Kerbala Journal of Pharmaceutical Sciences

ISSN: 2221-7207(Print) / **ISSN**: 3006-7189 (Online)

The Impact of the SLC5A2 Gene Polymorphism on the Treatment Efficacy of Empagliflozin in Iraqi Patients with Type 2 Diabetes

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Received: 20/01/2024
Accepted: 25/04/2024
Published: 31/12/2025

Keyword: Diabetes mellitus**,** SLC5A2 polymorphism, Sodium–glucose cotransporter 2 inhibitors, Empagliflozin.

DOI: 10.62472/kjps.v16.i27.259-267

Abstract

Background: Diabetes is a prevalent, life-threatening chronic disease and one of the leading causes of death worldwide. sodium glucose co-transporter 2, is the major co-transporter involved in renal glucose uptake, and its inhibition has been shown to be a relatively new strategy for managing diabetes. The gene encoding sodium glucose co-transporter 2 is located on chromosome 16 and is also known as soluble carrier family 5 member 2. Empagliflozin is a relatively new agent that improves glycemic control by increasing glucose excretion and inhibiting sodium–glucose cotransporter 2.

The aim of study: was to investigate the potential effects of common single nucleotide polymorphisms in the sodium–glucose cotransporter 2 encoding gene soluble carrier family 5 member 2 on diabetes-related metabolic characteristics in patients who are at risk of type 2 diabetes. Secondly, we investigated whether these single nucleotide polymorphisms have pharmacogenetic significance by potentially influencing the response of type 2 patients to empagliflozin treatment.

Methodology; The study participants included 50 healthy subjects and 110 diabetics who were selected during outpatient clinic visits with age range 30 to 65 years. Each subject underwent glycemic analysis, renal parameters, and genetic analysis of the soluble carrier family 5-member 2 reference SNP 121918621 polymorphism with blood samples that collected from different cities of Iraq. Each patient received 10 mg oral tablet of empagliflozin daily as monotherapy.

Results: The findings of the study revealed significant variability with P-value less 0.05 in each of the glycemic and renal parameters among the study groups, with diabetic patients having significantly higher glycemic and renal parameters than healthy samples. soluble carrier family 5 member 2 reference SNP 121918621genotype distribution showed that (Fasting Serum Insulin, Fasting blood glucose, Glycated hemoglobin) levels did not differ significantly. The results also showed significant differences in (Serum creatinine, blood Urea) levels.

Conclusion: The soluble carrier family 5-member 2 polymorphism is one of the genetic variables that contribute to heterogeneity in empagliflozin responsiveness in people with type 2 diabetes.

تأثير تعدد الأشكال الجينية لجين SLC5A2 على فعالية العلاج بالإمباغليفلوزين لدى المرضى العراقيين المصابين بداء السكري من النوع الثاني

بنين هادي سعيد ومحمد إسماعيل رسول

الخلاصة

المقدمة: مرض السكري هو مرض مزمن منتشر ومهدد للحياة وأحد الأسباب الرئيسية للوفاة في جميع أنحاء العالم. الناقل المشارك لجلوكوز الصوديوم 2، هو الناقل المشارك الرئيسي المشارك في امتصاص الجلوكوز الكلوي، وقد ثبت أن تثبيطه يمثل استراتيجية جديدة نسبيًا لإدارة مرض السكري. يقع الجين الذي يشفر الناقل المشترك لجلوكوز الصوديوم 2 على الكروموسوم 16 ويعرف أيضًا باسم عائلة الناقل القابلة للذوبان 5 عضو 2. إمباغليفلوزين هو عامل جديد نسبيًا يعمل على تحسين التحكم في نسبة السكر في الدم عن طريق زيادة إفراز الجلوكوز وتثبيط الناقل المشترك للجلوكوز الصوديوم 2.

الهدف من الدراسة: هو دراسة التأثيرات المحتملة لأشكال النوكليوتيدات المفردة الشائعة في الناقل المشترك للصوديوم والجلوكوز 2 الذي يشفر عائلة حامل الجينات القابلة للذوبان 5 عضو 2 على الخصائص الأيضية المرتبطة بمرض السكري في المرضى المعرضين لخطر الإصابة بداء السكري من النوع 2. ثانيًا، قمنا بالتحقق مما إذا كانت أشكال النوكليوتيدات المفردة هذه لها أهمية دوائية من خلال التأثير المحتمل على استجابة مرضى النوع 2 للعلاج بالإمباجليفلوزين .

