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Angiotensin II type 1 receptor (A1166C) gene polymorphism in Egyptian adult hemodialysis patients

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Abstract *Background:* Genetic variability in the genes of different components of renin-angiotensin system (RAS) is likely to contribute for its heterogenous association in renal diseased patients. Among the candidate genes of RAS, angiotensin II type 1 receptor gene polymorphism (AT_1R A1166C) seems to be particularly biologically and clinically relevant to renal diseases.
The aim: This study was to evaluate the association of AT_1R A1166C gene polymorphism in adult Egyptian hemodialysis (HD) patients. Its association with hypertension was also done.
Subjects and methods: The study was conducted on 202 adult Egyptian subjects: 100 controls and 102 HD patients. Determination of this polymorphism was done by PCR/RFLP strategy.
Results: It revealed that HD patients had significantly higher frequency in AC and CC genotypes than controls. But on dividing these patients to normotensive and hypertensive ones, no significant difference in the genotypes was found. The multiple regression analysis revealed no dependent variables on the AT_1R A1166C gene polymorphism.
Conclusion: This study suggested that CC/AC genotype could serve as a predictor of an early end-stage renal disease (ESRD) and could, in the future, become an important part of the clinical process of renal risk identification.

1. Introduction

Renal function and blood pressure are tightly linked. Physiologically, kidneys provide a key regulator of both blood pressure and body fluids, via renin-angiotensin–aldosterone system (RAAS).[1] This enzymatic cascade acts as an endocrine and paracrine system that results in the production of angiotensin II (AngII). [2,3] Angiotensin II type 1 receptor (AT_1R) mediates most of the action of AngII and therefore modulates the RAAS.[4,5] The role of RAAS in the pathogenesis of hypertension is well documented, but its contribution to chronic renal failure (CRF) is still debated.[6–8]

Hypertension is a major contributor in the progression of renal disease to renal failure.[7,9] It affects up to 90% of

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hemodialysis patients and is a risk factor for adverse cardiovascular outcomes.[10,11]

The impact of genetic variability on the development of renal failure emphasizes the need to elucidate its genetic basis.[12] Genetic polymorphism of RAAS has been associated with organ damage in essential hypertension.[13] A great number of AT_1R gene polymorphisms have been identified and the most studied one is the A1166C variant.[2–5,8,10–14] It is a polymorphism in the ′3 untranslated region of the gene encoding human AT_1R, corresponding to adenine (A) to cytosine (C) transversion at position 1166 (rs 5186).[15]

The AT_1R A1166C gene polymorphism was reported to be implicated as a risk factor for hypertension.[15–19] However, previous studies showed conflicting data concerning the association of AT_1R C 1166 allele with the progression of renal dysfunction and its influence on the patient's susceptibility to CRF or end-stage renal disease (ESRD).[4,5,12,15,20–23]

To our knowledge the influence of AT_1R A1166C gene polymorphism on the development of ESRD in adult Egyptian patients, especially those with hypertension has not yet reported. There was only one report on the association of this polymorphism in Egyptian hemodialysis children.[12] Therefore, the present study was designed to determine whether the AT_1 R A1166 C gene polymorphism, if present, is involved in the occurrence of ESRD in adult Egyptian hemodialysis patients; its relation to the presence of hypertension was also studied.

2. Subjects

After the approval of the Ethical Committee of the Medical Research Institute (MRI), this case control study was conducted on 102 adult Egyptian hemodialysis (HD) patients (50 males & 52 females) with a mean age of 50.5 ± 14.0 years. They were recruited from the Nephrology Unit of the MRI teaching hospital, Alexandria University. One 100 adult healthy volunteers (35 males & 65 females) with a mean age of 56.0 ± 10.2 years were also included. They had no family history of hypertension. Written informed consents were taken from all subjects included in this study, which was done from April to December 2013.

Etiology of ESRD was diabetic nephropathy (28 cases), hypertension (24 cases), primary glomerulonephritis (16 cases), obstructive uropathy (10 cases), lupus nephritis (8 cases), drug induced nephropathy (4 cases), eclampsia (3 cases), adult polycystic kidney disease (2 cases), congenital anomalies (2 cases), acute tubular necrosis (one case) and unknown etiology (4 cases).

The patients were under maintenance HD for more than one year (the median duration of HD was five years), as four hours thrice weekly, using polysulphone hollow fiber dialyzers and bicarbonate-buffered dialysate.

