Biochemical and hematological indices of normal human subjects, pre-treatment, and combined therapy (semi-vegan diet and metformin)-administered diabetic patients of Imo State University Teaching Hospital Orlu, Nigeria

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The aim of the study is to investigate the efficacy of the use of biochemical, and hematological indices as diagnostic parameters of type 2 diabetes mellitus disease, useful for monitoring recovery from the disease. The experimental units were twenty (n=20) pre-treatment diabetic patients (pre-treat D patients); the same twenty pre-treat D patients administered with metformin (glucophage: dose ≈1250 mg/day) and balanced semi-vegan diet (met-AD patients) for a six-week treatment period; and twenty normal human subjects (Norm-H subjects). The mean values (± standard error) of fasting blood sugar (FBS), serum glycated albumin (GA), fasting serum insulin (I), serum aspartate aminotransferase (AST), serum cholesterol (C), serum triglyceride (TG), glycated hemoglobin (HbAc1), serum Ca²⁺, were significantly higher (p<0.05), (p<0.01, for Ca²⁺), in the pre-treat D patients compared with the met-AD patients (73.98±1.15 mg/dl, 15.8±0.5%, 8.2±0.9 mU/L, 9.9±1.0 mg/dl, 80.1±1.5 mg/dl, 5.25±0.8%, and 2.29±0.10 mmol/l, respectively) and Norm-H subjects. The mean values of Mg²⁺ and Na⁺ were significantly lower (p<0.01) in the pre-treat D patients compared with the met-AD patients (0.99±0.05 mmol/l and 142.5±0.2 mmol/l), respectively, and Norm-H subjects. Multiple regression studies revealed that FBS regressed significantly (p<0.05) with C, TG, GA, and HbAc1 of met-AD patients. The correlation statistical analysis between C and TG of pre-treatment D patients was significant (p<0.05), r = 0.985. Analyzed indices are effective and sufficient for the diagnosis, and post-treatment monitoring of recovery from type 2 diabetes mellitus. Prognosis studies show that a combined semi-vegan dietary and drug (metformin/glucophage) therapy is very efficient and useful in the management and treatment of type 2 diabetes mellitus.

Key words: Serum, glycated, treatment, prognosis, rehabilitation.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by a chronic hyperglycemic condition resulting from defects in insulin secretion, and/or insulin action. Permanent neonatal diabetes is an inborn error of the glucose-insulin signaling pathway, caused by glucokinase deficiency (Njolstad et al., 2003). A staggering 53.1 million citizens will be affected by the diabetes disease according to experts who predicted that the incidence of diabetes is set to soar by 64% by 2025 (Rowley and Bezold, 2012). There are two main types of diabetes mellitus viz : Type 1 diabetes, also known as the
insulin dependent diabetes mellitus (IDDM), which is caused by lack of insulin secretion by beta cells of the pancreas, and Type 2 diabetes, known as non-insulin dependent diabetes mellitus (NIDDM), which is caused by decreased sensitivity of target tissues to insulin (Ozougwu et al., 2013).

Pathogenesis and Pathophysiology of Type 1 and Type 2 Diabetes Mellitus

The basic effect of lack of insulin or insulin resistance on glucose metabolism, in the disease condition of diabetes mellitus, is to prevent the efficient uptake and utilization of glucose by most cells of the body, except those of the brain. As a result of this, blood glucose concentration increases, cell utilization of glucose falls increasingly lower and utilization of fats and proteins increases. (Guyton and Hall, 2006a). Type 1 diabetes mellitus is a chronic autoimmune disease which facilitates selective destruction of insulin-producing pancreatic β-cells (Al Homsi and Lukic, 1992). Loss of insulin secretion, abnormal function of pancreatic α-cells, and excessive secretion of glucagon are evident in IDDM patients. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle (Raju and Raju, 2010).

The predominant form of diabetes is the type 2 diabetes and accounts for at least 90% of all cases of diabetes mellitus (Gonzalez et al., 2009). Impaired insulin secretion through a dysfunction of the pancreatic β-cell, and impaired insulin action through insulin resistance are the two main pathological defects in type 2 diabetes (Holt, 2004). Type 2 diabetes mellitus has a greater genetic association than type 1 DM. The pathogenesis of type 2 diabetes mellitus is characterized by impaired insulin secretion and insulin resistance. Pancreatic abnormalities in the islet of Langerhan's secretory cells in type 2 diabetes mellitus are noted in beta, alpha and delta cells of the islets. Relative decrease in basal secretion, decreased first and second phases of insulin response, glucose insensitivity and amino acid hypersensitivity of insulin release are defects associated with poor insulin secretion (Ozougwu et al., 2013).

Types 1 and 2 diabetes mellitus could lead to diabetic retinopathy, cataracts, and total loss of vision. Epidemiologic studies have demonstrated that cataracts are the most common cause of visual impairment in older-onset diabetic patients (Klein et al., 1985). Three molecular mechanisms seem to be involved in the development of diabetic cataracts: non-enzymatic glycation of lens proteins, oxidative stress and activated polyol pathway (Kyselova et al., 2004).

