Relationship between SGLT2 Inhibitor Use and Risk of Lower Limb Amputations: A Pharmacovigilance Study

By

Tahia Anjum Khandker
19146015

A thesis submitted to the School of Pharmacy in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy (Hons)

School of Pharmacy
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

Student's Full Name & Signature:

Tahia Anjum Khandker

ID: 19146015

Approval

The project titled "Relationship between SGLT2 Inhibitor Use and Risk of Lower Limb Amputations: A Pharmacovigilance Study" submitted by Tahia Anjum Khandker (19146015) of Spring, 2019 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy (Hons) on 9th March, 2023.

Supervised by:

Supervisor:

_____

Professor Dr. Eva Rahman Kabir
Dean
School of Pharmacy
Brac University

Approved by:

Assistant Dean & Program director:

_____

Professor Dr. Hasina Yasmin
Assistant Dean and Program Director,
School of Pharmacy
Brac University

Dean:

_____

Professor Dr. Eva Rahman Kabir
Dean
School of Pharmacy
Brac University

Ethics Statement

The project does not involve any kind of animal trial or clinical trial on humans.

Abstract

Type 2 Diabetes Mellitus is a chronic, complicated, multifactorial condition affecting millions of people worldwide. A variety of anti-diabetic pharmacological interventions are available for the condition. Amongst many such drugs, SGLT2 inhibitors have multiple benefits such as reno-protection, weight loss, and reduced hypertension. However, some harmful adverse effects also exist that need to be addressed and monitored to ensure patient safety. For instance, these drugs are suspected to be linked to an increased risk of limb amputations, although findings from previous studies remain inconclusive. The current study therefore collected post-marketing data from the FAERS database to establish or disprove a significant association between use of SGLT2 inhibitors and increased risk of limb amputations.

Keywords: SGLT2 inhibitors, canagliflozin, adverse events, type 2 diabetes mellitus, lower limb amputations, FAERS

Dedication

Dedicated to my respected faculty members, beloved parents and dear friends.

Acknowledgement

I want to thank Allah (SWT) for watching over me and for granting me the patience and perseverance to traverse this far. With that being said, the successful completion of this project is owed to the following individuals.

Firstly, I would like to convey my utmost gratitude to my respected supervisor, Dr. Eva Rahman Kabir (Professor and Dean, School of Pharmacy, Brac University) for her constant guidance and support throughout the entirety of this project. I am thankful to her for taking the time to proofread my thesis and for giving me this opportunity.

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List of Acronyms

T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
GDM	Gestational Diabetes Mellitus
NPH	Neutral Protamine Hagedorn
DPP-4	Dipeptidyl Peptidase-4
GLP-1	Glucagon Like Peptide-1
FDA	Food and Drug Administration
FAERS	FDA Adverse Event Reporting System
ROR	Reporting Odds Ratio
CI	Confidence Interval
SGLT1	Sodium-Glucose Co-transporter-1
SGLT2	Sodium-Glucose Co-transporter-2
ADE	Adverse Drug Events
MedDRA	Medical Dictionary for Regulatory Activities
HbAlC	Glycated Hemoglobin
SAR	Structure Activity Relationship
GLUT1	Glucose Transporter-1
GLUT2	Glucose Transporter-2

ADME	Absorption, Distribution, Metabolism, and Excretion
UGT	Uridine diphosphate-glucuronosyltransferases
CYP	Cytochrome P450
GFR	Glomerular Filtration Rate
FPG	Fasting Plasma Glucose
RTG	Renal Threshold for Glucose
ADR	Adverse Drug Reactions
MHRA	Medicines and Healthcare Products Regulatory Agency
UTI	Urinary Tract Infection
LEA	Lower Extremity Amputation
CANVAS	Canagliflozin Cardiovascular Assessment Study
CREDENCE	Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation
RAAS	Renin-Angiotensin-Aldosterone System

Chapter 1

Introduction

Diabetes is a chronic, complicated, multifactorial condition that results in inadequate secretion of insulin or insulin resistance or both, causing increased blood glucose concentration known as hyperglycemia (Husein et al., 2018). Diabetes can be classified into three main types, namely Type 1 Diabetes Mellitus, Type 2 Diabetes Mellitus and Gestational Diabetes Mellitus.

Type 1 Diabetes Mellitus

Type 1 Diabetes Mellitus (abbreviated as T1DM) is an auto-immune disorder, characterized by destruction of pancreatic β-cells that is mediated by T-cells, thereby causing a decrease in synthesis and secretion of the peptide hormone, insulin, (Akil et al., 2021). As T1DM has inadequate islet of Langerhans cell repair mechanisms, and leads to complete absence of insulin, it eventually leads to disturbances in glycemic control. Insulin injections are therefore the first-line treatment strategy for this condition (Pathak et al., 2019). Till date, T1DM therapy regimens aim to include a balance between food intake and the injected insulin while encouraging patients to make lifestyle changes such as daily exercise and maintaining a good sleep schedule. The basis of T1DM insulin centered therapy is combining basal and bolus insulin in accordance to caloric intake (Powers, 2021).

Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus (abbreviated T2DM), a heterogeneous disease characterized by elevated blood glucose levels results from decreased insulin production or insulin resistance (insensitivity), caused by defective β-cells of the pancreas. 90% of the diagnosed diabetes cases are T2DM, making it the most common type of diabetes worldwide. The two major risks for development of T2DM are obesity and inactive lifestyle in modern times (Dahlén et al., 2022). Hyperglycemia causes microvascular complications (retinopathy, neuropathy, nephropathy)

and macrovascular complications (cardiovascular disease, peripheral artery disease and stroke)(Chawla et al., 2016). Drug treatment is usually initiated by the time the disease has progressed to its mid-stage as the symptoms appear gradually over time. Treatment of T2DM focuses on continuous control of blood glucose levels. Most treatment regimens vary from patient to patient starting with recommended lifestyle changes such as encouraging frequent exercise, healthy diet and appropriate body weight. If changes in lifestyle are inadequate in achieving desired glycemic control, pharmacological measures are initiated (C. J. Bailey & Day, 2018). In most cases, the first line treatment for T2DM is the biguanide, metformin. Conversely, sulfonylureas (e.g. glimepiride) can act as a first-line treatment if the patient does not need to lose weight or is intolerant to metformin. Otherwise, sulfonylureas typically acts as a second-line treatment, in combination with metformin. Injectable insulin (NPH) acts as a third line treatment in combination with metformin and sulfonylureas. In cases where insulin is not an option, oral hypoglycemic agents such as dipeptidyl peptidase-4 inhibitors (DPP-4 inhibitors) e.g. sitagliptin can be used instead ("Optimal Second- and Third-Line Therapy in Type 2 Diabetes," 2013). Other second and/or third line treatment strategies include oral hypoglycemic agents such as meglitinides (e.g. nateglinide), thiazolidinedione (e.g. rosiglitazone), sodium-glucose cotransporter-2 inhibitors (e.g. empagliflozin), alpha-glucosidase inhibitors (e.g. acarbose) and subcutaneous injections such as GLP-1 receptor agonists e.g. liraglutide (C. J. Bailey & Day, 2018; Simos et al., 2021).