N.B.: Patients with malignancy, pulmonary hypertension, liver cirrhosis, ascites, heart failure, atrial fibrillation, or ischemic heart disease were excluded.

The HD patients were further subdivided according to the presence of hypertension (Ht) into: normotensive group (30 patients) and hypertensive group (72 patients) (their median duration of Ht was 10 years). The hypertensive group was further subdivided according to the type of antihypertensive therapy into: those taking RAS suppressors either angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II type 1 receptor blockers (ARBs)], either alone or in combination with others (34 patients). The other subgroup was those using RAS suppressors [e.g. β-blockers, calcium channel blockers and /or diuretics] (38 patients).

3. Methods

To all subjects, full clinical examination was done, with special stress in patients on age, duration of dialysis and detailed history of hypertension, diabetes and therapy. Predialysis measurement of blood pressure (BP) for three times was done after resting in a sitting position, according to the WHO/International Society of Hypertension Recommendation, then their mean was taken and the mean arterial pressure was calculated.[24] Hypertension (Ht) was defined as systolic BP(SBP) > 140 mmHg, diastolic BP (DBP) > 90 mmHg, or they were on maintenance use of antihypertensive therapy. Determination of body mass index (BMI) was also calculated.[25]

Following eight hours fasting period, five ml venous blood were withdrawn from all subjects. For patients, this was done immediately before midweek dialysis session. Two ml was dispended into K_3EDTA vacutainer and mixed gently for the genomic analysis. The rest were dispended into plain vacutainer and the obtained serum was used for estimation of glucose and creatinine levels as well as the alanine aminotransferase activity using Humaster 300 clinical chemistry analyzer (Human GmbH, Germany).

Detection of AT_1RA1166C gene polymorphism was done as follows:

1. Genomic DNA extraction from the peripheral blood leukocytes was done[26], using Gene JET™ Genomic DNA Purification kit (Fermentas). Then the integrity of the extracted DNA was assessed by two ways: 2% agarose gel electrophoresis[27] and estimation of its concentration and purity[28], using Thermo Scientific Nano Drop™ 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, Delaware, USA).
2. Detection of A1166C single nucleotide polymorphism (SNP) of the AT_1R gene was done by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) strategy[18], where the 850 bp fragment was generated by PCR amplification using a forward primer (5′-AAT GCT TGT AGC CAA AGT CAC CT-3′) and a reverse primer (5′-GGT TTT GCT TTG TCT TGT TG-3′) (Fermentas – Thermo, USA). The reaction mixture was performed where 5 µl extracted DNA was mixed with 0.5 µl of each primer and 12.5 µl Dream Tag PCR Green™ master mix (Fermentas-Thermo, USA) and completed to final volume of 25 µl with nuclease free sterile water. The PCR amplification of 5 µl genomic DNA was done on the S-96 thermo cycler (Quanta Biotech, UK) as initial denaturation for one cycle at 94 °C for 2 min, followed by 12 cycles amplification (denaturation at 94 °C for 1 min, annealing ''touchdown'' at 72 °C down to 60 °C for 1 min and extension at 72 °C for 2 min), then 28 cycles amplification (denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 2 min). The final extension was done by one cycle at 72 °C for 10 min. Then 12 µl of the PCR product was visualized by 2% agarose gel electrophoresis stained by ethidium bromide as bands at 850 bp.

3. Restriction digestion at 37 °C for 1–4 h of the PCR product, using 200 units (10 U/μl) Dde I enzyme (Promega, USA) which targeted specific sequence of amplified DNA product. It cuts the product into 2 pieces (600, 250 bp) long. An additional Dde I recognition site is present in C type variant to split the 250 bp into 110 and 140 bp fragments. Visualization of the digestion products was done on 2% agarose gel electrophoresis stained with ethidium bromide, where the homozygote AA produced 2 bands (600 & 250 bp long), the homozygote CC produced 3 bands (600, 140, 110 bp long) and the heterozygote AC produced all 4 bands (600, 250, 140 & 110 bp long).

