, after 4 weeks of starting the study (Lambers Heerspink et al., 2013).

The increase in EPO levels following dapagliflozin therapy could be driven by a variety of causes. A possible mechanism is that the inhibition of sodium reabsorption in the proximal tubule leads to the transfer of greater but less effective oxygen-consuming active sodium reabsorption transporters to the distal tubule causing the production of hypoxia-inducible factors that promote erythropoiesis (Packer, 2023). Additionally, the reduced burden of increased glucose reabsorption on proximal tubular cells may decrease cortical oxidative stress and enhance recovery from tubulointerstitial damage, and thereby restoring EPO synthesis (Ekanayake & Mudaliar, 2023).

The increase in RBC count for patients on dapagliflozin therapy in the current study may account for the higher HbA1c values in those patients. RBC count was demonstrated to be positively correlated with HbA1c in diabetic patients and an increase in RBC levels led to higher HbA1c readings (Esraa R et al., 2020).

WBC count also increased significantly in patients receiving dapagliflozin therapy compared to other groups. This result is consistent with Bas et al. (2022) who demonstrated an increased WBC count in diabetic patients on dapagliflozin (Baş & Müşerref Türkmen, 2022). The increased WBC count could be attributed to the effects of dapagliflozin on intracellular 1,5-anhydroglucitol-6-phosphate (1,5AG6P) levels. Dapagliflozin reduces the intracellular buildup of 1,5AG6P, a

substance that limits glucose phosphorylation by hexokinases and consequently, glycolysis, the primary energy source for mature neutrophils. (Wortmann et al., 2020). Nevertheless, more investigations are required about the impact of dapagliflozin therapy on WBC.

4.4. Correlation between uric acid, erythropoietin, and hematological parameters in patients involved in group 3

The data from the results have shown that SUA is strongly correlated with RBC, Hb, Hct, EPO, WBC, and RDW-SD. There is a significant positive correlation between SUA and these parameters in patients involved in group 3. SUA may act as a blood antioxidant, protecting RBCs from oxidative damage and potentially improving their lifespan and function leading to a rise in RBC count. As a result, Hb and Hct also increased with increasing RBC counts (Song et al., 2019). Conversely, the nucleoprotein that results from RBC metabolism participates in the synthesis of SUA (Su et al., 2016).

Additionally, UA may affect EPO through the increase in renal vasoconstriction which increases renal hypoxia and EPO synthesis (Sulikowska et al., 2012). Besides being a measure of catabolic rate, UA has been suggested to have a direct role in systemic inflammation. SUA promotes inflammation by activating the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K-Akt) pathways, inhibiting the adenosine monophosphate-activated protein kinase (AMPK) pathway, and lowering NO production. High levels of SUA activate the NLRP3 inflammasome and generate interleukin-1β, triggering an inflammatory cascade reaction (Y. Wang et al., 2023). On the other hand, some inflammatory cytokines such as interleukin-6 could activate the xanthine oxidase enzyme resulting

in a rise in SUA (L. L. W. and J. T. Wu, 2008). These effects illustrate the linear relationship between SUA and WBC in addition to other inflammatory markers.

Moreover, elevated RDW-SD may indicate inflammation and oxidative stress. Since increased SUA levels are linked to inflammatory conditions such as diabetes and both SUA and RDW-SD can be increased by similar factors, they exhibit a linear relationship with each other (Luo et al., 2014). Considering that, oxidative stress led to a higher value of RDW-SD and elevated levels of SUA. Measurement of the oxidative stress markers may confirm the positive correlation between RDW-SD and SUA shown in the current study (Kalaycı, 2023; Packer, 2020).

