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Full Length Research Paper

Butyrylcholinesterase activity in Nigerian type 2 diabetics with and without metabolic syndrome

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Type 2 diabetes mellitus is a chronic progressive disease typified by a loss of glycaemic control over time as the insulin secreting pancreatic β-cells lose their ability to compensate for the prevailing levels of insulin sensitivity. Several abnormalities are associated with diabetes and metabolic syndrome. Butyrylcholinesterase activity in diabetes and metabolic syndrome is generally under reported. Blood samples and demographic data were obtained from one hundred and five patients presenting at Specialist Hospital Sokoto, Nigeria. These were screened for type 2 diabetes according to the guidelines of the American Diabetes Association. Based on anthropometric indices and clinical data, patients were stratified into to 4 groups: Control (n = 44), type 2 diabetics with metabolic syndrome (n = 14), obese type 2 diabetics without metabolic syndrome (n = 21) and non-obese type 2 diabetics without metabolic syndrome (n = 26). Butyrylcholinesterase was assayed by kinetic and colorimetric method, other biochemical and clinical parameters were according to standard methods. Type 2 diabetics with or without metabolic syndrome have significantly higher activity of butyrylcholinesterase than control group. The higher activity of the enzyme may have been influenced by hyperglycemia, obesity and metabolic syndrome through enhanced transcription or catalytic mechanism of the enzyme or both. Butyrylcholinesterase activity may serve as marker to predict the development of type 2 diabetes and or metabolic syndrome.

Key words: Type 2 diabetes, metabolic syndrome, butyrylcholinesterase, Nigerian, diabetics.

INTRODUCTION

Type 2 diabetes mellitus is a chronic progressive disease typified by a loss of glycaemic control over time as the insulin secreting pancreatic β-cells lose their ability to compensate for the prevailing levels of insulin sensitivity. The hyperglycemia of type 2 diabetes is associated with an increased risk of micro vascular (retinopathy, neuropathy, nephropathy) and macro vascular (myocardiac infarction, stroke) events (Home, 2005; Stettler et al., 2006). Diabetes (together with obesity) represents a serious threat to the health of the populations of almost every country in the world. The World Health Organization estimates that 1.1 million people died of diabetes in 2005 (Nath et al., 2006). Although viewed as a gross underestimate (Nath et al., 2006), the figure is expected to rise by 50% during the next ten years (Amos et al., 1997; A.D.A. 2008). Metabolic syndrome is a complex condition associated with obesity, and characterized by a cluster of closely related clinical features such as dyslipidaemia, inflammation, insulin resistance or full blown diabetes and hypertension (Reaven, 1989). A meta- analysis of prospective studies has shown that presence of metabolic syndrome increases the risk of type 2 diabetes (Bakori et al., 1999). The prevalence of diabetes mellitus in Nigeria is reported to be one to four percent (Bakori et al., 1999; Akinkugbe, 2000) but that of metabolic syndrome has not been reported.

Butyrylcholinesterase (E.C. 3.1.1.8) (BuChE) is primarily associated with glial cells, endothelial cells and neurons (Darvesh, 2003). Its activity in healthy human brain is low compared with acetyl cholinesterase although it may likely have been underestimated (Li et al., 2000). BuChE,

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Abbreviations: BuChE, Butyrylcholinesterase; **BMI,** body mass index; **HDL-C,** high density lipoprotein cholesterol; **LDL,** low density lipoprotein; **VLDL,** very low density lipoprotein; **CVD,** cardiovascular disease.

Table 1. Demographic, anthropometric and blood pressure data of the study subjects.

Group	N	Age (yrs)	M	F	BMI (Kg/m^2)	W.C (Cm)	Blood pressure	
							Systolic	Diastolic
Non Obese, Non Diabetes (M.S absent)- Control	44	50 ±12	26	18	23 ± 4	83 ± 10	110 ± 5	70 ± 5
Obese type 2 Diabetes (M.S present)	14	56 ± 8	9	5	40 ± 3*	105 ± 11*	145 ± 5*	85 ± 5 *
Obese type 2 Diabetes (M.S absent)	21	56 ± 8	14	7	32 ± 2*	103 ± 8*	125 ± 5*	75 ±5*
Non-Obese type 2 Diabetes (M.S absent)	26	54 ± 9	19	7	22 ± 4	86 ± 10^{ns}	120 ± 5 *	60 ± 5^{ns}