Gestational Diabetes Mellitus

Gestational Diabetes Mellitus (GDM) develops in pregnant women, usually in the third trimester (after 24 weeks) and is characterized by elevated blood glucose levels and abnormal glucose tolerance. Though the condition, generally resolves after childbirth, patients with GDM are at a risk of developing T2DM during their later years (Mishra et al., 2021). Management of GDM primarily involves medical nutritional therapy and blood glucose

monitoring to achieve the desired glycemic target. Insulin therapy (along with lifestyle interventions) is considered as first-line treatment when there is failure to obtain required blood glucose levels. Alternatively, oral anti-hyperglycemic agents such as metformin or glyburide can be utilized if nutritional and insulin therapy prove to be insufficient (Sandu et al., 2021).

It is essential that a robust post-marketing surveillance system is established to ensure patient safety. According to the World Health Organization, pharmacovigilance is defined as "the science and activity related to the detection, assessment, understanding and prevention of adverse effects or any other possible drug-related problems". It has been widened to include products such as traditional and complementary medicines, herbal medicines, blood products, biologicals, medical devices and vaccines (World Health Organization. & WHO Collaborating Centre for International Drug Monitoring., 2002). Additional issues need to be assessed such as medication errors, inadequate reports of efficacy, use of substandard medicine, off-label use of medication (i.e. unapproved indication and lack of scientific evidence for use), reports of acute and chronic poisoning, drug related mortality, abuse and misuse of medicines and health products, along with adverse interactions of medicines occurring with chemicals, food or other drugs (Nour & Plourde, 2019).

Pharmacovigilance is vital to the process of drug development and remains relevant in clinical practice through post-marketing research and reporting by healthcare professionals (Thomas & Klika, 2019). Each country has its own mechanism for the post-marketing safety monitoring of drug products available in the market. In the USA, The FDA (Food and Drug Administration) manages its pharmacovigilance through its database, FAERS (FDA Adverse Event Reporting System), a spontaneous reporting system that conducts post-marketing safety monitoring of drug and bio-therapeutics by collection of world-wide reports of adverse events

(Zhou et al., 2021). FAERS database includes important information regarding adverse events, medication errors, and patient demographics. It collects reports from consumers, health-care professionals and manufacturers (Zhou et al., 2021) to detect and quantify signals between the drug and reported adverse event (Fang et al., 2014). Pharmacovigilance can be approached clinically, experimentally, epidemiologically, or diagnostically. Eventually, the primary goal of pharmacovigilance is optimization and characterization of the risk/benefit ratio of a drug through its entire product life cycle (Nour & Plourde, 2019).

Disproportionality analysis is a tool to establish a causal relation between drugs and adverse effects, by comparing between observed and expected numbers of reports, for any given combination of drug and adverse event (Caster et al., 2020). One such disproportionality method is use of case-non case studies, utilized for analysis of pharmacovigilance databases. The disproportionality studied can therefore generate signals for unknown adverse events experienced by patient after the drug is launched into the market. A signal in this case is a higher than expected number of adverse drug reactions being reported for a specific drug, thus generating a disproportionate rate of reporting compared to other reactions recorded in the database. Reports on the adverse reaction of interest for suspected drug are called cases and the other reports are called non-cases.

The reporting association between the adverse reaction of interest and suspected drug is based on analysis of a 2 × 2 contingency table. When there is no signal, the distribution of cases and non-cases is independent of exposure to the suspected drug. This means adverse reaction reports on exposure to the drug of interest and other drugs is similar. When there is a difference in this distribution, a ‘‘disproportionality’’ is said to be found, determined by calculating the reporting odds ratio (ROR).

The ROR measures the strength of disproportionality. The ROR represents the odds of the association of the adverse reaction with suspected drug versus the odds of the association of the same adverse reaction with all the other drugs in the database. When the ROR is 1, there is no signal as the adverse reaction of interest is being reported at the same rate with drug of interest compared to other drugs in database. Again, when the ROR is less than 1, there is no signal as the adverse reaction of interest is being reported less for the drug of interest compared to other drugs in database. A signal is generated when the ROR is greater than 1, i.e. more adverse reactions are being reported for the suspected drug compared to other drugs in the database. The higher the value of ROR the greater the disproportionality. The ROR is always interpreted with its 95% confidence interval. When the lower value of 95% CI is greater than 1, a positive signal is detected which means that the suspected drug has a greater association with the adverse reaction of interest compared to other drugs in the database. Conversely, when the lower value of the 95% CI is less than 1, no signal is generated (Faillie, 2019).

1.1 Aim of the project

The aim of the study was to investigate the incidence of adverse side effects in patients prescribed with FDA approved sodium-glucose cotransporter-2 (SGLT2) inhibitors.

1.2 Objectives of the project

1. To examine whether there is a link between SGLT2 inhibitor use and occurrence of lower limb amputations.

2. To explore whether or not drugs belonging to the therapeutic class of SGLT2 inhibitors, specifically canagliflozin, increase the risk of lower limb amputations in diabetic patients.

Chapter 2

Methodology

2.1 Literature search

A literature search was carried out on PubMed and Google Scholar database using key words such as “adverse effects of SGLT2 inhibitors”, “adverse effects of canagliflozin”, “adverse effects of empagliflozin”, “adverse effects of dapagliflozin”, “adverse effects of ertugliflozin”, “type 2 diabetes”, “lower limb amputation”, and “pharmacovigilance”.

The articles collected were restricted to those published during the year 2017-2022. Only articles related to FDA approved SGLT2 inhibitors and lower limb amputations were included.

Articles not written in the English language were excluded. Literature related to SGLT2 inhibitors not approved by the FDA currently were also excluded. Articles that provided inadequate and unreliable data were also not included.

The type of literature sources were included but not limited to original research, review articles, systematic reviews, meta analyses, brief reports, commentary, correspondence, patient case reports and others. The articles contained data from randomized clinical trials, observational studies, retrospective cohort study, population-based cohort study, disproportionate analysis, real-world retrospective cohort study, pharmacovigilance analysis, etc.

A total of 33 articles were finally selected for use in the project. These articles state that lower limb amputation may potentially be associated with canagliflozin use but the results remain inconclusive. This adverse event was therefore selected for further evaluation to bridge the research gap.

The FAERS database containing reports on Adverse Drug Events (ADEs) was then utilized. This involved collecting and compiling the type and number of adverse drug reaction cases reported for the four FDA approved SGLT2 inhibitors, “dapagliflozin”, “canagliflozin”, “empagliflozin” and “ertugliflozin” during January 2017- September 2022.

