Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Applied Sciences
3(1), 2015, 01-09

ISSN 2321 – 7456

Original Research Article

A COMPARATIVE STUDY OF *ANGIOTENSIN CONVERTING ENZYME* (ACE) GENE POLYMORPHISM OF EASTERN INDIAN BENGALI AND WESTERN INDIAN MEWARI TYPE 2 DIABETES PATIENTS IN INDIA

Pulakes Purkait[1*], Kalpataru Halder[2]; Abhishikta Ghosh Roy[1]; P N. Venugopal[3]; B.N. Sarkar[1]; J.M. Naidu[4],
[1]DNA Laboratory, Anthropological Survey of India, 27 Jawaharlal Nehru Road, Kolkata, India
[2]Brahmananda Keshab Chandra College, 111/2 B.T Road, Bon Hooghly, Kolkata, India
[3]Anthropological Survey of India, North West Regional Centre, Dehradun, India
[4]Department of Anthropology, Andhra University, Visakhapatnam, Andhra Pradesh, India

Abstract: Diabetes mellitus is a rapidly emerging global health problem and it is expected to cross the pandemic level within a span of another two decades. World-wide its frequency is expected to rise from 171 million to 366 million within the next two decades and India alone will take almost 25% shares from it to become the leading country with highest number of diabetic patients. For the present study 111 type 2 diabetes (59 Bangali and 52 Mewari) were enrolled. Anthropometric measurement and Clinical data- including information on duration of diabetes, presence of any complication, history of other disorders, systolic and diastolic blood pressure- were collected. The weight (kg) and height (cm) were recorded, the body mass index (BMI) was calculated and Genomic DNA was isolated from 5ml of peripheral blood samples of 111 type 2 diabetes individuals. Further, PCR has been performed to know the frequency of ACE I/D polymorphism. Our study reveals that anthropometric measurements and few bio-chemical parameters were significantly varying in type 2 diabetes patients among western Indian Mewari population and eastern Indian Bengali population. Distribution of ACE genotypes as well as their allele frequency doesn't differ significantly. It can presume that ACE gene I/D polymorphism, an *Alu* insertion polymorphism, can be used as a suitable marker for studying genetic variation among different human populations because of its stable nature as well as representing a unique evolutionary event.

Key words: Type 2 Diabetes, ACE polymorphism, Anthropometry, Bengali, Mewari

Introduction:

Diabetes mellitus is a complex heterogeneous group of disorders characterized by high levels of glucose in the bloodstream (IFD 2006), caused by impairment in both insulin secretion and action. Type 2 diabetes is the most common form of diabetes and accounts for 90 – 95 % of all cases of the disease and the most serious health problems of modern time (IFD 2006). Diabetes mellitus is a rapidly emerging global health problem and it is expected to cross the pandemic level within a span of another two decades. World-wide its frequency is expected

For Correspondence:
Pulakes.purkait28@gmail.com
Received on: November 2014
Accepted after revision: January 2015

to rise from 171 million to 366 million within the next two decades and India alone will take almost 25% share from it to become the leading country with highest number of diabetic patients (Wild 2004).

