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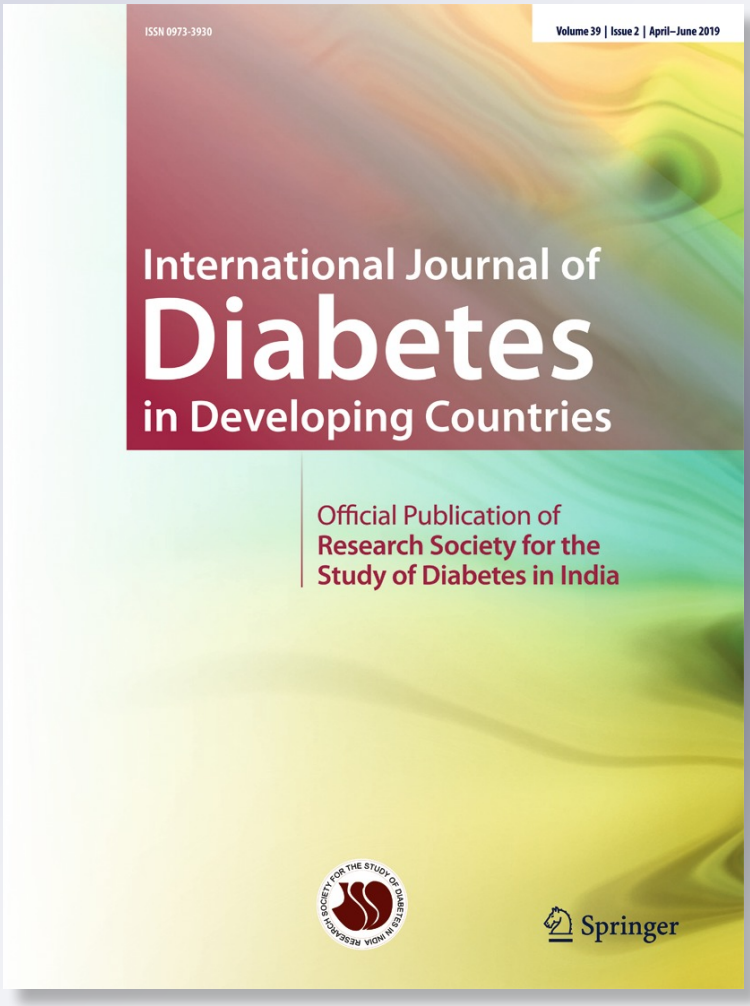

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ORIGINAL ARTICLE

Clinical and biochemical characteristics and the association of angiotensin type 1 receptor with normoalbuminuric chronic kidney disease among South Indian type 2 diabetes population

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Abstract

The aim of the study was to determine the clinical and biochemical characteristics especially cystatin C and the association of angiotensin type I receptor (AT1R) gene polymorphism with normoalbuminuric chronic kidney disease (NACKD) among South Indian type 2 diabetes (T2DM) population.

Material and methods The study comprised of 308 (M:F 190:118) subjects with T2DM categorized into three groups. Group I: T2DM patients without albuminuria (NA) and estimated glomerular filtration rate (eGFR) > 90 ml/min/1.73m^2 ($n = 110$); group II: T2DM patients with albuminuric chronic kidney disease (CKD) with eGFR < 60 ml/min/1.73m^2 ($n = 98$); and group III: T2DM patients with normoalbuminuric CKD (NACKD) and eGFR < 60 ml/min/1.73m^2 ($n = 100$). The eGFR was estimated using the Modification of Diet in Renal Disease (MDRD) formula. Cystatin C was measured by particle-enhanced immunoturbidimetric assay. AT1R gene polymorphism was analyzed using the PCR-RFLP method.

Results The biochemical parameters urea, creatinine, post prandial glucose, HbA1c, triglycerides, cystatin C levels, erythrocyte sedimentation rate (ESR), and diastolic blood pressure were lower and the levels of calcium were higher in normoalbuminuric CKD subjects as compared to albuminuric CKD subjects. The AT1R genotypic distribution of AA, AC, and CC was 80.2%, 19.8%, and 0% in NACKD, and 95.1%, 4.9%, and 0% in CKD subjects. The distribution of allelic frequencies of A and C allele in NACKD was 90.1% and 9.9%, and in CKD, it was 97.6% and 2.4% respectively. The relative risk of AC ($p = 0.08$) genotype and C ($p = 0.01$) allele in NACKD was 4 times higher as compared to CKD.

