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Evaluation of Biochemical Parameters of Patients with Type 2 Diabetes Mellitus Based on Age and Gender in Umuahia

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Abstract

Diabetes mellitus is a major public health issue. The study was done to determine the changes in some biochemical parameters between type 2 diabetes mellitus patients and apparently healthy adults in Umuahia. The fasting blood glucose was significantly higher in females than in males in the diabetic population. The table revealed a significantly ($P<0.05$) higher levels of serum total cholesterol, trialytglycerol, LDL-cholesterol, in females than males, except low HDL cholesterol ($P<0.05$) compared to male matched patients. Glycated haemoglobin was significantly ($P<0.05$) higher in female than male. Creatinine was significantly ($P<0.05$) higher in male. Urea was also significantly ($P<0.05$) higher in female. Potassium was significantly ($P<0.05$) higher in females. Bicarbonate was significantly ($P<0.05$) lower in female.The study showed a significantly ($P<0.05$) higher levels of total cholesterol, trialyglycerol, LDL- cholesterol in patient's aged between 57-74years, except low HDL-cholesterol ($P<0.05$) compared to those aged between 40-56years. Fasting blood glucose and HbAIC were significantly ($P<0.05$) higher in age limit 57-74years than 40-56years. Urea and creatinine were significantly ($P<0.05$) low in patients aged 57-74years when compared to age 40-56years. However, HDL concentration was significantly ($P<0.05$) high in the 40-56years when compared to 57-74years.Elevated levels of triglyceride (TG), total cholesterol (TC), and glycated haemoglobin (Glyc.Hb) and reduced levels of HD.

Keywords: Type 2 diabetes mellitus, lipid profile, kidney function, liver function, glycated haemoglobin, fasting blood sugar.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by high blood glucose. It is a carbohydrate metabolic disorder characterized by elevated blood glucose [1-4]. Frequently it is a consequence of deficient insulin secretion, insensitivity or both [5]. In 2013, the global estimate of diabetics was about 382 million, amounting to a prevalence of 8.3%. North America and the Caribbeans had the higher prevalence of 11% or 37 million people with diabetes, followed by the Middle East and North Africa with a prevalence of 9.2% or 35 million diabetics [6]. The incidence of known cases as reported is essentially the same for men and women except among the elderly and generally increases with age. The prevalence of diabetes rises from about 6% in people aged 45-64years to 12% in those aged 65years and above [7]. In the older group, more women than men are diagnosed with disease each year, with relatively higher risk in non-whites [8]. Three million two hundred thousand diabetes related deaths are reported annually, 8,700 deaths per day or 6 deaths each minute [8].

In the African continent, prevalence was estimated to be 3 million in 1994 and 7.1 million in 2000. This figure is expected to rise by 2030 to 18.6 million [6]. The prevalence of 23.4% was reported among oil workers in Port Harcourt Nigeria [9].

Diabetes as one of the five leading causes of death in most countries poses financial implication on the sector and patients. World Health Organization (WHO) estimated that as at 2007 diabetes alone accounted for $215-375 billion on cost [7].

Diabetes mellitus can be classified into 3 broad groups namely; Type 1 diabetes mellitus (formally called insulin dependent diabetes mellitus, IDDM) characterized by lack of insulin production due to autoimmune destruction of pancreatic beta cells and constitute 5-10% of cases. Type 2 (formally called non-insulin dependent diabetes mellitus, NIDDM) is characterized by impaired insulin action which accounts for 90-95% of diabetes mellitus [10]. The third category gestational diabetes is a form of glucose intolerance during pregnancy which resolves after the mother has given birth [11]. Diabetes can be associated with many complications. Nephropathy, neuropathy, retinopathy and cardiovascular diseases which are the most commonly encountered episodes [12]. Type 2 diabetes, the most common type is characterized by disorder of insulin resistance and insulin secretion, either of which may be the predominant feature. The important point is that insulin secretion does not cease when the disease develops but may be inadequate for the purpose for which it is secreted. There is usually too much glucose to be metabolized for the amount of insulin present.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PROCESSING

The blood samples were collected in the morning from subjects after overnight fasting from the left or right superficial vein of median cubital vein at *cubital fossa* by vein puncture. A tourniquet was applied at mid upper arm. The area of the suitable vein was cleansed with cotton wool soaked in 70% alcohol.

