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Effects of Sodium-glucose-co-transporter 2 (SGLT2) inhibitors on albuminuria and Glomerular filtration rate in patients with Type 2 Diabetic Nephropathy

A Thesis

Submitted to the Council of the College of Medicine , University of Babylon, as a partial fulfillment of the requirement for the degree of Master in Pharmacology/Pharmacology and Toxicology

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيم

يرفع الله الذين آمنوا منكم والذين

اوتوا

العلم درجات والله بما تعملون خبير

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Certification

*We certify that this thesis entitled "**Effects of sodium-glucose-co-transporter 2 inhibitors (SGLT2i) on albuminuria and glomerular filtration rate in patients with Type 2 diabetic nephropathy.**" was prepared by (**Samar Ali Mahmood**) under our supervision at the department of Pharmacology. College of Medicine, University of Babylon (Iraq) in partial fulfillment of the requirements for the master degree of sciences in pharmacology and toxicology.*

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Dedication

To my large family:

My parents, I could never have done anything in my life journey without your love, support and prays. Thank you for teaching me to trust Allah and believe in myself.

Mysiblings (Rasha,Yasser,Ghufran,raghad, Maisam,Kadom, Ahmed) the best gift my parents bring in my life, thanks for being my faithful friends. You give me the power to do my best.

To my small family

My husband, Ahmed Namaa thanks for your love, patience and friendship. Being always by my side makes everything possible.

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List of abbreviations:

Abbreviation key	Meaning
ACR	**Albumin to creatinine ratio**
ADA	**American Diabetes Association**
C max	**Maximum serum concentration**
CKD	**Chronic kidney disease**
DKD	**Diabetic kidney disease**
DN	**Diabetic nephropathy**
DPP-4	**Dipeptidyl peptidase-4**
FDA	**Food and drug administration**
g/dl	**Gram per deciliter**
GFR	**Glomerular filtration rate**
HbA1c	**Glycated hemoglobin**
hr	**Hour**
p.value	**Probability value**
SGLT2i	**Sodium-glucose co-transporter 2 Inhibitors**
SU	**Sulfonylurea**
T2DM	**Type 2 Diabetes mellitus**
UA	**Uric acid**
WHO	**World Health Organization**

Summary

Diabetic kidney disease is characterized by progressive kidney damage leading to increasing albuminuria, impairment in renal function (decline in glomerular filtration rate), elevated blood pressure, and excess morbidity and mortality due to risk of cardiovascular complications. Sodium-glucose co-transporter 2 (SGLT2) inhibitors are a new type of antihyperglycemic drugs, which can lower blood glucose by blocking glucose reabsorption via inhibiting (SGLT2) receptors located at the proximal renal tubule. SGLT2 inhibitors are becoming more and more popular because of their multiple actions.

The study aim to evaluate the effectiveness of SGLT2 inhibitors (Empagliflozin and Dapagliflozin) on the albuminuria and glomerular filtration rate in patients with type 2 diabetic nephropathy and to evaluate the effect of SGLT2i on weight, uric acid, HbA1c and blood pressure. This is prospective comparative study recruited patient with diabetic kidney disease were newly started SGLT2 inhibitors (empagliflozin 25mg once daily and dapagliflozin 10mg once daily) from November 2021 to January 2023. During their visits to diabetes center of the Marjan hospital, Hilla, and private clinic, Iraq. Blood samples were collected to measure HbA1C ,S. uric acid , and urine output for 24 hour to measure glomerular filtration rate and fresh urine sample to measure albumin to creatinine ratio . We collected two samples from each patient: pre-SGLT2-inhibitor therapy (baseline) and 4 months post- SGLT2-inhibitor therapy. Total patients was 132 divided into two group 66 (empagliflozin) 66 (dapagliflozin) , the number which continue the study was 100 : 39 patients taking empagliflozin and 61 taking Dapagliflozin. The average age of the participating patients was 54.1 (±7.3) years and had type 2 diabetes mellitus for 9.3 (±3.87) years. The participants included 47 male and 53 female and one-third of them (37%) had primary school degree or no formal

education, 61% of patients were also on insulin and oral antihyperglycemic agent (sulfonylurea and dipeptidyl peptidase 4 inhibitors)and 31%were only on oral antihyperglycemic agent(sulfonylurea and dipeptidyl peptidase 4 inhibitors)

Among patients with diabetic nephropathy, SGLT2 Inhibitors (empagliflozin and dapagliflozin) have positive effects on renal function as they significantly (*P-value <0.05*) improve glomerular filtration rate (by 10 ml/min) and reduce albumin to creatinine ratio (by 3.6mg/mmole) which prevent kidney deterioration. SGLT2 Inhibitors reduce body weight. SGLT2 Inhibitors significantly reduce hyperglycemia in patient with Type 2diabetis mellitus through significantly reduction in HbA1c level (by 1%). The use of SGLT2 Inhibitors can reduce blood pressure: both systolic and diastolic (by 20 and 5 mmHg respectively). SGLT2 Inhibitors also improve urinary output which probably due to improvement in renal function. SGLT2 Inhibitors significantly reduce serum uric acid concentration (by 2 mg/dl). There was non-significant (*P-value> 0.05*) difference in effect in all measured parameters between the two drugs (empagliflozin versus dapagliflozin).

In conclusions ,the results showed that using SGLT2 inhibitor (both Empagliflozin and Dapagliflozin) to treat patients with Type2 diabetic nephropathy has significant positive impact on enhancing renal function (by increase glomerular filtration rate and decrease albumin to creatinine ratio), improve HbA1c , reduces serum uric acid , reduce blood pressure (both systolic and diastolic) and reduce the body weight . The study findings support prescribing SGLT2 inhibitors for patients with type 2 diabetic nephropathy associated with hypertension, hyperuricemia,overweigh.

Chapter One Introductions and literature review

1.1. Introduction

Diabetes, also known as diabetes mellitus, is a group of metabolic disorders characterized by a high blood sugar level (hyperglycemia) over a prolonged period of time (Müller *et al*.,2016) . Symptoms include frequent urination, increased thirst and increased appetite. If left untreated, diabetes can lead to many organ complications. Acute complications can include diabetic ketoacidosis, hyperosmolar hyperglycemic state, or death. Serious long-term complications include cardiovascular disease, stroke, chronic kidney disease, foot ulcers, damage to the nerves, damage to the eyes, and cognitive impairment. The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger). Type 2 diabetes is primarily due to lifestyle factors and genetics (Kerner *et al*.,2011).

Diabetes is due to either the pancreas no longer generating enough insulin, or the cells of the body not responding well to the insulin produced with the aid of pancreas . Insulin is a hormone that is liable for assisting glucose from food get into cells for use for strength (Roden *et al*.,2012).

Diabetes mellitus have affected greater than 415 million adults worldwide. It is expected that more than 640 million adults will be affected by DM by 2040 (Ogurtsova K *et al*., 2017). About 35% of type 2 diabetes mellitus (T2DM) develop into diabetic kidney disease (de Boer *et al*.,2011). Diabetic nephropathy has largely increased the incidence and prevalence of the end stage renal disease (ESRD). However, multiple strategies in treating diabetic nephropathy including optimal glycemic control, blood pressure control and avoidance of nephrotoxic agents (Tomino *et al*.,2015).Therefore, it is essential to develop powerful

therapies to prevent development of diabetic nephropathy (Ahola and Groop .,2013).

Almost, 40% of diabetic patients will develop diabetic nephropathy during their lifetime (de Boer *et al*.,2011).

Sodium-glucose cotransporter-2 inhibitors (SGLT2i) receptors block sodium-dependent glucose transporter-2 (SGLT2) located in the early proximal renal tubule that responsible for reabsorption of most (80–90%) of the glucose filtered by the glomerulus (Vallon ,2015).

SGLT2 inhibitors are becoming more famous because of their multiple advantages over other antihyperglycemic agent .In addition to its main action in glycemic control, SGLT2 inhibitors can also lower body weight and blood pressure , uric acid and decrease relevant adverse cardiovascular consequences in T2DM patients with high cardiovascular risk (Wiviott *et al*.,2019).

A secondary-prespecified analysis of the EMPA-REG OUTCOME trial has shown that empagliflozin(SGLT2i) reduced the rate of the kidney deterioration (progression to macro albuminuria, doubling of serum creatinine accompanied by an eGFR of ≤45 mL/min/1.73 m2, initiation of renal replacement therapy, or death from renal complications) by 39% ($p<0.001$) (Wanner *et al*.,2016).

1.2. Aims of study:

1. Evaluate the effect and safety of SGLT2i both empagliflozin and dapagliflozin on the albuminuria and GFR in Iraqi patients with type 2 diabetic nephropathy .

2. Evaluate the effect of SGLT2i on weight, blood pressure, uric acid, HbA1c .

3. Compare the difference in effect between empagliflozin and dapagliflozin in regarding Hba1C ,weight Blood pressure ,GFR,ACR, uric acid

1.3. Type 2 Diabetes mellitus

Diabetes mellitus is a general term for heterogeneous disturbances of metabolism for which chronic hyperglycemia the main finding. Diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of many organs. Diabetes mellitus present with common symptoms such as thirst, polyuria, blurring of vision, and weight loss .The cause is either due to impaired insulin secretion or impaired insulin action or both. Diabetes mellitus (DM) has been recognized by the World Health Organization (WHO) as one of the four major non-communicable diseases that should need urgent interest from all key shareholders; seen as the third highest risk factor for worldwide premature mortality due to hyperglycemia (Duncan ,2015).

1.3.1. Etiology

Diabetes mellitus is classified mainly into three types by etiology and clinical presentation, type 1 diabetes, type 2 diabetes, and gestational diabetes. Other less common types of diabetes that include monogenic diabetes and secondary diabetes (Carrillo *et al*.,2019).

1. Type 1 DM is also called insulin-dependent diabetes or juvenile-onset diabetes because it often begins in childhood. And it is characterized by

autoimmune destruction of insulin-producing beta cells in the islets of the pancreas. As a result, there is an absolute deficiency of insulin (Vana *et al*.,2019).

Teplizumab-mzwv, a monoclonal antibody that delayed the onset of clinically diagnosed stage 3 type 1 diabetes by a median of about 2 years (Herold *et al*.,2019)

2. Type 2 DM, also called non-insulin-dependent or adult-onset diabetes, but it's become more common in young over the past 20 years, largely because more young people are overweight or obese. Type 2 diabetes mellitus (T2DM) accounts for around 90% of all cases of diabetes. In T2DM, the response to insulin is decrease, and this is defined as insulin resistance (IR). During this condition, insulin is ineffective and is initially countered by an increase in insulin production to maintain glucose homeostasis, but with time, insulin production will decreases, lead to T2DM, it is increasingly seen in children, adolescents, and younger adults due to increasing levels of obesity(sedentary lifestyle), physical inactivity, and energy-dense food (Khan *et al*.,2020).

3. Gestational Diabetes Mellitus also known as hyperglycemia in pregnancy is first detected during pregnancy, It complicates 7% of all pregnancies (Chiefari *et al*.,2017).

4. Specific types of diabetes due to other causes, e.g., monogenic diabetes syndromes, diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (Hattersley and Patel .,2017).

1.3.2 Signs and Symptoms of Diabetes

The more common symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger).(as shown in figure 1.1) ,Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they develop much more slowly and may be absent in type 2 diabetes (Rockefeller .,2015).

Figure 1.1 Overview of the most significant symptoms of diabetes (Rockefeller .,2015).

1.3.3. Epidemiology and incidence of type 2 DM

Epidemiological data show alarming values that expecting a worrisome projected future for T2DM. According to the International Diabetes Federation , in 2019, diabetes also caused 4.2 million deaths; and 463 million adults aged

between 20 and 79 years old were living with diabetes, a number that may be rise up to 700 million by 2045 (Xu *et al* .,2017).

In Iraq, Around 1.4 million of Iraqis people have diabetes (WHO.,2018).

1.3.4. Risk factor for T2DM

Complex combination of genetic, metabolic and environmental factors includes the risk factor of T2DM. Genetic predisposition plays an important part in the risk of developingT2DM (Fuchsberger *et al*.,2016).

Patients with diabetes can be educated about the complication of the disease and treatment protocol , dietary changes, and exercise, with the goal of keeping both short-term and long-term blood glucose levels with in normal range (as shown in figure 1.2)

In addition, given the associated with more risks of cardiovascular disease, lifestyle modifications and diet are recommended to control blood pressure (Haw *et al*.,2017).

Weight loss also can prevent progression from prediabetes to diabetes type 2, also decrease the risk of cardiovascular disease, or result in a partial remission in patient with diabetes (Evert *et al*.,2019).

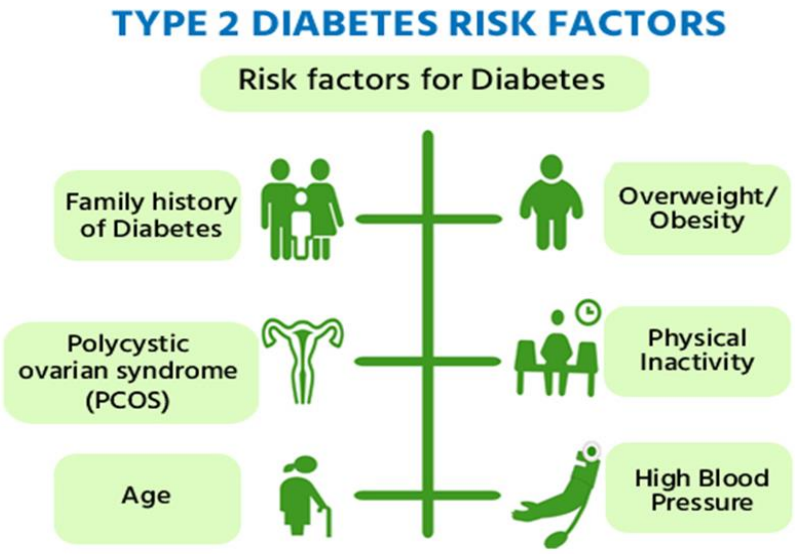

Figure1.2. risk factor of diabetes (Haw *et al*.,2017).