Genetic polymorphisms of the rennin-angiotensin-aldosteron system (*RAAS)* are interesting for investigation, because impairment of this system has shown to retard the development of diabetic complications, such as nephropathy and retinopathy (Lewis *et al.* 1993; Chaturvedi *et al.* 1998). Renin angiotensin system may play an important role in blood pressure regulation and acts as a key regulator of Sodium homeostasis. Angiotensin converting enzyme (ACE) is a major component of Renin-Angiotensin-Aldosterone System (RAAS).The gene coding for Angiotensin converting Enzyme *(ACE)* regulates vascular tone through the activation of angiotensin II, a potent vasoconstrictor (Timmermans *et al.* 1993), and inactivation of bradykinin (Atlas. 1998), a nonapeptide belonging to a class of active peptides (kinins) that are released from tissue to produce a variety of effects, including arterial vasodilatation and venoconstriction. ACE insertion (I) / deletion (D) polymorphism playing an effective role in hypertension and diabetes (Ruiz *et al.* 1994, Viswanathan *et al.* 2001). The ACE gene is located on chromosome 17q23, consists of 26 exons, 25 introns and it spans 21 kb. The polymorphism of ACE gene results from the insertion (I) or deletion (D) of a 287 bp Alu repeat sequence near the 3′ end of intron 16 which leads to three genotypes DD, II and ID (Rigat *et al.* 1992). The main function of ACE is the conversion of Angiotensin I to vasoactive, natriuretic octapeptide angiotensin II and is thus implicated in the pathogenesis of diabetic complication (Jeffers *et al.* 1997, Grzeszczak *et al.* 1998, Bedir *et al.* 1999, Estacio *et al.* 1998). However there are studies which suggest no association between the etiologies of diabetic with ACE genotype polymorphism (Mastana *et al.* 1997, Pasha *et al.* 2002). Therefore with the present state of knowledge the genotype-phenotype interaction between ACE gene polymorphism and diabetic have not yet been fully understood. An extensive interethnic variation in the distribution of ACE gene polymorphism appears to be one of the causes for such inconsistent findings.

The genetics of type 2 diabetes called polygenic or multifactorial is a result of the interaction between the environment and multiple genes. Alleles of these polymorphisms are present in both healthy individuals and type 2 diabetes patients with different frequencies.

Therefore, the aim of the present study was to investigate the distribution and comparison of ACE gene I/D polymorphism and its relationship with Type 2 Diabetes patients in Eastern Indian Bengali population and Western Indian Mewari population.

Materials and Methods

The present study on ACE gene I/D polymorphism was carried out among 59 Eastern Indian Bengali Type 2 Diabetes patients (EIT2DM) of both sex and 52 Western Indian Mewari Type 2 Diabetes (WIT2DM) patients. Registered patients were recruited from two ecological zones of India i.e. Calcutta Medical College and Hospital, Kolkata, West Bengal and Bhupalpura Govt. Dispensary, Udaipur, Rajasthan, detailed medical history of each patient was recorded accordingly. The detection of Type 2 diabetic patients were based on physician's recommendation. Prior to the recruitment of subjects the ethical committee clearance was obtained from the respective medical institutions and accordingly informed consent was obtained from all the participants.

Anthropometric measurement and Clinical data included information on duration of diabetes, presence of any complication, history of other disorders, systolic and diastolic blood pressure also collected. The weight (kg) and height (cm) were recorded and the body mass index (BMI) was calculated using the formula: weight (kg) divided by height (m) squared (kg/m^2).

10 ml venous blood was collected from each individual for this study. Biochemical tests were to determine Glucose(mg/dl), Cholesterol (mg/dl), HDL (mg/dl), LDL (mg/dl),Triglycerides (mg/dl) , BUN (mg/dl),

Uric Acid (mg/dl), Total Protein (g/dl), Albumin (g/dl) and Chloride (mmol/L) by using automated analyser (EM 360, TRANSASIA).

Molecular Analysis of the ACE Gene I/D polymorphism

Approximately 6 ml of venous blood was drawn from each of the subjects in EDTA vials and genomic DNA was extracted from whole fresh blood using standard salting out method using phenol-chloroform (Miller *et al.* 1988).

The ACE I and D alleles were identified by PCR amplification, using 20 pmol of each primer (flanking primer pair): oligonucleotide sense primer: 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and anti-sense primer : 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′ (Rigat *et al.* 1992) in a final volume of 10µl containing 50ng of genomic DNA, 20 pmol of each primer, 10X Taq PCR buffer, 25 mM MgCl2 ,100 mM of each dNTP and 1 U/uL of Taq polymerase (MBI Fermentas).

