RELATIONSHIP OF RAS GENE POLYMORPHISM TO TYPE-II DIABETES AND SYNDROME-X

Mohammed S. Fawzy , Ibrahim El-Maghawry, Nader M.M.A., Eman Abd Elkader Abd Elsalam and Osama A. Khalil*
Medical Biochemistry and Internal Medicine* Departments
Faculty of Medicine, Zagazig University

ABSTRACT

Metabolic syndrome, syndrome X, which is reaching epidemic proportions in population, is a cluster of insulin resistance and/or type-II diabetes mellitus with two or more of hypertension, dyslipidemia, central obesity and albuminuria in an individual patient. Genetic predisposition for metabolic syndrome was, to large extent, believed to be an important aspect in its pathogenesis. The renin-angiotensin system (RAS) genes are proposed as important genetic factors for diabetic complications. Therefore, the angiotensin converting enzyme (ACE) gene polymorphisms (II, ID or DD), which is an important component of RAS genes, might be included in the pathogenesis of metabolic syndrome and is a candidate gene for investigation in metabolic syndrome. We aimed to study the possible ACE genotyping-plasma ACE activity-metabolic syndrome relationship, and to assess the possible role of ACE genotyping in the pathogenesis of variable components of metabolic syndrome. This study is also a trial to take the distribution of ACE-I/D genotype among subjects as a possible risk marker for metabolic syndrome. ACE genotypes were determined by PCR amplification, and plasma ACE activity was measured by colorimetric method in 100 subjects (40 metabolic syndrome patients diagnosed according to WHO criteria, 30 type-II diabetic patients without any other criteria of metabolic syndrome, and 30 healthy controls). Insulin resistance was judged by homeostasis model assessment (HOMA) index after estimation of fasting blood glucose and plasma insulin. Moreover, HbA_{1c}, plasma lipids including total cholesterol, LDL-c, HDL-c, triglycerides and APO-A were assessed. Microalbuminuria was determined by dipstick method. The indices body mass index (BMI) and waist:hip ratio (WHR) were used to differentiate obese from non-obese subjects. ACE-DD genotype and D-allele were found more frequent among metabolic syndrome patients (Odds ratios were1.25 and 1.16 respectively) and among type-II diabetics (Odds ratios were1.25 and 1.10 respectively) than among healthy controls; and more frequent among metabolic syndrome patients than among type-II diabetic patients (Odds ratios were 1.10 and 1.32 respectively). The plasma ACE activity was found significantly higher in patient's groups compared to healthy subjects and in metabolic patients compared to diabetics. Also, it was significantly and positively correlated to HOMA index in both metabolic syndrome and diabetic patients. The plasma ACE also in overall studied subjects had direct significant correlation with FBG, HbA_{1c}, plasma insulin, HOMA index, TC, LDL-c, and TG; and indirect

significant correlation with HDL-c and APO-A. Moreover, in the three studied groups DD genotype subgroups had a statistically significant increase in plasma ACE activity, FBG, HbA_{1c}, plasma insulin, HOMA, total cholesterol, LDL-c, and triglycerides and a significant decrease in HDL-c and APO-A compared to II genotype subjects. Lastly, the ACE-DD genotype was associated with hypertension and with microalbuminuria than any of II genotype (Odds ratios were 3.50 and 6 respectively) and ID genotype (Odds ratios were 2.33 and 1.29 respectively); but not associated with obesity.

In conclusion, *ACE Deletion Polymorphism; DD genotype was associated with metabolic syndrome and type-II diabetes mellitus as well as with obvious increase in plasma ACE activity. All components of metabolic syndrome, except obesity were more aggressive when the ACE genotype was DD. Therefore, ACE may be a strong genetic risk factor that is involved in the pathogenesis of metabolic syndrome with type-II diabetes. Moreover, by detection of DD genotype, we can predict the higher possibility of occurrence of metabolic complications in type-II diabetics in the future and suggest early interventions to delay or prevent these complications.*

INTRODUCTION

WHO[1] proposed a working definition of metabolic syndrome or insulin resistance syndrome as an association of impaired glucose tolerance, diabetes mellitus and/or insulin resistance with two or more of the following components: hypertension, dyslipidemia, central obesity, and microalbuminuria. Then, the **NCEP and ATP III**[2] modified the WHO cut-off values and definition (required the presence of 3 of 5 of: abdominal obesity, hyper-triglycedidemia, low HDL-c, hypertension, and impaired glucose tolerance based on measuring FBG). **IDF**[3] again proposed that abdominal obesity is diagnostic of metabolic syndrome if associated with 2 or more of ATP III criteria**.**

Metabolic syndrome is a cluster of metabolic abnormalities including the most dangerous risk factors for cardiovascular diseases: glucose intolerance and diabetes, abdominal obesity, high cholesterol, and arterial hypertension[4,5].

Various genetic factors in combination with specific environmental factors are believed to attribute to metabolic syndrome. Genes for β_2- and β_3-adrenergic receptors, lipoprotein lipase, hormone sensitive lipase, peroxisome proliferator-activated receptor-γ, insulin receptor substrate-1, and glycogen synthase are among several candidate genes involved in the metabolic syndrome[6]**.**

Important genes that, by genetic studies, were proposed to be important factors for diabetic complications are those of renin-angiotensin system (RAS), the system that has been documented as an important regulator of blood pressure and renal homeostasis. Of these RAS genes, is the gene coding for the dipeptidyl carboxypeptidase (a zinc metallopeptidase) termed angiotensin-I-converting enzyme (ACE); or kininase II. This enzyme act to hydrolyze angiotensin-I to the potent

vasopressor and aldosterone-stimulating angiotensin-II, as well as to inactivate the vasodilator bradykinine[7].

The ACE gene has 26 exons and span 21kb on chromosome 17, with polymorphism consisting of either the presence (insertion; I allele) or absence (deletion; D allele) of 287 bp base pair DNA fragment within the intron 16, giving three ACE genotypes: DD genotype, II genotype, and the ID genotype[7].

As regard the ACE genotyping, it was found that the D allele is associated with higher serum ACE activity[8]; and I/D polymorphism is associated with: diabetes[9], hypertension[10], coronary heart disease[11] and with diabetic nephropathy[12]. Therefore, ACE might be a candidate gene for metabolic syndrome.

So, by comparing ACE genotyping and activity among healthy subjects, type-II diabetics, and those with the cluster of the metabolic syndrome, we tried to demonstrate if there is an association between metabolic syndrome and the ACE genotyping and activity, whether the ACE polymorphism participate in the pathogenesis of various metabolic syndrome's components, and to show the distribution of ACE genotypes among the studied groups.

SUBJECTS & METHODS

One hundred subjects, with the same social and economic levels, were enrolled in this study. They were termed as the following:

1. **Control group (Group I):** including 30, completely healthy, subjects (14 males and 16 females). Their ages ranged between 42 and 63 years with mean value ± SD of 52.03 ± 6.40.
2. **Type-II diabetic group (Group II):** comprising 30 patients with type-II diabetes mellitus, according to ADA criteria[13] without any other criteria of metabolic syndrome. The patients were 17 males and 13 with age ranging from 42 to 65 years and with a mean value ± SD of 52.20 ± 6.69.
3. **Metabolic syndrome group (Group III):** enrolling 40 patients (20 males and 20 females) between 47 and 63 years with a mean value ± SD of 52.97 ± 5.46. These patients were selected from endocrinology outpatient clinic according to WHO criteria of metabolic syndrome[1].
4. Patients with renal or hepatic disease, patients on ACE inhibitor medication and/or smoker were excluded from this study.

After full history was taken, complete clinical examination was performed and routine laboratory investigations were done. Fasting (12 - 14 hrs) blood samples were collected and divided into three tubes; EDTA tubes (whole blood for ACE genotyping and HbA_{1C} assay, and EDTA plasma for insulin determination), fluoride/oxalate tubes (plasma for glucose estimation) and plain tubes (sera for other investigations). Morning urine samples were collected for microalbumin assessment. Plasma, serum and urine and samples were stored at -20°C[14] until analysis.

