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***Prospective Study***

**Role of angiotensin converting enzyme and angiotensinogen gene polymorphisms on angiotensin converting enzyme inhibitor-mediated antiproteinuric action in type 2 diabetic nephropathy patients**

Aggarwal N *et al.*RAAS and ACE in diabetic nephropathy

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**Abstract**

***AIM***

To evaluate the role of genetic variants of angiotensin converting enzyme (ACE) and angiotensinogen gene (AGT) genes on the antiproteinuric efficacy of ACE inhibitor therapy in diabetic nephropathy (DN) patients.

***METHODS***

In the present study, total 270 type 2 diabetes mellitus patients with nephropathy were enrolled and treated with ACE inhibitor (ramipril) and followed-up at 6 mo for renal function and albumin excretion by estimating serum creatinine, end stage renal disease and albumin/creatinine ratio (ACR) in urine. Genotyping of ACE I/D and AGT M235T polymorphisms were performed by using primer specific polymerase chain reaction (PCR) and PCR-RFLP techniques respectively.

***RESULTS***

Forty eight percent of DN patients (responders) benefited with respect to proteinuria on ACE inhibitor therapy after 6 mo follow up. Significant reduction in ACR was observed after 6 mo treatment with ACE inhibitor irrespective of whether DN patients were micro-albuminuric (≥ 30 and < 300 mg/g creatinine) or macro-albuminuric (≥ 300 mg/g creatinine) at the time of enrollment. However, macro-albuminuric patients (55%) showed better response to therapy. Reduction in urinary ACR was found independent of genotypes of ACE I/D and AGT M235T polymorphisms although, macro-albuminuric patients having TT genotype showed statistically insignificant increased response (72%).

***CONCLUSION***

ACE inhibitor therapy reduced ≥ 30% urinary ACR in 50% of DN patients and the response is independent of ACE I/D and AGT M235T polymorphism.

**Key words:** Diabetic nephropathy; Angiotensin converting enzyme inhibitor therapy; Renin-angiotensin-aldosterone system gene polymorphisms; Responder; Urinary albumin/creatinine ratio,; Albuminuria

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**Core tip:** Angiotensin converting enzyme (ACE) inhibitors are used as standard therapy in patients with diabetic nephropathy (DN) and reported to have reno-protective effect in these patients; however the response to ACE inhibitor therapy is not uniform in all patients. We investigated whether ACE I/D and angiotensinogen gene (AGT) M235T polymorphism of genes of renin-angiotensin-aldosterone system is associated with variable response to ACE inhibitors in DN patients. ACE inhibitor treatment to DN patients caused significant reduction in urinary protein excretion and was found independent of ACE I/D and AGT M235T polymorphism.

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**INTRODUCTION**

Diabetic nephropathy (DN) is a clinical syndrome indicating kidney disease and occurs approximately in 20%-30% of patients with diabetes mellitus (DM). Nephropathy gradually progresses and makes the patient dependent on renal replacement therapy. Diabetic nephropathy is clinically presented with persistent micro-albuminuria (≥ 30 to 299 mg/g creatinine) which subsequently progress to macro-albuminuria (≥ 300 mg/g creatinine)[1]. Later severity of disease is characterized by fall in estimated glomerular filtration rate (eGFR) as a consequence of renal impairment, ultimately leading to end stage renal disease (ESRD)[2]. Various factors including poor glycemic control, family history of diabetes or hypertension etc. may predispose to the development of DN, however not all DM patients tends to develop nephropathy[3].

The renin-angiotensin-aldosterone system (RAAS), that plays important role in regulating blood pressure, is involved in the pathophysiology of renal complications including DN. Polymorphisms of various genes of RAAS particularly angiotensin converting enzyme (ACE) and angiotensinogen (AGT) have been strongly implicated in the development and progression of nephropathy[4,5]. ACE is a zinc-dependent di-peptidase enzyme which catalyzes the conversion of inactive angiotensin (angiotensin-I) to angiotensin-II[6]. ACE gene is located at locus 17q23. It is known to be associated with the pathogenesis of diabetic nephropathy, including progression to overt proteinuria. ACE gene is highly polymorphic in nature. Of the 160 polymorphisms known, insertion/deletion (I/D) polymorphism is the most studied as it affects ACE enzyme activity in blood. I/D polymorphism involve the presence or absence of a 287 bp Alu repeat in intron 16 of the gene. It has been observed that DD genotype is associated with higher ACE activity and II genotype with the lowest[7].

