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Serum Angiotensin Converting Enzyme Levels In Nigerian Type 2 Diabetic Patients In Port Harcourt.

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ABSTRACT

Angiotensin converting enzyme (ACE) regulates blood pressure and its increased level has been implicated in the microvascular and macrovascular complications of diabetes mellitus. These complications Arise from, which progressively cause the formation of Advanced glycation end product (AGE) generating free radicals that lead to tissue damage. The aim of the study was to assess the serum level of angiotensin converting enzyme in adults with type 2 diabetes mellitus. A cross-sectional study conducted on 120 confirmed cases of adult type 2 diabetes patients who were on hypoglycaemic medication (but not taking ACE inhibitors) and 120 apparently healthy age-matched control subjects. Blood was collected in plain and lithium heparin bottles (for ACE and other analytes) respectively. Serum ACE levels were measured with Eton Bioscience ELISA kits. Plasma electrolytes were analysed with ion selective electrodes while plasma urea and creatinine were Measured with Randox test kits, to assess renal function. Data generated was analysed with SPSS version 20.0. The mean ACE level in diabetic subjects was 25.61 ± 0.63 IU/L, and 23.69 ± 0.84 IU/L for control subjects (p = 0.01). There was a significant difference in ACE levels among the male diabetic subjects and control subjects (p = 0.04), while there was no difference among (P = 0.10). There was no association between the ACE levels and the parameters of renal function. The study found out that serum ACE was higher in diabetes male subjects. Despite the progressive effect of diabetes on renal function, the enzyme level was not affected by the duration of diabetes.

Keywords: Angiotensin converting enzyme, type 2 diabetes mellitus, Nigeria

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INTRODUCTION

Angiotensin converting enzyme (ACE) is a glycoprotein with a molecular weight of 130 – 160 KDa[1] discovered by Skeggs and co-workers in 1956. It is synthesized from the vascular endothelial cells of the pulmonary and renal tissues[2]. It catalyzes the cleavage of a variety of dipeptide substrates while inactivating kinins resulting in the regulation of blood pressure.[3] It was previously used for diagnosis of sarcoidosis.[4] Recently, it is advocated for studies in diabetic nephropathy[5]. Angiotensin converting enzyme has been discovered to play an important role in diabetic micro- and macro- vascular complications.[6,7] These complications arise by chronic hyperglycaemia progressively causing formation of advanced glycation end products generating free radicals that lead to tissue damage.[8,9]

Further research led to the discovery its homologue, ACE2.[10,11] Both enzymes (ACE and ACE2) have opposite physiological function,[12,13] as reported by Crackower *et al.*,, in a study done with animal heart when compared with human beings.[14]

However, genetic studies have revealed an association between a higher concentration of ACE and the vascular complications of diabetes, though with individual differences[7,15]

The study was designed to estimate the serum level of ACE and evaluate its ability to predict diabetic nephropathy among adult type 2 diabetic patients.

MATERIALS AND METHOD

The study is a cross-sectional study conducted among adult type 2 diabetic patients. Written informed consent was obtained from all the study subjects and healthy controls who were on routine clinic visit at the University of Port Harcourt Teaching Hospital. (UPTH). Ethical approval was obtained from the hospital. Sample size was calculated using Fischer's formula[16] and a proportion from a study on reference ranges[17]. A total of 240 subjects were used for the study. One hundred and twenty already diagnosed type 2 diabetic patients who were older than 21 years from the Metabolic Clinic and the General Out-Patients Clinic, and who were not on angiotensin converting enzyme inhibitors, were recruited for the study. One hundred and twenty controls matched for age were got from among the hospital workers and workers in the nearby University. Those with renal, respiratory, liver and cardiovascular diseases as well as subjects on steroid therapy were excluded from the study.