1- ACE genotyping[15]:

DNA was extracted from whole blood samples using E.Z.N.A. blood DNA kit (supplied by Omega Bio-Tek) according to the protocol provided by the manufacturer.

The ACE I/D gene polymorphism from DNA samples was determined by polymerase chain reaction (PCR) according to the method described by ***Lee and Tsai*[15]**. The primers sequences were (F:5'-CTG GAG ACC ACT CCC ATC CTT TCT-3') and (R:5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3').

The reactions were transferred to the thermal cycler PTC-100 machine (MJ Research, Inc., Watertown, Mass. USA) and subjected to an initial denaturation at 94º C for 10 minutes followed by 35 cycles of (94º C for 1 minute, 60º C for 1 minute, 72º C for 1 minute) then final extension step at 72º C for 5 minutes.

After PCR, the samples were separated by 2% agarose gel electrophoresis, stained with ethidium bromide and photographed for the amplification study. The agarose gel showed an amplification band of 190 bp in samples with the homozygous DD genotype, a band of 490 bp in samples with the homozygous II genotype and two bands of 490 and 190 bp in samples with the heterozygous ID genotype.

2- ACE activity assay: It was assessed by colorimetric method according to **Hurst and Lovell-Smith[16]** using Spectronic 3000 Array, Milton-Roy, USA. The kits were purchased from Buhlamann laboratories AG, Switzerland.

3- Lipid profile assay: Enzymatic method was applied for determination of triglycerides[17], total cholesterol[18] and high density lipoprotein cholesterol (HDL-c)[19]**.** According to **Friedwald et al.[20]**, low density lipoprotein cholesterol (LDL-c) was calculated by the equation that [LDL-c = total cholesterol – (HDL-c + TG/5)]**.** Apolipoprotien A-1, as an index of dyslipidemia, was also measured by immunoturbidometric assay[21].

4- Detection of microalbumiuria: This was done by Micral test, a test strip for immunochemical, semiquantitative determination for microalbuminuria[22] using strips, kit supplied by Roche Diagnostics Ltd.

5- Glycemic indices:

a- Fasting plasma glucose was determined by enzymatic oxidase method[23].

b- Glycosylated hemoglobin (HbA_{1C}) was assayed according to **Bisse and Abraham[24].**

c- Fasting plasma insulin was measured by ELISA using monoclonal antibodies directed against distinct epitopes of insulin according to **Temple, et al. [14]**. The kits are provided from BISOURCE, Belgium**.**

d- Assessment of insulin resistance by homeostasis model assessment (HOMA) index that is calculated from the following formula:

- HOMA index= fasting insulin (μU/ml) x fasting

plasma glucose (mmol/l)/22.5.

- HOMA index >1.46 is an indication of insulin resistance[25].

Statistical analysis:

Statistical analysis was performed using the computer software statistical package of social science (SPSS 10.0, 1999 SPSS Inc.); data were expressed as mean ±SD.

Comparison between data of different groups was done using one-way ANOVA followed by multiple comparison for significance of difference (Least significant difference; LSD), whereas the non parametric values were compared by the test Chi-square. Universal correlations were performed using Pearson correlation and p value <0.05 was considered statistically significant.

To detect the likelihood of an event between 2 groups the Odds ratio was calculated by the dividing the odds in the exposed group by the odds in the control group.

RESULTS

The ACE I/D genotyping was demonstrated in figure (1). Our results were shown in table (1) up to table (8) and in figure (2).

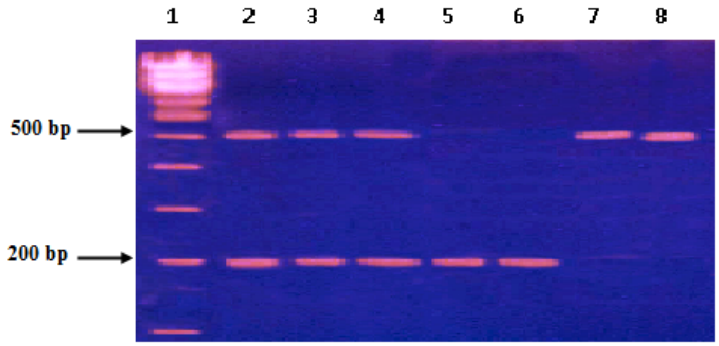

Figure (1): The Angiotensin converting enzyme I/D genotyping. The figure shows a 2% agarose gel, stained with ethidium bromide, of I/D PCR genotyping reactions. (Lane 1) DNA ladder. (Lanes 2,3 and 4) ID heterozygote, showing amplification of two bands. (Lanes 5,6) DD homozygote showing amplification of only the 190-bp band. (Lanes 7,8) II homozygote, identified by presence of 490-bp band and absence of 190-bp band

Table (1): Distribution of Angiotensin converting enzyme genotypes and allele frequencies in overall studied groups (above) and between studied groups (below)

Group	Genotype					Allele			
	II No (%)	ID No (%)	DD No (%)	X^2	P	I No (%)	D No (%)	X^2	P
All studied subjects	38 (38%)	43 (43%)	19 (19%)	9.62	<0.05	119(59.5%)	81 (40.5%)	7.22	<0.05

	Genotype			Allele	
	II No (%)	ID No (%)	DD No (%)	I No (%)	D No (%)
Group I (control)	13 (43.3%)	12 (40.0%)	5 (16.7%)	38 (63.3%)	22 (36.7%)
Group II (type 2 diabetics)	13 (43.3%)	11 (36.7%)	6 (20.0%)	37 (61.7%)	23 (38.3%)
Group III (metabolic syndrome)	12 (30.0%)	20 (50.0%)	8(20.0%)	44 (55%)	36 (45%)
X^2	0.53	3.39	0.73	0.72	4.51
P	>0.05	>0.05	>0.05	>0.05	>0.05

***Insertion: I allele** ***Deletion: D allele**

Table (2): Odds ratio (OR) of Angiotensin converting enzyme genotypes and allele frequencies between studied groups

Groups	Genotypes						Allele			
	II		ID		DD		I		D	
	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
Group I vs. group II (control vs. diabetics)	1	0.32-3.15	0.87	0.27-2.79	1.25	0.28-5.90	0.93	0.42-2.08	1.10	0.48-2.40
Group I vs. group III (Control vs. metabolic pts.)	0.77	0.18-1.69	1.5	0.52-4.38	1.25	0.31-5.47	0.71	0.34-1.48	1.16	0.67-2.97
Group II vs. group III (Diabetics vs. metabolic pts.)	0.56	0.18-1.69	1.73	0.59-5.10	1.10	0.45-2.38	0.76	0.36-1.59	1.32	0.63-2.67

***OR>1 means +Ve association or risk.** ***OR: Odds ratio.**
***CI: 95% confidence interval.** ***Insertion: I allele**

***Deletion: D allele**

Table (3): Effect of Angiotensin converting enzyme genotype on components of metabolic syndrome in the metabolic syndrome group

Genotype	N	Obesity N (%)	Hypertension N (%)	Microalbuminuria N (%)
II	**12**	**10 (83.3%)**	**8 (66.0%)**	**4 (30.0%)**
ID	**20**	**15 (75.0%)**	**15(75.0%)**	**14(70.0%)**
DD	**8**	**6 (75.0%)**	**7 (87.5%)**	**6 (75.0%)**
Chi-square		**0.58**	**1.18**	**6.93**
P		>0.05	<0.05	<0.05

***Insertion: I allele** ***Deletion: D allele**

Table (4): Association between nonparametric criteria of metabolic syndrome and the three Angiotensin converting enzyme genotype subgroups in metabolic syndrome group by Odds ratio (OR)

