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Detection of AGT Gene Polymorphism in Patient with Hypertension in Mosul City

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Abstract: Polymorphisms in the promoter region of the angiotensinogen (AGT) gene may affect AGT transcription and level of blood pressure. We determined the frequency of the AGT polymorphism in sample of Iraqi patients with primary hypertension. Using a molecular epidemiology approach, we also determined the relationship between primary hypertension and environmental-AGT polymorphism interactions of this study was to investigate the association between genetic polymorphisms of AGT M235T genes and Hypertension in Mosul city. Venous blood samples were collected from each subject in two separate test tubes: one was used for biochemical analysis. The other was collected in EDTA tube for DNA extraction. Genomic DNA was isolated from whole-blood samples of all the patients and control subjects. DNA concentration and purity were determined measuring by Bio drop and detected the optimum DNA concentration for PCR analysis. The quality of the DNA was determined using agarose gel electrophoresis stained with ethidium bromide, samples were stored at -20 °C until further use. The current study showed increase biochemical parameters in female patients compare with male patients. Regarding AGT/M235T gene polymorphism, In the present study, AGT/MT genotyping revealed that 47.5 % of patients, AGT/MM homozygous had 42.6 % and AGT/TT homozygous had 9.8 % in patients with Hypertension.

Keywords: Polymorphism, AGT, Hypertension, DNA.

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Introduction:

Hypertension is an important public health challenge because it is associated with morbidity and mortal (1). The cause of hypertension is “unknown” or essential in 90 % of people and genetics within this group is thought to contribute 40% while environment (lifestyle) is 60%. The remaining 10 % is due to underlying disease; renal diseases, renal arterial stenosis, tumors (brain, lung, kidney, adrenal), endocrine and medications3 Research on the molecular genetics of human hypertension developed less than 15 years ago (2). Its goal was to identify the loci involved, to detect gene variants at the loci identified, and associate them with intermediate phenotypes, and ultimately to estimate their quantitative effects on blood pressure and their interaction with the principal environmental factors. Some researchers shows that renin-angiotensin system genes are differentially regulated in human obesity and hypertension. The researchers are concentrated on the genes encoding the components of renin angiotensin system (R-A): Which includes Angiotensinogen (AGT) M235T, (3). Genetic polymorphisms of the renin- angiotensin system and complications of hypertension patients(4).

(AGT) constitutes a central component of the renin-angiotensin system that controls the systemic blood pressure and several other cardiovascular functions and may play an important role in atherosclerosis pathways. (5). The researchers are concentrated on the genes encoding the components of renin angiotensin system (R-A): Which includes Angiotensinogen (AGT) M235T, (ACE) I/D angiotensinogen II type 1

Polymorphisms of candidate genes have been studied in association and linkage studies to assess their potential role in essential hypertension in humans. The investigation shows polymorphisms of the angiotensinogen (AGT) gene and the angiotensin converting enzyme (ACE) gene in human essential hypertension is more common (6). Due to importance of AGT and ACE with reference to hypertension we included these two genes in this study (7). Were also included in the study. Blood was drawn after an informed consent.

The aim of this study was to investigate the association between genetic polymorphisms of AGT M235T genes and Hypertension in some Iraqi samples

Subjects and Methods:

Subjects:

A total of 30 adult hypertensions patients (15 male age 28-60 years and 15 female age 33-55 years) were recruited from the ibn sena hospital in Mosul city, Information regarding the demographic features such as age, marital history, parity, gestational age, family history, consanguinity detailed, weight and height were measured to calculate the body mass index (BMI), systolic BP (SBP) and diastolic BP (DBP) was measured in a sitting position using mercury column sphygmomanometer.

Angiotensin I- converting enzyme gene polymorphism and acute response to captopril in essential hypertension.

Method:

Venous blood samples were collected from each subject in two separate test tubes: one was used for biochemical analysis. The other was collected in EDTA tube for DNA extraction.

Biochemical measurements:

Serum total cholesterol, Triglyceride, high density lipoprotein, low density lipoprotein and fasting blood sugar were done using analyzer Commercial kits (Syrbio).

DNA Extraction:

Genomic DNA was isolated from whole-blood samples of all the patients and control subjects using a method described by (8). DNA concentration and purity were determined by measuring the absorbance of diluted DNA solution at 260 and 280 nm using (Bio drop Thermo scientific, England) and detected the optimum DNA concentration for PCR analysis. The quality of the DNA was determined using agarose gel electrophoresis stained with ethidium bromide, samples were stored at -20 °C until further use.

Evidence from combined segregation and linkage analysis.

