ORIGINAL ARTICLE

Genetic analysis: Therapeutic drug monitoring of metformin and glimepiride on diabetic patients' plasma including genetic polymorphism

Areen Ibrahim, Mohanad Odeh[1], Eyad Mallah, Luay Abu-Qatouseh, Ahmad Abu Awaad[2], Mohammad I. A. Ahmad[3], Amjad Shdifat[4], Soadad Saleh[5], Muwafaq Al Hyari[6], Ibrahim Khadra[5], Khaled W. Omari[7], Tawfiq Arafat[8]

Faculty of Pharmacy and Medical Sciences, University of Petra, [6]Center of Diabetes and Endocrinology, Diabetic Center, Prince Hamza Hospital, [8]Jordan Center for Pharmaceutical Research, Amman, [1]Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences, The Hashemite University, [4]Department of Medicine and Family Medicine, Faculty of Medicine, The Hashemite University, Zarqa, [2]Faculty of Pharmacy, Jerash University, Jerash, Jordan, [3]Department of Chemical Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal, [5]Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, Scotland, United Kingdom, [7]College of Engineering and Technology, American University of The Middle East, Kuwait, Jordan

J. Adv. Pharm. Technol. Res.

ABSTRACT

Diabetes is a widespread disease that needs to be controlled. Therapeutic monitoring of drugs is very helpful in maintaining desirable doses. To study a correlation between the blood level of metformin (to a lesser extent, glimepiride) and genotyping (mainly the SULT1A1 genotype). Determine drug levels using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) tool. A validated LC-MS/MS method was developed to determine metformin and glimepiride levels in human plasma. DNA extraction was performed using Jena Bioscience's Blood DNA preparation, in which a column kit was used to extract DNA for genetic polymorphism. The investigation was carried out using both medications in type 2 diabetes patients alongside the genetic polymorphism. One hundred and six patients were assessed. The prevalence of homozygosity for SULT1A1 and wild-type CYP2D6 * 4 were 72.6% and 73.6%, respectively. After adjustment for daily intake of metformin, three patients out of five with the highest levels of metformin had no homozygosity (SULT1A1 genotype). Statistically, variables that demonstrated an insignificant correlation with the level of metformin were body mass index (rs (87) = 0.32, $P = 0.011$) and age (rs (87) =0.26, $P = 0.017$). The homozygous (SULT1A1 genotype) correlation was moderate (rs (87) =0.21, $P = 0.052$). According to the findings, patients with the wt/wt CYP2D6 genotype had considerably greater levels of endoxifen than those with the v/v CYP2D6 genotype. The study's results reported a probable correlation between the blood level of metformin (to a lesser extent, glimepiride) and genotyping (mainly the SULT1A1 genotype). Genotype-guided drug therapy may provide a novel contribution to maximize drug efficacy and/or minimize toxicity.

Key words: Diabetes, drugs, mellitus, pharmacogenomics, therapeutic drug monitoring

Address for correspondence:
Prof. Eyad Mallah,
Faculty of Pharmacy and Medical Sciences, University of Petra, Amman, Jordan.
E-mail: emallah@uop.edu.jo
Dr. Khaled W. Omari,
College of Engineering and Technology, American University of the Middle East, Kuwait.
E-mail: khaled.omari@aum.edu.kw

Submitted: 15-Mar-2024 **Revised:** 09-Apr-2024
Accepted: 10-Apr-2024 **Published:** 22-Jul-2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/JAPTR

DOI: 10.4103/JAPTR.JAPTR_99_24

INTRODUCTION

Worldwide, diabetes mellitus affects around 463 million individuals. By 2030, the prevalence of diabetes is predicted to have increased thrice.[1] In the United States, the prevalence of diabetes has been on the rise by more than 54.9 million Americans from 2015 to 2030.[2] In the Middle

How to cite this article: Ibrahim A, Odeh M, Mallah E, Abu-Qatouseh L, Awaad AA, Ahmad MI, *et al*. Genetic analysis: Therapeutic drug monitoring of metformin and glimepiride on diabetic patients' plasma including genetic polymorphism. J Adv Pharm Technol Res 2024;15:150-5.

East and North Africa region, the predictable number of patients with diabetes is expected to be 76 million by 2030.[3]

According to the International Diabetes Federation, three countries out of the top 15 with the highest prevalence of diabetes are in the Middle East (24.9% in Kuwait; 20.9% in Egypt; and 19.5% in Qatar). Jordan is ranked third in the prevalence of diabetes in the Arab world.[4] A 31.5% increase in diabetes prevalence among the Jordanian population compared to a survey conducted in 1994. Type 2 diabetes mellitus prevalence is expected to rise to 16.0% in 2020 and 20.6% in 2050.