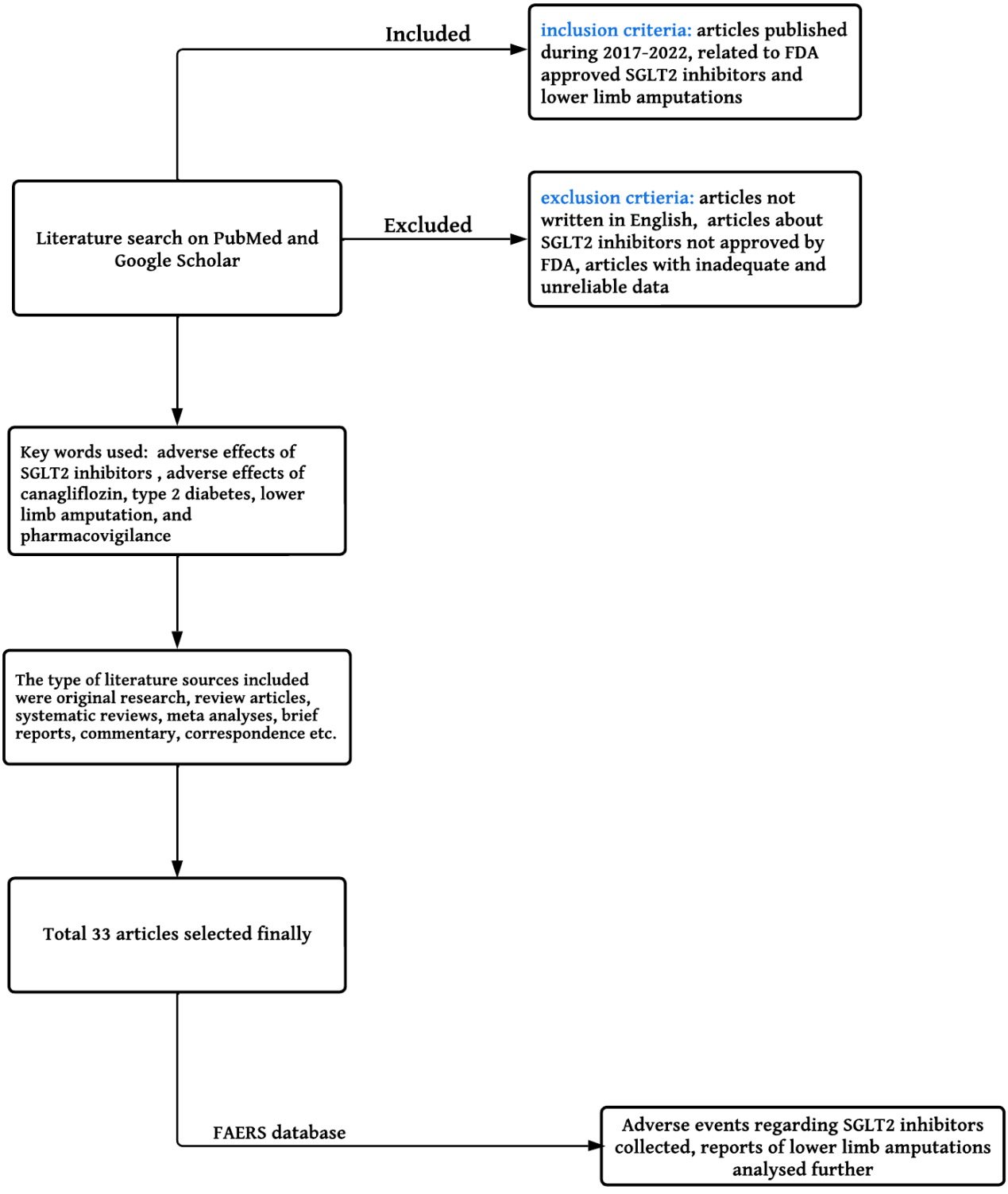


Figure 1: Research Methodology

2.2 Data collection and analysis

FAERS, a quarterly updated database was used as the source for collecting data on adverse events reported. It contains information on suspected drugs, concomitant drugs, events, reporter type, reporting country and outcome (Kvist et al., 2021). Data from January, 2017 to September, 2022 was extracted and analyzed in this study with the search being conducted in January, 2023. The search terms included the generic names for the drugs: canagliflozin, empagliflozin, dapagliflozin, ertugliflozin and different types of Insulin (Insulin Human, Insulin Lispro, Insulin Aspart, Insulin Glulisine, Insulin Detemir, Insulin Degludec and Insulin Glargine). The standard terminology of the Medical Dictionary for Regulatory Activities (MedDRA) were used to classify terms for the adverse events.

Adverse events reported in the FAERS database for canagliflozin, empagliflozin, dapagliflozin, ertugliflozin (drugs of interest) along with different types of insulin (reference drug) were included. Data was included from January, 2017 to September, 2022. Duplicated reports were excluded using case number, and also by matching age, sex and event date. Any data where a number of other suspected drugs were present was also excluded. The investigation was conducted using limb amputation as the endpoint. The end points of the adverse effect were identified using the standardized terminologies of the MedDRA.

Data was collected and analyzed in January 2023 using the FAERS database. A disproportionality analysis was conducted, with signals of reporting odds ratio (ROR) and its corresponding 95% confidence interval (CI) being generated to establish a reporting association between the suspected drug and limb amputation. The ROR and its 95% CI were calculated by two approaches. Firstly, the SGLT2 inhibitors were compared with the whole database in terms of ROR and CI to find which drug in that class has the highest reporting association. Secondly, drugs in the insulin class were used as a reference to remove disease bias since the treatment of diabetes is associated with higher risk of amputation (Hippisley-

Cox & Coupland, 2016). However, in this case, the indication was restricted to Type 2 diabetes only. All the data analyses were performed using R, version 4.2.1.

Chapter 3

Sodium-Glucose Co-transporter-2 (SGLT2) inhibitors

The kidneys play a vital role in glucose homeostasis. All glucose molecules once filtered goes through reabsorption at the proximal tubule of the kidneys to an extent, after which glucose appears in urine proportionally to the increase in plasma glucose levels. During the development of early anti-hyperglycemic agents, Type 2 diabetes was treated by increasing glucose uptake, reducing insulin sensitivity or restoring β-cell activity, to keep blood glucose levels within a normal range (Kshirsagar et al., 2020). Two sodium dependent-glucose transporters, SGLT1 and SGLT2, that facilitate glucose reabsorption at the proximal tubule, were discovered in 1950. As SGLT2 account for 90% of the glucose reabsorbed, it is a desirable potential target for antidiabetic drug development, most recently by a class of drugs called SGLT2 inhibitors (Kshirsagar et al., 2020; Simes & Mac Gregor, 2019).

Currently, the FDA approved SGLT2 inhibitors are

1. canagliflozin
2. dapagliflozin
3. empagliflozin
4. ertugliflozin

SGLT2 inhibitors work by increasing renal excretion of glucose and are independent of the action of insulin, thus hypoglycemia is avoided. Additional benefits of the drug include weight loss, reduced HbA1C levels, and a decrease in systolic blood pressure. The drugs also decrease vascular disease risk factors and provides reno-protection by decreasing albuminuria (Pittampalli et al., 2018).

3.1 Structure of SGLT2 inhibitors

The first non-selective SGLT inhibitor was phlorizin, derived from the bark of apple trees. Structurally, it is a β-d-glucoside, composed of a glucose moiety, an aglycone and two aromatic carbocycles held together by an alkyl group (Isaji, 2011). However, initially phlorizin could not be used as an antihyperglycemic agent due its short-half life, poor oral bioavailability and non-selectivity for SGLT1/SGLT2 transporter. These problems were overcome by the development of C-glycosylated phlorizin derivatives that prevented hydrolysis of the drug molecule, and are currently used as SGLT2 inhibitors in the treatment of Type 2 diabetes (Simes & Mac Gregor, 2019). These aromatic and heteroaromatic C-glycoside structures that were later developed, contained a glucose group which linked to aglycone directly via a carbon-carbon bond, for e.g. in Dapagliflozin. These drugs also have higher selectivity for SGLT2 receptors over SGLT1(Isaji, 2011; Wright, 2021).

