ACE Single Nucleotide Polymorphism I/D and Its Relationship to ACE Level in Egyptian Patients with Essential Hypertension

Seham A Khodeer[1], Dalia H Abou-Elela[1*], Yassein S Yassein[2]

[1]Clinical Pathology Department, Faculty of Medicine, Menoufia University.Shebein El-Kom, Egypt.[2]Internal Medicine Department, Faculty of Medicine, Menoufia University. Shebein El-Kom, Egypt.
*aboeleladalia@yahoo.com

Abstract: Background: Renin-Angiotensin system is one of the factors that have an important role in controlling blood pressure and sodium homeostasis. Angiotensin converting enzyme (ACE) has a role in hypertension pathogenesis. The ACE gene is located on chromosome 17 where more than 160 ACE gene polymorphisms have been reported. Although I/D polymorphism is located in a non-coding region of the ACE gene, several investigators have found that the D allele is related to the activity of plasma ACE. The aim of this workwas to investigate the association between ACE I/D polymorphism gene with plasma ACE level in patients with essential hypertension.Subjects and methods: The study was conducted on one hundred and fifty subjects who were divided into three groups: 53 prehypertensive & 58 hypertensive patients in addition to 39 normotensive gender and age matched subjects. Full clinical examination and history were taken, lipid profile & plasma ACE by ELISA were determined to all subjects. Also, polymerase chain reaction was used to determine the distribution of allele and genotype frequency of ACE I/D polymorphism. Results: Comparing with the normotensive & pre-hypertensive groups, the hypertensive patient group exhibited a higher distribution of the DD genotype (10.3%, 17.0% & 50%, respectively) & D allele frequency (10.3%7, 17.9% & 63.8%, respectively). By using logistic regression analysis, subjects with the DD genotype & D allele were at increased risk for hypertension (OR 1.41, 95% CI 0.68 –2.93 & OR 8.05, 95% CI 4.03 –16.08, respectively) compared with those having the II genotype and I allele. Comparing DD genotype with both II & ID genotypes in the hypertensive patient group, they had higher levels of ACE enzyme (P < 0.001 for both). Conclusion: The observed association of D/D genotype with essential hypertension emphasizes on the need for further prospective study that include larger sample size to confirm the results of the present study.
[Seham A Khodeer, Dalia H Abou-Elela, Yassein S Yassein. **ACE Single Nucleotide Polymorphism I/D and Its Relationship to ACE Level in Egyptian Patients with Essential Hypertension.** *J Am Sci* 2015;11(10):168-175]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 16. doi:10.7537/marsjas111015.16.

Key words: ACE gene –I/D polymorphism- Essential hypertension.

1. Introduction:

Essential hypertension (EH) is the clinical expression of a disordered interaction between the genetic, physiological, and biochemical systems that under usual conditions maintain cardiovascular homeostasis [1]. Angiotensin-converting enzyme (ACE), a key zinc metalloenzyme of the rennin-angiotensin system [2]. The ACE catalyzes the conversion of angiotensin I to the biologically active peptide, angiotensin II, which is an aldosterone-stimulating peptide with a direct, potent vasopressive effect on the peripheral vasculature, and plays a pivotal role in electrolyte and circulatory homeostasis [3].

The ACE gene is located on long arm of chromosome 17 (17q23.3). The gene is 21 kilo bases (kb) long and comprises 26 exons and 25 introns. More than 160 ACE gene polymorphisms have been reported so far and most of which are single nucleotide polymorphisms (SNPs). Only 34 of those polymorphisms are located in coding region of this gene [4 &5] were the first to report the insertion/deletion (I/D) polymorphism of ACE. This polymorphism is characterized by the presence (insertion) or absence (deletion) of a 287 bp AluYa5 element inside intron 16 producing three genotypes (II homozygote, ID heterozygote and DD homozygote). Although I/D polymorphism is located in a non-coding region (namely intron) of the ACE gene, several investigators have found that the D allele is related to the activity of ACE in blood[6].

The number of studies carried out around the world suggested the genetic predisposition of the ACE I/D polymorphism with several diseases including coronary heart diseases, stroke, hypertension and diabetes mellitus [7&8]. However, conflicting results have been reported regarding the association between ACE polymorphism and disease [9] Moreover, various reports were published suggesting inter-ethnic variations in the frequency of allelic forms of the ACE genes [10].

The aim of this work:

Was to investigate the association between ACE I/D polymorphism gene with plasma ACE level in patients with essential hypertension.

