ABCB1 C3435T genetic Polymorphism and response to Glibenclamide therapy in patients with type 2 diabetes mellitus

Surendiran A[1*], Pradhan SC[1], Subrahmanyam DKS[2], Aparna Agrawal[2], Umamaheswaran G[1], Rajan S[1], Adithan C[3]

[1]*Department of Pharmacology, JIPMER, Puducherry, India.*
[2]*Department of Medicine, JIPMER, Puducherry, India.*
[3]*Department of Clinical Pharmacology, JIPMER, Puducherry, India.*

ABSTRACT

Background: Glibenclamide is a substrate and an inhibitor of P-glycoprotein which is coded by the gene *ABCB1*. **Objective:** To study the influence of *ABCB1 C3435T* gene polymorphism on the therapeutic effect of glibenclamide, its plasma levels and hypoglycemic adverse effects. **Materials and Methods:** The study was done in Type 2 diabetes mellitus patients of South India (n = 80) who were on treatment with glibenclamide as a single agent or along with metformin. From a venous blood sample, *ABCB1 C3435T* genetic polymorphism and plasma levels of glibenclamide were determined. The parameters were compared between genotype groups. Patient characteristics across genotypes were analyzed using one way ANOVA and the association between glycemic status and genotype was studied using Chi Square test. The association between genotypes and parameters such as C/D values, hypoglycemic episodes were compared using Kruskal Wallis Test. **Results:**There were no significant differences in age, body mass index and duration of treatment between the genotype groups *ABCB1 CC, CT* and *TT*. There was no significant association between glycemic status of type 2 diabetes and presence of variant genotypes *ABCB1 CT* and *TT*. There were no statistically significant differences in plasma concentration of glibenclamide, number and severity of adverse effects between the genotype groups. **Conclusion:** *ABCB1 C3435T* genetic polymorphism did not produce any significant influence on the therapeutic response to glibenclamide, plasma glibenclamide levels and the occurrence of adverse events in South Indian patients with type 2 diabetes mellitus.

Key Words: *ABCB1 C3435T*, Diabetes Mellitus, Drug Transporters, Glibenclamide, MDR1, Personalized Medicine, Pharmacogenetics

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INTRODUCTION

Among non-communicable diseases, diabetes mellitus is one of the major causative factor for significant morbidity and reduction in quality of life among patients.[1] Type 1 diabetes mellitus occurs due to insufficient production of insulin and the main modality of therapy is replacement of insulin. In type 2 diabetes mellitus, ineffective utilization of insulin occurs resulting in elevated blood glucose levels even though insulin levels are not deficient. Oral antidiabetic medications are used for patients with Type 2 diabetes mellitus which have varied mechanisms such as decrease in gluconeogenesis, increased uptake of glucose, reversal of insulin resistance, and increase in secretion of insulin.[2-3] Sulfonylureas play a significant role in the treatment of this condition. They act by promoting the secretion of insulin from the β cells of pancreas. Glibenclamide, a long acting sulfonylurea is commonly prescribed for patients with type 2 diabetes mellitus as monotherapy or along with metformin as add-on therapy. It acts on sulfonylurea receptors on the β cells of pancreas and increases the secretion of insulin.[2] It also increases

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***Correspondence : surenmbbs@gmail.com**
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the sensitivity of liver to the action of insulin by increasing number of insulin receptors and post-receptor activation.[4-6] Hypoglycemia is a common adverse effect that occurs with glibenclamide owing to its ability to increase insulin secretion.[7] A significant variation has been reported in the therapeutic response to glibenclamide and the risk of hypoglycemic adverse effects. Among various contributory factors, genetic polymorphisms contribute significantly to such variations. The effect of genetic polymorphisms in the gene CYP2C9 which codes for the enzyme which metabolized glibenclamide has been demonstrated to cause significant variation in therapeutic response.[8-10] However, the genetic influence has been understated since genetic polymorphisms of genes coding for proteins other than enzymes have not been studied. We have studied the role of genetic polymorphism in the drug transporter, P-glycoprotein coded by the gene, *ABCB1*. Genetic variations in this gene can result in altered drug disposition within the body and can theoretically affect drug absorption and distribution, ultimately resulting in altered drug efficacy and safety. The objective of this study was to explore the influence of *ABCB1 C3435T* gene polymorphism on the therapeutic effect of glibenclamide, its plasma levels and hypoglycemic adverse effects .

