Research Article

Cardiology & Vascular Research

Increased Plasma Angiotensinogen Level, BMI and Its Association with the Angiotensinogen Gene M235T Polymorphism and Essential Hypertension in Myanmar

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Received: 14 November 2017; **Accepted:** 03 December 2017

Citation: Khin Sandar OO, May Pyone Kyaw, Mo Mo Than, et al. Increased Plasma Angiotensinogen Level, BMI and Its Association with the Angiotensinogen Gene M235T Polymorphism and Essential Hypertension in Myanmar. Cardiol Vasc Res. 2017; 1(1); 1-5.

ABSTRACT

Introduction: *There is alarming problem in increasing incidence of hypertension and its complications in Myanmar. The genetic background of hypertension is not known well in Myanmar population. The present study aimed to find out the association with angiotensinogen gene M235T polymorphism, plasma angiotensinogen level, BMI and essential hypertension in local area.*

Methods: *There were 144 subjects, 72 hypertensive and 72 normotensives from internal medicine unit of Mandalay General Hospital and Mandalay area. After getting informed consents, determination of blood pressure and BMI were done. The AGT M235T genotypes were determined by polymerase chain reaction followed by digestion of the products with Tth111I. The determination of plasma angiotensinogen level was done by ELISA method.*

Results: *The odd ratio for essential hypertension of TT genotype was 4.93 (95% CI- 1.97-5.40) and genotype frequency was statistically significant between hypertensive and normotensives, $p<0.001$. And subjects carrying T allele has 2.5 times greater chance for essential hypertension [OR=2.56 (95% CI-1.59-4.13)]. The plasma angiotensinogen level in hypertensive was 65.00 ± 27.73 ng/ml and 24.87 ± 15.06 ng/ml in normotensives ($p<0.001$). Moreover, subjects carrying TT genotype have increased plasma angiotensionogen than other genotypes in both hypertensive and normotensives ($P<0.001$). Determination of their BMI, there was found that significance difference between hypertensive than that of normotensives ($p<0.001$). In normotensives, 9.7% has TT genotypes and interestingly they have increased BMI than other genotypes.*

Conclusion: *In fact, the study noted that there was association between AGT M235T polymorphism and increased plasma angiotensinogen and increased BMI in essential hypertension.*

Keywords

Angiotensinogen gene polymorphism, Essential hypertension.

Introduction

Nowadays, there is alarming in increasing impact of hypertension affecting man-power in all sectors of Myanmar. Even though it

is a non-communicable disease, its prevalence is quite high in Myanmar. In Myanmar, the prevalence of hypertension was 30.1 % (95 % CI: 28.4-31.8) in males and 29.8 % (28.5-31.1) in females [1]. Moreover, hypertension is the tenth leading cause of death in Myanmar.

Essential hypertension is a multifactorial and complex disorder not only arising from the influences of several susceptible genes but also environmental stimuli and life style. The activation of Renin-Angiotensin-System (RAS) is one of the main pathogenesis of hypertension and there have been many studies about genes coding for the Renin-Angiotensin-System. Among the outstanding genes suspected for the pathogenesis of hypertension, AGT M235T polymorphism might be very likely prone to be a cause. Many studies have been found some correlations between this gene polymorphism and hypertension.

Observation of the neighboring countries of Myanmar, the study conducted with Malaysian [2], Taiwan [3], Hong Kong [4], Han Chinese [5], and South Indian populations [6] showed a strong association of AGT M235T polymorphism with essential hypertension. However, some populations have been challenged with no association between AGT M235T polymorphism and essential hypertension in the Tibet population [7] and in the Mongolian population [8]. The evidence supporting or refuting an association of AGT M235T polymorphism with essential hypertension is inconsistent and controversial. Therefore, this study firstly focused on the getting the confirmation of this gene polymorphism in Myanmar patients and then the results lead on the further correlation with the pathogenesis of essential hypertension and the treatment of the disease.