1.3.5. Complications

The complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves. Damage to the eyes, known as diabetic retinopathy and can lead to gradual vision loss and blindness. also increases the risk of having glaucoma, cataracts . other complication is Damage to the kidneys, known as diabetic nephropathy, eventually chronic kidney disease Damage the nerves of the body, known as diabetic neuropathy, is the most common danger complication of diabetes (Papatheodorou *et al*.,2018).(as shown in figure 1.3)

From the other hand, These complications are also related to a strong risk factor for severe COVID-19 illness (Kompaniyets *et al.*,2021).

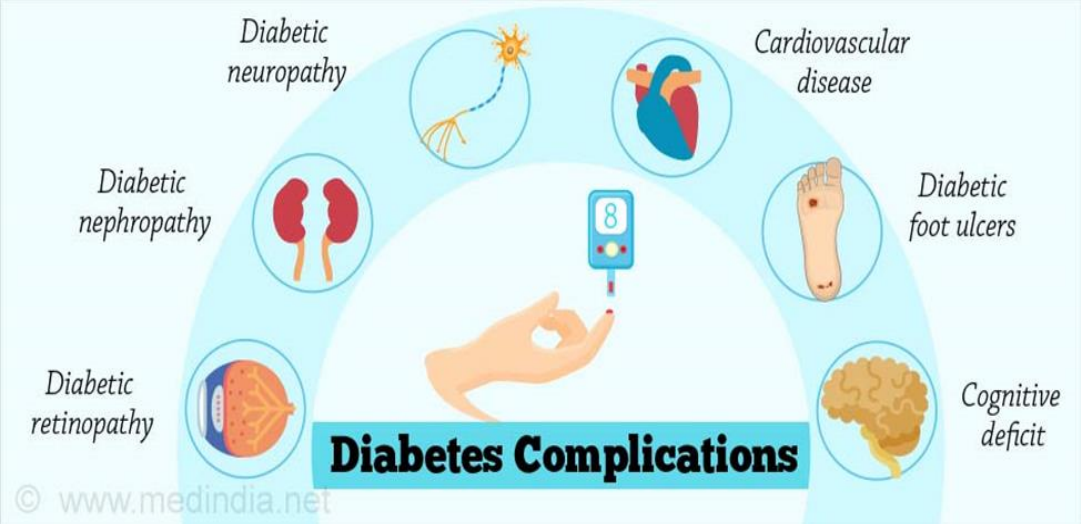

Figure 1.3: Complication of diabetes (Papatheodorou *et al*.,2018)

1.3.6. Diagnosis of T2DM:

The American Diabetes Association "Standards of Medical Care in Diabetes" includes ADA's current clinical practice recommendations, Diabetes can be diagnosed with any one of the following criteria (ADA,2019).

• Fasting plasma glucose (FPG) ≥ 126 mg/dL or

• Oral glucose tolerance test (OGTT) using 75 g of anhydrous glucose with fasting plasma glucose (FPG) ≥ 126 mg/dL and/or 2-hour plasma glucose ≥ 200 mg/dL

• Glycated hemoglobin (A1C) ≥ 6.5% or

• Random plasma glucose ≥ 200 mg/dL in the presence of classical diabetes symptoms

• Glycated hemoglobin is better and accurate than fasting glucose for determining risks of cardiovascular disease and death (Selvin *et al*.,2010).

1.3.7. Management of type 2 diabetes mellitus

1- Lifestyle modification

Weight loss and healthy diet with daily exercise can prevent progression from prediabetes to diabetes type 2 and must decrease Dietary salt intake (Evert *et al*.,2019).

2- Medications

There are a number of different classes of anti-diabetic medications describe briefly by (as shown in Table 1.1)

Table 1.1 .medication to treated DM (Evert *et al*.,2019).

Class	Drug
Biguanides	Metformin
Sulfonylureas	Glibenclamide, glipizide, gliclazide, glimipride
α-glucosidase inhibitor	Acarbose
DDP-IV Inhibitors	Sitagliptin, saxagliptin, vildagliptin, linagliptin
GLP1 receptor agonists	Liraglutide, exenatide, lixisenatide
SGLT2 Inhibitors	Dapaglifozin, empagliflozin, canagliflozin [pending]
Insulins	Human Insulin R, Mix 70/30, NPH, glulisine, insulin aspart, detemir, glargine, degludec

DDP-IV, dipeptidyl peptidase-4; GLP1, glucagon-like peptide 1; SGLT2, sodium glucose cotransporter-2.

Mounjaro (Tirzepatide) is a new drug for type 2 diabetic mellitus act as dual GIP/GLP-1 receptor agonist which means it works on the glucose-dependent

insulinotropic polypeptide (GIP) receptors and the glucagon-like peptide-1 (GLP-1) receptors. GLP-1 and glucose-dependent insulinotropic polypeptide (GIP) are two incretin hormones released by the gut to aid in blood glucose control. Increases in these hormones lead to

- insulin release
- increased satiety
- decreased glucose production(Salvon,2022)

1.4. Diabetic kidney disease

Diabetic kidney disease (DKD) is a serious complication of diabetes that can be life-threatening, DKD consider a major cause of morbidity and mortality in diabetes. Certainly, the excess mortality of diabetes occurs mainly in individuals with diabetes and proteinuria, and results not only from end-stage renal disease but also from cardiovascular disease, with the latter being particularly common in patients with type 2 diabetes (Tuttle *et al*.,2014). Clinically, diabetic kidney disease (DKD) is characterized by progressive kidney damage leading to increasing albuminuria, impairment in renal function (decline in glomerular filtration rate [GFR]), elevated blood pressure, and excess morbidity and mortality due to risk of cardiovascular complications. Diabetic kidney disease rarely occur in patients with type 1 diabetes before ten years following diagnosis with type 1 diabetes , whereas nearly 3% of patients with recently diagnosed with type 2 diabetes already have diabetic nephropathy (Afkarian *et al*.,2016).

1.4.1. Staging of DKD

Classically, DKD is divided into 5 stages as showed in table 1.1. Growing evidence suggests that proteinuria does not always occur preceding the loss of renal function in diabetes (Porrini *et al*., 2015).

Table1.2. Clinical staging (Fink *et al*., 2012).

CKD Stage	eGFR level (mL/min/1.73 m^2)
Stage 1	≥ 90
Stage 2	60–89
Stage 3	30–59
Stage 4	15–29
Stage 5	< 15

1.4.2. Prevalence of DKD

The International Diabetes Federation (IDF) in 2017 predicted that there were 451 million people with diabetes worldwide, and the number was may be to increase to 693 million by 2045 , In addition, the prevalence of diabetes in female is estimated to be 8.4%, which is slightly lower than the prevalence in male (9.1%) nearly (N.H. *et al*.,2017).

Among these diagnosed cases of diabetes, about 90% of patients have T2DM, and nearly half of T2DM patients eventually progress to chronic kidney disease (CKD) (R. Saran et al.,2017). it was estimated that, among people with aged 20–79 years, 425 million had diabetes, 50% were undiagnosed, and approximately 4.0 million died, which accounted for 14.5% of global all-cause mortality among people in this age range (S. Karuranga *et al*.,2017).

1.4.3. Pathophysiology

The disease progression of diabetic nephropathy involves various clinical stages: hyperfiltration, microalbuminuria, macroalbuminuria, nephrotic proteinuria to progressive chronic kidney disease leading to end-stage renal disease (ESRD). The damage is exerted on all compartments of the kidney: the glomerulus, the renal tubules, the vasculature (afferent and efferent renal arterioles) and the interstitium. Renal fibrosis is the final common pathway of DN. This fibrosis is a product of multiple mechanisms including renal hemodynamic changes, glucose metabolism abnormalities associated with oxidative stress as well as inflammatory processes and an overactive renin-angiotensin-aldosterone system (RAAS).The pathophysiology of diabetic nephropathy is thought to involve an interaction between hemodynamic and metabolic factors (Lin *et al*.,2018)

Hemodynamic factors include an increase in systemic and intraglomerular pressure, as well as the over-activation of the RAAS. Studies have shown that in the setting of diabetes, various factors stimulate the RAAS, which is one of the most important pathways in diabetic nephropathy pathophysiology. Due to the higher load of filtered glucose, there is an up-regulation in the sodium-glucose cotransporter 2 (SGLT2) in the proximal tubules, which cotransports sodium and glucose back into circulation. This leads to a decrease in the delivery of sodium chloride to the macula densa in the distal tubules, promoting the release of renin and over-activating RAAS. Hyperfiltration is one of the earliest features of DN. Several mechanisms have been proposed to cause hyperfiltration. One of these mechanisms is that as glomeruli becomes hypertrophied, filtration surface area initially increases. Another possible mechanism is that abnormal vascular control

in diabetic nephropathy leads to a reduction in afferent glomerular arteriolar resistance and an increase in efferent glomerular arteriolar resistance, leading to a net increase in renal blood flow (RBF) and glomerular filtration rate (Hostetter *et al*.,2003)

Metabolic factors include the formation of Advanced glycation end-products (AGEs), which have a central role in the pathophysiology of many of the complications of diabetes mellitus, including cardiovascular complications.AGEs are chemical groups that form when a reducing sugar (glucose in this case) reacts non-enzymatically with an amine group, predominantly lysine and arginine, which are attached on proteins, lipids and nucleic acids.These glycation products accumulate on the proteins of vessel wall collagen, forming an irreversible complex of cross-linked AGEs. An important way AGEs exert their effect is through a receptor-mediated mechanism, most importantly by the receptor for advanced glycation end products (RAGE). RAGE is a signal transduction receptor found on a number of cell types including macrophages, endothelial cells, renal mesangial cells and podocytes in the glomerulus. Bindings of AGEs to RAGE receptors enhances production of cytosolic Reactive Oxygen Species (ROS) as well as stimulates intracellular molecules such as Protein Kinase C (PKC), NF-κB and the activation of growth factors TGF-B and vascular endothelial growth factor (VEGF). These factors, along with the hemodynamic changes that occur, lead to podocyte injury, oxidative stress, inflammation and fibrosis. As injury worsens, kidney function decreases and glomerular basement membrane (GBM) become more permeable and less efficient at filtration. This is accompanied by a steady decline in kidney function (Soldatos *et al*.,2008).

1.4.4. Sign and symptom of DKD

The onset of symptoms of DKD is 5 to 10 years after the diabetic started . A common first symptom is frequent urination at night (nocturia) . Many other symptoms include tiredness, headaches, a general feeling of illness, nausea, vomiting, polyuria, lack of appetite, itchy and dry skin, and also leg swelling. (As shown in Figure 1.4)

The clinical presentation of diabetic nephropathy (DN) is characterized by the main clinical presentations

- Proteinuria (protein in the urine)
- Hypertension (increase both systolic and diastolic)
- Progressive loss of kidney function (decrease in GFR)

The process may be begin indolent, so regular and annual screening for diabetic nephropathy in patients with T2DM is highly importance to avoid end stage kidney failure (Mora-Fernández *et al*.,2014).

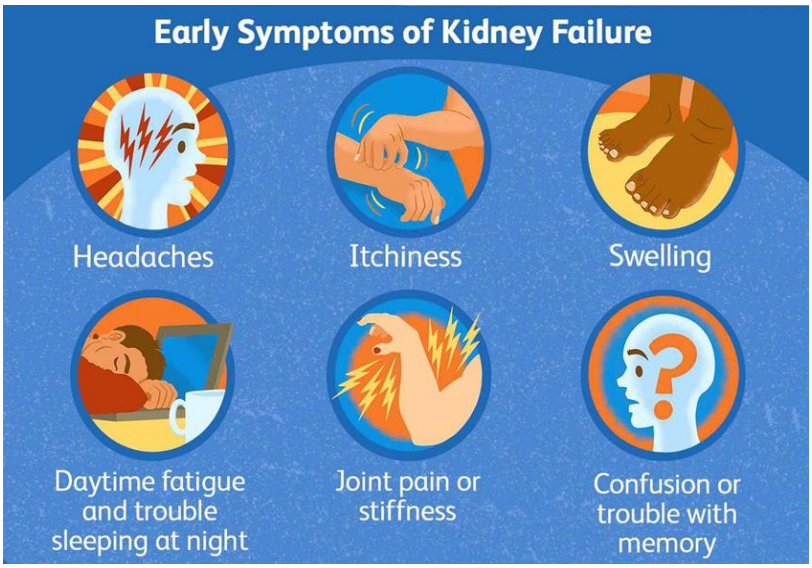

Figure 1.4.early symptom of diabetic nephropathy(Mora-Fernández *et al*.,2014).

1.4.5. Screening of DKD

Annual checking of all patients with diabetes is very critical to expose any abnormal levels of albuminuria and renal capacity for good filtration (GFR), so that early renoprotective treatment should be started as soon as possible.

Morning (spot) urine collections are enough for screening and monitoring, and it is easy for the entire patient. It should be noted that urinary albumin excretion may be elevated regardless of kidney disease or degree of kidney deterioration, the factors that also lead to increase albumin in urine such as severe exercise within twenty four hours, severe urinary tract infection, menstruation, heart failure, and marked hyperglycemia so albumin to creatinine(ACR) consider more correct and accurate for diabetic kidney disease (DKD) screening .

The other (second clinical test) that used in screening for diabetic kidney disease is glomerular filtration rate (GFR) that shows how well the kidneys are filtering, and it consider more accurate ,GFR decrease with age , normal results range from **90 to 120 mL/min/1.73 m^2** (as shown in figure 1.5), over use of non-steroidal anti-inflammatory drugs the most drug that reduce GFR (Levey *et al*., 2009).

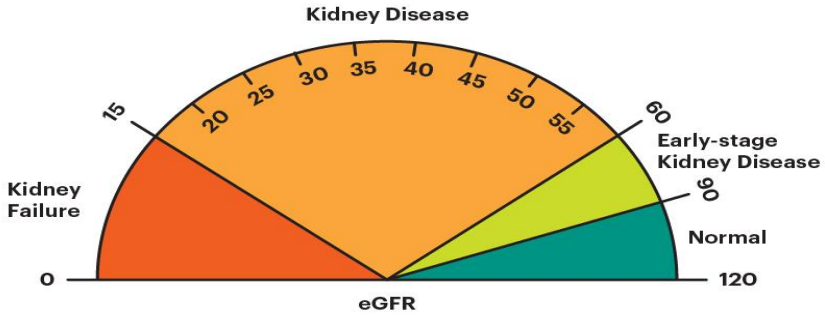

Figure 1.5.normal range of glomerular filtration rate(Levey *et al*., 2009).