N = Number of subjects; M = male; F = female; BMI = body mass index, W.C. = waist circumference; *, significantly different compared with control at P<0.05; ns, not significantly different compared with control at P< 0.05.

although discovered since 1932 (Stedman et al., 1932), its physiologic importance has been poorly understood. But the enzyme is thought to play a key role in compensating for the action of acetyl cholinesterase (Mesulam et al., 2002), a widespread group of enzymes present in both cholinergic and non-cholinergic tissues as well as in plasma (Randell et al., 2005). There is an urgent need for new approaches to address type 2 diabetes and its associated complications. In particular, understanding the various processes that result in the disease and dysfunction of molecular mechanisms will pave the way for the development of new treatment strategies (Nath et al., 2006). Although activities of BuChE have been reported in *in vitro* and animal models (Whittaker,1986; Darvesh, 2003), studies on human diabetics and metabolic syndrome have not been widely reported (Calderon-Margalit et al., 2006). We therefore report our findings of abnormally high activity of BuChE in a cohort of Nigerians with type 2 diabetes with and without metabolic syndrome.

MATERIALS AND METHODS

Subjects and study design

Blood samples and demographic data were obtained from subjects presenting as out-patients at the Diabetic Clinic of the Specialist Hospital, Sokoto, Nigeria, after obtaining institutional approval and study participants submitting signed consent forms. The subjects were screened for diabetes type 2 according to the guidelines of American Diabetes Association (A.D.A 2003). A total number of 105 subjects were enrolled into the study.

Sample collection and preparations

The study was conducted from April to July 2008. Three milliliters (mls) of fasting veinous blood samples were collected from the subjects at the Chemical Pathology Laboratory of the same hospital. Prior to blood collection, anthropometric and clinical data were collected and recorded. Blood pressure was measured with aneroid mercury sphygmomanometer (Accoson) using the non dominant arm and after ten minutes of rest. Patients were defined as hypertensive if they have blood pressure > 140 systolic/90 diastolic mmHg. Anthropometric indices including weight, height, waist and hip circumferences were measured with patients wearing light clothing and no shoes on. Body mass index (BMI), was calculated as ratio of weight (kg) divided by the square of the height (m^2). Weight status was classified using BMI into the following categories: normal, (18.5 - 24.9 kg/m^2), overweight (25 - 29.9 kg/m^2) and obesity ($\geq$30) (W.H.O. 1997). Waist circumference greater than 102 cm (male) or greater than 88 cm (female) was taken to be abdominal obesity.

Samples for biochemistry analyses were centrifuged at 3000 g for five minutes within thirty minutes of collection. The serum was extracted and batch analyzed immediately using Technicon SMAC (Technicon Instruments Corporation) for triglycerides (using enzymatic method), high density lipoprotein cholesterol (HDL-C) using modified enzymatic method of precipitating low density lipoprotein (LDL) and very low density lipoprotein (VLDL) with dextran magnesium sulfate, blood glucose using glucose oxidase method (Trinder, 1969) and plasma BuChE using kinetic colorimetric method employing S-butyryl-thiocholiniiodide (Sigma Chemical Co. M.A.) as substrate. Samples not analyzed immediately (except for glucose which was determined immediately) were stored in plain tubes at 2 - 8°C. Based on the lipid profiles and anthropometric indices, patients were allocated to groups. Group 1 (n = 44; 26 males and 18 females) served as control. Group II (n = 14; 9 males and 5 females) obese type 2 diabetics with metabolic syndrome characterized by fasting blood glucose level above 7 mmol/l, HDL-Cholesterol <1.0 mmol/L, blood pressure > 140/90 mmHg systolic/diastolic , triglyceride >1.7 mmol/L and waist circumference > 102 cm (male) or 88 cm (female). Group III (n = 21; 14 males and 7 females) characterized by obesity, type 2 diabetes without metabolic syndrome and Group IV (n = 26; 19 males and 7 females) non-obese, type 2 diabetic subjects without metabolic syndrome. All subjects were aged between 22 and 73 years.

