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REVIEW ARTICLE

Pharmacogenomic Studies of ACE and ARB Inhibitor Drug Therapy in Type-II Diabetic Nephropathy (T2DN) Patients with *GSTT1* and *GSTM1* Gene Polymorphisms

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ABSTRACT

Background and Objective: Diabetic nephropathy is one of the most common and serious complications of diabetes mellitus with pathological activation of Rennin Angiotensin Aldosterone System (RAAS) as the key pathogenic mechanism. The purpose of the present study was to investigate the pharmacogenomic involvement and genetic polymorphism of *GSTT1* and *GSTM1* genes in Type-II Diabetic Nephropathy (T2DN) patients taking ACE or ARB inhibitor drugs in Hyderabad Indian population. The Renin-Angotensin-Aldosterone System (RAAS) includes angiotensin converting enzymes-ACE-inhibitors and angiotensin -receptor blockers- ARBS, these are widely used in treatment of DN. **Materials and Methods:** This study was a case control study conducted on 40 T2DN patients taking ARB or ACE inhibitor drugs and 40 controls of Hyderabad population of India. The cases were reporting to hospital for checkup. Institutional Ethics Committee approved the conduct of this study. Patient's demographs were collected by direct interview. Blood samples were collected for GST genotyping by multiplex PCR-based methods. **Results:** It was observed that *GSTT1* gene polymorphism was associated with the risk of developing T2DN ($p = 0.01$) taking ARB or ACE inhibitor drug, but there was no clear evidence that *GSTT1* gene was directly involved in pharmacogenomic drug response in treated T2DN patients. Whereas *GSTM1* gene polymorphism was associated neither with the risk of developing T2DN (taking ARB or ACE inhibitor drug) nor any clear evidence that *GSTM1* gene was involved in pharmacogenomic drug response in treated T2DN patients. Understanding the biological differences in the activity of *GSTT1* relative to *GSTM1* helps to identify pathways involved in DN. **Conclusion:** *GSTT1* gene polymorphism appears to contribute to the development of T2DN in Hyderabad population. However the involvement of these detoxifying genes (GSTs) in the pharmacogenomic drug response in diabetic nephropathy patients (taking ARB or ACE inhibitor drugs) was not clearly understood due to the patient heterogeneity. This is a preliminary study which give us the lead to further investigate the role of these genes with reference to the two popular drugs ARB and ACE inhibitors, these findings will be confirmed with larger number of samples.

Key words: Diabetic nephropathy, *GSTM1*-Mu Glutathione S-Transferase, *GSTT1*-Theta Glutathione S-Transferase, ARB , ACE inhibitor drug, pharmacogenomics

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INTRODUCTION

In the world population, patients with Type 2 Diabetes Mellitus (T2DM) are reported to progress towards end-stage kidney disease over a long period of time. It is estimated that T2DM is the primary cause in 20-40% of people starting dialysis. Diabetic Nephropathy (DN) is estimated to affect one-third of individuals with DM and is associated with considerable cardiovascular morbidity and mortality. It is the leading cause of End-Stage Renal Disease (ESRD) worldwide, accounting for 42% of all patients on Renal Replacement Therapy (RRT)[1].

Hence, there is need to identify biomarkers for early detection, and possibly also detect disease progression or regression after drug therapy. Diabetic Nephropathy (DN) or diabetic kidney disease is a progressive disease caused by damage to the capillaries in the kidney's glomeruli. Figure 1 represents the normal kidney anatomy and represents the filtration units in the normal and diseased kidney the nephrons. Risk factors affecting progression of kidney disease include baseline albumin excretion, age factor, glycemic control, blood pressure, serum cholesterol and use of renin-angiotensin system blockers[2]. Present scenario presents that the study of DN has progressed with regards to its pathophysiology, stages of renal involvement, especially, with regard to the therapeutic options available. Early detection of DN, the multifactorial approach targeting the main risk factors (hyperglycemia, hypertension, dyslipidemia and smoking) and the use of reno-protective agents such as; the drugs that act on the Renin Angiotensin Aldosterone System (RAAS), may delay progression of kidney disease in DM, besides reducing cardiovascular mortality[3,4]. Modern therapies have benefitted a large number of diabetics; however there is still a need to develop new biomarkers for early diagnosis. To achieve this goal it needed to look at molecular diagnosis besides biochemical parameters such as identifying proteins in the urine or serum creatinine in the blood. Since, pathophysiology of DN affects the renin-angiotensin system or Renin Angiotensin Aldosterone System, (RAAS) which is a hormone system that regulates blood pressure and fluid and electrolyte balance, as well as systemic vascular resistance; this modulation is achieved by the ACE or ARB inhibitors[5].