1.5. Sodium-glucose co-transporter 2 Inhibitors (SGLT2i).

SGLT2 inhibitors, also called gliflozins or flozins, are a type of medications that act on sodium-glucose transport proteins in the nephron (the functional units of the kidney), unlike SGLT1 inhibitors (figure 1.6) that have same function in the intestinal mucosa so selective SGLT2i drug is require (Shubrook *et al*.,2015).

SGLT	Expressed in human tissues
SGLT1	Intestine, trachea, kidney, heart, brain, testis, prostate
SGLT2	Kidney, brain, liver, thyroid, muscle, heart

Figure (1.6) : expressed of SGLT in human tissue (Shubrook *et al*.,2015).

Sodium–glucose co-transporter 2 inhibitors (SGLT2is) , are group of drugs licensed for the treatment of T 2 diabetes mellitus (T2DM) when diet and lifestyle modification have failed to improved glycemic control very well , SGLT2i also recently licensed by both European Medicines Agency and US Food and Drug Administration for the treatment of heart failure with reduced ejection fraction in those with or without diabetes. In diabetes, they improve glycemic control by causing:

- Glycosuria
- Natriuresis
- Osmotic diuresis.

Their effect is totally independent of insulin and they are thought to exert beneficial action through pleiotropic mechanisms beyond improved glycaemia, including: favorable hemodynamic changes (Verma and McMurray .,2018).

The first SGLT2i discovered was phlorizin, a naturally occurring compound derived from apple tree bark. But due to its non-selective nature, it can lead to undesired side effect like severe gastrointestinal symptoms. Due to its non – selective nature and to its poor oral bioavailability, working on its development could not continue (Ehrenkranz *et al*.,2005).

1.5.1. Mechanism of Action

Sodium-glucose co-transporter-2 inhibitors work by inhibiting SGLT2 in the proximal convoluted tubules to prevent reabsorption of glucose and facilitate its excretion in urine ((glycosuria). when glucose is excreted, glucose plasma levels

will decrease and leading to an improvement in all other glycemic parameters (Hummel *et al*.,2011). (as shown in figure 1.7)

Pharmacologic inhibition of SGLT2 in the kidney will reduces 50% for the kidney capacity to reabsorption of glucose

The mechanism of SGLT2 inhibition occurs independently of insulin secretion, and its mechanism not affecting the following factors:

- pancreatic β-cells function
- The degree of insulin resistance (M.A. Abdul-Ghani *et al*.,2008).

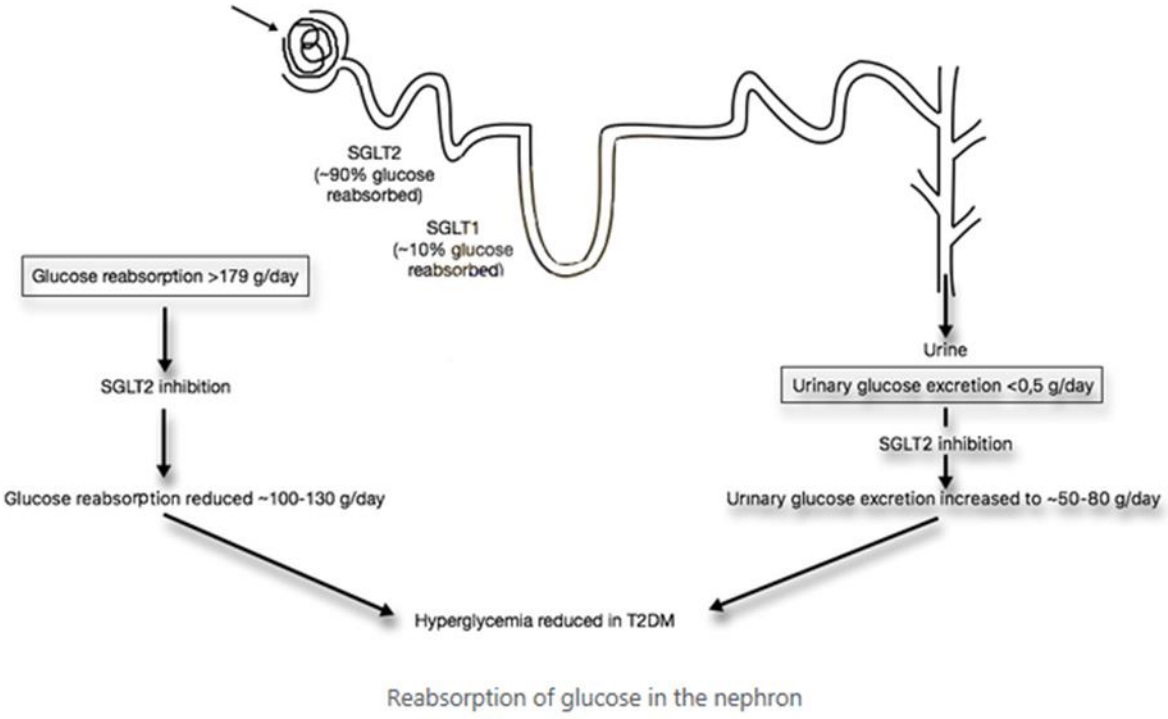

Figure (1.7).mechanism of action of SGLT2 i. SGLT2 I (sodium glucoseco-transporter inhibitors (Hummel *et al*.,2011).

1.5.2. Clinical Pharmacology

In 2022, American Diabetes Association standards of medical care in diabetes include SGLT2 inhibitors as a first line pharmacological therapy for type 2 diabetes (usually together with metformin), specifically in patients who suffer from diabetes complications like diabetic kidney disease, cardiovascular disease or heart failure

Pharmacokinetic parameters of various medications (as showed in table 1.3) of this class that include :

- ✓ Elimination half-life
- ✓ Bioavailability
- ✓ Protein binding
- ✓ The blood concentration Cmax at time tmax
- ✓ The SGLT2 selectivity over SGLT1.

These medications are excreted in the urine as inactive metabolites (Madaan *et al.*,2016)

Table 1.3: pharmacokinetic parameters of various SGLT-2i.(Madaan *et al*.,2016)

Name of drug	Bioavailability	Protein binding	tmax (hours)	t1/2 (hours)	Cmax	SGLT2 selectivity over SGLT1
Canagliflozin	65% (300 mg dose)	99%	1–2	10.6 (100 mg dose); 13.1 (300 mg dose)	1096 ng/mL (100 mg dose); 3480 ng/mL (300 mg dose)	250 fold
Dapagliflozin	78%	91%	1–1.5	12.9	79.6 ng/mL (5 mg dose); 165.0 ng/mL (10 mg dose)	1200 fold
Empagliflozin	90–97% (mice); 89% (dogs); 31% (rats)	86.20%	1.5	13.2 (10 mg dose); 13.3h (25 mg dose)	259nmol/L (10 mg dose); 687nmol/L (25 mg dose)	2500 fold
Ertugliflozin	70-90%	95%	0.5-1.5	11-17	268 ng/mL (15 mg dose)	2000 fold
Ipragliflozin (50 mg)	90%	96.30%	1	15–16 (50 mg dose)	975 ng/mL	360 fold
Luseogliflozin	35.3% (male rats); 58.2% (female rats); 92.7% (male dogs)	96.0 –96.3%	0.625±0.354	9.24±0.928	119±27.0 ng/mL	1650 fold
Tofogliflozin (10 mg)	97.50%	83%	0.75	6.8	489 ng/mL	2900 fold

- Cmax: Maximum serum concentration that drug achieves in body after the drug has been and administrated
- tmax: Time to achieve maximum plasma concentration
- t1/2: Biological half-life

In contrast with other anti-hyperglycemic diabetes medications, SGLT2 inhibitors increase, rather than suppress, gluconeogenesis (metabolic pathway that results in generation of glucose from non-carbohydrate carbon substrates) and ketogenesis (biochemical process through which organisms produce ketone bodies by breaking down fatty acids and ketogenic amino acids) , on the other hand SGLT2 inhibitors also can activate other substance sirtuin 1 , so they are more

cardioprotective and renoprotective than the other oral medications that used for treat diabetes patients with or with not heart problem (Packer ,2020).

Food consumption does not significantly affect the efficacy of SGLT2i (Kasichayanula *et al.*,2011)

1.5.3. Alternative pharmacological actions

The cardioprotective effects of SGLT2 inhibitors have been related to the elevated ketone levels (Kolb *et al*.,2021).

Gliflozins like empagliflozin and others have been posited to exhibit protective effects on many organ like the heart, liver, kidneys, anti-hyperlipidemia, anti-atherosclerotic, anti-obesity Pleiotropic effects of this class have been attributed to a variety of its pharmacodynamics actions such as :

- ✓ Natriuresis
- ✓ Hemoconcentration
- ✓ Deactivation of renin-angiotensin-aldosterone system
- ✓ Ketone body formation, alterations in energy homeostasis.
- ✓ Glycosuria
- ✓ Lipolysis
- ✓ Anti-inflammatory
- ✓ Antioxidative actions (Varzideh ,2020).

1.5.4. FDA approval of SGLT2 inhibitors.

• Dapagliflozin is the first SGLT2 inhibitor approved in the world by the European Union in (2012). It was approved for use in the United States under the brand name Farxiga by the Food and Drug Administration in January (2014).(Fujita and Inagaki .,2012)

• Empagliflozin, approved in the United States in August 2014, under the brand name Jardiance by Boehringer Ingelheim (FDA,2014) Of the gliflozins, empagliflozin and Tofogliflozin have the highest specificity for SGLT2 inhibition (Shubrook *et al*.,2015).

The FDA has approved recent SGLT2i bexagliflozin (Brenzavvy), a once-daily, oral, sodium-glucose co-transporter 2 (SGLT2) inhibitor as an adjunct therapy to diet and exercise for the improvement of glycemic control in adults with type 2 diabetes, treatment with bexagliflozin for patients with diabetic nephropathy who have GFR =30 and above (Shubrook *et al*.,2015).

1.5.5. Posology

Dapagliflozin is approved orally (tablet) as 10 mg once-daily drug, as monotherpy, or as add-on with other drugs like (metformin, sulfonylurea , dipeptidyl peptidase-4 (DPP-4) inhibitors, and/or insulin) . Also it can be prescribed alone as first line therapy to patient who do not tolerate or cannot take metformin safely.

A 5-mg dose is also available for patients who have hepatic insufficiency. In case of mild to moderate hepatic impairment, no dosage adjustment is required, while, in severe hepatic impairment, 5 mg of dapagliflozin dose is require.

Empagliflozin is oral tablet with daily doses of 10 and 25 mg. Trials have showed dose-dependent HbA1c reductions with empagliflozin monotherpy. Empagliflozin can be administered orally without relation to meal timings, in a once-daily frequency oral tablet (Fujita and Inagaki ,2014).

1.5.6. Adverse events.

The incidence of adverse events in clinical trials of SGLT2i is similar to that observed with other anti-diabetic drugs like sulfonylurea. The incidence of adverse events has Varity ranged from 57.3 to 83.0%, while other side effect that considered serious (more danger) have varied between 1.0% and 12.6% (Rossenwasser *et al*.,2013).

The most common adverse events in patients that recently on SGLT2i showed are uro-genital tract infections, especially in female gender and in uncircumcised male ,Common infections that occur with SGLT2i included the following ;

- vulvitis and vulvo-vaginitis in women
- Balanitis and balanoposthitis in men.

The infection that occur from SGLT2i can be prevented by :

- ✓ Maintaining adequate and good hygiene
- ✓ Treatment with oral antifungal therapy

The reason for these infection (genital infections) are thought to be caused by an increased glucose load in the urinary tract, which encourages and facilitate the fungal growth. The incidence of urinary tract infections does not increase with SGLT2i therapy (Kalra *et al*.,2014).

Due to they have a non-insulin-based mechanism of action so Hypoglycemia with SGLT2i is minimal. However, hypoglycemia may be occur when SGLT2i are used in combination with other anti-diabetic drugs, including Biguanides(metformin) (Rohwedder *et al*.,2014).

Sodium-glucose co-transporter-2 inhibitors use may be leads to a small reduction in bone formation and a rise in bone resorption markers, although there are no major changes on bone mineral density. On the other hand, other long-term studies have not demonstrated any significant increase in adverse drug reactions related with skeletal system with any one of the SGLT2i(Stenlof *et al*.,2014)

In August /2018, the food and drug administration (FDA) issued a warning of an increased risk of Fournier gangrene in patients using SGLT2 inhibitors (Isaacs *et al.,2018)*

In May 2015, the FDA issued a warning that SGLT2 i can increase risk of diabetic ketoacidosis (Hsia *et al*.,2016).

Diabetic ketoacidosis occur by reducing glucose blood circulation, gliflozins can cause less stimulation of endogenous insulin secretion or lower dose of exogenous insulin that results in diabetic ketoacidosis. They can specifically cause euglycemic diabetic ketoacidosis (euDKA, DKA where the blood sugar is not

elevated) because of the renal tubular absorption of ketone bodies (Isaacs *et al*.,2017).

The most high risk period for ketoacidosis is the perioperative period. SGLT2 inhibitors may need to be stopped before surgery, and only require when patients is not unwell, is adequately good hydrated and able to consume a regular diet (Milder *et al*.,2018).

After surgery, to decrease the risk of developing ketoacidosis (a serious condition in which the body produces high levels of blood acids called ketones), the FDA has approved changes to the prescribing information for SGLT2 inhibitor diabetes medicines to recommend, they must be stopped temporarily before surgery. Canagliflozin, dapagliflozin, and empagliflozin should be stopped at least three days before surgery(Milder *et al*.,2018).

1.5.7. Effect of Glomerular Health, as Measured by GFR, on the Efficacy of SGLT2 Inhibitors.

The ability of SGLT2i to increase the urinary glucose execration primarily depends on GFR, it is observed that their pharmacodynamics actions gradually decreases with increasing severity of renal dysfunction (Ferrannini *et al*.,2013) . many recent clinical studies showed the efficacy of SGLT2i in patients with varying degrees of renal impairment suggest that the glucose-lowering capacity of the SGLT2i, as assessed by a reduction in HbA1c, is maintained the same in patients with mild renal impairment (stage 2 CKD; estimated glomerular filtration rate [eGFR] 60–90 mL/min/1.73 m2). But The glucose-lowering capacity decreases in patients with moderate renal impairment (stage 3 CKD: eGFR ≥30 to

<60 mL/min/1.73 m2) and the glucose –lowering capacity limited in patients with severe renal impairment (stage 4 CKD: eGFR <30 mL/min/1.73 m2), but in renal failure, the use of SGLT2i with no glycemic improvement so the SGLT2i in renal failure will not recommended (Scheen ,2015).

