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Original Article

Association between UGT2B7 Genetic Polymorphism and Pharmacokinetics of Empagliflozin in Humans

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ABSTRACT

Empagliflozin is a pharmaceutical drug that is utilized in the treatment of type 2 diabetes in adult patients. The frequency of polymorphisms of UGT2B4, UGT2B7, and UGT2B15 may affect the pharmacokinetics of empagliflozin, which may have the potential to affect its safety and efficacy. The current study aimed to evaluate the possible effect of UGT2B7 genetic polymorphisms on the pharmacokinetics of empagliflozin. The study also investigated the demographic covariates that may affect empagliflozin's clearance and therapeutic effect. The results showed that there was no significant correlation between age, smoking status, or measured pharmacokinetic parameters. Additionally, BMI, age, gender, and race had a small impact on empagliflozin's clearance and area under the curve (AUC), but the values were considered not clinically relevant. On the other hand, the study findings did not reveal any significant association between UGT2B7 rs7438135, rs11740316, and the measured pharmacokinetic parameters ($p>0.05$), except for the $MRT_{0\text{-}inf}$ in the dominant model of UGT2B7 rs11740316 ($p= 0.03$). It can be concluded that the UGT2B7 polymorphism did not affect empagliflozin pharmacokinetics and had no significant effect on its clinical efficacy or therapeutic safety.

Keywords: *Empagliflozin; diabetes; pharmacokinetics; UGT2B7; polymorphism.*

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1. Introduction

Empagliflozin is an anti-diabetic medication used in adults with type 2 diabetes mellitus (T2DM). The drug received FDA approval in 2014. It can be used as a single therapy or in conjunction with other diabetes medications. Empagliflozin and linagliptin, as well as empagliflozin in conjunction with metformin, are present in a combination product. Although these newer treatments may be more expensive for patients, the American Diabetes Association (ADA) is advocating for empagliflozin and liraglutide, which have been shown to reduce mortality, to be used as second-line therapy following metformin **[1]**.

Empagliflozin acts by inhibiting the sodium-glucose co-transporter-2 (SGLT-2) enzyme, which is situated in the proximal tubules of the kidney. This action results in a reduction in the reabsorption of glucose by the renal system, leading to an elevation in the excretion of glucose in the urine via the inhibition of SGLT2. The drug's glucose-lowering impact is independent of insulin. Investigations showed that there was an observed elevation in urinary glucose excretion of about sixty-four grams/day in type 2 diabetes patients treated with empagliflozin 10 mg and by

seventy-eight grams/day in individuals treated with a 25 mg dose. Moreover, empagliflozin decreases salt and volume load, inducing intravascular contraction **[2]**.

It is available at a dose of 10 or 25 mg and is taken once per day. The suggested conventional dose is a single administration of 10 milligrams, to be taken in the morning, regardless of whether breakfast is consumed or not. If the dose is well tolerated at first, it may be raised to 25 mg. In individuals with renal impairment, no dosage change is required if eGFR is more than 45mL/min/1.73 m^2, although care is advised if it goes below this level **[3]**.

The effects of empagliflozin added to standard therapy on cardiovascular morbidity and death in T2DM patients at high cardiovascular risk were investigated. A cohort of 7020 individuals underwent treatment, with a median observation period of 3.1 years. The main endpoint was observed in 490 out of 4687 patients (10.5%) belonging to the empagliflozin group, while it was observed in 282 out of 2333 patients (12.1%) belonging to the placebo group (P= 0.04 for superiority). There were no significant differences in the rates of myocardial infarction or stroke across the groups. However, the empagliflozin group had notably lower rates of death from cardiovascular causes, hospitalization for heart failure, and death from any cause. In the group treated with empagliflozin, the relative risk of death from cardiovascular causes decreased by 38%, the risk of hospitalization due to heart failure reduced by 35%, and the overall risk of death from any cause dropped by 32% compared to the placebo group **[4]**.

There was an increase in genital infections among empagliflozin patients but no increase in other side events (placebo: 1 percent, empagliflozin 10 milligrams: 4.4 percent, empagliflozin 25 milligrams: 4.7 percent, and 'all comparators': 1.1 percent) **[5]**.

Data from clinical trials showed that individuals with T2DM who were at high risk for cardiovascular events who received empagliflozin had a decreased rate of the main composite cardiovascular outcome and death from any cause when empagliflozin was added to usual treatment when compared to placebo **[4, 6]**.

Empagliflozin appears to be a promising neuroprotective agent for diabetic peripheral neuropathy **[7]**. Moreover, findings from the EMPA-KIDNEY trial demonstrated that empagliflozin successfully reduced the likelihood of kidney disease advancement in individuals who were at risk of progressing to chronic kidney disease (CKD) **[8]**.

Pharmacokinetic studies showed that upon administration of single doses of 10 mg empagliflozin, peak plasma levels reached T_{max} at 1.42 h, C_{max} was 226 nmol/L, and half-life $t_{1/2}$ ranged from 8.57 to 13.1 h **[9]**.

A clinical study showed that after single or multiple peroral dose administration of empagliflozin within the dose range of 0.5 to 800 mg, the drug exhibited rapid absorption into the body and attained its maximum concentration in the plasma between 1.33 and 3.0 h, followed by a two-phase elimination. In investigations involving the administration of a single increasing dose, the average duration of terminal elimination half-life in the body ranged from 5.6 to 13.1 h. Trials involving multiple doses showed that the mean duration ranged from 10.3 to 18.8 h. After the administration of numerous oral doses, there was a proportional increase in exposure, and consistent trough concentrations were observed beyond day 6, indicating that a steady state was achieved **[10]**.