After obtaining informed written consent, questionnaires were administered to participants to determine inclusion and exclusion criteria. The weights and heights were measured with bathroom weighing scale (Camry) and a Stadiometer (Surgifriend Medical, UK) respectively. Body mass index was calculated for each participant by dividing the weight in kilograms by the square of the height in meters (Kg/m^2). Blood pressure was obtained using a standard mercury sphygmomanometer (Accoson, UK) with the subject on a sitting position, and the

cuff wrapped round the arm and then inflated. The reading was taken from the manometer while the cuff was deflated.

Ten milliliters (10ml) of venous blood was taken from all participants after an overnight fast. The blood was dispensed as follows: 2ml into a fluoride oxalate bottle for fasting plasma glucose, 3ml into a plain bottle for ACE measurement and 5 ml into lithium heparin bottles for electrolytes, urea and creatinine. Serum for ACE measurement was separated after 60 minutes; and plasma was separated within 30 minutes of collection by centrifugation at 3000g for 15 minutes and transferred into Eppendorf tubes. Fasting plasma glucose was analysed within two hours of specimen collection. Samples for other assays were batch-analysed and so were stored frozen at -20°C and analysed within two weeks of specimen collection.

Plasma glucose was analysed by glucose oxidase method using Randox kits.[18]. Angiotensin converting Enzyme level was measured using Eton Bioscience ELISA kits.[19] Sodium and potassium were analysed using ion selective electrode while urea and creatinine were analysed using enzymatic methods with Randox test kits. Quality control samples were analysed simultaneously with each batch of 10 samples to ensure analytical accuracy and precision. The intra-batch and inter-batch coefficient of variation for ACE measurement were 3.03% and 4.17% respectively.

Statistical analysis of data was done using the statistical package for social sciences (SPSS) version 20.0 (IBM, USA). Values were expressed as mean ± standard deviation. Student t-test, and Bar chart were used to compare the mean differences and establish associations. Confidence interval was 95%, while *p*-value less than or equal to 0.05 was taken to be significant.

RESULTS AND DISCUSSION

The diabetic group comprised 58 males and 62 females with a male-to-female ratio of 1:1.1, while the control group had 66 males and 54 females with ratio of 1:0.8. (Figure 1).

The mean (± SD) serum angiotensin converting enzyme (ACE) level in control subjects was 23.69 (± 0.84) IU/L. The lower and upper limits of the reference interval were 22.85 IU/L and 24.53 IU/L respectively. Also, the mean (± SD) serum ACE level for the diabetic subjects was 25.61(± 0.63) IU/L. The mean (± SD) ACE level in relation to sex showed male (diabetics) 26.12 (± 0.89) IU/L to male (control) 24.09 (± 0.49) IU/L; female (diabetic) 25.13 (± 0.88) IU/L to female (control) 23.20 (± 0.73) IU/L.

Of the 120 subjects respectively, 3 diabetics (2.5%) and 6 control subjects (5.0%) were underweight (BMI < 18.5kg/m^2). Forty eight diabetic subjects representing 40.0% and 66 control subjects (55.0%) had normal weight (BMI = 18.5-24.9kg/m^2). Forty two diabetics

(35.0%) and 35 control subjects (29.2%) were overweight (BMI= 25.0-29.9kg/m^2), whereas, 27 diabetics (22.5%) and 13 control subjects (22.5%) were obese (BMI > 30.0kg/m^2). (Figure 2).

Comparing the physical attributes of the diabetes and the control subjects, the mean differences in systolic BP and diastolic BP were statistically significant and higher in diabetics than in control subjects ($p \leq 0.05$). The differences between the diabetics and the controls for the height, weight and BMI were not statistically significant ($p \geq 0.05$). (Table 1).

Comparing the ACE level between the diabetes, and the control subjects, the mean difference in ACE level was statistically significant and higher in diabetics than the control subjects (p = 0.01). Similar statistically significant difference was observed between male diabetics and the male control subjects (p = 0.04). There was no statistically significant difference between the female diabetics and the female control subjects (p = 0.10).(Table 2).