PCR amplification was performed in a DNA thermo cycler (Gene Amp PCR 9700 - Applied Bio systems, USA and S1000^{TM} Thermo Cycler, BIO-RAD). PCR was carried out with a Gradient standardize PCR condition with an initial denaturing time at 95°C for 6min. Then the DNA was amplified for 35 cycles with denaturation at 94°C for 1 min, annealing at 60°C for 1:30 min and extension at 72°C for 2 min. Electrophoresis of PCR products were done in 2.5% Agarose gel with ethidium bromide staining and directly visualized in UV light. The amplified product is a 190 bp fragment in the presence of the deletion (D) allele and a 490 bp fragment in the presence of the insertion (I) allele. Therefore, there were three genotypes after electrophoresis: a 490 bp band (genotype II), a 190 bp band (genotype DD), or both 490 and 190 bp band (genotype ID).

Statistical analysis

All data were analyzed by SPSS (Statistical Package for social sciences, Version 19, SPSS). Data were presented as mean, standard deviation (SD) or as proportions. Genotype and allele frequencies of ACE gene polymorphism were compared with χ^2 analysis was used to investigate the results by MINITAB software. Statistical significance was assumed at the P <0.05 level.

"p" is level of significance. p > 0.05, NS = Not Significant; *p < 0.05, Significant at 5% significance level; **p < 0.01, more Significant at 1% significance level; ***p < 0.001, Highly Significant.

Result

Table1 shows the study was categorized into two groups with type 2 diabetes. **EIT2DM**-Type 2 diabetes subject born in Bengali family of Kolkata city and nearby area. **WET2DM**-Type 2 diabetes subject born in Mewari family of Udaipur city and nearby area.

Table 1. Classification of patients according to the ecologic zones and populations

No. of Subjects	Criteria
59	Born in Bengali Family of Kolkata and surrounding area with type 2 diabetes (West Bengal)
52	Born in Mewari Family of Udaipur and surrounding area with type 2 diabetes (Rajasthan)

Table2 Shows the statistical characteristics of the two studied group (EIT2DM and WIT2DM) with the mean values and standard deviations and the Table 3 shows mean differences, p values of basic anthropometric and clinical variables viz age, sex, duration of diabetes, height, weight, Body Mass Index , systolic blood pressure, diastolic blood pressure , Glucose, cholesterol, HDL, LDL, BUN, Uric-Acid, Total protein, albumin and Chloride respectively.

The frequency distribution of ACE genotypes as well as allele between two

different study groups along with their odds ratio has been presented in Table 3. The table suggests that the frequency of DD genotype is higher in the EIT2DM patients (22.03 %) compared to the WIT2DM (17.31 %). The same result is also found in case of II genotype where a higher frequency is found among the EIT2DM patients (35.6%) than the WIT2DM patients (34.62 %). The frequency of ID genotype is found to be higher in WIT2DM patients than the EIT2DM patients (48.07 %) and 42.37 % respectively).The frequency of D allele is found to be higher among the EIT2DM patients (0.43) than the WIT2DM patients (0.41). The frequency of I allele is found to be higher among the WIT2DM patients (0.59) than the EIT2DM patients (0.57) with an odds ratio (1.08). Overall it can be said that within the different study groups the distribution of ACE genotypes as well as their allele frequency don't differ significantly.

Table2. Basic anthropometric and clinical characteristics of studied Mewari and Bengali T2DM patients