Empagliflozin is mostly eliminated in its unaltered form. In an investigation that used [14C]-radiolabeled empagliflozin, it was

observed that empagliflozin in its unchanged form was responsible for 75.5% to 77.4% of plasma radioactivity. A notable amount of the administered dosage, specifically 34.1% and 23.7%, was excreted in the urine and feces, respectively **[11]**. Both glomerular filtration and active tubular secretion play an equal and significant role in the excretion of empagliflozin via the urinary tract **[12]**.

Metabolic processes are relatively insignificant in empagliflozin clearance. The primary metabolic products found in urine are 2 glucuronide-conjugates, which make up approximately 7.8% to 13.2% of the administered dosage. Meanwhile, the most prevalent metabolite detected in fecal matter is a tetrahydrofuran ring-opened carboxylic acid metabolite, constituting approximately 1.9% of the total administered dosage. The biosynthesis of glucuronide conjugates is facilitated through the involvement of UGT2B7, UGT1A3, UGT1A8, and UGT1A9. Empagliflozin, on the other hand, is a substrate of OAT3, OATP1B1, OATP1B3, P-gp, and BCRP transporters. However, it is not a substrate for OAT1 or OCT2 and there is not enough evidence that these transporters can affect the pharmacokinetics of empagliflozin **[13]**.

An in-vitro assessment found that empagliflozin exhibited no inhibitory or inducing effects on CYP isoforms. Additionally, it was observed that transporters such as OAT3, OATP1B1, OATP1B3, P-gp, and BCRP, were not inhibited by empagliflozin **[14]**.

A population pharmacokinetic model for empagliflozin was developed using data from five randomized trials in individuals with T2DM (N= 974). The results of the population estimate of oral apparent clearance were 9.87 liters per hour, the central and peripheral volumes of distribution were 3.02 and 60.4 liters, and inter-compartmental clearance was 5.16 liters per hour. Gender and ethnicity did not contribute to pharmacokinetic variability above allometric weight effects, other than a 25% higher oral absorption rate constant for Asian patients. The study indicated that age, total protein, smoking history, or alcohol intake did not affect the pharmacokinetic characteristics of empagliflozin **[15]**.

The absorption of empagliflozin was evaluated in relation to food intake by administering twenty-five milligrams of empagliflozin following a high-fat, high-calorie breakfast. The results indicated that there was a reduction in C_{max} and $AUC_{0\text{-}inf}$, but the reduction was not clinically significant indicating that empagliflozin can be administered with or without meals **[16]**.

An investigation was undertaken to assess the frequency of UGT2B4, UGT2B7, and UGT2B15 polymorphic forms in Caucasians and Asians from a pharmacogenomics perspective. The results of the study indicated significant differences in genotype and allele frequencies between the two populations for all polymorphisms. In particular, Asians exhibited homozygosity for common alleles, with a prevalence of wild-type alleles that was twice as high as that observed in Caucasians **[17]**. The correlation linking the pharmacokinetics and pharmacogenomics of empagliflozin has the potential to enhance its safety and efficacy.

Although the metabolic process of empagliflozin is not the major elimination route **[12, 13]**, the presence of UGT2B7 polymorphic forms may have an impact on the elimination process that may reflect on the drug pharmacokinetics. Till now, the effect of UGT genetic polymorphisms on the pharmacokinetics of empagliflozin has not yet been studied **[18]**.

It is worth mentioning that the interindividual variability in the pharmacokinetics of

empagliflozin can be attributed to the differences in absorption, distribution, metabolism, and excretion processes. Moreover, factors such as age, gender, body weight, renal and hepatic function, and concomitant medications can influence these processes. For instance, patients with renal impairment may exhibit altered pharmacokinetics due to reduced drug clearance **[10]**.

Genetic polymorphisms, particularly in genes encoding drug-metabolizing enzymes and transporters, can significantly impact the pharmacokinetics of empagliflozin. The SLC5A2 gene, which encodes the SGLT2 protein, and the UGT1A9 gene, involved in drug metabolism may have an impact. Variants in the SLC5A2 gene can affect the expression and function of the SGLT2 protein. For example, the SLC5A2 polymorphism rs9934336 has been associated with variations in HbA1c levels during glucose tolerance tests. However, general SLC5A2 variants do not show a significant effect on the pharmacokinetics or efficacy of empagliflozin **[19]**.

On the other hand, polymorphisms in the UGT1A9 gene can lead to differences in the metabolic rate of empagliflozin. The UGT1A9*3 allele, for instance, is associated with higher drug exposure compared to non-carriers **[19]**.

Since the impact of the UGT2B7 gene on the pharmacokinetics of empagliflozin was not studied before the purpose of this investigational work was to assess the potential impact of UGT2B7 genetic polymorphisms on the pharmacokinetics of empagliflozin which might affect both safety and therapeutic outcomes.

Finally, the current study addressed the association between association between UGT2B7 genetic polymorphism and pharmacokinetics of empagliflozin in healthy humans as well as the assessment of potential genetic variants in UGT2B7 which can contribute to interindividual variability in drug exposure and patients' response. This knowledge could guide dose adjustment and optimize therapeutic outcomes, particularly in populations with known genetic variations.

