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Original Research Article

Genetic analysis of SLC47A1, SLC22A1, SLC22A2, ATM gene polymorphisms among diabetics in an Indian population

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ABSTRACT

Background: Metformin is a first-line therapy for type 2 diabetes mellitus. However, the glycaemic response to metformin is likely to be affected by polymorphisms of transporter genes. Therefore, the study was done with the aim to assess demographic distribution of transporter genotypes involved in disposition and action of metformin.
Methods: This cross-sectional, observational, single centre, clinical study was conducted in 80 diabetic patients recruited from medicine OPD. Descriptive analysis was done for distribution of the four transporter genotypes viz. SLC47A1 (rs2289669), ATM (rs11212617), SLC22A2 (rs316019) and SLC22A1 (rs622342). Genotyping was determined by DNA extraction, agarose gel electrophoresis, estimation of DNA concentration, polymerase chain reaction, DNA sequencing, sequencing analysis.
Results: Transporter genotype analysis showed that for SLC47A1 (rs2289669) transporter, 31.25% and 26.25% were homozygous for AA and GG allele respectively, while 42.5% were heterozygous (AG). For ATM (rs11212617), SLC22A2 (rs316019) and SLC22A1 (rs622342) transporter, 45% and 10%, 1.25% and 80%, 58.75% and 7.50% were homozygous for AA and CC allele respectively; while 45%, 18.75%, 33.75% were heterozygous (AC) respectively. Interethnic differences in the genotype and allele frequencies of SLC22A1 (rs622342) and ATM (rs11212617) gene polymorphism were observed when compared with other major populations.
Conclusions: In the genotypic distribution of four transporter genotype study showed that there was an ethnic variation in allelic distribution of allele A and C of ATM (rs11212617) and SLC22A1 (rs622342) while AA genotype of SLC22A2 (rs316019) was rare genotype and allele 'A' was major allele found in our study. The study data observed would justify further pharmacogenetic studies to evaluate the role of gene polymorphism in the therapeutic efficacy of metformin.

Keywords: Diabetes, Metformin, Transporter gene, Polymorphism

INTRODUCTION

The most commonly prescribed anti diabetic drug worldwide is metformin and it is use as a first-line therapy for type 2 diabetes mellitus.[1] Although, metformin is very well tolerated by most of the diabetic patients, but glycaemic control to metformin considerably varies among the patients. Some patients showed significant response, whereas others are benefited to lesser extent/not at all.[2] Studies have shown that metformin monotherapy is not effective in ~30% patients and 20-60% users shows common side-effects, which

marginally showing non-compliance up to 30% of users and discontinuation of treatment in 5-10% of all cases. The most important factors that influence the effectiveness and response to metformin, apart from severity of hyperglycaemia, are the SLC22A1 and C11ORF65 genes. The efficacy of metformin is primarily affected by polymorphisms in these two genes.[3]

The SLC22A1 gene encodes the trans-membrane transporter1 (OCT1) important for the transport of metformin to the cells and outside of the cells, while the other polymorphism is located in the C11ORF65 gene. This gene regulates the activity of the ATM gene, which is responsible for the phosphorylation and the activation of the AMPK enzyme.[4]

The pharmacokinetics of metformin is affected by polymorphism in SLC22A2 gene.[5] This gene encodes Polyspecific organic cation transporters in the intestine, kidney, liver, and other organs. These transporters are also involved in elimination of many endogenous small organic cations, drugs and environmental toxins.

Metformin is not metabolized in the liver or kidney but rather excreted intact in the urine. The transport of Metformin has been recently reviewed by Gong, Goswami et al.[6] Several transporters, for which Metformin is a likely substrate, have been identified e.g. organic cation transporter (OCT) 1-3, plasma membrane monoamine transporter (PMAT), multidrug and toxin extrusion protein (MATE) 1-2, serotonin transporter (SERT) and high-affinity choline transporter (CHT).[7,8] OCTs are members of solute carrier family 22 (SLC22), encoded on chromosome 6q26. OCTs are expressed in several tissues, including the intestine, liver, kidney, brain, muscle and heart.[7] OCT1 is predominantly expressed in the liver, but plays an important role in the transfer of cations, including Metformin, from the gut lumen to the interstitium. Initial studies report localised OCT1 to the basolateral membrane, more recent studies found that OCT1 on the apical surface of intestinal epithelial cells. OCT2 is expressed mainly in the kidney and is partly responsible for the renal excretion of Metformin. OCT3 is mainly expressed in the skeletal muscle but is also expressed in the intestine.[9]