المرضى وطرق العمل: شمل المشاركون في الدراسة 50 شخصًا صحيًا و110 مرضى سكري تم اختيارهم خلال زيارات العيادات الخارجية والذين تتراوح أعمارهم بين 30 إلى 65 عامًا. خضع كل موضوع لتحليل نسبة السكر في الدم، ومؤشرات الكلى، والتحليل الجيني لعائلة الناقل القابلة للذوبان 5 أعضاء 2 مرجع SNP 121918621 تعدد الأشكال مع عينات الدم التي تم جمعها من مدن مختلفة في العراق. تلقى كل مريض 10 ملغ من قرص إمباغليفلوزين عن طريق الفم يوميًا كعلاج وحيد.

النتائج: كشفت نتائج الدراسة عن تباين كبير مع قيمة P أقل من 0.05 في كل من المعلمات نسبة السكر في الدم والكلى بين مجموعات الدراسة، مع مرضى السكري لديهم مؤشرات نسبة السكر في الدم والكلى أعلى بكثير من العينات السليمة. أظهرت عائلة الناقل القابلة للذوبان 5 أعضاء 2 مرجع SNP 121918621 توزيع النمط الوراثي أن مستويات (مصل الأنسولين الصائم، جلوكوز الدم الصائم، الهيموجلوبين السكري) لم تختلف بشكل كبير. كما أظهرت النتائج وجود فروق معنوية في مستويات (الكرياتينين في الدم، يوريا الدم).

الاستنتاج: يعد تعدد الأشكال من عائلة الحامل القابلة للذوبان المكونة من 5 أعضاء 2 أحد المتغيرات الوراثية التي تساهم في عدم التجانس في استجابة الإمباجليلوزين لدى الأشخاص المصابين بداء السكري من النوع الثاني.

1. Introduction

Diabetes mellitus (DM) is an incurable condition that can have significant adverse effects on a person's life. It ranks among the highest rates of death around the world and has a major role in heart attacks, strokes, kidney failure, blindness, and amputation of lower limbs (Mannino et al., 2019). There exist four distinct forms of diabetes: type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), and diabetes associated or produced by specific diseases, pathologies, and/or syndromes (Gojnic et al., 2022).Diabetes type 2 (T2D) is brought on by deficiencies in the production of insulin, problems with insulin signaling, and/or insulin resistance, all of which lead to chronic hyperglycemia. (Lima et al., 2022).Type 2 diabetes (T2DM) is a progressive, metabolic, long-term illness. The goal of treatment is to attain adequate glycemic control, as assessed by self-monitoring blood glucose (SMBG), continuous glucose monitoring (CGM), and hemoglobin A1C evaluation. In healthy people, all of the glucose filtered at the glomerulus (160–180 g daily) is reabsorbed and no glucose is detected in the urine (Vallon, 2020). The transport of glucose, amino acids, vitamins, osmolytes, and certain ions is accomplished by a family of membrane proteins known as SGLTs. There is a common core structure among these membrane proteins (Kaur et al., 2021). In the kidney, glucose reabsorption is significantly influenced by two sodium-glucose transporters (SGLT) that are members of the solute carrier family 5 (SLC5). Within the proximal tubule, SGLT2 (SLC5A2) reabsorbs approximately 90% of the glucose from the renal ultrafiltration in an insulin-independent manner in segments 1 and 2, while SGLT1 reabsorbs 10% in segment 3 (SLC5A1) (Klen et al., 2020). Sodium glucose co-transporter 2 (SGLT2) is the major cotransporter involved in the kidney's absorption of glucose, and its inhibition has been identified as a novel treatment approach for controlling diabetes. Several SGLT2 inhibitors were created and put through clinical trials because SGLT2 inhibition has been identified as a unique and safe method of decreasing elevated glucose levels in people with type 2 diabetes (Mascolo et al., 2022). Their remarkable ability to reduce blood glucose levels without causing hypoglycemia was accompanied by the demonstration of both cardioprotective and nonprotective characteristics (Heerspink et al., 2018). Many SGLT2 inhibitor compounds have advanced to the point of marketing approval; four of these are canagliflozin, dapagliflozin, empagliflozin, and ertugliflozin, which are currently licensed by both the FDA and the EMA. As an inhibitor of sodium-glucose cotransporter 2 (SGLT2), the relatively new medication empagliflozin increases the excretion of glucose in the urine, improving glucose metabolism, reducing glucotoxicity, and reducing insulin resistance. Empagliflozin looks to be a safe and generally well-tolerated medication. When empagliflozin is used alone or in conjunction with other therapies to treat patients with insufficient glycemic control, it effectively lowers glycated hemoglobin A1C (HbA1C), average daily glucose levels, postprandial blood glucose, fasting blood glucose, and significantly reduces weight in T2DM patients (Forycka et al., 2022). SLC2A, SLC5A, and SLC50A encode three different families of glucose transporters. In humans, SLC5A encodes SGLTs. SLC5A consists of 12 genes, each of which has been identified in a variety of tissues and has a distinct distribution and function (Sędzikowska and Szablewski, 2021). The SGLT2 gene, also known as SLC5A2 (solute carrier family 5 member 2) and located on chromosome 16, encodes GLT2. A number of mutations affecting membrane location, transporter function, or SGLT2 expression in the SLC5A2 gene have been connected to familial renal glucosuria, a disorder characterized by abnormally high urine glucose excretion while blood glucose levels are normal (Klen and Dolžan, 2021). It has been suggested that glucose homeostasis is impacted by genetic differences in SLC5A2. We examined the potential impact of the common SLC5A2 rs121918621 polymorphism on glycemic