A ten-milliliter syringe with needle was used. Nine milliliters of blood samples were collected from both the diabetic patients and control subjects and dispensed into the separate test tubes. An aliquot (2ml) of each sample was taken into a fluoride oxalate test tube for fasting blood glucose. A volume (7ml) of each sample was taken into a plain test tube for electrolytes, urea and creatinine(S/E/U/Cr) and lipid profiles estimations. Each sample bottle was labeled with the subject's/patient's name, date and serial number, placed mouth up in a rack and stored in freezer until used.

The blood samples for S/E/U/Cr and lipid profiles were allowed to clot at room temperature and centrifuged at 1,500 revolutions per minute (rpm) for 5 minutes to separate the serum from whole blood.

ESTIMATION OF PLASMA GLUCOSE

Plasma glucose was estimated by the glucose oxidaze method described by Trinder (1969). The reagent kit used was manufactured by Randox Laboratories Limited Ardmore, Diamond Road, United Kingdom.

PROCEDURE

Test tubes were placed in a rack and labelled blank, standard, test and control respectively. One milliliter of glucose reagent was dispensed into each tube. Ten microliters each of distilled water, standard, plasma and control were dispensed into the respective tubes. The contents of each tube were properly mixed and incubated for 10 minutes at room temperature. The absorbances of the standard, test and control were read on a spectrophotometer against a reagent blank at 540 nm within 30 minutes.

ESTIMATION OF GLYCATED HAEMOGLOBIN

Glycated hemoglobin was measured using NyocardHbAlcboronate affinity assay method described by Jeppson *et al.* (2002).

PROCEDURE

The reagent I (RI) was equilibrated at room temperature before use. Five micro liters (5ul) of whole blood was added to the test tube with 20ul RI/reagent. It was well-mixed and then incubated for 2 minutes. It was then mixed to obtain a homogenous suspension twenty-five micro liters (25ul) of the reaction mixture was applied to a test device (TD) by holding the pipette approximately 0.5cm above the test well. The content of the pipette was then emptied quickly into the middle of the test well. The reaction mixture was allowed to soak completely into the membrane. Twenty-five (*25ul*) microliters of the washing solution/reagent 2 mixture were applied to the test device (TD). The washing solution was also allowed to soak completely into the membrane by allowing it to stand for ten minutes. The test result was read within five minutes using the Nycocard Reader II.

ESTIMATION OF SERUM SODIUM

Serum sodium was estimated by colorimetric method of Henry et al. (1974). The reagent kit was manufactured by Obour Industrial Company, Cairo, Egypt.

PROCEDURE

Clean test tubes were placed in a rack and labelled blank, standard, test and control respectively. One milliliter of sodium reagent was dispensed into each tube. Ten microliters (10ul) each of standard, serum and control was dispensed into the respective tubes. The contents of each tube were thoroughly mixed and incubated at room temperature for 5 minutes and the absorbance of the Standard, test and control samples read against reagent blank on a spectrophotometer at 630nm.

ESTIMATION OF SERUM POTASSIUM

Serum potassium was estimated by the turbidimetrictetra pheylborate (TPB) method Hillman (1967). The reagent kit was manufactured by Obour industrial company, Cairo, Egypt.

PROCEDURE

Clean test tubes were placed in a rack and labelled blank, standard, test and control respectively. One milliliter of potassium reagent was dispensed into each tube. Twenty microlitres (20ul) of standard, sample and control were dispensed into the respective reaction tubes. The contents of each tube were thoroughly mixed and incubated at 37^0C for 3minutes and their absorbances read against reagent blank at 578nm.

ESTIMATION OF SERUM CHLORIDE

Serum chloride was estimated by the mercuric chloride method of Schonfeld [13]. The reagent kit was manufactured by Agape Diagnostics, Switzerland.

PROCEDURE

Clean test tubes were placed in a rack and labelled blank, standard, test and control respectively. One milliliter of chloride reagent was dispensed into each tube. Tenmicrolitres (10ul) of standard, sample and control were dispensed into the respective reaction tubes. The contents of each were thoroughly mixed and incubated at 37 C for a minute and their absorbances read against reagent blank at 505nm.