Conclusion The present study highlighted that the clinical and biochemical parameters showed significant differences especially cystatin C whose levels increased in normoalbuminuric CKD subjects as compared to normoalbuminuric subjects. Significant association of AGTR1 A1166C polymorphism was observed in normoalbuminuric CKD subjects as compared to CKD subjects with T2DM.

Keywords Chronic kidney disease · Normoalbuminuria · Type 2 diabetes mellitus · AGTR1 · South India

Introduction

One of the major complications of type 2 diabetes mellitus (T2DM) is diabetic renal disease (DKD) that predisposes individuals to excess morbidity and mortality resulting in renal failure and cardiovascular disease [24, 31]. A recent study from India reported that patients with chronic kidney disease (CKD) spend three times more than those without any other diabetic complications [33]. The diagnosis of DKD is an important issue in people with long-term T2DM. Persistent albumin excretion has been considered to be the hallmark for DKD and is believed to be the earliest marker of glomerular disease [16]. However, it has been now established that a non-excretion of albumin is a type of kidney disease quite prevalent in T2DM [16]. Approximately, one-third to half of the T2DM patients have CKD without proteinuria may be due to atubular glomeruli, renal microvascular atherosclerotic disease, analgesics, etc. [29, 34].

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Studies have reported a prevalence of 17% and 21.8% of normoalbuminuria among diabetes subjects with CKD [1, 3, 14, 20]. Diagnosing renal impairment without the evidence of albuminuria is a critical issue. At this stage, the glomerular filtration rate (GFR) is believed to be preserved. However, some individuals with T2DM present reduced GFR level with little or no albuminuria [12]. Several studies in adults have shown that cystatin C is a more sensitive marker of changes in the GFR than serum creatinine [9, 4, 26]. Studies have demonstrated that serum cystatin C is an early renal marker in patients with diabetes [22, 27, 30].

In the last decade, the beneficial effect of blockade of the renin-angiotensin system has been demonstrated in a wide variety of cardiovascular disease, from heart failure to stable coronary artery disease and diabetic as well as non-diabetic chronic nephropathies [7, 28]. The angiotensin II type 1 receptor (AT1R) gene is located on chromosome 3q 21-25 and has a length of > 55 kb, composed of five exons and four introns. It is expressed mainly in the heart and kidney tissues and its products are predominantly found in the vascular smooth muscle cells, heart, adrenal gland, and kidney. AT1 plays an integral role in blood pressure control and is implicated in the pathogenesis of hypertension. A1166C polymorphism in AT1R gene has shown to be associated with an increased risk of urinary albumin excretion [11] and a faster progression of DKD in patients with T2DM [35]. Many studies have highlighted the role of AGTR1 (A1166C) gene polymorphism and the risk of DKD of which two were from India and the results were inconsistent across different populations [5, 6, 11, 23, 25, 35]. There is limited data available on this association among the South Indian population. The aim of the present study was to determine the clinical and biochemical characteristics especially cystatin C and the association of angiotensin type1 receptor (AT1R) gene polymorphism with normoalbuminuric CKD among south Indian subjects with T2DM.

Materials and methods

We conducted a cross-sectional study of subjects with T2DM who were attending a tertiary care center for diabetes in South India. A total of 1177 patients (M:F 542:635) with diabetes were screened from Feb 2015 to May 2016. At the end of the study period, a total of 308 (M:F 190:118) subjects were included in the study. A pilot study was first carried out using 30 subjects per group. Based on these preliminary results, with a confidence interval of 95%, a prevalence of 72%, a 5% type 1 error, an estimated p value < 0.05, and a power of 80%, the present sample size was derived. In this study, the inclusion criteria were T2DM subjects with proved kidney complication and categorized into three groups based on the urinary albumin excretion rate (UAER) and their eGFR levels. A total of 869 patients were excluded due to the history of intake of angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB) in the last 6 months, eGFR > 60–89, T1DM, age > 65 years, DM duration < 5 years, and diabetic foot ulcer patients. Also, those who refused to participate or withdrew their consent were also excluded from the present study. The selected 308 subjects were categorized into 3 groups. Group I: T2DM patients without albuminuria (NA) UAER < 20 μg/mg creatinine and eGFR > 90 ml/min/1.73m^2 (n = 110); group II: T2DM patients with albuminuria (UAER > 20 μg/mg creatinine) and chronic kidney disease (CKD) eGFR < 60 ml/min/1.73m^2 (n = 98); and group III: T2DM patients with normoalbuminuria and CKD (NACKD) and eGFR < 60 ml/min/1.73m^2 (n = 100). CKD was based on eGFR < 60 ml/min/1.73m^2 for 3 months or more, irrespective of the cause. The eGFR was estimated with serum creatinine using the Modification of Diet in Renal Disease (MDRD) formula [17]. The written informed consent was obtained from all study participants in accordance with the principles of the declaration of Helsinki.