management and the likelihood of macro or microvascular complications in patients with type 2 diabetes (T2D) who take empagliflozin (Xu et al., 2023).The aim of this study was to investigate the potential effects of common single nucleotide polymorphisms (SNPs) in the SGLT2-encoding gene SLC5A2 on diabetes-related metabolic characteristics in patients who are at risk of type 2 diabetes. Secondly, we investigated whether these SNPs have pharmacogenetic significance by potentially influencing the response of type 2 patients to empagliflozin treatment. In the Iraqi population, we looked at the relationship between glycemic and renal parameters in 110 diabetes patients and 50 healthy individuals. We also performed genetic analysis to determine the genotyping distribution of the SLC5A2 rs121918621 polymorphism.

2. Materials and Methods

2.1. Samples Collections

Following an overnight fast, all patients and healthy controls had blood taken. The samples of blood were separated into two portions: the first, which weighed two milliliters and was stored in an EDTA tube for DNA extraction, and the second, which weighed three milliliters and was stored in a gel tube for serum isolation intended for biochemical testing.

2.2. Patients

In this study, 110 patients between the ages of 30 and 65 who visited a private clinic for medical care and case consultation were included. The period of this investigation was November 2022–May 2023. Based on diagnostic criteria, type 2 diabetes mellitus had already been diagnosed in all of the patients.50 healthy controls, ages 30 to 60, were also enrolled.

2.3. Study Design

This research is cross-sectional that included 110 Iraqi peoples with type 2 diabetes mellitus who were given 10 mg empagliflozin oral tablet (Jardiance 10mg Boehringer Ingelheim) daily for six months to two years. Fifty people who appeared healthy and had no illness served as the control group. Blood was drawn from fasting individuals who had previously taken empagliflozin for genetic research and biochemical testing (HB1AC, serum creatinine).

Ethical and scientific approval: The research proposal was reviewed and approved by the College of Pharmacy's scientific and ethical committee at Karbala University. Participants were asked to fill out a specially created questionnaire and signed a written consent form outlining the study's objectives before they could be considered for enrollment. Genotyping: Intron's total DNA extraction kit, G-spin, was utilized to extract genomic DNA from a whole blood sample, giving pure DNA appropriate for storage and quick application. The SLC5A2 gene rs121918621 was amplified using Polymerase Chain Reaction (PCR) with a specific primer Table1. Primer-BLAST was used to create this primer and was acquired in several picomol working concentrations from Bioneer in Korea as a lyophilized product (present study). The PCR reaction was tuned to denature the template for 4 minutes at 95 oC, then go through 35 cycles of initial denaturation for 40 seconds at 95 oC, annealing for 30 seconds at 58 oC, extension for 30 seconds at 72 oC, and final extension for 5 minutes at 72 oC. After the PCR product was electrophoresed and sized in an agarose gel with a 1.5% concentration for 90 minutes at 70 V, it was seen using an ultraviolet transillator and captured on camera using a digital camera.