1.5.8.Effect of SGLT2 inhibitors on Renal Function

The mechanism of action of SGLT2i mainly depends on filtration of glucose at the glomerulus. So , SGLT2i are less effective in patients with renal impairment. However, recent evidence suggests that SGLT2i have clear nephroprotective effect in patient with diabetic nephropathy (DN) . The effect of SGLT2i on the kidney have been shown to reduce the following:

- Glomerular hyperfiltration
- Systemic and intraglomerular pressure, and therefore, the progression of diabetic kidney disease DKD (Terami *et al*.,2014).

The causes for the usage of SGLT2i as a nephroprotective remedy is compelling as SGLT2i has the capability to regulate the pathophysiologic process suggested in the tubular hypothesis. So SGLT2 inhibitors increase the sodium shipping to the distal convoluted tubule in the kidney thereby increasing intracellular adenosine, main to afferent arteriolar vasoconstriction, which results in decreased 3 main actions:

1. Glomerular pressure
2. Hyperfiltration
3. Renal injury (Cherney *et al*.,2013).

1.5.9. Drug–Drug Interactions

- Sodium-glucose co-transporter-2 inhibitors do not exhibit any clinically drug–drug interactions, including with other anti-diabetic drugs and diuretics. SGLT2i also can be combined with metformin, sulfonylureas(SU) , pioglitazone, sitagliptin, and voglibose
- Interactions are important for SGLT2 inhibitors because most T2DM patients are taking many other medications.
- SGLT2i appear to increase the diuretic effect of thiazides, loop diuretics and related diuretics and so may increase the risk of the following effect :

✓ Dehydration

✓ Hypotension (Scheen ,2014)

1.5.10. Clinical Use

Sodium-glucose co-transporter-2 inhibitors can be used as initial monotherpy in persons in whom metformin is not indicated, or not tolerated. Examples include those with hepatic impairment and gastrointestinal intolerance. SGLT2i can also be used as second-line drugs, and third-line drugs, in persons inadequately controlled on a single or dual glucose-lowering therapy (Usman *et al* .,2018)

Their action that show glucose-lowering effect may help some people with diabetes to delay for need to insulin injections. Additionally, a significantly due to low risk of hypoglycemia with these agents can improve outcomes and compliance considerably.

The pleiotropic effects of SGLT2i : (as shown in figure 1.8)

- body weight loss
- Decrease Blood pressure(systolic and diastolic)
- Uricosuria

The pleiotropic effect of SGLT2i makes them attractive for use in patients with metabolic syndrome, and in those with mild fluid overload. Due to oral mode of administration may favor their use over the injectable glucagon-like peptide-1 receptor agonists like dulaglutide .

This class of drugs(SGLT2i) can also be used in combination with insulin, and its insulin-sparing effect allows for more effective, well-tolerated glycemic control, without causing weight gain additionally (Wilding *et al*.,2012).

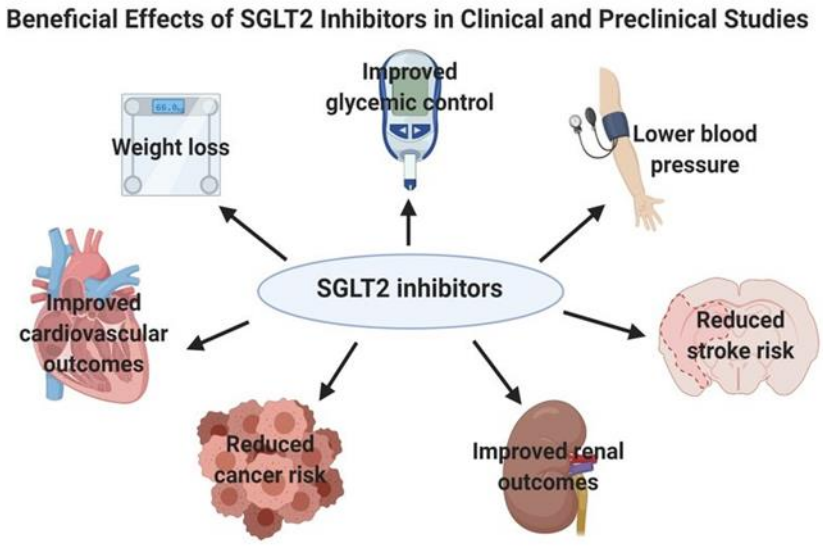

Figure (1.8) clinical effect of SGLT2i (Wilding *et al*.,2012)

1.5.11. Monitoring

- Due to the event of ketoacidosis, SGLT-2 inhibitor should be discontinued, and management for ketoacidosis should be started such as insulin, intravenous fluids, and carbohydrate replacement
- When diabetic patients on insulin therapy , so When an SGLT2 inhibitor require to be started with insulin so , dose reductions in insulin and insulin secretagogues may be required to avoid hypoglycemia
- Diabetic patients with SGLT2i therapy should also be monitored for any signs and symptoms of necrotizing fasciitis of the perineum, also have another termed "Fournier's Gangrene," as such cases have been reported with some patient that starting SGLT-2 inhibitors (Scheen ,2019).

1.5.12. Toxicity

The prescribe of SGLT-2 inhibitors during pregnancy for control blood sugar may cause inherent risk to the fetus, particularly during the second and third trimesters of pregnancy so its use during pregnancy not recommended, on the other hand SGLT2i therapy during lactation is also not recommended (Elkind-Hirsch *et al*.,2020).

2.1. Patients

The patients were enrolled in the study with T2DM and established with nephropathy , during their visit to diabetic center of Margan Hospital, Hilla, and private clinic, total patient was132 and only 100 patients complete the study aged 39- 65 years and have DM for at least 5 years and above .

2.1.1. Patient criteria

2.1.1. A. Inclusion Criteria

1. Patients whom they have been diagnosed with type 2 DM for at least 5 years or more and diagnosis with diabetic nephropathy diagnosis by measuring GFR and ACR
2. Female or male aged 18-65 year.

2.1.1.B .Exclusion criteria

Patients with type 2 diabetes mellitus who are:

1) Mentally unstable
2) History of kidney transplantation
3) Extreme ages(more than 65 years)
4) Type 1 diabetes.
5) Pregnant and lactated women

6) Patients with other causes of kidney disease like systemic lupus erythromatous.

2.1.1.C. Ethical and official approval

- The proposal of the research was discussed and approved by the scientific and ethical committee in the college of medicine –Babylon university
- All patients were verbally informed about the study and they were asked the permission to make them be part of the study and all personal information was kept anonymous.
- Approval from Diabetes Center in Al-Margan medical city also was granted.

2.1.2. Study design

This was prospective study design (comparative), to investigate the effect of two type of SGLT2 inhibitors(dapagliflozin and empagliflozin) on the albuminuria and GFR in patients with type 2 diabetes mellitus and established with nephropathy by measuring baseline value of GFR and ACR and then compared it with post treatment value , conducted in the Diabetes Center in Al-Murjan medical city, Hilla, Iraq and private clinic during the period from November, 2021 to January 2023

2.1.3. Study groups

The patients were assigned into two main groups:

Group 1: include 39 T2DM patients assigned to be treated with empagliflozin oral tablet 25 mg once daily at morning

Group 2 : include 61 T2DM patients assigned to be treated with dapagliflozin oral tablet 10mg once daily at morning

2.1.4. Demographic information

- ✓ Age
- ✓ Gender
- ✓ Smoking status
- ✓ level of education divided in to 3 level
  - Primary or less = 1
  - Middle and secondary =2
  - College or higher=3

2.1.5. Study intervention

Initially, the study recruited 132 diabetic patients with DKD who were assigned into two groups: 66 on dapagliflozin (10 mg once daily) and 66 on empagliflozin (25 mg once daily). After four months, 27 patients on empagliflozin discontinued the study and five patients on dapagliflozin discontinue the study. Five of the discontinued patients (dapagliflozin group) revealed the reasons behind their dropout from the study: four patients felt well and refused to continue the study and one patient had low income and were unable to purchase the SGLT2 inhibitor from the private sector. The discontinued patients (empagliflozin group) revealed the reasons behind their dropout from the study: 19 felt well, three patients lost

follow-up, three patients had nausea and two patients had low income and were unable to purchase the SGLT2 inhibitor from the private sector. (as shown in figure 2.1) .In the present study ,we measured seven parameters include (GFR,ACR,HbA1C,seum uric acid ,blood pressure ,weight and urine volume)

The parameters were measured pre-treatment (baseline value) and then measured post- treated with SGLT2i .the period took 4-months to re-measure all parameters.

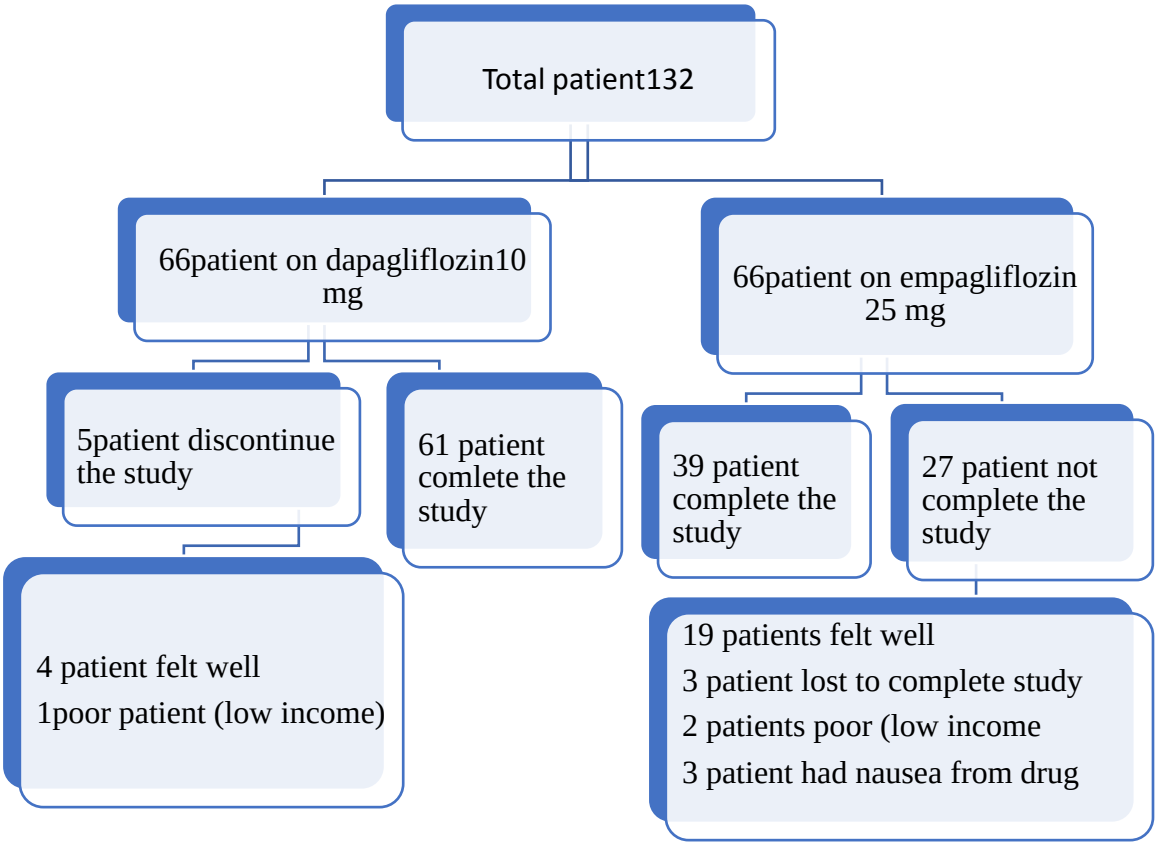

Figure (2-1) the flow chart of study groups

2.2. Materials

2.2.1. Drugs, kits and their suppliers

All drugs, kits and their suppliers utilized in the study are included in the table (2-1)

Table (2-1) drugs, kits and their suppliers

Kits and Drugs	Suppliers
Albumin bromcresol green	Dirui /China
Albumin reagent kit(BROMCRESOL GREEN METHOD)	Dirui /China
Creatinine reagent kit(enzyme method)	Dirui/China
Dapagliflozin 10mg oral tablet	Al-hikma/Jordan
ELUENT 80A 80B	Arkray factory /Japan
Empagliflozin 25mg oral tablet	Getz /Pakistan

2.2.2. Instruments

All instruments, equipment and their supplier used in the study are included in table (2.2)

Table (2.2) instrument, equipment and their supplier

Instrument / equipment	Suppliers
Arkray	Arkray(japan)
Blood collection plain tube	SAIL BRAND (China)
Centrifuge	Hettich(Germeny)
centrifuge tube	Hettich (Germany)
Cylinder	Wafi medical laboratory(iraq)
DIRUI	Durui company (china)
Disposable pipette tips	Eppendorf (china)
Disposable syringe (5ml)	Meheco (China)
EDTA tube	Wafi medical laboratories (Iraq)
Electronic weight scale	Camry (china)
Gel tube	SAIL BRAND (China)
Micropipette	Eppendorf (china)

2.3. Methods

2.3.1 . Sample collection

2.3.1.A. blood collection

Aplastic disposable syringe was used to collect 5 milliliter of venous blood from arm and placed in 3 disposable tube , 2 cc in gel tube ((special gel that separates blood cells from serum after 10 min , as well as particles to cause blood to clot quickly) to measure serum creatinine ,Then 2 cc in ethylene diamine tetra acetic acid (EDTA) tubes to measure HbA1c .1 cc in gel tube to measure uric acid.

2.3.1.B. urine sample collection (24 h)and fresh urine sample.

