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Full Length Research Paper

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 Ca^{2+} were significantly higher ($p<0.05$), ($p<0.01$, for Ca^{2+}), in the pre-treat D patients compared with the met-AD patients (73.98±1.15mg/dl , 15.8±0.5%, 8.2 ±0.9 mU/L, 9.9 ±1.0 U/l, 172.5 ± 11 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 Mg^{2+} 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

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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 glucagons 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 Dipeptidy 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 adenylyl 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 comparism 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 (Takhelmayum *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 (Ca^{2+}) and magnesium (Mg^{2+}) 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 Mg^{2+} 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 Mg^{2+} 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 Mg^{2+} 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 Ca^{2+} 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 Mg^{2+} level and reciprocally elevated free intracellular Ca^{2+} level (Barbagallo *et al*., 2007). Elevated serum Ca^{2+} 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 Ca^{2+} in muscle contractions, insulin secretion, and glucose uptake after the binding of insulin to muscle cell membranes (Bjornholm and Zierath, 2005).

The functions of Ca^{2+}-Mg^{2+}-ATPase, Na^+/Ca^{2+} exchanger and Ca^{2+} pump, which are located in the cell membrane, mitochondria or endoplasmic reticulum, are impaired in diabetes mellitus [Mikaelian *et al*. (2013), (2013), Dhalla *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 (Ca^{2+}), Magnesium (Mg^{2+}), and sodium (Na^+) as diagnostic indices of type 2 diabetes mellitus and for the monitoring of recovery (rehabilitation) from type 2 diabetes mellitus disease.

Table 1. Normal range of values of some diagnostic indices of diabetes mellitus.

Diagnostic index	Range of normal values
Fasting blood sugar	< 100mg/dl
Glycated hemoglobin	<5.7%
PCV%	40-53%
Insulin	2-20mU/l
Serum glycated albumin	11-16%
Aspartate aminotransferase	6- 40 I.U/l
Serum triglyceride	101-150mg/dl
Serum cholesterol	<200mg/dl
Serum calcium	2.08-2.6 mmol/l
Serum sodium	137-145 mmol/l
Serum magnesium	0.65-1.05 mmol/l

Reitman and Frankel (1957), Fairbanks and Tefferi (2000), Roohk and Zaidi (2008), Shenqi *et al*. (2013), Morris (2015).

- 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 :

$\hat{Y}_{ijk} = \mu + T_i + T_j + \epsilon_{ijk}$
$\hat{Y}_{ijk}$ = individual observation
μ = overall mean
T_i = ith type of drug treatment (oral administration of metformin).
T_j = jth type of dietary treatment (oral administration of semi-vegan diet).
ϵ_{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)-

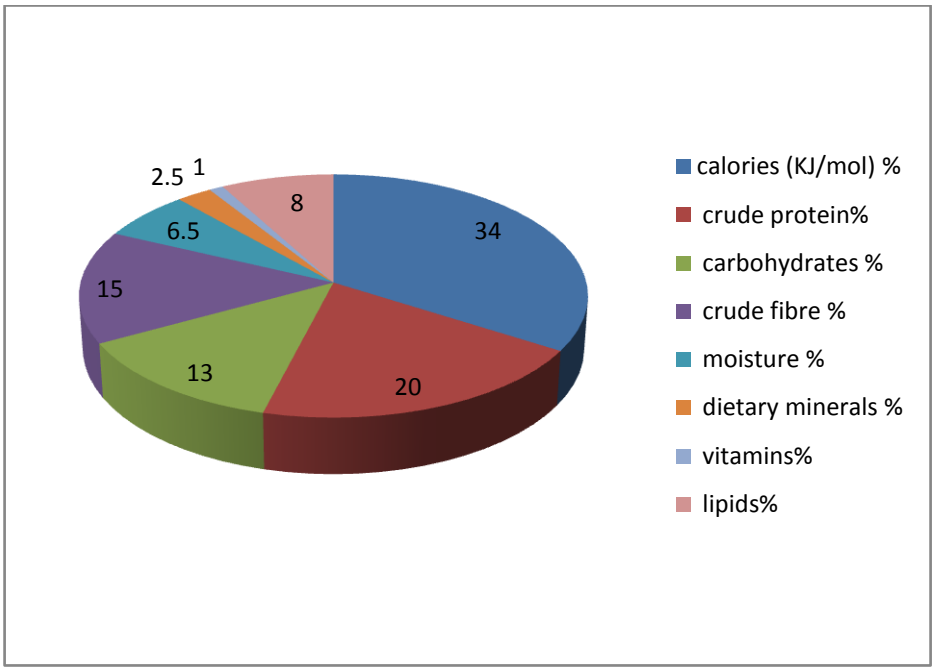

Figure 1. Proximate constituent and caloric value of the semi-vegan diet.
34% represents 2298KJ/100gram diet sample, derivable from 41 gram of crude protein, lipids and carbohydrates/100gram of diet

