A Comparative Study of Angiotensin Converting Enzyme Concentration In Hypertensive and Non-Hypertensive Adults with Type 2 Diabetes in South-South Nigeria

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ABSTRACT

Hypertension is the commonest cardiovascular co-morbidity of type 2 diabetes mellitus (DM). Obesity and dyslipidaemia contribute to increased peripheral resistance in obese hypertensive diabetic patients. The rising prevalence of hypertension among adults with type 2 diabetes mellitus in Nigeria calls for the evaluation of angiotensin converting enzyme (ACE) as blood pressure regulator. The aim is to measure and compare the serum concentration of ACE in a group of hypertensive and non-hypertensive patients with type 2 diabetes. A cross-sectional comparative study conducted in the University of Port Harcourt Teaching Hospital using confirmed adult type 2 diabetic hypertensive and non-hypertensive patients, selected by simple random sampling technique. Information obtained with a structured questionnaire. One hundred and twenty (120) adult type 2 diabetic patients were used for the study, of which 58 (48.3%) were males and 62 (51.7%) females. Of the 120 diabetics, the hypertensive and the non - hypertensive patients were 55 (45.8%), and 65 (54.2%), respectively. The reference interval of ACE is 22.85- 24.53IU/L. The mean ACE concentration for the hypertensive and the non-hypertensive diabetic patients were 26.8IU/L and 24.6IU/L respectively. There was no significant statistical difference in ACE concentration between both groups, ($p \geq 0.05$). Similarly, no positive association between the serum ACE concentration with the renal indices in both groups was observed. Rather, there were significant statistical differences in blood pressure Body mass Index and renal indices between both groups. There was no difference in the ACE concentration between the hypertensive and non- hypertensive diabetic patients. Also, no correlation between the concentration of ACE and other biochemical variables in both groups was observed, but the physical and biochemical variables showed significant differences.

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

INTRODUCTION

Angiotensin converting enzyme (ACE), a glycoprotein with a molecular weight of 130-160kDa was discovered by Skeggs *et al.* in 1956.[1] It exists in two genetic isoenzyme and physical forms respectively. The soluble form is found in the blood while the insoluble form is a component of the cell membrane.

The reference interval is 0-40 IU/L in individuals above 20 years of age, but is higher in age groups younger than 20 years. Its assay is unreliable in children and adolescents, and in patients suffering from granulomatous diseases.[2]

Genetically, the human ACE gene is located on chromosome 17q23 where polymorphism involving the insertion of 287 base-pair sequence in intron 16 and 26 exons of the ACE gene occurs. This forms the basis for the regulation of ACE function. Presence of the base-pair sequence, marks the allele as insertion allele (I) and its absence is termed, deletion allele (D). Studies have shown that insertion genotype favours lowest ACE levels while deletion genotype favours highest ACE level, and (ID) intermediate levels.[3] This forms the genetic basis for the pathology of several cardiovascular disorders and diabetes.

Physiologically, renal hypoperfusion and hyperkalaemia stimulate the renin-angiotensin-system, which in turn enhance the function of angiotensin converting enzyme in the renal vascular endothelial cells.[4]

Functionally, it regulates blood pressure and plays a role in diabetic micro- and macro-vascular complications.[5,6] Hypertension as a common vascular co-morbidity in diabetes affects about 20% of the world's adult population, including Nigeria.[7,8] Its homologue, angiotensin converting enzyme 2 (ACE2) has a protective function to the heart. And is not affected by ACE inhibitors.[9] In diabetes mellitus, certain mechanisms resulting from hyperglycaemia contribute to endothelial cell damage[10] causing elevated concentration of angiotensin converting

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enzyme. This manifests as the complications observed in these patients.

On account of this, pharmaceutical compounds (ACE inhibitors) were introduced to provide a renoprotective effect,[11] control of hypertension, regulates plasma glucose metabolism, and reduces the rate of development of atherosclerosis.[12] In diabetic patients, ACE inhibitor is a preferred antihypertensive agent for the management of hypertension with or without nephropathy, because it is safer and cost effective.[13]

The rational behind this study was to compare the serum concentration of angiotensin converting enzyme in adult type 2 diabetic patients who have hypertension and diabetic patients without hypertension in South- South Nigeria. A significant change would aid in the management of DM with cardiovascular co-morbidity.

MATERIALS AND METHODS

This is a cross sectional comparative study conducted at the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Rivers State in South-South Nigeria. Questionnaires and the management records were used for subject selection in all the treatment units of the hospital. Patients were adults with type 2 diabetes whose treatment did not include angiotensin converting enzyme inhibitors (ACEI). Informed written consent was obtained after disclosing the objective of the study and explaining the required tests to the patients.