Clinical characteristics	**EIT2DM-Bengali (n = 59)**	**WIT2DM-Mewari (n = 52)**
	Mean ±SD	Mean ±SD
Sex (M/F)	27 / 32	33 / 19
Age (YEAR)	55.63 ± 11.28	54.85 ± 10.90
Duration of Diabetes	8.1356 ± 4.42352	7.8462 ± 3.52795
Glucose(mg/dl)	143.3559 ± 41.40538	180.9808 ± 91.8288
Cholesterol (mg/dl)	179.3332 ± 37.01663	178.8846 ± 48.07578
Triglycerides (mg/dl)	176.1895 ± 67.31019	179.6731 ± 100.9408
HDL (mg/dl)	56.7708 ± 20.89284	44.4385 ± 9.944
LDL (mg/dl)	87.3245 ± 26.21899	110.0544 ± 32.28931
BUN (mg/dl)	15.2786 ± 6.60228	10.8267 ± 6.36224
Uric Acid (mg/dl)	5.7842 ± 0.98669	5.8438 ± 1.53876
Total Protein (g/dl)	8.6283 ± 1.03185	7.3402 ± 0.57284
Albumin (g/dl)	4.7351 ± 0.44094	3.9308 ± 0.26237
Chloride (mmol/L)	113.4949 ± 11.98599	115.3327 ± 14.78076
Height (cm)	155.4729 ± 8.8622	162.0135 ± 8.26697
Weight (kg)	58.3729 ± 11.9595	71.1538 ± 20.06498
Body Mass Index (BMI) (Kg/m^2)	24.1214 ± 4.56671	26.9628 ± 5.91127
SBP (mm of mercury)	133.5593 ± 18.1215	139.1154 ± 21.04771
DBP (mm of mercury)	82.5593 ± 8.02425	88.0385±10.97763

Table 3: statistical analysis for comparison between groups according to Mean difference of basic anthropometry and clinical characteristic variables

Clinical characteristics	Mean Difference	P
Glucose(mg/dl)	37.625	0.005
Cholesterol (mg/dl)	0.449	0.956
Triglycerides (mg/dl)	3.484	0.829
HDL (mg/dl)	12.332	0.000
LDL (mg/dl)	22.73	0.000
BUN (mg/dl)	4.452	0.000
Uric Acid (mg/dl)	0.059	0.806
Total Protein (g/dl)	1.288	0.000
Albumin (g/dl)	0.804	0.000
Chloride (mmol/L)	1.838	0.471
Height (cm)	6.541	0.000
Weight (kg)	12.781	0.000
Body Mass Index (BMI) (Kg/m^2)	2.841	0.005
SBP (mm of mercury)	5.556	0.138
DBP (mm of mercury)	5.479	0.003
Duration of Diabetes	0.289	0.706

Table 4: Genotype and allele distribution of ace gene insertion (I) / deletion (D) polymorphism in EIT2DM (Bengali) and WIT2DM (Mewari) patients.

ACE gene	EIT2DM-Bengali (N= 59)		WIT2DM-Mewari (N = 52)	
Genotype	N	%	N	%
DD	13	22.03	9	17.31
ID	25	42.37	25	48.07
II	21	35.6	18	34.62
X^2 =0.519 , df = 2 , p= 0.772				
Allele				
D	51	43.22	43	41.35
I	67	56.78	61	58.65
X^2 =0.080, df=1, p= 0.778 ; OR= 1.08 (1.84 - 0.63)				

Dicussion

Diabetic mellitus is a complex path physiological process which accounts for reduced life expectancy in various countries around the world and it involves the contribution of several aetiologies both genetic as well as environmental in nature. Genetic factors, lipid profiles, hypertension are potential risk factors to improve the complications in diabetes (Katsuya, *et al.* 1995). The World Health Organization recommends measurement of BMI as a universal criterion of overweight and obesity.

Anthropometric & Clinical Characteristics

Few Anthropometric measurements were factor that affects greatly towards metabolic risk. In fact, it was reported earlier too, that weight loss and or gain was related to increased risk for abdominal fat distribution and therefore metabolic risk profile (Pihl and Jurimae 2001). Hence higher values of anthropometric measurements which are important risk factors for diabetes which is already well known in many studies (Rimm *et al.* 1995; Montague and O'Rahilly 2000) and similar observations was found in our study. However, our study reveals that anthropometric measurements and few bio-chemical parameters were significantly varying in type 2 diabetes patients among western Indian Mewari population and eastern Indian Bengali population. The variations between two different populations are due to vary from one ethnic group to other, one geographical region to other and among different races. A recent study among Koreans found association between WHR, total cholesterol and LDL cholesterol in men. In the same study, inverse correlations were observed between WC and HDL in women (Sarah and Edwin 2011). In an another study from North India (Sandhu *et al.* 2008), reported positive correlations between WHR, total cholesterol, LDL cholesterol, and triglycerides in the 41 to 50 year age group in men. A recent Malaysian study in type II diabetics among three different ethnic groups has demonstrated significant differences in anthropometric parameters and lipid profile patterns (Blebil *et al.* 2011). These studies reveal that anthropometric parameters and lipid profile patterns may vary from one ethnic group to other, one geographical region to other and among different races. So the results of one study cannot be extrapolated to other studies and generalized conclusions cannot be drawn for all populations.