3.1. Statistical analysis[29]

It was done using SPSS program (version 20) (Chicago, USA). D'Agostino–Pearson K-squared test for normality was done to test for the deviation from the normal distribution. For normally distributed quantitative variables, mean ± S.D., unpaired *t*-test and Pearson's correlation were used. While median and range (min.–max.), Mann Whitney test and Spearman correlation were used for data that were abnormally distributed. For data of nominal variables (frequency or percentage), the Chi–Square test (χ^2 test) with Monte Carlo estimate of the exact *p* value and Fisher's exact test were used. Hardy–Weinberg Equilibrium[30] was done to calculate the equilibrium expected genotypic proportions. Odd's ratio (OR) with 95% confidence intervals (CI) were estimated for the effects of high risk alleles. Multiple linear regression analysis was done to analyze the relationship between a dependent variable (mean arterial blood pressure) and several other anticipated independent predictor variables. A p value of <0.05 was considered statistically significant. The generalization of Casagrande's method proposed by Fleiss was used to estimate the statistical power of our study.

4. Results

The whole HD patients showed significantly higher levels of glucose, creatinine, total cholesterol, LDL-C and triglycerides than their corresponding values in controls. In addition, they showed significantly lower levels of BMI and HDL-cholesterol than their corresponding values in controls (Table 1).

The AT_1R A 1166C genotyping in controls and whole HD patients were in agreement with H.W. equilibrium (Table 2). The HD patients showed decreased frequency of the wild (AA) genotype and increased frequency of heterotype (AC) and mutant (CC) type than the corresponding control values ($P = 0.024$) (Table 2).

In whole HD patients, the frequency allele A showed significantly lower percentage and the frequency of C allele showed significantly higher percentage than their corresponding percentage in controls ($p^{FE} = 0.03$) (Table 3).

On dividing the HD patients according to the presence of hypertension, there were no significant differences in either the frequency of genotypes or in the allele frequency between normotensive and hypertensive HD patients ($P^{FE} = 0.612$ & 0.258) (Table 4).

Table 1 Some clinical and laboratory data of the studied groups.

	BMI (kg/m^2)	Glucose (mg/dl)	Creatinin (mg/dl)	ALT (U/L)	Chol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	TG (mg/dl)
Controls ($n = 100$): mean ± S.D./min.–max & median	28.6 ± 4.13	88.9 ± 8.79	0.5–1.4	6–69	188.3 ±	30–80	121.0 ±	34–240
			0.9	19.5	39.2	41	38.1	88.5
HD patient ($n = 102$): mean ± S.D./min.–max. & median	16–39.5	57–377	3.4–16.8	6–139	98–384	22–68	39–282	43–400
	24.0	99.0	9.7	16	218.5	35	182	162
P^{MW}	0.000	0.000	0.000	N.S.	0.05	0.001	0.001	0.000

Table 2 Comparison of AT_1R(A1166C) genotype among the studied groups and their agreement with H.W. equilibrium.

	Observed frequency		H.W. expected frequency		Statistical analysis (observe. # H.W. expect	
	n.	%	*n.*	%	χ^2	*P*
Controls (n = 100):					$\chi^2 = 1.86$	$P = 0.173$ (N.S.)
AA (wild)	76	76	77.44	77.44		
AC (hetero)	24	24	21.12	21.12		
CC (mutant)	0.0	0.0	1.44	1.44		
HD patients (n = 102):					$\chi^2 = 1.34$	$P = 0.246$ (N.S.)
AA (wild)	67	65.7	65.1	63.8		
AC (hetero)	29	28.4	32.8	32.1		
CC (mutant)	6	5.9	4.1	4.1		
Fisher exact *P* value	0.024					

Table 3 Comparison of allele frequency among the studied groups.

	Controls (n = 100)		HD patients (n = 102)		Statistical analysis		
	n.	%	n.	%	χ^2	P^{FE}	OR
A allele	176	88	163	80	4.91	0.03	OR = 1.84 95% C.I. = 1.07–3.19
C allele	24	12	41	20			

Based on Fisher's exact-test results, the relative risk approximated by an Odds ratio of (1.84) and our minor allele frequency of (0.12) the power of our sample size to detect association was estimated to be (0.544).

Table 4 Comparison of AT_1R(A1166C) genotype among normo-and hypertensive HD patients.

	Normotensive HD pts. (n = 30) (MAP 88 ± 9.8 mmHg)		Hypertensive HD pts. (n = 72) (MAP 98 ± 13.7 mmHg)		Statistical analysis		
	n.	%	n.	%	χ^2	P^{FE}	OR
AA	22	73.4	45	62.5		0.612 (N.S.)	
AC	7	23.3	22	30.6			
CC	1	3.3	5	6.9			
A allele	51	85	112	78	1.38	0.258 (N.S.)	OR = 1.69 95% C.I. = 0.7–3.6
C allele	9	15	32	22			

Table 5 Comparison of AT_1R(A1166C) genotype among hypertensive patients receiving different antihypertensive therapy.