Ask the patient to urinate into the toilet when get up in the morning(start with empty bladder) .then start collecting all urine in a special container for the next 24 hour .then after finished collection we must measure the volume of urine(total) that collects during 24 hour by cylinder to know the total volume Then draw 3 cc from total urine for centrifuge (3000 rpm) in to centrifuge tube for 5 min then draw 50 micron from urine sample and diluted with distal water 20 time (950 micron d w) then the result multiply by dilution factor(20) this will measure creatinine (during 24 hour) by using DIRUI apparatus ,and then calculated GFR according to the equation (Levey AS *et al*.,2009).

$$\text{GFR} = \frac{Urin\ Creatinine(\ 24hour\ ) \times Urine\ volume\ (24\ hours)}{Serum\ Creatinine\ \times 1440}$$

While fresh sample to measure of ACR (spot urine sample and put it sterile, screw-top container to measure creatinine and albumin .ACR is calculated by dividing albumin concentration in milligrams by creatinine concentration in gram (Levey AS *et al*.,2009).

2.3.2. Principle of Measurement of albumin

Albumin binds with the dye Bromocresol Green in a buffered medium to form a green coloured complex. The intensity of the colour formed is directly proportional to the amount of albumin present in the sample.

2.3.3. Principle of Measurement of creatinine

Creatine can be generated by hydrolyzing creatinine amido with hydrolase in sample .creatine can be hydrolyzed at the function of creatine amidine hydrolase, and generate urea and sarcosine. In the role of sarcosine oxidase, creatine can produce glycine and hydrogen peroxidase which can react with 4 –aminoantipyrine and the chromogen compounds in the role of peroxidase and generate quinine imine pigment .thereafter creatinine content in sample can be calculated by monitoring the produced volume of Quinone imine pigment at the specified wavelength point. Reagent include the integrands and mechanism that exclude the containing creatine interfere in sample with the reaction principle

2.3.4. Principle of Measurement of HbA1C

The HA-8180 measures HbA1C in the blood using reversed –phase cation exchange chromatography. Blood sample diluted with the hemolysis washing solution is sent to the column which fractionates the sample in to several hemoglobin components based on high performance liquid chromatography (HPLC).

2.3.5. Principle of measurement of uric acid

Uric acid is oxidized by uricase to produce allatoin and hydrogen peroxide. Then The hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 3,5-dichloro-2-hydroxybenzene sulfonate (DCHBS) in a reaction catalyzed by peroxidase to produce a colored product.

2.3.6. Blood pressure measurement:

Recommendations: patient must

- Patients do not talk ,eat ,drink while blood pressure is started to measured.
- Sit in a comfortable chair with back supported for at least 5 minutes before reading.
- Put both feet flat on the ground and also keep the legs uncrossed.
- Rest the arm with the cuff on a table at chest height.
- Make sure the blood pressure cuff is snug but not too tight. The cuff should be against the bare skin(not over clothing.).

First , wraps an inflatable cuff around patients arm, then inflates the cuff, which gently tightens on the arm. The cuff has a gauge on it that will measure blood pressure, then will slowly let air out of the cuff while that listening to the pulse with a stethoscope and watching the gauge. The gauge uses a unit of measurement called millimeters of mercury (mmHg) to measure the pressure in the blood vessels.we measured blood pressure two time .

2.3.7. Weight measurement.

1. First Place the scale on a flat surface.
2. Step onto the scale (Stand still on the scale with both feet even and flat. without touch or hold on to anything) then read the number.
3. The same scale type must be used to measure the weight pre and post treatment**.**

2.4. Statistical analyses

Data was analyzed using Statistical Package for the Social Sciences (SPSS) software version 25 (IBM, USA). GraphPad Prism 7.04 was used to develop the figures. Descriptive statistics (means, standard deviation, medians, frequencies and percentages) were conducted for the study measures. We conducted normality (Shapiro–Wilk) test for continuous variables. Because the P-value of Shapiro-Wilk test for all continuous variable was significant (P-value< 0.05), this means they were not normally distributed (skewed) . Thus, non-parametric tests were used for differential analyses. Wilcoxon Signed Ranks Test (alternative to paired T-test) was used to measure the difference in the parameter levels before and after the SGLT2-inhibitor treatment. Mann-Whitney Test (alternative to Independent T-test) was used to measure the difference in the continuous parameters between the two treatment groups. Kruskal Wallis Test (alternative to One-way ANOVA) and Pairwise comparisons were used to measure the differences in the continuous parameter levels according to the three levels of the patient education. Spearman's correlation was used to measure the relationships among the continuous parameters

for all patients. P-value of less than 0.05 was considered statistically significant (Shapiro,1972) .

2.5. APPENDIX

A questionnaire had been applied to all patients in this study to collect needed information (table 2.4). The questionnaire was filled through direct interview with the patient .the time taken with each patient nearly 15 minutes , it was used to gather the necessary information as the following:

Table (2.4) The questionnaire used in this study:

Patient name: / mobile number:
Numbering of patients :
Sex: male female / urban rural
Age: /smoking status : yes no
Weight: pre ( ) post ( )
Duration of DM :
Level of education : 1 -primary 2-secondary 3-high
On insulin : yes no
GFR pre( ) post( )
ACR pre ( ) post( )
HbA1c pre ( ) post( )
Blood pressure pre ( ) post ( )
Urine volume pre ( ) post ( )
Uric acid pre ( ) post ( )

3.1. Demographic and disease characteristics of the participants.

The total participants were 100 patient with type 2 diabetic nephropathy who have started taking Sodium-glucose co-transporter 2 (SGLT2) inhibitors: 39 patients taking Empagliflozin (25mg once daily) , 61 patients taking Dapaglifolzin (10mg once daily) included 47 male and 53 female and 51% were smoker 59% patient from urban and 41 % patient from rural one-third of them (37%) had primary school degree or no formal education ,21% had college or higher ,Sixty-one percent of the patients were also taking insulin and oral antihyperglycemic agents the other was on oral antihyperglycemic agent only (as shown in table 3.1) . The average age of the participating patients was (54.1 ±7.3) years and the duration of their T2DM 9.3 (±3.87) years .

Table 3.1: Patient categorical demographic characteristics

Parameter	subcategories	Frequency	Percent
Gender	Male	47	47.0
	Female	53	53.0
Smoking	Yes	51	51.0
	No	49	49.0
Resident area	Urban	59	59.0
	Rural	41	41.0
Education level	Illiterate or Primary school	37	37.0
	Middle or high school	42	42.0
	College or higher degree	21	21.0
SGLT2 inhibitors	Empagliflozin	39	39.0
	Dapaglifolzin	61	61.0
Treatments	Insulin+ oral antidiabetic(SU+DDP-4 i)	61	61.0
	Oral only(SU+DDP-4 i)	39	39.0

3.2. The measured parameters before and after SGLT2i treatment (both empagliflozin and dapagliflozin groups).

Regarding the values of the all measured parameters (GFR ,ACR,HbA1C , weight ,urine output , uric acid ,systolic and diastolic BP) empagliflozin results showed significant difference (p value ≤ 0.05) found between the baseline (before treatment) and 4-month after SGLT2i treatment. as shown in table (3.2.A) and figure (3.1) ,(3.2),(3.3) ,(3.4) ,(3.5) ,(3.6) ,(3.7),(3.8)

Groups with dapagliflozin the values of the measured parameters (GFR ,ACR,HbA1C ,urine output , uric acid ,systolic and diastolic BP) results showed significant difference (p value ≤ 0.05) found between the baseline (before treatment) and 4-month after SGLT2i treatment ,only weight of this group have not significant difference . as shown in table (3.2.B) and figure (3.1) ,(3.2),(3.3) ,(3.4) ,(3.5) ,(3.6) ,(3.7),(3.8)

Table 3.2.A: Comparison of the measured parameters before and after SGLT2i (treatment with empagliflozin)

Parameters /units		Median	Mean±SD	P-value
Pair 1	Body weight 1	86	91.41±18.85	0.005*
	Body weight 2	84	89.18±17.11	
Pair 2	HbAIC1 % 1	8.5	8.50±1.25	0.001*
	HbAIC2 % 2	7.5	7.72±1.02	
Pair 3	GFR1 (ml/min) 1	90	87.71±26.49	0.001*
	GFR2 (ml/min) 2	92	95.71±28.46	
Pair 4	ACR1(mg/mmol)1	7.2	10.21±9.12	0.001*
	ACR2 (mg/mml) 2	2.5	6.72±9.10	
Pair 5	urine volume 1 (ml/24hr)	2000	2036.13±707.01	0.003*
	urine volume 2 (ml/24hr)	2200	2232.69±720.24	
Pair 6	Uric acid (mg/dl) 1	7.0	8.7±2.2	0.001*
	Uric acid (mg/dl) 2	5.0	6.4±1,9	
Pair 7	Systolic BP 1	150	150±8,4	0.001*
	Systolic BP 2	130	135±6.7	
	Diastolic BP 1	90	95±6,5	0.001*
	Diastolic BP 2	85	90±6.4	

1=before treatment / 2= after treatment

*=The difference is significant (*P*-value≤ 0.05).
GFR=glomerular filtration rate / ACR =albumin to creatinine ratio

Table 3.2.B: Comparison of the measured parameters before and after SGLT2i (treatment with dapagliflozin)

Parameters/ units		Median	Mean±SD	*p-value*
Pair 1	Body weight 1(Kg)	83	85.28±11.53	0.512
	Body weight 2(kg)	83	84.80±11.34	
Pair 2	HbAIC1 %	8.7	8.82±1.48	0.001*
	HbAIC2 %	7.6	7.59±o.94	
Pair 3	GFR1 (ml/min)	71	77.13±26.91	0.001*
	GFR2 (ml/min)	78.1	85.40±27.85	
Pair 4	ACR1(mg/mmol)	5.4	12.25±20.12	0.001*
	ACR2(mg/ mmol)	1.8	6.59±12.50	
Pair 5	urine volume 1 (ml/24hr)	1900	2022.13±832.19	0.003*
	urine volume 2 (ml/24hr)	2100	2138.69±783.17	
Pair 6	Uric acid 1(mg/dl)	8.0	7.96±2.45	0.001*
	Uric acid 2(mg/dl)	7.0	6.39±1.91	
Pair 7	Systolic BP 1	150	149.8±9.4	0.001*
	Systolic BP 2	130	132.9±7.8	
	Diastolic BP 1	95	93.5 ±4.5	0.001*
	Diastolic BP 2	90	85.3 ±4.9	

1=before treatment / 2=after treatment

***=** The difference is significant (*P*-value≤ 0.05).

GFR=glomerular filtration rate / ACR =albumin to creatinine ratio /BP=blood pressure

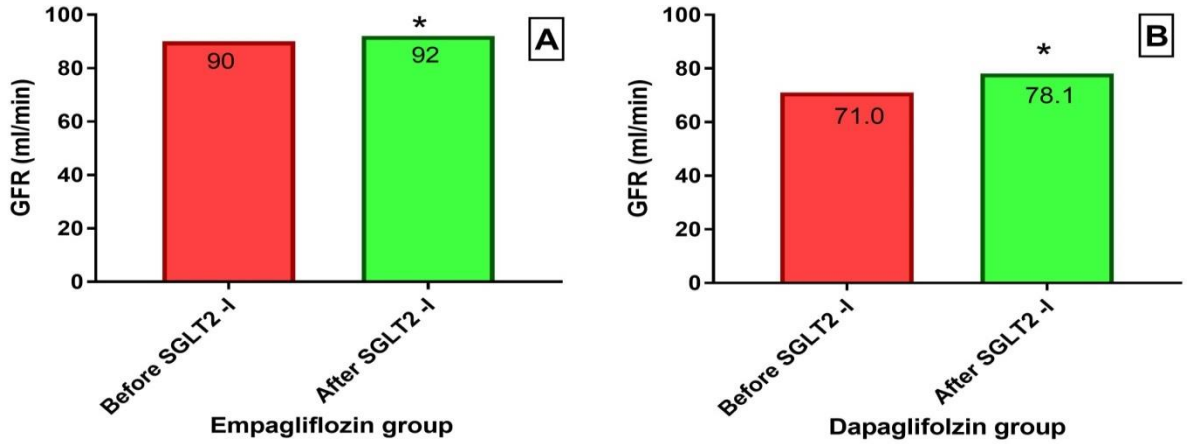

Figure 3.1: The medians of body weight before and 4-months after SGLT2 inhibitors' treatment: A- Empagliflozin group; B- Dapaglifolzin group

*Significant difference (*P*-value≤ 0.05).

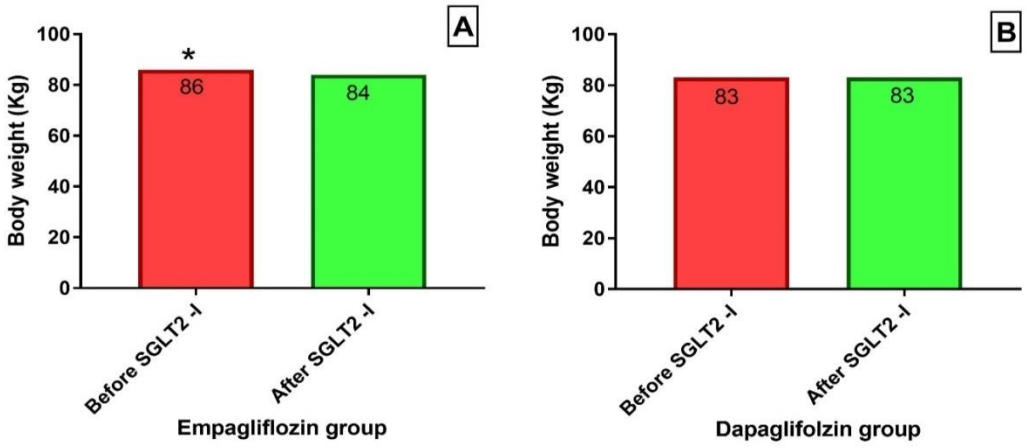

Figure 3.2: The medians of body weight before and 4-months after SGLT2 inhibitors' treatment: A- Empagliflozin group; B- Dapaglifolzin group