The exact genetic model underlying DN susceptibility is uncertain, but theoretically few genes with a major contribution and some with minor interaction with the environment could cause DN[6,7]. Association studies of candidate genes have been performed aiming to identify polymorphic variants associated with DN or with different degrees of renal disease. Often, genes that play a role in the expression of proteins that are related to the modulation of cytokines, proteins involved in the glycol and lipid metabolism, in the formation of extracellular matrix, in blood pressure homeostasis and in insulin sensitivity have been considered candidates for the development of DN[8,9]. However, the studies have not been successful in identifying genes that consistently show an association with DN. In an earlier study reported the role of an inflammatory marker TNF-α in chronic diabetic patients progressing towards nephropathy, this investigation was a small study which did not include other complications of DM[10]. In this investigation, the aim was to study the role of some drug metabolizing genes like GSTs and their SNPs in promoting diabetic nephropathy.

Glutathione-S-transferases (GSTs) are a multigene family of phase-II metabolic enzymes, which catalyze the conjugation of reduced glutathione with avariety of endogenous and exogenous electrophilic compounds, including several potentiallytoxic carcinogens and chemotherapeutic drugs, thereby reducing thereactivity of the compounds by making them water soluble and favoring their elimination from the body[11].

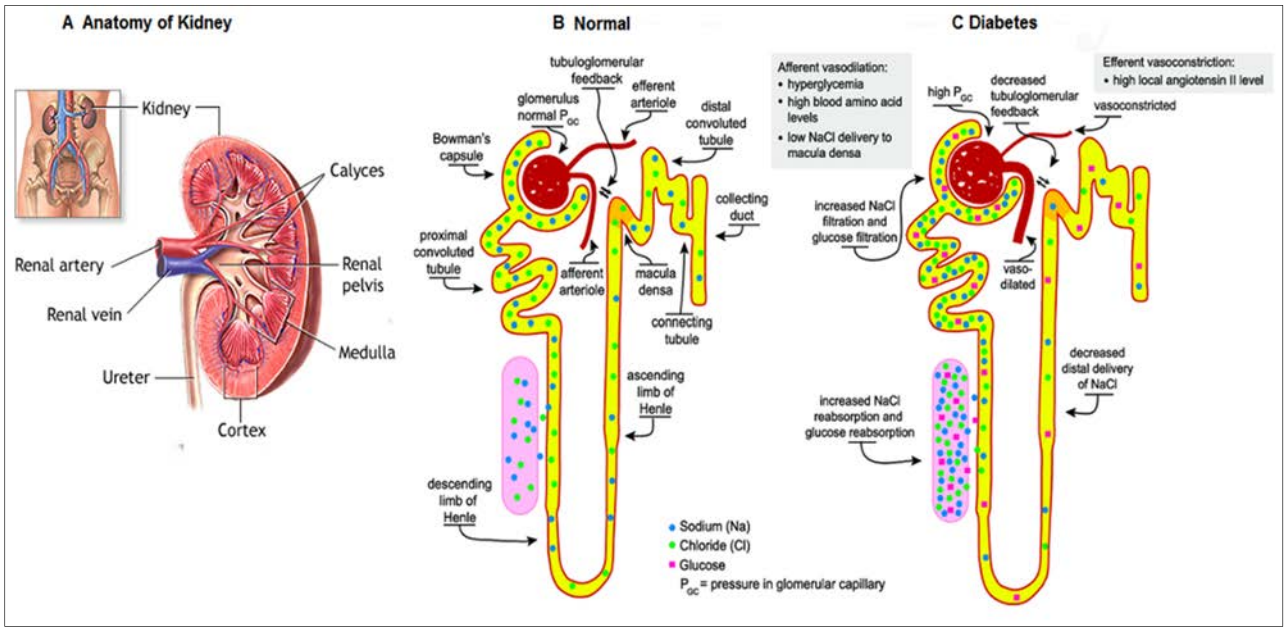

Fig. 1: Schematic representation (a) Dissection showing the Anatomy of normal kidney, (b) Nephron, the functional unit of the kidney showing filtration in normal kidney and (c) Diabetic kidney (Adapted from internet sources)