The American Diabetes Association treatment algorithm for Type 2 diabetes recommends comprehensive lifestyle changes as the first steps in the treatment.[5] Metformin is used for most diabetic patients.[6] Alternatively, glimepiride can be used as a single therapeutic agent in patients who cannot tolerate metformin.[7] Glimepiride is the most recent second-generation Sulfonylurea (SU) agent and is sometimes referred to as a third-generation SU due to its higher substitution rate and fewer side effects than other second-generation agents. It has been approved by the US Food and Drug Administration for the treatment of type 2 diabetes since 1995 as a monotherapy agent or in combination with other agents, including metformin and insulin.[8]

The therapeutic window for metformin and glimepiride, even though metformin is a relatively safe drug, laboratory monitoring is recommended to avoid complications such as anemia and lactic acidosis. Accordingly, Vitamin B12 levels should be monitored every 2–3 years, and hematologic parameters should be monitored at baseline and annually.[9] To avoid lactic acidosis, metformin plasma levels must not exceed 5 μg/mL, as studies have suggested that plasma levels of 5 μg/mL or greater metformin were a high indication of lactic acidosis.[10] Thus, even at maximum doses, metformin plasma concentrations do not exceed 5 μg/mL in controlled clinical trials.[11]

The first pharmacogenetic study focused on the role of metformin transporters. However, the most comprehensive study to date is the genome-wide association study conducted by Shu *et al*.[12,13] to investigate the effect of genetic variation in the SLC22A1 gene, which codes for the OCT1 transporter, and the glucose-lowering effect of metformin in both animal models and healthy volunteers.

The current study aims to perform a validated simple LC-MS/MS method for the determination of metformin and glimepiride in human plasma. Determine diabetic patients' plasma levels of metformin and glimepiride using LC-MS/MS for therapeutic drug monitoring (TDM) purposes. Investigate genetic polymorphism effect on metformin and glimepiride response in diabetic patients.

METHODOLOGY

Patient recruitment

The eligible patients were identified as those who meet the following inclusion criteria: type 2 diabetic patients, adults (18–60 years), treated with metformin and/or glimepiride.

Patients who voluntarily accepted to enroll signed the consent form and proceeded with the study protocol.

Sample size and individuals' plasma sample preparation for analysis

To detect the difference between the groups of patients based on their genotype and level of metformin in their blood, the following sample size calculations were carried out, 74 patients will be needed to detect a 0.2 difference between the groups, a power of 80%, a two-sided level of significance of 5%.

Before the LC-MS/MS analysis, the individuals' plasma samples were treated in the same way that the validation samples were treated. LC-MS/MS analytical method for metformin and glimepiride determination in plasma was validated according to the European Medicines Agency guideline.[14] Linearity, coefficient of determination (R^2) was ca. 0.999 over the range (0.5–30) μg/mL for metformin and (0.05–3) μg/mL for glimepiride. The accuracy was 93%–106%. The relative standard deviation (SD) was 2%–8%. Supplementary Tables 1-6 depicts method validation.

Genotyping

DNA extraction method: DNA was extracted using the Blood DNA preparation - Column kit from Jena Bioscience (Germany). Following the manufacturer's instructions with minor changes to optimize the process. Amplification using polymerase chain reaction (PCR), the desired genes were amplified by PCR using Labnet® PCR System TC6000-G-230V. A mixture of 20 μL total volume was prepared for each DNA sample and negative samples.

PCR-restriction fragment length polymorphism (PCR-RFLP) was used to digest the previously amplified DNA fragments. The PCR reaction products were incubated in a 25 L reaction mixture containing 0.5 units of CutSmart HaeII for the SULTA1 gene and 0.5 units of BstN1 for all the CYPs genes, as well as the appropriate reaction buffer supplied by the manufacturer. For SULT1A1, the mixture was incubated at 37°C for 20 min for enzyme digestion before being heat-inactivated at 80°C for 20 min, while the CYPs were incubated for 15 min at 60°C. The PCR RFLP products were subsequently analyzed using 2% agarose gel electrophoresis, and the resultant bands were assigned to the specified lengths by comparison to the standard 100 bp DNA ladder as shown in Figure 1.

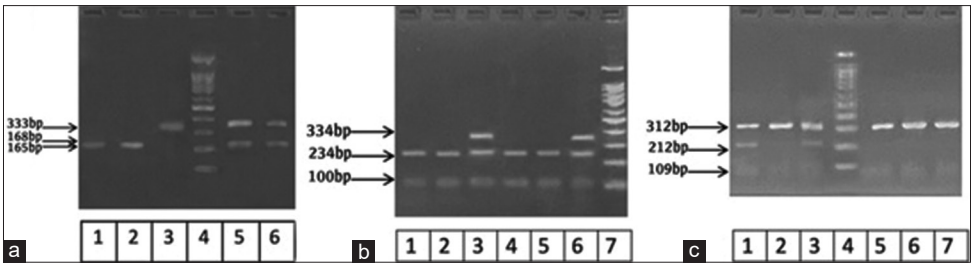

Figure 1: (a) SULT1A1 restriction gel-imaging results where bands 1, 2 heterozygous, band 3 homozygous wild type, and bands 5, 6 homozygous mutant. (b) CYP2D6*4 restriction gel-imaging results were bands 1, 2, 4, 5 homozygous wild type, bands 3, 6 heterozygous. (c) CYP2D19*2 restriction gel-imaging results where bands 2, 5, 6, 7 homozygous wild type, bands 1, 3 heterozygous

Genotype for the recruited patients, Sult1A1, was involved in the metabolism of a wide range of compounds, including metformin and glimepiride. However, the mechanism needs to be proved. Two-third of the recruited patients were homozygous for SULT1A1 (72.6%) and homozygous wild type for CYP2D6 * 4 (73.6%). On the other hand, <30% of recruited patients were found to be heterozygous abnormal for SULT1A1, CYP2D6 * 4, CYP2C19 * 2 (24.5%, 26.4%, and 12.10%, respectively).