*Significant difference (*P*-value≤ 0.05

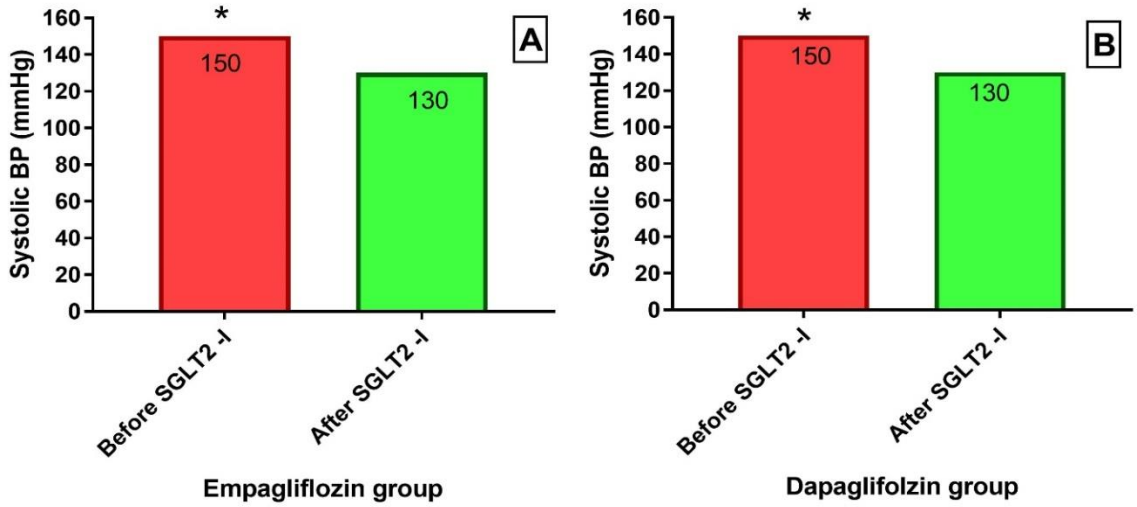

Figure 3.3: The medians of systolic blood pressure before and after SGLT2i treatment: A- Empagliflozin group; B- Dapaglifolzin group

*= Significant (*P*-value< 0.05) ,Bp=blood pressure ,SGLT2i=Sodium-glucose co-transporter-2 inhibitor

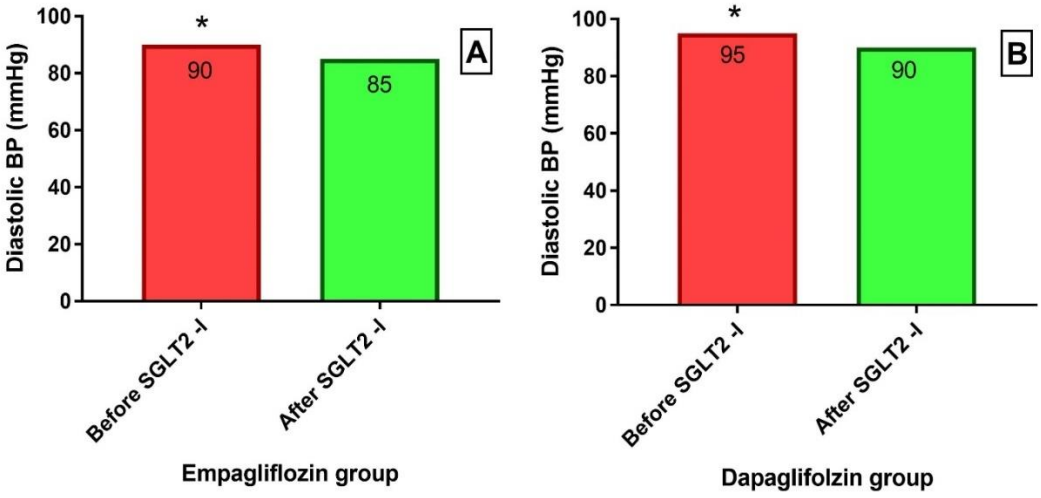

Figure 3. 4: The medians of diastolic blood pressure before and after SGLT2i treatment: A- Empagliflozin group; B- Dapaglifolzin group

*=Significant difference (*P*-value ≤ 0.05), SGLT2i=Sodium-glucose co-transporter-2 inhibitor

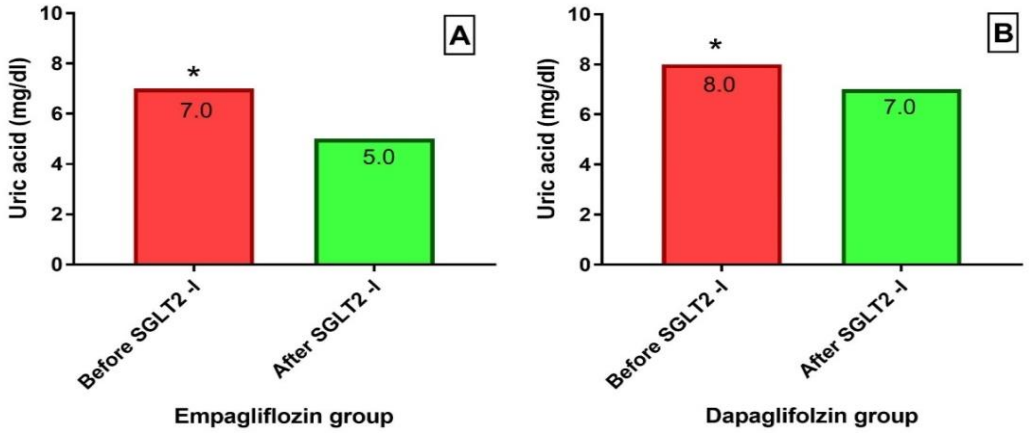

Figure 3. 5: The medians of uric acid before and 4-months after SGLT2i treatment: A- Empagliflozin group; B- Dapaglifolzin group / *=Significant difference (*P*-value ≤ 0.05

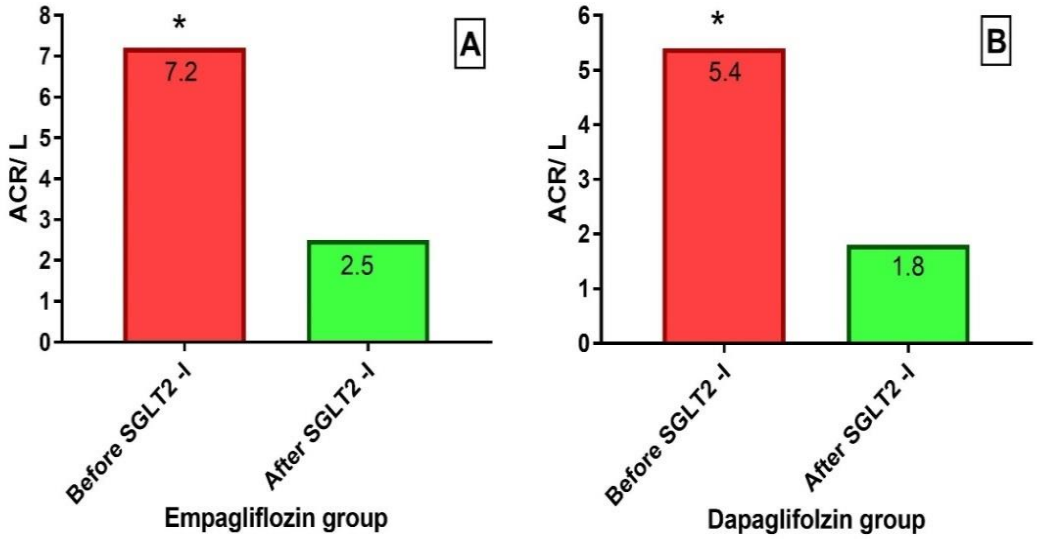

Figure 3.6: The medians of ACR before and 4-months after SGLT2i treatment: A- Empagliflozin group; B- Dapaglifolzin group

*=Significant according to (*P*-value≤ 0.05)/ ACR=albumin to creatinine ratio, SGLT2i=Sodium-glucose co-transporter-2 inhibitor

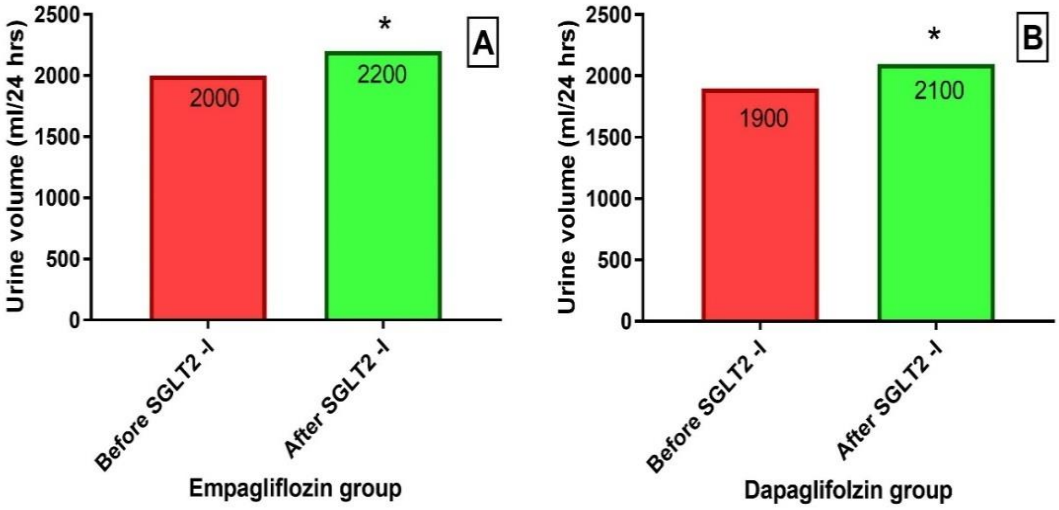

Figure 3.7: The medians of urine volume before and 4-months after SGLT2i treatment: A- Empagliflozin group; B- Dapaglifolzin group

*=Significant difference (*P*-value≤ 0.05). SGLT2i = Sodium-glucose co-transporter-2 inhibitor

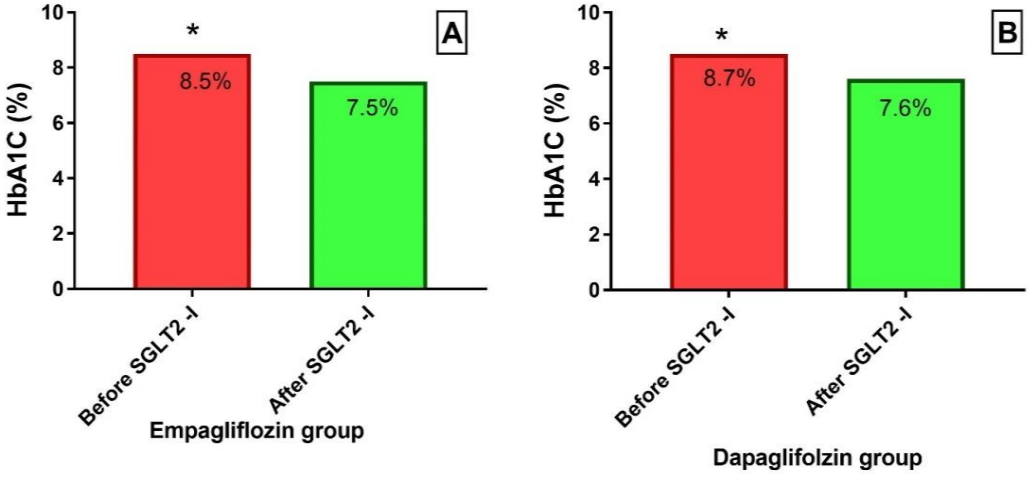

Figure 3.8: The medians of HbA1C before and 4-months after SGLT2i treatment: A- Empagliflozin group; B- Dapaglifolzin group

*=Significant difference (*P*-value ≤ 0.05). SGLT2i=Sodium-glucose co-transporter-2 inhibitor

3.3. The difference between the pre- and post SGLPT2 measurements across the two study groups:

Results showed Non-significant difference (*P*-value >0.05) in the effect between Empagliflozin and Dapagliflozin groups in all measured parameters as shown in table (3.3)

Table 3.3. The difference between the pre- and post SGLT2i measurements across the Two study groups

Study variable	medicine	No	Mean ±SD	*P-value*
Body weight difference Kg	Empagliflozin	39	2.23±5.10	.127
	Dapagliflozin	61	0.84±3.87	
	Total	100		
HbA1c_difference %	Empagliflozin	39	0.78±1.06	.035
	Dapagliflozin	61	1.23±1.00	
	Total	100		
GFR_difference (ml/min)	Empagliflozin	39	-8.15±13.80	.962
	Dapagliflozin	61	-8.28±11.81	
	Total	100		
ACR_difference (mg/mmol)	Empagliflozin	39	3.49±9.49	
	Dapagliflozin	61	5.66±14.06	.398
	Total	100		
Urine_output_difference (ml/24 hr)	Empagliflozin	39	-195.10±629.8	.508
	Dapagliflozin	61	-116.56±102.5	
	Total	100		
Uric_acid_difference (mg/dl)	Empagliflozin	39	1.77±1.55	.242
	Dapagliflozin	61	1.45±1.19	
	Total	100		
SBP_difference	Empagliflozin	39	1.62±1.02	.537
	Dapagliflozin	61	1.74±0.93	
	Total	100		
DBP_difference	Empagliflozin	39	0.82±0.48	.936
	Dapagliflozin	61	0.81±0.59	
	Total	100		

Non-significant difference *(P*-value >0.05)

Difference=pre-measurement – post GLPT2i measurement

SBP=systolic blood pressure / DBP =diastolic blood pressure

3.4. Impact of SGLT2i –insulin combined therapy on the measured parameters.

The GFR and uric acid levels were significantly better improved *(P*-value ≤ 0.05) in patients who were not taking insulin compared to those taking insulin .On other hand, patients with insulin group had non-significant statistical (*P*-value> 0.05) impact on the levels of HbA1C, ACR, urine output, body weight and blood pressure (as shown in table 3.4)

Table 3.4: Impact of SGLT2i –insulin combined therapy on measured parameters.