Ethical clearance was obtained before the study was carried out, and at no cost and minimal risk to the consented patients. The study was carried out over six (6) months.

Type 2 diabetic patients (hypertensive and non-hypertensive) who were 21 years and above were included in the study. The diabetic patients were further stratified according to gender. Those excluded from the study were: type 1 DM patients, type 2 diabetic patients who were less than 21 years old and who were on angiotensin converting enzyme inhibitors (ACEI), individuals with renal disease on treatment, tuberculosis, asthma, lung cancer, emphysema, alcoholic hepatitis, chronic liver disease, sarcoidosis, pregnancy and those on steroid therapy.

Biodata, blood pressure and anthropometric measurements were variables used for the study. Body Mass Index (BMI) was calculated for the patients. 10mls of blood was collected and shared into plain bottle without anticoagulant for ACE, fluoride/oxalate bottle for glucose, lithium heparin bottle for electrolyte, urea, creatinine, albumin and ethylene diamine tetra acetic acid (EDTA) bottle for lipids. Urine was collected into urine plain bottle. ELISA kit (Eton Bioscience, Lot number: 2631128313) was used for the ACE measurement; Ion selective electrode (URIT-910 Electrolyte Analyzer), for electrolytes, and the respective methods of analysis for glucose, creatinine, urea.[14,15,16] Microalbuminuria[17] and lipid profile were also determined in the study. Safety measures adopted included the wearing of laboratory coats, gloves, shoes and observation of laboratory instructions. Quality control samples were analysed simultaneously in each batch of 10 samples to ensure analytical accuracy and precision. The intra-batch and inter-batch coefficient of variation for ACE were 3.03% and 4.17% respectively.

Statistical Package for Social Sciences (SPSS version 20.0) software was used for analysis of data generated. Variables were compared using Pie-chart and Student's t-test, and Pearson's Correlation. Confidence interval was 95%, while p-value of ≤ 0.05 was considered statistically significant.

The BMI, physical and biochemical variables were compared in both hypertensive and non-hypertensive diabetic patients.

RESULTS

Characteristics of study population

One hundred and twenty (120) confirmed adult type 2 diabetic patients who met the inclusion criteria were screened for the study. The patients comprised 58 males (48.3%) and 62 females (51.7%). Of the 120 diabetic patients, 55 (45.8%) were hypertensive while 65 (54.2%) had no hypertension. According to gender, equal number of male patients (29) were hypertensive and non-hypertensive. Thirty six (36) female diabetic patients were non-hypertensive and 26 had hypertension. (Figure 1).

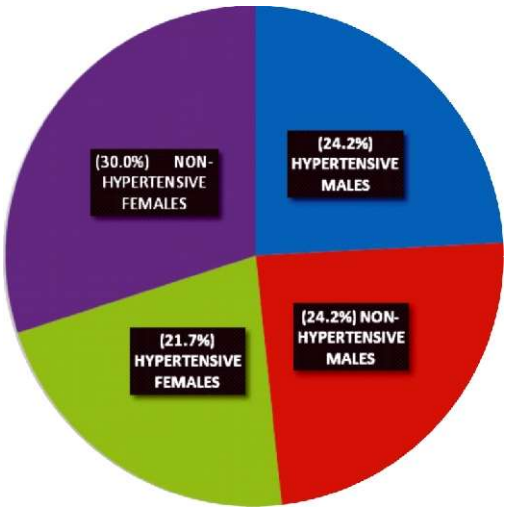

Figure 1. Distribution of diabetic patients according to gender

All the patients used for the study were aged between 26 years and 78 years. The mean age was 42.70 years in hypertensive and 41.23 years in non-hypertensive diabetic patients. Hypertensive patients were not significantly older than the non-hypertensive diabetic patients (p =*0.18*). Both groups of patients were averagely overweight, with BMI of 28.26 and 25.20 respectively. This was significantly higher in hypertensive than the non-hypertensive diabetic patients (p = 0.001). However, the blood pressure of the hypertensive diabetic patients were significantly different from those of the group of non-hypertensive DM patients. (Table 1).

The lower and upper reference limits of the serum ACE were 22.85 IU/L and 24.53 IU/L, respectively. The mean (± SD) serum ACE concentration for the overall diabetic patients was 25.61(± 0.63) IU/L. The mean statistical difference in angiotensin converting enzyme (ACE) concentration between male hypertensive (26.48) and male non-hypertensive diabetic (25.75) was not significant (p = 0.69). The ACE concentration in female hypertensive was 27.07, when compared with female non-hypertensive diabetic patients (23.73). This was equally not statistically significant (p = 0.06). (Table 2).