	Criteria					
	Obesity		Hypertension		Microalbuminuria	
Genotypes	**OR**	**CI**	**OR**	**CI**	**OR**	**CI**
DD vs. II	0.64	0.05-8.6	3.56	0.25-63.3	7.88	0.82-16.1
DD vs. ID	1.0	0.11-10.4	2.29	0.17-63.8	1.35	0.15-13.4
ID vs. II	0.64	0.06-5.31	1.56	0.24-10.4	5.85	0.97-39.7

***OR>1 means +ve association or risk.** ***OR: Odds ratio.**
***CI: 95% confidence interval.** ***Insertion: I allele**
***Deletion: D allele**

Table (5): Mean ± SD of some studied parameters among the different groups

	Group			Statistical values	
Parameter	**Group I (mean±SD)**	**Group II mean±SD**	**Group III (mean±SD)**	**F**	**P**
ACE activity (U/l)	14.43±3.96	31.93±8.20 **a**	40.48±11.20 **a,b**	77.31	<0.001
FBG (mg/dl)	87.90±8.91	192.67±37.83 **a**	192.53±38.17 **a**	110.92	<0.001
HbA_{1c} (%)	5.08±0.69	10.19±1.86 **a**	9.75±1.66 **a**	105.76	<0.001
Fasting insulin (µU/ml)	3.68±0.67	13.48±3.96 **a**	14.65±5.38 **a**	68.33	<0.001
HOMA index	1.06±1.29	6.07±2.11 **a**	6.39±2.02 **a**	2.18	<0.001
Total cholesterol (mg/dl)	165.67±11.86	204.03±18.82 **a**	240.83±22.54 **a,b**	138.06	<0.001
LDL-c (mg/dl)	91.08±18.97	130.92±18.25 **a**	163.79±21.29 **a,b**	116.44	<0.001
HDL-c (mg/dl)	49.51±4.36	42.16±3.15 **a**	36.62±2.91 **a,b**	118.15	<0.001
Triglycerides (mg/dl)	89.60±13.01	164.73±29.60 **a**	199.10±32.90 **a,b**	139.66	<0.001
APO-A (mg/dl)	150.57±6.45	140.27±4.81 **a**	132.98±5.32 **a,b**	68.36	<0.001

***a ,b :** point to significant difference using LSD (P<0.01) **where:**

a: points to significant difference in comparison to control group (group I)

b: points to significant difference in comparison to type 2 diabetic group (group II)

Table (6): Mean ± SD of some parameters among the study groups in the different genotype subgroups

Parameters of genotypes subgroups		Group I mean±SD	Group II mean±SD	Group III mean±SD	F	P
II	ACE activity	15.85±3.36	32.57±5.07[a]	37.84±5.52[a,b]	77.07	<0.001
	FBG	87.85±7.50	196.15±38.92[a]	186.33±36.95[a]	47.73	<0.001
	HbA_{1c}	5.07±0.68	10.01±1.83[a]	9.19±1.43 [a]	46.34	<0.001
	Plasma insulin	3.45±0.68	10.95±1.91[a]	11.27±3.20 [a]	54.27	<0.001
	HOMA index	0.70±0.14	4.55±0.83[a]	5.11±0.39 [a,b]	255.08	<0.001
	Total cholesterol	163.53±10.83	200.31±13.46[a]	228.83±20.30[a,b]	57.92	<0.001
	LDL-c	86.29±8.61	126.39±14.8[a]	153.46±15.09[a,b]	77.82	<0.001
	HDL-c	51.87±2.38	43.03±2.75[a]	38.11±1.79[a,b]	110.12	<0.001
	Triglycerides	83.00±5.94	149.69±21.78[a]	181.58±27.77[a,b]	76.56	<0.001
	APO-A	151.62±4.93	142.85±2.76[a]	137.75±4.07[a,b]	38.34	<0.001
ID	ACE activity	15.86±3.36	32.57±5.07[a]	37.85±5.52[a,b]	77.07	<0.001
	FBG	90.00±9.04	199.18±40.36[a]	186.80±22.24[a]	67.47	<0.001
	HbA_{1c}	5.21±0.69	9.91±1.96[a]	9.45±1.49[a]	39.37	<0.001
	Plasma insulin	4.17±0.52	13.65±0.86[a]	14.44±4.25[a]	49.19	<0.001
	HOMA index	0.95±0.12	6.41±1.46[a]	6.40±0.80[a]	152.67	<0.001
	Total cholesterol	162.75±11.91	198.27±18.68[a]	240.15±4.93[a,b]	64.79	<0.001
	LDL-c	87.25±25.41	123.36±19.80[a]	161.52±21.19[a,b]	43.19	<0.001
	HDL-c	49.53±3.88	42.89±2.44[a]	36.80±2.62[a,b]	69.13	<0.001
	Triglycerides	89.33±14.09	165.82±30.96[a]	197.05±31.27[a,b]	57.81	<0.001
	APO-A	152.83±5.69	139.91±4.66[a]	132.25±3.96[a,b]	73.02	<0.001
DD	ACE activity	19.10±2.18	39.45±4.45[a]	59.58±7.60[a,b]	78.44	<0.001
	FBG	83.00±11.79	173.1±28.72[a]	216.13±61.93[a]	13.91	<0.001
	HbA_{1c}	4.82±0.77	11.10±1.73[a]	10.46±2.20[a]	20.38	<0.001
	Plasma insulin	4.22±0.36	18.63±5.66[a]	20.26±6.34[a]	15.86	<0.001
	HOMA index	2.29±3.08	8.77±2.21[a]	8.31±3.69[a]	7.24	<0.001
	Total cholesterol	178.20±6.38	222.67±19.94[a]	260.50±13.19[a]	49.87	<0.001
	LDL-c	110.14±6.79	150.05±12.53[a]	184.99±15.47[a,b]	52.59	<0.001
	HDL-c	43.3.±3.63	38.93±3.44	33.96±3.34[a]	11.62	<0. 01
	Triglycerides	107.40±6.07	195.33±17.64[a]	230.50±22.41[a,b]	72.44	<0.001
	APO-A	142.40±6.11	135.33±5.05[a]	127.63±3.99	14.18	<0.001

***a ,b :** point to significant difference using LSD (P<0.01) **where:**
a: points to significant difference in comparison to control group (group I)
b: points to significant difference in comparison to type 2 diabetic group (group II)
***Insertion: I allele**
***Deletion: D allele**

Table (7): Mean ± SD of some parameters among the genotype subgroups in the different study groups