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 hydrazone 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 Ibegbulem 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^{+}, Ca^{2+}, Mg^{2+} 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

Table 2. Results on the fasting blood sugar (FBS) of the pre-treatment diabetic patients, Met-administered diabetic patients and normal human subjects.

Gender	Pre-treatment diabetic patients	Met-administered diabetic patients	Normal human subjects
Male	127±2.5[a]	73.98±1.15[b]	69.4±3.49[b]
Female	126.3±2.0[a]	72.36±2.67[b]	70.96±2.68[b]

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.

	Serum glycated albumin (%)	Fasting Serum insulin (mU/L)	Serum aspartate aminotransferase (U/l)
Normal human subjects	15.5±0.7[a]	8.5±1.7[a]	9.2 ±1.9[a]
Met-administered diabetic patients	15.8±0.5[a]	8.2 ±0.9[a]	9.9 ±1.0[a]
Pre-treatment diabetic patients	28.1±1.7[b]	17.7±1.0[b]	30.5 ±1.0[b]

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.

	Packed cell volume (PCV%)	Hemoglobin concentration (Hb) (g/dl)	Glycated hemoglobin (HbAc1)(%)
Normal human subjects	45.1±0.075[a]	15.5±0.1[a]	5.15±0.8[a]
Met-administered diabetic patients	44.2±0.155[a]	14.85±0.12[a]	5.25±0.8[a]
Pre-treatment diabetic patients	43.2±0.15[a]	14.85±0.12[a]	8.35±0.6[b]

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.

	Mg^{2+} (mmol/l)	Ca^{2+} (mmol/l)	Na^{+}(mmol/l)
Normal human subjects	1.01±0.10[a]	2.30±0.11[a]	143±0.10[a]
Met-administered diabetic patients	0.99±0.05[a]	2.29±0.10[a]	142.5±0.2[a]
Pre-treatment diabetic patients	0.85±0.10[b]	2.41±0.13[b]	140±0.05[b]

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^{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.

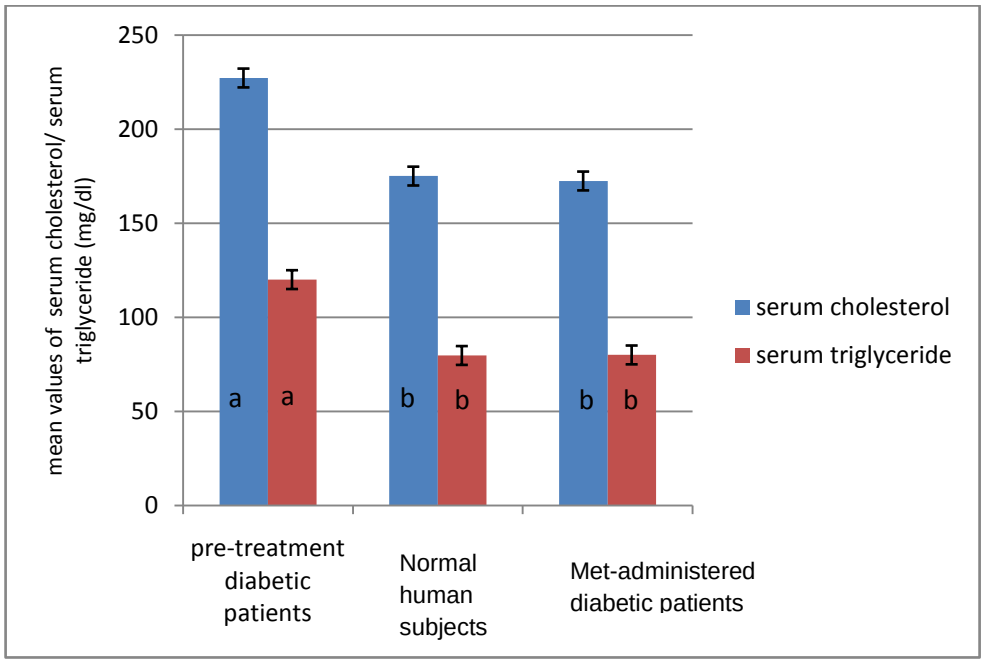

Figure 2. Graphical results on the biochemical indices : serum cholesterol and serum triglyceride of the pre-treatment diabetic patients, normal human subjects and Met-administered diabetic patients.
Statistical results are expressed as mean ± standard error (mg/dl) (n = 20). Error bars represent values 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 ($p<0.05$).