Causative agents and factors of obesity are genetic as well as environmental, and have a strong effect on the development of type 2 DM (Bjorntorp (1992); Haffner et al. (1992)). Aging, obesity, insufficient energy consumption, alcohol drinking, smoking, etc are independent risk factors of pathogenesis of type 2 diabetes. An inability to produce or release antidiuretic hormone (ADH) from the posterior pituitary can be caused by head injuries or infections, or it can be congenital. In this condition, the distal tubular segments cannot reabsorb water in the absence of ADH, resulting in a peculiar disease called ‘central diabetes insipidus’, which is characterized by the formation of a large volume of dilute urine, with urine volumes that can exceed 15 L/day (Guyton and Hall, 2006b).

Prophylaxis and Treatment of Types 1 and 2 Diabetes Mellitus

Drinking a barley extract–enriched beverage may help to improve insulin sensitivity and prevent type 2 diabetes (Bays et al., 2011). Individuals who consume red meat—especially processed types (such as deli meats, bacon, and sausage), are at a risk of developing type–2 diabetes (Pan et al., 2011). Calcium is a nutritious metal/mineral which increases insulin secretion and may reduce insulin resistance. Dairy milk, cheeses, and yogurts are rich sources of calcium. Men and women who walk briskly for 30 minutes for five days of a week, lower their fat and calorie intake, and achieve a 7% body weight reduction over a three–year period, eliciting a 58% reduction of their risk of developing type 2 diabetes mellitus.

Insulin analogs (e.g insulin lispro, insulin glargine and insulin aspart), constructed by changing the structure of the native protein, improved the therapeutic properties of insulin, without an increase in hypoglycemic events (Vajo et al., 2001). Human insulin synthesized by means of plasmid vectors of E coli, cloned with the human insulin, is used in treating diabetes. Stem cell therapy for diabetes was developed using molecular biology technology, molecular immunology and cell biology. Self activation of the islets of Langerhan stem cell and stem cell transplantation are effective in curing diabetes. Embryo stem cells, and the insulin type of cells which originate in the embryo and develop into bone mesenchymal stem cells are used as transplantation cells in diabetes therapy (Chu, 2013). A major therapeutic strategy for blood glucose control in type 2 diabetes is the regulation of glycogen metabolism. A compound labeled, CP-316819, binds at a regulatory inhibitor pocket site, some 33 Å from the catalytic site (where glucose binds), of the less-active b form of glycogen phosphorylase, so preventing its transformation to the more active a form of the enzyme (Baker et al., 2005). A number of medicinal plants have been studied for the treatment of diabetes. Cinnamon has blood sugar-lowering properties (Yeh et al., 2003). Extracts from Australian Sandalwood and Indian Kino tree slow down two key enzymes in carbohydrate metabolism, essential to the cure of diabetes. Isoorientin is the main hypoglycemic component in Gentiana olivieri (Sezik et al., 2005). Grape
seed extracts decreased cardiovascular risk in type 2 diabetic human subjects, because they significantly improved markers of inflammation, glycaemia and oxidative stress in obese Type 2 diabetic subjects at high risk of cardiovascular events over a 4-week period (Kar et al., 2009).

The biochemical mechanisms involved in the lowering of blood sugar by several classes of type 2 diabetes medicines include: stimulating the pancreas to produce and release more insulin [e.g Meglitinides (Prandin), and Sulfonylureas (Glucotrol)], inhibiting the production and release of glucose from the liver [e.g Dipeptidyl peptidase-4 (DPP-4) inhibitors (Onglyza)], blocking the action of stomach enzymes that break down carbohydrates and improving the sensitivity of cells to insulin [e.g Biguanides (glucophage)] [Verdonck et al. (1981), Rendell (2004), Eurich et al. (2007)].

Metformin suppresses glucose production by the liver by directly reducing hepatic glucose production, but slightly increases sensitivity to insulin by reducing hyperglycemia. Metformin acts on the mitochondria, causing increased AMP. Elevated cellular AMP levels inhibit membrane bound adenyl cyclase, causing a reduction in cellular cAMP levels and decreased protein kinase A (PKA) activation and target phosphorylation (Miller et al., 2013). Bloating, fullness, nausea, cramping, diarrhea, vit B12 deficiency, headache, metallic taste, agitation, and lactic acidosis are side effects associated with the administration of metformin. Contraindications to the administration of metformin include: Diabetic ketoacidosis (DKA), alcoholism, binge drinking, kidney or liver disease, congestive heart failure, pregnancy, surgery, and heart attack.

A non-pharmacologic approach to the treatment of diabetes may require nutritional/dietary therapy. The recommended balanced diet should be low in saturated fat, rich in fibre, and is designed to bring about progressive weight loss (Hallé, 2001). The diet often recommended for diabetic patients is high in dietary soluble fiber, low in saturated fat and sugar, but moderate in some essential fatty acids (EFAs).

High fiber diet induced lower fasting blood glucose levels (p<0.01), and decreased the ratio of low-density lipoproteins to high density lipoproteins (p<0.025), in comparison with low fiber diet, in non-insulin-dependent diabetes mellitus (NIDDM) patients (Hagander et al., 1988).

**Diagnostic Indices of Diabetes**

Significant increase (p< 0.05) in fasting and post prandial glucose concentrations was observed of type 2 diabetic mellitus patients in the age range (13-39 years) as compared with control although insulin concentration was normal by (Bahgat et al., 2010).

Significant increase (p<0.05) in serum cholesterol and serum triglyceride level were observed of women suffering from gestational diabetes mellitus compared with healthy pregnant women (Khan et al., 2012).