Parameters of genotypes subgroups		Genotype II (mean±SD)	Genotype ID (mean±SD)	Genotype DD (mean±SD)	F	P
Group I(control subjects)	ACE activity	11.32±2.10	15.86±3.36[a]	19.10±2.18[a,b]	17.89	<0.001
	FBG	87.85±7.50	90.00±9.03	83.00±11.79	1.09	>0.05
	HbA_{1c}	5.07±0.68	5.21±0.69[a]	4.82±0.77	0.55	>0.05
	Plasma insulin	3.45±0.68	4.17±0.52[a]	4.22±0.36[a]	5.94	<0.01
	HOMA index	0.70±0.14	0.95±0.12	2.29±3.08	3.32	>0.05
	Total cholesterol	163.54±10.83	162.75±11.91	178.20±6.38[a]	4.08	<0.05
	LDL-c	87.28±8.98	87.25±25.41	110.14±6.79[a]	3.57	<0.05
	HDL-c	51.87±2.38	49.53±3.88	43.30±3.63[a]	12.51	<0.001
	Triglycerides	83.00±5.94	89.33±14.09	107.40±6.07[a]	10.54	<0.001
	APO-A	151.62±4.93	152.83±5.69	142.40±6.11[a]	6.94	<0.01
Group II (type 2 diabetics)	ACE activity	27.14±7.99	32.57±5.06[a]	39.45±4.45[a,b]	7.69	<0.01
	FBG	196.15±38.92	199.18±40.36	173.17±28.72	1.02	>0.05
	HbA_{1c}	10.01±1.83	9.91±1.96	11.10±1.73	0.91	>0.05
	Plasma insulin	10.95±1.91	13.65±0.86[a]	18.63±5.66[a,b]	15.53	<0.01
	HOMA index	4.55±0.83	6.41±1.46[a]	8.77±2.21[a,b]	18.82	<0.001
	Total cholesterol	200.31±13.46	198.27±18.68	222.67±19.94[a]	4.64	<0.05
	LDL-c	128.49±13.07	123.36±19.80	150.05±12.53[a]	5.79	<0.01
	HDL-c	43.03±2.75	42.89±2.44	38.93±3.44[a]	5.03	<0.05
	Triglycerides	149.69±21.78	165.82±30.96	195.33±17.63[a]	6.88	<0.01
	APO-A	142.85±2.76	139.91±4.66	135.33±5.05[a]	7.24	<0.01
Group III (metabolic syndrome pts.)	ACE activity	32.13±3.07	37.85±5.52[a]	59.58±7.60[a,b]	66.21	<0.01
	FBG	186.33±36.95	186.80±22.24	216.13±61.93	2.01	>0.05
	HbA_{1c}	9.19±1.43	9.45±1.49	10.46±2.20	1.57	>0.05
	Plasma insulin	11.27±3.20	14.44±4.25	20.26±6.34[a]	9.79	<0.01
	HOMA index	5.11±0.39	6.40±0.80[a]	8.31±3.69[a,b]	8.34	<0.01
	Total cholesterol	228.83±20.30	240.15±22.05	260.83±22.54[a]	5.96	<0.01
	LDL-c	153.46±15.09	161.52±21.19	184.99±15.47[a]	7.25	<0.01
	HDL-c	38.11±1.79	36.80±2.62	33.96±3.34[a]	6.33	<0.01
	Triglycerides	181.58±27.77	197.05±31.27	230.50±22.41[a]	7.05	<0.01
	APO-A	137.75±4.07	132.25±3.96[a]	127.63±3.99[a,b]	16.04	<0.001

***a ,b :** point to significant difference using LSD (P<0.05) **where:**

a: points to significant difference in comparison to control group (group I)

b: points to significant difference in comparison to type 2 diabetic group (group II)

***Insertion: I allele *Deletion: D allele**

Table (8): Correlation between plasma angiotensin converting enzyme activity and different studied parameters among diseased subjects and overall studied subjects

	Diseased subjects		Overall subjects	
Parameter	**R**	**P**	**R**	**P**
Fasting blood glucose (mg/g)	0.044	>0.05	0.635	<0.001
HbA_{1c} (%)	0.087	>0.05	0.641	<0.001
Fasting plasma insulin (µU/ml)	0.509	<0.001	0.785	<0.001
HOMA index	0.480	<0.001	0.774	<0.001
Total cholesterol (mg/g)	0.529	<0.001	0.784	<0.001
LDL-c (mg/g)	0.530	<0.001	0.779	<0.001
HDL-c (mg/g)	-0.537	<0.001	-0.778	<0.001
Triglycerides (mg/g)	0.560	<0.001	0.818	<0.001
APO-A (mg/g)	-0.598	<0.001	-0.771	<0.001

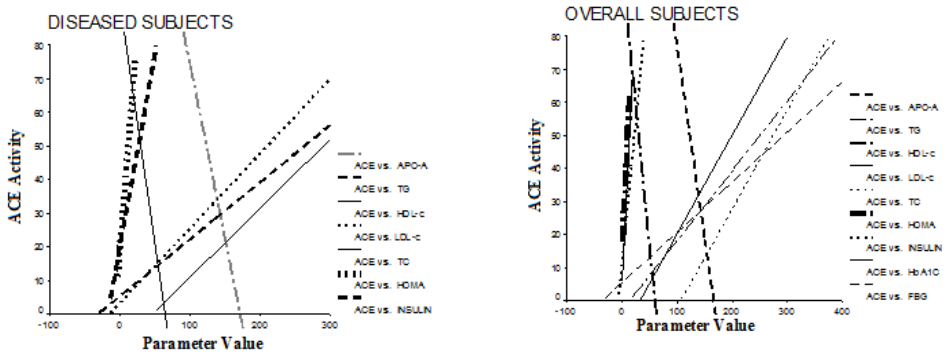

Figure (2): Significant correlation between plasma angiotensin converting enzyme activity and different studied parameters in the diseased and overall studied subjects

DISCUSSION

Metabolic syndrome is defined as impaired glucose regulation or diabetes mellitus and/or insulin resistance, associated with hypertension, obesity, dyslipidemia and/or albuminuria[26].

RAS, by genetic studies, has been proposed as an important genetic factor for diabetic complications and metabolic syndrome[27]. Among RAS genes, is the gene encoding for ACE, a key enzyme in the RAS system that play an important documented role in the regulation of body's internal environment. The ACE gene is characterized by insertion or deletion (I/D) polymorphism based on presence or absence of 278-bp repeat sequence within the intron 16 located on chromosome 17q23 respectively[28].

The current study revealed that ACE genotypes distribution among overall included subjects was 38%, 43% and 19 % for II, ID and DD

respectively, whereas allele frequencies were 59.5 and 40.5 for I and D alleles respectively, with significant differences between these percentages. The distribution of each ACE genotype and each allele did not differed significantly between the three groups of study.

Moreover we revealed that one carrying DD genotype and D allele among the type-II diabetes group were 1.2 and 1.1 times more than subjects carrying DD genotype and D allele among control subjects respectively. As regard the metabolic syndrome it was found that the presence of DD genotype and D allele in metabolic syndrome subjects was 1.3 and 1.4 times more than in control subjects respectively; and 1.1 and 1.3 times more than in type-II diabetic subjects respectively.

These observations suggest a significant association between ACE DD genotype and both type-II diabetes mellitus and metabolic syndrome as well as suggesting D allele as a more risk factor, than I allele, for both morbidities.

The results of the current study agreed with those reported by **Stephens and his coworkers**[29] who have found an association between ACE DD genotype and type-II Caucasian diabetics with study included non diabetic controls. This association was, also, reported by **Feng et al.** [30] and by **Daimon et al.** [31].

Opening a field of controversy, **Lee and Tsai**[15] carried out a study on Chinese and reported that although the ACE I/D polymorphism was not associated with type-II diabetes, it was associated with metabolic syndrome.

In contrast to the results of the present study, **Costa et al.**[32] concluded that, in Brazilians, there was no an effect of ACE genotyping on metabolic syndrome with type-II diabetes. They attributed these controversies to different ethnic factors. Also, **Sinorita et al.**[33] stated that there was no association between metabolic syndrome and the component of metabolic syndrome and variants of the ACE gene among the type-II diabetes patients.

The results of present study revealed significant differences in plasma ACE activity levels between the three studied groups and also between the three genotype subgroups. The levels were significantly higher among patient's groups compared to controls and also in patient's genotype subgroups compared to the corresponding control genotype subgroups. Also, plasma ACE activity levels were significantly higher among metabolic groups and its genotype subgroups than among type-II diabetic groups and its corresponding genotype subgroups. Furthermore, in all studied groups there was a significant increase in plasma ACE level in subjects with DD genotype (highest levels) than in subjects with ID or II genotypes; and in subjects with ID genotype (intermediate levels) than in subjects with II genotype (lowest levels).

These observations suggested that increased plasma ACE activity levels among metabolic syndrome and type-II diabetes patients is possibly the high prevalence of D-allele containing ACE genotypes. The pathogenetic effect of increased ACE activity results from increased conversion of

angiotensin I to angiotensin II with subsequent elevation of blood pressure, stimulation of renal mesangial cell proliferation, activation of synthesis and inhibition of breakdown of collagen, inhibition of fibrinolysis, and stimulation of both cytokines and growth factor secretion[27]. So, D-allele can predict who person will pass to complications.