2. Methods

2.1. Design of the study and setting

The study was designed as a single-dose, one-period pharmacokinetic study of empagliflozin 10mg tablets on 32 adult and healthy male subjects in a fasting state. The recruitment of subjects included about 55 subjects, of which 32 were selected to participate in the study. The study was conducted at the Drug Research Center, Cairo, Egypt which is licensed by the Ministry of Health (MOH).

2.2. Eligibility criteria

The participants in the study were individuals whose ages ranged from 18 to 55 years. Additionally, their body mass index (BMI) was in the range of 18.5 to 30 kg/m^2. The results of their normal physiological examinations and laboratory data were found to be within the expected limits. It was required that the individuals selected for the study did not have a history of alcohol or drug abuse, nor had they previously taken part in any other clinical studies. Furthermore, a preference was given to non-smokers over smokers. In the case of smokers, it was specified that their daily consumption of cigarettes should not exceed eight.

Moreover, subjects should not have any of the various medical conditions and factors that may affect the subject's safety or study outcome. These include hypersensitivity reactions to empagliflozin, gastrointestinal complications, abnormalities in blood cell counts, renal disorders, autoimmune disorders, cardiovascular diseases, diabetes, hepatic impairments,

respiratory ailments, prior history of alcohol consumption and substance abuse, positive HIV status, atypical laboratory test results, recent administration of medications within a two-week timeframe before the study initiation, blood donation, and participation in other clinical trials necessitating substantial blood loss exceeding 500 ml within the preceding six weeks before the onset of the pharmacokinetics study.

2.3. Ethics Committee Approval

The methodology employed in this study was approved by the Faculty of Pharmacy at Ain Shams University Ethics Committee under serial number (277) and the Drug Research Center Ethics Committee under number (EMP-RES-BS-0120/0013). The investigational work was registered at ClinicalTrials.gov (Code: NCT05036421).

2.4. Planned study period

Healthy volunteers were confined at the clinical site and monitored for a period of 3 days.

2.5. Study procedures

The timing of blood sample collection was determined based on the pharmacokinetic characteristics of empagliflozin. After a minimum of 10 hours of fasting overnight, the 32 subjects were given a single dose of empagliflozin tablets, each containing 10 mg, and maintained their fasting state for approximately 4 h following the administration of the medication.

To avoid the occurrence of hypoglycemic episodes, the medication was administered with a 240-mL solution of aqueous glucose at a concentration of 20%. This was then followed by the administration of a 60-mL glucose solution at 15-minute intervals for a duration of up to 4 hours post-dose administration **[20]**.

Seventeen blood samples were collected from the subjects at the following intervals: 0 (prior dosing), 20 min, 40 min, 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, and 48 h after dose administration. The blood samples were obtained within tubes that contained an anticoagulant (EDTA disodium) and were promptly subjected to centrifugation at a speed of approximately 4000 revolutions per minute for a duration of 10 minutes. Subsequently, plasma samples were successfully separated in a 5 mL Wassermann plastic tube. The collected plasma samples were immediately preserved at a temperature of - 80 °C until the time of analysis. The study code, subject number, study period, and time interval were documented on the tubes. The total quantity of blood withdrawn throughout the whole study did not exceed 85 milliliters.

Subjects who met the specified criteria for inclusion were allowed to enter the study facility and were monitored for a minimum of 10 h before the administration of the drug dose. Subsequently, they were observed until the time of collection of the 48-hour blood sample.

Thirty-two volunteers were enrolled in the pharmacokinetics investigation, in which they underwent a comprehensive physical examination, neurological, and laboratory analysis (urine, biochemical, and complete blood picture) evaluation by a licensed physician. The selected subjects had no previous record of drug or alcohol misuse and did not have any immediate or long-term gastrointestinal, cardiac, vascular, hepatic, or renal disorders.

Concomitant medication was prohibited throughout the duration of the investigation; no meals, drinks, coffee, or tea were permitted for a period of four hours following the administration of the study dose **(Fig. 1.).**

