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RESEARCH ARTICLE

ANGIOTENSINOGEN GENE POLYMORPHISM (M235T) IN SUDANESE HYPERTENSIVE PATIENTS.

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Key words:-

Angiotensinogen M235T Gene Polymorphism, essential hypertension, renin, aldosterone, Sudan.

Abstract

Background and objectives: Essential hypertension is common in the general population by its increasing incidences, thus recently researchers suggested that AGT (M235T) polymorphism may be the functional genotype in hypertensive patients. Therefore the study aimed to evaluate Angiotensinogen (AGT) gene polymorphisms (M235T) as well as its relation to serum level of renin and aldosterone among Sudanese hypertensive patients.

Materials and Methods: In a case control study, 96 patients with essential hypertension and 79 healthy apparently controls were enrolled. The clinical data were obtained and serum renin and aldosterone levels were measured using ELISA. The AGT gene polymorphism(M235T) alleles and genotypes were identified by PCR–RFLP analysis.

Results: Analysis of allele frequency showed that, MM, MT and TT genotypes of Angiotensinogen (AGT) gene polymorphism (M235T)for patients were 91.7%, 8.3%, and 0% respectively and that for the control group was 96.2%, 3.8%, and 0%. Statistically no significant difference was found ($p=0.219$).There were significant increase in both serum levels of renin and aldosterone among essential hypertensive patients p -value(0.001and 0.005) respectively.

Conclusion: The study concluded that there is no association between the polymorphism (M235T) of the AGT gene and essential hypertension, whereas association observed with serum renin and aldosterone levels.

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Introduction:-

Essential hypertension is a leading cause for human cardiovascular mortality and morbidity with a prevalence rate of 25-30% among adults of industrialized societies⁽¹⁾. Blood pressures are known to be regulated by the renin-angiotensin system by controlling peripheral vascular resistance fluid and electrolyte homeostasis^(2,3).

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Angiotensinogen (AGT) is a major precursor of the renin-angiotensin-system and its plasma levels have been shown to correlate with blood pressure⁽⁴⁾. Variation in AGT gene and other genes of the RAS regulate blood pressure smooth muscle cell growth and cardiac remodeling⁽⁵⁾. AGT variants coding region missense polymorphisms M235T the Methionine substituted to Threonine amino acid at the 235th position in the exon 2 of the AGT gene. It is suggested that AGT (M235T) polymorphism may be the functional genotype, as it affects the basal transcription rate of AGT, which could explain the association of the M235T genotypes with the plasma AGT concentration⁽⁶⁾. Genetic polymorphisms in components of the RAS including AGT (M235T) are suggested to be associated with the pathogenesis of essential hypertension⁽⁷⁾. The variant allele 235T is the most frequent one among African populations and it is not significantly associated with hypertension^(8,9). It is reported that M235T mutants leads to small increase in concentration of polymorphic AGT rather than changes in function^(10,11).

Frequency distribution and disease association of M235T has been shown to vary between different ethnic groups and also within large ethnic groups. To identify the specific frequency distribution and to explaining the role of these variants in Red sea State of essential hypertension in West Sudan, we screened the AGT gene for M235T variants among hypertensive patients and controls.

Materials and Methods:-

In analytical case control study, ninety six Sudanese patients with essential hypertension mean of age (52.98±9.9) years, and seventy nine healthy controls were enrolled in this study. Essential hypertension was diagnosed according to World Health Organization criteria⁽¹²⁾. Consent was obtained from each participant. All information regarding risk factors was explained to all participants under the study. Blood specimens was collected and transferred to the laboratory under the standard conditions.

Ethical consideration:-

Permission of this study was obtained from the local authorities in the area of the study. The objectives of the study were explained to the local authorities in the area of the study (in Red sea State) and to all individual in the study. A written consent was obtained from each participates in this study.