The AGT gene (*rs 699*) is located at chromosome 1 and consists of five exons. There are more than 23 variants of AGT gene[8]. The common polymorphism of AGT gene is (M235T) that encodes threonine instead of methionine at position 235 in exon 2[9]. T allele of the M235T variant is associated with higher plasma circulating AGT level[10].

A number of drugs that block renin-angiotensin-aldosterone system like ACE inhibitors, angiotensin receptor blockers (ARB) are often prescribed to control hypertension; in addition these drugs are known to control proteinuria either alone or in combination in DN patients[11]. However, the reno-protective response of ACE inhibitor therapy is not uniform in all patients. The reasons behind the uneven response of these drugs towards antiproteinuric effect are not completely understood. The polymorphisms of genes of RAAS may possibly involve in this process.

Despite several studies on association of ACE and AGT gene polymorphisms with ACE inhibitor treatment in type 2 DM patients with nephropathy, no substantial data on the role of ACE and AGT gene polymorphism on antiproteinuric efficacy of ACE inhibitors with regard to Indian context are available. In the present study the association of ACE and AGT gene polymorphism on antiproteinuric response to ACE inhibitor therapy in north Indian type 2 diabetic patients with nephropathy has been carried out.

**MATERIALS AND METHODS**

***Subjects***

The study was designed as a single arm prospective-longitudinal study to evaluate antiproteinuric effect of ACE inhibitor therapy based on change in albumin/creatinine ratio (ACR) and the baseline data served as reference values (control). The required number of cases for 80% power at 5% Type I error in detecting a reduction of proteinuria to at least 30% of pretreatment value for a given odds ratio of 1.5 were 221, based on the frequency of mutant ACE gene allele in the Asian population as 40%[12]. In order to accommodate drop out during the course of the study, we recruited 270 patients with type 2 diabetes mellitus (T2DM) having persistent microalbuminuria (30-300 mg/g creatinine) or overt albuminuria (> 300 mg/g creatinine), of which 18 patients could not complete the follow-up. The patients were enrolled from Department of Medicine, Diabetic and Nephrology clinic at Guru Teg Bahadur Hospital, Delhi, India. Patients having age between 30 to 65 years, duration of diabetes ≥ 5 years; with the evidence of diabetic retinopathy and chronic kidney disease (CKD) stage 1 to 3 were recruited. Patients intolerant to ACE inhibitors, pregnant or lactating women, patients taking aspirin or other non-steroidal anti-inflammatory drugs (NSAID) were excluded from the study. Diagnosis of DM was based upon American Diabetes Association (ADA) guidelines 2012. Patients having evidence of 1+ or more proteinuria by urinary dipstick test were included in the study. In addition, patients with dipstick negative proteinuria were screened by urinary dipstick for presence of microalbumin. Patients with evidence of micro-albuminuria or overt proteinuria on two separate occasions at least 6 weeks apart were included in the study and assessed for urinary ACR.

The study was approved by Institutional Ethics Committee-Human Research (IEC-HR) of University College of Medical Sciences and written informed consent was obtained from all patients. All enrolled patients were under satisfactory glycemic control and were under well-controlled blood pressure. The patients were followed-up after 6 mo of initiation of ACE inhibitor therapy. All were treated initially with ramipril 5 mg/d along with anti-diabetic therapy. The dose was up-titrated to a maximum of 20 mg/d. Patients receiving ramipril > 5 mg/d, equal dosage were given at 12 hourly interval.

***Clinical response point***

The decrease in urinary ACR was calculated by using the following formula. Decrease in urinary ACR% = (baseline value - follow up value) × 100/baseline value.