Statistical analysis

Microsoft Excel was utilized to electronically gather patient data and associated variables. Following variable coding, the data were imported into SPSS for Windows, version 25, which was developed by SPSS Inc., (Chicago, IL, USA) for statistical analysis. The initial step in the data analysis process was descriptive analysis, which presented the variables related to the patient's characteristics: median, maximum–minimum range, mean, and SD for continuous data, and frequency (%) for categorical data. Data were tested for normal distribution using the Shapiro–Wilk test. Based on the results of distribution, either Pearson's correlation or Spearman's rank-order correlation (Spearman's correlation) was used to assess the strength and direction of the relationship between tested variables. Spearman's coefficient (rs was reported, along with the two-tailed significance level (*P* value), and the cases/participants included in correlation, where degrees of freedom, were reported to be N – 2. The following descriptions were considered for interpretation of the correlation strength based on absolute coefficient value >70, very strong; 0.4–0.69, strong; 0.3–0.39 moderate; 0.2–0.29 weak, and finally <0.2, negligible.[15]

RESULTS

Demography and patient characteristics, data were collected from 106 DM patients. The ages of the patients ranged from 32 to 82 years (mean = 59.4 years, SD = 11.1). Out of the 106 participants, 55 (51.9%) were males. Their average weight was 88.01 ± 18 kg, their average height was 165 cm, and their BMI was 32.75 ± 7 kg/m^2. Regarding HbA1c (hemoglobin A1C), the average of repeated HbA1c was above 7. Normal weight (18.6–24.9 kg/m^2), overweight (25–29.9 kg/m^2), obese grade I (30–34.9 kg/m^2), obese grade II (35–35.9 kg/m^2), and obese grade III (≥40 kg/m^2). Good glycemic control for glycosylated hemoglobin (HbA1C) <7%.[5]

For patient polypharmacy profiles, no statistically significant correlations with LC-MS/MS levels of glimepiride or metformin were noted, [Supplementary Table 7]. However, amlodipine was the only medication that reported a statistically significant correlation with glimepiride level rs (71) = 0.34, *P* = 0.003.

Metformin

The results of metformin plasma levels analyzed by LC-MS/MS are shown in Table 1. Groups were divided into three categories: subtherapeutic (<0.5 μg/mL), within the therapeutic window (0.5–5) μg/mL, and more than the minimum toxic concentration (more than 5 μg/mL). The percentage of patients within each group was 44.3%, 50.9%, and 5.6%. The highest value found for metformin plasma level was 11.965 μg/mL and the lowest was 0.020 μg/mL. The reference range of metformin is (0.5–5) μg/mL. There is no clear mechanism explaining the relationship between metformin and plasma levels in the literature. The authors back up the phase II drug-metabolizing enzyme theory.

Related to LC-MS/MS metformin and the daily intake, Table 2 shows that about 42.2% of the participants' daily dose intake of metformin was 1700 mg/day, and only 17.9% of their daily dose intake of metformin was 850 mg/day.

Related to metformin TDM results in relationship to genotyping and other variables, details of patients' characteristics who had the highest and lowest metformin based on the therapeutic window categories are presented in Supplementary Table 8. It is worth mentioning that results showed that younger patients reported relatively lower plasma levels of metformin. Findings showed that 4 out of 5 patients with the highest plasma level of metformin who were taking 850 mg/day had no Homozygous Mutation of the SULT1A1 Gene. The other patients with the lowest plasma level of metformin who were taking 850 mg/day had Homozygous Mutation in the SULT1A1 Gene. This was only noted in the patient group who took 850 mg/day of metformin.

Three patients with the highest level of metformin reported values were to 11.97 μg/mL (2250 mg/day intake), 5.45 μg/mL (850 mg/day intake), and 5.92 μg/mL (2250 mg/day intake). The median value for metformin plasma levels for those who exceeded the minimum toxic dose was 5.3 μg/mL (5.04–87), the median for those taking 2250 mg/day, and 850 mg/day was 2.3 μg/mL (1.38–3.24), and 0.94 μg/mL (0.24–1.6), respectively. Those three patients had no Homozygous (SULT1A1 Genotype).

A Spearman's rank-order correlation was run to assess the relationship between metformin plasma concentration and patients' variables [Supplementary Table 9]. Statistically, significant correlation between metformin plasma concentration and the Body Mass Index rs (87) = 0.32, P = 0.011 as well as statistically significant correlations were demonstrated with the daily dose of metformin and age (weak correlations rs (87) = 0.26, P = 0.017 and rs (87) = 0.25, P = 0.011, respectively. Another correlation was noted with Homozygous Mutant (SULT1A1 Genotype) rs (87) = 0.21, yet its statistically P = 0.052. After adjustment for daily intake, it shows that the main driver of the results were the patients who had a daily intake of metformin of 850 mg.

Glimepiride

The levels in the recruited patients and the results of glimepiride analyzed by LC-MS/MS are shown in Table 3. There were three categories: subtherapeutic (<0.05 μg/mL), within the therapeutic window (0.05–0.6) μg/mL, and more than the minimum toxic concentration (more than 0.6 μg/mL). The percentage of patients within each group was 79.2%, 19.8%, and 0.9%, respectively. The highest value found for glimepiride plasma level was 0.92 μg/mL and the lowest was 0.0004 μg/mL. The reference range of glimepiride is (0.1–0.60) μg/mL.

Related to LC-MS/MS glimepiride and the daily intake, Table 4 shows that half of the participants did not use glimepiride as an antidiabetic drug, 26.4% of their daily dose intake of glimepiride was 4 mg/day, and 23.6% of their daily dose intake of glimepiride was 8 mg/day.