To date, numerous mutations have been shown to affect type 2 diabetes risks[12]. The contribution of each gene is generally small. However, each additional mutation seems to increase the risk leading to micro or macro-complications. In such cases consulting diabetologists recommend a combination of drugs.

It is now a scientifically proven fact that genes are amongst the major contributors to diabetic nephropathy apart from the environmental factors involved. In this context, a wide range of genes have been assessed to see their association with diabetic nephropathy along with a number of single-nucleotide polymorphisms in diabetic nephropathy susceptibility genes. It is seen that different ethnic groups may have variable risk associated with a specific gene in individuals suffering from a particular disease like diabetic nephropathy.

Among all classes of GSTs, *GSTM1*, *GSTT1* and *GSTP1* polymorphisms are extensively studied worldwide. Homozygous deletions of *GSTM1* and *GSTT1* genes are common and result in a complete loss of enzyme activity. The frequencies of *GSTM1* null alleles display race and ethnic variations, being highest in Europeans (42-60%) and Asians (41-63%) compared with that of Africans (16-36%)[13]. However, the frequency of *GSTT1* null genotypes is somewhat less in Europeans (13.31%) compared with that of Africans (14-57%) and in Asians (35-48%). Almost all population-based studies have reported a *GSTM1* prevalence ranging from 16% to 60%. Asians and Caucasians have the highest frequencies (50-53%) while black populations including Africans, African-American and black populations of Brazil have the lowest ones[14,15].

Since treatment of T2DM patients can delay the development of micro albuminuria it was important to identify patients who are under treatment. For example, the ACE inhibitor or angiotensin-converting enzyme inhibitors such as trandolapril[16] and the Angiotensin-Receptor Blocker (ARB) such as olmesartan[17] have both shown to delay the T2DM-related microalbuminuria. The ACE inhibitors, which also include lisinopril (Zestril), benazepril (Lotensin) and enalapril (Vasotec) and ARBs include losartan (Cozaar), valsartan (Diovan) and irbesartan (Avapro) are also used widely by diabetologists. The current study was conducted to investigate

the pharmacogenomic involvement and genetic polymorphism of *GSTT1* & *GSTM1* genes in type-II diabetic nephropathy patients taking ACE or ARB inhibitor drug.

MATERIALS AND METHODS

This study was conducted in Bhagwan Mahavir Medical Research Centre, Hyderabad, Telangana, India. Male or female Type II DM subjects aged 30-80 years with diabetic nephropathy (high B.P, albuminuria of >300 mg dL^{-1} from past 3 years were included in the study). Subjects with drug induced nephropathy, type-I diabetes, CKD and gestational diabetes were excluded in this study. About 2 mL venous blood samples were collected in EDTA vials (Vacuette®) from 40 type-II DN patients taking ACE or ARB inhibitor drug and 40 controls with no drugs and no diabetes. The study was approved by the Institutional Ethics Committee (Ref. No. 517/BMMRC/2015/EC approval) and signed informed consent was obtained from the participants before the study initiation.

Genomic DNA was isolated from blood samples by DNA salting out procedure[18]. The presence of genomic DNA was detected by running the isolated samples on 1% Agarose gel electrophoresis at 60V. A multiplex Polymerase Chain Reaction (PCR) was performed for the identification of GST polymorphisms at annealing temperatures of 56°C for *GSTM1*, 59°C for *GSTT1* using the primers described below:

GSTM1
Forward: 5'- GAA CTCCCT GAA AAG CTA AAG C -3'
Reverse: 5'- GTT GGG CTC AAA TAT ACG GTG G -3'

GSTT1
Forward: 5'- TTC CTT ACT GGT CCT CAC ATC TC -3'
Reverse: 5'- TCA CCG GAT CAT GGC CAG CA-3'

Other details were collected using a questionnaire and also from patients records.