In agreement with the current results, **Rigat et al.**[8] reported a progressive increase in plasma ACE activity levels in patients with DD, ID and II genotypes; whereas **Tsutaya et al.**[34] reported increased plasma and tissue ACE levels among DD genotype subjects. Positive association between D-allele and plasma ACE levels was also reported by **Agerholm-Larsen et al.**[35].

Degirmenci et al.[36]**,** in agreement with the results of this study, found that Turkish type-II diabetics with ACE DD genotype had a higher ACE activity levels than those with ID and II genotypes.

The present study's results disagree with those reported by **Nagi et al.**[37] who stated that plasma ACE concentrations were not significantly different between diabetic and non-diabetic subjects.

In this study, the indices of insulin resistance and glucose metabolism were significantly higher in patient's groups and subgroups compared to the control group and corresponding subgroups, but no significant differences in these indices between metabolic group and diabetic groups and their corresponding genotype subgroups.

As regard the differences between the three ACE genotypes, it was found that, in the patient's groups, subject with DD genotype have a statistically significant increase in insulin resistance indices compared to subjects with ID and II genotypes; although no significant differences were found between the three ACE genotype subgroups in fasting blood glucose and HbA_{1c} among all studied groups.

Moreover, there was a significant direct correlation between plasma ACE activity (which was found to be highest among DD genotype subgroups) and FBG, HbA_{1c}, plasma insulin, and HOMA index in overall studied subjects. In diseased subjects plasma ACE activity was found to be directly and significantly correlated to both plasma insulin and HOMA index. In type 2 diabetics and in metabolic syndrome subjects, the contribution of increased plasma ACE activity to insulin resistance was suggested by significant direct correlation between plasma ACE level and both fasting plasma insulin and HOMA index.

The above mentioned results suggest that ACE gene DD genotype and D-allele contribute, possibly via increasing plasma ACE activity, to the pathogenesis of insulin resistance in type 2 diabetes mellitus and in metabolic syndrome[9].

The mechanism of contribution of RAS in insulin resistance could be explained by the fact that RAS and insulin signaling shared signal transduction pathways. Thus activation of RAS may inhibit metabolic action of insulin via phosphatidylinositol 3-kinase (PI3-K).

Anigiotensin II has been suggested to upregulate TNF-α that may mediate insulin resistance via interfering with phosphorylation of insulin receptors, inhibition of action of glucose transporter protein GLUT-4, inhibition of glucose stimulating insulin release from pancreatic β-cells and inhibition of action of lipoprotein lipase[38,39].

In agreement to our results, the significant association between insulin resistance and ACE DD genotype was reported by **Viitanen et al.**[40] and, in hypertensives, by **Perticone et al.**[41]; whereas the absence of significant difference in HbA_{1c} and blood glucose between ACE genotypes was reported by **Hsieh et al.**[9] and by **Lee and Tsai**[15]. In contrast, **Zingone et al.**[42] found a significant increase in fasting blood glucose levels among subjects with DD polymorphism than among those with II polymorphism.

Moreover, our results disagreed with those of **Ryan et al.**[43] who concluded a higher plasma insulin and insulin resistance with ACE II genotype than with DD genotype in healthy overweight. **Paolisso et al.**[44] also reported higher insulin resistance in aged healthy Italians with II ACE genotype than in those with ID and DD genotypes. On the other hand, absence of association between insulin levels and/or insulin resistance and ACE genotyping has been reported in studies of **Huang et al.**[45] in control and type-II diabetic subjects.

The results of present study represent significant differences in all studied parameters of lipid profile (total cholesterol, LDL-c, HDL-c, triglycerides and APO-A) between the three groups of the study. The levels of total cholesterol, LDL-c and triglycerides were significantly higher, whereas the levels of HDL-c and APO-A were significantly lower in patient's groups compared to control group and in metabolic syndrome group compared to diabetic group. Also, when the corresponding genotype subgroups in each studied group were compared to each other the differences were the same as when the whole groups were compared.

Moreover, with focusing on different ACE genotypes, the study revealed that, in the three studied groups, subjects with DD genotype have a significant increase in plasma total cholesterol, LDL-c and triglycerides levels; and a significant decrease in plasma HDL-c and APO-A levels compared to subjects with ID or II genotype.

The correlation study revealed that plasma ACE activity has a significant direct correlation with total cholesterol, LDL-c and triglycerides levels and a significant inverse correlation with HDL-c and APO-A levels among overall subjects, diseased subjects and metabolic syndrome subjects. In diabetic subjects plasma ACE was significantly and inversely correlated to HDL-c and APO-A, whereas in controls ACE directly correlated to LDL-c and indirectly to HDL-c significantly.

These results would suggest that ACE gene deletion polymorphism and increased ACE activity are obvious risk factors for dyslipidemia particularly the patterns of dyslipidemia observed in metabolic syndrome and in type-II diabetes[4].

As regard the differences in plasma lipids between diabetics and non-diabetics, the results of **Hsieh et al.**[9] and **Lee and Tsai**[15] were in agreement with our results. They revealed significantly higher plasma total cholesterol and triglycerides in diabetics than in non diabetics. Also, studies of **Frenais et al.**[46] and **Dionyssiou-Asteriou et al.**[47] reported significantly lower APO-A among diabetics than among non-diabetics.

As regard differences in plasma lipids with different ACE genotypes **Lee and Tsai**[15] similar to our results, reported a higher prevalence of dyslipidemia and higher serum triglycerides in diabetic patients with DD genotype than in those with II genotype. Also, **Oren et al.**[48] stated that the mean LDL-c level was highest in DD genotype subjects and lowest in II subjects.

Controversial to our results **Nagi et al.**[37] did not find an association between these lipid parameters and ACE genotyping in Pima Indians. But with respect to the effect of ACE activity on lipid profile, they revealed a significant direct correlation between the plasma ACE levels and both total cholesterol and triglycerides levels in diabetics and non-diabetics.

Our study concluded a higher incidence of microalbuminuria among metabolic syndrome patients with ACE D-allele containing genotypes than those with insertion polymorphism. By application of Odds ratio, it was found that the occurrence of microalbuminuria among DD genotype patients was 6 and 1.29 times higher than its occurrence among II and ID genotypes respectively. Also, the incidence of microalbuminuria with ID genotype was 4.66 times more than with II genotype. These results showed that ACE gene deletion may be associated with increased susceptibility to albuminuria in metabolic syndrome and in type 2 diabetic patients. Therefore, ACE gene deletion may play a role in the development and progression of nephropathy in these patients, predicting who will develop these complications.

The contribution of ACE DD genotype to this albuminuria may be attributed to our results that ACE DD genotype was associated with increased plasma ACE activity; with subsequent increase in activation of angiotensin I to angiotensin II Angiotensin II then may promote glomerular measngial and renal tubular cell growth as well as enhancement of collagen accumulation with subsequent development and progression of albuminuria[49].

Current study's results agree with those revealed by **Ohno et al.**[50] who stated that, in type 2 diabetic patients, the D allele of the ACE gene was more frequent in micro- and macro-albuminuric groups than in normoalbuminuric group. As well, our results are in accordance that of **Hashimoto et al.**[12] who viewed an association between ACE DD genotype and proteinuria in Japanese overweight men.

There was a discrepancy between our results and those of **Nakajima et al.**[51] who found that the distribution of ACE genotypes did not differed significantly between normo-, micro-,

and macro- albuminuric patients, although the distribution of D allele was slightly higher in protinuric than in non-protinuric males but not females.

The results of Odds ratio revealed that the occurrence of hypertension in metabolic syndrome patients carrying DD genotype was 3.50 and 2.33 times more than its occurrence in those carrying II or ID genotypes respectively. Thereafter, ID hypertensive subjects were 1.50 times increased than II hypertensive subjects. These results would suggest the ACE DD genotype to be associated with increased susceptibility to hypertension in metabolic syndrome patients with type-II diabetes. The predisposition of ACE DD genotype to hypertension could be explained by presence of high ACE activity, as showed in our results, with subsequent elevation in arterial blood pressure via well documented mechanisms of angiotensin converting enzyme[49].