Empagliflozin, another SGLT2 inhibitor has a C-glucoside resembling the one in phlorizin. The glucose and aglycone moieties are joint via a carbon-carbon bond that allows the drug to bypass degradation in the gastrointestinal tract and get rapidly absorbed (Kshirsagar et al., 2020). Canagliflozin is a C-glycosylic compound used in its hemihydrate form (*Canagliflozin | C24H25FO5S - PubChem*, 2023). Lastly, the structure of Ertugliflozin is a unique bridged ketal ring system that makes the drug SGLT2 selective (Cinti et al., 2017).

Overall, as C-glycosides are more potent, and provide prolonged glucose excretion during day and nighttime, they are suitable for once daily dosing. On the other hand, O-glycosides, are short acting, so patients are less likely to be compliant, however these drugs have the better safety profile. Presently, pharmaceutical companies are trying to develop drugs that have a long duration of action, are highly potent and SGLT2 selective (Isaji, 2011).

3.2 Structure activity relationship (SAR) of SGLT2 inhibitors

A drug or a ligand binds to its corresponding active site in a particular way to achieve its desired biological activity. The structure activity relationship is a way to establish a relationship between the chemical structure of a drug/ligand with its biological activity, that is it links chemical structure to chemical property or biological activity. The assumption is that structurally similar molecules will therefore exhibit similar biological activity(Penta, 2016).

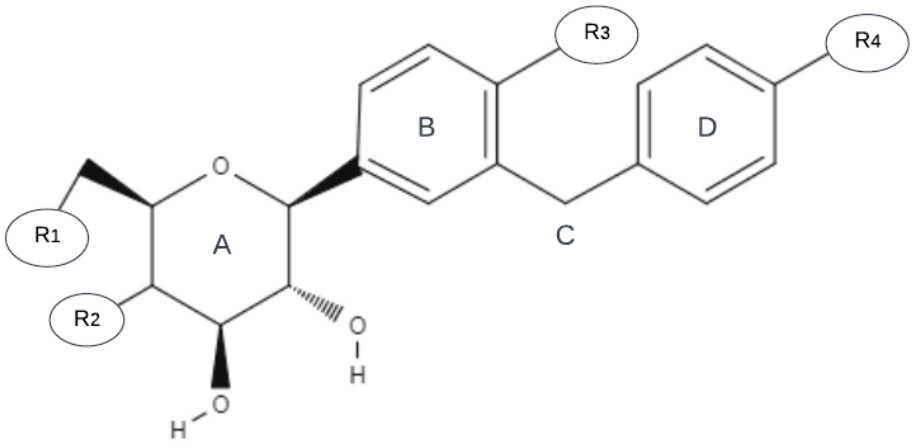


Figure 2: Structure activity relationship of SGLT2 inhibitors adapted from (Bhattacharya et al., 2020)

The structure of the SGLT2 inhibitors shown in Figure 2 has 4 distinct regions:

- A glucose moiety (A)
- A central benzene ring (B)
- A methylene bridge (C)
- A distal benzene ring (D)

3.3 General mechanism of action of SGLT2 inhibitors

In order to understand the mechanism of action of SGLT2 inhibitors, we also need to be familiar with the physiology of SGLT1 and SGLT2, the primary sodium-glucose transporters in the body. These co-transporters help to facilitate the transport of various solutes through a positive gradient. SGLT1, is a high capacity, low-affinity glucose transporter that is primarily located in the small intestines, heart, skeletal muscle and kidneys whereas SGLT2 is a low capacity, high affinity glucose transporter expressed mainly in the kidneys. Together, SGLT1 and SGLT2 are responsible for reabsorbing 100% of filtered glucose in the proximal tubules of the kidney, thereby preventing energy lost through glucose excreted in urine. SGLT2 cotransporters are located in the S1 and S2 segment of the proximal tubule, lining the brush border of renal tubular cells and facilitate reabsorption of up to 90- 97% of filtered glucose. The rest of the glucose is reabsorbed through the SGLT1 cotransporter situated in the S3 section of the proximal tubule(Fonseca-Correa & Correa-Rotter, 2021).

Reabsorbed glucose exits the renal tubular cells and goes back into systemic circulation through glucose transporter 2 (GLUT2) for SGLT2 and glucose transporter 1 (GLUT1) for SGLT1 transporters located in the basolateral membrane. The one way transportation of sodium and glucose is mediated by Na-K-ATPase pump present in the basolateral membrane. Glucose reabsorbed in the proximal tubules changes according to deviations in serum glucose levels in normal physiological conditions (Fonseca-Correa & Correa-Rotter, 2021; Hsia et al., 2017).

SGLT2 inhibitors block SGLT2 co-transporters that are located in the proximal tubules. This prevents glucose reabsorption and enhances urinary glucose excretion, resulting in lower blood glucose levels. The SGLT2 inhibitor mechanism of action is insulin-independent and relies primarily on blood glucose levels. Insulin-independence has the advantage of the drug being utilized in advanced stages of Type 2 Diabetes Mellitus, particularly when pancreatic beta cells have been completely depleted. It also poses minimum risk for hypoglycemia and does not overstimulate beta cells. However, SGLT2 inhibitors have a reduced efficacy in patients with renal impairment as it solely depends on normal functioning of kidney cells (Kalra, 2015).

