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Activities of Pancreatic Enzymes and Anthropometric Indices in Type 2 Diabetic Patients Attending Nnamdi Azikiwe University Teaching Hospital Nnewi, South Eastern, Nigeria

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ABSTRACT: Type 2 diabetes mellitus is the most common type of diabetes, having high blood glucose, reduced insulin secretion and / or inadequate glucagon secretion. The study is to assess the activities of pancreatic enzymes and anthropometric indices. 81 participants comprised 38 diabetic and 43 control were randomly recruited. Informed, oral and written consent was obtained from the participants. Ethical approval was obtained.6mls of fasting blood samples were collected. Plasma glucose; amylase and lipase activities were analyzed using standard laboratory methods. Body mass index (BMI) of participant was determined from height and weight. The results showed significantly increased plasma glucose level in the diabetic participants than in control at p=0.000 and in male diabetics than in female diabetics at p=0.048 in each case. But the activities of lipase, amylase, the mean age and BMI level were the same in both diabetic and control groups at p>0.05 respectively. BMI level, lipase and amylase activities were the same in both genders (p>0.05). Stronger positive correlation exist between Weight Vs BMI (r=0.834; p=0.000), followed by Lipase Vs Amylase (r=0.767; p=0.000), least between Age Vs BMI (r=0.353; p=0.022) but weaker negative associations exist between Height Vs BMI (r=-0.490; p=0.001) and Weight Vs FBS (r=-0.325; p=0.036) in the diabetic subjects. The significant higher level of blood glucose; stronger positive correlation between Lipase and Amylase; Weight and BMI may likely revealed pancreatic exocrine function abnormality in diabetes mellitus type 2.

KEYWORD: Amylase, Diabetes, Glucose, Lipase, Mellitus, Type 2

INTRODUCTION

Type 2 diabetes mellitus has been so problematic in the health of individuals both in Nigeria and in the world. WHO has predicted that diabetes will become the seventh leading cause of death in the world by the year 2030 (1). In 2016, WHO estimated a 4.3% prevalence of diabetes in Nigeria (2).Ajikobi reported that about 4.7 million Nigerians had type 2 diabetes 3). The prevalence of diabetes in the six geographical regions in Nigeria is least in North-West 3.0 %, followed by 3.8 % in the North-Central, 4.6 % in the South-East, 5.5 % in the South-West, then 5.9 % in the North- East and highest 9.8 % in the South- South (4). Individuals with diabetes have a 2–3 folds risk of all-cause mortality (5). Presence of diabetes is associated with increased mortality from infections, cardiovascular disease, stroke, chronic kidney disease, chronic liver disease, and cancer (6,7). Researchers have reported a relationship between type 2 diabetes mellitus and pancreatic dysfunction (8,9).

The pancreas contains the insulin-producing beta cells that are affected in both type 1 and type 2 diabetes mellitus (10). It is divided into three main parts; head, body and tail, that are composed of lobes and smaller 1-10 mm lobules (11).The islets consist of cells with different endocrine functions; the beta cells produce insulin, alpha cells produce glucagon, delta cells produce somatostatin, PP cells produce pancreatic polypeptide, and epsilon cells produce ghrelin. It also secrets pancreatic enzymes. The beta cell mass is the largest (~55%), followed by alpha cells (~33 %), while the other endocrine cells comprise a lesser part of the islet. This relative distribution is, however, varying in different parts of the pancreas (12).

Amylase breaks down starch and glycogen to maltose. It is present at a high concentration in pancreatic juice and in saliva and may be extracted from other tissues, such as gonads, fallopian tubes, skeletal muscle and adipose tissue. Estimation of plasma amylase

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activity is mainly requested to help in the diagnosis of acute pancreatitis, in which the plasma activity may be very high (13). It has become expedience and necessary to ensure continuous and effective clinical monitoring of patients with type 2 diabetes mellitus, so to achieving better management and reduced mortality, hence the need to assessing the activities of pancreatic enzymes and anthropometric indices in this subjects.

MATERIALS AND METHODS

This is a case controlled study designed for the assessment of pancreatic enzymes activities in type 2 diabetic patients attending Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, South Eastern, Nigeria. The formular of Naing *et al*, (14) for sample size calculation, was used and a total of eighty one (81) participants with mean age of 56.32 ±8.61 years were randomly recruited for the study and categorized into two (2) groups as: group A: diabetic patients (n= 38) and group B: control participants (n= 43).

The study protocol was explained to the willing and intending participants and those who gave oral and written informed consent to the study were recruited. Six (6) milliliters of blood sample was collected from each participant into plain containers for the estimation of serum Amylase and lipase enzyme activities respectively. The serum samples were stored at -20°C until analyzed. The anthropometric indices were measured and recorded accordingly. A structured questionnaire was used on the participants, for their bio-data. This research was carried out in the Chemical Pathology laboratory of Nnamdi Azikiwe University Teaching Hospital, Nnewi.