Diabetic patients have significantly higher levels of mean plasma cholesterol (p = 0.03), LDL triglycerides (p = 0.003), and HDL triglycerides (p= 0.02) compared with normal control subjects (Manzato et al., 1993). Cell membrane disruption at high concentration, mitochondrial dysfunction, toxin formation and activation, and inhibition of key steps in the regulation of metabolism account for free fatty acids found in the insulin-resistant state which is directly toxic to hepatocytes resulting in elevated levels of transaminases.

Thirty-nine (39) out of one hundred and fifty (150) patients had significantly elevated levels (p=0.0001) of aspartate aminotransferase (AST) in a liver dysfunction analyses of diabetic patients carried out in a Referral hospital (Tkakelmayum et al., 2014). Mean values of aspartate amino transferase (AST), and serum glucose were significantly higher (p<0.001) in type 2 diabetes mellitus patients compared with normal individuals (control) (Idris et al., 2011).

Production of free radical is increased during diabetes. Serum albumin is a major antioxidant agent but becomes structurally modified by glucose or free radicals, thus impairing its antioxidant properties, in diabetes mellitus (Faure et al., 2008). The total plasma proteins were significantly increased (p<0.05), while serum albumin was significantly decreased (p<0.05), in type 1 and type 2 diabetics compared with normal, healthy, non-diabetics (Rehman et al., 2012).

Serum albumin concentrations were significantly lower (p<0.01) in type 2 diabetes mellitus patients compared with normal individuals (control). However, the means of AST, and albumin fell within the normal range of values (Idris et al., 2011).

Serum glycated albumin (GA) is hypothesized to be an alternative marker for glycemic control in patients with diabetes, which is not affected by changes in the survival time of erythrocytes as is the case with type 2 diabetes characterized by hemoglobinopathy (Kosecki et al., 2005). Measurement of glycated albumin is free of interference by endogenous glycated amino acids, and is unaffected by changes in albumin concentration (Kouzuma, 2004). A 3% increase of GA is equal to a 1% increase of HbA1c, for this reason, it was suggested that an increase of GA might be more highly indicative of diabetes than that of HbA1c (Inaba et al., 2007). The mean value of glycated albumin was significantly increased (p<0.05) in hemodialysis patients with diabetes compared with hemodialysis patients without diabetes (Inaba et al., 2007).

Hyperinsulinemia and insulin resistance are important risk factors for the future development of type 2 diabetes mellitus. Serum insulin was significantly higher (p < 0.001) in type 2 diabetes mellitus patients compared with control (Kim et al., 2000).

In nondiabetic patients, glycosylated hemoglobin levels were within the normal range (4.0% to 6.8% of total blood
hemoglobin levels), and correlated significantly with fasting blood glucose levels, serum urea levels, and serum total carbon dioxide content (Tzamaloukas et al., 1989).

The A1C (HbA1c, glycated or glycosylated hemoglobin) test result reflects the value of the average blood sugar level for the past two to three consecutive months. Specifically, the A1C test measures what percentage of hemoglobin is coated with sugar (glycated). The higher the A1C level, the poorer the blood sugar control and the higher the risk of diabetes complications. A non-diabetic human subject has about five percent glycated hemoglobin. A value of glycated hemoglobin that is 6.5 percent or above is indicative of diabetes; A value of glycated hemoglobin that is 5.7 to 6.4 percent is indicative of prediabetes (Manfred, 2014). The 2010 American Diabetes Association Standards of Medical Care in Diabetes stated that A1c ≥ 48 mmol/mol (≥6.5 DCCT %) is another criterion for the diagnosis of diabetes (Cefalu, 2010).

In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, and retinopathy (Larsen et al., 1990).

The longer hyperglycemia occurs in blood, the more glucose binds to hemoglobin in the red blood cells and the higher the glycated hemoglobin (Sidorenkov et al., 2011).

Sodium (Na⁺), potassium (K⁺), calcium (Ca²⁺) and magnesium (Mg²⁺) are serum electrolytes and play an important role in intermediary metabolism and cellular functions, including enzyme activities and maintenance and regulation of electrical gradients [Lobo (2004), Hall and Guyton (2006)]. Alterations in the levels of some serum electrolytes are associated with changes in plasma glucose levels and diabetes mellitus [DeFronzo et al. (1976), Katz (1973)].

Serum Mg²⁺ level in African Americans and Caucasians was significantly lower (p= 0.017) in subjects with prevalent cardiovascular disease and diabetes (Ma et al., 1995). Serum Mg²⁺ depletion is associated with the symptoms of peripheral artery disease (PAD), such as foot ulcers, in subjects with type 2 DM (Rodriguez-Moran and Guerrero-Romero, 2001). Plasma Mg²⁺ concentrations were inversely related to plasma glucose levels in human subjects with diabetes mellitus (Yajnik et al., 1984). Hypomagnesemia may lead to a higher incidence of diabetes mellitus (Pham et al., 2007). Oral magnesium supplementation reduces fasting plasma glucose levels in diabetes mellitus patient (Song et al., 2006).