2. Subjects&Methods:

The present study was carried out at Clinical Pathology Department in collaboration with Medical Biochemistry& Internal Medicine Departments, Faculty of Medicine, Menoufia University in the period between March, 2012 & September 2013. The study was conducted on one hundred and fifty subjects who were divided into three groups: fifty three prehypertensive subjects (thirty two males and twenty one females) with a mean age ±SD of 54.09±10.33, fifty eight hypertensive patients(thirty four males and twenty four females) with a mean age ±SD of 57.55±10.07in addition to thirty nine gender and age matched normotensive subject (sixteen males and twenty three females) with a mean age ±SD of 54.10±8.59 out of the thirty nine controls, Ten subjects had previous history of smoking and; twenty one subjects had family history of hypertension. Thirty one of prehypertensive subjects had previous history of smoking and twenty two hadn't; thirty nine subjects had family history of hypertension and fourteen subjects hadn't. Thirty one of hypertensive subjects had previous history of smoking and twenty seven hadn't; twenty eight subjects had family history of hypertension and thirty subjects hadn't. Blood pressures were measured in all three groups of the study subjects using sphygmomanometer at least three times at an interval of five minutes in accordance with the procedures recommended by JNC VII (The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High blood pressure) criteria. The subjects were seated in a chair with back support and their feet on the ground and the arm placed comfortably on a table at heart level. There was 5 min of rest in a quiet room preceding the blood pressure measurements. The appropriate-sized cuff, based on the patient's arm circumference was placed on the upper arm, 5 cm above the ulnar head. Hypertension was defined as a sustained diastolic blood pressure ≥90 mmHg that is accompanied by an elevated systolic blood pressure ≥140 mm Hg. Prehypertension was defined as between 120/80 mmHg and 139/89 mmHg. Exclusion criteria:chronic kidney disease (creatinine>1.5 mg/dl), serum cholesterol >240 mg/dl as secondary hypertension was excluded using detailed health questionnaire and clinical evaluation. Subjects with a history of diabetes mellitus, major infectious disease were excluded. They had no metabolic or endocrine disorder or any acute illness. The normotensive subjects were recruited from employee in the hospital. They had no history of hypertension.

For all the subjects the followings were done: history and clinical examination, body mass index (calculated by dividing body weight in kilograms by the square of the height in meters). Laboratory investigations including fasting blood sugar,serum creatinine& lipid profile (total cholesterol, triglycerides, LDL-C& HDL-C), plasma ACE & polymerase chain reaction was used to determine the distribution of allele and genotype frequencies of the I/D polymorphism in ACE gene. Written informed-consents were provided by all participants.

Sampling:

Under complete aseptic conditions, 5 ml of venous blood were collected after 12 hour fasting & divided as follows: Tube A, 1ml of blood collected in citrate (to prevent clotting and DNA degradation) for DNA extraction and kept immediately at -20°C. Tube B, 2 ml were collected, left to clot and serum was separated and used for immediate assay of lipid profile& fasting blood glucose. Tube C, 2 ml were collected on citrate, plasma was separated and kept at -20 C° for assay of ACE.

Laboratory investigations:

Serum creatinine, fasting blood sugar, total cholesterol, triglycerides, and HDL-C concentrations were determined by using an enzymatic colorimetric assay on SynchronCx9 (Beckman Instrument. Inc. Fullerton, California USA). LDL-C concentration was calculated according to the Friedewald formula [11], plasma ACE level was measured by kit supplied by Human ACE ELISA (MyBiosource USA).

DNA analysis:

DNA extraction: DNA was purified from whole blood using Axygen Prep Blood Genomic DNA Miniprep Kit for the purification of genomic DNA from whole blood. This method was based on the efficient release of genomic DNA from anti-coagulated whole blood by a special cell lysis and heme/protein precipitation buffer coupled with the selective adsorption of the genomic DNA to a special AxyPrep column. The purified genomic DNA was eluted in a low salt Tris buffer containing 0.5 mM EDTA which enhanced DNA solubility and helped to protect the high molecular weight DNA against subsequent nuclease degradation. The eluted genomic DNA was kept immediately at -20°C subjected to PCR.

PCR amplification:

The DNA was then subjected to nested PCR amplification of the insertion/deletion (I/D) polymorphic site of the ACE gene DreamTaq™ Green PCR Master Mix (Fermentas)was a ready to use solution containing DreamTaq™ DNA polymerase, optimized DreamTaq™ Green buffer, $MgCl_2$ and dNTPs. The master mix was supplemented with two tracking dyes and a density reagent thatallow for direct loading of the PCR product on a gel.