INCLUSION AND EXCLUSION CRITERIA

Know diabetic Participants and apparently healthy participants aged between 40 and 70 years were included in this study.
Pregnant women, and subjects who has history of smoking, hypertension, heart and renal diseases and any other clinical condition apart from type 2 diabetes mellitus were excluded from the study.

Quality control measures: Quality control sera were analyzed along tests samples in each batch of analysis these were compared with the reference values of the control sera. Also, pooled sera were included as control; mean, standard deviation and coefficient of variation were calculated on them.

Ethical Clearance: Ethical approval for the study was obtained from the Ethics Review Committee, Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. Written and oral informed consent was also obtained from the participants and they were assured of anonymity and confidentiality.

METHODS OF ASSAY

Determination of serum lipase activity

This was determined according to the method described by Kurooka and Kitamura (15).

Principle of the Assay

Lipase catalyzes the hydrolysis of ester bonds on the glycerol backbone of a lipid substrate. In humans, pancreatic lipase is the key enzyme responsible for breaking down fats in the digestive system by converting triglycerides to monoglycerides and free fatty acids. Human pancreatic lipase and its related protein 2 are the main lipases secreted by the pancreas. In acute pancreatitis, lipase levels can rise 5 to 10-fold within 24 to 48 hours. Increased activities have also been associated with pancreatic duct obstruction, pancreatic cancer, kidney disease, salivary gland inflammation, bowel obstruction, and other pancreatic diseases. Decreased levels may indicate permanent damage to lipase-producing cells in the pancreas. Simple, direct and automation-ready procedures for measuring lipase activity are very desirable. Lipase Assay Kit is based on an improved dimercaptopropanol tributyrate (BALB) method, in which SH groups formed from lipase cleavage of BALB react with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to form a yellow colored product. The color intensity, measured at 412 nm, is proportionate to the enzyme activity in the sample.

Determination of plasma glucose level

This was determined using glucose oxidase peroxidase method described by Bergmeyer and Bernt (16) using Randox test kit.

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Principle

Glucose present in the sample is acted upon by glucose oxidase to produce gluconic acid and hydrogen peroxide. Hydrogen peroxide is broken down to water and oxygen by peroxidase. The oxygen reacts with 4-aminophenazone and phenol to give a pink colored complex which is measured spectrophotometrically and the concentration of glucose determined accordingly.

Procedure:

Two (2) tests tubes were set up as follows: test and standard.10ul of glucose standard was added to the standard. 10ul of the patient's serum was added to the test tube for test using an automatic micropipette**.** 0.5mls of glucose reagent was added to both test tubes**.** The test tubes were mixed well and incubate at 37^0C for 10 minutes. Absorbance was read at 500nm.2ml of distilled water was usually added to both test and standard to increase the volume.

DETERMINATION OF PLASMA AMYLASE ACTIVITY

This was determined using the modified Caraway method described by Cheesbrough (17).

Principle

The Iodometric (amyloclastic) method is based on the ability of iodide to form a vivid blue color in combination with starch. The starch is hydrolyzed by amylase in the sample to liberate smaller molecules such as dextran, maltose, and some glucose molecules. The smaller sugar molecules do not form a complex with iodide and do not give a blue color. As more starch is broken down, the intensity of the blue color decreases. Substrate (starch) and sample (amylase) are mixed together and incubated for a fixed time. Iodide color solution is added and the starch/iodide color complex is formed. A spectrophotometric measurement is made to determine color intensity (absorbance). The lower the final absorbance (the greater the difference between the blank and test solutions), the higher the serum amylase activity.

Statistical Analysis

The values were expressed as mean ± standard deviation. The significant difference between the mean value of control and experimental group was determined by independent student t-test and pearson r correlation. $P<0.05$ was considered as statistically significant.

RESULTS

Table 1: Comparison of mean ± SD of Anthropometric indices, Lipase and Amylase activities

Parameters	Diabetics (n=38)	Control (43)	t-value	p-value
Age (years)	57.57 ± 8.15	55.07 ± 9.04	1.289	0.201
Height (m)	1.65± 0.09	1.67±0.01	-0.98	9.33
Weight (kg)	69.05±11.99	69.04±10.23	0.007	0.995
BMI (kg/m2)	25.57±5.21	24.81±3.46	0.77	0.443
FBS (mg/dl)	138.35±37.68	82.40±8.61	9.463	0.000
Amylase (iu/l)	81.41±17.37	79.13±8.42	0.758	0.451
Lipase (iu/l)	45.20±30.11	52.05±21.19	-1.186	0.239

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Table 2: Comparison of mean ± SD of Anthropometric indices, Lipase and Amylase activities in both male and female diabetic subjects