4.5. Correlation between uric acid, erythropoietin, and hematological parameters in patients involved in group 2

SUA was negatively correlated with EPO, RBC, Hct, and Hb in patients involved in group 2. Metformin has been implicated in various metabolic pathways and produced a variety of side effects. It has been demonstrated to be associated with vitamin B12 deficiency that leads to increased homocysteine levels which are involved in increased hemolysis in diabetic patients (Li, Fang, et al., 2021). Accordingly, the increased levels of homocysteine with metformin therapy may exacerbate erythrocyte hemolysis, thereby augmenting serum uric acid levels (Cristiana et al., 2012). This hypothesis aligns with the observed negative correlation between SUA and RBC, Hb, and Hct, in patients receiving metformin as monotherapy. However, further studies are required to elucidate the precise mechanisms involved in this correlation.

On the other hand, the addition of dapagliflozin as an add-on therapy to metformin modified the negative correlation of SUA with RBC, Hb, and Hct shown in patients treated with metformin-only therapy. The proposed mechanism beyond these effects is that dapagliflozin has been reported to reduce the level of homocysteine and may be involved in mitigating its deleterious effects on erythrocyte integrity and function, thereby attenuating the propensity for RBC hemolysis (Yu et al., 2023).

Furthermore, the negative correlation that has been demonstrated in the present study between SUA and EPO in patients who received metformin as a monotherapy may require more future investigations to illustrate this relationship. Finally, it is essential to mention the limitations of this study, which included a relatively small sample size of participants, and the absence of a group that contains patients on dapagliflozin as a monotherapy. Additionally, the study was conducted in a single center, which requires future multi-center trials to further generalize the obtained results. However, the study has given a clear insight into the effects of dapagliflozin on hematological parameters and EPO levels, in addition to its effects on renal function in patients with T2-DM.

Chapter five

Conclusions and Recommendations

5. Conclusions and recommendations

5.1. Conclusions

Several conclusions have been drawn from the results of this retrospective cohort study, which are outlined below.

1. Concerning glycemic status, there are neutral effects on FSG when using dapagliflozin as an add-on therapy to metformin in patients with T2-DM. HbA1c levels increased unfavorably with dapagliflozin, representing a need for further investigation into these effects.
2. Regarding the effects of dapagliflozin on renal function tests, the urea levels and creatinine clearance remained unchanged with dapagliflozin, while there was a significant reduction in creatinine levels. The reduction in creatinine levels observed in this study highlights its beneficial effects on the kidneys, supporting the use of dapagliflozin in diabetic patients with renal disease
3. The levels of SUA were significantly reduced with dapagliflozin, as an add-on therapy, which suggests its beneficial impact in diabetic patients with hyperuricemia.
4. The upregulation of renal GLUTs transporters in the kidneys of diabetic patients may result in reduced efficacy of dapagliflozin in those patients that require more investigation.
5. Patients on dapagliflozin experienced notable higher levels of their EPO, RBC, Hb, and Hct which may give additional benefits when using dapagliflozin to control blood glucose in diabetic patients with anemia.
6. The addition of dapagliflozin as an add-on therapy to metformin changes the negative correlation between SUA, EPO, and hematological parameters to a

positive relationship between these parameters that may be related to reduced homocysteine levels which increased with metformin therapy.

5.2. Recommendations

1. Investigating the effects of dapagliflozin on reducing or treating hyperuricemia in diabetic patients with gout.
2. Beyond glycemic effects, exploring the beneficial impacts of dapagliflozin, in comparison to other SGLT2 inhibitors on EPO, RBC, and Hb levels in diabetic patients with anemia.
3. Exploring the impact of diabetes on renal GLUTs transporters and the effects of these transporters on the action of dapagliflozin and other SGLT2 inhibitors.
4. Investigating the effects of increased sodium excretion on the function of GLUTs transporters.
5. Measurement of oxidative stress markers with the aim of further revealing the positive correlation obtained between RDW-SD and SUA in diabetic patients on dapagliflozin.
6. Investigating the molecular mechanism of how dapagliflozin reduces homocysteine levels in diabetic patients.