	Pts. Taking non RAS blockers (n = 38)		Pts. Taking RAS blockers (n = 34)		Fisher Exact with Freeman Halton
	n.	%	n.	%	P value
AA	24	63.2	21	61.8	0.855 (N.S.)
AC	12	31.5	10	29.4	
CC	2	5.3	3	8.8	

Table 6 Comparison of AT_1R SNP in relation to sex.

	Controls (n = 100)		HD patients (n = 102)	
	F (n = 65)	M (n = 35)	F (n = 52)	M (n = 50)
AA	51(78.5%)	25(71.4)	31(59.6%)	36(72%)
AC	14(21.5%)	10(28.6%)	18(34.6%)	11(22%)
CC	0.0(0%)	0.0(0%)	3(5.8%)	3(6%)
$P^{F.E.}$-value & Freeman Halton	0.111 (N.S.)		0.469 (N.S.)	

Table 7 Comparison of AT_1R SNP in relation to age.

	Controls (n = 100)		HD patients (n = 102)	
	⩽50y (n = 30)	>50y (n = 70)	⩽50y (n = 52)	>50y (n = 50)
AA	27(88.5%)	50(71.2%)	37(71.2%)	30(60%)
AC	3(11.1%)	20(28.8%)	12(23.0%)	17(34%)
CC	0.0(0%)	0.0(0%)	3(5.8%)	3(6%)
$P^{F.E.}$-value & Freeman Halton	0.111 (N.S.)		0.469 (N.S.)	

When dividing the hypertensive HD patients according to the type of anti-hypertensive therapy given, no significant difference in the AT_1RA 1166C genotype was found between those receiving RAS suppressors antagonists and those receiving other antihypertensive drugs (P^{FE} = 0.866) (Table 5).

No significant difference in the frequency of AT_1R genotype according to change in sex in either controls or HD patients (Table 6). Also, no significant changes in it between those ⩽50 years and those >50 years in either controls or HD patients (Table 7).

Table 8 Final multiple regression analysis of MAP.

	Beta	T	Significance
Constant		13.743	0.000
LDL-C	−0.204	−2.178	0.032*
Duration of analysis	−0.217	−2.272	0.025*
BMI	−0.294	−3.065	0.003*
Age	0.162	1.704	0.092*
AT_1R(A1166C) genotype	0.082	0.853	0.396 (N.S.)

* Significant.

The final results of multiple linear regression analysis with mean arterial blood pressure as the dependent variable revealed that it is not dependent on AT_1RA1166C genotype, but they ended up with 4 independent variable (LDL-cholesterol, duration of dialysis, age and BMI (Table 8).

5. Discussion

The RAS genes have been investigated as potential etiologic candidates for cardiovascular[11,14] and renal diseases.[4,5] Most of the known effects of Ang II, the powerful effector peptide of RAS, are mainly mediated by Ang II type 1 receptor (At_1R).[31]

The AT_1R is a member of G-protein coupled receptor superfamily that is expressed in most tissues.[14] Human AT_1R gene is located on chromosome 3q21–25. The AT_1R activation leads to vasoconstriction and water retention.[32] It may also regulate cell proliferation and vascular extra cellular protein synthesis, with effects on renal vasculature and glomerular fibrosis.[4]

It was reported that the c-allele of AT_1RA1166C gene polymorphism may be associated with a faster decline in renal functions.[33] It has been suggested that the renal and systemic Ang II activity would be augmented in subjects with c-allele of AT_1RA1166C gene polymorphism.[5]

Conflicting results were reported about the relation between the presence of AT_1R C-1166 allele and kidney damage: Buraczynska et al. in 2002[20] and in 2006[34] found an association between this allele and the progression to ESRD. Others[5] reported that the underlying diagnosis may modify the association of genetic polymorphism and dialysis dependent ESRD. On the other hand, Coll et al.[21] in China and Lee et al.[4] in Spain found that the faster progression of renal damage was associated with AA genotype.