Serum Ca²⁺ level correlated positively with glucose level in diabetic patients (Shenqi et al., 2013). Resnick ionic hypothesis suggests that diseases such as hypertension, metabolic syndrome, and diabetes mellitus, share a common, altered intracellular condition, characterized by decreased Mg²⁺ level and reciprocally elevated free intracellular Ca²⁺ level (Barbagallo et al., 2007). Elevated serum Ca²⁺ levels observed in type 2 diabetes mellitus is caused by insulin resistance and the impairment of insulin secretion, and is as a result of the critical role played by Ca²⁺ in muscle contractions, insulin secretion, and glucose uptake after the binding of insulin to muscle cell membranes (Bjornholm and Zierath, 2005).

The functions of Ca²⁺-Mg²⁺-ATPase, Na⁺/Ca²⁺ exchanger and Ca²⁺ pump, which are located in the cell membrane, mitochondria or endoplasmic reticulum, are impaired in diabetes mellitus [Mikaelian et al. (2013), (2013), Dhall et al. (2012)].

Metformin has the ability to slow down the accelerated basal metabolic rates of hepatic gluconeogenesis without significant, apparent effect on lactate turnover for gluconeogenesis or increases in insulin secretion by decreasing the amount of phosphoenolpyruvate carboxykinase and glucose 6-phosphate [Cusi et al. (1996), Mithieux et al. (2002)]. Metformin reduces glycated hemoglobin (HbA1c) by 1.4-2% [Cusi et al. (1996), DeFronzo and Goodman (1995)]. Metformin has been proposed as a treatment for cancer (Quinn et al., 2013). Metformin increases number of insulin receptors on muscle and fat cells.

Metformin markedly lowered plasma total cholesterol and triglyceride levels, due, mostly to a decrease in very low density lipoprotein-triglyceride (Geerling et al., 2014). The full improvement in glycemic control and cholesterol levels by the administration of metformin to diabetic patients, may not be seen until 4 to 6 weeks of use have passed.

In type 2 diabetic patients who are intensively treated with insulin, the combination of insulin and metformin results in superior glycemic control compared with insulin therapy alone, while insulin requirements and weight gain are less (Wulffelé et al., 2002).

The more common adverse effects of metformin are gastrointestinal symptoms (Krentz et al., 1994), which may be relieved by reduction of dosage and may rarely require discontinuation of treatment (Hermann and Melander, 1992).

Table 1 shows the range of values of normal levels of some diagnostic indices of type 2 diabetes mellitus disease.

The aim of this research is

- To investigate the efficacy of the use of biochemical, and hematological indices: fasting blood sugar (FBS), serum glycated albumin (GA), Glycated hemoglobin (HbAc1), fasting serum insulin (I), serum aspartate aminotransferase (AST), serum triglyceride (TG), serum cholesterol (C), and serum Calcium (Ca²⁺), Magnesium (Mg²⁺), and sodium (Na⁺) as diagnostic indices of type 2 diabetes mellitus and for the monitoring of recovery (rehabilitation) from type 2 diabetes mellitus disease.
To determine the efficacy of the use of combined semi-vegan dietary and drug (metformin/glucophage) therapy in the management and treatment of type 2 diabetes mellitus disease.

MATERIALS AND METHODS

Selection of Human Subjects

Twenty (n = 20) clinically confirmed type 2 diabetes patients (10 male and 10 female), of age bracket 40-70 years and twenty normal (normal glucose regulation, NGR) or healthy human subjects (n=20, 10 male and 10 female) of the same age bracket, voluntarily participated in this study, at Imo State University Teaching Hospital, Orlu, Imo State, Nigeria. The subjects were randomly selected between September and October 2014.

Inclusion criteria at screening were: age 40–70 years of age and initial pre-treatment, glycosylated haemoglobin level (HbA1c) between 6.5% and 8.5%, and fasting blood sugar ≥ 126mg/dl. All diabetic human subjects had to be on stable dose of metformin of ≥1250 mg/day for 6-weeks. The met-administered diabetic patients were simultaneously administered with a balanced semi-vegan diet, high in dietary soluble fiber, low in saturated fat and sugar, but moderate in essential fatty acids (EFAs), prepared primarily from processed catfish, processed soya bean (Glycine max) seeds, processed groundnut (Arachis hypogaea) seeds, fluted pumpkin leaves, low-sugar banana (Musa acuminata) fruit, palm oil and vitamin-dietary mineral premix. Figure 1 shows the proximate constituent and caloric value of the semi-vegan diet.

Exclusion criteria included: gastrointestinal tract infection, protein energy malnutrition, hepatitis, obstructive jaundice, cancer, hypertension, obesity, smoking, alcoholism, persons living with HIV, patients taking drugs other than metformin.

EXPERIMENTAL DESIGN

The experimental design is a two-factor completely randomized design (CRD). The statistical linear equation is:

\[ \hat{Y}_{ijk} = \mu + T_i + D_j + E_{ijk} \]

\( \hat{Y}_{ijk} \) = individual observation
\( \mu \) = overall mean
\( T_i \) = ith type of drug treatment (oral administration of metformin).
\( D_j \) = jth type of dietary treatment (oral administration of semi-vegan diet).
\( E_{ijk} \) = error which is independently, randomly and normally distributed with zero mean and constant variance.

SPSS for windows (version 17.0, SPSS, Chicago, IL, USA) was used to perform the statistical analyses. The significance levels were p<0.05, p<0.01.

The research was given Ethical approval from the Department of Biochemistry, School of Science, Federal University of Technology Owerri, because it was carried out in compliance with the Declaration on the Right of the Patient (WMA, 2000).