Plasma monoamine transporter (SLC29A4) and organic cation transporter 3 (SLC22A3) are responsible for metformin uptake from the intestine, then metformin is transported into the bloodstream by OCT1 (SLC22A1) and finally other members of the OCT family are responsible for target tissues uptake. Multi-antimicrobial extrusion protein 1 (MATE1) which is encoded by SLC47A1 gene removed metformin actively from target tissues and metformin is excreted from proximal tubule cells into the urine by MATE1 and MATE2 (SLC47A2) About 50% of an orally administered dose is absorbed into the systemic circulation. The half-life of the drug measured in plasma is between 4 and 8 h in individuals without renal dysfunction, and the clearance exceeds glomerular filtration rate, consistent with tubular secretion.[10]

PMAT was originally identified as a monoamine transporter from the equilibrative nucleoside transporter (ENT) family, found predominantly in the brain and central nervous system. However latter studies found PMAT in many tissues throughout the body and it transports metformin from the intestine with similar affinity to the OCTs. Now, it was found that PMAT located at the tips of the mucosal epithelial layer and involved in metformin uptake.[11]

Genetic variabilities of metformin transporters in the gut, liver and kidney have been shown to affect the pharmacokinetics and thus indirectly affecting the efficacy of metformin in T2D patients.[12]

Thus, metformin shows pharmacokinetic as well as pharmacodynamics variations by virtue of the genetic variations in transporters. Therefore, it is apparently essential to study the distribution of the genotypes of the various transporters involved disposition and action of metformin, which may then help in predicting the responsiveness to metformin. Though there is western literature on distribution of genotype, there is no such data on the Indian population.

The objectives of our study were to analyse and compare the demographic distribution of four transporter genotypes SLC47A1, SLC22A1, ATM and SLC22A2 involved in disposition and action of metformin in Indian population.

METHODS

This study was a prospective, observational, single centre, clinical study. The study was conducted in a tertiary care hospital where patients attending the medicine outpatient department (OPD)/diabetes clinic were recruited for the study. The study period was from January 2017 to December 2018. A synopsis of the study protocol was submitted to the Institutional Ethics Committee and approval was obtained. 80 subjects were recruited for genotype analysis.

Patient inclusion criteria

Newly diagnosed cases (male/female) of diabetes mellitus type 2 (fasting plasma glucose >126 mg% or 2 hours postprandial plasma glucose >200 mg%); and HbA1c ≥6.8% included in the study, who, as per the clinician, are deemed fit to receive metformin monotherapy.

Exclusion criteria

Exclusion criteria were age less than 18 years or more than 60 years of age and type 1 diabetes mellitus.

Estimation of genotype

5 ml of EDTA blood was collected from study patients and was stored at 4-8º c. Then EDTA blood was transported to the Genesupport laboratory.

DNA extraction was done by using DN easy blood and tissue DNA kit (Qiagen, Germany). Gel photograph was recorded using gel Doc-XR gel documentation system the Qubit® (previously known as Quant-I T™) dsDNA BR assay kit which is designed specifically for use with the Qubit® fluorometer was used to quantify DNA concentration. Polymerase chain reaction (PCR) of the human DNA was performed using primers specific for each mutation locus. The purified PCR product was then checked by gel electrophoresis and then used for DNA sequencing. Sequencing of the purified PCR product (~200 ng/reaction) was carried out using 1.6 p/moles of a sequencing primer and applied biosystems big dye terminator v3.1 cycle sequencing kit in a total volume of 10 µl. The sequence data was retrieved in FASTA format and QC checked using quality value bars. The chromas pro v3.1 software was used for sequence assemble. Sequence alignment of reference and samples was done using clustalw online sequence alignment tool (http://www.genome.jp/tools/clustalw/) (Figures 1-4).[13-16]