Patients were classified as responders when decrease in urinary ACR ≥ 30% and as non-responders when decrease in urinary ACR > 30% at the end of 6 mo follow up[13,14].

***Biochemical parameters estimation***

Blood sample of 5 mL was collected for biochemical analysis and genotype study. Blood was centrifuged at 1000 g for 15 min for serum separation. Serum samples were frozen at -80 ℃until assayed. All parameters were determined within a month after sample collection. Morning spot urine samples were collected for urine albumin and urine creatinine test.

The plasma glucose level was measured immediately by glucose oxidase-peroxidase method and quantified spectrophotometrically at 500 nm. HbA1c was estimated by micro-column based technique and quantified spectrophotometrically at 500 nm. Total cholesterol (TC), serum sodium, potassium and hemoglobin were determined using routine clinical assays in hospital laboratory. Average of three blood pressure readings 15 min apart, were taken as B.P. and all patients underwent fundus examination for the detection of diabetic retinopathy.

Urine and serum creatinine were estimated by alkaline picrate Jaffe’s method (kinetic method). Urine albumin was measured by an immuno-turbidometric assay (Nephelometer, Nephstar®) after calibration of the instrument by the standard provided. The minimum sensitivity is 10 mg/L. The result was expressed as ACR in terms of mg/g creatinine.

***Determination of genotypes***

**ACE *I/D* gene polymorphism:** The ACE gene(*rs 4646994*) I/Dpolymorphism was determined by polymerase chain reaction (PCR) using a flanking primer pair that recognizes insertion-specific sequence. The 25 μL PCR reaction mixture contained: 100 ng of genomic DNA and amplification buffer containing 20 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 200 µmol/L of dNTPs, 10 pmol of each primer, and 1.0 U of Taq DNA polymerase (RBC, India). The DNA was amplified by cycling at 94 ℃for 2 min, at 60 ℃ for 45 s, and at 72 ℃ for 2 min (Eppendorf PCR machine, Germany). After 30 cycles, the reaction was extended for an additional 8 min at 72 ℃. The oligonucleotide sequences of the primers were: 5-‘CTGGAGACCACTCCCATCCTTTCT-3’ and 5’-GATGTGGCCATCACATTCGTCAGAT-3’. The PCR products were separated by 1.5% agarose gel electrophoresis, and 490 bp with insertion (I allele) and 190 bp with deletion (D allele) were visualized with ethidium bromide staining in the UVP Bio-Documentation System.

**AGT *M235T* gene polymorphism:** The AGTgene (*rs 699*) M235T polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The 25 μL PCR reaction mixture contained: 100 ng of genomic DNA and amplification buffer containing 20 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 200 µmol/L of dNTPs, 10 pmol of each primer, and 1.0 U of Taq DNA polymerase (RBC, India). The DNA was amplified by cycling at 94 ℃ for 1 min, at 68 ℃ for 45 s, and at 72 ℃ for 2 min (Eppendorf PCR machine, Germany). After 30 cycles, the reaction was extended for an additional 10 min at 72 ℃. The oligonucleotide sequences of the primers were: 5’-CCGTTTGTGCAGGGCCTGGCTCTCT-3’ and 5’-CAGGGTGCTGTCCACACTGGACCCC-3’.

The PCR product was digested with restriction enzyme *Tth111* I (Fermentas) to identify the M/T polymorphism at 37 ℃ for 16 h. Digested DNA fragment products were separated by electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The presence of uncut 165 bp fragment band indicated homozygous MM genotype, 141 bp and 24 bp fragment band indicated TT homozygous genotype, and 165 bp, 141 bp and 24 fragment band indicated MT heterozygous genotype.

***Statistical analysis***

The statistical methods of this study were reviewed by Department of Biostatistics, UCMS and GTB Hospital, Delhi, India. Data of all the parameters were collected on enrollment and at 6 mo after ramipril treatment. Analysis of obtained data was performed by using SPSS, version 20.0. *P* value < 0.05 was considered significant. Chi-square test was applied to compare genotype data of ACE and AGT genes with antiproteinuric response to therapy in all groups. For biochemical parameters, paired student’s *t*-test was applied to compare the baseline values with the values obtained at 6 mo. ACR values follows the skewed distribution, hence we applied non-parametric method: Wilcoxon-signed rank test to compare the baseline ACR values with the values at 6 mo.