ESTIMATION OF SERUM BICARBONATE

Serum bicarbonate was estimated by spectrophotometric method of Norris [14]. The reagent kit was manufactured by Chema Diagnostica Monsano-Italy.

PROCEDURE

Clean test tubes were placed in a rack and labeled blank, standard, test and control respectively. One milliliter of bicarbonate reagent was dispensed into each tube. Ten microlitres (10ul) each of water, standard, serum and control were dispensed into the respective reaction tubes. The contents of each tube were thoroughly mixed and incubated at 37 C for 2 minutes.

ESTIMATION OF SERUM CREATININE

Serum creatinine was estimated by the buffered kinetic Jaffe reaction without deproteinization method of Bowers and Wong [15]. The reagent kit was manufactured by

Obour industrial company Cairo, Egypt.

CREATININE

Clean tubes were placed in a rack and labelled standard, test and control respectively. One milliliter of creatinine reagent was dispensed into each tube. One hundred microlitres

(100uL) each of standard, test and control reagents were dispensed into the respective tubes. The contents of each tube were properly mixed. After 30 seconds, the respective

absorbances (AI) of the standard, test and control tubes were read. Exactly 2 minutes later, the absorbances (A2) of the standard, test and control tubes respectively were read again.

A2-A1=A specimen or A standard

ESTIMATION OF SERUM UREA

Serum urea was estimated by the urease method of Wheatherburn [16]. The reagent kit was manufactured by Chema Diagnostica, Italy.

PROCEDURE

Clean test tubes were placed in a rack and labelled blank, standard, test and control respectively. One milliliter of urea reagent A was dispensed into each test tube. Ten microlitres

(10uL) each of distilled water, standard, test control was dispensed into the respective tubes.

The contents of each tube were thoroughly mixed and incubated at 37^0C for 5 minutes.

Next, one milliliter of urea reagent B was dispensed into the each of the tubes.

The contents were mixed and incubated at 37^0C for 5 minutes. The absorbances of the standard (AC), test(AX) and control(AV) were read against a reagent blank at 600nm.

ESTIMATION OF SERUM CHOLESTEROL

Serum cholesterol was estimated by the cholesterol oxidase peroxidase method of Arntz [17]. The reagent kit was manufactured by Agape Diagnostics, Switzerland.

PROCEDURE

Clean test tubes were placed in a rack and labelled blank, standard, test and control respectively. One milliliter (1ml) of cholesterol working reagent was dispensed into each tube. Ten microlitres (10ul) each of standard, test and control reagent was dispensed into the respective tubes.

The contents were mixed and incubated for 5minutes at 37°C. The absorbance of each tube was read at 540nm against a reagent blank.

Cholesterol conc (mg/dl)= $\dfrac{\text{Absorbance of test or control}}{\text{Absorbance of standard}}$ ×200

ESTIMATION OF SERUM TRIACYLGLYCEROL

Serum triacylglycerol was estimated by the glycerol -3-phosphate oxidase-TOPS method of Schettler and Nussel(1975). The reagent kit was made by Agape Diagnostics, Switzerland.

PROCEDURE

Clean test tubes were placed in a rack and labelled blank, standard, test and control respectively. One milliliter (1ml) of triacylglycerol reagent was dispensed into each tube. Ten microliters(10ul) of standard, test and control reagents were dispensed into each tube mixed and incubated for 5 minutes at 37^0C. Change in absorbance was measured at 546nm against a reagent blank for each sample.

ESTIMATION OF HDL-CHOLESTEROL

Serum HDL –Cholesterol was estimated by the precipitation. The reagent kit was made by Agape Diagnostics, Switzerland

PROCEDURE

1. Precipitation

Clean test tube was placed in a rack and 30uL of HDL reagent and 30uL of test sample added to the tube. The content was mixed, well, allowed to stand for 10 minutes at room temperature, mixed again and centrifuged for 10 minutes at 4000 rpm. After centrifugation, the clear supernatant was separated from the precipitate within one hour using a Pasteur pipette. HDL cholesterol concentration was determined using

cholesterol reagent.