Statistical analyses: The data obtained was tabulated and analyzed. The mean/median±SD. were computed for quantitative data. The distribution of the genotype frequencies of *GSTT1* and *GSTM1* was determined by Hardy-Weinberg stats for controls compared to patients. Observed frequencies of genotypes are for Hyderabad population. The analysis was done by using SPSS software. To evaluate the p-value of the observations, t-test (tails = 2 and type = 2) was applied. The analysis by ANOVA (Analysis Of Variance) was performed for the confirmatory results for the "significance testing" of the clinical raw data obtained. A P-value <0.05 was considered significant.

RESULTS

This study included 40 type-II diabetic nephropathy patients taking ARB or ACE inhibitor drug and 40 healthy volunteers. The demographic characteristics of the subjects are presented in Table1. The duration of disease (diabetes) in all diabetic nephropathy cases varied from five to fifteen years. A significantly higher BMI mean value was observed in all diabetic nephropathy cases compared to the control group (Table 1). The study found the age of onset of diabetes DN was between 30-40 years in 10 patients, 41-50 years in 8 patients, 51-60 years in 10 patients, 61-70 years in 6 patients and 71-80 years was also observed in 6 patients of DN. Also median values of both systolic and diastolic blood pressure were significantly higher in DN cases. The clinical characteristics of the subjects are presented in Table 2.

Table 1: Demographic characteristics in between T2DN patients (taking ARB or ACE inhibitor drug) and controls

Parameters	Controls (n = 40) Mean/median ± SD	Treated Type-II diabetic nephropathy patients (n = 40) Mean/median ± SD
Gender		
Male	n = 28 (70%)	n = 22 (55%)
Female	n = 12 (30%)	n = 18 (45%)
Smoking		
Smoker	n = 26 (65%)	n = 22 (55%)
Non-smoker	n = 14 (35%)	n = 18 (45%)
Alcoholism		
Alcoholic	n = 21 (52.5%)	n = 14 (35%)
Non-alcoholic	n = 19 (47.5%)	n = 26 (65%)
Obesity		
Obese	n = 21 (52.5%)	n = 11 (27.5%)
Non-obese	n = 19 (47.5%)	n = 29 (72.5%)
Age		
Mean Age(years)	47.57±13.19	53.42±10.86
30-40	n = 06 (15%)	n = 18 (45%)
41-50	n = 09 (22.5%)	n = 08 (20%)
51-60	n = 14 (35%)	n = 03 (7.5%)
61-70	n = 07 (17.5%)	n = 08 (20%)
71-80	n = 04 (10%)	n = 03 (7.5%)
BMI(kg m^{-2})*	23.53±2.99	24.40±3.15

*P<0.05 is considered significant

Table 2: Comparison of clinical characteristics in between T2DN patients (taking ARB or ACE inhibitor drug) and controls

Parameters	Controls (n = 40) Mean/median ± SD(Min-Max)	Treated Tpe-II diabetic nephropathy patients (n = 40) Mean/median ± SD(Min-Max)	P-value*
Serum (Fasting)			
Glucose (mg dL^{-1})	90 (74-99)	168 (78-448)	0.018
Creatinine (mg dL^{-1})	0.9 (0.6-1.2)	1.0 (0.7-5.1)	0.04
ALT (U/L)	15±7	26±12	0.242
Total cholesterol (mg L^{-1})	174±22	207±12	0.084
HDLcholesterol (mg L^{-1})	55±12	55±12	0.417
LDLcholesterol (mg L^{-1})	100±19	130±45	0.347
TG (mg dL^{-1})	94±31	172±77	0.178
Blood pressure			
Systolic (mmHg) 115(90-135)	130 (90-135)	140 (110-180)	0.04
Diastolic (mmHg) 70 (60-85)	80 (60-85)	90 (60-110)	0.05