Several studies focused on the relation between ACE gene polymorphism and hypertension with variable subject selection and with controversial results. Of these, many agreed with our results including studies of **Fornage et al.**[10] and **O'Donnell et al.**[52] who found the association in hypertensive males; studies of **Duru et al.**[53] and **Barley et al.**[54] who revealed the association in hypertensive individuals; as well as studies of **Morise et al.**[55] and **Maeda et al.**[56] who reported the association in Japanese hypertensives. In all these studies the association was detected between DD genotype or D allele and hypertension.

On the other hand, lack of association between ACE genotyping and hypertension was reported by **Lee and Tsai**[15] in type-II diabetics and by **Hsieh et al.**[9] who detected no differences in blood pressure between ACE II, ID and DD genotypes.

With the respect of obesity, the current study denies presence of association between ACE genotyping and obesity in metabolic syndrome patients. Our results detected increased distribution of ACE II genotype among obese metabolic syndrome patients, meanwhile it was not significant. The lack of association between ACE genotypes and obesity seems to be obscure.

In close to our results **Ryan et al.**[43] reported an association between II genotype of ACE and obesity. **Lee and Tsai**[15] and **Nagi et al.**[37] found no differences in obesity markers (BMI and WHR) between the three ACE genotypes. Also, close to our results,

In contrast to the present study, **Strazullo et al.**[57] revealed a higher prevalence of obesity in Italian males with D/D allele compared to those with I/D or I/I allele, although the differences was not statistically significant.

Genotype II which was relatively abundant in obese subjects may be responsible for production of different ACE phenotype that responsible for ACE activity in adipose tissue.

From these results we can conclude that the ACE gene, one of RAS genes, DD polymorphism was more abundant among metabolic syndrome and type-II diabetic patients; the result that provide an evidence of genetic susceptibility to

the metabolic syndrome. The Deletion polymorphism of ACE gene was, moreover, associated with the components of metabolic syndrome: hypertension and microalbuminuria but not with the obesity. Also, the activity of the ACE gene enzyme was more among these patients and associated with insulin resistance as well as with atherogenic hyperlipidemia. These results suggested that the ACE DD genotype, via increasing plasma ACE activity, may be involved in the pathphysiology of metabolic derangement and type-II diabetes. Moreover, by detection of ACE DD genotype, we can predict the possibility of occurrence of complications in diabetic patients with metabolic syndrome and this can suggest early intervention to delay or prevent these complications..

REFERENCES

1- **World Health Organization (1999):** Report of the WHO consultation: definition of metabolic syndrome in definition, diagnosis and classification of diabetes and its complications. I. diagnosis and classification of diabetes mellitus. Geneva, World Health Organization, Department of Noncommunicable disease Surveillance.

2- **National Cholesterol Education program and Adult Treatment Panel III (2001):** Expert panel on detection, evaluation and treatment of high blood cholesterol in adults J.A.M.A.; 285 (19): 2486.

3- **International Diabetes Federation (2005):** IDF worldwide definition of the metabolic syndrome. Permission from Medscape Diabetes & Endocrinology 2005; 7(2): http://www.medscape.com/viewarticle/514211 © 2005 Medscape.

4- **Lukšienė, D.I.; Bacevičienė, M.; Tamošiūnas, A.; Černiauskienė, L.R.; Margevičienė L. and Rėklaitienė, R. (2010):** Prevalence of the metabolic syndrome diagnosed using three different definitions and risk of ischemic heart disease among Kaunas adult population. Medicina (Kaunas); 46(1): 61.

5- **Tong, W.; Yi, H.; Xin-hua, X.; Duen-mei, W.; Cheng-ming, D.; Feng, Z.; Ling-ling, X.; Yong-biao, Z.; Wen-hui, L.; Li-li, Z.; Yun, Z.; Xiao-fang, S.; and Qian, Z. (2010):** The association between common genetic variation in the FTO gene and metabolic syndrome in Han Chinese. Chin Med J; 123(14):1852.

6- **Grundy S.M., Cleeman J.I., Daniels S.R., Donato K.A., Eckel R.H., Franklin B.A., Gordon D.J., Krauss R.M., Savage P.J., Smith S.C., Spertus J.A., and Costa F. (2005):** Diagnosis and management of metabolic syndrome. Circulation; 112 (17): 2735.

7- **Groop L.C. (2000):** Genetics of the metabolic syndrome. Br. J. Nutri.; 83 (1): S39.

8- **Rigat B., Hubert C., Alhenc-Gelas F., Cambien F., Corvol P. and Soubrier F. (1990):** An

insertion deletion polymorphism in angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J. Clin. Invest.; 86 (4): 1343.

9- **Hsieh M.C., Lin S.R., Hsieh T.C., Hsu H.C., Chen H.C., Shin S.J. and Tsai J.H. (2000):** Increased frequency of angiotensin-converting enzyme DD genotype in patients with type 2 diabetes in Taiwan. Neprol. Dial. Transplant.; 15 (7): 1008.

10- **Fornage M., Amos C.I., Kardia S., Sing C.F., Turner S.T. and Boerwinkle E. (1998):** Variation in the region of angiotensin-converting enzyme gene influences inter-individual differences in blood pressure levels in young white males. Circulation; 97: 1773.

11- **Tiret L., Nicaud V., Kee F., Evans A., Cambou J.P., Arveiler D., Luc G., Amouyel P., Poirier O., Lecerf L. and Cambien F. (1993):** Deletion polymorphism in the angiotensin-converting enzyme gene associated with parental history of myocardial infarction. Lancet; 341 (8851): 991.

12- **Hashimoto Y., Futamura A., Nakara H. and Yokota H., Omura M., Tsukamoto K., Togo M., Sato H., Hara M., Isoo N., Taguchi J. and Nakahara K.(2001):** Association between deletion polymorphism of angiotensin converting enzyme gene and proteinuria in Japanese overweight men. J. Occup. Health; 43: 80.

13- **American Diabetes Association (2005):** Standards of Medical Care. Classification and diagnosis of diabetes. Diabetes Care; 28 (1): s4.

14- **Temple R.C., Clarck P.M. and Hales C.N. (1992):** Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Diabetic Medicine; 9 (6): 503.

15- **Lee Y.J. and Tsai J.C.R. (2002):** ACE gene insertion/deletion polymorphism associated with 1998 World Health Organization definition of metabolic syndrome in Chinese type 2 diabetic patients. Diabetes Care; 25 (6): 1002.

16- **Hurst P.L and Lovell-Smith C.J. (1981):** Optimization assay for serum angiotensin converting enzyme activity. Clin. Chem..; 27 (12): 2048.

17- **Wahlefeld A.W. (1974):** Triglycerides: determination after enzymatic hydrolysis. In: Bergmeyer H.U., ed. Method of Enzymatic Analysis. 3rd ed. Weinheim: Verlag Chemie, New York, NY: Academic Press; PP. 1878.

18- **Allain C.A., Poon L.S., Chan C.S.G., Richmond W. And Fu P.C. (1974):** Enzymatic determination of serum total cholesterol. Clin Chem; 20 (4): 470.

19- **Burstein M., Scholnick H.R. and Morfin R. (1970):** Rapid method for the isolation of lipoproteins from human serum by pricipitation with polyanion. J Lipid Res; 11 (6): 583.

20- **Friedwald W.T., Fredrickson R.I. and Fredrickson D.S.**

(1972): Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem.; 18 (6): 499.

21- **Mount J.N., Kearney E.M., Rosseneu M. and Slavin B.M. (1988):** Immuno-turbidometric assays for serum apoprotein A-I and B using Cobas Bio centrifugal analyzer. J. clin. Pathol.; 41 (4): 471.

22- **Adamson C.L., Kumar S., Sutcliffe H., France M.W. and Boulton A.J.M. (1993):** Screening strategies in the detection of micro-albuminuria in insulin-dependent diabetic patients. Practical Diabetes, 10 (4): 142**.**

23- **Trinder P. (1969):** Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann Clin Biochem; 6: 24.