For glimepiride TDM results concerning genotyping and other variables, it was observed that the five patients with

Table 1: Description of the metformin level measured by LC-MS/MS based on the therapeutic window categories

Therapeutic window categories	*n* (%)		Mean LC-MS/MS results 95% CI	Median LC-MS/MS results (IQR)	Minimum and maximum	Normality test Shapiro–Wilk test significant (*P**)
Below the calibration curve	47 (44.3)	17 (16)	0	0	0	Not normal distribution (<0.001)
		30 (28.3)	0.2 (0.15–0.77)	0.22 (0.04–0.34	0.02–0.47	
Within therapy window	54 (50.9)		1.85 (1.6–2.1)	1.46 (1.1-2.5)	0.53–4.46	Not normal distribution (<0.001)
More than mini toxic conc	5 (4.7)		6.5 (4.8–10.9)	5.3 (5.04–87)	5.04–11.97	Not normal distribution (<0.001)

*P < 0.05. CI: Confidence interval, IQR: Interquartile range, LC-MS/MS: Liquid chromatography-tandem mass spectrometry

Table 2: Description of the metformin level measured by LC-MS/MS based on the daily medication intake

Metformin daily dose (mg per day)	*n* (%)		Mean LC-MS/MS results 95%CI	Median LC-MS/MS results (Range)	Minimum and Maximum	Normality test Shapiro–Wilk test significant (*P*)
0	9 (8.5)	7	0	0		Normal distribution (<0.001)
		2	0.02 (0.005–0.06)	0.03 (0.02–0.04)	0.02–0.04	
850	19 (17.9)		0.94 (0.24–1.6)	0.36 (0.19–1.3)	0.02–5.45	Not normal distribution (<0.001)
1700	45 (42.4)		1.37 (1.0–1.73)	1.24 (0.5–1.67)	0.02–5.04	Not normal distribution (<0.001)
2250	33 (31.2)		2.3 (1.38–3.24)	1.65 (0.45–3.82)	0.03–11.97	Not normal distribution (<0.001)

LC-MS/MS: Liquid chromatography-tandem mass spectrometry

Table 3: Description of the glimepiride level measured by LC-MS/MS based on the therapeutic window categories

Therapeutic window categories	*n* (%)		Mean LC-MS/MS results 95% CI	Median LC-MS/MS results (IQR)	Minimum and Maximum	Normality test Shapiro–Wilk test significant (*P**)
Below the calibration curve	84 (79.2)	33 (31.1)	0	0	0	Not normal distribution (<0.001)
		51 (48.1)	0.007 (0.004–0.01)	0 (0.0–0.1)	0–0.04	
Within therapy window	21 (19.8)		0.21 (0.17–0.26)	0.2 (0.1–0.3)	0.07–0.4	Normal distribution (>0.05)
More than mini toxic conc	1 (0.9)		NA	NA	NA	NA

*P < 0.05. NA: Not available, CI: Confidence interval, IQR: Interquartile range, LC-MS/MS: Liquid chromatography-tandem mass spectrometry

the lowest LC-MS/MS reading for glimepiride within the therapeutic window had a Homozygous Mutant (SULT1A1 Genotype) and Homozygous Wild-Type (CYP2D6*4 Genotype), [Supplementary Table 10].

Spearman's rank-order correlation was run to assess the relationship between glimepiride concentration and patients' variables. There was a statistically significant, strong positive correlation between its concentration and the daily dose of glimepiride rs (71) = 0.62, $P < 0.001$. Another statistically significant, weak correlation was demonstrated with age (rs[71] = 0.26, $P = 0.019$. This could be related to the fact that as people get older, their renal excretion and function decline, resulting in higher plasma concentrations [Supplementary Table 11].

DISCUSSION

Al Eitan *et al*. examined the impact of 21 single-nucleotide polymorphisms in the genes SLC22A1, SLC22A2, and SLC22A3 on the pharmacogenetics of metformin in patients with type 2 diabetes who were diagnosed in Jordan.[16] A significant ($P = 0.05$) correlation was seen between the SLC22A3 gene's rs12194182 single-nucleotide polymorphisms and lower mean HbA1c levels; this correlation was particularly prominent in patients with the CC genotype. Metformin pharmacology was observed to be affected by the SLC22A1, SLC22A2, and SLC22A3 genes. These factors end up affecting the way the patient reacts to the medication.

Tamoxifen levels of metabolites and CYP/SULT genotypes in concern in patients with breast cancer ($n = 135$) were associated with each other, according to a study that was carried out.[17] The findings demonstrated that patients with the wt/wt CYP2D6 genotype had noticeably greater levels of endoxifen than those with the v/v CYP2D6 genotype.

Other statistically significant correlations were demonstrated with the daily dose of metformin and age (weak correlations rs (87) = 0.26, $P = 0.017$ and rs (87) = 0.25, $P = 0.011$, respectively. This is consistent with the findings of a study of 82 patients, which discovered that age was a predictor of metformin pharmacokinetic profile.[18] This can be explained by the fact that in older age, renal excretion and function decreased, which led to increased plasma concentration. The weak correlation could be because this study excluded elderly patients (those over the age of 60).

Homozygous for SULT1A1 (72.6%) and homozygous for wild type for CYP2D6*4 (73.6%). On the other hand, <30% of recruited patients were found to be heterozygous abnormal for SULT1A1, CYP2D6*4, and CYP2C19*2 (24.5%, 26.4%, and 12.10%, respectively).