Comparing the duration of diabetes with the biochemical parameters, 99 (82%) of the subjects had diabetes for more than one year and 21 (17.5%) for less than one year duration after diagnosis. The markers of renal function (urea and creatinine) were significantly higher in those with diabetes of more than one year duration. Other variables including the ACE level were not affected by the duration of diabetes (Table 3).

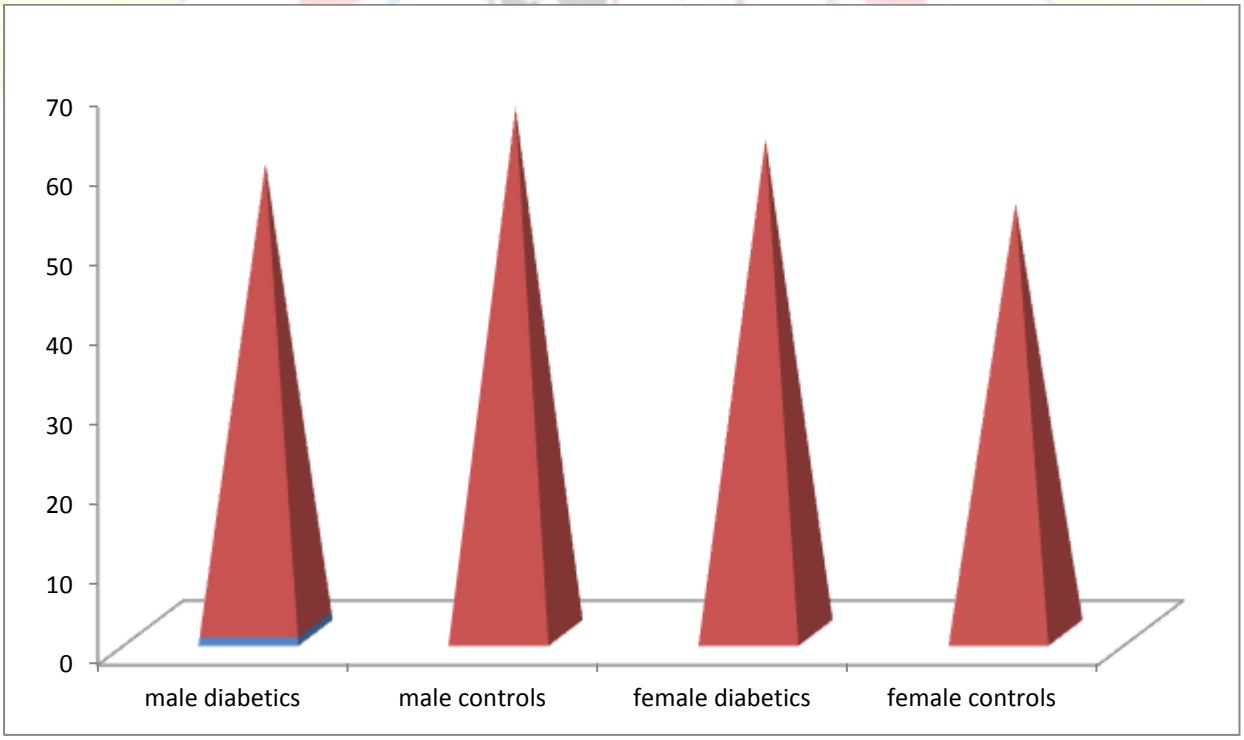

Figure 1: Sex distribution of diabetics and controls (in numbers)