There was no significant statistical difference between the calculated mean (sum of ACE concentration divide by number of diabetic patients) for the hypertensive and the non-hypertensive diabetic patients (p=0.09).In contrast, there were higher and statistically significant differences in sodium (p = 0.04, potassium (p = 0.005, urea (p = 0.001), creatinine (p = 0.001) and microalbuminuria (p = 0.003) between the hypertensive and the non-hypertensive diabetic patients. In the index study, concentration of ACE, total cholesterol, triglyceride, low density lipoprotein cholesterol in hypertensive were 26.76IU/L, 4.91mmol/L, 1.15mmol/L and 3.23mmol/L respectively; these values were comparatively higher than values in non- hypertensive patients. The HDL-cholesterol was significantly lower in hypertensive patients compared to the non-hypertensive diabetic patients (p = 0.04). (Table 3).

Table 4 shows correlation between ACE concentration and other biochemical variables. In this study, there was no significant relationship between the ACE and other biochemical variables in both groups of patients. The variables were similar except microalbuminuria which was evidently higher in diabetic patients with hypertension than diabetic patients without hypertension.

Table 1: Demographic and Clinical Characteristics of diabetic subjects grouped by the presence of hypertension

Characteristic	Mean(SD) Hypertensive (n=55)	Mean(SD) Non-hypertensive (n=65)	p-value
Age (years)	42.70(1.10)	41.23(0.84)	0.18
Body mass index(kg/m^2)	28.26 (0.75)	25.20 (0.50)	0.001*
Systolic blood pressure (mmHg):			
Male	139.31	122.07	0.001*
Female	144.00	118.14	0.001*
Diastolic blood pressure (mmHg)			
Male	85.38	77.24	0.009*
Female	85.12	76.81	0.004*

*Significance (p < 0.05); SD- Standard Deviation

Table 2: Comparing mean differences in ACE concentration in accordance with gender

	ACE concentration Mean (SD)	p-value
Hypertensive males (n = 29)	26.48 (1.24)	0.69
Non-hypertensive males (n = 29)	25.75 (1.29)	
Hypertensive females (n = 26)	27.07 (1.52)	
Non-hypertensive females (n = 36)	23.73 (1.01)	0.06

*Significance ($p < 0.05$); SD - Standard Deviation

Table 3: Comparing mean biochemical variables in diabetic subjects with and without hypertension

	Hypertension N=55 Mean(SD)	No hypertension N=65 Mean(SD)	p-value
ACE concentration (IU/L)	26.76(0.96)	24.63(0.81)	0.09
FPG(mmol/L)	9.03(0.60)	9.18(0.49)	0.85
Sodium(mmol/L)	139.51(0.43)	138.18(0.45)	0.04*
Potassium(mmol/L)	4.15(0.07)	3.93(0.04)	0.005*
Urea(mmol/L)	4.67(0.23)	3.68(0.12)	0.001*
Creatinine(µmol/L)	95.64(2.87)	82.31(2.85)	0.001*
TC(mmol/L)	4.91(0.15)	4.70(0.12)	0.25
TG(mmol/L)	1.15(0.07)	1.05(0.07)	0.34
HDL-c(mmol/L)	1.16(0.03)	1.26(0.03)	0.04*
LDL-c(mmol/L)	3.23(0.15)	2.97(0.11)	0.16
Microalbuminuria (ACR) (µg/mg)	1.88(0.04)	1.65(0.07)	0.003*

*Significance ($p < 0.05$); SD - Standard Deviation

Table 4: Correlation between the ACE concentration and the biochemical variables

	DM without hypertension ACE concentration	DM with hypertension ACE concentration
FPG	.102	.102
sodium	.004	.004
potassium	.160	.160
Urea	.068	.068
Creatinine	-.065	-.065
TC	-.051	-.051
TG	-.033	-.033
HDL	-.055	-.055
LDL	-.034	-.034
microalbuminuria	-.056	.009

*Significance

DISCUSSION

Angiotensin converting enzyme is an enzyme that plays a central role in blood pressure regulation ranging from vascular volume to constriction.[4] It was known as hypertension converting enzyme initially.[1] It is recently identified by its function.[5]

This study compared the concentration of ACE in two different groups of adults having type 2 DM- a group with hypertension and another without hypertension. The female gender was more than the males in distribution. The males had equal distribution, while the female diabetic patients without hypertension were more than the female diabetic patients with hypertension. The recruitment of more females in this study indicates a higher prevalence of female diabetic patients than the males in Nigeria. This is similar to the observation made by Chinenye *et al.*[18] In addition, it could be adduced to the fact that women are likely to be more sensitive to their health, thus they seek health care early compared to men.