After 4 months of SGLT2-I therapy	on insulin	N	Median	Mean±SD	*P-value*
Body weight (Kg)	Yes	61	85	86.26±12.75	.692
	N	39	80.5	86.32±15.91	
HbA1C 2(%)	Yes	61	7.5	7.62±1.00	.470
	No	39	7.7	7.69±0.92	
GFR2 (ml/min)	Yes	61	77.5	84.57±29.12	.008*
	No	39	94	97.01±25.79	
ACR 2 (mg/mmol)	Yes	61	3.7	7.12±6.91	.729
	No	39	2.2	5.88±4.23	
Urine output 2 (ml/24hour)	Yes	61	2050	2109.67±770.60	.275
	No	39	2200	2277.44±733.10	
Uric acid 2(mg/dl)	Yes	61	7.2	6.77±1.97	0.024*
	No	39	5.4	5.79±1.67	
Systolic BP 2	Yes	61	130	13.31±0.74	0.567
	No	39	130	13.26±0.85	
Diastolic BP 2	Yes	61	90	8.60±0.48	0.076
	No	39	80	8.42±0.48	

*=Significant difference (P-value≤ 0.05) / 2=post treatment , GFR=glomerular filtration rate , ACR =albumin to creatinine ratio /Yes=on insulin and oral therapy / no = on oral therapy only

3.5. Impact of patient's educational level on the measured parameters after SGLT2i treatment

Patient education level had significant (P-value ≤ 0.05) effects on the levels of (GFR , ACR and uric acid) after SGLT2i treatment , the patients with higher education had significantly better disease measures including higher GFR and lower ACR , uric acid compared to patients with lower education levels .table (3.6)

According to the patients educational levels results showed that the GFR was significantly increased (p value ≤0.05) in patient with level 3of education i (college or higher) in comparison to those with level 1 and 2 of education .table (3.6)

Similarly, the ACR was significantly decreased (*p*- value ≤ 0.05) in patients with level 2 and 3 of education in comparison to these with level 1 of education as shown in table (3.7)

Regarding to the uric acid level results showed that it was significantly decrease (*p value ≤0.05*) in patient with level 3 of education in comparison to those with level 1 and 2 of education, although uric acid level was decreased in patients with level 2 of education in comparison to those with level 1 of education significant difference were not found (*p- value ≥0.05*) as shown in table (3.5)

Table 3.5: Impact of patient's educational level on the measured parameters after SGLT2i treatment

Parameters/unit	Education level	No	Means±SD	*P-value*
Body Weight – post- SGLT2i	1.00	37	88.49±12.86	.366
	2.00	42	85.36±15.93	
	3.00	21	84.26±11.67	
HbAIc %- post- SGLT2i	1.00	37	7.81±0.99	.058
	2.00	42	7.71±0.91	
	3.00	21	7.21±0.94	
GFR- post- SGLT2i	1.00	37	80.07±28.01	.009*
	2.00	42	90.80±25.85	
	3.00	21	103.14±29.11	
ACR- post- SGLT2i	1.00	37	8.38±7.29	.002*
	2.00	42	5.15±4.12	
	3.00	21	6.54±5.10	
Urine output (ml//24hr)- post- SGLT2i	1.00	37	2076.22±688.28	.115
	2.00	42	2126.19±855.70	
	3.00	21	2447.14±617.63	
Uric acid – post- SGLT2i	1.00	37	6.99±1.87	.025*
	2.00	42	6.29±1.84	
	3.00	21	5.52±1.83	
Systolic BP – post- SGLT2i	1.00	37	13.41±0.76	.158
	2.00	42	13.31±0.84	
	3.00	21	13.05±0.67	
Diastolic BP – post- SGLT2i	1.00	37	8.55±0.50	.927
	2.00	42	8.51±0.46	
	3.00	21	8.52±0.54	

*=Significant difference (P-value≤ 0.05). 1=Illiterate or Primary school; 2=Middle or high school; 3=College or higher degree

3.6. Correlations between the measured parameters (post SGLT2-I treatment) and patient characteristics

Post-treatment with SGLT2i results showed significant (p value ≤0.05) positive correlation between the age of patient and duration of DM.

Regarding to the GFR results showed significant negative correlation between the GFR and duration of DM and with ACR. Whereas the correlation was significant positive between ACR and urine output

The results also showed significant negative correlation between GFR and with (uric acid, systolic and diastolic BP).Similarly the correlation was negative between uric acid and urine output (as shown in table 3.6)

Table 3.6: The correlations between the measured parameters (post SGLT2i treatment) and patient characteristics

Parameters	Spearman's correlation	Duration Of DM	HbAIC2 %	GFR2	ACR2	Urine output 2 (ml/24hr)
Age	Correlation Coefficient	.430	.082	-.157	.018	-.088
	P-value	**.000***	.419	.118	.857	.381
	N	100	100	100	100	100
Body Weight 2	Correlation Coefficient	-.086	.134	.023	.146	.004
	P-value	.396	.182	.823	.148	.966
	N	100	100	100	100	100
Duration Of DM	Correlation Coefficient	1.000	.037	-.265	.049	-.049
	P-value	.	.715	**.008***	.627	.627
	N	100	100	100	100	100
HbAIC2 %	Correlation Coefficient	.037	1.000	-.101	.114	.151
	P-value	.715	.	.319	.259	.134
	N	100	100	100	100	100
GFR2	Correlation Coefficient	-.265	-.101	1.000	-.247	.324
	P-value	**.008***	.319	.	**.013***	**.001***
	N	100	100	100	100	100
ACR2	Correlation Coefficient	.049	.114	-.247	1.000	-.044
	P-value	.627	.259	**.013***	.	.665
	N	100	100	100	100	100
Urine output 2 (ml/24hr)	Correlation Coefficient	-.049	.151	.324	-.044	1.000
	P-value	.627	.134	**.001***	.665	.
	N	100	100	100	100	100
Uric acid 2	Correlation Coefficient	.175	.042	-.876	.101	-.319
	P-value	.082	.680	**.000***	.318	**.001***
	N	100	100	100	100	100
Systolic BP Post- SGLT2_I	Correlation Coefficient	.108	.307	-.330	.158	-.042
	P-value	.284	**.002***	**.001***	.117	.682
	N	100	100	100	100	100
Diastolic BP Post- SGLT2_I	Correlation Coefficient	-.066	-.073	-.291	.008	-.185
	P-value	.514	.472	**.003***	.938	.065
	N	100	100	100	100	100

*=Correlation is significant at the 0.05 level/ +=positive correlation. _=negative correlation

In general, this is the first study in Iraq aimed to evaluate effect of SGLT2i on kidney function in patients with diabetic nephropathy.

DM is strongly related with DKD owing to hyperglycemia and many metabolic and hemodynamic alterations in kidney function (Soldatos *et al*.,2008) .

Diabetic nephropathy has become the leading cause of end-stage renal disease (ESRD),on the other hand , Patients with both diabetes and hypertension have approximately twice the risk of heart attack and stroke as non-diabetic people with hypertension, Hypertensive diabetic patients are also at increased risk for complication including kidney disease, on the other hand, glucose lowering therapy or glycemic control have an effect of lowering GFR , optimal glycemic control have protective effect on kidney function (Cynda *et al*., 2004).

It has been found that up to 50% of the patients with diabetes may develop chronic kidney disease due to uncontrolled blood glucose (Thomas *et al*., 2016).

Iraqi patients have high risk for kidney dysfunction due to several factors, like:

- used NSAID without prescriptions so over use of NSAID lead to kidney dysfunction,
- Routine high salty food (high in sodium) also have bad effect on kidney
- hot weather countries with high rate incidence of early kidney dysfunction so well hydrated helps your kidneys clear sodium and toxins from the body
- smokers are more likely to have protein in the urine – a sign of kidney damage

- eating Foods High in Sugar (Sugar increase obesity which increases risk of developing high blood pressure and diabetes, two of the leading causes of kidney disease
- Eating Too Much Meat

In the present study showed that using SGLT2i (both empagliflozin and dapagliflozin) to treat patients with type 2 diabetic nephropathy has significant positive impact to enhances renal function (by increasing GFR and decrease ACR) also SGLT2i have good effect on blood glucose control (by reducing HbA1C), reduce body weight , reduce uric acid and reduces blood pressure (both systolic and diastolic).

4.1. Demographic and disease characteristics of the participants.

In the present study, all participating patients had type 2 DN (N =100) initially not receive either empagliflozin 25mg or dapagliflozin 10mg. Although, the study started with equal number of participants in each group (N=66), more patients (n=27) in the empagliflozin dropped out the study (i.e. did not come back for the follow-up visit) compared to those in Dapagliflozin group (n=5).

Consequently, the two groups had unequal number of patients who completed the study: 39 patients in Empagliflozin group and 61 in Dapagliflozin group.

In addition to SGLT2i, insulin was already prescribed to 61% of the participating patients. The patients were prescribed insulin in addition to oral anti-diabetic medications because they experienced uncontrolled glucose level in addition to the comorbidities such as inadequate renal function.

The average age of the participants was 54.1 (±7.3) years. The patients had comparable percentages in terms of gender male =47% and female 53% and residential geographical (urban 59% or rural 41%) areas distribution, patients had diabetic kidney disease according to results of both GFR and ACR.

It is worth mentioning that more than three-quarters (79%) had secondary school or lower education levels. As indicated by several studies, the low-education could negatively impact the clinical status of patients with chronic diseases because they may have inadequate self-care, not understand the instructions of their healthcare providers, less adhered to their medication and have inadequate health literacy about their diseases(Al-Jumaili *et al*., 2015; Kim & Lee, 2016; Marciano *et al*., 2019).

4.2. The impact of SGLT2i (Empagliflozin and Dapagliflozin) on the measured parameters.

4.2.1. The effect of empagliflozin and dapagliflozin on GFR, ACR and urine output.

In the present study, using of (empagliflozin or dapagliflozin) was associated with statistically significant improvement in GFR, increment in urine output and reduction in ACR after treatment with empagliflozin and dapagliflozin

These findings are agree with that reported by Heerspink *et al*., (2020) a large clinical trial in 21 countries which indicated the renoprotective effects of

dapagliflozin (10 mg once daily in 4304 diabetic patients with diabetic kidney disease .

Also , Herrington *et al*., (2023) another large randomized clinical trial (RCT) recruiting 6609 diabetic patients with DKD at 241 centers in eight countries found that patients treated with empagliflozin were at lower risk of kidney disease progression and this finding also agree with this study findings .

Other study that agree with our results , Liu *et al*., (2015) a previous meta-analysis up to December 2014 has concluded that SGLT2 inhibition does not have a significant effect on the glomerular filtration rate (GFR) and urine output in patient with T2diabetic nephropathy but associated with reduction in albumin to creatinine ratio in type 2 diabetic patients by indirect mechanism .

SGLT2i suggested to exert nephroprotective effects through glucose-dependent and glucose-independent mechanisms The mechanisms of renal protection with SGLT2 inhibitors are multifactorial and it is likely that reduction in the glomerular capillary pressure, and reduction in an initial hyperfiltration, can contribute to the reduced albuminuria and prevention of renal impairment (Wanner, 2017).

SGLT2i also have indirect effect on GFR , when SGLT2i reduce ACR that effect will reduce risk of kidney deterioration, Meta-analyses of large clinical trials have suggested that a 30% reduction in albuminuria might lead into a 30% reduction in the risk of end stage renal disease (Lambers *et al*.,2014).

4.2.2. Effect of empagliflozin and dapagliflozin on the level of HbA1c.

In the present study, after treatment with SGLT2 inhibitors (empagliflozin or dapagliflozin) were associated with statically significant reduction in HbA1c. Despite these positive clinical outcomes , a relatively small percentage of patients achieved the glycemic target (HbA1c ≤7) after SGLT2 addition and this finding agree with Saisho,(2020) that showed using SGLT2 inhibitor by diabetic patients for few months was associated with lowering HbA1c by about 1% on average . It has been revealed that SGLT2 inhibitors can reduce Glycated hemoglobin (HbA1c) without raising hypoglycemia risk

Another Emirati study Alaaeldin Bashier *et al*., (2017) also in line with this study finding . The baseline HbA1c was 8.9±1.7%, which dropped significantly to 8±1.5% at 6 months (P = 0.0001).

Another study that agree with our finding D. Vasilakou *et al*.,(2013) a meta-analysis of 45 randomized trials that show empagliflozin reduces HbA1c by 0.79% with monotherpy and 0.61% with add-on treatments to other glucose-lowering medications ,the

Rosenstock *et al*.,(2012) another study that showed Dapagliflozin have good effect in reducing glucose when add-on therapy .

Due to its effect on blocking SGLT2 in the proximal convoluted tube this lead to reduce renal tubular glucose reabsorption, producing a reduction in blood glucose without stimulating insulin that's why SGLT2i have no hypoglycemic effect (Nauck, 2014).

4.2.3. Effect of empagliflozin and dapagliflozin on body weight

In the present study, empagliflozin was associated with statically significant reduction in body weight and this agree with Vasilakou *et al*., (2013), a systematic review including 58 studies found that SGLT2 inhibitors reduce the body weight and lead to excretion of 60–80 g of glucose per day through the urine, SGLT2i lead to a significant loss of 240–320 calories per day, resulting in weight loss and enhance short-term clinical outcome of patients with type 2 diabetes .

On the other hand, there was non-significant changes in body weight among the patients in Dapagliflozin group .this result disagree with Bolinder *et al*., (2014) that show Treatment with dapagliflozin for six months significantly reduced body weight without reducing muscle mass in T2DM patients and this is may be short period we used and our patient without daily exercise and unhealthy food

Another Korean study that also disagree with our study Won *et al* .,(2018) showed that dapagliflozin can effectively reduce body weight in T2DM patients but need special condition include regularly exercising, having a normal kidney function, and combining with metformin . The other benefits from weight loss are effective in improving blood pressure, fasting blood glucose, and cholesterol level.

In general , weight lose result from SGLT2i due to increase glucose excretions in the kidney proximal tubule (calorie loss), weight loss have many advantages in patients with diabetic ,the patients will have more energy and the risk of serious complications will reduced, Weight loss also increase the blood glucose control , in the DiRECT randomized controlled trial in the U.K., 149 participants who had

type 2 diabetes for less than 6 years , The DiRECT researchers found that diabetes remission was correlated with the amount of weight lost , patients who lost 15 kg or more during the study achieved diabetes remission (Lean *et al*.,2018).

In Iraq, Many factors contribute to increase weight gain like sedentary life style and fatty food , stress the most important factor that cause high glucose level.

Stress will increase Cortisol that stimulates the fat and carbohydrate metabolism, creating a surge of energy in the body. While this process is essential for survival situations, it also increases appetite. Additionally, elevated cortisol levels can cause cravings for sweet, fatty and salty foods (Dallman *et al.*,2004).

The factors mentioned above may be explain why dapagliflozin have non-significant effect on weight reductions and small sample size may be the reason

4.2.4. Effect of empagliflozin and dapagliflozin on Uric acid level

In the present study both Empagliflozin and Dapagliflozin have significantly reduce the serum uric acid level. This result agree with (Xin *et al*., 2019) a systematic review including 31 clinical trials with 13,650 patients found that SGLT2 inhibitors significantly decreased serum uric acid levels compared with placebo.