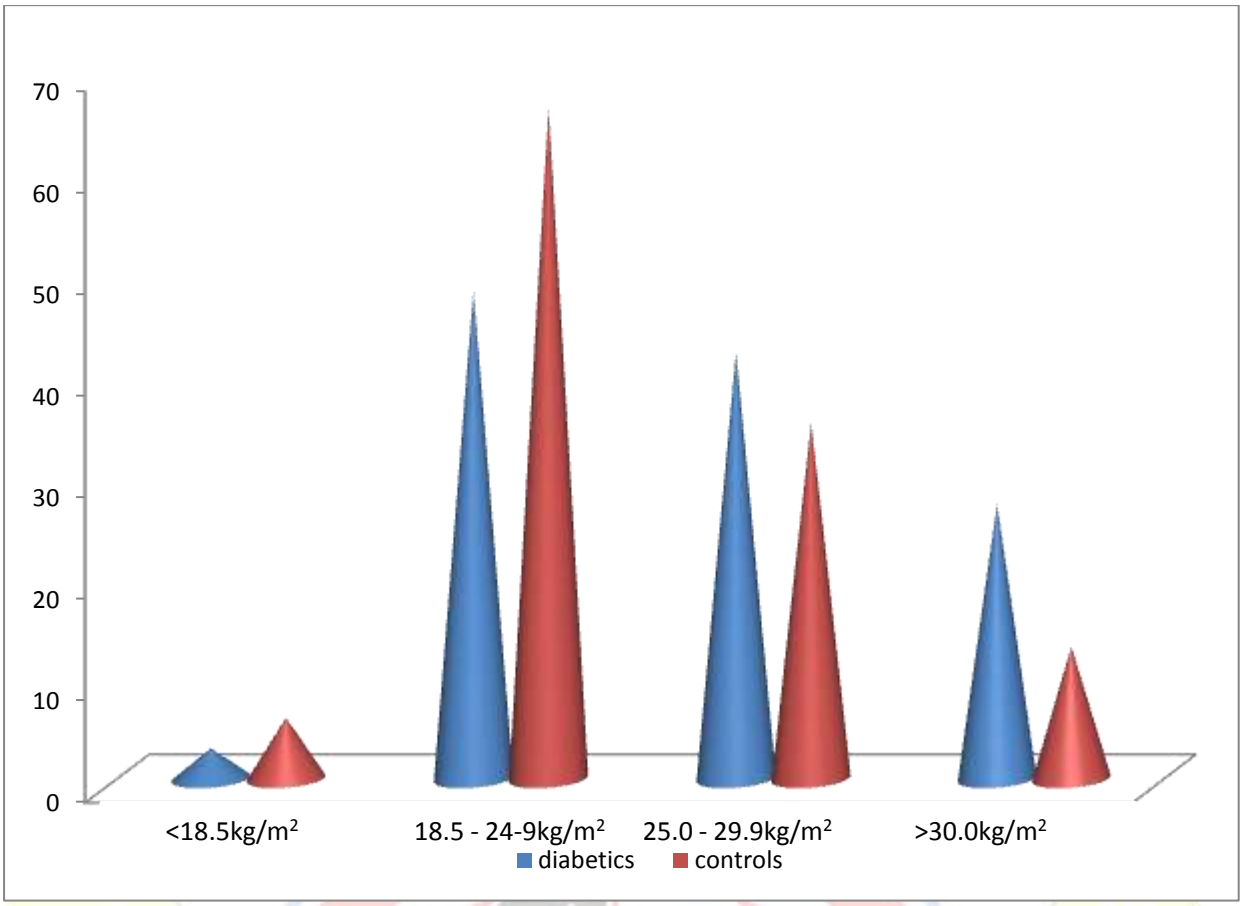

Figure2: BMI distribution of diabetics and controls (in numbers)

Table 1: Mean differences in age and physical variables of diabetic and control subjects

	Diabetics N=120 Mean(sem)	Controls N=120 Mean(sem)	t	p-value
Age(years)	32.70(1.10)	31.23(0.84)	1.49	0.18
Systolic BP (mmHg)	129.81(1.92)	115.17(1.01)	6.75	0.001*
Diastolic BP (mmHg)	80.78(1.07)	68.17(0.74)	9.73	0.001*
Height (metres)	1.68(0.008)	1.69(0.007)	1.19	0.23
Weight(kg)	70.23(1.28)	69.63(1.16)	1.06	0.08
Body Mass Index(BMI)(kg/m^2)	24.88(0.46)	24.36(0.35)	3.87	0.06