Anthropometric and biochemical investigations

Age, sex, height, and weight measurements were obtained, and body mass index (BMI) was calculated using the formula weight in kilograms divided by the square of height in meters. Blood pressure was recorded in the sitting position in the right arm to the nearest 2 mmHg with a mercury sphygmomanometer. The JNC VII criteria for hypertension were considered for classification of subjects having hypertension. Venous blood sample (whole blood, plasma, serum) was collected for biochemical investigations and genetic studies. Biochemical investigations were done on BS 400 autoanalyzer using DiaSys kits. Fasting plasma glucose (glucose oxidase-peroxidase [GOD-POD] method), serum cholesterol (cholesterol oxidase-phenol4-amino antipyrene peroxidase [CHOD-PAP] method), serum triglycerides (glycerol phosphatase oxidase−phenol4-amino antipyrene peroxidase [GPO-PAP] method), high-density lipoprotein cholesterol (direct method–polyethylene glycol-pre-treated enzymes), and low-density lipoprotein cholesterol (direct homogenous method) were measured using auto-analyzer. Glycosylated hemoglobin (HbA1c) was measured by high-performance liquid chromatography method using the Turbo Variant machine (Bio-Rad, Hercules, CA). The CV for in-house quality control was less than 2.5%. Serum creatinine was measured using the Jaffe method. Calcium was measured by arsenazo dye method. Urinary albumin excretion (UAE) was measured by immunoturbidimetric method. Serum cystatin C was measured by particle-enhanced immunoturbidimetric assay using DiaSys Cystatin C FS kit.

Isolation of genomic DNA from peripheral blood and PCR amplification

Genomic DNA was isolated from peripheral blood (anticoagulated EDTA-blood stored at − 20 °C) using standard phenol–chloroform extraction method with minor modification [19]. The isolated DNA was subjected to PCR amplification using T100 Thermal Cycler (Bio-Rad, USA). Genomic DNA (~ 50 ng) was incubated in a total reaction volume of 20 μL containing equal concentration of the forward primer 5′-ATA ATG TAA GCT CAT CCA CC-3′ and reverse primer 5′-GAG ATT GCA TTT CTG TCA GT-3′ (Bioserve Biotechnologies, India) ~ 10 picomoles and 2× solution of Prime Taq Premix (Genet Bio, Chungnam, Korea). DNA was initially denatured at 94 °C for 5 min prior to amplification. PCR amplification was accomplished using 35 cycles consisting of 1 min denaturation at 94 °C, 30 s annealing at 58 °C, and 45 Sec extensions at 72 °C. The final extension was carried out for 5 min at 72 °C. After PCR, 10 μL of the reaction mixture was digested with 4 U *DdeI* restriction enzyme (C*TNAG) (New England Biolabs) and Tris-HCl buffer (100 mM KCl, 1 mM DTT, 1 mM EDTA, pH 7.4 at 25 °C) in a 20 μL reaction volume for overnight at 37 °C. The digested products were subjected to electrophoresis on a 2.0% agarose gel and visualized under UV light using gel documentation system (BioRad).

Statistical analysis

All statistical analyses were performed using SPSS 20.0 version software (SPSS Inc., IL). Mean and standard deviation for continuous variables and proportions for categorical variables are reported. One-way ANOVA was used to test continuous variables and a chi-square test was used to compare categorical variables. Correlation analysis of cystatin C with clinical, anthropometric, and biochemical parameters was done in the study groups. A p value of < 0.05 was considered to be statistically significant. The relationship between genotypes and alleles was determined by obtaining the odds ratio (ORs) (OR, 95% confidence interval (CI)) using the Primer 6.0 software.