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Appendices

I. published reviews

REVIEWS

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The regulatory role of erythropoietin in diabetes mellitus: a focus on oxidative and glycemic statuses

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ABSTRACT

Background and objectives. *One of the leading causes of disability and death among the population is type 2 diabetes mellitus (T2DM), which is characterized by persistent hyperglycemia. The disease is associated with disorders including oxidative stress and resistance to insulin action, which are involved in the progression of other diabetic complications. Such complications strongly rationalize the need for advanced treatment strategies. Considering the antioxidant, and anti-inflammatory in addition to cytoprotective properties of erythropoietin (EPO), it may offer a new potential strategy for the management of these disorders in diabetic patients. This review aims to explore the effects of EPO on oxidative stress and insulin resistance and its impact on glucose metabolism to guide healthcare professionals to a novel approach for controlling diabetes complications.*

Materials and methods. *This review was conducted by analyzing studies involved with oxidative stress, resistance to insulin, and glucose metabolism in T2DM published in Cochran Library, PubMed, and Google Scholar until February 2024.*

Results. *EPO shows promising beneficial effects in the management of these diabetes-associated disorders.*

Conclusion. *With this beneficial effect of EPO, it is considered a strong candidate and area of development for the creation of new choices in the management of diabetes.*

Keywords: diabetes mellitus, erythropoietin, insulin resistance, oxidative stress

Abbreviations:

AGEs – Advanced Glycation End Products
Akt – Protein Kinase B
DM – Diabetes Mellitus
EPO – Erythropoietin
EPO-R – Erythropoietin Receptor
GLUT-4 – Glucose Transporter-4
HOMA-IR – Homeostatic Model Assessment for Insulin Resistance
IRS-1 – Insulin Receptor Substrate-1
NF-κB – Nuclear Factor Kappa B
PC-1 – Plasma Cell Differentiation Antigen-1
PI3K – Phosphatidylinositol 3 Kinase
POMC – Hypothalamic Pro-Opiomelanocortin
RBC – Red Blood Cell
ROS – Reactive Oxygen Species
T2DM – Type 2 Diabetes Mellitus

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REVIEW ARTICLE

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The Impact of Anti-Diabetics on Platelets Function and Cardiovascular Outcomes in Type 2 Diabetes Mellitus: A Narrative Review

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ABSTRACT

Increased atherosclerosis is a major risk factor for atherothrombotic disorders in patients with type 2 diabetes mellitus (T2DM). Changes in platelet metabolism and alterations in intra platelet signaling pathways are the main contributors to the pathogenesis of atherothrombotic complications of diabetes. In addition, advances in understanding the action of some anti-diabetics have demonstrated probable effects of these agents on platelet function and thrombotic state in T2DM. This review aimed to explore the possible mechanistic association between anti-diabetic agents and the development of thromboembolic disorders, considering the effect of drugs on platelet function and their impact on cardiovascular outcomes in diabetic patients. The cochran Library, PubMed, and Google Scholar were searched to analyze related publications. The relationship between anti-diabetic medications and the alterations in platelets function, in addition to the impact of the drugs on the occurrence of thromboembolic disorders in diabetic patients and their relation to cardiovascular events, were the main targets. Ninety-three published articles from November 1999 until February 2024 met the requirements for inclusion in this review. We realized various mechanisms responsible for enhanced platelet aggregation in T2DM, represented by immature, large, or activated platelets in the altered metabolic environment, against the background of vascular damage in DM. In parallel, analyzing the impact of anti-diabetics on platelet aggregation revealed that most of them are believed to have favorable protective effects against thrombotic disorders via reducing platelet activation and aggregation. Conversely, some anti-diabetics may exhibit negative consequences by exacerbating platelet hyperactivity and possibly predisposing to a higher incidence of thrombotic events in diabetic patients. When prescribing anti-diabetic agents to patients with T2DM, especially those at high risk of developing cardiovascular problems, we should consider these outcomes with proper monitoring of coagulation status.

