Research Article

Frequency of Cyp2c93* allele in patients with type 2 diabetes mellitus on sulfonylurea therapy

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Abstract

Background: Sulfonylureas are mainstay of pharmacotherapy for type 2 diabetes mellitus (T2DM). Individual variability exists in pharmacokinetic and pharmacodynamic responses adverse effects to sulfonylurea.

Objective: To determine frequency of cytochrome P450 2C9 mutant allele CYP2C93*, in T2DM patients on sulfonylurea therapy, and to ascertain the frequency of adverse drug reactions (ADR) with respect to particular allelic distribution.

Materials and Methods: A hospital-based prospective observational study was carried out in a **t**ertiary-care teaching hospital. Study included 136 T2DM patients on sulfonylurea therapy (83 with ≥1 ADR and 53 without ADR). DNA was isolated from the blood samples taken from all 136 patients by DNA isolation. PCR-RFLP (restriction fragment length polymorphism) technique was used for detection of CYP2C93* (Ile359Leu) allele and the wild type allele CYP2C91* by digestion with restriction enzyme. Data were analyzed using Statistical Package for Social Survey (SPSS) for Windows, version 16.0 and Microsoft Excel to determine descriptive statistics.

Result: Allele CYP2C93* was detected in 11 patients. All alleles negative for the nucleotide substitutions at position 42614 (*3) were presumed to be wild type CYP2C9*1. Among the patients with CYP2C93* allele 11 patients experienced hypoglycemia and one patient experienced acute visual disturbances. No CYP2C93* was detected in the subjects without ADR.

Conclusion: In our study CYP2C93* was identified in 11 patients experiencing hypoglycaemia and in one patient experiencing acute visual disturbances. In view of the existence of such polymorphisms and its effects on sulfonylurea therapy further studies are required to assess the magnitude of such problems in T2DM.

KEY WORDS: Sulfonylurea, adverse drug reaction, type 2 diabetes mellitus, frequency, C CYP2C93* allele.

Introduction

The prevalence of diabetes has increased rapidly during the past few decades worldwide, from 35 million in 1985 to nearly 387 million in 2014.[1,2] In adult, type 2 diabetes mellitus (T2DM) is the most prevalent form accounting for 90%–95% of worldwide cases of diabetes.[3] Sulfonylureas have been a mainstay of T2DM pharmacotherapy for a long duration of time. It is well-established that inter-individual variability exists in the pharmacokinetic and pharmacodynamic responses to sulfonylurea and also its adverse effects. The evolving field of pharmacogenomics is being increasingly applied to sulfonylureas to elucidate the genetic background of this response variability. The identification of such genetic biomarkers that predict antidiabetic treatment response can advance current clinical practice in diabetes.

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The cytochrome P450 enzyme CYP2C9 is primarily responsible for the oxidative metabolism of many sulfonylureas including glibenclamide, glimepiride, and glipizide.[4] CYP2C9 isoenzyme is highly polymorphic. Till date more than 34 different alleles for CYP2C9 have been discovered of which CYP2C91*, CYP2C92*, and CYP2C93* seem to be important because of their high allele frequency.[5] These three alleles, *CYP2C9*1*, **2* and **3*, are present in most ethnic populations

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and decreased *CYP2C9* function has been reported in individuals with the *CYP2C9*2* and **3* mutant alleles.[6,7] As the metabolism of these drugs is influenced by the genetic variability of such allelic distributions, the doses of these drugs may need to be adjusted according to *CYP2C9* genotype. While the observations that CYP2C9 polymorphisms influence sulfonylurea metabolism and adverse effects are intriguing, the overall published data on frequency of Cytochrome P450 2C9 mutant allele CYP2C93* in T2DM patients who are on sulfonylurea therapy, are very limited. Therefore, this study was carried out in a tertiary care teaching hospital situated in north east India to find out the frequency of Cytochrome P450 2C9 mutant allele CYP2C93*, in T2DM patients under sulfonylurea therapy and to ascertain the frequency of adverse drug reactions (ADR) with respect to particular allelic distribution.