Angiotensinogen contributes to arterial pressure regulation through extravascular fluid volume control. A correlation between plasma angiotensinogen concentration and blood pressure has been observed and this observation suggests a direct involvement of plasma angiotensinogen in pathogenesis of essential hypertension. A positive association was found between the AGT M235T polymorphism and plasma angiotensinogen concentration with about a 20% increase in plasma angiotensinogen in subjects carrying the “T” allele [9,10].

The aim of present study targeted on the association between the AGT M235T polymorphism and plasma angiotensinogen level in essential hypertension in Myanmar subjects. This study gives information that the influence of the AGT M235T polymorphism upon its functional gene product, plasma angiotensinogen level and emphasize the influence of gene variant predisposing into essential hypertension in Myanmar population.

Methods

This case control study included 72 essential hypertensive patients (28 male, 44 female) with mean age of 49.70 ± 6.27years old. Also 72 healthy subjects (28 male, 44 female) matching in age and sex (48.75 ± 6.61years old) were included as control group. The patients were selected from the outpatient department of Medicine, Mandalay General Hospital and control group from Aungmyaetharzan Township.

A written consent was obtained from all subjects accepting to participate in the study for both the clinical part and the genetic study. All subjects under study were subjected to thorough history taking and the determination of BP and BMI were done. Peripheral blood samples were collected from all subjects and routine investigations were done for determination of plasma angiotensinogen level and genotyping.

In the present study, essential hypertensive subject was defined as the patients who have been diagnosed and treated by physician based on the following criteria: a systolic blood pressure (SBP) >140 mmHg and/or a diastolic blood pressure (DBP) >90 mmHg, for at least two consecutive blood pressure measurements or taken antihypertensive drugs in which no clearly defined aetiology. The normotensive controls were healthy individuals with a negative history of hypertension and with a SBP ≤ 120 mmHg and DBP ≤ 80mmHg, also all study subjects were non diabetic.

Genotyping

DNA samples were isolated from whole blood by the salting out DNA extraction method [11]. The AGT M235T polymorphism was detected by RFLP- PCR using the following primers [12] 5′-CAG-GGT-GCT-GTC-CAC-ACT-GGA-CCC-C-3′as forward primer and 5′-CCG-TTT-GTG-CAG-GGC-CTG-GCT-CTC-T-3′as reverse primer. Master mix was prepared by containing distilled water 37.3 µl, 5 µl 10X PCR buffer with (NH4)2SO4, 2.5 µl MgCL2, 0.2 µl of Taq polymerase (Thermo Scientific), 1 µl of each deoxynucleotide triphosphate, 1 µl of each primer and 1 µl of genomic DNA in a final volume of 50 µl. Individual pipette tips were used for all additions to prevent cross- contamination of the samples. Heat denaturation was done at 94°C for 4 minutes. Then, 35 cycles of 94.C 1min, 69°C 1 min, 72°C 1.5 min were followed by final extension at 72°C for 8 min and were hold at 4°C. And then, checking the PCR products with 3% agarose gel electrophrosis was done.

Amplification resulted in a 165-bp product which was digested using restriction enzyme Tth 111I. Electrophoresis on a 3% agarose gel containing ethidium bromide and UV transillumination were used for analysis. Determination of plasma angiotensinogen level was done by ELISA method. Statistical Analysis was carried out using SPSS version 15.0 software and the following tests were used for data analysis: Chi squared (χ^2) test, t-test was used for comparison between means, Odds ratio (OR) was used for the measurement of association.