Reaction mixture:

For a total 50 µl reaction volume: DreamTaq™ Green PCR Master Mix (2X) 25 µl, GIIS Primer 2µl, GAS Primer 2 µl, Extracted DNA 10 µl, Water nuclease-free 11 µl.

Primers:

The lyophilized primers (Fermentas Life Sciences) were reconstituted by addition of sterile water to a final concentration of 50 pmoles/µl and distributed in aliquots and stored at –20°C. The primer sequences were as follow:

Forward primer (GIIS) 5′CTCAAGCACGCCCCTCACAGGACTG-3′.

Reverse primer (GAS)5′-GATGTGGCCATCACATTCGTCATCAGAT-3′.

Nested primer (FYM) 5′-ATCACGAGGTCAGGAGATCGGGAGAC-3′.

The forward and reverse primers span the insertion in intron 16 of the ACE gene, yielding two bands of different lengths, depending on the presence or absence of the insertion. Since this PCR reaction may fail to amplify the I polymorphism across the insertion, and may thus potentially lead to misclassification of an I allele as a D allele, a nested FYM primer that extends the insertion segment was used.

Protocol of amplification:

The reaction mixture was heated to 95° C for 1.5 min and then amplified for an additional 20 cycles of 95° C for 30 s, 62° C for 30 s, and 72° C for 30 s. The tubes were cooled to 4° C after 20 cycles, and 1µl of GIIS and FYM primers (5′-ATCACGAGGTCAGGAGATCGGGAGAC-3′) were added to the reaction mixture. The PCR was then continued for an additional 20 cycles. The FYM primer is internal to the insertion in intron 16. As a result, the second PCR reaction amplifies only the I allele. The amplification products were separated by electrophoresis on 2% agarose gel stained with ethidium bromide and visualized on a UV trans-illuminator, ACE I/D genotyping was determined based on the discriminating band size pattern where band sizes of approximately 561-bp results from the extension of the I allele between the GIIS and GAS primers, and a 274-bp product results for the D allele. The extension between the FYM and GIIS primers results in a 376-bp product for the I allele only. Hence, the II genotype is indicated by presence of the 376-bp and 561-bp bands, the DD genotype is represented only by the 274-bp band and the ID genotype is represented by the presence of all three bands.

Statistical analysis:

The statistical analysis was undertaken using SPSS software (version 17; SPSS Inc., Chicago, IL, USA). Descriptive statistics in the form of mean and standard deviation for parametric data were used. Chi-square test ($\chi 2$) was used for qualitative variables. T-test for comparison between the two groups having quantitative variables normally distributed, man-witteny test for comparison between two groups having quantitative variables not normally distributed, ANOVA test for comparison between the three groups having quantitative variables normally distributed followed by LSD (least significant difference) and Kruskal-Wallis test for comparison between three groups not normally distributed having quantitative variables. Odd ratios (ORs) and 95% confidence intervals (CI) were calculated by logistic regression analysis. The significance level was set at 0.05 or less.

3. Results:

Comparing with the normotensive & pre-hypertensive groups, the hypertensive patient group exhibited a higher distribution of the DD genotype (10.3%, 17.0% & 50%, respectively) & D allele frequency (10.3% 7, 17.9% & 63.8%, respectively). By using logistic regression analysis, subjects with the DD genotype & D allele were at increased risk for hypertension (OR 1.41, 95% CI 0.68 –2.93 & OR 8.05, 95% CI 4.03 –16.08, respectively) compared with those having the II genotype and I allele. When the hypertensive patient group was subclassified according to genotype, the DD genotype exhibited higher frequency of BMI >25 and higher levels of ACE enzyme compared with both II & ID genotypes. While no statistical significant difference was found between the different genotypes regarding other studied parameters in the same group.

Table1: Baseline characteristics of the studied subjects.