*Significance (p< 0.05)

Table 2: Comparing ACE level in diabetic and control subjects

	Diabetics N=120 Mean(sem)	Controls N=120 Mean(sem)	t	p-value
ACE level(IU/L)	25.61(0.63)	23.69(0.42)	2.53	0.01*
Male (ACE level)	26.12(0.89)	24.09(0.49)	2.06	0.04*
Female(ACE level)	25.13(0.88)	23.20(0.73)	1.65	0.10

*Significance (p < 0.05)

Table 3: Comparing Mean of biochemical variables with duration of DM

	<1year duration of DN=21 Mean(sem)	>1year duration of DM N=99 Mean(sem)	t	p-value
ACE level(IU/L)	23.72(1.15)	26.007(0.71)	1.39	0.17
FPG(mmol/L)	8.64(0.85)	9.21(0.42)	0.57	0.57
Sodium(mmol/L)	136.81(1.03)	139.21(0.30)	2.98	0.004*
Potassium(mmol/L)	3.97(0.08)	4.04(0.04)	0.70	0.48
Urea(mmol/L)	3.56(0.18)	4.26(0.15)	2.06	0.04*
Creatinine(µmol/L)	77.14(4.39)	90.81(2.32)	2.52	0.01*

*Significance ($p < 0.05$)

DISCUSSION

Angiotensin converting enzyme (ACE), a glycoprotein and metallopeptidase is synthesized in vascular endothelial cells of the lungs and kidneys[1,2,20.] It exists in both soluble and insoluble forms.[21] The gene location in humans is on chromosome17q 23.[22] However, being a proteolytic enzyme, it catalyses the cleavage of angiotensin I (a decapeptide) to angiotensin II (octapeptide) dipeptide, leading to vascular constriction and blood pressure elevation[3]. ACE is said to be high in diabetic patients. It also contributes to the chronic complications observed in these subjects[6,7].

Angiotensin converting enzyme level is measured quantitatively by different methods. Enzyme linked immunosorbent assay (ELISA) was used in this study[19].

This study assessed the level of ACE in a cross - section of adults with type 2 diabetes mellitus and comparing with apparently healthy subjects who were age and sex - matched. The blood pressure of the diabetic subjects was significantly higher than those of the control subjects. This observation may be due to synergistic (or combined) effect from the defect in the transcription of contractile proteins, resulting in the stiffening of the arterial wall, as well as the vasopressor effect of ACE.[3,8]

Despite the universal use of angiotensin converting enzyme inhibitors in the management of high blood pressure, to the best of our knowledge, this work is the first to assess the level of ACE in adults with type 2 diabetes in Nigeria.

The level of ACE was significantly higher in diabetes subjects. This agrees with independent studies of Schenthaner *et al.*,, Troup *et al.*,, and Wong *et al.*,, who observed similar significant differences.[23,24,25]

The male diabetic subjects showed more ACE levels than the female diabetic subjects. This observation may be due to the diabetes and other social behaviours, as observed on elderly men in a study by Ljunberg and co-workers.[26] The inter-individual genetic differences also contribute to elevated ACE level.[27]

This study observed that some male diabetes subjects were obese (BMI ≥ 30.0 kg/m^2). In related separate studies, Barton *et al.*, as well as Mao and colleagues observed significant contribution of obesity to higher ACE level in diabetic subjects.[28,29]