In the present study, the heterozygous prevalence for SULT1A1 was 24.5%, which is very similar results to another study in Jordan, where the analysis revealed that 24.7% of Jordanian cancer patients and 25.3% of controls were heterozygous for the SULT1A1*1 allele (SULT1A1*1/SULT1A1*1). Similarly, the present results regarding CYP2D6 are in line with previously published results in Jordan and the Middle East.[19,20]

According to literature studies, metformin plasma levels >5 g/mL are typically found when metformin is implicated as the cause of lactic acidosis.[10] Metformin plasma concentrations do not exceed 5 μg/mL during controlled clinical trials, even at maximum doses.[11,21] In the present study, five of the 106 patients had exceeded minimum toxic metformin plasma levels despite having a prescribed metformin dose within the recommendation.

Further investigations are always needed to optimize concepts. A room for improvement is a sensible way to highlight discoveries from additional research. Reaching these objectives can be accomplished by expanding the sample size and diversity, looking into more type 2 diabetes-specific medications, providing long-term follow-up, and controlling cost-effectiveness, among other things.

CONCLUSION

The study indicates a potential link between the SULT1A1 Genotype and blood levels of metformin and glimepiride, suggesting that genotype-guided drug therapy could enhance drug effectiveness and minimize adverse effects. Because of this relationship, blood analysis should be used to track the drug levels (glimepiride and metformin)

Table 4: Description of the glimepiride level measured by LC-MS/MS based on the daily medication intake

Glimepiride daily dose (mg/day)	*n*, (%)		Mean LC-MS/MS results 95% CI	Median LC-MS/MS results (range)	Minimum and Maximum	Normality test Shapiro–Wilk test significant (*P*)
0	53 (50)	31	0	0	0	Normal distribution (<0.001)
		22	0.0004 (0–0.0008)	0	0	
4	28 (26.4)		0.034 (0.019–0.049)	0.017 (0.001–0.04)	0–0.16	Not normal distribution (<0.001)
8	25 (23.6)		0.32 (0.2–0.43)	0.27	0.1–0.92	Not normal distribution (<0.001)

CI: Confidence interval, LC-MS/MS: Liquid chromatography-tandem mass spectrometry

during treatment to obtain the most possible advantages. Through the ability to customize treatment plans based on each patient's unique genetic profile, such as adjusting dosages or minimizing side effects, genotype-guided drug therapy holds great promise to transform clinical practice in the management of type 2 diabetes and improve long-term outcomes for patients by optimizing treatment efficacy.

Acknowledgment

We express our gratitude for the efforts that enabled the completion of this study, particularly to the University of Petra, Hashemite University, Jordan Center for Pharmaceutical Research, and Prince Hamza Hospital.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, *et al.* Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the international diabetes federation diabetes Atlas, 9(th) edition. Diabetes Res Clin Pract 2019;157:107843.
2. Rowley WR, Bezold C, Arikan Y, Byrne E, Krohe S. Diabetes 2030: Insights from yesterday, today, and future trends. Popul Health Manag 2017;20:6-12.
3. Al Ma'aitah OH, Demant D, Jakimowicz S, Perry L. Glycaemic control and its associated factors in patients with type 2 diabetes in the Middle East and North Africa: An updated systematic review and meta-analysis. J Adv Nurs 2022;78:2257-76.
4. Meo SA, Usmani AM, Qalbani E. Prevalence of type 2 diabetes in the Arab world: Impact of GDP and energy consumption. Eur Rev Med Pharmacol Sci 2017;21:1303-12.
5. American Diabetes Association. 5. Lifestyle management: Standards of medical care in diabetes-2019. Diabetes Care 2019;42:S46-60.
6. Ahmad E, Sargeant JA, Zaccardi F, Khunti K, Webb DR, Davies MJ. Where does metformin stand in modern day management of type 2 diabetes? Pharmaceuticals (Basel) 2020;13:427.
7. Kalra S, Bahendeka S, Sahay R, Ghosh S, Md F, Orabi A, *et al.* Consensus recommendations on sulfonylurea and sulfonylurea combinations in the management of type 2 diabetes mellitus – International task force. Indian J Endocrinol Metab 2018;22:132-57.
8. Basit A, Riaz M, Fawwad A. Glimepiride: Evidence-based facts, trends, and observations (GIFTS). Vasc Health Risk Manag 2012;8:463-72.
9. Kim J, Ahn CW, Fang S, Lee HS, Park JS. Association between metformin dose and Vitamin B12 deficiency in patients with type 2 diabetes. Medicine (Baltimore) 2019;98:e17918.
10. DeFronzo R, Fleming GA, Chen K, Bicsak TA. Metformin-associated lactic acidosis: Current perspectives on causes and risk. Metabolism 2016;65:20-9.
11. Dumitrescu R, Mehedintu C, Briceag I, Purcărea VL, Hudita D. Metformin-clinical pharmacology in PCOs. J Med Life 2015;8:187-92.
12. Shu Y, Sheardown SA, Brown C, Owen RP, Zhang S, Castro RA, *et al.* Effect of genetic variation in the organic cation transporter 1 (OCT1) on metformin action. J Clin Invest 2007;117:1422-31.
13. Shu Y, Brown C, Castro RA, Shi RJ, Lin ET, Owen RP, *et al.* Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics. Clin Pharmacol Ther 2008;83:273-80.
14. ICH Guideline M10 on Bioanalytical Method Validation and Study Sample Analysis. Guideline on Bioanalytical Method Validation; 2022. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-guideline-m10-bioanalytical-method-validation-step-5en.pdf. [Last accessed on 2023 Nov 18].
15. Dancey CP, Reidy J. Statistics without Maths for Psychology. 8th ed. London UK: Pearson; 2020.
16. Al Eitan LN, Almomani BA, Nassar AM, Elsaqa BZ, Saadeh NA. Metformin pharmacogenetics: Effects of SLC22A1, SLC22A2, and SLC22A3 polymorphisms on glycemic control and HbA1c levels. J Pers Med 2019;9:17.
17. Fernández Santander A, Gaibar M, Novillo A, Romero Lorca A, Rubio M, Chicharro LM, *et al.* Relationship between genotypes Sult1a2 and Cyp2d6 and tamoxifen metabolism in breast cancer patients. PLoS One 2013;8:e70183.
18. Ningrum VD, Ikawati Z, Sadewa AH, Ikhsan MR. Patient-factors associated with metformin steady-state levels in type 2 diabetes mellitus with therapeutic dosage. J Clin Transl Endocrinol 2018;12:42-7.
19. Khalaj Z, Baratieh Z, Nikpour P, Khanahmad H, Mokarian F, Salehi R, *et al.* Distribution of CYP2D6 polymorphism in the middle Eastern region. J Res Med Sci 2019;24:61.
20. Alali M, Ismail Al Khalil W, Rijjal S, Al Salhi L, Saifo M, Youssef LA. Frequencies of CYP2D6 genetic polymorphisms in Arab populations. Hum Genomics 2022;16:6.
21. Frid A, Sterner GN, Löndahl M, Wiklander C, Cato A, Vinge E, *et al.* Novel assay of metformin levels in patients with type 2 diabetes and varying levels of renal function: Clinical recommendations. Diabetes Care 2010;33:1291-3.