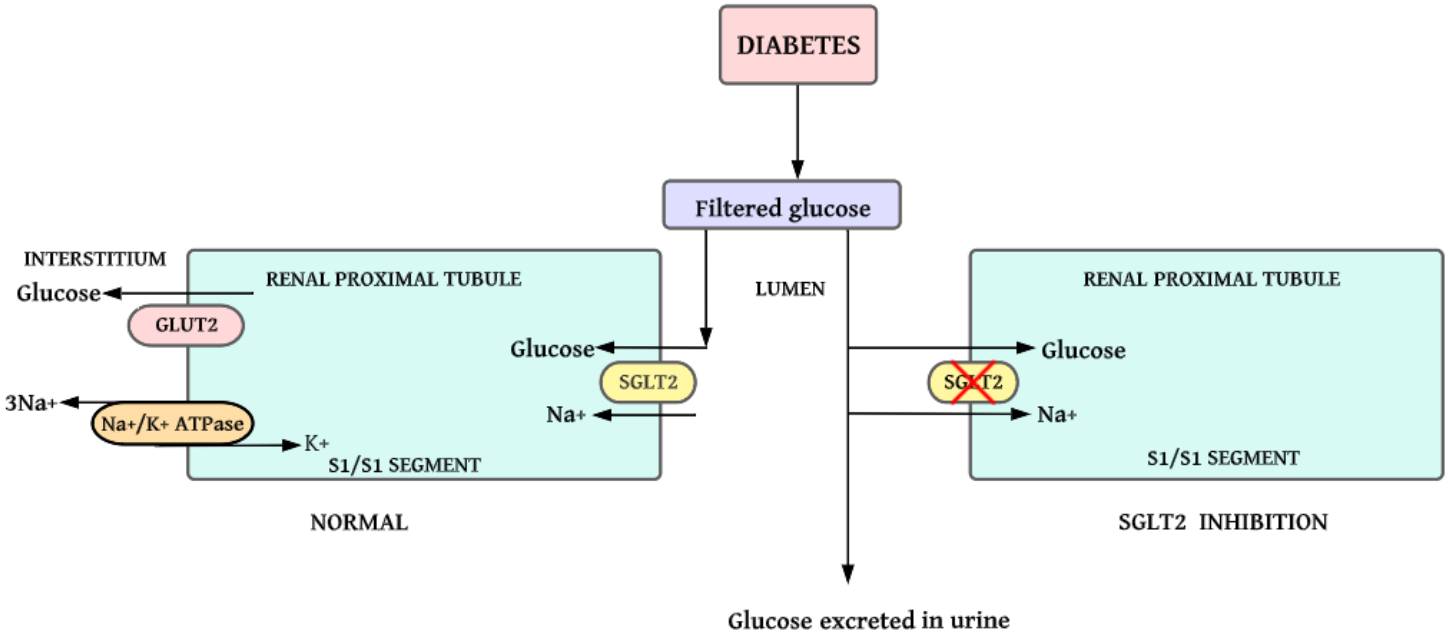


Figure 3: Mechanism of Action of SGLT2 inhibitors, adapted from (Kaur et al., 2021)

3.4 Pharmacokinetics of SGLT2 inhibitors

Pharmacokinetics (PK) studies the interaction of the body with the drug administered for the entire period of exposure. The four main pharmacokinetic parameters are absorption, distribution, metabolism, and excretion (ADME). Familiarity with pharmacokinetic principles aids physicians in prescribing and administering, safe and efficacious medicine while making changes as needed depending on patient lifestyle and physiology (Chappell & Payne, 2022).

Dapagliflozin

Dapagliflozin is 1200 times more selective for SGLT2 than SGLT1. It undergoes oxidation metabolism and glucuronidation in the liver to form the metabolites, Dapagliflozin 3-O-glucuronide (primary) and Dapagliflozin 2-O-glucuronide (secondary). The drug is removed by biliary, renal and intestinal clearance (Kaur et al., 2021).

Canagliflozin

Canagliflozin is less selective for SGLT2 compared to dapagliflozin (250 fold selectivity for SGLT2 over SGLT1). The enzymes involved in canagliflozin metabolism are UGT1A9 and UGT2B4 hepatic enzymes along with the cytochrome P450 enzyme, CYP3A4. A total of 93% of canagliflozin is eliminated, 30% through urine, and 60% through feces (Kaur et al., 2021).

Empagliflozin

Empagliflozin has a 2500 fold selectivity for SGLT2 compared to SGLT1, making it the most selective SGLT2 inhibitor. Around 55% of the drug is eliminated by renal pathways, another 40 % is eliminated by fecal routes (Garcia-Ropero et al., 2018). A total 95% of empagliflozin is eliminated through urinary (54%) and fecal routes (41%). The enzymes specifically involved in its metabolism are UGT1A3, UGT1A8, UGT1A9, and UGT2B7 (Kaur et al., 2021).

Ertugliflozin

Ertugliflozin is metabolized into the pharmacologically inactive metabolites, 2-O-β-glucuronide and 3-O-β-glucuronide primarily by hepatic metabolic enzymes, UGT1A9, UGT2B7 and also by CYP3A4 enzyme (Kaur et al., 2021).

3.5 Pharmacodynamics of SGLT2 inhibitors

Pharmacodynamics studies the molecular, biochemical or physiological action of a drug molecule on the body. Pharmacodynamic goals in therapeutic settings are to exert maximum pharmacological action at lowest required dose while minimizing drug interactions that result in an adverse event (Yue et al., 2022).

SGLT2 inhibitors have not yet been reported to show any clinically significant drug-drug interactions. They can be given in combination with anti-diabetic agents like sulfonylureas, metformin, pioglitazone, and sitagliptin. However, there is potential risk of volume depletion, diuresis and dehydration when they are concomitantly prescribed with loop diuretics and thiazides. This combination is therefore avoided (Kalra, 2015). Additionally, patients with baseline renal impairment, or those on ACE inhibitors or potassium-sparing diuretics are exposed to a greater risk for hyperkalemia. This is due to changes in potassium transport at the renal tubules due to SGLT2 inhibition (Thynne & Doogue, 2014).

Dapagliflozin

No drug interactions of dapagliflozin with other anti-diabetic agents used for T2DM have been reported. The drug is not recommended with patients older than 75 years old, individuals who are pregnant or breastfeeding and those with moderate to severe renal impairment. The initial dose of dapagliflozin is lowered to 5mg once daily for patients with severe hepatic impairment (Garcia-Ropero et al., 2018).

Canagliflozin

Canagliflozin is not recommended in patients with severe hepatic impairment or patients with a glomerular filtration rate (GFR) of less than < 45 mL/min/1.73 m2. A maximum dose of 100mg is prescribed in the older population. The drug is not usually prescribed in pregnancy. (Garcia-Ropero et al., 2018). Concomitant administration of canagliflozin with inducers of UGT enzyme e.g. ritonavir, phenytoin, can increase its metabolism, leading to decrease in peak plasma levels and efficacy. Therefore an increase in dose from the initial 100 mg to 300mg of canagliflozin is recommended (Kalra, 2015). The plasma concentration of digoxin may increase with co-administration of canagliflozin. Therefore when initiating or stopping canagliflozin therapy, digoxin plasma concentrations are monitored (Thynne & Doogue, 2014).

Empagliflozin

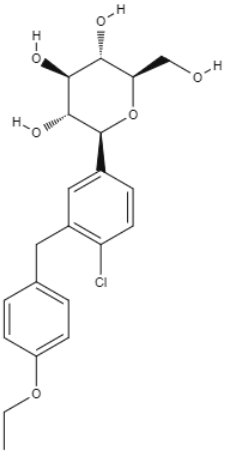
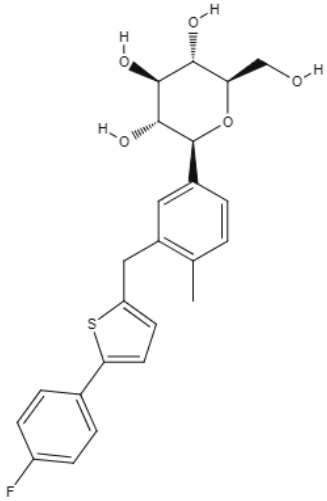
Empagliflozin is rapidly absorbed post oral administration, however rate drops in patients with renal dysfunction (Kaur et al., 2021). The drug, reportedly has no interactions with other anti-diabetic agents used in T2DM. It is not usually prescribed for pregnant or breastfeeding patients. The drug is well tolerated in stages 2-3 of chronic kidney disease and may induce hypoglycemia in the fourth stage (Garcia-Ropero et al., 2018).