**RESULTS**

***Demographic and biochemical data at baseline and at 6 mo after ACE inhibitor therapy***

The demographic and biochemical data are listed in Tables 1 and 2. The age of the patients ranged from 30 to 60 years. The duration of diabetes ranged between 5 years to 20 years and mean duration of diabetes was 8.31 years. 39% of enrolled patients had family history of diabetes and 23% had family history of hypertension. Biochemical data before treatment and after 6 mo of treatment with ramipril are listed in Table 2. There was no significant change in blood urea, serum sodium, serum potassium, fasting plasma glucose, post prandial plasma glucose, systolic and diastolic B.P., hemoglobin and HbA1c level after follow up. Also, the difference in serum creatinine and eGFR levels after treatment was not statistically significant.

***Antiproteinuric effect of ACE inhibitor therapy***

The Antiproteinuric effect of ACE inhibitor therapy was evaluated by urinary ACR values. A decrease of more than 30% in ACR values was considered as responder (R) to ACE inhibitor treatment. ACR values of enrolled patients at baseline varied widely and ranged between 30 to 14573 mg/g creatinine. An overall significant decrease in ACR values was observed on ACE inhibitor treatment as compared to baseline values (Table 3). Taken together 48% of enrolled patients were found as responder to ACE inhibitor therapy. Subsequently, based on the ACR, patients were grouped as micro-albuminuric (ACR ≥ 30 and ≤ 300 mg/g creatinine) and macro-albuminuric (ACR > 300 mg/g creatinine). A significant decrease in ACR was observed in both micro- and macro-albuminuric DN groups. In micro-albuminuric DN group (*n* = 170) responders were found to be 45% whereas in macro-albuminuric group (*n* = 82) responders were found to be 55% after 6 mo follow up.

***Distribution of genotypes of ACE and AGT gene***

ACE I/D polymorphism was studied by sequence specific PCR method and AGT M235T polymorphism was studied by PCR-RFLP method. Genotype distribution and allele frequency for ACE and AGT genes are listed in Table 4. Distribution of all genotypes was in Hardy-Weinberg equilibrium for all the subgroups of ACE and AGT genes. For ACE gene the genotype frequency of II, ID, DD was found to be 31%, 53% and 16% respectively. For AGT gene MM, MT, TT genotypes were found to be 25%, 53% and 22% respectively.

***ACE and AGT polymorphisms and response to ACE inhibitor therapy***

Table 5 shows the genotype distribution of DN patients based on the response to ACE inhibitor therapy. No significant change in the genotype distribution was observed among responder and non-responder with regard to ACE and AGT gene. When the patients were grouped as micro- and macro-albuminuric based on their ACR values (Table 6), no inter-genotype differences were observed in subgroups. Though macro-albuminuric patients carrying ACE I/D genotypes are responding in a better way to therapy with regard to micro-albuminuric patients. Seventy two percent of macro-albuminuric patients having TT genotype responded to therapy, although not significant statistically.