24- **Bisse E. and Abraham E.C. (1985):** New less temperature-sensitive microchromato-graphic method for the seperation and quantitation of glycosylated haemoglobin.. J Chromatog; 344: 81.

25- **Matthews D.R., Hosker J.P., Rudenski A.S., Naylor B.P., Treacher D.F. and Turner R.C. (1985):** Homeostatic method assessment: Insulin resistance and cell function from fasting plasma glucose and insulin concentration in man. Diabetologia; 28 (7): 412.

26- **Thomas G.N., Tomlinson B., Chan J.C.N., Sanderson J., Cockram C.S. and Critchley J.A.J.H. (2001):** Renin-angiotensin system gene polymorphism, blood pressure, dyslipidemia, and diabetes in Chinese. A significant association of the ACE insertion/deletion polymorphism with type 2 diabetes. Diabetes Care; 24 (2): 356.

27- **Crisan D. and Carr J. (2000):** Angitensin I-converting enzyme genotype and disease association. J.Mol,Diagn.; 2 (3): 105.

28- **Taute B.M., Glaser C., Taute R. and Podhaisky H. (2002):** Progression of atherosclerosis in patients with peripheral arterial disease as a function of angiotensin-converting enzyme gene insertion/deletion polymorphism. Angiology.; 53 (4): 375.

29- **Stephens J.W., Dhamrait S.s., Copper J.A., Acharya J., Miller G.J., Hurel S.J. and Humphries .S.E. (2005):** The D allele of the ACE I/D common gene variants is associated with type 2 diabetes mellitus in Caucasian subjects. Mol. Genet. Metab.; 84 (1): 83.

30- **Feng Y., Niu T., Xu X., Chen C., Li Q., Qian R., Wang G. and Xu X. (2002):** Insertion/deletion polymorphism of the ACE gene is associated with type 2 diabetes. Diabetes; 51 (6): 1986.

31- **Daimon M., Oizumi T., Saitoh T. and Kameda W., Hirata A., Yamaguchi H., Ohnuma H., Igarashi M., Tominaga M. and Kato T. (2003):** The D allele of the angiotensin-converting enzyme insertion/deletion (I/D) polymorphism is a risk factor for type 2 diabetes in a population-

based Japanese sample. Endocrine Journal; 50 (4): 393.

32- **Costa L.A., Canani L.H., Maia A.L. and Gross J.L. (2002):** The ACE insertion/deletion polymorphism is not associated with the metabolic syndrome (WHO definition) in Brazilian type 2 diabetic patients. Diabetes Care; 25 (12): 2365.

33- **Sinorita H., Madiyan M., Pramono R. B., Purnama L. B., Ikhsan, M. R. and Asdie A.H. (2010):** ACE gene insertion/deletion polymorphism among patients with type 2 diabetes, and its relationship with metabolic syndrome at Sardjito Hospital Yogyakarta, Indonesia. Acta Med Indones-Indones J Intern Med; 42 (1): 12.

34- **Tsutaya S., Kitaya H., Saito Y., Nakata S. Takamatsu H., And Yasujima M. (1997):** Angiotensin converting enzyme gene polymorphism and its enzyme activity in serum in young Japanese females. Tohoku J. Exp. Med.; 182 (2): 151.

35- **Agerholm-Larsen B., Nordestgaard B.G. and Tybjaerg-Hansen (2000):** ACE gene polymorphism in cardiovascular disease. Meta-analysis of small and large studies in whites. Arterioscler. Thromb. Vasc. Boil.; 20 (2): 484.

36- **Degirmenci I., Kebapci N., Basaran A., Efe B., Gunes H.V., Akalin A., Kurt H., Urhan M.and Demirustu C. (2005):** Frequency of angiotensin-converting enzyme gene polymorphism in Turkish type 2 diabetic patients. Int. J. Cli. Pract.; 59 (10): 1137.

37- **Nagi D.K., Foy C.A., Mohamed-Ali V., Yudkin J.S., Grant P.J. and Knowler W.C. (1998):** Angiotensin-1-convertenig enzyme (ACE) gene polymorphism, plasma ACE levels, and their association with the metabolic syndrome and electrocardiography coronary artery disease in Pima Indians. Metabolism; 47 (5): 622.

38- **Ukkola O. and Santaniemi M. (2002):** Adiponectin: a link between excess adiposity and associated comorbidities? J. Mol. Med.; 80 (11): 696.

39- **Hu F.B., Meigs J.B., Li T.Y., Rifai N., and Manson J.E. (2004):** Inflammatory markers and risk of developing type 2 diabetes in women. Diabetes; 53 (3): 693.

40- **Viitanen L., Pihlajama'ki, Halonen P., Lehtonen M., Kareinen A., Lehto S. and Laakso M. (2001):** Association of angiotensin converting enzyme and plasminogen activator inhibitor-1 promote gene polymorphisms with features of the insulin resistance syndrome in patients with premature coronary heart disease. Atherosclerosis; 157: 57.

41- **Perticone F., Ceravolo R., Iacopino S., Cloro C., Ventura G., Maio R., Gulletta E., Perrotti N. and Mattioli P.L. (2001):** Relationship between angiotensin-converting enzyme gene polymorphism and insulin resistance in never-treated

hypertensive patients. J. Clin. Endocrinol. Metab; 86 (1): 172.

42- **Zingone A., Dominicans A., Melee E. Marasco O., Melina F., Minchella P., Quaresima B., Tiano M.T., Gnasso A., and Pujia A. (1994):** Deletion polymorphism in the gene for angiotensin-converting enzyme is associated with elevated fasting blood glucose levels. Hum. Genet.; 94 (2): 207.

43- **Ryan A.S., Nicklas B.J., Berman D.M. and Ferrell R.E. (2001):** The insertion/deletion polymorphism of the ACE gene is related to insulin sensitivity in overweight women. Diabetes Care; 24 (9): 1646.

44- **Paolisso G., Tagliamonte M.R., DeLucia D. and Pal,ieri F., Manzella D., Rinaldi C., Bossone A., Colaizzo D., Margaglione . and Varricchio M. (2001):** ACE gene polymorphism and insulin action in older subjects and healthy centenarians. J. Am. Geriatr. Soc.; 49 (5): 610.

45- **Huang X.H, Rantalaiho V., Wirta O., Pasternack A., Koivula T., Hiltunen T., Nikkari T. and Lehtimäki T. (1998):** Relationship of the angiotensin-converting enzyme gene polymorphism to glucose intolerance, insulin resistance, and hypertension in NIDDM. Hum. Genet.; 102 (3): 372.

46- **Frenais R., Ouguerram K., Maugeais C., Mahot P., Maugère P., Krempf M., and Magot T. (1997):** High density lipoprotein and apolipoprotein AI kinetics in NIDDM: a stable isotope study. Diabetologia; 40 (5): 578.

47- **DionyssiouAsteriou A., Papastamatiou M., Vatalas I.A. and Bastounis E. (2002):** Serum apolipoprotein A-I levels in atherosclerotic and diabetic patients. Eur. J. Vasc. Endovasc. Surg; 24 (2): 161.

48- **Oren I., Brook J.S., Greshoni-Baruch R, Kepten I., Tamir A., Linn S., Wolfovitz E. (1999):** The D allele of the angiotensin-converting enzyme gene contributes towards blood LDL-cholesterol levels and the presence of hypertension. Atherosclerosis; 145 (2): 167.

49- **Schmitz A. (1993):** Renal function changes in middle aged and elderly Caucasian type 2 (non-insulin dependent) diabetic patients. Diabetologia; 36 (10): 985.

50- **Ohno T., Kawazu S. And Tomono S. (1996):** Association analysis of the polymorphism of angiotensin-converting enzyme and angiotensinogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. Metabolism; 45 (2): 218.