Results

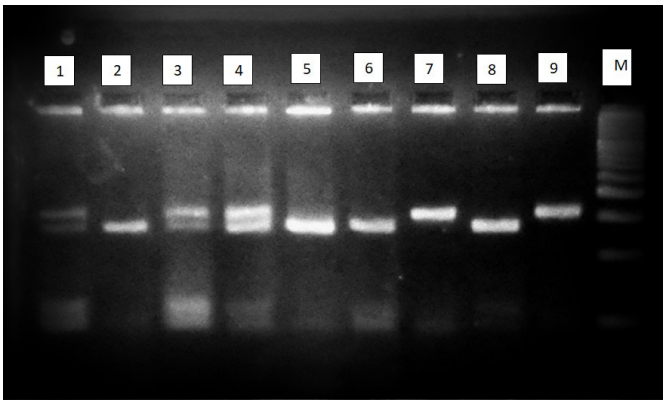
In this study, blood samples were taken from both hypertensive and normotensives according to the inclusion criteria. There were a total of 144 subjects recruited in the study, consisting of age, sex matched 72 hypertensive (28 male and 44 female) and 72 normotensives (28 male and 44 female).

Variables	Hypertensive	Normotensives
Age (Years)	49.70 ± 6.27	48.75 ± 6.61
SBP (mmHg)	140.27 ± 10.84	108.82 ± 6.46
DBP (mmHg)	93.12 ± 8.68	70 ± 5.03
BMI	28.53 ± 7.96	23.73 ± 3.33

Table 1: General characteristics and clinical data of hypertensive and normotensives.

The clinical characteristics of the study population were shown in table 3. The mean age of both hypertensive and normotensives were 49.70 ± 6.27years and 48.75 ± 6.61years. The systolic blood pressure of hypertensive was 140.27 ± 10.84 mmHg and the diastolic blood pressure of hypertensive was 93.12 ± 8.68 mmHg. The systolic blood pressure and diastolic blood pressure of normotensives were 108.82 ± 6.46 mmHg and 70 ± 5.03 mmHg. BMI of hypertensive was 28.53 ± 7.96 and BMI of normotensives was 23.73 ± 3.33.

Genotypes and allele frequencies



MT TT MT MT TT TT MM TT MM 50 bp

Figure 1: Agarose gel with electrophrosis showing amplification of the 165 fragment after enzymatic digestion with the Tth 111I restriction endonuclease enzyme.

Lane 1, 3 and 4 are 141 and 165 bp fragments showing heterozygous (MT). Lane 2, 5, 6 and 8 are 141 bp digested fragments showing homozygous mutant (TT), and lane 7 and 9 are 165 bp fragments showing homozygous wild type (MM) respectively.

AGT Genotype	Noemotensives (n=72)		Hypertensive (n=72)		χ^2 ρ value
	No.	%	No.	%	
MM	17	23.61	2	2.78	13.01
MT	48	66.67	45	62.50	p<0.001*
TT	7	9.72	25	34.72	

Table 2: The distribution of genotype frequencies of AGTM235T in normotensives and hypertensive.

χ^2: Chi square test; *Statistically significant at p<0.05.

In this study, the TT genotypes is more common in hypertensive than those of normotensives (χ^2=13.01, p<0.001). Moreover, the heterozygous MT was the most common type in both hypertensive and normotensives.

Allele frequency	Normotensives (n=72)		Hypertensive (n=72)		χ^2 ρ value
	No.	%	No.	%	
M	82	56.94	49	34.02	15.24
T	62	43.05	95	65.97	p<0.001*

Table 3: The distribution of allele frequencies of AGT M235Tin normotensives and hypertensive.

χ^2: Chi square test; *Statistically significant at p<0.05

The frequency of T allele was significantly increased in hypertensive (χ^2=15.24 and p<0.001).

AGT Genotype	Normotensives (n=72)		Hypertensive (n=72)		Odd ratio (OR)	95% (CI)
	No.	%	No.	%		
MM	17	23.61	2	2.78	0.1092	0.02-0.49
MT	48	66.67	45	62.50	0.83	0.43-1.65
TT	7	9.72	25	34.72	4.93	1.97-3.40

Table 4: The risk of having AGT M235T polymorphism in relation to essential hypertension.

Allele frequency	Normotensives (n=72)		Hypertensive (n=72)		Odd ratio (OR)	95% (CI)
	No.	%	No.	%		
M	82	56.94	49	34.02		
T	62	43.05	95	65.97	2.56	1.59-4.13

Table 5: The risk of having T allele in relation to essential hypertension.