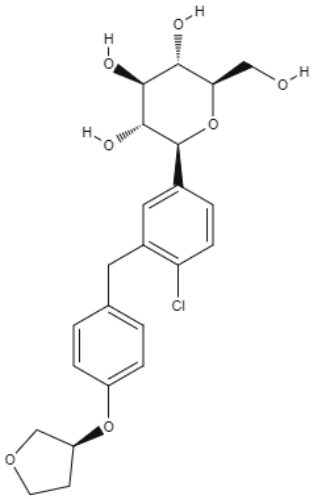
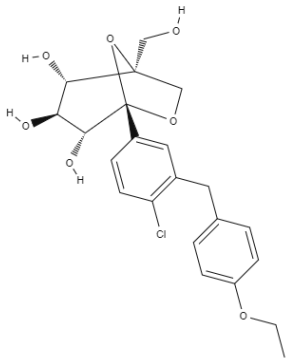
Ertugliflozin

Ertugliflozin is contraindicated for patients with a glomerular filtration rate of less than 30 mL/min/1.73 m2 and the therapy must not be initiated in patients with a glomerular filtration rate of less than < 60 mL/min/1.73 m2. The drug does not have any serious drug-drug interactions, and exhibits a similar precaution profile as other drugs in the SGLT2 inhibitor class (Garcia-Ropero et al., 2018).

Table 1 gives an overview of the structure, and important pharmacokinetic and pharmacodynamic properties of the SGLT2 inhibitors.

Table 1: Overview of SGLT2 inhibitors

Drug	**Structure (all are aryl C-glycosides)**	**Pharmacokinetics**	**Pharmacodynamics**
Dapagliflozin Brand name: Farxiga FDA approved in January, 2014		**Oral bioavailability:** 78% **Half-life:**13 h **Tmax:** 1-1.5 h **Volume of distribution:** 118 L **Protein binding:** 78% **SGLT2 inhibitory concentration:** 1.2 nM	Increases urinary glucose excretion Reduces blood pressure and serum uric acid concentrations
Canagliflozin Brand name: Invokana FDA approved in March, 2013		**Oral bioavailability:** 65% **Half-life:** 11 h **Tmax:** 1-2 h **Volume of distribution:** 83.5L **Protein binding:** 99% **SGLT2 inhibitory concentration:** 2.7 nM	Increases urinary glucose excretion Decreases body weight and systolic blood pressure Decrease in HbA1c (glycated hemoglobin) Decrease in fasting plasma glucose (FPG) and renal threshold for glucose (RTG)

Empagliflozin Brand name: Jardiance FDA approved in August 2014		**Oral bioavailability:** 75% **Half-life:** 13 h **Tmax:** 1.5h **Volume of distribution:** 73.8L **Protein binding:** 86% **SGLT2 inhibitory concentration:** 3.1 nM	Increases urinary glucose excretion Decreases body weight HbA1c and systolic blood pressure Decrease in fasting plasma glucose (FPG) and RTG
Ertugliflozin- Brand name: Steglatro FDA approved in December 2017		**Oral bioavailability:** 90-100% **Half-life:** 11-17 h **Tmax:** 0.5-1 h **Volume of distribution:** 86L **Protein binding:** 93% **SGLT2 inhibitory concentration:** 0.9 nM	Increases urinary glucose excretion Decreases systolic blood pressure Decrease in FPG

Chapter 4

Adverse drug events of SGLT2 inhibitors

Adverse drug events (ADEs) can be defined as an unintentional injury or harm that results from use of a medication. Adverse drug reactions (ADR) fall under this category and are defined as noxious and unintended responses to drugs administered at human doses for therapeutic, diagnostic or prophylactic means. Detection, documentation and reporting of ADRs are key to pharmacovigilance which involves reporting rare, perhaps dangerous adverse events that were not detected in clinical trials before the drug gained market approval. The dataset required for valid reporting includes stating the ADE, suspected medication/ drug product, an identifiable patient and reporter (C. Bailey et al., 2016).

Regulatory bodies all around the globe have established their own adverse drug reporting system. For instance, in the United Kingdom, the regulatory body is the Medicines and Healthcare Products Regulatory Agency (MHRA) that has developed the "yellow card scheme" as an ADR form. Health Canada has developed the database Canada Vigilance that collects reports through Canada Vigilance Reporting form. Similarly, the USA has the FAERS database that collects reports through MedWatch (Kumar & Khan, 2015).

As with all drug products, it is essential to monitor the ADEs of SGLT2 inhibitors. Table 2 is a summary of the adverse events of SGLT2 inhibitors reported during January 2017-September 2022 in FAERS database.

Table 2: Adverse events of SGLT2 inhibitors as reported in FAERS

Adverse event reported	**Adverse event as reported in FAERS (2017-2022)**			
	Canagliflozin	Empagliflozin	Dapagliflozin	Ertugliflozin
	(FDA approved,2013)	(FDA approved, 2014)	(FDA approved, 2014)	FDA approved,2017)
urinary tract infections (UTI) (Donnan et al., 2019)	301	702	184	1
Lower limb amputation (Donnan et al., 2019)	4,038	178	69	1
Toe Amputation	2,142	94	40	1
Leg Amputation	752	41	15	-
Foot Amputation	562	20	9	-
Amputation	319	14	5	-
Limb Amputation	261	9	-	-
Limb Traumatic Amputation	2	1	-	-
Finger Amputation	19	2	4	-
Hand Amputation	2	1	-	-
Arm Amputation	2	1	-	-
Spontaneous Amputation	-	-	1	-
volume depletion (Qiu et al., 2021).	14	57	13	-
Genito-Urinary Tract Infection (Garofalo et al., 2019)	1	15	2	-
Acute Kidney Injury- more strongly acute nephrotoxicity	1,563	480	257	-
renal insufficiency	467	156	73	-
dehydration	218	526	174	1
orthostatic hypotension	8	18	8	-
euglycemic DKA	210	990	434	-
Diabetic keto acidosis (Musso et al., 2020)	2,147	2,611	1,116	-

metabolic acidosis	221	282	136	1
lactic acidosis	60	132	117	-
ketoacidosis(Pereira & Eriksson, 2019).	431	953	486	3
Genital mycotic infections (Engelhardt et al., 2021)	20	71	4	-
Myocardial infarction (Han et al., 2021)	277	248	64	1
Stroke	23	24	40	1
Severe hypoglycemia	70	178	137	-
Bone fracture	106	152	72	2
Fournier gangrene (Scheen, 2019)	233	518	96	5

The most common ADE reported are:

- Diabetic ketoacidosis. (Musso et al., 2020)
- urinary tract infections (Donnan et al., 2019)
- volume depletion (Qiu et al., 2021)
- Bone fracture
- Acute kidney injury
- Dehydration (Garofalo et al., 2019)
- Lower limb amputation (Donnan et al., 2019)

This study focused on establishing an association between SGLT2 inhibitor use and lower limb amputations.

The lower extremities are divided into the thigh, lower legs and foot. Lower limb amputation is surgical removal of one part or several parts of the lower limb, also known as lower extremity amputation (LEA). The types of lower limb amputation are above-knee amputation, below-knee amputation, through-knee amputation, amputation at the ankle, amputation at the hip, and foot-amputation including toes. Amputation is indicated depending on the degree of tissue

viability and necrosis (Sexton & Fleming, 2022). The most common cause leading to amputation is Type 2 Diabetes Mellitus, with diabetic patients 30 times more likely to undergo amputation than those without the disease. Other causes could be due to peripheral vascular disease, neuropathy and traumatic injury.