51- **Nakajima S., Baba T. and Yajima Y. (1996):** Is ACE gene polymorphism a useful marker for diabetic albuminuria in Japanese NIDDM patients? Diabetes Care; 19 (12): 1420.

52- **O'Donnell C.J., Lindpaintner K., Larson M.G., Rao V.S. Ordovas J.M., Schaefer E.J., Myers R.H. and Levy D. (1998):** Evidence for association and genetic linkage of the

angiotensin-converting enzyme locus with hypertension and blood pressure in men but not in women in the Framingham Heart Study. Circulation; 97 (18): 1766.

53- **Duru K., Farrow S., Wang J.M., Lockette W. and Kurtz T. (1994):** Frequency of a deletion polymorphism in the gene for angiotensin converting enzyme is increased in African-Americans with hypertension. Am. J. Hypertens; 7 (8): 759.

54- **Barley J., Blackwood A., Miller M., Markandu N.D., Carter N.D., Jeffery S., Cappuccio F.P., MacGregor G.A. and Sagnella G.A. (1996):** Angiotensin converting enzyme gene I/D polymorphism, blood pressure and the renin angiotensin system in Caucasian and Afro-Caribbean peoples. J. Hum. Hypertens.; 10 (1): 31.

55- **Morise T., Takeuchi Y. and Takeda R. (1994):** Angiotensin-converting enzyme polymorphism and essential hypertension. Lancet; 343 (8889): 125.

56- **Maeda Y., Ikeda U., Ebata H, Hojo Y. Seino Y., Nakagami H. and Shimada K. (1997):** Angiotensin converting enzyme gene polymorphism in essential hypertension based on ambulatory blood pressure monitoring. Am. J. hypertens.; 10 (7-1): 786.

57- **Strazullo P., Iacone R., Iacoviello L., Russo O., Barba G., Russo P., D'Orazio A. and Barbato A. (2003):** Genetic variation in the renin-angiotensin system and abdominal adiposity in men: the Olivetti Prospective Heart Study. Ann. Intern. Med.; 138 (1): 17.

علاقة التحور الشكلي الجيني للراس بالنوع الثاني من مرض البوال السكري وبمتلازمة إكس

محمد سامي فوزي، إبراهيم المغاوري، نادر محمود محمد علي،
إيمان عبد القادر عبد السلام و أسامة خليل*
قسمي الكيمياء الحيوية الطبية والباطنة العامة*
كلية الطب، جامعة الزقازيق

متلازمة إكس (المتلازمة الأيضية) هي تجمع مقاومة الأنسولين أو النوع الثانى من مرض البوال السكرى مع إثنين أو أكثر من: إرتفاع ضغط الدم، إرتفاع دهون الدم، السمنه الجذعية و وجود زلال بالبول. ويعتقد أن التحور الجينى من أهم مظاهر الاصابه بالمتلازمة ويحتمل أن جينات جهاز الرينين–أنجيوتنسين من أهم العوامل الوراثية لمضاعفات مرض البوال السكرى ولذلك يحتمل أن التحور الشكلى الجينى للإنزيم المحول للأنجيوتنسين له دور فى الاصابه بهذه المتلازمة مما يؤهل الكشف عن هذا الجين كفحص للمتلازمة.

وقد هدف البحث الى دراسة العلاقة المحتملة بين التحور الشكلى الجينى للإنزيم المحول للأنجيوتنسين وكل من نشاط هذا الإنزيم ومتلازمة إكس كما يهدف البحث الى تقييم دور التحور الشكلى الجينى لهذا الانزيم فى حدوث الاصابة بهذة المتلازمة، بالاضافة إلى ذلك فان هذة الدراسه تعتبر محاولة لمعرفة هل النوع الجينى (DD) لهذا الإنزيم هو عامل المخاطرة المحتمل للمتلازمة.

وأشتملت الدراسة على ١٠٠ شخاصاً (٤٠ مريضا بالمتلازمة – ٣٠ مريضا بالنوع الثانى من مرض البوال السكرى – ٣٠ شخصا صحياً خالياً من الامراض) ، و قد تم تعيين التحور الجينى لهذا الإنزيم بطريقة التفاعل التسلسلى عديد البلمرة، كما تم قياس نشاط الإنزيم المحول للأنجيوتنسين بطريقه الإليزا. وقد تم تشخيص المقاومة للأنسولين بتطبيق مؤشر هوما بعد تحديد نسبة السكر الصائم بالدم ومستوى الانسولين بالبلازما مع قياس نسبة الهيموجلوبين السكرى ودهون الدم (الكوليسترول الكلى – الكوليسترول ذات الكثافة العالية - الكوليسترول ذات الكثافة المنخفضة – الدهون الثلاثية – أبو إيه)، وقد تم الكشف عن الزلال فى البول بطريقة غمس الشرائط كما تم تطبيق معامل كتلة الجسم وحساب نسبة الخصر إلى الحوض للفصل بين الاشخاص السمنيين والغير سمنيين.

وقد أتضح أن الجين (DD) للإنزيم المحول للأنجيوتنسين يوجد بصورة اكثر شيوعا فى مرضى المتلازمة ومرضى البوال السكرى (النوع التانى) مقارنة بالاشخاص الاصحاء ، كما انه يوجد فى مرضى التلازمة اكثر من مرضى البوال السكرى، وقد وجد ان هناك ارتفاع ذو دلالة إحصائية فى نشاط الإنزيم المحول للأنجيوتنسين فى بلازما دم مجموعات المرضى مقارنة بالاصحاء، وكذلك فى مرضى المتلازمة مقارنة بمرضى البوال السكرى. كما وجد أن نشاط هذا الإنزيم له علاقة إيجابية ذو دلالة إحصائية مع مؤشر هوما فى كل من مرضى المتلازمة ومرضى البوال السكرى وأيضا له علاقه ايجابية ذو دلالة إحصائية مع نسبة السكر الصائم بالدم والهيمجلوبين السكرى ومستوى الأنسولين بالبلازما والكوليسترول الكلى وكوليسترول منخفض الكثافة والدهون الثلاثية ، بينما له علاقة عكسية ذو دلالة إحصائية مع الكوليسترول عال الكثافة والأبو إيه. بالاضافة الى ذلك قد وجد زيادة ذو دلالة إحصائية فى نشاط الإنزيم المحول للأنجيوتنسين فى بلازما الدم ومستوى السكر الصائم بالدم والهيمجولبين السكرى والأنسولين بالبلازما ومؤشر هوما والكوليسترول الكلى والكوليسترول المنخفض الكثافة والدهون الثلاثية فى مجموعات النوع الجينى (DD) وكذلك نقص ذات دلالة إحصائية فى الكوليسترول عال الكثافة والأبو إيه فى هذه المجموعات بالمقارنه مع مجموعات النوع الجينى (II). و أخيرا فان النوع الجينى (DD) كان مصحوباً بإرتفاع ضغط الدم وزلال فى البول أكثر من النوع الجينى (II) و (ID) ولكن ليس مع السمنة.

خلاصةً، فان التحور الشكلى الجينى للإنزيم المحول للأنجيوتنسين كان مصحوباً بالمتلازمة الأيضية ومرض البوال السكرى (النوع الثانى) مع زيادة فى نشاط الإنزيم المحول للأنجيوتنسين، وكذلك فان كل مكونات المتلازمة ما عدا السمنة كانت أكثر ظهوراً مع النوع الجينى (DD)، ولذلك يعتبر الإنزيم المحول للأنجيوتنسين عامل مخاطرة محتمل للاصابة بالمتلازمة وبمرض البوال السكرى (النوع الثانى) بالاضافة الى ذلك فإنه باكتشاف النوع الجينى (DD) نستطيع أن نتوقع امكانية أكبر للاصابة بمضاعفات المتلازمة ومرض البوال السكرى (النوع الثانى) فى المستقبل ونقترح التدخل المبكر لمنع أو تأخير التعرض الى هذه المضاعفات.