Results

Anthropometric, clinical, and biochemical characteristics of the study groups

The anthropometric, clinical, and biochemical characteristics of the study groups are shown in Table 1. As expected, significant differences were noted in age ($p < 0.001$), systolic blood pressure ($p = 0.01$), diastolic blood pressure ($p = 0.008$), duration of DM ($p < 0.001$), urea levels ($p < 0.001$), creatinine levels ($p < 0.001$), eGFR ($p < 0.001$), UAER ($p < 0.001$), uric acid levels ($p < 0.001$), hemoglobin levels($p < 0.001$), and erythrocyte sedimentation rate (ESR) ($p < 0.001$) in the CKD group as compared to NA group. In NACKD group, age ($p < 0.001$), duration of DM ($p = 0.03$), urea levels ($p < 0.001$), eGFR ($p < 0.001$), calcium levels ($p = 0.01$), uric acid levels ($p = 0.02$), hemoglobin levels ($p = 0.001$), and ESR ($p < 0.001$) showed significant differences as compared to NA subjects.

The subjects having history of hypertension was significantly higher in CKD ($p = 0.008$) and NACKD ($p = 0.03$) as compared to NA group. The duration of hypertension was significantly longer in NACKD ($p < 0.001$) and CKD ($p < 0.001$) groups as compared to NA group. The presence of dyslipidemia was not statistically significant among the groups but a higher percentage of subjects had dyslipidemia in NACKD (51.9%) group than NA (36.4%) and CKD (34.3%) groups. The presence of retinopathy was higher in CKD group ($p = 0.004$) than NA and NACKD groups ($p = 0.7$). The presence of cardiovascular disease was found to be non-significant among the groups.

The NACKD subjects were older ($p = 0.02$), and urea ($p < 0.001$), creatinine ($p = 0.001$), eGFR($p < 0.001$), UAER ($p = 0.002$), diastolic blood pressure ($p = 0.009$), post prandial glucose levels ($p = 0.04$), HbA1c % ($p = 0.05$), calcium levels ($p < 0.001$), triglyceride levels ($p = 0.04$), hemoglobin levels ($p = 0.001$), ESR ($p < 0.001$), and cystatin C levels ($p < 0.001$) showed significant differences when compared among NACKD and CKD subjects.

Determination of AT1R genotyping

The AA, AC, and CC genotypes were identified by PCR-RFLP and were visualized using gel documentation system as shown in Figs. 1 and 2. Table 2, 3 and 4 show the distribution of genotypic and allelic frequencies of AT1R SNP. In NA groups, 76.5% and 23.5% of the subjects had AA and AC genotype, respectively, while none of the subjects had CC genotype. On the other hand, subjects with CKD had 95.1% of AA genotype followed by 4.9% of AC genotype and 0.0% with CC genotype. The NACKD group had 80.2% of AA genotype and 19.8% of AC genotype and none of them had CC genotype. The distribution of allelic frequency of A and C allele in NA was 88.2% and 11.8%, respectively, 97.6% and 2.4% in CKD, and 90.1% and 9.9% in NACKD.

Levels of cystatin C in study groups

The cystatin C levels in NA, CKD, and NACKD were 1.06 ± 0.30, 2.70 ± 1.31, and 1.68 ± 0.58 mg/L respectively as shown in Fig. 3. The levels of cystatin C were significantly increased in CKD ($p < 0.001$) and NACKD ($p < 0.001$) compared to