Keywords: Anti-diabetic; Cardiovascular Diseases, Diabetes mellitus; Platelets; Thrombosis; Coronary artery disease.

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INTRODUCTION

Cardiovascular disease is a life-threatening complication of type 2 diabetes mellitus (T2DM). Among the most common complications of this condition, are the microvascular and macrovascular complications [1]. Macrovascular consequences are presented as peripheral vascular disease, cerebrovascular illness, and premature coronary artery disease (CAD), all of which are due to accelerated atherosclerosis [2]. CAD incidence is similar across patients with T2DM and those without diabetes who were previously presented with heart attacks. However, individuals with T2DM usually show a 2-4 fold higher risk of CAD when compared to people who have not presented previously with heart attacks. Furthermore, T2DM is associated with a poor prognosis after myocardial infarction (MI), a higher risk

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Review Article

Antidiabetic Agents and Bone Quality: A Focus on Glycation End Products and Incretin Pathway Modulations

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Diabetes mellitus is associated with inadequate bone health and quality and heightened susceptibility to fractures, even in patients with normal or elevated bone mineral density. Elevated advanced glycation end-products (AGEs) and a suppressed incretin pathway are among the mechanisms through which diabetes affects the bone. Accordingly, the present review aimed to investigate the effects of antidiabetic medications on bone quality, primarily through AGEs and the incretin pathway. Google Scholar, Cochrane Library, and PubMed were used to examine related studies until February 2024. Antidiabetic medications influence AGEs and the incretin pathway directly or indirectly. Certain antidiabetic drugs including metformin, glucagon-like peptide-1 receptor agonists (GLP-1RA), dipeptidyl-peptidase-4 (DDP-4) inhibitors, α-glucosidase inhibitors (AGIs), sodium-glucose co-transporter-2 inhibitors, and thiazolidinediones (TZDs), directly affect AGEs through multiple mechanisms. These mechanisms include decreasing the formation of AGEs and the expression of AGEs receptor (RAGE) in tissue and increasing serum soluble RAGE levels, resulting in the reduced action of AGEs. Similarly, metformin, GLP-1RA, DDP-4 inhibitors, AGIs, and TZDs may enhance incretin hormones directly by increasing their production or suppressing their metabolism. Additionally, these medications could influence AGEs and the incretin pathway indirectly by enhancing glycemic control. In contrast, sulfonylureas have not demonstrated any obvious effects on AGEs or the incretin pathway. Considering their favorable effects on AGEs and the incretin pathway, a suitable selection of antidiabetic drugs may facilitate more protective effects on the bone in diabetic patients.

Key Words: Antidiabetic agents · Bone · Diabetes Mellitus · Glycation · Incretin

INTRODUCTION

Skeletal fragility frequently coexists with type 1 and type 2 diabetes, where it is regarded as a pathological consequence of this condition. While low bone mass in type 1 diabetes mellitus (T1DM) can greatly increase the risk of fractures, individuals with type 2 DM (T2DM) also experience an increased incidence of fractures, even when normal or high bone mineral density (BMD) and a higher body mass index are present (factors that protect against fractures in non-diabetic individuals). Therefore, diabetes may be linked to a reduction in the strength of bone that

II. Consent form

You are invited to participate in clinical scientific research on **the investigation of hematological parameters, erythropoietin level, and renal function in type II diabetic patients on dapagliflozin**. Please take the appropriate time to read the following information carefully before deciding whether you wish to participate or not. You can request further clarifications or additional information about any matter mentioned in the form or about the study from your doctor. Provided that patients do not bear any financial consequences for the tests.