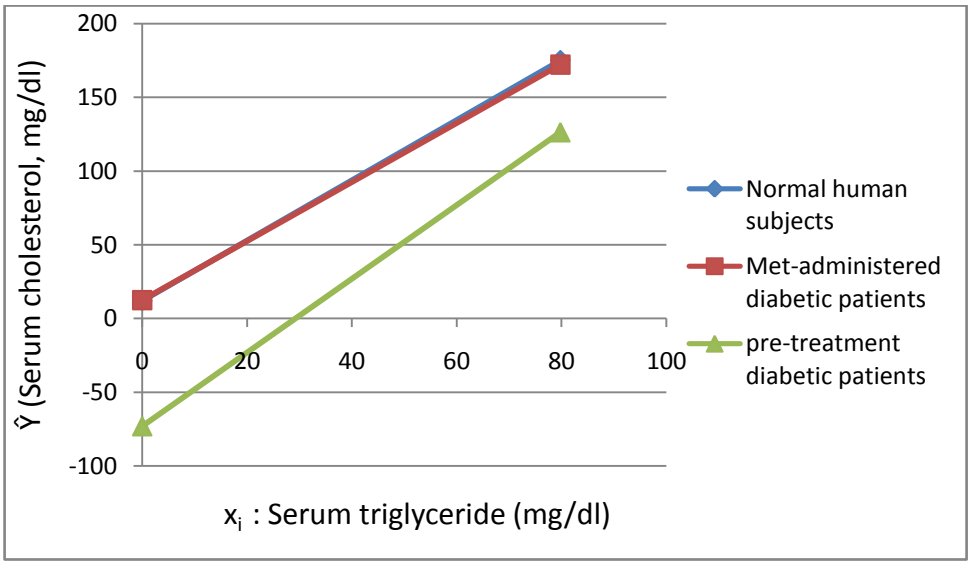

Figure 3. Regression curve of serum cholesterol and serum triglyceride of the pre-treatment diabetic patients, normal human subjects and Met-administered diabetic patients.
(Normal human subjects) : $\hat{Y} = 12 + 2.05x_i$mg/dl
(Met-administered diabetic patients) : $\hat{Y} = 12.5 + 2x_i$mg/dl
(Pre-treatment diabetic patients) : $\hat{Y} = -73.05 + 2.5x_i$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

(227.2±10.28 and 120.1±1.2), normal human subjects (175.1±11.3 and 79.8±2.3) and Met-administered diabetic patients (172.5±11.0 and 80.1±1.5). The mean values of these biochemical indices (serum cholesterol and serum triglyceride) 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.

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). Meformin 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 (Viollet *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-naive 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 HbA(1c) 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) = $-73.05 + 2.5x_i$mg/dl (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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REFERENCES

Al Homsi MF, Lukic ML (1992). An Update on the pathogenesis of Diabetes Mellitus, Department of Pathology and Medical Microbiology (Immunology Unit) Faculty of Medicine and Health Sciences, UAE University, Al Ain, United Arab Emirates.