Table 1 Biochemical and anthropometric parameters of study groups

Parameters	NA ($n = 110$)	CKD ($n = 98$)	NACKD ($n = 100$)
Male	72 (66%)	69 (70%)	49 (49%)
Female	38 (34%)	29 (30%)	51 (51%)
Age (years)	52.5 (13.2)	60.5 (11)***	63 (8)***#
Body mass index (kg/m^2)	26.1 (4.5)	26.0 (4.4)	26.4 (5.7)
Systolic BP (mm Hg)	120 (12)	130 (30)**	130 (30)**
Diastolic BP (mm Hg)	80 (10)	80 (20)	80 (10)
Duration of DM (years)	10 (7)	13 (10)***	10 (7)
Fasting plasma glucose (mg/dL)	154 (91)	152 (90)	125 (67)
Post prandial glucose (mg/dL)	214 (123)	247 (148)	190 (105)
Glycated hemoglobin (HbA1c) (%)	8.4 (2.7)	8.8 (2.4)	7.7 (2.2)#
Urea (mg/dL)	20 (7)	37 (38)***	41 (14)***###
Creatinine (mg/dL)	0.8 (0.2)	1.7 (0.8)***	1.3 (0.3)#
Albumin/creatinine ratio (mg/g)	7.5 (9.5)	52.5 (48.3)*	11 (10)#
eGFR (ml/min/1.73 m2)	95 (12)	42 (24)***	55 (9)***###
UAER μg/min	7 (4.2)	28 (4)***	7 (9)
Triglycerides (mg/dL)	123 (78)	119 (97)	113 (76)#
Total cholesterol (mg/dL)	165 (60)	143 (57)	145 (70)
HDL-cholesterol (mg/dL)	40 (13)	36 (12)	39 (12)
LDL-cholesterol (mg/dL)	94 (39)	80 (37)	78 (42)
VLDL-cholesterol (mg/dL)	30 (14)	28 (21)	25 (16)
Non-HDL cholesterol (mg/dL)	122 (53)	110 (56)	103 (60)
Calcium (mg/dL)	8.8 (7.6)	8.6 (0.9)	9.3 (0.6)**###
Uric acid (mg/dL)	4.6 (1.6)	6.2 (3.3)***	5.3 (3.9)**
Hemoglobin (g/dL)	13.3 (2.4)	10.8 (2.9)***	12.3 (2.3)***##
ESR (mm in 1st hour)	20 (31)	71 (65)***	38 (39)***###
History of hypertension (%)	26.6	54.1**	44*
Duration of hypertension (years)	1 (1)	1 (2)	2 (2)***
Retinopathy (%)	22.9	48.1**	31.9
History of dyslipidemia (%)	36.4	34.3	51.9
History of neuropathy (%)	45. 7	75.0***	68.8
History of CVD (%)	1.3	7.9	4

*($p < 0.05$), **($p < 0.01$), ***($p < 0.001$) versus NA group. #($p < 0.05$), ##($p < 0.01$), ###($p < 0.001$) versus CKD group. *UAER*, urinary albumin excretion rate

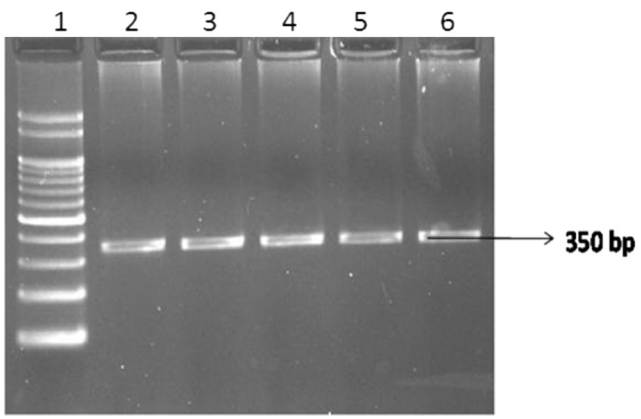

Fig. 1 PCR amplification of AT1R gene. 2% agarose gel electrophoresis of the amplified DNA of AT1R region. Lane 1, 100 bp DNA ladder, lanes 2–6 amplified 350 bp of AT1R region

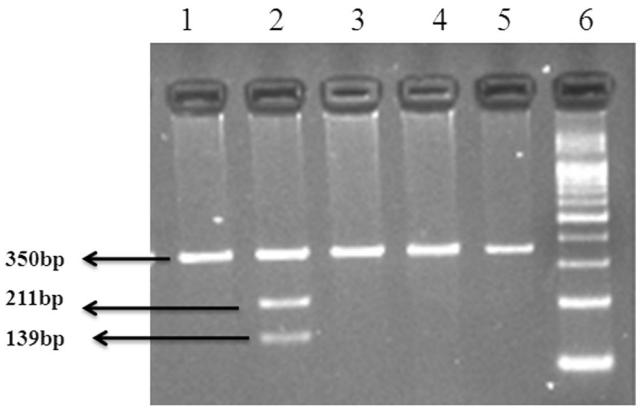

Fig. 2 A1166C polymorphism of the AT1R gene in 2% agarose gel. Lanes 1, 3, 4, and 5 show the presence of AA genotype (350 bp), lane 2 shows the presence of AC genotype (350, 211, and 139 bp), and lane 6 shows the 100 bp DNA ladder

Table 2 Odds ratio—relative risk between NA vs CKD. This table represents the comparison of genomic and allelic frequencies of NA and CKD

Genotype		NA (94) *n* (%)	CKD (82) *n* (%)	RR (95% CI)	*p* value
AA		72 (76.5)	78 (95.1)	5.9 (1.9–18.1)	0.001
AC		22 (23.5)	4 (4.9)	0.16 (0.05–0.5)	0.001
CC		0	0	–	–
Allele	A	166 (88.2)	160 (97.6)	5.3 (1.8–15.7)	0.02
	C	22 (11.8)	4 (2.4)	0.189 (0.06–0.6)	0.02

NA. The levels were significantly decreased in NACKD as compared to CKD ($p < 0.001$).