Parameters	male diabetics (n=17)	female diabetics (21)	t-value	p-value
Age (years)	57.35 ± 7.98	57.57 ± 8.32	-0.082	0.935
Height (m)	1.65± 0.09	1.63±0.08	1.690	0.100
Weight (kg)	68.88±11.70	69.14±12.22	-0.067	0.947
BMI (kg/m2)	24.70±5.01	26.19±5.27	-0.887	0.381
FBS (mg/dl)	154.24±46.27	129.14±28.87	20.045	0.048
Amylase (iu/l)	80.84±20.46	81.86±14.91	-0.177	0.861
Lipase (iu/l)	44.29±26.26	45.93±33.53	-1.165	0. 870

Table 3: Levels of association between parameters studied in the diabetic subjects (n=38)

Parameters	Correlation Pearson r coefficient	p-value
Age vs BMI	0.353	0.022
Weight vs BMI	0.834	0.022
Weight vs FBS	-0.325	0.036
Height vs BMI	-0.49	0.001
Lipase vs Amylase	0.767	0

DISCUSSION

In this study, the mean weight (Kg) and body mass index (Kg/m^2) of the diabetic participants were not significantly different from those observed in the non - diabetic participants. This finding is similar with the report of Al-Dahhan (18) that recorded no significant differences in mean age and BMI of diabetic patients on drug. However, the diabetic participants had a mean BMI of 25.57±5.21Kg/m^2 typical of an overweight population. The BMI is the most commonly used index of weight status (19) and is used commonly to classify overweight and obesity in adults where normal weight is a BMI 18.5−24.9 kg m^{-2}; overweight is a BMI 25.0−29.9 kg m^{-2}; obese a BMI >30.0 kg m^{-2} (20). It has since been known that overweight and obesity is an important risk factor for the development and progression of type 2 diabetes mellitus (21). Excess weight and physical inactivity are associated with an increased risk of developing various diseases, particularly type 2 diabetes (22). Hence, it is important to target lowering excess weight gain through physical exercise, moderations in dietary patterns and life style in order to avert or reduce the possible diabetic complications arising from such factors.

The present study revealed significantly increased mean plasma glucose level in the diabetic participants than in the non - diabetic participants. This increase is attributable to the prevailing insulin resistance in the diabetics, arising from the destruction of beta cells of the islet of langerhans. β-cells play a very important role in ensuring that in healthy subjects, concentrations of blood glucose

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are stable within a relatively normal physiological range. In a normal healthy subject, there is a continuous feedback relationship between the β-cells and the insulin-sensitive tissues (23). If the adipose tissue, liver, and muscles demand glucose, this will lead to increased insulin supply by the β-cells. If the glucose levels require stability, changes in insulin sensitivity must be matched by a relatively opposite change in circulating insulin levels. Failure of this process to take place results in a deregulation of glucose levels and the development of diabetes mellitus. If the β-cells are healthy, there is an adaptive response to insulin resistance, which leads to the maintenance of normal levels of glucose. By contrast, when pancreatic β-cells are impaired, abnormal glucose tolerance or abnormal fasting glucose may develop, and it may even be followed by the development of type 2 diabetes (24). A continued decline in β-cell function is one of the main causes leading to type 2 diabetes. This finding is consistent with the reports of previous studies (25). Therefore, monitoring glycemic index in diabetic persons is vital to reducing the risk of diabetic complications.

In this study, Lipase (IU/L) and Amylase (IU/L) activities were the same in the diabetic participants than in control participants respectively. Some studies have shown significant decrease in serum amylase and lipase in type 2 diabetic patients which signifies alterations in the exocrine function of the pancreas (26, 27) unlike the current results.

More so, this study showed no statistically significant differences in the mean age, weight and BMI levels, although the mean plasma glucose level was significantly higher in male diabetic participants when compared to the female diabetic participants respectively. This is partly in agreement with the findings of (28) which reported significantly higher mean age in diabetic males than in female; significantly higher BMI in females than in male diabetic participants (28). They further recorded significantly higher mean fasting blood glucose level in female diabetic participants than in their male counterpart; a report which is in contrast to the finding in this study (28). There were stronger positive correlation between Weight Vs BMI (r=0.834; p=0.000), followed by Lipase Vs Amylase (r=0.767; p=0.000), least between Age Vs BMI (r=0.353; p=0.022) but weaker negative associations exist between Height Vs BMI (r=-0.490; p=0.001) and Weight Vs FBS (r=-0.325; p=0.036) in the diabetic subjects.

CONCLUSION

The significant higher level of blood glucose; stronger positive correlation between Lipase and Amylase; Weight and BMI may likely revealed pancreatic exocrine function abnormality in diabetes mellitus type 2.

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