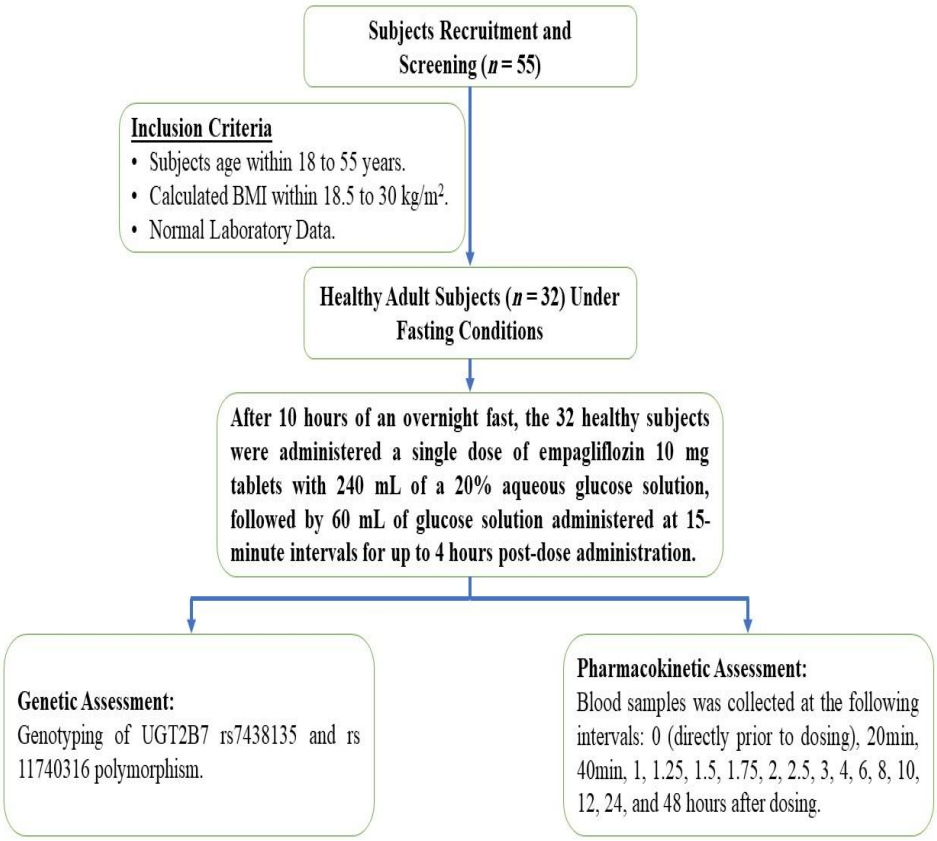

Fig. 1. Study flow diagram

2.6. Bioanalysis of empagliflozin

The quantification of empagliflozin in plasma samples from the participants was carried out via liquid chromatography-tandem mass spectrometry (LC/MS/MS) employing a bioanalytical method that was both developed and validated following international guidelines. The established procedure was designed to allow for the quantification of empagliflozin within a calibration range of 0.5–200 ng/mL, utilizing a liquid-liquid extraction technique. In brief, a simple extraction method was employed to extract the target analyte from plasma, employing a 3 mL extraction solvent consisting of a 70% diethyl ether and 30% dichloromethane mixture (v/v), along with the addition of 50 µL of dapagliflozin (IS) at a concentration of 2500 ng/mL. Following a 1-minute vortex and subsequent centrifugation at 3500 rpm for 5 min, the organic layer (approximately 2.5 mL) was separated. The organic extract was then evaporated to dryness using a Concentrator Plus/Vacufuge® Plus instrument (Eppendorf, Germany). The resulting residue was reconstituted using a mobile phase (100 µL) and subsequently injected (0.3 µL) into an LC/MS/MS.

2.7. Validation of Method of Analysis

The peak area ratios of different concentrations of empagliflozin in plasma within the specified range of concentrations must show a high linear relationship (r^2 not less than 0.999). The outcomes of intraday precision, expressed as the coefficient of variation (C.V.%), should align with the most recent recommendations provided by the Food and Drug Administration (FDA) **[21]**. The evaluation of accuracy and precision

was conducted on three distinct concentrations within the anticipated range of drug concentrations, with consideration given to both intra-day and inter-day variations. The lower limit of quantitation (LLOQ) must show adequate sensitivity to quantify small concentrations of drugs during the phase of elimination. The drug should manifest a satisfactory level of stability in plasma under the conditions that were studied.

2.8. DNA Extraction and Genotyping

For the extraction of DNA and genotyping of UGT2B7 selected single nucleotide polymorphisms (SNPs), a blood sample of 5 mL was acquired from each participant using EDTA vacutainers before drug administration. The DNA extraction process involved the utilization of the QIA amp® DNA mini and blood mini handbook 05/2016 (Qiagen AB, Sollentuna, Sweden) manual **[22]**. The concentration of the extracted DNA was subsequently determined using the Nano Drop® (ND-1000) Spectrophotometer (Nano Drop Technologies Inc., Washington, USA). The DNA isolates were used in the SNP analysis without any dilution.

2.9. Genotyping for UGT2B7 rs7438135 and rs 11740316 Single Nucleotide Polymorphism (SNPs)

Taq-man™ predesigned probes (Thermo Fisher Scientific, USA) and the Rotor-Gene QTM real-time PCR apparatus (QIAGEN Hilden, Germany) were used to genotype UGT2B7 rs7438135 and rs11740316. The reaction plate was prepared using TaqMan™ GTXpress™ Master Mix (Thermo Fisher Scientific, U.S), gDNA, and RNAse-free water. The thermal profile was as follows: denaturation of the DNA strand at 95 °C for 20 sec, hybridization of the primers and probes at 92°C for 40 seconds, then elongation at 60 °C for 30 sec.

2.10. Safety and Tolerability Assessment

The evaluation of empagliflozin's safety and tolerability involved monitoring of side effects and/or unfavorable events frequency between the subjects throughout the duration of the investigation. Moreover, subject medical histories, physical examinations, and laboratory results were reported. In addition, vital signs such as blood pressure and heart rate were assessed before drug administration and subsequently at intervals of 2, 4, 6, 10, 12, 24, and 48 h.

2.11. Pharmacokinetics Parameter Assessment

The following empagliflozin pharmacokinetic characteristics were evaluated

-Maximum drug concentration in plasma (C_{max}).

-Time to peak drug concentration (T_{max}).

-Drug elimination half-life during the terminal phase ($t_{1/2e}$).

-Elimination rate constant (K_e).