2. HDL cholesterol determination

Three clean test tubes were placed in a rack and labelled blank, standard, test and control respectively. One thousand microliters (1000uL) of cholesterol reagent were dispensed into each tube. Fifty mircoliters (50uL) of HDL standard and the HDL supernatant were dispensed into standard and test tubes respectively. The contents were mixed incubated for 5 minutes at 37C. The absorbances of the standard and test samples were measured at 630nm against a reagent blank.

STATISTICAL ANALYSIS

The data generated were analyzed using statistical software SPSS version 16.0. Students t- test was used for analysis of difference between means for two groups at $P<0.05$ level of significance. Pearson correlations were conducted to determine associations among different variables.

RESULT

BIOCHEMICAL PARAMETERS BETWEEN MALES AND FEMALES

In table 1, fasting blood glucose was significantly higher in females than in males in the diabetic population. The table revealed a significantly ($P<0.05$) higher levels of serum total cholesterol, trialytglycerol, LDL-cholesterol, in females than males, except low HDL cholesterol ($P<0.05$) compared to male matched patients. Glycated haemoglobin was significantly ($P<0.05$) higher in female than male. Creatinine was significantly ($P<0.05$) higher in male. Urea was also significantly ($P<0.05$) higher in female. Potassium was significantly

($P<0.05$) higher in females. Bicarbonate was significantly ($P<0.05$) lower in female.

Table 1: Comparing the biochemical parameters between males and females

TREATMENTS	MALE	FEMALE
Sodium	144.23±47.34	123.08±32.11
FBS	129.54±10.73	133.71± 9.42
Potassium	3.95 ± 5.71	4.53± 0.60
Chloride	87.50± 9.58	90.33± 9.90
Bicarbonate	21.50± 3.35	22.25± 3.19
Urea	32.6923 ± 14.33532	29.1250 ± 14.02579
Creatinine	0.85± 0.26	0..89± 0.21
Total Cholesterol	210.77±24.09	191.92±30.15
High Density Lipoprotein	41.62± 8.23	46.21± 14.26
TG	134.54±21.99	140.08±26.02
Glycated Haemoglobin	6.54± 0.54	6.49± 0.64
LDL	136.58±25.88	114.10±41.64

Table 2: Comparing of biochemical parameters between age ranges 57-74 years and 40- 56 years

AGE	57 – 74 YEARS	40 – 56 YEARS
Bicarbonate	21.86 ± 3.65	21.86 ± 2.8
Sodium	128.92 ± 16. 28	132.24 ± 6.91
Potassium	4.88 ± 0.70	4.70 ± 0.57
Chloride	87.86 ± 11.02	89.06 ± 9.33
FBS	160.38 ± 59.14	127.68 ± 27.89
Urea	33.79 ± 20.48	30.72 ± 13.33
Creatinine	0.93 ±0. 27	0.86 ±0. 24
Cholesterol	205.58 ± 32.02	202.50 ± 33.74
HDL	43.19 ± 11.45	45.43 ± 12.38
TG	151.43 ± 35.29	146.11 ± 19.85
HBA/C	6.64 ± 53	6.47 ± 0.61
LDL	126.9429 ± 38.82065	124.9806 ± 39.92871

Table 2 showed a significantly (P<0.05) higher levels of total cholesterol, trialyglycerol, LDL- cholesterol in patient's aged between 57-74years, except low HDL-cholesterol (P<0.05) compared to those aged between 40-56years.

Fasting blood glucose and HbAIC were significantly (P<0.05) higher in age limit 57-74years than 40-56years. Urea and creatinine were significantly (P<0.05) low in patients aged 57-74years when compared to age 40-56years. However, HDL concentration was significantly (P<0.05) high in the 40-56years when compared to 57-74years.

Result for HbAIC showed an increase in 57-74years when compared to the 40-56years group.

DISCUSSION

In this study, fasting blood glucose was significantly higher in females than in males in the diabetic population. This may be as a result of the fact that after menopause, there is loss of ovarian function.