Chapter 5

Results and Discussion

Table 2 shows that during 2017-September 2022, the total number of lower limb amputations reported to the FAERS database for the SGLT2 inhibitor drug class was 4286. Out of these, 4038 cases were attributed to Canagliflozin. Empagliflozin, dapagliflozin and ertugliflozin, had 178, 69 and 1 cases reported, respectively. Recently, randomized clinical trials (RCTs) conducted have had inconclusive and differing findings. Firstly, the CANVAS program (two trials involving 10,142 volunteers with T2DM and cardiovascular risk) reported a significant association between canagliflozin use and increased risk of lower limb amputations in comparison to a placebo (Neal et al., 2017). Conversely, in the CREDENCE trial (involving participants with T2DM and chronic kidney disease), there was no significant difference in Canagliflozin use and risk of lower limb amputations (Perkovic et al., 2019).

Table 3: ROR and 95% CI values for SGLT2 inhibitors with whole database as reference.

Drug	ROR (Reporting odds ratio)	CI (Confidence Interval)
Canagliflozin	1923	1817.5 to 2034.7
Empagliflozin	19.7	16.8 to 23.1
Dapagliflozin	26.7	20.6 to 34.6
Ertugliflozin	100.4	13.5 to 746.8

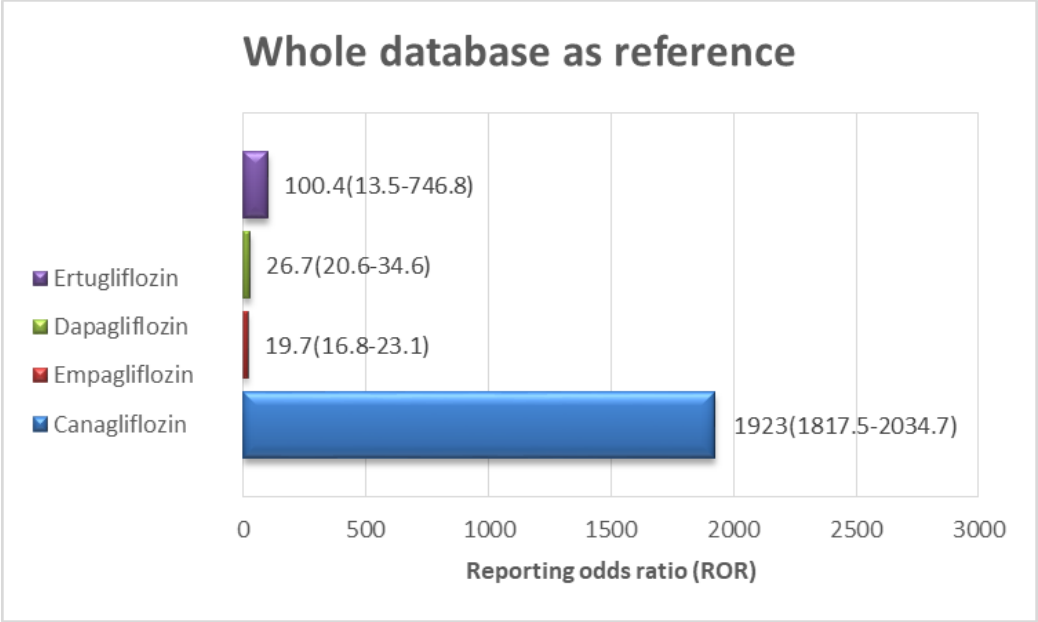


Figure 4: Comparison of SGLT2 inhibitors with other drugs in the database in terms of ROR and 95% CI shown in brackets.

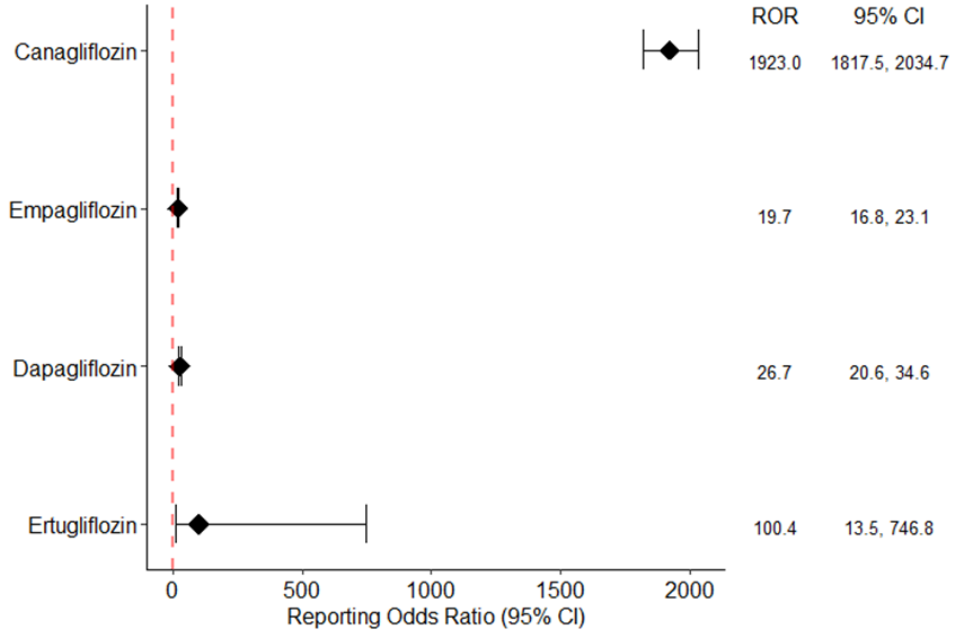


Figure 5: Forest plot for each drug compared to whole database

This study used two approaches for signal detection. The first approach involved comparing the drugs in the SGLT2 inhibitor class with other drugs in the database, i.e. the whole database was used as a reference. The results are displayed in Table 3 where all four drugs in the class exhibited strong signals. Canagliflozin had the highest signal detected amongst the four drugs (ROR 1923, 95% CI 1817.5-2034.7) and this was followed by ertugliflozin (ROR 100.4, 95% CI 13.5-746.8). Dapagliflozin had the third highest signal (ROR 26.7, 95% CI 20.6-34.6), and empagliflozin had the lowest signal (ROR 19.7, 95% CI 16.8-23.1). Based on the results from these findings, a link between treatment with drugs in the SGLT2 inhibitor class and increased risk of lower limb amputations has been established. When the whole database was used as a reference, all four drugs in this class exhibited an ROR greater than 1 and therefore count as a positive signal. These results are represented graphically in Figure 4.

Figure 5 shows these findings in a forest plot for each drug compared to whole database. The vertical red line is the line of null effect, placed at the value where there is no association between the exposure (to drug) and outcome (adverse reaction endpoint). The null effect value is usually 0 or 1 and in this case for odds ratio, it is 1. The diamond represents the pooled estimate from all four studies combined and the horizontal line through the diamond indicates the 95% confidence interval range (Dettori et al., 2021). Figure 5 shows ertugliflozin having the widest CI, followed by canagliflozin.