MATERIALS AND METHODS

Study Design

The study was conducted after obtaining approval from Institute Review Board and Institute Ethics Committee (Human Studies), JIPMER, Puducherry. The study participants were recruited after obtaining written informed consent for participation in the study. The study was done in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Inclusion and exclusion criteria

The study participants include 80 patients with type 2 diabetes mellitus who were on treatment with glibenclamide for a minimum period of 3 months. Patients belonging to South Indian origin were selected for the study. The nativity was confirmed with the history of successive three generations staying in any of the South Indian states and speaking the native language as the mother tongue. Patients who had liver or renal dysfunction, chronic alcoholism, drug therapy that are substrates or inhibitors of ABCB1 were excluded from study participation. Similarly pregnant and lactating mothers were excluded from study participation.

Drug therapy and parameters assessed

The study participants were prescribed a uniform brand of glibenclamide at doses recommended by the physician for the purpose of the study for a period of 3 weeks. Five ml of venous blood was collected at the end of second and third week for the purpose of estimation plasma levels of glibenclamide and ABCB1 genotype. The blood samples were collected 2 hrs 30 mins after the morning dose of glibenclamide. Capillary finger prick method was used for estimation of fasting blood glucose. Due to variations in drug dose, the plasma levels of glibenclamide were normalized to the drug dose by calculating concentration of the drug per unit dosage per kg body weight (C/D ratio per kg body weight).

Plasma levels of glibenclamide were estimated by reversed phase High Pressure Liquid Chromatography (rHPLC) using the method by Rajendran *et al*[11] with minor modifications. DNA was extracted from the cellular fraction of the venous blood by means of Phenol Chloroform extraction procedure.[12] Genotyping for *ABCB1 C3435T* gene polymorphism was done using Quantitative Real Time Polymerase Chain Reaction (qRT-PCR). The allelic discrimination analysis for *MDR1* was performed using 7300 SDS Software (Version 1.3.1).

The patients were classified based on their genotypes as normal (CC genotype), heterozygous mutant (CT genotype) and homozygous mutant (TT genotype). Reduction of fasting blood glucose value to less than 110 mg/dl was considered as controlled glycemic status.[13] Failure to achieve the target was considered as uncontrolled glycemic status. Hypoglycemic episodes were documented in terms of number of episodes per week, severity of episodes as assessed by Hartwig and Siegel Scale of Severity, and overall severity per week measured as the product of number of episodes and severity score of hypoglycemic episodes.

Statistical Analysis

The patient characteristics across genotypes were analyzed using one way ANOVA and the association between glycemic status and genotype was studied by Chi Square test. The association between genotypes and parameters such as C/D values, number and severity of hypoglycemic episodes were compared using Kruskal Wallis Test. Statistical analysis were done using Graph Pad In Stat Ver.3.06

RESULTS

The study participants did not vary significantly in

Table 1: Patient characteristics

Parameters	*ABCB1 CC* (n=13)	*ABCB1 CT* (n=38)	*ABCB1 TT* (n=29)
Age (years)	57.7 ± 7.5 (53.1-62.2)	52.4 ± 8.6 (49.5–55.2)	52.1 ± 9.1 (48.6–55.6)
Body mass index (kg/m²)	24.9 ± 3.6 (22.6–27.0)	24.8 ± 3.4 (23.6 – 25.9)	23.5 ± 3.8 (3.3–6.2)
Treatment duration (years)	6.2 ± 5.5 (2.9–9.5)	5.5 ± 3.9 (4.2–6.8)	4.8 ± 3.8 (3.3–6.2)
Drug, (n) Glibenclamide monotherapy	13	36	29
Glibenclamide and metformin	0	2	0

Values given as mean ± SD (95% confidence interval). p value >0.05 for all parameters across the three groups as measured using one way ANOVA.

Table 2: Relationship between *ABCB1 C3435T* genotypes and glycemic status in type 2 diabetes mellitus.