Correlation of cystatin C with anthropometric and biochemical parameters

The levels of cystatin C were correlated with anthropometric and biochemical parameters. In NA subjects, cystatin C had a significant positive correlation with urea ($r = -0.227$, $p = 0.004$) and a significant negative correlation with fasting plasma glucose ($r = -0.269$, $p = 0.01$) and HbA1c ($r = -0.253$, $p = 0.02$) while other parameters did not show any correlation. In CKD subjects, there was a negative correlation between eGFR and cystatin C level ($r = -0.420$, $p < 0.001$). In NACKD subjects, none of the parameters correlated with cystatin C levels (Table 5).

Discussion

Normoalbuminuric renal impairment has much significance in the management of patients with T2DM. Only a limited number of studies have been reported on the clinical characteristics of T2DM subjects with reduced eGFR and normoalbuminuria. The main focus of the study was to determine the clinical and biochemical parameters and AT1R polymorphism association with normoalbuminuric CKD subjects in South Indian population.

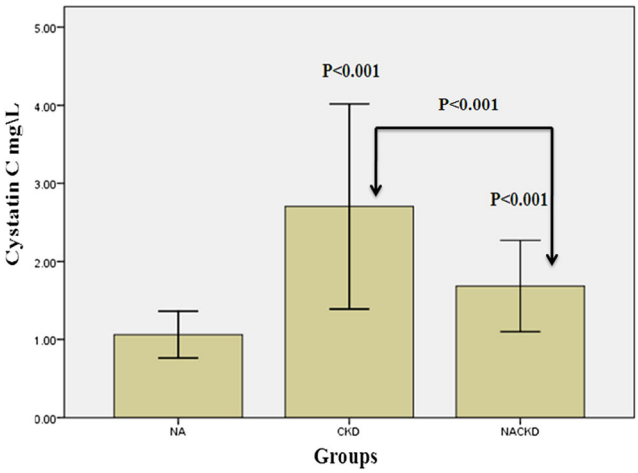

Fig. 3 The levels of cystatin C in study groups

Normoalbuminuric renal impairment has a different pathophysiology, in addition to the classical pathology. The presence of both kinds of pathology was associated with worse metabolic control, suggesting that hyperglycemia may cause different patterns of renal injury in T2DM subjects [10]. In addition to this, other possible causal factors associated with normoalbuminuric CKD are hypertension nephrosclerosis, renovascular disease, tubulointestinal fibrosis, cholesterol microemboli, and classical diabetic nephropathy effectively treated with ACE/ARBs [10]. In the present study, apart from their relatively low eGFR levels, normoalbuminuric CKD patients displayed a number of specific characteristics, they were older in age than NA and CKD groups, a higher prevalence of hypertension than NA group, a high prevalence of dyslipidemia than NA and CKD groups, a higher duration of hypertension than CKD and NA group, a greater duration of diabetes than NA group, a slightly higher levels of calcium than NA and CKD groups, a higher ESR than NA group, a higher level of uric acid than NA group, and a lower hemoglobin level than NA group.

In this regard, the lack of association of high blood pressure with NACKD could be related to extremely tight blood pressure control. The occurrence of dyslipidemia in NACKD is 51.9% which was higher than NA (36.4%) and CKD (34.3%)

Table 3 Odds ratio—relative risk between NA vs NACKD. This table represents the comparison of genomic and allelic frequencies of NA and NACKD

Genotype		NA (94) *n* (%)	NACKD (81) *n* (%)	RR (95% CI)	*p* value
AA		72 (76.5)	65 (80.2)	1.241 (0.6–2.6)	0.68
AC		22 (23.5)	16 (19.8)	0.80 (0.4–1.7)	0.68
CC		0	0	–	–
Allele	A	166 (88.2)	146 (90.1)	1.209 (0.6–2.4)	0.708
	C	22 (11.8)	16 (9.9)	0.827 (0.4–1.6)	0.708

Table 4 Odds ratio—relative risk between NACKD vs CKD. This table represents the comparison of genomic and allelic frequencies of NA and CKD