Table1: Primer Sequences of SLC5A2 rs121918621

Primer		Sequences	Product size (bp)
Primer sequences of SLC5A2 rs121918621	Forward Allele G	5-ATCGTGGTAGTGTCGGTGGCCTGG-3	400
	Forward Allele A	5-ATCGTGGTAGTGTCGGTGGCCTGA-3	
	Reverse common	5- TGAGGAGGCCAGAGCAGAAGAACA-3	

2.4. Statistical Analysis

The study participants' data were input into an electronic database, verified for accuracy and consistency that subsequently handled, processed and examined using IBM's Version 26 of the statistics program for social sciences (SPSS), US. Age and BMI are examples of scale variables are given as mean and standard deviation (SD).A parametric test was employed since these variables have a statistical normal distribution. To be considered significant, a difference or connection has to have a p-value of less than 0.05. Lastly, tables and/or figures with an explanatory paragraph for each were used to present the results and findings.

3. Result

In this investigation, the SLC5A2 gene (rs121918621) polymorphisms were evaluated using genetic analysis; GG (reference allele), AG (heterozygous allele), and AA (mutant allele); on the therapeutic response of empagliflozin in 110 patients with type 2 diabetes. Our study included 110 patients with T2D, 50 healthy control persons without any disease, and an age ranged of (30-65) years old, all patients were treated with empagliflozin oral tablet as monotherapy at a daily dose (10 mg). The result of this study showed significant differences in all glycemic and renal parameters between study groups, the glycemic parameters (FBG, HbA1c) and kidney parameters (S. creatinine, B. urea) in individuals with diabetes were significantly higher than those in the healthy group. however, as shown by the data in the table (2), the fasting serum insulin (FSI) of diabetic patients was significantly lower than that of the healthy group.

Table2: mean and standard deviation of the type 2 diabetic group's kidney parameters and glycemic control group's

Parameters	Groups		P-value
	Control (n = 50) Mean ± SD	Diabetics (n = 110) Mean ± SD	
FBG (mg/dl)	117± 42.33	183.55 ± 45.02	0.032
FSI (mg/dl)	19.45± 5.73	13.7 ± 3.58	< 0.001
HBA1C	4.21 ± 0.11	8.223 ± 1.42	< 0.027
S. creatinine	0.64± 0.289	1.007± 0.745	0.027
B. Urea	15.45± 6.87	76.42± 9.48	0.042
The data is shown as mean±SD, with p< 0.05 indicating a significant difference.			

3.1. Genotyping Analysis

The SLC5A2 polymorphism (rs121918621) depicted in Fig.1. One patient's allelic specific PCR study revealed one band, 400 bp, in two different tubes. SLC5A2 rs121918621 genotype distribution was as follows: Sixty-six patients had the wild GG genotype hemizygously, 21 were AG heterozygous polymorphism and 23 were AA homozygous mutant genotype as shown in Fig.2.

Table3 displayed the results, which included the level of (FBG, FSI, and HbA1c) were no significant differences. The results also showed the levels of (S. creatinine, B. Urea) were significantly differences.