*P<0.05 is considered significant

Genotyping results: In type-II diabetic nephropathy patients (taking ACE or ARB inhibitor drug) with *GSTM1* had a frequency distribution of 20% for null genotypes and 35% distribution of *GSTT1* null genotypes. In case of control, *GSTM1* had a frequency distribution of 25% for null genotype and 30% distribution of *GSTT1* null genotype. Presence of *GSTM1* genotype in type-II diabetic nephropathy patients with drug was found to be 80% of studied population and 65% distribution in *GSTT1* genotypes. In case of controls, presence of *GSTM1* genotype had a frequency distribution of 75% and 70% distribution in *GSTT1* genotype. *GSTT1* gene

Table 3: Distribution of *GSTM1* and *GSTT1* genotype frequency among the cases andcontrols

GST gene polymorphism genotypes	Control (n=40)		Treated diabetic nephropathy cases (n = 40)		
	Number	%	Number	%	p-value
***GSTM1* gene**					1.00
Present	30	75	32	80	
Deleted (Null)	10	25	8	20	
***GSTT1* gene**					0.01*
Present	28	70	26	65	
Deleted (Null)	12	30	14	35	

*P<0.05 is considered significant

polymorphism was found to be significant in T2DN (p = 0.01) patients taking ARB or ACE inhibitor drug (Table 3). *GSTM1* (chromosome 1p13.3) and *GSTT1* (chromosome 22q11.2) catalyze the conjugation of glutathione to numerous potentially genotoxic compounds, including aliphatic aromatic heterocyclic radicals, epoxides or arene oxides. Hence, 40 DN cases and 40 controls were studied to evaluate the risk of these two markers individually and in combination. Also, since *GSTM1* and *GSTT1* have distinct structures, kinetic properties, and substrate specificities

DISCUSSION

Renin Angiotensin Aldosterone System (RAAS) inhibitors are a group of drugs that act by inhibiting the RAAS which include Angiotensin Converting Enzyme (ACE) inhibitors, Angiotensin Receptor Blockers (ARBs), and direct renin inhibitors. The most important system involved in the regulation of renal blood flow and glomerular filtration rate is the Renin Angiotensin Aldosterone System (RAAS). Further, biological differences in the activity of *GSTT1* relative to *GSTM1* may further help to identify pathways involved in DN.

It is reported by Radica *et al.*[19], that diabetic kidney disease develops in approximately 40% of patients who are diabetic and is the leading cause of CKD worldwide. Type-II DN patients with hypertension typically require multiple agents to control BP. Therapies that target the Rennin Angiotensin Aldosterone System (RAAS) offer particular benefit to hypertensive, proteinuric patients with kidney disease because these agents reduce proteinuria as well as BP[20]. Reduction of proteinuria by >30% of baseline within the first 3-6 months of treatment in patients with kidney disease has been shown to predict long-term renal and cardiovascular (CV) outcomes.

The ACE inhibitor drugs inhibit competitively the activity of ACE (also termed kinase II) to prevent formation of the active octapeptide, angiotensin II from the inactive decapeptide, angiotensinI[21]. This occurs in blood and tissues including kidney, heart, blood vessels etc. Angiotensin II is a potent vasoconstrictor, promotes aldosterone release, facilities sympathetic activity and has other potentially harmful effects on the cardiovascular system. The ACE inhibitors bind to tissue and plasma protein. Whereas free drug is eliminated relatively rapidly by the kidney predominantly by glomerular filtration, binding to tissue sites means that the plasma concentration-time profile shows a long lasting terminal elimination phase. Nitric Oxide Synthase 3 (NOS) is also considered as one of the potential candidate gene for diabetic nephropathy susceptibility. The G894T variant of NOS was reported to increase the risk of macroalbuminuria and progression from microalbuminuria to macroalbuminuria, with declining glomerular filtration rate as serum creatinine value rises progressively, culminating in nephropathy.

Among other gene variants and SNPs associated with diabetic nephropathy, inflammatory cytokines are involved in pathogenesis of diabetic nephropathy and the genetic variability in the genes encoding these cytokines may predispose a person to diabetic nephropathy. It observed that drug metabolizing genes like the GSTs play a significant role by making the drugs non-effective, hence leading to the gradual progress of the disease to nephropathy.