Another study that agree with this study Zhao Y *et al*., (2018) A meta-analysis of 62 clinical trials including a total of 34 941 with T2DM patients showed a consistent effect of empagliflozin in reducing UA levels. The Empagliflozin effect remained stable for up to 2 years of follow-up.

SGLT2 inhibitors decrease UA levels by increasing urinary excretion and, possibly, via the reduction of reactive oxygen species leading to a decrease in the activity of the enzyme xanthine-oxidase (which catalyses the oxidation of xanthine to UA) (Zhao *et al*., 2018).

SGLT2i as antigout drugs, antigout treatments such as allopurinol may precipitate acute gout flares early after their introduction, which is not known to happen with SGLT2 inhibitors (Zhao *et al* .,2 018) .

Hyperuricemia has been shown to be a risk factor for the progression of diabetic peripheral neuropathy, diabetic nephropathy, increased mortality rate, cancer incidence, atrial fibrillation, and metabolic syndrome in patients diagnosed with T2DM. Both blood glucose and uric acid are reabsorbed at the same site in the kidney so when uric acid increase lead to increase glucose level (Kodama *et al* ., 2009).

A study with 1938 patients indicated that high plasma uric acid was associated with greater risk of insulin resistance (Hu *et al*., 2021) .

4.2.5 .Effect of empagliflozin and dapagliflozin on blood pressure.

In the present study there is significant difference in systolic and diastolic blood pressure before and after treatment with SGLT2i and the results agree with Vasilakou *et al*., (2013) a systematic review and meta-analysis including 58 clinical trials comparing SGLT2 inhibitors with placebo or other medication for T2DM also found that SGLT2 inhibitors reduce systolic blood pressure .

Likewise , Heerspink *et al*.,(2020) a randomized clinical trial In which 4304 patients with DKD found that receiving dapagliflozin (10 mg once daily) can reduce the risk of death from cardiovascular causes and decrease blood pressure compared to placebo.

Another Chinese study Tikkanen, I *et al*.,(2015) showed Empagliflozin was associated with significant and clinically meaningful reduction in BP versus placebo in Chinese elderly patients with type 2 diabetes and hypertension.

Multiple mechanism of blood pressure lowering including osmotic diuresis, mild natriuresis, body weight loss, local inhibition of the renin-angiotensin aldosterone, and nitric oxide release.

Uncontrolled hypertension in patient with T2DM increase the risk of heart attack, heart failure, kidney disease, stroke, and cognitive decline (Yoon *et al*., 2015)

4.3. The difference between the before and after SGLPT2 I measurements across the two study groups

In the present study , results show empagliflozin had the same effect with dapagliflozin in all measured parameters and this result disagree with Ku et al.,(2019) that showed empagliflozin was more effective in reducing HbA_{1c} and other parameters of kidney function than dapagliflozin .

Shao *et al.,(*2021) another Korean study agree with our study that showed There were no significant differences in clinical outcomes between dapagliflozin and empagliflozin.

4.4. Impact of insulin-SGLT2i combination on the measured parameters.

Many patients have achieved their normal HbA1c depending on the replacement therapy of insulin**,** the disadvantage of insulin therapy its hypoglycemic effect and weight gain especially in large dose .Some oral hypoglycemic agent will aggravate effect of insulin (hypoglycemia and weight gain) while SGLT2i in combination with insulin therapy have advantages in decrease hypoglycemic effect and prevent weight gain (Bell ,2015).

The addition of sodium–glucose co-transporter 2 (SGLT2) inhibitors to a regimen of insulin therapy in the patient population has the potential to mitigate insulin-related weight gain and risk of hypoglycemia with the added benefit of insulin dose reduction (John *et al*.,2016).

According to post- SGLT2i treatment, there was no significant difference in (HbA1C, ACR, urine output, weight and blood pressure (both systolic and diastolic) between insulin group and group without insulin when used SGLT2i in combination with insulin

On the other hand, the GFR and uric acid levels were significantly better improvement in patients were not taking insulin compared to those with insulin treatment .

This may be due to SGLT2i have better effect in patients with early stages of DKD and its effect decrease in patients with decrease kidney function .

. In other words, the insulin group may have uncontrolled DM for long-term; thus, this patient group has more risk factors for complications like chronic kidney disease (Behradmanesh,2013)

4.5. Impact of patient education level on the measured parameters

According to the result of present study the patient educational level showed significant positive effect on renal function (increase d GFR) and this result is consistent with that reported by Al-Jumaili *et al*.,(2015) which showed significant positive effect between educational level of iraqi patients and their health literacy(patient ability to understand healthcare providers' instructions)

While ACR was better improved in primary school patients better than high school or college this result can explain by patients with primary school almost poor and related with malnutrition and vitamins deficiency and this lead to decrease albumin level

On the other hand, there is no significant difference after treatment with SGLT2i between the three levels of education in the other parameters

People who are well educated showed better health as reflected in the high levels of self-reported health and low levels of morbidity, mortality, and disability. On the other hand, low educational attainment is associated with self-reported poor

health, shorter life expectancy, and shorter survival when sick. The relationship between education and health is related to three general classes of mediators:

1. economic
2. social, psychological, and interpersonal
3. behavioral health

- ✓ Economic variables such as income and occupation mediate the relationship between education and health by controlling and determining access to acute and preventive medical care.
- ✓ Social, psychological, and interpersonal resources allow people with different levels of education to access coping resources and strategies, social support, and problem-solving and cognitive abilities to handle ill-health consequences such as stress.
- ✓ Healthy behaviors enable educated individuals to know symptoms of disease health in a timely manner and search for appropriate medical help (Cutler *et al*.,2006)

4.6. The correlation between the measured parameters (post SGLT2i treatment) and patient characteristics.

There were several significant correlations among the measured parameters of the total patients in our study. Patient age has significant positive correlation with the duration of diabetes disease,

In the present study the GFR level has significant negative correlations with uric acid concentration that mean when GFR decrease will lead to increase uric acid and this agree with Siu YP *et al*., (2006) that showed Increased uric acid levels have a pathogenic role in the progression of diabetic kidney disease (DKD), rather than solely reflecting decreased renal uric acid excretion.

GFR have negative significant correlation with ACR (p value≤0.05) this finding comparable with Meguro S *et al*., (2009) that showed diabetic patients with micro albuminuria have a significantly increased rate of decline of GFR (five times greater) compared to patient with normalbuminuria.

MacIssac *et al., (2004)* another comparable study in a single-center outpatient population (*n* = 301) showed a minor influence of the degree of proteinuria on progression decline in GFR at 2.8 mL/min/year in people with diabetes.

Hirayama *et al*., (2015) another inline study found that the annual decline of GFR correlated with the annual increase in albuminuria.

HbA1c have negative correlation with GFR in line with Takagi M *et al*., (2015) that showed DM2 patients the worst glycometabolic control as indicated by values of HbA1c ≥8% predicted the annual decline of GFR.

Age have negative correlation with GFR and those result in line with O'Hare AM *et al*.,(2006) that showed The age-related decline in glomerular filtration rate (GFR) is the most important leading cause of chronic kidney disease (CKD) in old age, which showed affects 70% at eighty years of age

5.1. **Conclusions**

From the finding of the study it can be conclude the following:

1. Among patient with diabetic nephropathy ,SGLT2i (empagliflozin and dapagliflozin) have good effect on renal function and it will improve (GFR , ACR and urine volume) and prevent kidney from deterioration
2. Empagliflozin has good effect on body weight reduction.
3. The uses of SGLT2i improve blood pressure (both systolic and diastolic)
4. SGLT2i have clear effect in reducing serum uric acid concentration.
5. SGLT2i can be combined effectively and safely with insulin therapy
6. Empagliflozin have the same effect in compared with dapagliflozin in improvement of kidney function and all other measured parameters in this study.

5.2. Recommendations

1) We recommend prescribing SGLT2 inhibitor(s) for type 2 diabetic patients
2) Study the use of SGLT2 inhibitor(s) as first line therapy for treatment of DM
3) We recommend prescribing SGLT2 inhibitors for type 2 diabetic patients and have hypertension
4) We recommend prescribing SGLT2 inhibitor(s) for patients with hyperuricemia (as antigout therapy)
5) Study the effect of SGLT2 inhibitors on weight reduction and used as medicine for weight reduction in obese individuals with or without of DM.

6) Study the difference between Dapagliflozin and Empagliflozin to know which one better than others in improve (GFR, ACR ,Hba1c , uric acid) in study with large sample size and equal number .
7) Study the use of SGLT2 inhibitor(s) to reduce uric acid concentration in patient with renal stone if the cause of stone was uric acid

5.3. Study limitations

This study had several limitations including inequal number of participants in each group due to imbalanced patient dropout rate. The high dropout rate was probably due to the tedious requirement to collect urine output for 24-hours by patients at home and keep it refrigerated and bring it to the researcher. Additionally, the sample size was not large. Finally, the study was limited to one center in one province.

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الخلاصه:

يتميز مرض اعتلال الكلى السكري بتلف الكليه تدريجيا مما يودي الى زياده الزلال في البول وضعف في وظيفه الكليه (انخفاض في معدل الترشيح الكبيبي) ,ارتفاع ضغط الدم وزياده معدل الوفيات

ويرجع السبب لذلك الى حدوث مضاعفات القلب والاوعيه الدمويه. حديثا اكتشف مثبطات الناقل المشترك للجلوكوز والصوديوم SGLT2i وهي فئه جديده من الادويه الخافضه لنسبه السكر بالدم والتي تخفض الجلوكوز في الدم عن طريق منع اعاده امتصاص الكلوكوز عن طريق تثبيط SGLT2 في الانابيب الكلويه القريبه . حاليا اصبحت مثبطات SGLT2 اكثر شيوعا وذلك بسبب فوائدها المتعدد.

هدفت الدراسه الى تقييم فعاليه مثبطات SGLT2 (امباجليفلوزين و داباكليفلوزين) على معدل الترشيح الكبيبي وقياس معدل الزلال الى الكرياتنين في المرضى المصابين باعتلال الكلى السكري / النوع الثاني وايضا تقييم تاثيرها على الوزن ,حامض اليوريك ,معدل السكر التراكمي وضغط الدم .تم اخيار مرضى مصابين باعتلال الكلى السكري /النوع الثاني وتم اضافه مثبطات الناقل المشترك التي كانت (امباكلوفازين و داباكليفلوزين) , كان وقت جمع العينات من تشرين الثاني 2021 الى كانون الثاني 2023 , من خلال زياره المرضى لمركز السكري /مستشفى مرجان والعياده الخاصه ,العراق .

تم جمع عينات دم لقياس معدل السكر التراكمي ,حمض اليوريك, الكرياتنين وتم ايضا اخذ عينه ادرار (تجميع لمده 24 ساعه) لقياس معدل الترشيح الكبيبي بالاضافه الى عينه ادرار جديده لقياس نسبه الالبومين الى الكرياتنين. تم جمع العينات قبل العلاج وبعد 4 اشهر من استخدام العلاج والمقارنه بينهم .

الدراسه شملت 132 شخص تم تشخيصهم باعتلال الكلى السكري /النوع الثاني قسموا الى مجموعتين 66 شخص تناولو دواء الامباكليفلوزين و 66 شخص تناولوا دواء الامباكليفلوزين وكان عدد المرضى الذين اكملوا الدراسه 100 شخص 39 (امباكليفلوزين) و 61 (داباكليفلوزين).كان معدل العمر 54.1 (±7.3) سنه وكان معدل مده السكري 9.3 (±3.87) سنه وكان عدد الرجال 47 وعدد النساء 53 ,ثلثهم (37%) كانوا بدون تعليم .61% كانوا على علاج الانسولين بالاضافه الى ادويه خافضه للسكر عن طريق الفم,39% كانوا على ادويه خافضه للسكر عن طريق الفم فقط.

ان مثبطات الناقل المشترك للجلوكوز والصوديوم لها تاثير ايجابي على وظائف الكلى لانها تحسن بشكل ملحوظ قيمه معدل الترشيح الكبيبي (بمقدار 10 مل/ دقيقه)وتقلل نسبه الالبومين الى الكرياتنين (بمقدار 3.6)

مما يمنع تدهور الكلى .كما تقلل مثبطات الناقل المشترك للجلوكوز والصوديوم من الوزن ,تقلل ارتفاع ضغط الدم الانقباضي والانبساطي (بمقدار 20 و 5 مم زئبق) ,تقلل من معدل السكر التراكمي (بنسبه 1%) ,تحسن الاخراج البولي والذي ربما يرجع الى التحسن في وظائف الكلى ,يقلل من تركيز حمض اليوريك في الدم (بمقدار 2 ملغ/ديسيلتر). لا يوجد هنالك اختلاف معنوي بين الدوائين من ناحيه التاثير على جميع المتغيرات المقاسه.

استنتجت الدراسه ان استخدام مثبطات الناقل المشترك للجلوكوز والصوديوم لعلاج مرضى اعتلال الكليه السكري النوع الثاني له تاثير ايجابي على تعزيز وظائف الكلى (عن طريق زياده معدل الترشيح الكبيبي وتقليل نسبه الالبومين الى الكرياتنين وايضا يقلل من معدل السكر التراكمي ,تقليل حمض اليوريك ,تقليل ضغط الدم الانقباضي والانبساطي ,بالاضافه الى تقليل الوزن.نتائج الدراسه تدعم استخدام مثبطات الناقل المشترك للجلوكوز والصوديوم في علاج السكري نوع الثاني في الاشخاص المصابين باعتلال الكلى السكري.

جهوية العراق
وزاره التعليم العالي والبحث العلمي
جامعة بابل
كليه الطب
فرع الادوية والسموم

فعالية مثبطات الصوديوم- الجلوكوز - الناقل المشترك 2 (SGLT2) على نسبه زلال البول ومعدل الترشيح الكبيبي بين المرضى المصابين باعتلال الكلى السكري من النوع الثاني.

رسالة

مقدمة الى مجلس كلية الطب في جامعه بابل كجزء من متطلبات نيل درجة الماجستير في علم الادويه والسموم

من قبل

سمر علي محمود درويش

بكلوريوس صيدله (2013)

كليه الصيدلة/ الجامعة المستنصرية

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