Materials and Methods

This study was a hospital-based prospective study, carried out between July 2012 and July 2014 in patients attending the outpatient departments of medicine and endocrinology departments of a tertiary care teaching hospital in the north eastern region of India catering to several north eastern states of the country. A total of 136 consecutive patients with T2DM, who were on sulfonylurea therapy for a duration of minimum 1 month were selected for the study. The sample consisted of two groups: (1) one group of 83 patients on sulfonylurea therapy experiencing at least one ADR - 38 patients on glimepiride (1–2 mg/day), 19 patients on gliclazide (80 mg/day), 20 patients on glipizide (2.5–5 mg/day), and 6 patients on glibenclamide (1.25–5 mg/day); and (2) the other group of 53 patients also on sulfonylurea therapy but who did not have any documented ADRs. Due to constraints of time and resources we adopted a method of consecutive sampling taken within a fixed period of time, thereby including all cases encountered during the given study period. The participants were enrolled in the study after having satisfied the selection criteria and having given consent for participation in the study. Ethical clearance was obtained from the Institutional Ethical Committee.

Patients with T2DM diagnosed as per American Diabetic Association (ADA)[8] and aged ≥35 years, who have been on sulfonylurea therapy for a duration of minimum of 1 month were included for the study. Patients who were seriously ill, those with chronic kidney disease and/or liver disease, and those receiving any other concomitant medications likely to inhibit or induce CYP2C9 activity are excluded from the study.

Procedure

Blood was withdrawn from both groups of patients and DNA was isolated from the blood samples by DNA isolation kit (Hipure blood genomic DNA mini preparation kit, Himedia). The extracted DNA samples were kept at –80 °C. PCR-RFLP (restriction fragment length polymorphism) technique was used for detection of CYP2C93* (Ile359Leu) allele and the wild type allele CYP2C91* by digestion with restriction enzyme.[9] For CYP2C9*3, two forward primers (F1 and F2) and one reverse primer (R) were used.

F1 (5'–AATAATAATATGCACGAGGTCCAGAGATGC–3)
F2 (5'-AATAATAATATGCACGAGGTCCAGAGGTAC–3')
R (5'–GATACTATGAATTTGGGGACTTC–3').

Forward primer F1 and F2 together with the reverse primer R lead to two different amplicons for the wild type allele and two different amp icons for the CYP2C9*3 allele in two separate restriction reactions. The PCR products were then digested with either NsiI or KpnI restriction enzyme.[9]

Statistical Analysis

The data were analyzed using Statistical Package for Social Survey (SPSS) for Windows, version 16.0 and Micrsoft Excel to determine descriptive statistics.

Result

The distribution of CYP2C9*1/*1, CYP2C9*1/*3, and CYP2C9*3/*3 in both the groups with and without ADRs is depicted in Table 1. The allele (CYP2C91*, CYP2C93*) distribution frequency noted among 83 patients who had suspected adverse drug reaction due to sulfonylurea treatment is shown in Table 2. The individual ADRs with different types of sulfonylurea observed in our study are shown in Table 3.

Table 1: Allele (CYP2C91*, CYP2C92*, CYP2C93*) frequency in T2DM patient with and without suspected adverse drug reactions (ADRs) taking sulfonylurea

Allele	ADRs *n* (%)		No. of patients
	With	Without *n*	
CYP2C91*	72(52.94)	53(38.97)	125(91.91)
CYP2C92*	0(0.00)	0(0.00)	0(0.0)
CYP2C93*	11(8.09))	0(0.00)	11(8.09)
Total	83(61.03)	53(38.97)	136(100)

Table 2: Frequency of allele (CYP2C91*, CYP2C93*) distribution among 83 patients with suspected adverse drug reaction due to sulfonylurea treatment

Drug	Frequency of adverse drug reaction			
	CYP2C91*		CYP2C93*	
	Male	Female	Male	Female
Glimepiride	25(30.12)	7(8.43)	5(6.02)	1(1.20)
Gliclazide	9(10.84)	8(9.63)	2(2.41)	0(0.0)
Glipizide	12(14.58)	6(7.22)	2(2.41)	0(0.0)
Glibenclamide	5(6.02)	0(0.0)	1(1.20)	0(0.0)
Total	51(61.44)	21(25.30)	10(12.05)	1(1.20)