-Area under the plasma concentration-time curve from zero to time t (AUC_{0-t}).

-Area under plasma concentration-time curve from zero to infinity (AUC_{0-inf}).

2.12. Statistical Evaluation

Analysis was carried out using R software version 4.1.1. Two-sided p-values of less than 0.05 were deemed statistically significant. To evaluate normality across genotype groups, Shapiro-Wilk's test was used, normally distributed data were compared using one-way ANOVA or student’s t-test, and non-normally distributed data were compared using the Kruskal-Wallis test or Mann-Whitney's U test. Pearson’s correlation was implemented to investigate the correlation between age and pharmacokinetic parameters, and Fisher’s exact test was implemented to compare gender and smoking status distributions across genotypic groups. The codominant, dominant, and recessive genetic models were all considered. Genetic parameters for each SNP including minor allele

frequency (MAF), Heterozygosity, and Polymorphism Information Content (PIC) were reported for each SNP. The Concordance of the observed and expected genotype frequencies per the Hardy-Weinberg Equilibrium (HWE) for each SNP was checked using the Chi-Squared test.

Sample size calculation was done based on Schuirman's two one-sided *t*-tests procedure using the ±20 rule. The sample size should be large enough to provide a power (ϕ= 1-β) of 80% for the detection of a difference of the magnitude Δ at least 20% of the unknown reference mean. Significance level α (type I error) equal to 0.05 and β (type II error) equal to 0.2. Therefore, the sample size to provide a power of 80% for the detection of a difference of the magnitude at least 20% of the unknown reference mean should be equal to/or greater than 28 subjects putting into consideration about 5% to 10% dropout thus, a total of 32 subjects was sufficient to acquire a study power of 80% at a level of significance of 5% **[23]**.

3. Results and Discussion

3.1. Clinical Findings (Safety and Tolerability)

Empagliflozin was tolerated by the participants. There were no adverse effects or laboratory abnormalities associated with the medication. Throughout the trial, blood samples were taken at the appropriate times. There were no individuals who dropped out of the trial due to pharmacological adverse effects.

3.2. Pharmacokinetics Parameters

The results of pharmacokinetic parameters data shown in **(Table 1)** and **(Fig 2)** revealed that the mean values for C_{max} were 125.795±34.387 ng/mL, t_{max} was 2.078±0.817 h, $t_{1/2e}$ 7.488±1.013 h, AUC_{0-t} 955.708±190.991 ng. h/mL, which corresponded to those mentioned in the literature.

Table 1. Pharmacokinetics' parameters after administration of empagliflozin 10 mg tablets for 32 healthy subjects

Parameters	T_{max} (h)	C_{max} (ng/mL)	AUC_{0-t} (ng.h/mL)	AUC_{0-inf} (ng.h/mL)	K_{el} (h^{-1})	$T_{1/2}$ (h)	MRT_{0-inf} (h)
Mean	2.078	125.795	955.708	973.556	0.094	7.488	9.709
CV%	39.302	27.336	19.984	20.117	12.518	13.532	11.053
Range (Median)	1.00 - 4.00 (1.875)	79.320 - 194.495 (116.680)	664.913 - 1460.788 (940.817)	670.392 - 1487.497 (953.565)	0.069 - 0.113 (0.096)	6.136 - 10.060 (7.213)	7.936 - 12.553 (9.699)

T_{max}, Time to peak drug concentration; C_{max}, Maximum drug concentration in plasma; AUC_{0-t}, Area under the plasma concentration-time curve from zero to time t; AUC_{0-inf}, Area under the plasma concentration-time curve from zero to infinity; K_{el}, Elimination rate constant; $T_{1/2}$, Drug elimination half-life during the terminal phase; MRT_{0-inf}, Mean Residence time from time zero to infinity.

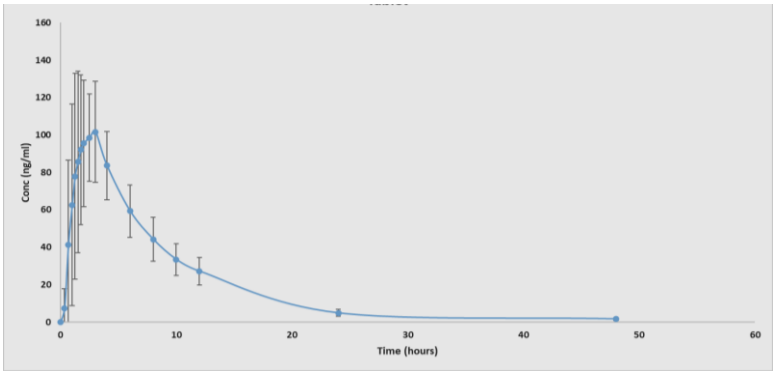

Fig. 2. Mean plasma concentration (±S.D.) after single-dose administration of 10 mg empagliflozin tablets for 32 healthy subjects.

3.3. Demographic Data and Genotyping Groups

The study included thirty-two subjects. The median age of participants was 26.5 years (range: 19–54 years), 12.5% of subjects were females, and 56.3% were smokers. Across all genotype groups of rs7438135 and rs11740316, subjects were matched to age, gender, and smoking status. The baseline demographic features of our study subjects are shown in **(Table 2)**.