**DISCUSSION**

In the present study, we examined the antiproteinuric effect of ACE inhibitor (ramipril) in DN patients by following urinary ACR. Angiotensin converting enzyme inhibitors are commonly used for inhibition of the RAAS and are known to have renoprotective efficacy in both diabetic and non-diabetic kidney disease[15] and antiproteinuric efficacy of ACE inhibitors are more pronounced than any other antihypertensive drugs[16]. However, there is variable response regarding antiproteinuric efficacy of RAAS blockers among patients and 20%-80% reduction is observed[17]. In the present study, overall we observed 36% of reduction in ACR values and about 48% of patients responded to therapy. Our finding is in accordance with previous studies showing overall decrease in albumin excretion after treatment with ACE inhibitor[13,18-20]. According to NKF KDOQI Guidelines[14], ACE inhibitors reduced protein excretion by approximately 35% to 40% which is greater than other antihypertensive agents, when effect of blood pressure has been taken into account. Hence, in the present study patients with ACR change ≥ 30% were considered as responders to ACE inhibitor therapy. On subdividing our study subjects as micro- and macro-albuminuric it was observed that 55% patients with macro-albuminuria responded in a better way to ACE inhibitor therapy. Earlier anti-proteinuric effect of ACE inhibitor has been shown to be more pronounced in macro-albuminuric patients[21,22]. The mechanism leading to antiproteinuric effect of ACE inhibitor has not been elucidated fully. However, it is thought that ACE inhibitor causes efferent arteriolar vasodilation of glomerulus and thereby decreasing the intraglomerular hypertension leading to anti-proteinuric effect[23]. Recently it has been shown that ACE inhibitor ameliorates the glomerular membrane size-selective dysfunction resulting anti-proteinuric effect[24].

In order to find out the reason behind differential response to ACE inhibitor therapy in DN patients we studied the polymorphisms of two genes namely ACE and AGT as these polymorphisms are strongly associated with the progression of DN. The genotype distribution of ACE gene observed in our study subjects are in line with most of the previous studies on Indian population[25,26].

In the present study, the percentage of responders did not differ significantly with regard to ACE I/D genotypes indicating that the antiproteinuric effect of ACE inhibitor is independent of ACE genotype. Similar to our finding that the anti-proteinuric effect of ACE inhibitor is independent of ACE genotypes have been reported by several authors[27,28,14]. Cheema *et al*[29] have reported that ACE II genotype with a cumulative genetic risk score of < 1 in normoalbuminuric type 2 DM patients, is coupled with better response to ACE inhibitor but no significant difference found in renoprotective effect of ACE inhibitor therapy based on ACE I/D genotypes after 3 years follow up. The antiproteinuric effect of RAAS inhibitors in patients with macro-albuminuria is also found to be independent of ACE I/D genotypes[30]. However, there are number of controversies about the association of ACE I/D genotypes with the therapeutic efficacy of ACE inhibitors. In Korean and Caucasian patients, DD genotype has been shown to be more responsive to ACE inhibitor therapy[31,32]. While, Japanese, European and Caucasian DN patients carrying II allele, exhibit better reno-protection to ACE inhibitor therapy[33-35].

Another important gene of renin-angiotensin-aldosterone system is AGT and M235T polymorphism influence the risk of nephropathy in type 2 DM patients[36,37]. Frequencies of M/T genotypes of AGT gene in our study are similar to several other studies in different populations[36,38-41]. We observed that the percentage of responders did not differ significantly in different genotypes of AGT gene, as compared to non-responders. This indicates that the antiproteinuric effect of ACE inhibitor is independent of genotypes of AGT gene. When patients were subdivided as micro- and macro-albuminuric; we observed that macro-albuminuric patients carrying TT genotype showed better antiproteinuric response to ACE inhibitor therapy, however results were not found statistically significant. No significant reports are available on AGT M235T gene polymorphism and antiproteinuric response to ACE inhibitor therapy. Similar to our finding, reports by several authors failed to show any significant association between AGT polymorphism and diabetic[40,41] chronic kidney disease. Also no association between AGT M235T genotypes and reduction in albumin excretion after ACE inhibitor treatment was reported[29]. However, Narita *et al*[41] concludes that the therapeutic efficacy of ACE inhibitor or ARBs is influenced by AGT M235T genotypes in patients with IgA nephropathy.

Our study has few limitations. Patients were given different doses of ramipril as per their requirement of dose titration. In addition, short duration of follow-up period as well as heterogeneity in gender may also have hindered the significant association of ACE I/D or AGT M235T genotypes.

In conclusion, ACE inhibitor treatment to DN patients appears to cause significant reduction in urinary protein excretion and macro-albuminuric patients exhibit better response. The antiproteinuric effect of ACE inhibitor therapy in patients is independent of ACE I/D and AGT M235T genotypes. Long term follow-up of larger populations with ACE inhibitor therapy may validate the present findings.