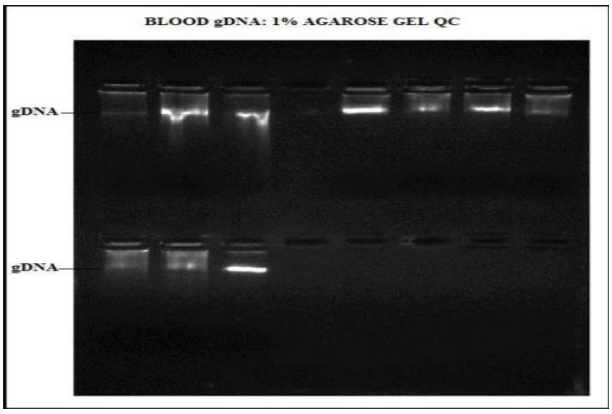

Figure 1: Blood gDNA extraction from blood samples, 5 µl gDNA loaded on 1% agarose gel.

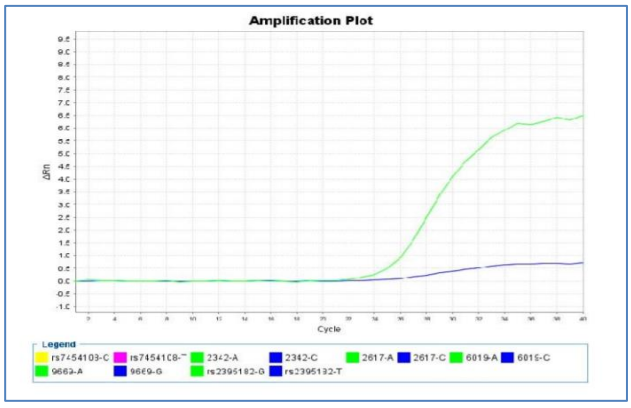

Figure 2: AA genotype (green colour amplification) of the sample.

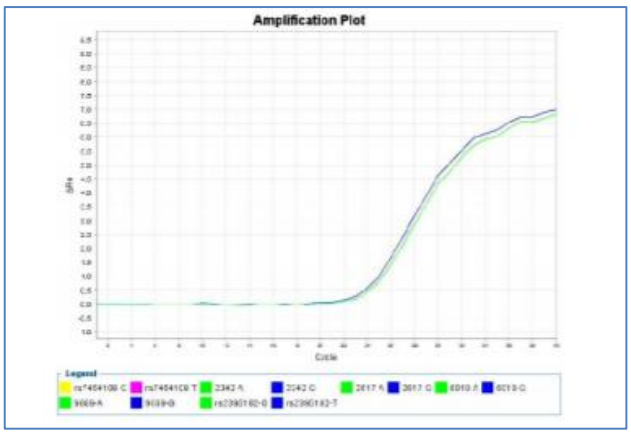

Figure 3: AG genotype (green/blue colour amplifications) of the sample.

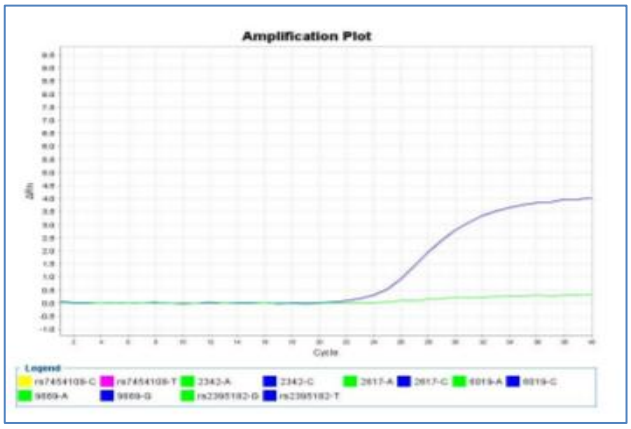

Figure 4: GG genotype (blue colour amplification) of the sample.

Statistical analysis

Descriptive analysis was performed for distribution of each of the 4 transporter genotypes. The direct gene counting method was used to determine the frequency of genotypes and alleles (Table 1).

Table 1: Gene transporter and single nucleotide polymorphism included in statistical analysis.