ACE Polymorphism

The ACE gene polymorphism has been implicated as a risk factor for a number of pathologies, such as type 2 diabetes, hypertension, stroke, myocardial infarction and CVD, but the frequency of this genetic polymorphism in different ethnic groups is unknown (Ruiz *et al.* 1994, Viswanathan *et al.* 2001, Singh *et al.* 2006, Cambien *et al.* 1992, Marian *et al.* 1993, Schunkert *et al.* 1994). The ACE gene was proposed to be one of the first candidate genes for developing diabetic complication (Uddin *et al.* 2007). Ng *et al.* 2005 noticed that this genotype-phenotype association is more marked among the Asians than the Caucasians.

Several previous studies have reported that individuals with the ACE gene DD genotype are more sensitive to insulin. Baroudi *et al.* 2009 reported that DD genotype and D allele were associated with type 2 diabetes in Tunisian population and another studies in non Caucasian populations have suggested an association between the D allele and type 2 diabetes.

In this study we have presented the distribution of ACE genotype and allele frequency among the Type 2 diabetes in Bengali population of Eastern India as well as Mewari population of Western India. The study population as a whole shows a high frequency of ID genotype (42.37%, 48.07%) than II genotype (35.6%, 34.62 %) and DD genotype (22.03%,17.31%) respectively.

Some studies from different ethnic background suggest that the DD genotype is associated with an increased susceptibility to type 2 diabetes (Ergen *et al.* 2004, Hsieh *et al.* 2000, Feng *et al.* 2002). Studies conducted in India as well as outside India have showed significant relatonship between the presence of

DD genotype and an increased risk of nephropathy among Type2 diabetic patients (Jeffers, B.W *et al.* 1997, Bhabani *et al.* 2005, Naresh *et al.* 2009, Yoshida *et al.* 1996, Nikzamir *et al.* 2006).Previous studies from India as well as outside of India have shown that null association between ACE genotype and type 2 diabetes (Mastana *et al.* 1997, Pasha *et al.* 2002).

Our study finds that there is almost similar distribution of ACE genotypes and its allele frequency between the two study groups.

Conclusion

Therefore while studying the susceptibility of ACE gene with the etiology of diabetes in a community or a language group, ecologic zone we have to take care of the ethnic background of the population. At this stage we can presume that ACE gene I/D polymorphism, an *Alu* insertion polymorphism, can be used as a suitable marker for studying genetic variation among different human populations because of its stable nature as well as representing a unique evolutionary event. Further studies of diabetes with ethnic background from other parts of India will definitely add more information to this issue.

Acknowledgements

We would like to thank the members of the study populations and Doctor's (Dr. P. Raychoudhury, Calcutta Medical College and Hospital, Kolkata ; Dr. V.K Purohit, Dr. Lalit K. Gandharve , Bhupalpura Govt. Dispensary, Udaipur,) for their cooperation during data collection. We wish to express our deep gratitude to the Director, Anthropological Survey of India, for his kind permission to initiate the work and also for providing financial support.