**COMMENTS**

***Background***

Angiotensin converting enzyme (ACE) inhibitors are the standard therapy for patients with hypertension, proteinuria and kidney diseases. The use of ACE inhibitors delays the progression of diabetic and non-diabetic kidney disease. Various polymorphisms of renin-angiotensin-aldosterone system (RAAS) have been implicated in the pathology of diabetic nephropathy. Of these, polymorphism of ACE gene is the most important. The current study was designed to evaluate the therapeutic efficacy of ACE inhibitor in terms of proteinuria and the role of ACE and AGT gene polymorphisms in ACE inhibitor-mediated antiproteinuric response in diabetic nephropathy patients.

***Research frontiers***

Patients on ACE inhibitor therapy have improved proteinuria. In this study, we observed that ACE and AGT gene polymorphisms do not have any role in reducing albuminuria in patients with diabetic nephropathy.

***Innovations and breakthroughs***

The literature suggests a mixed role of ACE gene polymorphisms in renoprotective action in diabetic patients. However, the present study suggests no role of ACE *I/D* and AGT *M235T* gene polymorphisms in modulating the renoprotective efficacy of ACE inhibitor in terms of reducing albuminuria in diabetic nephropathy patients.

***Applications***

The authors’ study serves as additional evidence supporting the therapeutic role of ACE inhibitors in reducing albuminuria. They conclude that genotypes of various genes of RAAS are not responsible for non-uniform response to ACE inhibitor in DN patients.

***Terminology***

Diabetic nephropathy: It is the damage to kidneys due to diabetes. Polymorphism: The presence of genetic variation within a population.

***Peer-review***

This is a good paper.

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**Table 1 Demographic and clinical characteristics of patients**

|  |  |
| --- | --- |
| Parameters | Type 2 diabetes mellitus with nephropathy |
| Number of patients (n) | 270 |
| Gender (Male/Female) | 128/142 |
| Age (years) | 52.23 ± 6.011 |
| Duration of diabetes (years) | 8.31 ± 3.091 |
| Family history of diabetes (Yes/No) | 105/165 |
| Family history of hypertension (Yes/No) | 63/207 |
| Medications: |  |
| * Insulin (Yes/No) | 115/155 |
| * Metformin (Yes/No) | 153/117 |
| * Glimiperide (Yes/No) | 129/141 |

1Data represented as Mean ± SD.

**Table 2 Biochemical parameters before and after treatment with angiotensin converting enzyme inhibitor**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | Baseline1 | 6 mo1,2 | *P*-value |
| No. of patients | *n* = 252 | *n* = 252 |  |
| Blood urea (mmol/L) | 2.22 ± 0.86 | 2.01 ± 0.77 | 0.661 |
| Serum creatinine (µmol/L) | 95.47-30 | 99-28.28 | 0.068 |
| Serum sodium (mmol/L) | 139.47 ± 4.11 | 135.14 ± 3.88 | 0.512 |
| Serum potassium (mmol/L) | 4.32 ± 0.65 | 4.30 ± 0.52 | 0.141 |
| eGFR (MDRD) mL/min per 1.73 m2 | 73.65 ± 24.71 | 68.90 ± 24.44 | 0.081 |
| eGFR (EPI) mL/min per 1.73 m2 | 73.40 ± 22.8 | 70.56 ± 21.30 | 0.07 |
| Fasting plasma glucose (mmol/L) | 7.63 ± 0.60 | 6.693 ± 0.81 | 0.08 |
| Post-prandial plasma glucose (mmol/L) | 10.33 ± 1.62 | 8.52 ± 1.3 | 0.076 |
| HbA1c (%) | 6.52 ± 1.71 | 6. 1± 1.14 | 0.06 |
| Hemoglobin (g/L) | 123.8 ± 23 | 111.2 ± 31 | 0.65 |
| Systolic blood pressure (mm Hg) | 132.30 ± 13.67 | 130.12 ± 10.46 | 0.71 |
| Diastolic blood pressure (mm Hg) | 86.10 ± 10.03 | 84.07 ± 8.32 | 0.68 |

1Data represented as Mean ± SD; 2*P* > 0.05; HbA1c: Hemoglobin A1c.