A study on the effect of the duration of diabetes mellitus on renal function was carried out by measuring sodium, potassium, urea, and creatinine. The study showed that sodium, urea and creatinine were significantly higher in subjects whose diabetes lasted for more than one year than those whose diabetes lasted less than one year duration. This confirms the observation that diabetes affects renal function as the duration increases according to a study by Looker *et al.*, while looking at the relationship involving adiponectin, duration of diabetes and the renal function.[30] Despite this finding, there was no difference in the ACE levels in relation with the duration of diabetes. Similar observation occurred in other studies.[23,31] This shows that the duration of diabetes seams not to have any effect on the ACE level. As such the enzyme levels remain high from the beginning of the illness. However, assessment of the glycaemic control was not done to observe the differences in both controlled and uncontrolled diabetic subjects, but a study by Schernthaner and co-workers observed no correlation between ACE level and the level of glycaemic control.[23]There was also no correlation between the ACE levels and the biochemical parameters measured. There was an inverse relationship between plasma potassium and sodium. This may be due to the worsening glycaemic level as the diabetes progresses, insulin resistance, increased plasma osmolality and electrolyte movement across cell membranes.[32]

Limitations of the Study

1. This study did not include the assessment of glycaemic control for comparison with the level of the ACE in the studied subjects.
2. A larger sample size may offer more weight to any conclusion drawn from observations made.

CONCLUSION

It was observed from this study that the angiotensin converting enzyme levels were higher in diabetic subjects than their age-matched control subjects, and that this higher level was more pronounced in male diabetic subjects. The study also observed that though the biochemical parameters showed worsening renal function with duration of diabetes, the ACE levels did not show similar increase.

REFERENCES

1. Don BR, Lo JC. Endocrine hypertension. In: Gardner DG, Shoback DA. Editors. Greenspan's Basic and Clinical Endocrinology, 8th edition, Toronto, McGraw Hill Medical. 2007; 411.

2. Zhang Xu X, Chen T, Li L, Rao P. An assay for angiotensin converting enzyme using capillary zone electrophoresis. 2000; 280: 286-290.
3. Hoogwerf B.J., Young J.B. The HOPE study. Ramiprillowere cardiovascular risk, but Vit. E did not. Cleve Clin J Medicine. 2000; 67: 287-293.
4. Scultz T., Miller W.C., Bedrossian C.W. Clinical aapplication measurement of angiotensin convertinmg enzyme level. JAMA 1979; 242(5): 439-441.
5. Nicola W., Sidhom G., El KhyatZ.,Ibrahim S., Salah A., ElSayed A. Plasma angiotensin II, renin activity and serum ACE level in non-insulin dependent DM patients with diabetic nephropathy. Endocr J. 2001; 48: 25-31.
6. Ustundaq B., Cantan H., Cinkilinc N., Halifeogiu I., Bahcecioglu H. ACE level in insulin-independent DM and effect of ACE levels on diabetic patients with nephropathy. Cell Biological function 2000; 18: 23-28.
7. Juo S.H. Genetics of carotid atherosclerosis. Front Biosci 2009; 14: 4525-4534.
8. Brownlee M. The Pathology of Diabetic Complications: A unifying mechanism. Diabetes 2005; 54: 1615 -1625.
9. Odum EP, Ejilemele AA, Wakwe VC. Antioxidant status of type 2 diabetic patients in Port Harcourt, Nigeria. Nigerian Journal of Clinical Practice 2012; 15: 55 – 58.
10. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, *et al.,*. A novel angiotensin-converting enzyme-related carboxy- peptidase (ACE2) converts angiotensin I to I-9. *Cir Res 2000; 87:E I-E9.*
11. Keidar S, Kaplan M, Gamliel-Lazarovich A. ACE2 of the heart: From angiotensin I to angiotensin(I-7). *Cardiovasc Res 2007;73:463-469.*
12. Boehm M, Nabel EG. ACE2 – A new cardiac regulator. *N Eng J Med 2002; 347:1795-1797.*
13. Xiao F, Hiremath S, Knoll G, Zimpelmann J, Srivaratharajah K, Jadhav D *et al.,*. Increased urinary angiotensin-converting enzyme 2 in renal transplant patients with diabetes. *PLoS One. 2012;7:e37649.*
14. Crackower MA, Sarao R, Oudit GY. Angiotensin converting enzyme 2 is an essential regulator of heart function. *Nature 2002; 417:822-828.*
15. Nagi DK, Mansfield MW, Stickland MH, Grant PJ. Angiotensin converting enzyme insertion/deletion(I/D) polymorphism, and diabetic nephropathy in subjects with IDDM and NIDDM. *Diabet Med 1995;12:997-1001.*
16. Araoye M.O. Research Methodology with Statistics for Health and Social Sciences. Nathadex Publichers, Ilorin. 2003; 115-129