Genotype		NACKD (81) *n* (%)	CKD (82) *n* (%)	RR (95% CI)	*p* value
AA		65 (80.2)	78 (95.1)	0.2 (0.06–0.65)	0.008
AC		16 (19.8)	4 (4.9)	4.8 (1.53–15.0)	0.008
CC		0	0	–	
Allele	A	146 (90.1)	160 (97.6)	0.2 (0.07–0.7)	0.01
	C	16 (9.9)	4 (2.4)	4.3 (1.43–13.4)	0.01

Table 5 Correlation analysis of cystatin C with clinical parameters in the study groups

Clinical parameters	NA r	$n = 80$ p	CKD r	$n = 66$ p	NACKD r	$n = 80$ p
Age (years)	0.407	< 0.001	− 0.073	0.56	0.124	0.27
BMI (kg/ m^2)	− 0.020	0.864	− 0.045	0.719	0.131	0.245
Systolic BP (mmHg)	− 0.081	0.476	0.019	0.879	− 0.005	0.962
Diastolic BP (mmHg)	0.083	0.465	0.171	0.170	− 0.111	0.328
Duration of DM (years)	0.032	0.778	− 0.181	0.145	0.063	0.580
Urea (mg/dL)	0.227	0.04	0.352	0.004	0.19	0.07
Creatinine (mg/dL)	− 0.016	0.88	0.195	0.11	0.185	0.10
eGFR (MDRD)	− 0.041	0.717	− 0.420	< 0.001	− 0.181	0.108
FPG (mg/dL)	− 0.269	0.016	0.003	0.978	− 0.031	0.785
PPG (mg/dL)	− 0.115	0.367	− 0.031	0.828	− 0.037	0.752
HbA1c (%)	− 0.253	0.024	− 0.128	0.321	− 0.105	0.353
Tot cholesterol (mg/dL)	− 0.171	0.139	0.279	0.027	0.018	0.877
Triglycerides (mg/dL)	− 0.219	0.05	0.06	0.620	− 0.106	0.350
HDL cho (mg/dL)	− 0.132	0.255	0.085	0.505	0.041	0.719
Non-HDL cho (mg/dL)	− 0.130	0.265	0.264	0.037	0.075	0.506
LDL cho (mg/dL)	− 0.156	0.178	0.233	0.066	0.081	0.475
VLDL cho (mg/dL)	− 0.141	0.223	0.197	0.122	0.056	0.622
Calcium (mg/dL)	− 0.168	0.312	− 0.172	0.373	− 0.89	0.783
Uric acid (mg/dL)	0.225	0.163	0.095	0.588	0.379	0.120

*($p < 0.05$), **($p < 0.01$), and ***($p < 0.001$)

groups. The cholesterol microemboli result in inflammation and in turn which elevates erythrocyte sedimentation rate (ESR) as reported in a study by Modi and Rao [21]; in the present study, ESR rate was significantly increased in NACKD group than NA.

Many studies have observed that HbA1c is an independent correlate of albuminuria, but not of impaired renal function, thus indicating that non-albuminuric renal disease is probably less related to hyperglycemia and microvascular disease than the classic proteinuric form of kidney disease [1, 29]. In the present study, NACKD subjects had lower HbAlc percentage and post prandial glucose levels than the subjects with albuminuric CKD. Serum creatinine was significantly lower in NACKD compared to CKD subjects. Similar results were obtained in a study done by et al. [3].

Lim et al. highlighted that low serum calcium level (< 9.0 mg/dL) is an independent prognostic marker for rapid renal function progression in CKD subjects in Taiwan [18]. In this study, we observed mean serum calcium levels approximately 9 mg/dL in normoalbuminuric CKD subjects and no significant difference in CKD group as compared to NA group, but the mean values of calcium in the study groups are lower than the upper limits (10.3 mg/dL) and seem to be within the normal range (8.6–10.3 mg/dL). So far, no studies have reported the levels of calcium in normoalbuminuric CKD subjects.

In the present study, the levels of serum cystatin C were significantly higher in NACKD subjects (1.68 ± 0.58 mg/L) when compared to normoalbuminuric subjects (1.06 ± 0.30 mg/L). In a recent report by Dayanidhi et al. [8], similar results were reported in microalbuminuria T2DM subjects (1.74 ± 0.66) and normoalbuminuric subjects (1.19 ± 0.62) ($p < 0.05$). The levels of serum cystatin C were significantly higher in CKD subjects (2.70 ± 1.31 mg/L) in our study. Similarly, levels of serum cystatin C (2.04 ± 1.19 mg/L) were observed in macroalbuminuric subjects in a Korean population in a study by Jeon et al. [15].