This results in adverse changes in glucose and insulin metabolism [18]. Nutrition may also be a contributory factor of higher glucose levels in females. Females have a higher body mass index than males. The results revealed a significantly (P<0.05) higher levels of serum total cholesterol, triacylglycerol, LDL-cholesterol, in females than males, except low HDLcholesterol (P<0.05) compared to male matched patients. The higher prevalence of hyperlipidaemia in females was due to their higher body mass index. A number of changes occur in the lipid profile after menopause [19]. Lack of estrogen is an essential factor in this mechanism. A part from maintaining friendly lipid profile, estrogen change vascular tone by increasing nitric oxide production. Glycated haemoglobin was significantly (P<0.05) higher in female than male.

Bicarbonate was significantly (P<0.05) lower in female and male. This may be due to hormonal changes which lead to increased cardiovascular risk 19.

This study observed a significantly (P<0.05) higher levels of total cholesterol, trialyglycerol, LDL- cholesterol in patient's aged between 57-74years, except low HDL-cholesterol

(P<0.05) compared to those aged between 40-56years. This may be due to hormonal changes and aging.

Fasting blood glucose and HbA₁C were significantly (P<0.05) higher in age limit 5774years than 40-56years. The ensuing hyperglycernia lead to increase protein glycation hence high levels of glycated haemoglobin serum [20]. The increased levels may be due to diminishing body function because of aging.

Urea and creatinine were significantly (P<0.05) low in patients aged 57-74years when compared to age 40-56years. This can be attributed to the rapid break down of proteins during aging. Also, consulting the activity of muscle mass, the concentration of creatinine may be affected.

Generally, in diabetic conditions, high level of cholesterols is observed. From the result obtain from this study, cholesterol concentration was significantly (P<0.05) higher in the 57-74years group when compared to the 40-56years. This can be attributed to reduction in physical activity in aged patients and also the impairment in her to carry out its metabolic function during aging. TG and LDL were also high in the 57-74 years when compared to the 40-56years and this corroborated on result for total cholesterol.

However, HDL concentration was significantly (P<0.05) high in the 40-56years when compared to 57-74years. HDL has been reported to transport cholesterol, from the walls of the artery to the heart degradation. The increase in HDL in the 40-56years group can be attributed to high physical activities and also proper functioning of the liver. Diabetic complication may increase as aging progresses.

Result for HbA₁C showed an increase in 57-74years when compared to the 40-56years group. As diabetes progresses, hyperlipidemia or hypercholesteronaemia may set in.

Increase in cholesterol concentration is related to the onset of obesity.

From the result of the study, cholesterol concentration was higher in the older patients. Obesity has been linked to the increase in HbA₁C and this was ending net in our study.

Difference in electrolytes in the study can be attributed to the psychological change that is bond to occur during aging and also membrane disorder associated with aging.

Diabetes is a group of conditions linked by an inability to produce enough insulin and/or to respond to insulin. This causes high blood glucose levels (hyperglycemia). Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycaemic condition resulting from defects in insulin secretion, insulin action or both. Permanent neonatal diabetes is caused by glucokinase deficiency, and is an inborn error of the glucose-insulin signaling pathway [8]. The estimated worldwide prevalence of diabetes among adults in 2010 was 285 million (6.4%) and this value is predicted to rise to around 439 million (7.7%) by 2030 [11].

The body tries to rid the blood of excess glucose by flushing it out of the system with increased urination. This process can cause dehydration and upset the body's electrolyte balance as sodium, potassium is lost in the urine. The statistical result above (bar chart), compares the lipid profile and kidney function parameters in diabetic and normal patients. A sharp increase in fasting blood sugar is seen in diabetic patients (test group) compared with non-diabetic patients (control group).

This is as a result of the body not being able to process glucose, the body's primary energy source, effectively. Normally, after meal carbohydrates are broken down into glucose and other simple sugars. This causes blood glucose levels to rise and stimulates the pancreas to release insulin

into the blood stream. The basic effect of insulin lacks or insulin resistance on glucose metabolism is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain [6].

CONCLUSION

Elevated levels of triglyceride (TG), total cholesterol (TC), and glycated haemoglobin (Glyc.Hb) and reduced levels of HDL with either normal or elevated levels of LDL compared with non-diabetic patients was noted.

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