Data analysis

Data are presented as mean ± standard deviation. Differences between group means and control group mean were tested using student's t-test for two group comparisons and P values less than 0.05 were regarded as statistically significant. Statistical analyses were performed using Graph Pad Instat (Graph Pad Software, San Diego, USA).

RESULTS

The anthropometric and clinical data of the study subjects are presented in Table 1. The mean ages of the subjects in all the Groups (I - IV) were not statistically significant (P>0.05) but the control group had lower mean ages (50 ± 12years) followed by Group IV (54 ± 9years) then Group II and III (56 ± 8years), respectively. The difference in the BMI of group II (40 ± 3 Kg/m^2) and the

Table 2. Biochemical data of type 2 diabetics and control subjects in Sokoto, Nigeria.

Groups	TG (mmol/L)	HDL-C (mmol/L)	FBG (mmol/L)	BuChE (IU/L)
I	1.69 ± 0.18	2.17 ± 0.23	5.20 ± 0.87	2.7± 0.3 *
II	5.90 ± 0.71*	0.58 ± 0.11 *	13.50 ± 4.00 *	8.9 ± 0.3*
III	1.47 ± 0.39	1.44 ± 0.32 *	11.6 ± 3.3 *	8.2 ± 0.2*
IV	2.26 ± 0.39 *	1.12 ± 0.37 *	13.80 ± 4.60 *	8.6 ± 0.3 *

M.S = Metabolic syndrome; TG = triglyceride, HDL-C = high density lipoprotein-cholesterol, FBG = fasting blood glucose; BuChE = butyrylcholinesterase; *, statistically significant when compared to the control group at P<0.05; Group I, Non-obese, non diabetes (M.S absent)-control ; Group II, Obese type 2 Diabetes (M.S present); Group III, Obese type 2 Diabetes (M.S absent) ; Group IV, Non-obese type 2 diabetes (M.S absent).

control group - Group I (23 ± 4 Kg/m^2) and that of Group III (32± 2 kg/m^2) and the control group were statistically significant (P< 0.05). The difference in the BMI of Group IV (22 ± 4 kg/m^2) and Group I (23 ± 4 Kg/m^2) were not statistically significant (P> 0.05). Group II had higher mean BMI (40 ± 3 kg/m^2) followed by Group III (32 ± 2 kg/m^2). The differences in the mean waist circumference between Group II and I and Group III and I were statistically significant (P<0.05). The difference in the mean waist circumference of Group IV and I was not statistically significant (P>0.05). Group II had higher mean waist circumference followed by Group III (103 ± 8 cm), then Group IV.

The difference in the mean blood pressure of Group II (145/85 ± 5 mm) and that of Group I (110/70 ± 5 mmHg) and Group III (125/75 ± 5 mmHg) and Group I were statistically significant (P<0.05). The difference in the BMI of group IV (22±4kg/m2) and group I (23±4 Kg/m2) was not statistically significant (P> 0.05). The biochemical data of the study subjects are presented in Table 2. The differences in the mean triglyceride level of Group II (5.90 ± 0.71 mmo1/L) and Group I (1.69 ± 0.18 mmol/L) and that of Group IV (2.26+0.39 mmol/L) were statistically significant (P<0.05), that of Group III (1.47 ± 0.39 mmo1/L) and Group I (1.69 ± 0.18 mmo1/L) was not statistically significant (P>0.05). There exists a statistically significant difference in the mean HDL-cholesterol level between Group II (0.58 ± 0.11), III (1.44 ± 0.32 mmo1/L), IV (1.12 ± 0.37 mmo1/L) and I (2.17 ± 0.23 mmo1/L). The differences in the mean fasting blood glucose level of Group II (13.50 ± 4.00 mmo1/L), III (11.6 ± 3.30 mmoi1/L), IV (13.80 ± 460 mmo1/L) and that of Group I were also statistically significant (P<0.05). Group I showed a significantly lower activity of BuChE (2714 ± 332 IU/L) compared to all other groups. Comparing Group II and III, there exists a statistically significant difference in the activity of the enzyme although they were both obese type 2 diabetics. The difference in the mean activity of the enzyme between Group III (8169 ± 278 IU/L) and Group IV (8642 ± 315 IU/L) was also statistically significant, although they were both type 2 diabetics and devoid of metabolic syndrome. There is also a statistically significant difference in the mean activity of the enzyme between Group IV and I