**Table 3 Responders and non-responders before and after treatment with angiotensin converting enzyme inhibitor therapy**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Patients | Urinary ACR  at baseline1 | Urinary ACR  at 6 mo | *P-*value | R2  (%) | NR (%) |
| Overall (*n* = 252) | 185.97  (55.66-222.20) | 118.64  (96.24-146.26) | < 0.001 | 121 | 131 |
| Micro-albumin  (*n* = 170) | 78.79  (71.30-87.07) | 53.67  (44.46-64.79) | < 0.001 | 76 | 94 |
| Macro-albumin  (*n* = 82) | 1068.70  (879.62–1298.28) | 596.45  (451.60-787.68) | < 0.001 | 45 | 37 |

R: Responders; NR: Non-responders; ACR: Albumin:creatinine ratio; 1Median (IQR); 2A decline of > 30% in ACR value at 6 mo, is considered as R or NR.

**Table 4 Genotype distributions and allele frequency for angiotensin converting enzyme and angiotensinogen gene polymorphisms**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | *n* = 252 | Genotype/allele | Percentage (%) |
| ACE (*I/D*) | Genotypic frequencies | II | 31 |
| ID | 53 |
| DD | 16 |
| Allele frequencies | I | 57 |
| D | 43 |
| AGT (*M235T*) | Genotypic frequencies | MM | 25 |
| MT | 53 |
| TT | 22 |
| Allele frequencies | M | 51 |
| T | 49 |

ACE: Angiotensin converting enzyme; AGT: Angiotensinogen.

**Table 5 Genotypic distribution of responders and non-responders**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Gene | Genotype | No. of Patients (*n* = 252) | After 6 mo follow-up  R (%) NR (%)  (*n* = 121) (*n* = 131) | | *P*-value1 |
| ACE (*I/D*) | II | 78 | 38 (49) | 40 (51) | .893 |
|  | ID | 133 | 62 (47) | 71 (53) |  |
|  | DD | 41 | 21 (51) | 20 (49) |  |
| AGT (*M235T*) | MM | 61 | 34 (56) | 27 (44) | .369 |
|  | MT | 134 | 59 (44) | 75 (56) |  |
|  | TT | 57 | 28 (49) | 29 (51) |  |

1*P* > 0.05; comparison between R or NR; ACE: Angiotensin converting enzyme; AGT: Angiotensinogen; R: Responders; NR: Non-responders.

**Table 6 Genotypic distribution of responders and non-responders having micro-/ macro-albuminuria**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | Genotype  (*n* = 252) | Micro-albuminuric group (*n* = 170) | | | Macro-albuminuric group (*n* = 82) | | |
|  |  | R (%) (*n* = 76) | NR (%)  (*n* = 94) | *P-*value1 | Rc (%)  (*n* = 45) | NR (%)  (*n* = 37) | *P-*value2 |
| ACE  (I/D) | II | 23 (45) | 28 (55) | 0.974 | 15 (56) | 12 (44) | 0.636 |
|  | ID | 42 (44) | 53 (56) |  | 20 (53) | 18 (47) |  |
|  | DD | 11 (49) | 13 (54) |  | 10 (59) | 7 (41) |  |
| AGT  (M235T) | MM | 25 (60) | 17 (40) | 0.110 | 9 (47) | 10 (53) | 0.201 |
|  | MT | 36 (40) | 53 (60) |  | 23 (51) | 22 (49) |  |
|  | TT | 15 (38) | 24 (62) |  | 13 (72) | 5 (28) |  |

1*P* > 0.05: comparison between responder and non-responder to therapy in micro-albuminuric group; 2*P* > 0.05: comparison between responder and non-responder to therapy in macro-albuminuric group; ACE: Angiotensin converting enzyme; AGT: Angiotensinogen; R: Responders; NR: Non-responders.