Reference

- Atlas SA 1998. The renin-angiotensin system revisited: classical and nonclassical pathways of angiotensin formation. *Mt Sinai J Med*, **65:** 87-96.
- Baroudi T, Bouhaha R., Moran-Moguel C, Sanchez-Corona J, Ben-Maiz H, Kammoun, AH and Benammar- Elgaaied A. 2009. Association of the insertion/deletion polymorphism of the angiotensin-converting enzyme gene with type 2 diabetes in two ethnic groups of Jerba Island in Tunisia. J Renin Angiotensin Aldosterone Syst 10(1): 35-40.
- Bedir A, Arik N, Adam B, Kilinc K., Gumus T, Guner E. 1999. Angiotensinconverting enzyme gene polymorphism and activity in Turkish patients with essential hypertension. Am J Hypertens 12:1038-43.
- Bhabani BA, Pama T, Sastry BK, KrisnaReddy N, Nausheen K. 2005. The insertion/ deletion D polymorphism of Angiotensin converting enzyme (ACE) gene increase susceptibility to hypertension and/or diabetes. Int Jr Hum.Genet 5: 247-252.
- Blebil AQ, Hassan Y, Dujaili JA. 2011. Differences in demographics, lipid profile and other clinical characteristics among type 2 diabetic patients in the state of penang, Malaysia according to gender and races. Asian J Pharm Clin Res (Suppl 2) 4:130-133.
- Cambien F, Poirier O, Lecerf L, *et al.* 1992. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 359(6396): 641-44
- Chaturvedi N, Sjolie AK, Stephenson JM, Abrahamian H, Keipes M, Castellarin A et al 1998. Effect of lisinopril on progression of retinopathy in normotensive people with type 1 diabetes. *Lancet*, **351:** 28–31.
- Ergen HA, Hatemi H, Agachan B, Camlica H, Isbir T. 2004. Angiotensin-I converting enzyme gene polymorphism in Turkish type-2 diabetic patients. Exp Mol Med 36:345-350.
- Estacio RO, Jeffers BW, Hiatt WR, Biggerstaff SL, Gifford N, Schrier RW. 1998. The Effect of Nisoldipine as Compared with Enalapril on Cardiovascular Outcomes in Patients with Non-Insulin-Dependent Diabetes and Hypertension. N Engl J Med 338:645-652.
- Feng Y, Niu T, Xu X *et al.* 2002. Insertion/deletion polymorphism of the ACE

gene is associated with type 2 diabetes. Diabetes 51: 1986–1988.

- Grzeszczak W, Zychma MJ, Lacka B, Zukowska-Szczechowska E. 1998. Angiotensin I converting enzyme gene polymorphisms. Relationship nephropathy in patients with non-insulin dependent diabetes mellitus. J Am Soc Nephrol 9:1664-1669.
- Hsieh MC, Lin SR, Hsieh TJ, Hsu CH, Chen HC, Shin SJ, Tsai JH. 2000. Increased frequency of angiotensin converting enzyme DD genotype in patients with type II diabetes in Taiwan. Nephrol Dial Transplant 15:1008-1013.
- International Diabetes Federation (IFD). 2006. Diabetes Atlas, 3rd Edition.
- Jeffers BW, Extacio RO, Raynolds MV, Schrier RW.1997. Angiotensin converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus and its relationship with diabetic nephropathy. Kidney Int 52:473-477.
- Katsuya T, Koike G, Yee TW, Sharpe N, Jackson R, Norton R, Horiuchi M, Pratt RE, Dzau VJ, MacMahon S. 1995. Association of angiotensinogen T 235 variant with increased risk of coronary artery disease. Lancet 345:1600-1603.
- Lewis EJ, Hunsicker LG, Bain RP, Rohde RD 1993. The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Eng J Med*, **329:** 1456–1462.
- Marian AJ, Yu QT, Workman R, *et al.* 1993. Angiotensin-converting enzyme polymorphism in hypertrophic cardiomyopathy and sudden cardiac death. Lancet 342(8879): 1085-6.
- Mastana S, Nunn B. 1997. Angiotensin-converting enzyme deletion polymorphism is associated with hypertension in a Sikh population. Hum Hered 47:250– 253.
- Miller SA, Dykes DD, Polesky HF. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:12-15.
- Montague, CT and O'Rahilly, S. 2000. The perils of portliness: Causes and consequences of visceral adiposity. Diabetes 49: 883-888.
- Naresh VVS, Reddy ALK, Sivaramakrisna G, Sharma PVGK, Vardhan RV, Siva Kumar V. 2009. Angiotensin converting enzyme gene polymorphism in type-II diabetics with nephropathy. Indian J of Nephrology 19:145-148.
- Ng DP, Tai BC, Koh D, Tan KW, Chia KS. 2005. Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: a meta-analysis of studies reported between 1994 and 2004 and comprising 14,727 subjects. Diabetologia 48:1008-16.
- Nikzamir A, Esteghamati A, Feghhi M, Nakhjavani M, Rashidi A, Reja JZ. 2006. Angiotensin converting enzyme gene polymorphism in Iranian patients with type-II diabetes. Iranian Journal of Emmunology 3:23-29.
- Pasha MAQ, Khan AP, Kumar R, Ram RB, Grover SK, Srivastava KK, Selvamurthy W, Brahmachari S 2002. Variations in angiotensin-converting enzyme gene insertion/deletion polymorphism in Indian populations of different ethnic origins. J Biosci, 27: 67-70.
- Pihl E and Jurimae, T. 2001. Relationship between body weight change and cardiovascular risk factors in male former athletes. Inernational Journal of Obesity Related Metabolic Disorders 25: 1057-1062.
- Rigat B, Hubert C, Corvol P, Soubrier F. 1992. PCR detection of the insertion/deletion polymorphism of the human Angiotensin converting enzyme gene (DCP1) (dipeptidylcarboxypeptidase. Nucleic Acid Research 20:1433.
- Rimm EB, Stampfer MJ and Giovannucci E. 1995. Body size and fat distribution as predictors of coronary heart disease among middle-aged and older US men. American Journal of Epidemiology 141:1117-1127.
- Ruiz J, Blanche H, Cohen N, Velho G, Cambien F, Cohen D, et al 1994. Insertion/deletion polymorphism of the angiotensin-converting-enzyme gene is