Table 4: ROR and 95% CI values for SGLT2 inhibitors with insulin as reference

Drug	ROR (reporting odds ratio)	Confidence Interval (CI)
Canagliflozin	417.98	(320.02 to 545.93)
Empagliflozin	4.28	(2.95 - 6.22)
Dapagliflozin	10.73	(7.19 - 16.03)
Ertugliflozin	Inadequate data	Inadequate data

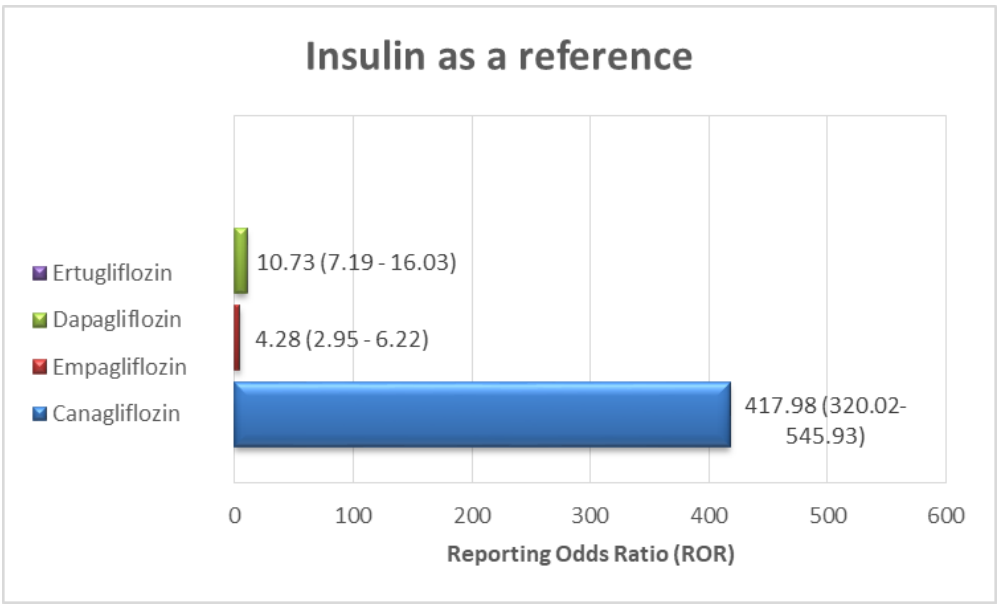


Figure 6: Comparison of SGLT2 inhibitors with insulin in terms of ROR and 95% CI interval in brackets

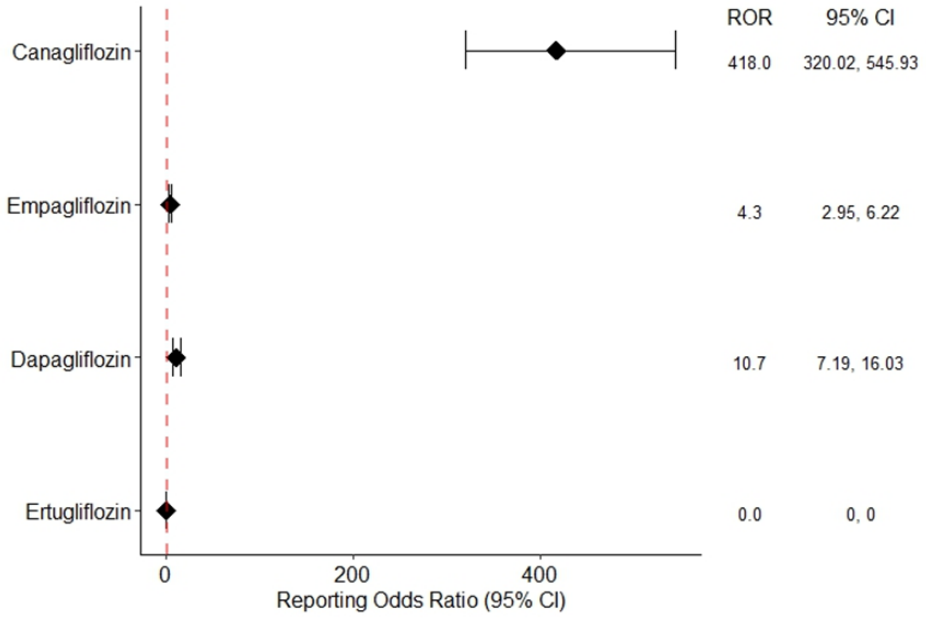


Figure 7: Forest plot for each drug compared to Insulin class

On the other hand, the second approach used insulin as the reference drug to eliminate disease bias from Type 2 Diabetes Mellitus. Insulin is initiated in T2DM usually when the hypoglycemia remains uncontrolled even with oral antidiabetic therapy and an initial A1C greater than 9% (Petznick, 2011). This approach will therefore determine whether the adverse effect cases reported are due to pathological changes caused by the disease itself or are linked to the antidiabetic drug in question. The results from this analysis show that Canagliflozin, empagliflozin and dapagliflozin exhibited strong signals (Table 4). The highest signal detected was for canagliflozin (ROR 417.98, 95% CI 320.02-545.93), followed by dapagliflozin (ROR 10.73, 95% CI 7.19 - 16.03) and empagliflozin (ROR, 4.28 95% CI 2.95 - 6.22). The number of cases for ertugliflozin reported during 2017-2022 were 0 when insulin was used as a reference as it is relatively newly approved drug (FDA approved in 2017). These results are represented graphically in figure 6. Therefore, for the other 3 drugs in this class, we can rule out diabetes as a confounding factor to the adverse effect reported. Patient history is taken into account when data is entered into the FAERS database, so it is unlikely that patients with co-morbid conditions were included. Figure 7 shows these findings in a forest plot where each drug in the SGLT2 inhibitor class is compared to the insulin class. The figure also represents the 95% CI graphically, depicted by the horizontal lines on either sides of the diamond and it is seen that canagliflozin has the widest CI.

The exact mechanism by which SGLT2 inhibitor are associated with amputations is unknown. One hypothesis indicates that hypovolemia caused by these drugs reduces blood flow to lower extremities and as these drugs increase hematocrit levels, they increase blood viscosity. This leads to ischemia and eventually amputation, more likely in patients with already decreased lower limb blood perfusion. Another hypothesis dictates that SGLT2 inhibitors activate the renin-angiotensin-aldosterone system (RAAS), which causes a decrease in nitric oxide, a vasodilator important for regulating tone of the afferent arteriole. If the arterioles of the lower

limbs also experience a decrease in nitric oxide, it could be a cause of amputation. However, if these theories were correct, a significant association between all drugs in this class and lower limb amputations would be established in previous studies, not just canagliflozin. The high ROR signal for association between canagliflozin use and risk of lower limb amputations could be attributed to it being the least selective inhibitor (Papadokostaki et al., 2020).

Furthermore, canagliflozin is different from dapagliflozin and empagliflozin as it activates adenosine monophosphate protein kinase via inhibition of complex I of the respiratory chain, resulting in increased cellular adenosine monophosphate or diphosphate (Papadokostaki et al., 2020).

Chapter 6

Conclusion

SGLT2 inhibitors have proved to be beneficial and effective in the treatment of Type 2 Diabetes Mellitus. However these drugs also come with their own set of side effects, one of which is limb amputations. Previous clinical trials and observational studies conducted state that though empagliflozin and dapagliflozin do not significantly increase the risk of limb amputation, the results for canagliflozin remain inconclusive. The current study detected a signal for all four FDA approved SGLT2 inhibitors using disproportionate analysis. Therefore the study establishes that an association exists between SGLT2 inhibitor use and increased risk of lower limb amputations. Healthcare professionals and patients therefore need greater awareness of this adverse event to subsequently monitor it. Patient history, such as existence of comorbid conditions and authenticity of the reports are also important factors that need to be included in further studies on this matter.

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