DISCUSSION

The present cross sectional study of middle-aged and elderly Nigerian subjects with type 2 diabetes with and without metabolic syndrome is to the best of our knowledge the first to report the activity of BuChE in a community-based sample. Several Nigerian (Sokoto - based) studies have reported on the demographic and clinical correlates of metabolic syndrome in type 2 diabetics. From the studies by Isezuo et al. (2003) and Isezuo and Ezunu (2005), incidence of full blown metabolic syndrome was 20.5% in 52 patients, about 72.4% were dyslipidaemic, and 54.3% were hypertensive. Concurrent hypertension and dyslipidaemia, obesity and dyslipidaemia, and hypertension and obesity occurred in 44.4, 42. 5 and 33.1% of type 2 diabetes, respectively. The connection between type 2 diabetes and dyslipidaemia in humans has been well recognized and reported from other countries (Astrup and Finer, 2000; Abbasi et al., 2002) and its link with mortality (Calderon-Margalit et al., 2006).

Metabolic syndrome is found to be common in diabetic population. Its presence is associated with high levels of plasma butyrylcholinesterase activity. From the results of this study, Group II only fulfilled the diagnostic criteria for metabolic syndrome. According to World Health Organization standard criteria, a person must have fulfilled three or more of the following criteria to be classified as having metabolic syndrome: increased waist circumference with population specific cut off values, increased triacylglycerol levels, low HDL-cholesterol concentration, elevated blood pressure or hypertension and elevated glucose concentration or treatment with a hypoglycaemic agent. It has been observed that these criteria have not been validated for their ability to discriminate optimally for individuals with both metabolic syndrome and related increase in cardiovascular disease (CVD) risk particularly critical for assessment of CVD risk associated with excess visceral adiposity in non-Caucasian populations. Much work is needed in this area (Desperes and Lemieux, 2006). Subjects in Group III

although are obese and have type 2 diabetes, did not satisfy the above criteria. Group IV subjects were type 2 diabetic, not obese and did not have the metabolic syndrome. This finding corroborates the findings from other reports (Isezuo et al., 2003; Isezuo and Ezunu, 2005) that the incidence of metabolic syndrome increases or correlates more closely with abdominal obesity. Plasma BuChE activity is significantly higher in all subjects with type 2 diabetes whether or not metabolic syndrome is present. Abbot et al. (1993) have reported similar finding. The activity of BuChE could serve as a marker for type 2 diabetes (Sridhar and Nirmala, 2002; Randell et al., 2005). However, its relevance as a marker for metabolic syndrome is equivocal since high activity occurs both in patients with metabolic syndrome and those without it but are equally obese. Measurement of the patients′ plasma BuChE level could be incorporated as a routine mass screening programme for type 2 diabetes and metabolic syndrome in the community. There is an urgent need for new approaches to address obesity and type 2 diabetes and their associated complications (Nath et al., 2006). In particular, understanding the various processes that give rise to the characteristics of metabolic syndrome and its attendant risks-from abnormal regulation of energy metabolism through to dysfunction of molecular mechanism (Nath et al., 2006) thus paving the way for the development of new treatment strategies.

Patients with type 2 diabetes and metabolic syndrome exhibit higher levels of plasma butyrylcholinesterase and higher scores of anthropometric indices. The higher activity of the enzyme may have been influenced by hyperglycemia, obesity and metabolic syndrome through enhanced transcription or catalytic mechanism of the enzyme. Butyrylcholinesterase activity may serve as a marker to predict the development of type 2 diabetes and or metabolic syndrome. A question worth asking is what is the molecular basis of the increased activity of the enzyme in type 2 diabetes? Butyrylcholinesterase K variant allele is more common among type 2 diabetics than in non-diabetic subjects suggesting a close association between the butyrylcholinesterase gene (3q26) and the disease which could be related to an identified susceptibility locus on chromosome position 3q27 but independent of islet function (Hashim et al., 2001). Further studies show the positive correlation between activity of the enzyme and risk factors for coronary heart disease such as Apo B , triglycerides and diabetes (Hashim et al., 2001), hence the association between increased activity of the enzyme, lipoprotein synthesis, hypertension and diabetes (Alcantra et al., 2005).

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