Measurement of all the biochemical and hematological parameters were carried out on whole blood or serum specimen (as applicable) collected from the normal human subjects, pre-treatment diabetic patients, the same pre-treatment diabetic patients administered with metformin (glucophage) (met-administered diabetic patients), after a six-week treatment period.

Blood was obtained by veni-puncture carried out by a Phlebotomist nurse.

The method described by Thavasu et al. (1992) was used in obtaining the serum. Whole blood was collected in a covered test tube, and allowed to clot by leaving it undisturbed for 15-30 minutes at room temperature. The clot was removed by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge, to obtain the blood serum. Citrate phosphate dextrose - adenine 1 (CPDA-1)-
stored whole blood was used for whole blood analysis.

**In Vitro Quantitative Determination of Serum Fasting Blood Sugar (FBS)**

Serum was obtained from patient/individual who had not taken any victual (food or drink, except water), for an 8-hour period. Glucose oxidase catalyses the oxidative transformation of β D-glucose present in the serum to D glucono-1,5-lactone with the formation of hydrogen peroxide. The lactone is slowly hydrolysed to D-gluconic acid.

The hydrogen peroxide produced is broken down to oxygen and water by a peroxidase enzyme. Oxygen reacts with ortho-toluidine to produce a coloured complex, the intensity of which is proportional to the concentration of the D-glucose in the serum, and measurable at 540nm.

**In Vitro Quantitative Determination of the Fasting Serum Insulin Concentration**

The method described by Kwame et al. (1993) was used in the determination of the fasting serum insulin concentration. The fasting serum insulin concentration was measured by a standard double antibody RIA technique. The sensitivity of the insulin assay was 2.5 mU/L. The inter and intra assay CVs were 6 and 10 % respectively.

**In Vitro Quantitative Determination of Serum Glycated Albumin**

Serum glycated albumin (GA) was measured according to methods described by Inaba et al. (2007). The *in vitro* quantitative determination of the serum glycated albumin was carried out by an enzymatic method using the Lucica GA-L kit (Asahi Kasei Pharma Corp., Tokyo, Japan) (Kouzuma, 2004). GA was hydrolyzed to amino acids by albumin-specific proteinase and then oxidized by ketoamine oxidase to produce hydrogen peroxide, which was measured quantitatively. The GA value was calculated as the percentage of GA relative to total albumin, which was measured with new bromocresol purple method using the same serum sample (Kouzuma, 2004). GA assay was not influenced by the physiologic concentrations of ascorbic acid, bilirubin, and up to 1000 mg/dl glucose (Nagamine et al., 2004).

**In Vitro Quantitative Analysis of Serum Aspartate Amino Transferase (AST)**

Quantitative *in vitro* determination of serum aspartate amino transferase (AST) was carried out using the method employed by Reitman and Frankel (1957). The test based on the reaction in which L-aspartate and α-ketoglutarate are converted to L-glutamate and oxaloacetate by the catalytic activity of AST. The oxaloacetate so formed, forms a complex known as oxaloacetate hydrzone with 2,4-dinitrophenyl hydrazine.
The intensity of the colour of the hydrazone complex, which is measurable with a colorimeter at 578 nm is directly proportional to the AST enzyme activity.

Lipid Profile Assays

Serum cholesterol (C), and serum triacylglycerol (TG) were determined using commercial kits (Randox Laboratory Ltd., UK), in conformity with the methods employed by Ibegbulum and Chikezie (2012); Chikezie and Okpara (2013).

Determination of Hemoglobin Concentration

The cyanmethemoglobin method described by Rosenblit et al. (1999) was employed in the determination of hemoglobin concentration. Whole blood was mixed with Drabkin's solution, (a solution that contains ferricyanide and cyanide). The ferricyanide oxidized the iron in the hemoglobin, thereby changing hemoglobin to methemoglobin. Methemoglobin reacted with the cyanide to form cyanmethemoglobin. Cyanmethemoglobin produced a color which was measured in a colorimeter at 540nm. The intensity of the colour of cyanmethemoglobin complex is directly proportional to the concentration of hemoglobin, and was determined from the serial dilution standard concentration calibration curve of hemoglobin.

Packed Cell Volume (PCV%)

Analysis of packed cell volume (PCV%) was carried out according to the method described by Ovuakporaye (2011). A plain capillary tube was filled with whole blood in an EDTA container by capillary action. It was sealed using plasticine or bunsen burner flame and placed in the haematocrit centrifuge for 10mins and the value of PCV% was obtained using haematocrit reader.

In Vitro Quantitative Determination of Serum Glycated Hemoglobin

Serum glycosylated hemoglobin (HbA1c) was measured using high-performance liquid chromatography, according to the method employed by Shenqi et al. (2013).

In Vitro Quantitative Determination of Serum Electrolytes

Serum electrolytes were determined consistent with methods described by NKF (2002) and Shenqi et al. (2013). The serum electrolytes were analyzed using an autoanalyzer (Hitachi 7600 analyzer, Hitachi, Japan). The inter- and intra-assay coefficients of variation for Na⁺, Ca²⁺, Mg²⁺ were 0.77% and 1.13%; 1.80% and 3.00%; and 1.15% and 1.92% respectively.