Gene	SNP	Patient genotype
SLC47A1	rs2289669	AA/AG/GG
SLC22A1	rs622342	AA/AC/CC
ATM	rs11212617	AA/AC/CC
SLC22A2	rs316019	AA/AC/CC

SNP- single nucleotide polymorphism.

RESULTS

To study the distribution of transporter genotypes, a total of 80 subjects were enrolled in this study. Out of 80 patients 51 (63.75%) were males and 29 (36.25%) were females. None of the baseline characteristics showed

statistically significant difference and were comparable in both the groups (Table 2).

Table 3, illustrates the genotype frequencies of four gene transporter obtained in the study population. Among 80 patients' that had been enrolled and seen for SLC47A1 (rs2289669) transporter genotypes, 42.5% were found to be heterozygous allele (AG). AG was the predominant genotype in case of SLC47A1 (rs2289669) gene transporter. CC genotype (10%) was the less frequently observed genotype as compared to other genotype in case of ATM (rs11212617) gene transporter. AA genotype (1.25%) was the rare while CC genotype (80%) was the predominant genotype found in case of SLC22A2 (rs316019) gene transporter. While in case of SLC22A1 (rs622342), CC genotype was the infrequent genotype.

Table 2: Baseline characteristics of the patients (n=80).

Variables	Males (n=51) Range (mean ±SD)	Females (n=29) Range (mean ±SD)
Age (years)	24-60 (45.80 ±8.86)	32-58 (44.172±7.474)
Weight (kg)	51-89 (68.76±9.82)	49-85 (68.13±10.48)
Height (cm)	149-179 (162.41±8.9)	150-173 (161.10±6.65)

Table 4, illustrates the comparisons among four transporter gene allele frequencies in worldwide populations. In case of SLC47A1 (rs2289669) gene transporter there was no statistically significant difference seen in allele frequencies when compared between the Indian diabetic patients and Chinese T2DM patients ($p>0.05$).

While in case of ATM (rs11212617) there was a statistically significant difference seen among other ethnicities but similar result was seen in south Indian population.

Table 3: Frequency of transporter genotype among diabetes Indian population (n=80).

Gene (SNP)	Genotype	Frequency of transporter genotype (%)
SLC47A1 (rs2289669)	AA	25 (31.25)
	GG	21 (26.25)
	AG	34 (42.5)
ATM (rs11212617)	AA	36 (45)
	CC	8 (10)
	AC	36 (45)
SLC22A2 (rs316019)	AA	1 (1.25)
	CC	64 (80)
	AC	15 (18.75)
SLC22A1 (rs622342)	AA	47(58.75)
	CC	6(7.5)
	AC	27 (33.75)

SNP- single nucleotide polymorphism.

Bangladeshi population showed the similar pattern of SLC22A2 (rs316019) genotypic distribution of homozygous allele (79.2%) when compared to present study population (81.25%). And finally, in the case of SLC22A1 (rs622342) similar frequency (A-75.6%, C-24.4%) of allele is seen when compared with Indian Tamilian population (A-75.5%, C-24.5%). But there was significant difference seen among other ethnicities such as Netherlands ($p<0.05$).

Table 4: Frequency of four transporter gene variants in different ethnicities as compared with the study population (Indian).

Population	N	Allele frequency (%)							
		SLC47A1 (rs2289669)		ATM (rs11212617)		SLC22A2 (rs316019)		SLC22A1 (rs622342)	
		A (%)	G (%)	A (%)	C (%)	A/G (%)	C/T (%)	A (%)	C (%)
Indian population#	80	52.5	47.5	67.5	32.5	10.6	89.4	75.6	24.4
Indian Tamilian[13]	112	ND	ND	ND	ND	ND	ND	75.5	24.5
Netherlands[13]	102	ND	ND	ND	ND	ND	ND	63	37*
Chinese T2DM patients[14]	267	52.81	47.19	ND	ND	ND	ND	ND	ND
Caucasians[15]	113	ND	ND	53.3	46.5*	ND	ND	ND	ND
Chinese[15]	43	ND	ND	31.4	68.6*	ND	ND	ND	ND
Japanese[15]	86	ND	ND	38.4	61.6*	ND	ND	ND	ND
Africans[15]	113	ND	ND	18.6	81.4*	ND	ND	ND	ND
South Indians[15]	118	ND	ND	65.2	35.8	ND	ND	ND	ND
Bangladeshi population[16]	125	ND	ND	ND	ND	88.62	11.37	ND	ND

#Present study, ND- not determined, *$p<0.05$ is considered statistically significant.