Serological tests:-

Sera were separated from blood for serological tests which were done by enzyme linked immunosorbent assays (ELISA), Serum rennin activity test was assayed by using the DEMEDITEC Renin ELISA Kit. Serum aldosterone test was assayed by aldosterone ELISA DE4128 Kit, both serum rennin and Serum Aldosterone activity expressed as pg/mL.

Genetic analysis:-

Genomic DNA was extracted by DNA Pure Link™ Genomic DNA Kits. The obtain DNA was stored at -20°C until used.

DNA samples were genotyped by using Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) using Quick-load PCR master mix kit (Biolab, New England), positive control and negative controls, 2µl was added to PCR tube and the following solutions were placed in a total volume of 25 µl; 12.5 µl green master mix(promga), 1 µL from each prime10 pmol/µl primer F, 10 pmol/µl primer R, 100 ng of genomic DNA template, 2.5iuTaq Polymerase and nuclease free water (up to the total volume of 25µl) . PCR amplification was performed using the following primers described by Laura et al., (2008)⁽¹³⁾ as F: 5- GAT GCG CAC AAG GTC CTG TC -3 and R: 5- CAG GGT GCT GTC CAC ACT GGA CCC C -3 which produces PCR product size 303 bp which cut by restriction enzyme PflFI (New England Biolabs, UK). The presence one fragment of 303 bp showed the presence of the AGT-M allele, to be MM (polymorphic homozygote) genotype, the presence of 279 pb fragments showed the TT (wild type) genotype; while the MT (polymorphic heterozygote) genotype was shown by fragments of 303, 3279 bp. Cycling condition were as follows; initial denaturing at 94°C for 3min, followed by 35 cycles at 95°C for 30 s, annealing at 57for 30 s, extension at 72°C for 30 s, and a final extension at 72° C for 5 min. PCR and RFLP were examined on 2% agarose electrophoresis stained with ethidium bromide.

Statistical data analysis:-

Data were recorded and then were analyzed using independent t-test and chi-square test by SPSS software, version 16.0. All tests were two-tailed, and a p-value of ≤0.05 was considered statistically significant.

Results:-**Table 1:-** Anthropometric and biochemical parameters of patients versus control subjects.

Characteristic	Patients (n=96) Mean \pm SD	Control (n=79) Mean \pm SD	p-value
Age/ years	52.98 \pm 9.9	50.19 \pm 11.5	
BMI (kg/m ²)	27.7 \pm 4.9	24.4 \pm 5.1	0.001
SBP(mmHg)	139.4 \pm 20.39	114.4 \pm 7.97	0.001
DBP(mmHg)	83.9 \pm 10.59	75.95 \pm 6.10	0.001
MAP	102.7 \pm 12.62	88.76 \pm 6.01	0.001
Renin (pg/mL)	94.3 \pm 43.8	16.3 \pm 14.4	0.001
Aldosterone (Pg/ml)	26.7 \pm 12.9	19.5 \pm 10.5	0.050

Results expressed as Mean \pm SD and significant differences considered as p-value \leq 0.05.

Table 2:- Correlation of AGT genotypes and allelic distribution among patients and control subjects.

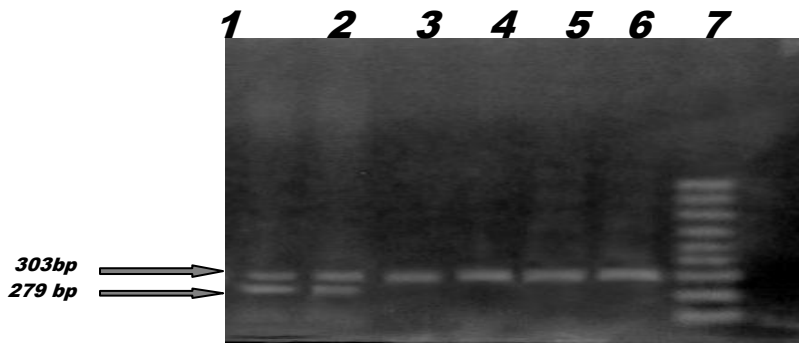
	Genotype distribution			Allele Frequency		p -value
	MM (n)%	MT (n)%	TT (n)%	M (n)%	T (n)%	
Normotensive	76 96.2%	3 3.8%	0 0%	79 96.3%	3 3.7%	0.219
Hypertension	88 91.7%	8 8.3%	0 0%	96 92.3%	8 7.6%	

TT: wild type genotype; MM: homozygous genotype; MT: heterozygous genotype. p \leq 0.05, statistical significant .