RESULTS

A major diagnostic parameter of diabetes mellitus is the fasting blood sugar. Therefore, results on the fasting blood sugar were statistically analyzed with a view to making valid inferences not only on the use of fasting blood sugar as a diagnostic tool of diabetes mellitus, but also to establish the experimental design of the present research.

From Table 2 it could be inferred that there was no significant difference (p<0.05) in fasting blood sugar between: the male and female pre-treatment diabetics; the male and female met-administered diabetics; and the male and female normal human subjects, respectively. The mean value of fasting blood sugar was significantly higher (p<0.05) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but significantly equal (p<0.05) between the Met-administered diabetic patients and normal human subjects.

This observation indicates that the male and female experimental units are homogenous, and not heterogenous, and as such the male and female replicates are considered as a single block in experimental design. The experimental design is a two-factor (gender and type of treatment), completely randomized design (CRD).

Table 3 shows the results on the biochemical indices: serum glycated albumin, fasting serum insulin and serum aspartate aminotransferase (AST) of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects. The mean values of these biochemical indices were significantly higher (p<0.05) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but were significantly equal (p<0.05) between the Met-administered diabetic patients and normal human subjects.

Table 4 shows the results on the hematological indices: packed cell volume (PCV%), hemoglobin concentration (Hb), and glycated hemoglobin (HbAc1) of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects. No significant difference (p<0.05) was observed of the mean values of the packed cell volume (PCV%), and hemoglobin concentration (Hb), among the corresponding pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects. The mean value of glycated hemoglobin (HbAc1) was significantly higher (p<0.05) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects.
Table 2. Results on the fasting blood sugar (FBS) of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Pre-treatment diabetic patients</th>
<th>Met-administered diabetic patients</th>
<th>Normal human subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>127±2.5\textsuperscript{a}</td>
<td>73.98±1.15\textsuperscript{a}</td>
<td>69.4±3.49\textsuperscript{a}</td>
</tr>
<tr>
<td>Female</td>
<td>126.3±2.0\textsuperscript{a}</td>
<td>72.36±2.67\textsuperscript{b}</td>
<td>70.96±2.68\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard error (mg/dl) (n = 20). Values labeled with the same superscript in the same row and column are not significantly different (p<0.05).

Table 3. Results on the biochemical indices: Serum glycated albumin, fasting serum insulin and serum aspartate aminotransferase (AST) of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects.

<table>
<thead>
<tr>
<th></th>
<th>Serum glycated albumin (%)</th>
<th>Fasting insulin (mU/L)</th>
<th>Serum aspartate aminotransferase (U/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human subjects</td>
<td>15.5±0.7\textsuperscript{a}</td>
<td>8.5±1.7\textsuperscript{a}</td>
<td>9.2±1.9\textsuperscript{a}</td>
</tr>
<tr>
<td>Met-administered diabetic patients</td>
<td>15.8±0.5\textsuperscript{a}</td>
<td>8.2±0.9\textsuperscript{a}</td>
<td>9.9±1.0\textsuperscript{a}</td>
</tr>
<tr>
<td>Pre-treatment diabetic patients</td>
<td>28.1±1.7\textsuperscript{b}</td>
<td>17.7±1.0\textsuperscript{b}</td>
<td>30.5±1.0\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard error (S.E) (unit) (n = 20). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

Table 4. Results on the Hematological indices: Packed cell volume (PCV%), hemoglobin concentration (Hb), and glycated hemoglobin concentration (HbAc1) of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects.

<table>
<thead>
<tr>
<th></th>
<th>Packed cell volume (PCV%)</th>
<th>Hemoglobin concentration (g/dl)</th>
<th>Glycated hemoglobin (HbAc1)(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human subjects</td>
<td>45.1±0.075\textsuperscript{a}</td>
<td>15.5±0.1\textsuperscript{a}</td>
<td>5.15±0.8\textsuperscript{a}</td>
</tr>
<tr>
<td>Met-administered diabetic patients</td>
<td>44.2±0.155\textsuperscript{a}</td>
<td>14.85±0.12\textsuperscript{a}</td>
<td>5.25±0.8\textsuperscript{a}</td>
</tr>
<tr>
<td>Pre-treatment diabetic patients</td>
<td>43.2±0.15\textsuperscript{a}</td>
<td>14.85±0.12\textsuperscript{a}</td>
<td>8.35±0.6\textsuperscript{b}</td>
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</tbody>
</table>

Results are expressed as mean ± standard error (S.E) (unit) (n = 20). Values that are labeled, in the same column, with the same superscripts, are not significantly different (p<0.05).

Table 5. Results on serum electrolytes of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects.

<table>
<thead>
<tr>
<th></th>
<th>Mg\textsuperscript{2+} (mmol/l)</th>
<th>Ca\textsuperscript{2+} (mmol/l)</th>
<th>Na\textsuperscript{+} (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human subjects</td>
<td>1.01±0.10\textsuperscript{a}</td>
<td>2.30±0.11\textsuperscript{a}</td>
<td>143±0.10\textsuperscript{a}</td>
</tr>
<tr>
<td>Met-administered diabetic patients</td>
<td>0.99±0.05\textsuperscript{a}</td>
<td>2.29±0.10\textsuperscript{a}</td>
<td>142.5±0.2\textsuperscript{a}</td>
</tr>
<tr>
<td>Pre-treatment diabetic patients</td>
<td>0.85±0.10\textsuperscript{b}</td>
<td>2.41±0.13\textsuperscript{b}</td>
<td>140±0.05\textsuperscript{b}</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± standard error (unit) (n = 20). Values labeled with the same superscript in the same column are not significantly different (p<0.01).

human subjects, but significantly equal (p<0.05) between the Met-administered diabetic patients and normal human subjects.