The index study revealed that the mean average age of the patients was greater than forty (40) years as a major predictor of hypertension, in diabetic patients. The prevalence of hypertension in these patients was in relation to the older age and weight. With the insignificant difference in age, there is a possibility of the development of hypertension in due course among those who are yet to be hypertensive as they advance in age. Overweight is an established risk factor in the development of hypertension. Similar observations were made in two independent studies carried out in the Southern and Northern Nigeria where age, and weight were implicated in the development of hypertension.[7,19] In a review conducted in 2011, it was estimated that up to 40% of patients with hypertension have a body mass index (BMI) in the obese range.[20] This study did not stratify the BMI in order to specifically identify the subclass of the obese class. Rather, the observation was collectively reported in the study above. In a study on the prevalence of type 2 Diabetes (DM) and hypertension in overweight and obese people, it was reported that obesity contributes immensely to the development of hypertension in DM.[21] A similar observation occurred in this study. This shows that the prevalence of type 2 DM and hypertension rises with the BMI. In related studies, obesity has been implicated in the development of diabetes, and its co-morbidities.[22,23,24] On the other hand, elevated ACE concentration has been reported in obesity, which is a major risk factor in diabetic complications.[25]

In the index study, the ACE concentration was observed to be higher in male than female diabetic patients. Studies have shown similar observations with elevated blood pressure in hypertensive and non-hypertensive males and females.[26] However, the systolic blood pressure in this study was found to be higher in females than the male hypertensive diabetic patients contrary to the study above. This observation may be due to the effect of DM on reducing the elasticity of blood vessels, and increasing body water in females. The overall study did not observe any significant difference in ACE concentration among the different genders.

Many controversial hypotheses centering on the renin-angiotensin system have been elucidated; however, a study has shown a connection between elevated glucose concentration and renin release from the juxtaglomerular apparatus.[27] This finding is further corroborated by a study done in Austria where a relationship between diabetes and higher concentration of angiotensin converting enzyme was established.[28]

Furthermore, the role of angiotensin converting enzyme in blood pressure control cannot be over-emphasized. Consequently, its concentration was expected to be increased in diabetic patients with hypertension though not significantly different from that of the non-hypertensive diabetic patients.

The glucose concentration is an evidence of the level of understanding of diabetic management by the groups of studied patients. The regularity of glucose check was not considered in the course of the study.

This study observed an insignificant relationship between the hypertensive and the non-hypertensive diabetic subjects in terms of the glucose level. The glucose concentration is an evidence of the level of understanding of diabetic management by the groups of studied patients. A strong basis for this argument would have been established if regularity of glucose check and assessment of glycaemic control were considered in the course of the study.

The significant mean differences among the markers of renal function in both studied patients proved the relationship between cardiovascular complications of diabetes and the renal function. ACE genetic studies have shown the correlation between elevated blood pressure and sodium sensitivity at older age.[29] Any associated renal co-morbidity worsens the cardiovascular complication as seen in elevated low density lipoprotein cholesterol (LDL-c).The diabetic patients without hypertension showed an elevated concentration of alphalipoproteins which seems to reduce the risk for hypertension.

The microalbuminuria showed a higher risk of developing nephropathy in hypertensive than non-hypertensive patients though both groups showed a significant relationship. It is an evidence of long standing diabetes. Odum *et al.* in their study showed similar observation.[30]

There was no significant correlation between angiotensin converting enzyme and other biochemical variables measured in both the hypertensive and non-hypertensive diabetic patients recruited in the study. The studied population and other factors such as awareness, duration of diabetes, onset of hypertension and compliance to treatment may be the contributing factors to the lack of correlation.

CONCLUSION

The ACE concentration in adults with type 2 diabetes was relatively not different irrespective of the associated co-morbidity. It did not correlate with other biochemical variables in both groups studied. However, diabetic patients with hypertension had higher blood pressure, BMI, indices of renal function (plasma creatinine, urea, sodium and potassium), lower HDL-c, and higher index of diabetic nephropathy (microalbuminuria) compared to patients without hypertension.

LIMITATIONS OF THE STUDY

It is a cross-sectional study. The regularity of glucose check and the assessment of glycaemic control was not considered. The glycaemic control was not compared with the ACE concentration due to financial constraint. The patient population was small and may be the cause of the insignificant result.

CONFLICT OF INTEREST: None declared.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Benjamin Aleme, Mr. Olugbenga Bamigbowo, Amos Gbogbo and all Chemical Pathology residents doctors in Department of Chemical Pathology for their contribution.

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