DISCUSSION

Number of studies have shown that polymorphism in different transporters affect the metformin pharmacokinetics and indirectly the efficacy. The major transporters responsible for metformin transport across membrane and uptake in different tissues are OCT1, OCT2, MATE 1 and ATM. Hence, study was plan to study the genotypic distribution of transporters SLC22A1 rs622342 (OCT-1), ATM rs11212617, SLC22A2 rs316019 (OCT2) and SLC47A1 rs2289669 (MATE 1) which are associated with disposition of metformin in Indian diabetic population.

Among 80 patients' that had been enrolled and seen for SLC47A1 (rs2289669) transporter genotypes, 31.25% and 26.25% were found to be homozygous for major allele (AA) and minor allele (GG) respectively; while 42.5% were found to be heterozygous allele (AG). AG was the predominant genotype in case of SLC47A1 (rs2289669) gene transporter. These results are similar in the study conducted by Xiao, Guo, and Li et al, where they found the 267 T2D patients in Chinese population, AA genotype were 28.84%, GG genotype were 23.22% and AG genotype was predominant 47.94%.[17] So it is probable that there is no ethnic variation present for SLC47A1 (rs2289669) transporter gene among the different populations. But there is need further study to conclude this. Among 80 patients' that had been enrolled and seen for ATM (rs11212617) transporter genotypes, 45% and 10% were found to be homozygous for major allele (AA) and minor allele (CC) respectively; while 45% were found to be heterozygous allele (AC). CC genotype was the less frequently observed genotype as compared to other genotype in case of ATM (rs11212617) gene transporter. Similar results were found in the study conducted in south Indian population by Vilvanathan et al, where among 118 T2D patients 48.4% had heterozygous allele AC. The allelic distribution of ATM (rs11212617) was 67.5% for A allele and 32.5% for C allele, while the allelic distribution among different ethnicities reported were Caucasians (A=53.3%, C= 46.5%), Chinese (A=31.4%, C=68.6%), Japanese (A= 38.4%, C=61.6%), and Africans (A=18.6%, C=81.4%), this suggest that there is a wide variation seen in genotypic distribution of ATM (rs11212617) transporter gene.[18] Then among 80 patients' that had been enrolled and seen for SLC22A2 (rs316019) transporter genotypes, 1.25% and 80% were found to be homozygous for allele (AA) and allele (CC) respectively; while 18.75% were found to be heterozygous allele (AC). AA genotype was the rare while CC genotype was the predominant genotype found in case of SLC22A2 (rs316019) gene transporter. In the study conducted on the Bangladeshi diabetic patients (n=125), 79.62% had the homozygous allele GG.[19] So it is probable that homozygous allele is the predominant allele in case of SLC22A2 (rs316019) transporter genotypes among other ethnicities.

And finally, among 80 patients' that had been enrolled and seen for SLC22A1 (rs622342) transporter genotypes, 58.75% and 7.50% were found to be homozygous for major allele (AA) and minor allele (CC) respectively; while 33.75% were found to be heterozygous allele (AC). AA genotype was the predominant genotype while; CC genotype was the infrequent genotype found in case of SLC22A1 gene transporter. Similar results were seen in the study conducted by Umamaheswaran et al, in Tamilian population (n=112), where 59% had AA genotype, 33% had AC genotype and 8% had CC genotype at SLC22A1 rs622342 gene variant. Thus, it is most likely that similar genotypic distributions for SLC22A1 rs622342 present among Indian population.[20]

CONCLUSION

We found that there was wide variation in genotypic distributions of SLC22A1 (rs622342) and ATM (rs11212617) among different ethnicities but there was no variation in genotypic distributions seen in different Indian population. For SLC47A1 (rs2289669) genotypic distributions no ethnic variation seen among the major population of world viz China and India. In SLC22A2 (rs316019) genotypic distributions, homozygous allele is the predominant allele.

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