- **Description of the research project and its objectives:** The research project is concerned with studying and evaluating the hematological parameters, erythropoietin levels, and kidney function in patients with type 2 diabetes on dapagliflozin therapy.
- **Potential positive benefits to the participant that may result from this research:** Knowing the levels of hematological parameters and erythropoietin level, as well as the efficiency of kidney functions for diabetic patients who use dapagliflozin.
- **Possible negative effects or side effects that you will experience during the research:** There are no negative or side effects that the patient may be exposed to while participating in the research.
- If you agree to participate in this study, your name will remain confidential. No one is allowed "unless stipulated by law" to view your medical file, except for the doctor responsible for the study, his assistants, and independent professional ethics committees. If any negative symptom occurs as a result of participation, there will be no financial compensation if it is not mentioned in the research.

- **If you agree to participate in the research, please sign**

Researcher name:	**Participant name:**
Signature:	**Signature:**
Date: / /	**Date**: / /

استمارة موافقة المريض على المشاركة بالبحث

أنت مدعو للمشاركة ببحث علمي سريري في (**تقييم العوامل الدموية ومستوى الاريثروبويتين ووظائف الكلية لدى مرضى السكري من النوع الثاني الذين يتناولون داباجليفلوزين**) يرجى أن تأخذ الوقت المناسب لقراءة المعلومات الآتية بتأنٍ قبل أن تقرر إذا ما كنت راغباً بالمشاركة أم لا. وبإمكانك طلب مزيداً من الإيضاحات أو المعلومات الإضافية عن أي أمر مذكور بالاستمارة أو عن الدراسة من طبيبك. على ان لا يتحمل المرضى اية تبعات مالية للفحوصات.

- **وصف مشروع البحث وأهدافه:**

مشروع البحث يختص بدراسة ونقييم تقييم العوامل الدموية ومستوى الاريثروبويتين ووظائف الكلية لدى مرضى السكري من النوع الثاني الذين يتناولون داباجليفلوزين.

- **الفوائد الايجابية المحتملة للمشارك التي قد تنتج من هذا البحث:**

معرفة مستوى العوامل الدموية ومستوى الارثروبويتين وأيضا كفاءة وظائف الكلية لمرضى السكري الذين يستعملون دواء الداباجليفلوزين.

- **التأثيرات السلبية أو الأعراض الجانبية المحتملة التي ستتعرض لها أثناء البحث:**

. ليس هناك تاثيرات سلبية او جانبية قد يتعرض لها المريض اثناء المشاركة بالبحث

- في حال موافقتك على المشاركة في هذه الدراسة سيبقى أسمك قيد الكتمان. ولا يسمح لأي شخص "ما لم ينص عليه القانون" حق الاطلاع على الملف الطبي الخاص بك باستثناء الطبيب المسؤول عن الدراسة ومعاونيه ولجان الأخلاق المهنية المستقلة. وإذا حصل أي عارض سلبي من جراء المشاركة لن يكون هناك أي تعويض مالي إذا لم يذكر في البحث.
- **في حالة الموافقة على المشاركة في البحث يرجى التوقيع ادناه**

أسم المشارك: **أسم الباحث:**

توقيع المشارك: **توقيع الباحث:**

التاريخ: / / **التاريخ:** / /

III. Ethical approval

Ministryof Higher Education
and Scientific Research
University of Mosul
Collegiate Committee for Medical
Research Ethics

No.: 54
Date: 8/11/2023
Code: CCMRE - phA – 23 - 17

Ethical Approval

To/ Muthanna k. Zaki
Dr. Mohammed N. Abed
Dr. Fawaz A. Alassaf

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Dear Researchers:
Protocol Title:(**Investigation of hematological parameters , erythropoietin levels , and renal function tests in type II diabetic patients on dapagliflozin**)

We are Pleased to inform you that the collegiate committee for medical research ethics / University of Mosul has reviewed your research documents and has approved your protocol.