Table 5 shows results on serum electrolytes of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects. The mean value of serum calcium (Ca\textsuperscript{2+}) was significantly higher (p<0.01) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but significantly equal (p<0.01) between the Met-administered diabetic patients and normal human subjects.
Figure 2. Graphical results on the biochemical indices: serum cholesterol and serum triglyceride of the pre-treatment diabetic patients, normal human subjects and Met-administered diabetic patients.
Statistical results are expressed as mean ± standard error (mg/dl) (n = 20). Error bars represent values of standard error (1.2 – 11.3 mg/dl). Corresponding bars labeled with the same letters represent mean values of serum cholesterol or serum triglyceride which are not significantly different (p<0.05).

Figure 3. Regression curve of serum cholesterol and serum triglyceride of the pre-treatment diabetic patients, normal human subjects and Met-administered diabetic patients.

(Normal human subjects): $\hat{Y} = 12 + 2.05x_{mg/dl}$
(Met-administered diabetic patients): $\hat{Y} = 12.5 + 2x_{mg/dl}$
(Pre-treatment diabetic patients): $\hat{Y} = -73.05 + 2.5x_{mg/dl}$

The mean values of serum Magnesium (Mg$^{2+}$) and sodium (Na$^+$) were significantly lower (p<0.01) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but significantly equal (p<0.01) between the Met-administered diabetic patients and normal human subjects.

Figure 2 shows the graphical results on the biochemical indices (mg/dl): serum cholesterol (C) and serum triglyceride (TG) of the pre-treatment diabetic patients.
DISCUSSION

A significance of biochemistry relevance is that significant difference (p<0.05) in fasting blood sugar between diabetics and non-diabetics, irrespective of the gender difference, is not necessarily, a criterion or yardstick for the diagnosis of diabetes mellitus, if the diabetic patients are placed on metformin (glucophage) drug therapy because the drug functions primarily to decrease/lower the blood sugar level, which in turn affects significantly, other diagnostic indices of diabetes mellitus. Metformin reduces fasting blood glucose levels by 44-53mg/dl [Cusi et al. (1996), DeFronzo and Goodman (1995)].

The mean value of fasting blood sugar shown in Table 2 was significantly higher (p<0.05) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but significantly equal (p<0.05) between the Met-administered diabetic patients and normal human subjects: a finding, corroborated by Freemark and Bursey (2001), who observed that metformin caused a progressive significant decline (p<0.05) in fasting blood glucose, and concluded that metformin could complement the effects of dietary and exercise counseling and reduce the risk of type 2 diabetes mellitus in patients.

Consumption of a low-glucose diet (including legumes) or high-fiber diet improved markers of glycemic control (including fasting blood glucose, insulin, fructosamine, and HbA1c levels) in diabetic patients (Sievenpiper et al., 2009): A finding consistent with the postulate of the present study.

The mean values of serum glycated albumin, fasting serum insulin and serum aspartate aminotransferase (AST) shown in Table 3, listed in order of consecutive significant decrease (p<0.05) were as follows: pre-treatment diabetic patients, Met-administered diabetic patients/normal human subjects. In otherwords, the mean values of these diagnostic indices were significantly equal (p<0.05) between the Met-administered diabetic patients and normal human subjects. Consistent with these findings, the administration of 1500mg/day of metformin to type 2 diabetic patients for a twenty-four week period, significantly decreased (p<0.05) their serum glycated albumin (Sumitani et al., 2015). Free radical production is increased during diabetes. Structural modification of albumin induced by glucose, in diabetes mellitus, increases the concentration of serum glycated albumin, and in consequence, reduces and impairs, the antioxidant properties of serum albumin (Faure et al., 2008). Metformin functions to reduce oxidative stress by modulating the glycation processes [including those of albumin and hemoglobin (Luna and Feinglos, 2001)], and thus significantly decreasing serum glycated albumin (Violett et al., 2012).

Akin to the finding that fasting serum insulin was significantly decreased (p<0.05), in the Met-administered diabetic patients is the postulate that Metformin significantly reduced (p<0.05), fasting serum insulin in non-obese type 2 diabetic patients compared with the non-significant reduction observed of the repaglinide administered type 2 diabetic patients (Lund et al., 2008).

Elevated transaminases in insulin-resistant states could be as a result of oxidant stress from reactive lipid peroxidation, peroxisomal beta-oxidation, and recruited inflammatory cells (Harris, 2005). Steato-hepatitis patients administered with a combined therapy of probiotics and metformin had significantly reduced (p<0.05) aspartate aminotransferase activity compared with normal human subjects (Shavakhi et al., 2013).

The mean value of glycated hemoglobin (HbAc1) in the present study, shown in Table 4 was significantly higher (p<0.05) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but significantly equal (p<0.05) between the Met-administered diabetic patients and normal human subjects, in keeping with the postulate that metformin glycinate administration caused significant decrease (P = 0.008) of glycated hemoglobin (Hb A1C) concentrations in drug-naïve adult patients with Type 2 Diabetes mellitus (González-Ortiz et al., 2012). Also, in conformity with the present study, significantly greater reduction (p<0.05) of HbA1c was observed of patients with type 2 diabetes mellitus, who were administered with high doses of metformin compared with those administered with low doses of metformin, with no significant increase in side effects (Hirst et al., 2012).