It has been also suggested that serum cystatin C is a more sensitive marker for detecting early changes in glomerular filtration in T2DM subjects than creatinine-based measurements [36]. In CKD subjects, the eGFR had a significant negative correlation with cystatin C. The correlation of cystatin C with eGFR confirms that the cystatin C could be one of the earlier markers representing CKD in T2DM subjects. In concordant to the above results, the urea levels also have a significant positive correlation with cystatin C level ($r = 0.22$, $p = 0.04$). It is known that serum cystatin C levels increase with advancing age and the serum cystatin C level has a significant positive correlation with age ($r = 0.40$, $p < 0.001$). There was no correlation found with serum cystatin C and biochemical parameters in NACKD subjects.

To our knowledge, the present study is first of its kind, to determine the polymorphism, in normoalbuminuric CKD subjects of AT1R in South Indian population. The relative risk of AC genotype ($p = 0.08$) and C allele ($p = 0.01$) in NACKD was 4 times higher compared to CKD which was significant.

There was a significant association of AGTR1 A1166C polymorphisms observed in normoalbuminuric CKD compared to CKD subjects. Ahluwalia et al. showed that the frequency of C allele of AGTR1 A1166C was higher in North Indian subjects with T2DM and DKD [2].

In this study, we found that the AA genotype was most frequent when compared to AC and CC genotypes in CKD (95.1%) and NACKD (80.2%) groups compared to NA (76.5%) group, while CC genotype was not found in any of the groups. Similar results were noted in the Chinese population without the frequency of CC genotype in normoalbuminuria, microalbuminuria, and macroalbuminuria subjects [37]. Jacobsen et al. reported that "A" allele of the AGTR1 A1166C polymorphisms is a risk allele in nephropathy subjects which was also confirmed in the survival analysis of time to doubling of serum creatinine or development of ESRD [13]. Similarly, AA genotype and "A" allele as a risk allele was observed in the present study as well in CKD subjects compared with normoalbuminuric subjects.

The conflicting results in the studies were ascribed to different ethnic population, difference in sample size, and a more complex model consisting of still poorly understood combinations of several AGTR1 gene variants that may affect disease susceptibility [25, 32]. Some of the limitations of this study include, owing to the cross-sectional design, it was difficult to clarify the causal relationship between the risk factors and the natural history of normoalbuminuric chronic kidney disease. Moreover, the subjects with normoalbuminuria and eGFR < 60 mL/min/1.73 m^2 might need more evaluation with other markers along with cystatin C to diagnose DKD. Second, the level of cystatin C was not measured in urine along with the albumin. The significant difference among the patient groups in terms of age can be seen as another factor which limits our study.

Conclusion

The present study highlighted that clinical and biochemical parameters showed significant differences especially cystatin C whose levels increased in normoalbuminuric CKD subjects as compared to normoalbuminuric subjects. Therefore, other markers, such as serum and urine NGAL, urine KIM-1, IgG, and transferrin, may add to the early diagnosis of diabetic kidney disease along with serum cystatin C. AGTRI, A1166C polymorphism showed significant association in normoalbuminuric CKD subjects. Relying on albumin excretion as first sign for renal involvement may be too late in diagnosing and modifying the progression of the disease and better markers for monitoring of renal function that can detect early renal damage are much needed. Early use of RAS blocking agents before the detection of urinary albumin excretion or renal impairment may be beneficial in preserving the renal function in diabetics.

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Authors' contribution Dr. Vijay Viswanathan conceived and designed the experiment, Ms. Anju Soni and Ms. Bliss screened and collected the samples, Ms. Ezhilarasi performed the experiment and drafted the manuscript, and Dr. Vijay Viswanathan and Dr. Satyavani contributed to the discussion and reviewed the manuscript.

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Compliance with ethical standards

The study protocol was approved by the institutional ethics committee (Ref ECR/51/Inst/TN/2013/MVDRC/30) and all methods were performed in accordance with the relevant guidelines and regulations of the institution. The written informed consent was obtained from all study participants in accordance with the principles of the declaration of Helsinki.

Conflict of interest The authors declare that they have no conflict of interest.

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