Supplementary Table 1: Calibration points accuracy results for metformin

Calibration point	Concentration (μg/mL)	Metformin area	IS area	Area ratio	Calculated amount (μg/mL)	Accuracy (%)
STD1	0.50	208	9783	0.021	0.510	102
STD2	1.00	528	15,174	0.035	0.997	100
STD3	2.50	1111	15,576	0.071	2.313	93
STD4	5.00	926	6454	0.143	4.908	98
STD5	10.00	2229	7682	0.290	10.187	102
STD6	20.00	4782	8687	0.550	19.551	98
STD7	30.00	6835	7995	0.855	30.502	102

STD: Standard, IS: Internal Standard

Supplementary Table 2: Calibration points accuracy results for glimepiride

Calibration point	Concentration (μg/mL)	Glimepiride area	IS area	Area ratio	Calculated amount (μg/mL)	Accuracy (%)
STD1	0.05	48	9783	0.005	0.053	106
STD2	0.10	115	155,174	0.008	0.100	100
STD3	0.25	241	15,576	0.016	0.239	96
STD4	0.50	194	6454	0.030	0.496	99
STD5	1.00	439	7682	0.057	0.972	97
STD6	2.00	1056	8687	0.122	2.104	105
STD7	3.00	1350	7995	0.169	2.937	98

STD: Standard, IS: Internal Standard

Supplementary Table 3: Accuracy results for 3 days for metformin

QC (μg/mL)	Day	Average accuracy (%)	3 days average accuracy (%)
L (1.5)	1	100	100.6
	2	102	
	3	100	
M (15)	1	102	99.3
	2	99	
	3	97	
H (24)	1	103	101
	2	99	
	3	101	

Supplementary Table 4: Accuracy results for 3 days for glimepiride

QC (μg/mL)	Day	Average accuracy (%)	3 days average accuracy (%)
L (0.15)	1	100	100.3
	2	101	
	3	100	
M (1.5)	1	100	100
	2	100	
	3	100	
H (2.4)	1	101	101.3
	2	100	
	3	103	

Supplementary Table 5: Precision results of metformin for 3 days

QC (μg/mL)	Day	Average concentration (μg/mL)	RSD%	3 days average RSD%
L (1.5)	1	1.49	4	4.3
	2	1.52	2	
	3	1.50	7	
M (15)	1	15.28	6	6
	2	14.91	5	
	3	14.53	7	
H (24)	1	24.75	7	5.6
	2	23.83	4	
	3	24.15	6	

RSD: Relative standard deviation

Supplementary Table 6: Precision results of glimepiride for 3 days

QC (μg/mL)	Day	Average concentration (μg/mL)	RSD (%)	3 days average RSD (%)
L (0.15)	1	0.15	8	5.3
	2	0.15	3	
	3	0.15	5	
M (1.5)	1	1.50	7	5.3
	2	1.49	5	
	3	1.50	4	
H (2.4)	1	2.43	8	5.6
	2	2.39	5	
	3	2.48	4	

RSD: Relative standard deviation

Supplementary Table 7: Polypharmacy profile for recruited patients (*n*=106) and the associations with LCMS level of glimepiride and metformin