17. Lott J.A., Mitchell L.C., Moeschbenger M.L. Estimation of reference ranges: How subjects are needed. Clin. Chem. 1992; 38: 648-652.
18. Randox laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom).
19. (Eton Bioscience,5820 Oberlin Drive Suite 108 San Diego USA); Lot number: 2631128313
20. Turner AJ. Exploring the structure and function of zinc metallopeptidases: Old enzymes and new discoveries. *Biochem Soc Trans 2003;3:723-727.*
21. Igic R, Behnia R. Properties and distribution of angiotensin I converting enzyme. *Curr Pharm Des 2003;9:697-706.*
22. Rieder MJ, Taylor SL, Clark AG. Sequence variation in the human angiotensin converting enzyme. *Nature Genet 1999; 22:59-62.*
23. Schernthaner G., Schwarzer C., Kuuzmits R., Muller M.M., Kleman U.,Freyler H. Increased angiotensin converting enzyme activity in diabetes mellitus: Analysis of diabetic type, state of metabolic control and occurrence of diabetic vascular disease. J. Clin. Pathol. 1984; 37: 307-312.
24. Toop M.J., Dallinger K.J., Jennings P.E., Barnette A.H. Angiotensin converting enzyme relationship of insulin dependent diabetes and microangiopathy. Diabet. Med. 1986; 3: 455-457.
25. Wong TY, Szeto CC, Chow KM, Chan JC, Li PK. Prognostic role of serum angiotensin converting converting enzyme activity on outcome of type 2 diabetic patients on chronic ambulatory peritoneal dialysis. *Am J kidney Dis 2002;39:1054-1060.*
26. Ljungberg L, Alehagen U, Lanne T, Bjorck H, De Basso R, Dahlstrom U, *et al.,*. The Association between circulating angiotensin converting enzyme and cardiovascular risk in the elderly. *Journal of the renin – angiotensin – aldosterone system 2011;3:281-289.*
27. Alhenc-Gallas F, Richard J, Courbon D, Warnet JM, Corvol P. Distribution of plasma angiotensin converting enzyme in healthy men; relationship to environmental and hormonal parameters. *J Lab Clin Med 1991;1173:33-39.*
28. Barton M, Carmona R, Morawietz H, d'Uscio LV, Goettsch W, Hillen H, *et al.,*. Obesity is associated with tissue-specific activation of renal angiotensin converting enzyme in vivo. *Hypertension 2000; 35:329-336.*
29. Mao S, Huang S. A meta-analysis of the association between angiotensin converting enzyme insertion/deletion gene polymorphism and the risk of overweight/obesity.

Journal of Renin – Angiotensin – Aldosterone system 2013; doi: 10.1177/1470320313501218.

30. Lookere HC, Krakoff J, Funahashi T, Matsuzawa Y, Tanaka S, Nelson RG, *et al.*,. Adiponectin Concentrations Are Influenced by Renal Function and Diabetes Duration in Pima Indians with Type 2 Diabetes. *Journal of Clinical Endocrinology and Metabolism 2004; 89: 4010 – 4017.*
31. Nicola W,Sidhom G, El Khyat Z, Ibrahim S, Salah A, El Sayed A.Plasma angiotensin II, rennin activity, and serum angiotensin converting enzyme In non-insulin dependent diabetes mellitus patients with nephropathy. *Endocr J. 2001;48:25-31.*
32. Saito T, Ishikawa S, Higashiyama M, Nakamura T, Rokkaku K, Hayashi H, *et al.*,. Inverse distribution of serum sodium and potassium in uncontrolled in-patient with diabetes. *Endocr J. 1999;46:75-80.*