3.4. Genotyping of the participants

The call rate for both SNPs was 100%. Observed genotype frequencies were the following expected genotypes (rs7438135: $\chi2$= 0.04, p= 0.84, rs7438135: $\chi2$= 2.80, p= 0.10) for both SNPs. Additionally, both SNPs were moderately informative, with a PIC of 0.37. The minor allele for both SNPs was the A allele, with an MAF of 0.41. The genetic parameters for both SNPs are shown in **(Table 3)**. Neither rs7438135 nor rs11740316 significantly affected any of the pharmacokinetic parameters in the codominant and recessive genetic models **(Table 4)**. However, in the dominant model, subjects harboring the rs11740316 wild-type G/G variant had significantly higher MRT0-inf than their counterparts harboring either the G/A or A/A genotypes (10.2 vs. 9.33 h, p= 0.03).

Table 2. Baseline demographic data of the 32 enrolled subjects

	rs7438135				rs11740316			
	GG (N=11)	AG (N=16)	AA (N=5)	p	GG (N=9)	AG (N=20)	AA (N=3)	p
Age (years): Median (Range)	35 (19-54)	30.5 (19-51)	21 (19-49)	0.48[1]	31 (19-48)	22 (19-54)	40 (22-52)	0.41[1]
Gender:				1.00[2]				1.00[2]
Females	2 (18.2%)	2 (12.5%)	0 (0.0%)		1 (11.1%)	3 (15.0%)	0 (0.0%)	
Males	9 (81.8%)	14 (87.5%)	5 (100.0%)		8 (88.9%)	17 (85.0%)	3 (100.0%)	
Smoking status:				0.72[2]				0.87[2]
Non-smokers	5 (45.5%)	6 (37.5%)	3 (60.0%)		5 (55.6%)	8 (40.0%)	1 (33.3%)	
Smokers	6 (54.5%)	10 (62.5%)	2 (40.0%)		4 (44.4%)	12 (60.0%)	2 (66.7%)	

1. Kruskal-Wallis rank-sum test; 2. Fisher's Exact test.; Significant difference (P < 0.05).

Table 3. Genetic parameters and HWE testing for rs7438135 and rs11740316

	rs7438135	rs11740316
MAF (A)	0.41	0.41
He	0.48	0.48
Ho	0.50	0.62
PIC	0.37	0.37
HWE $\chi2$	0.04	2.80
PHWE	0.84	0.10

He: Expected Heterozygosity, Ho: Observed Heterozygosity, HWE: Hardy-Weinberg Equilibrium, MAF: Minor Allele Frequency, PIC: Polymorphism Information Content.

Table 4. Association between rs7438135, and rs11740316 variants and measured pharmacokinetic parameters per the codominant, dominant, and recessive genetic models

	Co-dominant				Dominant			Recessive		
rs7438135:	**G/G (N=11)**	**A/G (N=16)**	**A/A (N=5)**	**P**	**G/G (N=9)**	**G/A-A/A (N=23)**	**p**	**G/G-G/A (N=27)**	**A/A (N=5)**	**p**
T_{max} (h)	2.00 (1.00-4.00)	1.75 (1.00-3.00)	2.50 (1.75-3.00)	0.51[1]	2.00 (1.00-4.00)	1.75 (1.00-3.00)	0.94[1]	1.75 (1.00-4.00)	2.50 (1.75-3.00)	0.26[1]
C_{max} (ng/ml)	107 (79.3-195)	128 (83.5-193)	98.8 (96.2-185)	0.11[1]	114 (±34.9)	132 (±33.3)	0.17[2]	120 (79.3-195)	98.8 (96.2-185)	0.34[1]
AUC_{0-t} (ng.h/ml)	928 (±189)	972.07 (±160)	964 (±306)	0.85[2]	928 (±189)	970 (±195)	0.56[2]	954 (±171)	964 (±306)	0.92[2]
K_e (h^{-1})	0.09 (±0.01)	0.09 (±0.01)	0.10 (±0.01)	0.54[2]	0.09 (±0.01)	0.09 (±0.01)	0.76[2]	0.09 (±0.01)	0.10 (±0.01)	0.27[2]
MRT_{0-inf} (h)	9.88 (±1.03)	9.67 (±1.20)	9.44 (±0.83)	0.75[2]	9.88 (±1.03)	9.62 (±1.11)	0.52[2]	9.76 (±1.12)	9.44 (±0.83)	0.55[2]
rs11740316:										
T_{max} (h)	2.00 (1.00-3.00)	1.88 (1.00-4.00)	1.25 (1.25-3.00)	0.81[1]	2.00 (1.00-3.00)	1.75 (1.00-4.00)	0.78[1]	2.00 (1.00-4.00)	1.25 (1.25-3.00)	0.60[1]
C_{max} (ng/ml)	123 (79.3-168)	116 (81.0-195)	105.51 (93.9-112)	0.38[1]	123 (79.3-168)	112 (81.0-195)	0.72[1]	128 (±35.3)	104 (±9.01)	0.25[2]
AUC_{0-t} (ng.h/ml)	939 (±213)	971 (±199)	907.09 (±38.3)	0.83[2]	939 (±213)	962 (±186)	0.76[2]	961 (±200)	907 (±38.3)	0.65[2]
K_e (h^{-1})	0.09 (±0.01)	0.10 (±0.01)	0.10 (±0.01)	0.11[2]	0.09 (±0.01)	0.10 (±0.01)	0.09[2]	0.09 (±0.01)	0.10 (±0.01)	0.12[2]
MRT_{0-inf} (h)	10.2 (7.94-12.0)	9.49 (8.11-12.6)	9.21 (8.25-9.30)	0.05[1]	10.2 (7.94-12.0)	9.33 (8.11-12.6)	0.03[1*]	9.79 (±1.09)	8.92 (±0.58)	0.19[2]

1. Data represented as medians and ranges and compared using the Kruskal-Wallis rank-sum test or Mann-Whitney's U test.
2. Data represented as means and standard deviations and compared using one-way ANOVA or Student's T-Test.