Drug	Availability	*n* (%)	LCMS level of glimepiride		LCMS level of metformin	
			Coefficient	*P*	Coefficient	*P*
Aspirin	No	39 (45.9)	0.05	0.67	−0.2	0.063
	Yes	46 (54.1)				
Beta blockers	No	44 (51.8)	0.2	0.09	0.14	0.2
	Yes	41 (48.2)				
Statins	No	36 (42.4)	0.21	0.08	0.02	0.82
	Yes	49 (57.6)				
ACE inhibitors	No	61 (71.8)	0.03	0.81	−0.06	0.56
	Yes	24 (28.2)				
Miconazole	No	48 (56.5)	0.07	0.59	−0.11	0.3
	Yes	37 (43.5)				
DPP-4 inhibitors ("Gliptins")	No	68 (80.0)	0.001	0.99	−0.01	0.91
	Yes	17 (20.0)				
Levothyroxine	No	70 (82.4)	−0.08	0.51	0.015	0.89
	Yes	15 (17.6)				
Furosemide	No	71 (83.5)	−0.13	0.26	0.016	0.88
	Yes	14 (16.5)				
Isosorbid	No	76 (89.4)	−0.14	0.24	−0.14	0.18
	Yes	9 (10.6)				
Hydrochlorothiazide	No	67 (78.8)	0.1	0.45	−0.08	0.49
	Yes	18 (21.2)				
Famotidine	No	67 (78.8)	0.05	0.67	−0.11	0.29
	Yes	18 (21.2)				
Amlodipine	No	70 (82.4)	0.34	0.003	0.14	0.21
	Yes	15 (17.6)				
Gabapentin	No	69 (81.2)	0.11	0.37	0.004	0.98
	Yes	16 (18.8)				
Allopurinol	No	71 (83.5)	−0.01	0.91	0.09	0.41
	Yes	14 (16.5)				
Clopidogrel	No	73 (85.9)	0.13	0.26	−0.041	0.71
	Yes	12 (14.1)				
ARBs	No	58 (68.2)	0.05	0.69	0.05	0.65
	Yes	27 (31.8)				

ARBs: Angiotensin II receptor blockers, ACE: Angiotensin-converting enzyme, LCMS: Liquid chromatography with tandem mass spectrometry

Supplementary Table 8: Description of the metformin level measured by LCMS based on the therapeutic window categories

Metformin levels	Patient number	Value	Daily dose metformin	Gender	Age	BMI	Homozygous mutant (SULT1A1 Genotype)	Homozygous wild type (CYP2D6*4 Genotype)	Homozygous wild type (CYP2C19*2 Genotype)	Number of comedications (polypharmacy)	Do the patient use glimepiride	Hba1c the most recent level
Below the calibration curve												
Highest												
1	85	0.47	3400	Female	65	33.1	X	X	X	7	No	6.4
2	3	0.45	2250	Female	43	25.9	X	X	X	3	No	5.5
3	72	0.45	1700	Male	56	32.3	X	X	X	5	No	7.6
4	52	0.44	NA	Female	72	NA		X	X	5	Yes	7.4
5	51	0.36	2250	Male	59	NA	X		X	2	No	10.5
Lowest												
1	90	0.02	1700	Male	40	37.9	X	X	X	2	No	7.8
2	23	0.02	850	Male	67	31.9	X	X	X	6	No	5.3
3	60	0.02	NA	Male	73	34.2	X	X	X	6	Yes	6.6
4	11	0.03	2250	Male	63	26.7		X	X	5	Yes	5.4
5	100	0.03	NA	Male	57	33.6	X			1	Yes	7.3
Within therap window												
Highest												
1	8	4.46	2250	Female	73	31.3	X	X	X	6	Yes	7.3
2	65	4.04	2250	Female	52	26.6	X		X	1	No	6.2
3	28	3.97	1700	Female	56	34.4	X		X	4	Yes	9.19
4	1	3.84	2250	Male	50	29.4	X			2	No	6
5	92	3.67	1700	Male	74	30.4		X	X	6	Yes	8.5
Lowest												
1	14	0.53		Female	46	NA	X	X	X	2	Yes	11.3
2	80	0.54	1700	Female	63	34		X	X	4	Yes	9.1
3	68	0.59	850	Male	52	NA	X	X	X	2	Yes	5.4
4	33	0.67	2250	Male	42	43.4	X	X	X	0	Yes	NA
5	106	0.68	1700	Female	35	36.6	X	X	X	10	No	5.9
More than mini toxic conc												
Highest												
1	94	11.97	2250	Male	69	22.4		X	X	1	No	NA
2	84	5.45	850	Female	72	27.7			X	7	No	6.7
Lowest												
1	18	5.04	1700	Male	65	NA	X		X	8	No	5.8
2	36	5.04	2250	Male	68	30.3	X	X	X	2	Yes	9

NA: Not available, BMI: Body mass index, Hba1c: Glycated hemoglobin, LCMS: Liquid chromatography with tandem mass spectrometry

Supplementary Table 9: Correlations between the metformin LCMS levels and the patient's variables

Metformin levels	Daily dose metformin	Gender	Age	BMI	Homozygous mutant (SULT1A1 Genotype)	Homozygous wild type (CYP2D6*4 Genotype)	Homozygous wild type (CYP2C19*2 Genotype)	Number of comedications (polypharmacy)	Do the patient use glimepiride	Hba1c the most recent level
As assessed by Shapiro–Wilk's test	Ordinal categories	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)
Correlations with the metformin concentration	Spearman's rho=0.26, $P=0.017$	Spearman's rho=0.16, $P=0.13$	Spearman's rho=0.25, $P=0.019$	Spearman's rho=−0.32, $P=0.011$	Spearman's rho=−0.21, $P=0.052$	Spearman's rho=−0.12 , $P=0.52$	Spearman's rho=−0.07, $P=0.51$	Spearman's rho=−0.016, $P=0.88$	Spearman's rho=0.17, $P=0.11$	Spearman's rho=0.096, $P=0.39$
Correlations with the metformin concentration when the daily dose 850 mg per day	1	0.2 $P=0.45$	0.43 $P=0.083$	−0.65 $P=0.03$	−0.47 $P=0.054$	−0.28 $P=0.27$	−0.42 $P=0.08$	0.13 $P=0.62$	0.04 $P=0.23$	0.38 $P=0.15$
Correlations with the metformin concentration when daily dose 1700 mg per day	1	0.1 $P=0.52$	0.17 $P=0.29$	−0.14 $P=0.5$	−0.17 $P=0.27$	−0.13 $P=0.42$	−0.14 $P=0.38$	0.01 0.93	0.09 $P=0.29$	−0.14 $P=0.4$
Correlations with the metformin concentration when daily dose 2250 mg per day	1	0.12 $P=0.52$	0.26 $P=0.16$	−0.29 $P=0.19$	−0.09 $P=0.65$	−0.05 $P=0.78$	−0.04 $P=0.82$	0.001 $P=0.99$	0.06 $P=0.2$	0.24 $P=0.25$