Variables	Pre-hypertension N = 53	Hypertension N = 58	Normotensive controls N = 39
Age X ± SD0	54.09±10.33	57.55±10.07	54.10±8.59
Gender Male Female	 32 (60.4%) 21 (39.6%)	 34 (58.6%) 24 (41.4%)	 16(41%) 23 (59%)
Residence Rural Urban	 23 (43.4%) * 30 (56.6%)	 26 (44.8%) 32 (55.2%)	 25 (64.1) 14 (35.9)
Smoking Smoker Non smoker	 31 (58.5%) * 22 (41.5%)	 31 (53.4%) * 27 (46.6%)	 10 (25.6) 29 (74.4)

Variables	Pre-hypertension N = 53	Hypertension N = 58	Normotensive controls N = 39
Family history Positive Negative	 39 (73.6%) * 14 (26.4%)	 28 (48.3%) € 30 (51.7%)	 21 (53.8%) 18 (46.2%)
BMI < 25 > 25	 17 (32.1%) 36 (67.9%)	 9 (15.5%) *€ 49 (84.5%) *	 14 (35.9 %) 25(64.1 %)
Systolic X ± SD(mmHg)	131.81±4.70*	177.87±20.66*€	109.35±7.44
DiastolicX ± SD(mmHg)	83.77±2.65*	92.60±17.45*€	70.0±6.68
Serum creatinine X ± SD(mg/dl)	0.98±0.23*	0.83±0.19*€	0.75±0.13
TriglyceridesX ± SD(mg/dl)	154.98±27.75*	268.79±75.56*€	135.15±7.91
Total cholesterol X ± SD(mg/dl)	185.96±47.71*	237.22±56.20*€	144.33±23.03
HDL-CX ± SD (mg/dl)	46.75±8.36*	41.05±8.47*€	66.20±5.37
LDL-CX ± SD(mg/dl)	108.45±49.50*	142.05±50.23*€	50.38±21.25
ACE plasma level X ± SD(mg/dl)	448.96±81.72*	618.41±150.85*€	287.69±67.60

* = Significant with control group
€ = Significant with prehypertension group

Table 2:Genotypic and allelic distributions of I/D polymorphism of ACE gene in the studied groups

Variables	Pre-hypertension N = 53	Hypertension N = 58	Controls N = 39	χ	P value	Odds ratio 95% CI
Genotype **DD** **DI** **II**	 9 (17.0%)a 7 (13.2)a 37 (69.8)d	 29 (50.0%)b 16 (27.6)c 13 (22.4)c	 4 (10.3%)a 6 (15.4)a 29 (74.4)d	 0.86* 26.89** 25.39***	 0.65 <0.001 <0.001	1.41 (0.68 – 2.93)
Allele **D** **I**	**N = 106** 25 (23.6)a 81 (76.4)c	**N = 116** 74(63.8)b 42 (36.2)d	**N = 78** 14(17.9)a 64 (82.1)c	0.85 39.55 36.24	0.35 <0.001 <0.001	8.05 (4.03 – 16.08)

* = comparison between control group and pre hypertension group
** = comparison between control group and hypertension group
*** = comparison between pre hypertension group and hypertension group
- different letters means significance

Table (3):Baseline characteristics of hypertensive group classified by genotype.

Variables	DD N=29		ID N=16		II No = 13		Test (P value)	Post–hoc test
	No	%	No	%	No	%		
Gender Male Female	 15 14	 51.7 48.3	 11 5	 68.8 31.3	 10 3	 76.9 23.1	2.84 (0.24)	>0.05* >0.05** >0.05***
Residence Rural Urban	 11 18	 37.9 62.1	 11 5	 68.8 31.3	 4 9	 30.8 69.2	5.3 (0.07)	>0.05* >0.05** >0.05***
Smoking Smoker Non smoker	 15 14	 51.7 48.3	 8 8	 50.0 50.0	 8 5	 61.5 38.5	0.45 (0.79)	>0.05* >0.05** >0.05
Family history Positive Negative	 13 16	 44.8 55.2	 7 9	 43.8 56.2	 8 5	 61.5 38.5	1.18 (0.55)	>0.05* >0.05** >0.05***

Variables	DD N=29		ID N=16		II No = 13		Test (P value)	Post–hoc test
	No	%	No	%	No	%		
BMI < 25 > 25	 00 29	 00 100	 2 14	 12.5 87.5	 04 09	 31.8 69.2	 6.7 (0.03)	<0.05* <0.05** <0.05***
Triglyceride X ± SD(mg/dl)	282.44±73.4		257.43±84.37		252.30±68.79		2.12 (0.35)	>0.05* >0.05** >0.05***
Total cholesterol X ± SD(mg/dl)	235.14±36.36		255.62±88.24		219.23±37.07		3.24 (0.20)	>0.05* >0.05** >0.05***
HDL-c X ± SD(mg/dl)	41.41±8.64		41.37±8.32		39.85±8.83		0.34 (0.84)	>0.05* >0.05** >0.05
LDL-c X ± SD(mg/dl)	137.17±38.30		161.56±73.16		128.92±33.44		2.05 (0.36)	>0.05* >0.05** >0.05***
ACE plasma level X ± SD (mg/dl)	751.65±55.47		537.18±64.13		421.15±43.78		46.83 (<0.001)	<0.001* <0.001** <0.001***

4. Discussion:

Incidence of hypertension is increasing alarmingly in various populations of developing nations. It is universally accepted that systemic hypertension is a distinct risk factor for various cardiovascular emergencies, particularly left ventricular failure, myocardial infarction, and stroke [12].