Thank you
Yours Sincerely

Prof. Dr. Basil Mohammednathir Saeed
Chairman University Committee for
Ethics of Medical Researches

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IV. The used questionnaire

Investigation of Hematological Parameters, Erythropoietin Level, and Renal Function in Type II Diabetic Patients on Dapagliflozin					
Name		**Age**		**Gender**	
Date		**Weight**		**Group**	
Code		**Height**		**Duration of DM**	
Other diseases				**Drug**	
Other medications				**Disease**	

Hematology				**Renal function**	
RBC		**MCV**		**S. urea**	
Hb		**MCH**		**S. creatinine**	
Hct		**MCHC**		**S. uric acid**	
WBC		**RDW-SD**		**Glycemic status**	
PLT		**RDW-CV**			
EPO		**S. ferritin**		**FBG**	
				HbA1c	

V. Reference values of the parameters

Parameter	Normal range	Reference
Fasting serum glucose	70-110 mg/dL	(Güemes et al., 2016)
Hemoglobin A1c	Non- diabetic 4.5-6.3 Diabetic with good glycemic control < 7 Diabetic poor control > 7	(Haghighatpanah et al., 2018)
Serum urea	15-45 mg/dL	(Botewad et al., 2023)
Serum creatinine	0.7-1.4 mg/dL (for men) 0.6-1.1 mg/dL (for women)	(Delanaye et al., 2017)
Serum uric acid	3.5-7.2 mg/dL	(Desideri et al., 2014)
Hemoglobin	8-17 g/dL (for men) 8-16 g/dL (for women)	(Karakochuk et al., 2019)
Red blood cells	3.5-5.5 ($\times 10^6$ / µL)	(Karakochuk et al., 2019)
Hematocrit	36-50 % (for men) 36-48 % (for women)	(Karakochuk et al., 2019)
Mean corpuscular volume	80-99 fL	(Karakochuk et al., 2019)
Mean corpuscular hemoglobin	26-32 (for men) 26-38 (for women)	(Karakochuk et al., 2019)
Platelets	150-410 ($\times 10^9$/L)	(Daly, 2011)
While blood cells	4-12 ($\times 10^3$/ µL)	(Seo & Lee, 2022)

Parameter	Normal range	Reference
Red cell distribution width-standard deviation	37-54 %	(Caporal & Comar, 2013)
Ferritin	20-250 ng/mL (for men) 20-200 ng/mL (for women)	(Koperdanova & Cullis, 2015)
Erythropoietin	3.8-200 pg/mL	SUNLONG kit

الخلاصة:

داء السكري من النوع الثاني هو مرض مزمن يرتبط بمجموعة متنوعة من المضاعفات اذا لم يتم السيطرة عليه بما في ذلك مشاكل الدم والكلى مما يستلزم أهمية تقييم تأثير مضادات السكر في تقليل هذه المضاعفات والوقاية منها. كان الهدف من الدراسة هو دراسة آثار داباجليفلوزين على معايير الدم والإريثروبويتين ووظائف الكلى لدى المرضى الذين يعانون من داء السكري من النوع الثاني لتقييم سلامة وفعالية والفوائد المحتملة للداباجليفلوزين في هؤلاء المرضى. كان تصميم الدراسة عبارة عن مجموعة بأثر رجعي، وتم إجراؤها في الفترة ما بين تشرين الثاني ٢٠٢٣ ونيسان ٢٠٢٤ في عيادة خاصة في الموصل، محافظة نينوى. تضمنت الدراسة ثلاث مجموعات، المجموعة الضابطة (١) ضمت ٤١ فردًا سليمًا، المجموعة (٢) ضمت ٤٠ مريضًا مصابًا بـداء السكري من النوع الثاني يتلقون العلاج بالميتفورمين، والمجموعة (٣) ضمت ٣٠ مريضًا مصابًا بداء السكري من النوع الثاني يتلقون علاجًا مركبًا من داباجليفلوزين والميتفورمين. وشملت الخصائص الأساسية للدراسة ، العمر والجنس ومؤشر كتلة الجسم ومدة الإصابة بمرض السكري. تم قياس ومقارنة تعداد الدم الكامل ، ومستوى الإريثروبويتين، وحامض اليوريك في الدم ، واختبارات وظائف الكلى بين المجموعات الثلاث. كان مستوى الكرياتينين في الدم أعلى بشكل ملحوظ في المجموعة (٢) مقارنة بالمجموعة (١) ($p > 0.013$)، في حين أن إضافة داباجليفلوزين كعلاج إضافي للميتفورمين قلل من مستويات الكرياتينين إلى أن تكون مقاربة للمجموعة (١). وكانت مستويات اليوريا في الدم مرتفعة بشكل ملحوظ في المجموعة (٢) والمجموعة (٣) مقارنة بالمجموعة (١) ($p > 0.001$) في حين لم تكن هناك تغييرات كبيرة بين المجموعة (٢) والمجموعة (٣). علاوة على ذلك، انخفض مستوى حامض اليوريك في الدم بشكل ملحوظ مع داباجليفلوزين مقارنة بالمجموعة (٢) ($p> 0.04$). ارتفع الإريثروبويتين بشكل ملحوظ في المرضى الذين تلقوا علاج داباغليفلوزين ($p > 0.01$) مقارنة بالمجموعة (١). بالإضافة إلى ذلك، زادت أيضًا بشكل ملحوظ خلايا الدم الحمراء ، والهيموجلوبين ، والهيماتوكريت في هؤلاء المرضى. وعلاوة على ذلك، زادت بشكل ملحوظ خلايا الدم البيضاء مع العلاج بالداباغليفلوزين. بالاضاقة لذلك، هناك علاقة إيجابية بين حامض اليوريك والإريثروبويتين والمعلمات الدموية لدى المرضى الذين تلقوا داباجليفلوزين. من ناحية أخرى، هناك علاقة سلبية بين حامض اليوريك والإريثروبويتين والمعلمات الدموية لدى المرضى المشاركين في المجموعة (٢) .وخلصت الدراسة إلى أن العلاج بالداباغليفلوزين أثبت آثاره المفيدة على الكلى عن طريق خفض مستويات الكرياتينين في الدم. تم أيضًا تقليل مستوى حامض اليوريك في الدم باستخدام دواء داباجليفلوزين. ويرتبط أيضًا بتأثير مفيد على مستويات الاريثروبويتين و كريات الدم الحمراء والهيموكلوبين والهيماتوكريت.

الكلمات المفتاحية: داباجليفلوزين، داء السكري، اضطرابات الدم، مثبطات SGLT2.

قرار لجنة المناقشة

نشهد بأننا اعضاء لجنة التقديم والمناقشة قد اطلعنا على هذه الاطروحة الموسومة بـ " **تاثير الداباجليفلوزين على العوامل الدموية ووظائف الكلية لدى مرضى السكري من النوع الثاني** " وناقشنا طالب الدراسات العليا **مثنى كنعان زكي** في محتوياتها وفيما له علاقة بها بتاريخ / / ٢٠٢٤ وأنها جديرة لنيل شهادة الماجستير في الصيدلة.

التوقيع

أ. د.

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عضوا

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قرار مجلس الكلية

اجتمع مجلس كلية الصيدلة بجلسته ..... و المنعقدة بتاريخ / / ٢٠٢٤ و قرر منح

الطالب **مثنى كنعان زكي حسن** شهادة الماجستير في الصيدلة بناء على اكماله متطلبات الشهادة بنجاح.

عميد كلية الصيدلة

أ. د. زينة عبدالمنعم عبدالمجيد

/ / ٢٠٢٤

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة الموصل
كلية الصيدلة

تأثير الداباجليفلوزين على العوامل الدموية ووظائف الكلية لدى مرضى السكري من النوع الثاني

رسالة مقدمة لمجلس

كلية الصيدلة / جامعة الموصل

كجزء من متطلبات نيل شهادة الماجستير في الصيدلة

من قبل الطالب

مثنى كنعان زكي حسن

(بكالوريوس صيدلة ٢٠١٧)

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