In the present study, the genotype distribution of AT_1RA1166C among controls and HD patients was in agreement with Hardy–Weinberg equilibrium ($P = 0.173$, 0.246; respectively) (Table 2). In addition, the HD patients showed significantly higher frequency of CC (5.9%) and significantly lower frequency of AA (65.7%) of AT_1R genotype than the corresponding values (0%, 76% respectively) in controls ($p = 0.024$) (Table 2). Also, HD patients showed higher percentage (20%) of c-allele than that (12%) in controls ($P = 0.03$) (Table 3). Similar results was reported by El-Shamaa et al.[12] as they found significantly higher frequency of the c-allele and of homozygous CC genotype in Egyptian HD children than those in controls. Also, the same findings were shown in adult HD in 2002 by Buraczynska et al.[20] They also reported in 2006 an association between AT1R A/C polymorphism and progression of renal disease.[34] Therefore, the c-allele appeared to be a possible predictor of fast progression to ESRD.[20]

In the present study, no significant differences in the AT_1R A1166C gene polymorphism in relation to either age or sex (Tables 6 and 7). In addition, the multiple regression analysis did not reveal any dependency of serum creatinine or duration of dialysis on the presence of this polymorphism.

In the multiple linear regression analysis of El-Shamaa et al.[12], the c-allele and serum urea were variables that were independently associated with systolic blood pressure (SBP). So in hypertensive HD patients, the AC/CC genotype of AT_1R gene polymorphism could contribute in a synergistic way to renal damage. Fabris et al.[35] found an increase progression to renal disease among hypertensive patients with AT_1R A1166C gene polymorphism.

Studies concerning the association of this polymorphism and hypertension in different ethnic patients yielded conflicting results: some studies found an association.[14–16,19,36,37] While others did not show such association[13,17,38–42], Others found such association in only male subjects.[43]

In the present study, on dividing the HD patients according to the presence of hypertension, there were no significant differences in either the AT_1R A1166C genotyping or in the allele frequency between normotensive and hypertensive HD patients (Table 4).This non significant difference may be due to small sample sizes when dividing HD patients to normotensive and hypertensive. Therefore, on pooling those with AC and CC genotypes together, 37.5% of hypertensive versus 26.6% of normotensive HD patients had both AC and CC genotypes. In addition, 22% of hypertensive versus 15% of normotensive had c-allele (Table 4). However, also no significant difference was found.

It could be suggested that the discrepancies in the results of the association of this polymorphism with hypertension may be ethnic group-related and the possibility that this polymorphism predisposed to hypertension still to be proved.[16,18,37,41] In addition, it could be due to the use of different methods and techniques in determining this polymorphism, beside the use of different (or even inappropriate) statistical methods for analyzing the data. The presence of linkages disequilibrium between AT_1RA1166C gene and other polymorphism may be another explanation.[15,44]

Whatever the role of AT_1R polymorphism in developing hypertension, regimen inhibiting AT_1R expression resulted in greater antihypertensive efficacy and organ protection.[45] Therefore, control of blood pressure (BP) is important to improve the outcome of these patients.[46]

In the present study, no significant differences were found in AT_1R A1166C genotyping between hypertensive HD patients receiving angiotensin antagonists (RAS blockade) and those receiving non-angiotensin antagonists (Table 5). Also, the mean arterial pressure (MAP) showed no significant difference between the two subgroups. Absence of this difference may be due to the fact that most of hypertensive HD patients took combined antihypertensive therapy to achieve adequate BP control.[47] Also, inter-individual variation in the responses to therapy have been reported.[12] It seems that RAS blockade in these patients would provide significant survival benefits,[48] however, no such effect was found in other study.[49]

In the present study, the final multiple regression analysis revealed that when using mean arterial pressure (MAP) as independent predictor, the LDL-chol, duration of dialysis, BMI and age; but not the AT_1RA1166C SNP were the only four significant dependent variables (Table 8).

In conclusion, as this study proved an association between AT_1RA1166 C gene polymorphism and ESRD progression, it may be suggested that CC/AC genotype could serve as a predictor of an early ESRD and could, in the future, become an important part of the clinical process of renal risk identification. However, this suggestion needs to be confirmed in another study on larger Egyptian patients. Also, the mechanism(s) by which this polymorphism affects the progression of ESRD remain to be elucidated.

The study did not prove the association of this polymorphism with hypertension. This may be due to the relatively small number of subjects enrolled in the study.

Conflict of interest

The authors declare that there are no conflict of interest.

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