Table 3:- Mean serum renin and Aldosterone level of genotype MM in comparison with MT among patients.

Parameters	Genotype MM	Genotype MT	p-value
Serum renin	95.5 \pm 45.7	82.1 \pm 12.4	0.184
Serum Aldosterone	26.8 \pm 13.4	25.3 \pm 4.30	0.632

Results expressed as Mean \pm SD and significant differences considered as p-value \leq 0.05.

**Fig 1:-** Agarose gel electrophoresis. Lane 7 is a 100 bp linear DNA ladder.

Lanes 1,2 correspond to RFLP pattern of heterozygous(MT), lane 3,4,5, 6 homozygous wild-type (MM).

Figure 1 shows the results of the PflFI digestion on PCR products. AGT +704 T \rightarrow C missense mutation (lead to amino acid substitution of AGT M235T) created a new restriction site with the sequence recognition: GACN NNGT \downarrow C for PflFI. PflFI digested the fragment into 2 parts, the longer fragment; 279 bp and the shorter 24 bp. However, the 2% agarose gel was unable to retain the shorter fragment and it was suspected to have migrated out of the gel. Therefore, a band at 303 bp indicates homozygous wild-type (MM), a band at 279 bp indicates homozygous mutated (TT), and two bands at 303 bp and 279 bp indicates heterozygous mutation (MT).

Discussion:-

Interactions between individual genetic and environmental factors determine the onset of the Essential hypertension as for the genetic factors there are only a few studies. The present study aimed to screen the Angiotensinogen gene (M235T) polymorphism and its relation to essential hypertension.

In this study conducted in the Red sea state in Sudan, we found no evidence of an association between the M235T polymorphism of the AGT gene and blood pressure in either hypertensive or normotensive subjects. Our findings similar with those of Rotimi et al.⁽¹⁴⁾ No associations between the M235T polymorphism and hypertension ($X^2=0.02$) was found in a case-control study (57 cases and 130 controls) The same lack of association between the M235T polymorphism and hypertension ($P>0.41$) was seen in a combined linkage and case-control study by Caulfield et al.⁽¹⁵⁾ and in a nested family-based study ($n=628$) by Whitfield et al.⁽¹⁶⁾ investigating the effect between M235T and hypertension ($P=0.25$).

The results of independent t-test analysis provide evidence that, serum renin level of hypertensive patients significantly increase in comparison with control group p-value 0.001. This finding is in agreement with that reported by Gordon⁽¹⁸⁾, who found a positive correlation between blood pressure measurements and renin concentrations among human beings as well as in animal experiments. This positive correlation between renin substrate and high levels of blood pressure suggests that increased renin substrate concentration may be a causal factor in cases of hypertension⁽¹⁷⁾.

This study showed that mean of plasma aldosterone levels was significantly higher among hypertensive patients compared with those in the control group p-value 0.05. This finding is in accordance with several reports of the effects of aldosterone on smooth muscle, skeletal muscle, colonic epithelial cells, and myocardial cells⁽¹⁹⁾. Freel and Connell⁽²⁰⁾ linked these effects to the development of increased systemic vascular resistance and therefore, may contribute to development of hypertension and cardiovascular disease.

Conclusion:-

The study concludes that, there is no association between the M235T polymorphism of the AGT gene and essential hypertension, whereas association observed with serum renin and aldosterone levels.

Competing of interest:-

The authors declare they have no competing of interest.

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