* Significant difference ($p < 0.05$)

MRT_{0-inf}: Mean Residence time from zero time to infinity, T_{max}: Time to peak drug concentration, C_{max}: Maximum drug concentration in plasma, AUC_{0-t}: Area under the plasma concentration-time curve from zero to time t, K_{el}: Elimination rate constant.

3.5. Statistical Evaluation

There was no remarkable correlation between the age of the subjects, their smoking status, or any of the measured pharmacokinetic parameters **(Fig. 3)**. On the other hand, there was a significant correlation between gender and

exposure to empagliflozin **(Table 5)**; female subjects had significantly higher C_{max} and AUC_{0-t} (C_{max}: 169 vs. 112, p = 0.01, AUC_{0-t}: 1130 vs. 931, p<0.05).

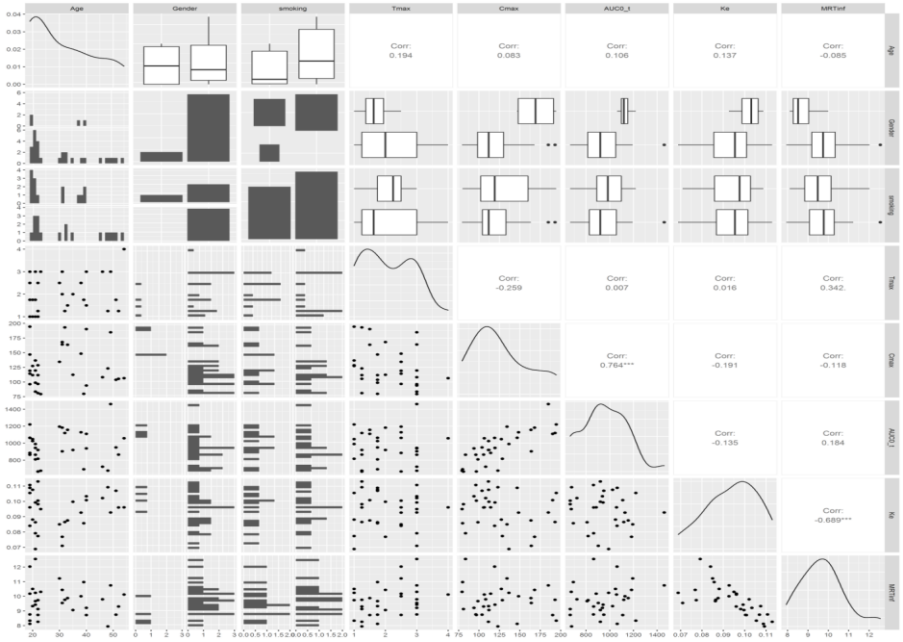

Fig. 3. Correlation matrix of age, gender, smoking status, and empagliflozin pharmacokinetic parameters

Table 5. Associations between gender, smoking status, and empagliflozin-measured pharmacokinetic parameters

Parameter	**Gender**		
	Females (N=4)	**Males (N=28)**	**P[1]**
T_{max} (h)			0.301
Median (Range)	1.62 (1.00-2.50)	2.00 (1.00-4.00)	
C_{max} (ng/mL)			0.011*
Median (Range)	169 (147-195)	112 (79.3-193)	
AUC_{0-t} (ng. h/mL)			< 0.052*
Mean (±SD)	1130 (±65.6)	931 (±190)	
K_e (h^{-1})			0.162
Mean (±SD)	0.10 (±0.01)	0.09 (±0.01)	
MRT_{0-inf} (h)			0.072
Mean (±SD)	8.79 (±0.85)	9.84 (±1.05)	
	Smoking		
	Non-smokers (N=14)	**Smokers (N=18)**	**P[1]**
T_{max} (h)			0.401
Median (Range)	2.25 (1.00-3.00)	1.62 (1.00-4.00)	
C_{max} (ng/mL)			0.392
Median (Range)	132 (±36.9)	121 (±32.6)	
AUC_{0-t} (ng. h/mL)			0.662
Mean (±SD)	973 (±172)	942 (±209)	
K_e (h^{-1})			0.972
Mean (±SD)	0.09 (±0.01)	0.09 (±0.01)	
MRT_{0-inf} (h)			0.682
Mean (±SD)	9.62 (±1.06)	9.78 (±1.11)	

1. Pearson's correlation test; * Significant difference ($p < 0.05$); T_{max},Time to peak drug concentration, C_{max}, Maximum drug concentration in plasma; AUC_{0-t}, Area under plasma concentration-time curve from zero to time t; K_{el}, Elimination rate constant; MRT_{0-inf}, Mean Residence time from time zero to infinity.