strongly associated with coronary artery disease in non-insulin dependent diabetes mellitus. ProctNatlAcadSci USA, 91: 3662-665.

- Sandhu HS, Koley S, Sandhu KS. 2008. A study of correlation between lipid profile and waist to hip ratios in patients with diabetes mellitus. Anthropologist 10:215-8.
- Sarah C, Edwin T. 2011. Anthropometric measures and lipid CHD risk factors in Korean Immigrants with Type 2 Diabetes. Cardiovasc Nurs 26:414-22.
- Schunkert H, Hense HW, Holmer SR, *et al.* 1994. Association between a deletion polymorphism of the angiotensin-converting-enzyme and left ventricular hypertrophy. New England Journal of Medicine 360(23): 1634-8.
- Singh PP, Naz I, Gilmour A, Singh M, Mastana S. 2006. Association of APOE (Hha1) and ACE (I/D) gene polymorphism with type 2diabetes mellitus in North West India. Diabetes Res Clin Pract 74: 95–102.
- Timmermans PBMWM, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ et al 1993. Angiotensin II receptor and angiotensin II receptor antagonists. Pharmacol Rev, 45: 205-251.
- Uddin M, Azam M, Chowdhury N, Akhteruzzaman S. 2007. Angiotensin I-converting enzyme gene polymorphism in type 2 diabetic patients with nephropathy. Journal of Medical Sciences 7:682-5.
- Viswanathan V, Zhu Y, Bala K, Dunn S, Snehalatha C, Ramachandran A, Jayaraman M, Sharma K. 2001. Association between ACE gene polymorphism and diabetic nephropathy in South Indian patients. J Pancreas 2:83-87.
- Wild S, Roglic G, Green A, Sicree R., King H. 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047–1053.
- Yoshida H, Kuriyama S, Atsumi Y, Tomonari H, Mitarai T, Hamaguchi A, Kubo H, Kawaguchi Y, KonV, Matsuoka K, Ichikawa I, Sakai O. 1996. Angiotensin I converting enzyme gene polymorphism in non-insulin dependent diabetes mellitus. Kidney int 50:657-664.