Genotyping:

AGT M235T polymorphism by RFLP-PCR:

100 ng of DNA was amplified using Gene Amp PCR system,The primers that used were F-5'CCGTTTGTGCAGGGCC TGGCTCTCT3' ´; and R-5'CAGGGTGCTGTCCACACTGGAC CCC3', PCR conditions were optimized for initial denaturation for 94°C for 5 min; then denaturation at 94°C for 1 min and 64 °C for 1 min and extension at 72°C for 1 min over 30 cycles and final extension was done at 72°C for 7 minutes. The PCR products were then digested with restriction endonucleases *Tth 111I*, for 3 hours at 37°C and were analyzed by electrophoresis through a 2% agarose gel (6).

Statistical analysis:

(Results were analyzed based on T-test ($P \leq 0.05$))

Result and discussion:

Demographic data of studied groups are shown in (Table 1).

Table (1): Demographic data of study participants—hypertension patients

Characteristics	Male (means)	Female (means)
Age (years)	55	59
Family history	+ 70 %	+ 65 %
Fasting blood sugar (FBS)	180 mg / dl	200 mg / dl
Duration of hypertension (years)	13	11
Systolic blood pressure (mm/Hg)	15	14
Diastolic blood pressure (mm/Hg)	9	9
Weight (Kg)	84	90

Table (2): Biochemical parameters of study participants—hypertension patients.

Biochemical measurement	Male(means±S.E)	female(means±S.E)
Fasting blood sugar (mmol/L)	10	11
Cholesterol (mg/dl)	182	185
Triglycerides (mg/dl)	135	144
HDL cholesterol (mg/dl)	39	40
LDL cholesterol (mg/dl)	125	124

Hypertension is a complex disorder accounting for about 90–95% of all heart disease. Despite numerous reports suggesting a substantial genetic contribution to the susceptibility of hypertension, no major susceptibility genes have been identified so far (9). The aim of the current study was to investigate the association between genetic polymorphisms of AGT M235T genes and hypertension in some Iraqi patients. To achieve this goal, AGT M235T genotyping was performed by PCR and PCR-RFLP technique Genetic variation in the Renin-Angiotensin system and progression of diabetic nephropathy (10). The current study showed increase biochemical parameters in female patients compare with male patients (Table 2).

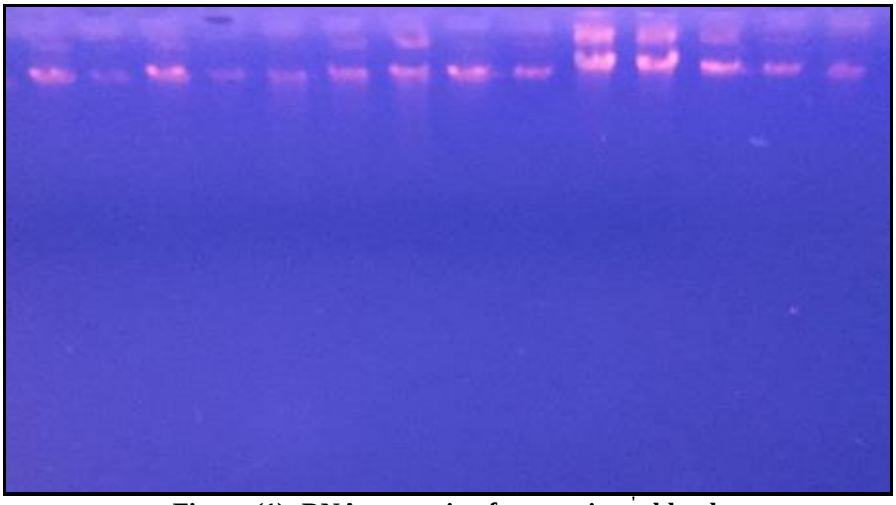

Figure (1): DNA extraction from patient's blood.

We are determined DNA concentration and purity by measuring the absorbance of diluted DNA solution using Bio drop technique and fixed the concentration (25 ng / µic) to used it in PCR amplification.

In the current study regarding AGT/M235T gene polymorphism, AGT/MM homozygous was significantly higher in hypertension patients, raising a protective role against occurrence of hypertension. Our results have shown an association between AGT M235T polymorphism and hypertension. Then, the risk of developing this cronic disease seems to be higher for TT genotype and T allele compared to MM genotype and M allele(4,11).

AGT M235T polymorphism genotype is caused by replace threonine to methionine substitution at position 235 (M235T) transition was not present in hypertension in some Iraqi patients, Thus it may have a protective role against susceptibility to hypertension. No statistically significant difference(3,12).