Diabetic status[#]	*ABCB1 CC* (n=13)	*ABCB1 CT* (n=38)	*ABCB1 TT* (n=29)
Controlled diabetes (n=24)	6 (25)	12 (50)	6 (25)
Uncontrolled diabetes (n=56)	7 (12.5)	26 (46.4)	23 (41.1)

ABCB1 CC vs *TT*: p=0.0456; *ABCB1 CC* vs *CT* and *CT* vs T: p>0.05 p value estimated by using chi square test; [#]Controlled diabetes-Fasting plasma glucose ≤ 110 mg/dl; Uncontrolled diabetes-fasting plasma glucose > 110 mg/dl; Values given as number of patients, with percentage in parenthesis.

Table 3: Influence of ABCB1 C3435T genotypes on C/D values of glibenclamide and hypoglycemic adverse effects

Parameters	*ABCB1 CC* (n=13)	*ABCB1 CT* (n=38)	*ABCB1 TT* (n=29)
C/D values[a]	2481.4 (634.6–3972.4)	1732.7 (966.4–3167.8)	1538.8 (1208.6–3062.7)
ADR events[b]	1 (1-2)	1 (0-2)	1 (0.25-2)
ADR severity[c]	1 (1-4)	2 (1-4)	1 (0.25-2)

ADR – adverse drug reaction. All values are given as median (interquartile range). [a]C/D – Concentration of drug/Drug dose. All C/D values are multiplied by 106 for convenience. [b]ADR events – No of ADR events in a week per patient. [c]ADR severity – No of ADR in one week x Severity score of the ADR. p value > 0.05 for all parameters between the three genotype groups.

their demographic parameters and treatment duration. Among 80 study subjects, 78 were on monotherapy with glibenclamide and only 2 patients were on therapy with both glibenclamide and metformin. Both the patients belonged to *ABCB1 CT* genotype. (Table 1)

There were no significant differences in the distribution of ABCB1 *C3435T* genotypes between controlled and uncontrolled status of diabetes mellitus (P > 0.05). Hence, the study did not identify any significant association between *ABCB1 C3435T* genotypes CC, CT and TT and the distribution of controlled and uncontrolled status of diabetes mellitus. (Table 2) Similarly, there were no significant differences in the C/D ratios of glibenclamide, number of events and severity of hypoglycemic adverse effects between the genotype groups. (Table 3)

DISCUSSION

To the best of our knowledge, the current study is the first in India to evaluate the role of genetic variations in *ABCB1* on the response to therapy with an antidiabetic medication. The study has not shown any significant association between *ABCB1 C3435T* variantgenotypes and controlled or uncontrolled diabetic status among South Indian type 2 diabetes mellitus patients when compared to patients with normal genotype. Glibenclamide has been shown to be a substrate and an inhibitor of ABCB1 drug transporter.[14] Variation in genetic sequence encoding the drug transporter can affect the protein and result in aberrant functioning of the transporter. This can in turn result in altered drug disposition which can manifest and altered drug efficacy and safety.

There were no published literature where studies where the influence of *ABCB1 C3435T* genetic polymorphism on the clinical response to glibenclamide has been documented. The current study does not find a significant association between the genotypes and the clinical response. Further there were no significant associations between the genotypes and plasma levels of glibenclamide, frequency and severity of hypoglycemic adverse effects. The results of the current study will be useful for future studies of meta-analysis where pooling of available published literature can be done to establish a definitive association.

The lack of association can also be attributed to the fact that glibenclamide is also significantly transported by organic anion transporter protein (OATP) at several tissues.[15] It is also a substrate for Breast Cancer Resistance Protein (BCRP).[16] Hence, the effect of polymorphism in ABCB1 alone may not have a significant impact on the pharmacokinetics of glibenclamide. In the current study, the polymorphisms of OATP and BCRP have not been studied. Single Nucleotide Polymorphisms (SNPs) in genes coding for OATP and BCRP may also contribute for variation in pharmacokinetics and

pharmacodynamics of glibenclamide. Further studies involving haplotypes in genes coding for OATP, ABCB1 and BCRP and on a larger number of subjects may provide information on the cumulative influence of genetic polymorphisms on the efficacy of glibenclamide. In conclusion, the study did not identify any significant influence of *ABCB1 C3435T* genetic polymorphism on the response to therapy with glibenclamide, plasma glibenclamide concentration and the occurrence of hypoglycemic adverse events in South Indian patients with type 2 diabetes mellitus. This study may be done in a larger sample to confirm the findings. Further, this study can be used in future studies of meta-analysis or systematic reviews to confirm the role of ABCB1 genetic polymorphism on glibenclamide therapy.

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