4. Discussion

Understanding the interplay between pharmacokinetics and genetic polymorphisms enables healthcare providers to predict patient responses more accurately. For example, in the case of antidiabetic drugs such as empagliflozin, genetic variations in SGLT2 transporters can influence drug efficacy and the risk of adverse effects. By integrating pharmacogenomic data, clinicians can tailor treatments to individual genetic profiles, optimizing therapeutic outcomes and minimizing adverse reactions. The previously mentioned information implies undergoing research similar to the current study to investigate the association type between both pharmacokinetics and genetic polymorphism of different medications, including antidiabetic medication, which might lead to negative clinical outcomes.

Regarding the results of the pharmacokinetics parameters in the present study, it was found that the obtained values of C_{max}, T_{max}, $AUC_{0-t,}$ and $T_{1/2}$ were 125.795 ng/mL, 2.078 h, 955.708 ng. h/mL, and 7.488 h, respectively which were consistent with those mentioned in the literature for empagliflozin 10 mg dose **[9, 10]**.

The results of the current study showed that plasma levels of empagliflozin were within the therapeutic range which is an important issue from a drug efficacy and safety point of view coinciding with the literature which stated that insufficient antidiabetic levels can lead to hyperglycemia, increasing the risk of diabetic complications, while excessive levels can cause hypoglycemia, potentially leading to a coma **[24, 25]**.

In the current study, it was preferred to undergo the study under fasting conditions to alleviate food effect on the pharmacokinetics behavior of the candidate drug although it is well known that food effect on empagliflozin pharmacokinetics was found to be non-significant **[26]** and is prescribed to be taken with or without food.

The effect of various covariates like ideal body weight, age, gender, and race was assessed in a population pharmacokinetic analysis. According to the analysis results, the outcomes revealed that age, gender, body mass index, or ethnicity (Asians versus predominantly whites) did not show a clinically relevant influence on empagliflozin clearance **[24]**. This was following our findings which revealed no significant correlation between the subject's age, smoking status, or any of the measured pharmacokinetic parameters ($p>0.05$). Furthermore, there was no clinically relevant change in empagliflozin's therapeutic effect coinciding with literature findings **[5, 15, 27]**.

On the other hand, our clinical study results indicated that there was a remarkable correlation between gender and empagliflozin plasma levels showing that female subjects had significantly higher C_{max} and AUC_{0-t} than males contrary to that mentioned in the literature **[5, 15, 27]**. The significant difference in C_{max} and AUC_{0-t} may not be inducing a clinically relevant empagliflozin hypoglycemic effect. However, this significance may be attributed to the inadequate sample size of females versus males in our study, as their sample size is relatively small compared to males (4 females versus 28 males).

Since the impact of UGT genetic polymorphisms on the pharmacokinetics of empagliflozin has not been studied **[18]**, to our knowledge the current study was the first study to address this effect. The present findings revealed the absence of a significant association between

UGT2B7 rs7438135, rs11740316, and the measured pharmacokinetic parameters ($p>0.05$), except for the $MRT_{0\text{-}inf}$ in the dominant model of UGT2B7 rs11740316 ($p= 0.03$). The results may sound relevant since empagliflozin metabolism plays a minor role in its elimination (7.8–13.2% of the dose eliminated as glucuronide conjugates) **[13]** and nearly 50% of empagliflozin is eliminated unchanged in urine and feces **[12]**.

Although smoking affects metabolic enzymes like cytochrome P450 (CYP) and uridine diphosphate-glucuronosyltransferases (UGTs) **[28]**, our study results showed a non-significant difference ($p>0.05$) between empagliflozin pharmacokinetics in smokers versus non-smokers. Additionally, factors including smoking status showed no influence on the pharmacokinetics of empagliflozin **[5]** which follows the obtained results in the present study. The reason for the non-significant difference may be attributed to the minor role of metabolic contribution in eliminating empagliflozin from plasma **[13]** since the metabolic conjugates of empagliflozin constitute less than 10% of the total drug in the blood circulation **[29]**.

Furthermore, since adults and young patients with type 2 diabetes mellitus (T2DM) exhibit similar exposure-response relationships at different doses of empagliflozin **[30]**, similar study findings are expected upon conducting our study in the future using different empagliflozin doses.

Conclusion

It can be concluded that the UGT2B7 polymorphism did not affect empagliflozin pharmacokinetics. Furthermore, subjects' demographics did not show any significant change in target drug pharmacokinetics except for gender, which showed significant changes in pharmacokinetics in females. These gender-related changes may not be clinically significant.

Recommendations

Conducting large-scale studies with a more diverse population to validate the findings and improve the generalizability of the results.

Exploring the influence of other potential factors, such as transporters, on the absorption of empagliflozin.

Conducting a similar study on diabetic patients.

Declarations

Consent to publish

All authors have read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Pharmacy, Ain Shams University under serial number (277) and the Ethics Committee of Drug Research Center under number (EMP-RES-BS-0120/0013).

Availability of data and material

The authors confirm that the data supporting the findings of this study are available and its supplementary materials upon request.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

Funding statement

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Author's contribution

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Heba Mohamed Yousef and Sara Mahmoud Shaheen. The first draft of the manuscript was written by Heba Mohamed Yousef and Sara Mahmoud

Shaheen. Writing–review and editing were performed by Heba Mohamed Yousef, Nagwa Ali Sabri, and Sara Mahmoud Shaheen. Supervision and project administration were done by Nagwa Ali Sabri and Sara Mahmoud Shaheen. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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