In the current study (Table1), no statistical significant differences were found between the studied groups regarding to age and gender. While, there were statistical significant differences between both hypertensive & prehypertensive patient groups comparing with normotensive control group as regard smoking. The highest BMI were observed in the hypertensive patient group which exhibited a higher statistical significant difference comparing with the normotensive group. From the result of this study, it could be reported that there is a relationship between BMI and hypertension(BMI>25 91.4%) which is similar to Lu et al.[13].

In the present study as regard lipid profile, the hypertensive patient group had a higher statistical significant differences regarding total cholesterol, triglycerides, LDL-C than both prehypertensive and normotensive control groups. While, the hypertensive patient group had a lower statistical significant differences regarding HDL-C comparing with the other studied groups. The study informed that serum cholesterol, Triglycerides & LDL-C in prehypertension subjects were statistically higher than those of normotension ones. Sposito et al.[14]reported that there was a significant association between hypercholesterolemia and hypertension. This relationship was influenced by some mechanisms, such as decreased bioavailability of nitric oxide, enhanced activity of vasoconstrictor (angiotensin II and endothelin-1), decreased salt sensitivity, enhanced oxidative stress, etc.

The renin-angiotensin system has been identified by many studies to be the most important of the endocrine systems that affect the control of blood pressure as reported by Sipahi et al.([15].Regarding ACE enzyme level in the current study, there were higher statistical significant differences between hypertensive group (618.41±150.85pg/ml) and both pre hypertensive (448.96±81.72 pg/ml) and the control groups (287.69±67.60 pg/ml, p<0.001 for both). Also, a higher statistical significant difference was found between prehypertension and the normotensive control groups (p<0.001). ACE catalyses the conversion of angiotensin I to angiotensin II, a potent vasoconstrictor in a substrate concentration-dependent manner[16]. Also, ACE degrades bradykinin, a potent vasodilator, and other vasoactive peptides [17]resulting in high blood pressure.

Hypertensive group had a statistical significant higher distribution of the DD genotype andD allele frequency (50 %, 63.8 %) than those of prehypertension group (17 %, 23.6%) & the normotensive controls (10.3%, 17.9 %). Meanwhile, there was no statistical significance between prehypertension group & the normotensive control group regarding DD genotype and D allele frequencies. The highest frequency of II genotype and I allele were observed in the healthy normotensive controls, then

prehypertensive group and lastly the hypertension group. Hypertension is a multifactorial disorder and evidence indicates that certain synergistic risk factors accelerate its incidence. In the current study by using logistic regression analysis revealed that hypertension group with the DD genotype as well as D allele were at increased risk for hypertension (OR 95% CI 1.41(0.68-2.93), OR=95%CI 8.05 (4.03-16.08), respectively) compared with those having the II genotype and I allele.

Recent studies of the association of hypertension with ACE genotype have shown a positive correlation of the D allele with hypertension in a south Indian population, juvenile American, Turkish or Sardinian populations. These findings suggest that in some populations, presumably in the face of various environmental variables, the D allele of the ACE gene, which elevates the activity of ACE, may be associated with hypertension [(18)].In accordance with these results a study was done in Han Chinese population by Ji et al.[(19)] demonstrated a positive association between the Dgeno type of the ACE gene and hypertension. Also, the results of Saab and colleagues[(20)], in the Lebanese population found a significant difference between hypertensive and normotensive groups across genotypes for the DD genotype. Also, D allele frequency was significantly different between the hypertensive and normotensive groups. Moreover, Bawazier and co-workers[(21)] found that ACE ID+DD genotype has significant relationship with hypertension in Melati population, Sleman, Yogyakarta, Indonesia. To explore the relationship between the ACE gene I/D polymorphism and essential hypertension in the Chinese population, 67 separated studies were analyzed in a meta-analysis including 21,058 participants by Li [(22)], stated that there was a significant association between distribution frequency of the D allele and essential hypertension risk in Han, Kazakh, Tibetan, Zhuang and unclassified nationalities. Also, a study done by Qingfang and co-workers[(2)] indicating that the ACE gene I/D polymorphism appeared strong evidence to support the role of the D-allele and/or DD genotype as a risk factor for hypertension. According to Rasyid et al.[(10)], the distribution of ACE genotypes is different among ethnic groups all over the world. Therefore, it is important that studies of ACE gene polymorphism be conducted in genetically homogenous populations.