Table 3: Various types of adverse drug reactions (ADRs) associated with drugs

Type of ADRs	Drug used			
	Glimeperide	Glipizide	Glibenclamide	Gliclazide
	N = 38	*N* = 20	*N* = 6	*N* = 19
Hypoglycemia	8(20.1%)	4(20%)	2(33.33)	4(21.05)
Vomiting	0(0.0)	1(5%)	0(0.0)	1(5.26)
Urinary retention	3(7.8%)	2(10%)	0(0.0)	0(0.0)
Abdominal discomfort	4(10.5%)	3(15%)	0(0.0)	0(0.0)
Diarrhea	0(0.0)	0(0.0)	0(0.0)	2(10.52)
Elevation of liver enzymes	1(2.6%)	0(0.0)	0(0.0)	0(0.0)
Cholestasis	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Jaundice	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Allergic reaction	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Generalized weakness	0(0.0)	1(5%)	0(0.0)	0(0.0)
Edema	3(7.8%)	5(25%)	0(0.0)	1(5.26)
Flautulence	0(0.0)	3(15%)	0(0.0)	0(0.0)
Agranulocytosis	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Anemia	1(2.6%)	0(0.0)	0(0.0)	0(0.0)
Aplastic anemia	0(0.0)	0(0.0)	0(0.0)	7(36.84)
Paresthesia	9(23.6%)	0(0.0)	2(33.33)	0(0.0)
Hyponatremia	1(2.6%)	0(0.0)	0(0.0)	0(0.0)
Visual disturbances	8(21%)	1(5%)	2(33.33)	4(21.05)

Allele CYP2C93* was detected in 11 patients. All alleles negative for the nucleotide substitutions at position 42614 (*3) were presumed to be wild type CYP2C9*1. The amplicon of the wild type allele defined by primer F1 carries the recognition site for NsiI and can therefore be cut by this enzyme, whereas the amplicon defined by primer F2 has no complete recognition site for KpnI and cannot be cut. Digestion with NsiI leads to a product (126bp) only for the wild-type allele. Digestion with KpnI leads to a product (126 bp) only for the CYP2C9*3 allele. Allele for CYP2C93* showed kpn1 digestion at 126 base pair, others showed NsiI digestion at 126bp which indicated wild type CYP2C91. Allele and ADRs showed substantial shared variation and were mutually correlative in our study. The odds of outcome with ADR were found to be significant (OR = 0.57; 95% CI: 0.48–0.66).

Discussion

To determine the influence of these CYP2C9 polymorphic mutant alleles in T2DM patients undergoing ADR, the first step was to find the frequency of the prevalence of ADR due to sulfonylurea treatment in the diabetic population under study. ADR was defined as "an appreciably harmful or unpleasant reaction, resulting from an intervention related to the use of a medicinal product, which predicts hazard from future administration and warrants prevention or specific treatment, or alteration of the dosage regimen, or withdrawal of the product."[10] We followed this by detecting frequency of the mutant CYP2C93* in both the groups with ADR and without ADR. Subsequently we studied the frequency distribution of the variant form of CYP2C9 in the T2DM population experiencing ADR due to sulfonylurea. CYP2C93* was identified in 11 patients experiencing hypoglycemia and in one patient experiencing acute visual disturbances. No CYP2C93* was detected in the subjects without ADR. Presence of allele CYP2C93* in the subjects with hypoglycemic events probably explains the reduced metabolism of sulfonylurea resulting in higher bioavailability and lower clearance of the drug in the subjects and resulting in ADRs.

With respect to drug-metabolizing enzyme polymorphisms, CYP2C9*3 (Ile359Leu) influences the pharmacokinetics of many sulfonylureas.[11] Oral clearance of drugs such as tolbutamide, glibenclamide, glipizide, and glimepiride is reduced in patients carrying a CYP2C9*3 allele, resulting in decreased clearance and increased plasma drug exposure of most of these agents. An earlier study showed that carriers of a CYP2C9*3 allele required significantly lower tolbutamide doses than wild-type homozygote.[12]

Polymorphisms of CYP2C9 gene significantly affects the response of diabetic patients to sulfonylureas because of its diminished metabolism followed by increase in drug bioavailability and consequent risk of adverse drug reaction. In allele CYP2C93* there is a change of nucleoside from adenine to cytosine at gene position 42614 which results in the amino acid substitution of isoleucine by leucine in protein position

359 resulting in a 70% reduction in enzyme activity compared to wild type.[13] This gives an insight into the fact that individuals with genetically determined low CYP2C9 activity can be at a risk for sulfonylurea-associated ADR.

Conclusion

In our study CYP2C93* was identified in 11 patients experiencing hypoglycemia and in one patient experiencing acute visual disturbances as an ADR to sulfonylurea therapy. No CYP2C93* was detected in the subjects without ADR. This study thus opens new avenues for greater and serious investigations in light of existence of polymorphisms to handle clinical situations systematically and attain improved standards of diabetic care.

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