Subjects having TT genotypes have more risk of the developing essential hypertension and the results were statistically significant [OR = 4.93 95% CI (1.97-3.40)]. Moreover, subjects having T allele were higher risk to essential hypertension and the results were statistically significant [OR = 4.93 95% CI (1.97-3.40)].

	Hypertensive (n=72)	Normotensives (n=72)	ρ value
Plasma angiotensinogen level (ng/ml)	65.00 ± 27.73	24.87 ± 15.06	<0.001

Table 6: Plasma angiotensinogen level of hypertensive and normotensives.

Results were shown as Mean ± SD.

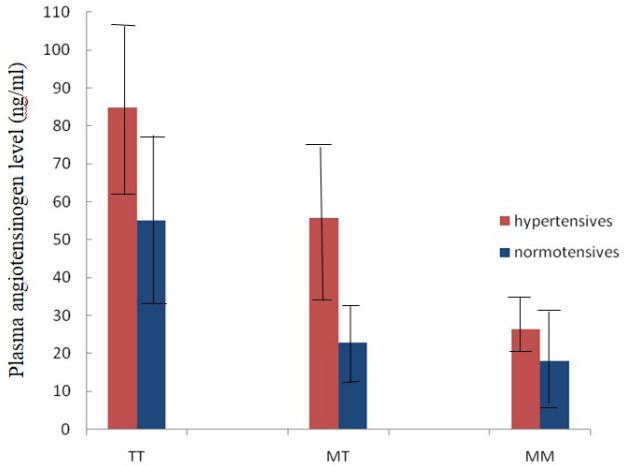


Figure 2: Comparison of plasma angiotensinogen level in hypertensive and normotensives with different genotypes of AGT M235T.

The plasma angiotensinogen level was significantly increased in hypertensive than those of normotensives ($p<0.001$).

The comparison of plasma angiotensinogen level in TT genotype and MT, the plasma angiotensinogen level was statistically increased in hypertensive than those of normotensives ($p<0.5$ and $p<0.001$).

Discussion

Hypertension is a complex multifactorial disorder with genetic, environmental and demographic factors contributing to its prevalence. The genetic element contribution to blood pressure variation ranges from 30 to 50%. Therefore, identifying hypertension susceptibility genes will help understanding the pathophysiology of the disease [13].

The main objective of this study was to get the confirmation of the association of AGT M235T polymorphism and essential hypertension in Myanmar population. Our results showed the TT genotypes is more common in hypertensive than those of normotensives ($\chi^2=13.01$, $p<0.001$). Interestingly, the heterozygous MT was the most common type in both hypertensive and normotensives. Subjects having TT genotypes have more risk of the developing essential hypertension and the results were statistically significant [OR = 4.93 95% CI (1.97-3.40)]. The frequency of T allele was significantly increased in hypertensive ($\chi^2=15.24$ and $p<0.001$). Moreover, subjects having T allele were higher risk to essential hypertension and the results were statistically significant [OR = 4.93 95% CI (1.97-3.40)].

The present study was similar to association between AGT M235T polymorphism and essential hypertension in China population [14]. The AGT M235T was associated with hypertension and independent risk factors of hypertension in the Japanese (OR=1.59 (1.25-2.01) p=0.0001) [15]. Studies from European population, there was inconsistent finding of association between AGT M235T polymorphism and essential hypertension. In the meta-analysis of study in Germany, there was no association between AGT M235T and essential hypertension [OR=0.52 (0.28-0.96)] [16]. However, there was found association between AGT M235T polymorphism and essential hypertension in the study of meta-analysis in European [OR=1.52 (1.08-2.12)] [17]. Furthermore, the results of highly statistically significant association between the AGT M235T polymorphism and essential hypertension in Egyptian patients were reported odd ratio was 36.217, $p<0.001$ for TT genotype compared to MM genotype [18].