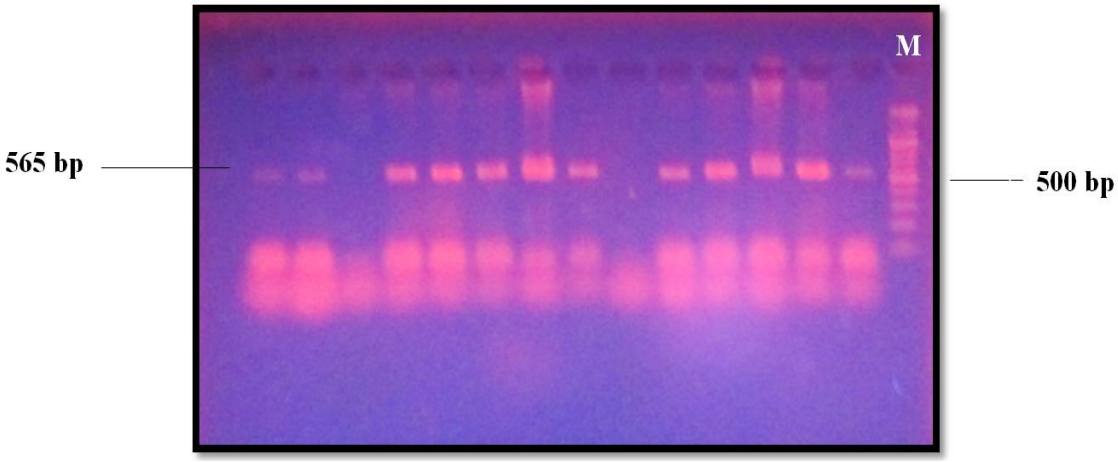

Figure (2): Detection AGT M235T polymorphism by the RFLP – PCR, undigested PCR product (565 bp).

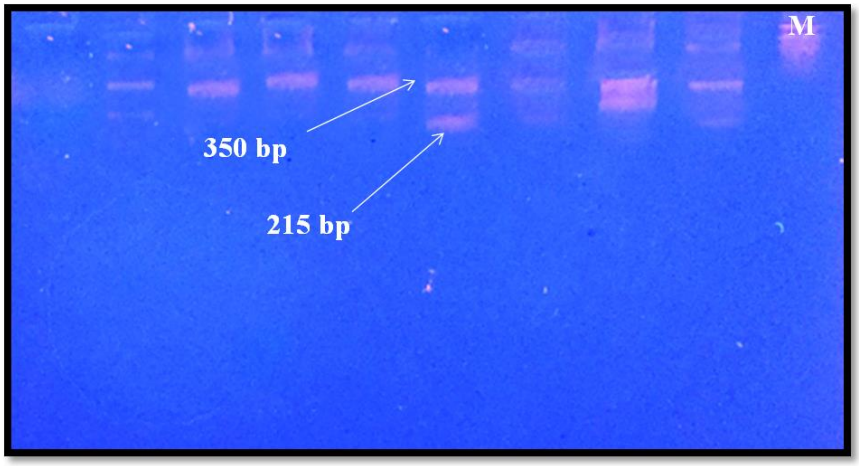

Figure (3): Detection AGT M235T polymorphism by the RFLP – PCR, M ladder, PCR products digested with endonuclease *Tth 111I* enzyme and present M235T mutation in some cases.

Table (3): The frequency of ACE genotypes in T2DM patients

Genotype	Frequency % in all patients	Freq. in male %	Freq. in female %
AGT \ MM (Homozygous)	42.6 %	48.1 %	41.1%
ACE \ MT (Heterozygous)	47.5 %	48.1 %	41.1%
ACE \ TT (Homozygous)	9.8 %	3.8%	14.7%

In the present study, AGT/MT genotyping revealed that 47.5 % of patients, AGT/MM homozygous had 42.6 % and AGT/TT homozygous had 9.8 % in patients with Hypertension. AGT/MM homozygous was higher in male (48.1 %) compared to female (41.1%). AGT/MT genotyping allele were detected (48.1%) in male compared to (44.1%) of female with no statistically significant difference between the two groups, AGT/TT homozygous was higher in female T2DM (14.5%) than male hypertension patients (3.8%) suggesting a protective role for the AGT gene with decreasing incidence for hypertension (Table 3).

The limitation of our study was the relatively small sample size. The present study could have yielded more consistent results if included more patients and if treatment results were followed in relation to the genotyping(13). In conclusion, our study provides further evidence of a role for genetic variation in AGT M253T polymorphism with risk of hypertension in some Iraqi patients. AGT M253T polymorphic markers may raise the hope to individualize therapy in order to optimize its effectiveness and to reduce adverse effects for genetically different subgroups. Hypertension remains an important focus of investigation.

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