To the opposite of our results, in a sample of Brazilian women, theI allele has been seen to be associated with a systolic blood pressure [(18)]. A study done in Pakistan by Ismail et al.[(23)] they found that the frequency of the ACE I/I genotype was significantly higher in hypertensive patients, aged 20-40 years, than in normotensive controls of the same age group. In amore recent study in India, Srivastava and co-workers[(24)], findings suggested that the I allele of ACE I/D polymorphism is associated with essential hypertension in their population.

On the other hand, Mondry et al.[(25)] who found that there is no significant association of essential hypertension with the ACE gene I/D polymorphism in German population. Also, a study done by Danková et al.[(26)],including two ethnic population samples from Slovakia -Romany and Slovaks- subjects demonstrated that the frequency of the mutant D allele was higher in the Slovak subjects than in the Romany subjects, but the difference was not significant. Also, Kabadou[(27)] revealed that the ACE I/D polymorphism is not significant factor for hypertension in the Tunisian population. Moreover, a study done by Li[(22)] in China, found that in the national minorities, such as Mongolian, Uigur the association between distribution frequency of the D allele and essential hypertension risk was not significant. Also, In a cross sectional study, Rasyid and co-workers[(10)] did not support the association of the I/D polymorphism at the ACE gene locus with hypertension in Makassar, South Sulawesi, Indonesia.

These discrepancies in results may be explained by variable sample sizes; gender or publication bias (positive results being easier to publish).Also, the different populations represent different gene pools. This suggesting that gene-disease relationships can be expected to be different between populations due to the differences in a compound genetic set.

The results obtained by this study (Table 3) revealed that in hypertension group stratified by ACE I/D gene polymorphism showed no statistical significant differences regarding the studied parameters except for, BMI > 25 kg/m2 and ACE enzyme levels($p<0.05$ for all).In accordance to the present study, Samani et al.[(28)] found that individuals with II genotype had the lowest circulating ACE levels as compared to DDgenotype, which are known to had high ACE levels. Moreover, Mehri et al.[(29)]stated that ACE DD genotype associated with higher serum ACE activity. In contrast to the present results Qingfang and co-workers[(2)] showed that both in hypertensive cases, ACE activities were highest in II carriers, then in DD carriers, and lowest in ID carriers.

It has been speculated that ACE and the components of the RAS are expressed in adipose tissue and therefore might be associated with obesity. Wacker et al.[(30)] indicated that the ACE I/D polymorphism may be differentially associated with obesity depending on multiple factors. However, Cooper et al.[(31)] pointed out that the I/D polymorphism of the ACE gene was associated with variation in the levels of ACE, but inconsistently with BMI. Therefore, the investigators suggested that obesity may alter the levels of ACE and angiotensinogen, and provide a potential pathway

through which obesity leads to elevation of blood pressure.

In Yogyakarta, Indonesia and to the opposite of the present study, Sinorita et al.[32] investigation showed no significant association between distribution of ACE I/D genotype and component of metabolic syndrome such as central obesity, hypertension, and dyslipidemia. Also, In a study done by in Saudian subjects, the hypertensive cases associated with obesity and cases with hypertension alone showed higher frequencies of the mutant D allele carriage than controls, but this was statistically non-significant[33].

Recognition of genetic marker as potential risk factor for essential hypertension will assist in stratifying our population into a high risk group that might develop hypertension in their course of life. By using logistic regression analysis, the results showed that the genetic marker ACE DD is associated with EH. This could mean that persons exposed to a cluster of variables have the highest propensity for development of EH. Genetic system being the non changeable factor in causation of any disease, more consideration should be given to correct the modifiable environmental factors. The clinicians can categorize a susceptible group of patients based on ACE genotypes that require intensive monitoring and adjustment of modifiable riskfactors like high BMI etc., for prevention of future development of hypertension.

Conclusion:

The observed association of D/D genotype with essential hypertension emphasizes on the need for further prospective study that include larger sample size to confirm the results of the present study.

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10/25/2015