Hba1c: Glycated hemoglobin, LCMS: Liquid chromatography with tandem mass spectrometry

Supplementary Table 10: Description of the glimepiride level measured by LCMS based on the therapeutic window categories

New group for glimepiride levels	Patient number	Value	Daily dose glimepiride	Gender	Age	BMI	Homozygous mutant (SULT1A1 Genotype)	Homozygous wild type (CYP2D6*4 Genotype)	Homozygous wild type (CYP2C19*2 Genotype)	Number of comedications (polypharmacy)	Do the patient use Metformin	Hba1c the most recent level
Below the calibration curve												
Highest												
1	83	0.04	4	Female	51	26.8	X		X	1	Yes	5.4
2	84	0.04	0	Female	72	27.7			X	7	No	6.7
3	82	0.03	0	Female	58	37.6	X	X	X	4	No	6.1
4	81	0.02	0	Female	66	34.2	X			5	No	6.4
5	79	0.02	0	Male	54	27.1	X		X	1	No	8.8
Lowest												
1	59	0.00	8	Male	75	NA	X			4	Yes	7.7
2	58	0.00	8	Male	66	24.6	X		X	11	Yes	8
3	57	0.00	0	Male	58	NA	X	X	X	9	No	6.07
4	56	0.00	0	Male	58	NA	X	X	X	4	No	6.3
5	55	0.00	0	Male	64	NA	X	X	X	6	No	6.4
Within therap window												
Highest												
1	105	0.40	4	Female	77	27.1	X	X		4	Yes	6.6
2	104	0.37	4	Male	41	40.6	X		X	5	Yes	12.4
3	103	0.36	0	Female	47	27.4	X	X		1	No	5.8
4	102	0.36	4	Male	44	32.7		X		1	Yes	8.7
5	101	0.31	4	Male	69	25.9		X	X	7	Yes	10.5
Lowest												
1	85	0.07	0	Female	65	33.1	X	X	X	7	No	6.4
2	86	0.09	0	Female	60	33.6	X	X	X	3	No	6.3
3	87	0.09	4	Female	47	33.6	X	X		7	Yes	7.9
4	88	0.10	4	Female	53	30.1	X	X	X	4	Yes	8.8
5	89	0.11	4	Male	82	27.7	X	X	X	2	Yes	6.9
More than mini toxic conc												
1	8	0.92	8	Female	73	31.3	X	X	X	6	Yes	7.3

NA: Not available, BMI: Body mass index, Hba1c: Glycated hemoglobin, LCMS: Liquid chromatography with tandem mass spectrometry

Supplementary Table 11: Correlations between the glimepiride LC-MS/MS levels and the patient's variable

New group for glimepiride levels	Daily dose glimepiride	Gender	Age	BMI	Homozygous mutant (SULT1A1 Genotype)	Homozygous wild type (CYP2D6*4 Genotype)	Homozygous wild type (CYP2C19*2 Genotype)	Number of comedications (polypharmacy)	Do the patient use metformin	Hba1c the most recent level
As assessed by Shapiro–Wilk's test	Ordinal categories	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)	Not all normally distributed, ($P<0.05$)
Correlations with the glimepiride concentration	Spearman's rho=0.62, $P<0.0001$	Spearman's rho=0.024, $P=0.84$	Spearman's rho=0.27, $P=0.019$	Spearman's rho=–0.40, $P=0.004$	Spearman's rho=–0.17, $P=0.15$	Spearman's rho=–0.10, $P=0.42$	Spearman's rho=0.038, $P=0.75$	Spearman's rho=0.16, $P=0.18$	Spearman's rho=0.14, $P=0.19$	Spearman's rho=0.36, $P=0.002$
Correlations with the glimepiride concentration 4 mg/day	1	0.12 $P=0.51$	0.15 $P=0.4$	–0.48 $P=0.02$	–0.24 $P=0.2$	–0.13 $P=0.49$	0.12 $P=0.53$	0.14 $P=0.44$	0.3 $P=0.12$	–0.07 $P=0.71$
Correlations with the glimepiride concentration 8 mg/day	1	0.03 $P=0.91$	0.35 $P=0.014$	–0.36 $P=0.017$	–0.24 $P=0.33$	–0.087 $P=0.72$	0.29 $P=0.23$	0.22 $P=0.36$	0.34 $P=0.19$	0.04 $P=0.848$

BMI: Body mass index, Hba1c: Glycated hemoglobin, LC-MS/MS: Liquid chromatography with tandem mass spectrometry