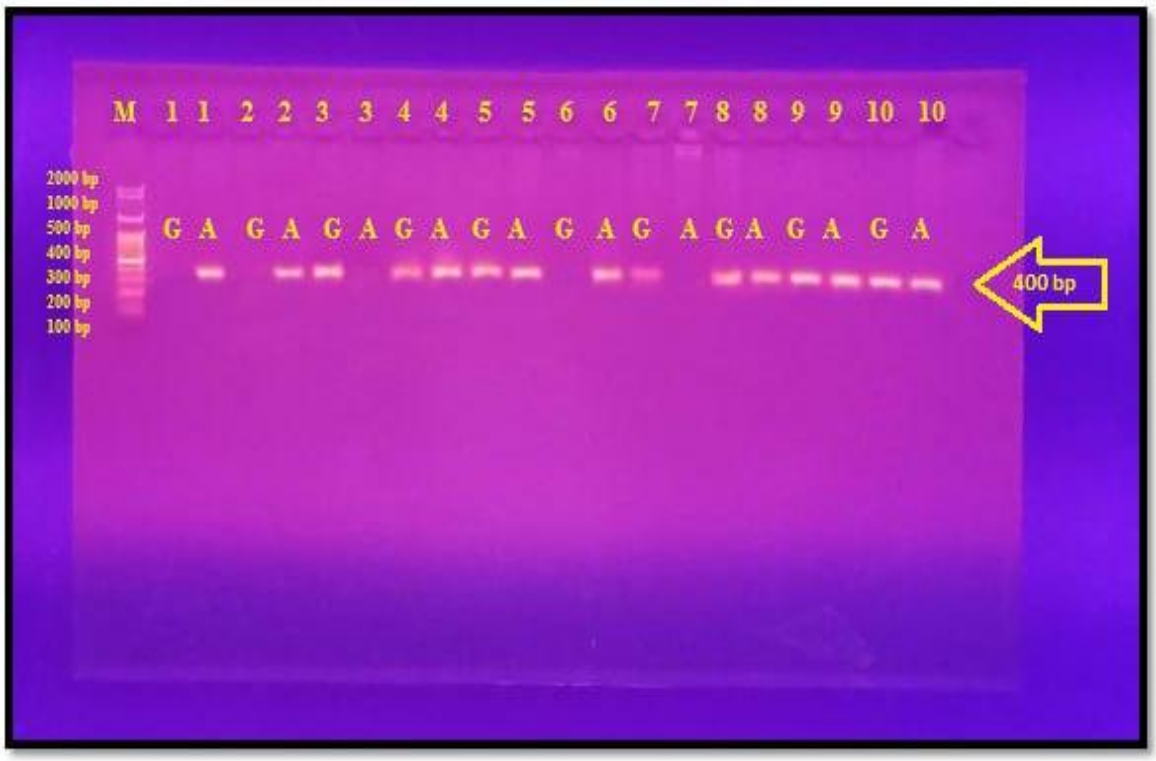

Figure1: Agarose gel electrophoresis showing PCR amplification of the target gene fragment. Lane **M** represents the DNA molecular weight marker (100–2000 bp). Lanes **1–10** correspond to the studied samples. A clear PCR product of approximately **400 bp** was observed (indicated by the arrow), confirming successful amplification. Genotype patterns are indicated above each lane (G/G, G/A, and A/A).

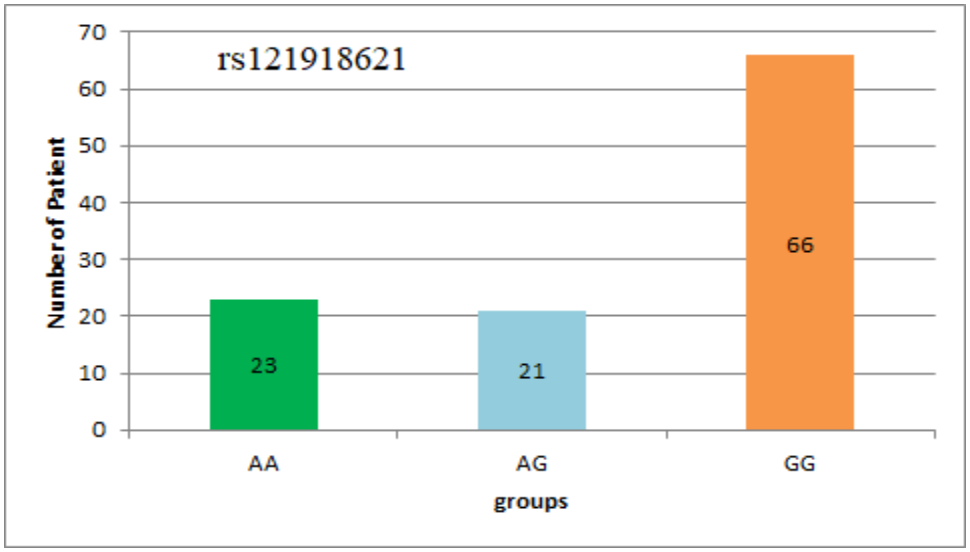

Figure2: Genotype Distribution of The Rs121918621 **Polymorphism Among Patients with Type 2 Diabetes Mellitus.** The frequencies of the **AA**, **AG**, and **GG** genotypes are shown, with the number of patients indicated within each bar.