The allele frequencies and genotype distribution of the M235T variant were not in the Hardy-Weinberg equilibrium in either data set for hypertensive ($\chi^2 = 11.06$, df = 2, $p<0.5$) and for normotensives ($\chi^2 = 10.07$, df = 2, $p<0.5$) subjects. There was significant difference in genotype and allele frequencies between hypertensive and normotensive groups. It may be due to random chance. There may be natural selection or something else going on. Another point was due to the small sample size in the present study.

The second objective of the study is the presence of polymorphism of angiotensinogene gene (M235T) that may effect on its phenotype that is increased level of plasma angiotensinogen level in essential hypertension. In this study, the plasma angiotensinogen level of the whole study was 29.99 ± 9.54 ng/ml and 65.00 ± 27.73 ng/ml in the hypertensive and 24.87 ± 15.05 ng/ml in normotensive control group. There was found that significantly increased in plasma angiotensinogen level in hypertensive than those of normotensives ($p<0.001$).

AGT M235T was in complete linkage disequilibrium with -6 upstream of transcription initiation site that controls the expression of AGT gene. Doing in vitro tests of promoter activity and DNA binding with nuclear proteins, the nucleotide substitution affects the basal transcription rate of AGT gene in various cell lines. Therefore, this underlying mechanism directs the association between AGT "T" allele and increased plasma angiotensinogen level in essential hypertension [19].

Comparison of plasma angiotensinogen level with different genotypes of AGT M235T in hypertensive and normotensives, there was significantly increased in TT and MT genotypes of hypertensive than those of normotensives ($p = 0.005$ and $p<0.001$). But among the MM genotypes between hypertensive and normotensive, there was no difference ($p = 0.3$).

Presence of "T" allele is associated with increased blood pressure. Increased plasma AGT level and its association with M235T gene polymorphism and hypertension in Nigeria, the absorbance values of plasma angiotensinogen were significantly higher in the patients (0.71) with "T" allele than in the controls (0.53) [20].

Determination of their BMI, there was found the significance difference between the hypertensive than that of the normotensives ($p<0.001$). In normotensives, 9.7% have TT genotypes and interestingly they have increased BMI rather than others BMI of MM and MT genotypes.

In this study, the heterozygous MT genotype was more common in both hypertensive and normotensives 62.5% and 66.7% respectively and risk for hypertension is 0.83 (95% CI=0.43-1.65). Increased numbers of carrier allele in both groups has notified in the present study. Moreover, risk allele carriers had increased plasma angiotensinogen level in hypertensive than those of normotensives (55.77 ± 23.22 and 22.89 ± 9.23 ng/ml and p value was <0.001). In this study, the heterozygous MT genotype was more common in both hypertensive and normotensives 62.5% and 66.7% respectively and risk for hypertension is 0.83 (95% CI=0.43-1.65). Increased numbers of carrier allele in both groups has notified in the present study. Moreover, risk allele carriers had increased plasma angiotensinogen level in hypertensive than those of normotensives (55.77 ± 23.22 and 22.89 ± 9.23 ng/ml and p

value was <0.001).

In fact, the study has been noted that there was association between angiotensinogen gene M235T polymorphism and increased plasma angiotensinogen level that found in the homozygous TT genotype of AGT M235T and essential hypertension and notified risk allele carrier, heterogygous MT genotype in normotensives in Myanmar.

Conclusion

This study identified the significant association of the AGT M235T variant with essential hypertension and has shown that higher frequencies of "T"allele carrier genotypes in this study. Homozygous TT genotype and "T" allele was significantly associated with essential hypertension. Plasma angiotensinogen level was increased in hypertensive than normotensives. There was significant association between AGT genotypes and plasma angiotensinogen level. Moreover, TT genotype was associated with increased level of plasma AGT in both hypertensive subjects and normotensives, suggesting that AGT variants might be play a critical role in the pathogenesis of essential hypertension.

Therefore, this study gave information that AGT M235T is an important gene for determining susceptibility to essential hypertension. By studying the AGT M235T gene, normal subjects with risk allele carrier have higher plasma agiotensinogen level and increased risk to essential hypertension.

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