Furthermore, it was observed that diabetics fed on vegan diets had lowered glycated hemoglobin and LDL levels. Vegan diets may lower advanced glycation end-products such as glycated hemoglobin and glycated serum albumin (McCarty, 2005).

The mean values of serum Magnesium (Mg^{2+}) and sodium (Na^+) were significantly lower (p<0.01), but the mean value of serum calcium (Ca^{2+}) was significantly higher (p<0.01), in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but significantly equal (p<0.01) between the Met-administered diabetic patients and normal human subjects, as shown in Table 5. The finding in the present study concurred with a similar submission that serum sodium and magnesium levels in
Chinese diabetic patients were significantly decreased (p<0.01), and serum calcium level was significantly increased (p<0.01) compared with normal human subjects (control group) (Shenqi et al., 2013). In keeping with the findings in the present study, administration of the combination therapy of metformin and glibenclamide to type 2 diabetic patients have been shown to restore normal serum calcium, potassium, and sodium levels in the patients (Javaid et al., 2007).

The mean values of serum cholesterol and serum triglyceride shown in figure 2 were significantly higher (p<0.05) in the pre-treatment diabetic patients compared with the Met-administered diabetic patients and normal human subjects, but were significantly equal (p<0.05) between the Met-administered diabetic patients and normal human subjects. Credence is given to this observation by the work in which were recorded, markedly lowered plasma total cholesterol and triglyceride levels, due, mostly to a decrease in very low density lipoprotein-triglyceride, in metformin-administered diabetics of (Geerling et al., 2014). In concurrence with the finding in the present study is the postulate that metformin significantly lowered (p=0.032) total cholesterol in diabetic patients, compared with placebo (Robinson et al., 1998).

Vegan diet significantly decreased serum cholesterol and triglyceride values of diabetic patients compared with normal human subjects [Simpson et al. (1981) and Anderson (1980)]: a trend observed in the present study.

A rapid catch-up recovery/rehabilitation rate measure was observed of the diagnostic indices: fasting blood sugar (FBS), serum glycated albumin (GA), Glycated hemoglobin (HbAc1), fasting serum insulin (I), aspartate aminotransferase (AST), serum triglyceride (TG), serum cholesterol (C), and the serum electrolytes: Calcium (Ca\(^{2+}\)), Magnesium (Mg\(^{2+}\)), and sodium (Na\(^+\)), for which there was no observed significant difference (p<0.05) between the met-administered diabetic patients and the normal human subjects (control).

Significant differences/alterations (p<0.05) in mean values of FBS, GA, HbAc 1, I, AST, TG, C, Ca\(^{2+}\), Mg\(^{2+}\) (p<0.01), and Na\(^+\) (p<0.01), observed among the pre-treatment diabetic patients, the met-administered diabetic patients and the normal human subjects (control), show that the measurement of these biochemical and hematological diagnostic indices are of prime importance in the diagnosis, treatment and monitoring of recovery from type 2 diabetes mellitus.

Multiple regression studies revealed that fasting blood sugar concentration regressed significantly (p<0.05) with serum cholesterol concentration, serum triglyceride concentration, serum glycated albumin, and glycated hemoglobin of met-administered diabetic patients. The correlation statistical analysis between serum cholesterol and serum triglyceride of pre-treatment diabetic patients was significant (p<0.05) with a Pearson’s product moment correlation coefficient of 0.985. The concentration of serum cholesterol could be predicted with high and efficient precision from observed values of concentration of serum triglyceride of pre-treatment diabetic patients, from the equation, \( \hat{Y} \) (predicted value of serum cholesterol) = -73.05 + 2.5x (figure 3). Furthermore, the regression curves of the met-administered diabetic patients and normal human subjects are approximately, co-located on the graph plane, indicating that a combined semi-vegan dietary and drug (metformin/glucophage) therapy achieved an almost complete and total recovery from diabetes mellitus, of the diabetic patients.

CONCLUSION

Significantly elevated levels (p<0.05) of mean values of fasting blood sugar (FBS), serum glycated albumin (GA), Glycated hemoglobin (HbAc1), fasting serum insulin (I), serum aspartate aminotransferase (AST), serum triglyceride (TG), serum cholesterol (C), and serum Calcium (Ca\(^{2+}\)), and significantly reduced levels (p<0.01) of mean values of serum Magnesium (Mg\(^{2+}\)) and sodium (Na\(^+\)), in pre-treatment diabetic patients compared with normal human subjects, indicate that these biochemical and/or hematological parameters are effective and sufficient for the diagnosis of type 2 diabetes mellitus.

Absence of significant difference (p<0.05) or (p<0.01), as applicable, in all the biochemical and hematological diagnostic indices measured of the met-administered diabetic patients and normal human subjects suggest that these diagnostic indices serve as efficient criteria for the monitoring of recovery (rehabilitation) from type 2 diabetes mellitus. Prognosis studies show that a combined semi-vegan dietary and drug (metformin/glucophage) therapy is very efficient and useful in the management and treatment of type 2 diabetes mellitus.

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