A number of epidemiological studies have tested possible associations between polymorphisms of the GST isoforms particularly deletions in the *GSTM1* and *GSTT1* genes (null genotypes) with disease risk or therapy outcome in different types of pathologies.Almost all population-based studies have reported a *GSTM1* prevalence ranging from 16-60%. Asians and Caucasians have the highest frequencies (50-53%) while black populations including Africans, African-American and black populations of Brazil have the lowestones[22,23]. No significant difference was noted in the frequency of *GSTM1* null genotype polymorphism between treated diabetic nephropathy patient group and control group. Such finding is in agreement with results reported by some studies[24-27], whereas other studies showed a significant association between the frequency of *GSTM1* genotype and T2DN[28]. The most important system involved in the regulation of renal blood flow and glomerular filtration rate is RAAS, known regulator of Blood Pressure (BP) and determinant of target-organ damage. It controls fluid and electrolyte balance through coordinated effects on the heart, blood vessels and kidneys. In diabetic nephropathy this is regulated by ACE or ARB inhibitors hence our studies have attempted to find if a correlation exists between genotypes and pharma[29].

As regards the *GSTT1* polymorphism among controls in our study, it was demonstrated a frequency rate of *GSTT1* null genotype (30%) that did not vary too much from European and Mediterranean that ranged from 10.4-42.5%[30]. being the highest among Chinese (64%), followed by Koreans (60%), African-Americans (22%), Caucasians (29%) and Asian-Indians (16%) and the lowest among Mexican-Americans (10%)[31].

In South India, Ramprasath *et al*.[32] found that T2DM patients were significantly associated between T2DM and both null genotypes of *GSTM1* and *GSTT1*. Amer *et al*.[33] also indicated that the *GSTM1* and *GSTT1* genotype distributions significantly differed between patients and controls in Egypt. In addition, they reported that the combined genotypes of *GSTM1*-null/*GSTT1*-null may increase the risk of T2DM development. However, in a study on Indian patients, significant differences were observed in the distribution of both *GSTM1* and *GSTT1* polymorphisms between T2DM patients and asymptomatic carriers. Fujita *et al*.[34] suggested that *GSTM1* null genotype was not contributive to the development of diabetic nephropathy in Japanese type 2 diabetic patients. A study carried out by Ramprasath *et al*.[32] demonstrated that *GSTT1*-null genotype had a moderately higher occurrence in T2DM with Coronary Artery Disease (CAD) patients than T2DM patients without CAD. Going by the mode of action of the drugs Physicians have a choice to use both the ACE and ARB inhibitors or only ACE or ARB. Since, ACE inhibitors work by blocking an enzyme called angiotensin I from being converted to angiotensin II that narrows blood vessels. This makes blood vessels relax and widen, reducing blood pressure. The ARBs also work by blocking angiotensin II from binding to receptors on the blood vessels that affect blood vessel constriction. It is observed that generally, Physians prescribe an Angiotensin Converting Enzyme (ACE) inhibitor and an Angiotensin Receptor Blocker (ARB) for patients at high risk of vascular events or renal dysfunction. The combination does not reduce poor outcomes, and leads to more adverse drug-related events than an ACE inhibitor or ARB alone. So far no one has discussed the role of drug detoxifying genes in the progress of the

disease or resistance to the disease. This investigation suggested that genotyping risk factor must be included in to look for more useful associations.

The present study did not relate the risk of developing diabetic vascular complications to the presence of the null genotype polymorphisms in the *GSTM1* or *GSTT1* genes. On the contrary, the *GSTT1* null genotype polymorphism showed a significantly decreased frequency in those suffering from neuropathy, retinopathy and nephropathy[35]. Hence identifying genotype could be very useful in proceeding with treatment strategies.

CONCLUSION

The present study investigated the role of GST gene polymorphism among the population of Hyderabad (South India). This study may be useful to understand how genetics can help to observe the association of *GSTM1* and *GSTT1* polymorphism in T2DN disease and the risks involved in diabetic patients. *GSTT1* gene polymorphisms may contribute to the development of T2DN ($p = 0.01$) taking ARB or, ACE inhibitor drugs. However, our data suggests that there is no clear evidence of *GSTM1* and *GSTT1* genes involvement in pharmacogenomic drug response in T2DN patients taking ARB or ACE inhibitor drug. This is a preliminary study and the findings will be confirmed with larger number of samples.

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