Table3: Chemical Indicators in Individuals with Diabetes, As Reported By rs121918621

parameters	*rs121918621*			*P-value*
	AA n=23 mean ±SD	**AG n=21 mean ± SD**	**GG n=66 mean ± SD**	
FBG (mg/dl)	142.92 ± 24.98	146.72 ± 28.82	161.43 ± 26.3	**0.062**
HBA1C	6.22 ± 3.25	6.87 ± 3.56	8.09 ± 3.36	**0.054**
FSI (mg/dl)	13.03 ± 2.41	11.52 ± 2.89	13.19 ± 1.26	**0.078**
S. creatinine	0.711 ± 0.133	0.95 ± 0.203	1.42 ± 0.156	**0.014**
B. Urea	69.82± 10.15	67.31±9.55	75.83± 10.19	**0.042**
Results are presented as mean ± SD, p<0.05 considered significantly different a; AA vs. AG, b; AA vs. GG c; AG vs. GG				

4. Discussions

Diabetes mellitus type 2 (T2DM) is a metabolic disorder that is complex and chronic that is typified by persistent hyperglycemia resulting from inadequate insulin signaling due to insulin resistance or impaired insulin production. Severe complications and early mortality may be caused by T2DM. Oral medications known as sodium-glucose cotransporter-2 (SGLT2) inhibitors, such as empagliflozin, are used to lower hyperglycemia in people with type 2 diabetes. The newest medication to lower blood sugar levels is an inhibitor of sodium-glucose co-transporter 2 (SGLT2) called empagliflozin (Zhao et al., 2019). Finding of this study show that significant response (P< 0.05) of empagliflozin that influenced fasting blood glucose, HbA1c, fasting serum insulin, serum creatinine and blood urea levels in T2D patients as shown in Table2.The result in table (3) that show no significant differences in (FBG, FSI, and HbA1c), while the levels of (S. creatinine, B. Urea) were significantly differences. The key conclusion of our

research was that blood urea and serum creatinine levels in T2D patients were significantly ($P<0.05$) raised by the SLC5A2 rs121918621 polymorphism. Even more, as table (3) demonstrates, we found that carriers of the polymorphic SLC5A2 rs121918621 GG wild allele had higher mean and standard deviation of S. creatinine and B. urea than mutant allele AA and heterozygote allele AG. While that SLC5A2 rs121918621 polymorphism non-significant influenced in fasting blood glucose, HbA1c and fasting serum insulin levels in T2D patients. Zimdahl H. et al. (2017) stated that there was no significant connection between the tested SNP and HBC1A, fasting blood glucose, or fasting blood insulin in a cross-sectional investigation. The results of our study were agreement with their findings. Additionally, there was no clinically significant impact on the response of type 2 diabetes patients to the SGLT2 inhibitor empagliflozin, which was a finding that contradicted our findings (Zimdahl et al., 2017).This disagreement may be attributed to dosage form of drug and use of combination drug with empagliflozin that may be impact on pharmacological effect as well as number of sample size. A prior study that used a single dosage of the SGLT2 inhibitor empagliflozin in a pharmacogenetic analysis found no substantial and clinically meaningful effects of the prevalent SLC5A2 SNPs on HbA1c, fasting glucose, body weight, or systolic blood pressure (Zimdahl et al., 2017). According to a meta-analysis by Zhao et al. (2018), empagliflozin lowers fasting plasma glucose, uric acid, and HbA1c.These results point to empagliflozin's advantageous impact on renal and glycemic indices (Zhao et al., 2019)

5. Conclusion

The SLC5A2 gene polymorphism (rs121918621) was linked to response with empagliflozin drug in type 2diabetes patients. The SLC5A2 polymorphism is one of the genetic variables that contribute to heterogeneity in empagliflozin responsiveness in individuals with Type 2 diabetes.

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