Anderson JW (1980). High fiber diets in diabetes and hypertriglyceridemia. Can. Med. Assoc. J., 123:975–9.
Bahgat MM, Maghraby AS, El Sayed MEM, Amany AEI, Naglaa HM (2010). Type 2 Diabetes Mellitus in Egyptian Diabetics Under Fourty Years. J. Genet. Eng. Biotechnol., 8(2): 27-34.
Baker DJ, Timmons JA, Greenhaff PL (2005). Glycogen Phosphorylase Inhibition in Type 2 Diabetes Therapy: A Systematic Evaluation of Metabolic and Functional Effects in Rat Skeletal Muscle. Diabetes 54(8): 2453-2459.
Barbagallo M, Dominguez LJ, Resnick LM (2007). Magnesium metabolism in hypertension and type 2 diabetes mellitus. Am. J. Ther., 14(4):375-385.
Bays H, Frestedt JL, Bell M, Williams L, Kolberg, Schmelzer W, Anderson JW (2011). Reduced viscosity Barley β-Glucan versus placebo: a randomized C controlled trial of the effects on insulin sensitivity for individuals at risk for diabetes mellitus. Nutr. Metab., 8: 58.
Bjornholm M, Zierath JR (2005). Insulin signal transduction in human skeletal muscle: identifying the defects in Type II diabetes. Biochem. Soc. Trans. 33(Pt 2):354-357.
Bjorntorp P (1992). Abdominal fat distribution and disease: an overview of epidemiological data. Annals Med. 24(1): 15-18.
Cefalu WT (2010). Executive summary: Standards of medical care in diabetes—2010". Diabetes Care 33 (Suppl 1): S4–10.
Chikezie PC, Okpara RT (2013). Serum Lipid Profile and Hepatic Dysfunction in Moderate Plasmodium Falciparum Infection. Global J. of Med. Res. Dis., 13 (4): 14-20.
Cusi K, Consoli A, DeFronzo RA (1996). Metabolic effects of metformin on glucose and lactate metabolism in noninsulin-dependent diabetes mellitus. J. Clin. Endocrinol. Metab., 81(11):4059-67.
DeFronzo RA, Goldberg M, Agus ZS (1976). The effects of glucose and insulin on renal electrolyte transport. J. Clin. Invest., 58(1): 83-90.
DeFronzo RA, Goodman AM (1995). Efficacy of metformin in patients with non-insulin-dependent diabetes mellitus. The Multicenter Metformin Study Group. N. Engl. J. Med. 333(9):541-9
Dhalla NS, Rangi S, Zieroth S, Xu YJ (2012). Alterations in sarcoplasmic reticulum and mitochondrial functions in diabetic cardiomyopathy. Exp. Clin. Cardiol., 17(3): 115-120.
Eurich, McAlister FA, Blackburn DF, Majumdar SR, Tsuyuki RT, Varney J, Johnson JA (2007). "Benefits and harms of antidiabetic agents in patients with diabetes and heart failure: systematic review". BMJ (Clinical research ed.) 335 (7618): 497.
Fairbanks VF, Tefferi A (2000). Normal ranges for packed cell volume and hemoglobin concentration in adults: relevance to 'apparent polycythemia'. Eur. J. Haematol., 65(5): 285-96.
Faure P, Wiernsperger N, Polge C, Favier A, Halimi S (2008). Impairment of the antioxidant properties of serum albumin in patients with diabetes: protective effects of metformin. Clin. Sci. (Lond). 114(3): 251-6.
Freemark M, Bursey D (2001). The effects of metformin on body mass index and glucose tolerance in obese adolescents with fasting hyperinsulinemia and a family history of type 2 diabetes. Pediatrics.107(4): E55.
Geerling JJ, Boon MR, van der Zon GC, van den Berg SA, van den Hoek AM, Lombès M, Princen HM, Havekes LM, Rensen PC, Guigas B (2014). Metformin lowers plasma triglycerides by promoting VLDL-triglyceride clearance by brown adipose tissue in mice. Diabetes. 63(3): 880-91.
González-Ortiz M, Martínez-Abundis E, Robles-Cervantes JA, Ramos-Zavala MG, Barrera-Durán C, González-Canudas J (2012). Effect of metformin glycinate on glycated hemoglobin A1C concentration and insulin sensitivity in drug-naive adult patients with type 2 diabetes mellitus. Diabetes Technol. Ther., 14(12): 1140-4.
González EL, Johansson S, Wallander MA, Rodríguez LA (2009). Trends in the prevalence and incidence of diabetes in the UK: 1996 – 2005. J. Epidemiol. Community Health. 63: 332-336.
Guyton AC, Hall JE (2006a). Insulin, Glucagon and Diabetes Mellitus. In: Textbook of Medical physiology. Schmitt W, Gruliow R (Eds.). 11th Edition. Elsevier Inc, New Delhi: 961-977.
Guyton AC, Hall JE (2006b). Regulation of Extracellular Fluid Osmolarity and Sodium concentration. In: Textbook of Medical physiology. Schmitt W, Gruliow R (Eds.). 11th Edition. Elsevier Inc, New Delhi: pp. 348-364.
Haffner SM, Mitchell BD, Stern MP, Hazuda HP, Patterson JK (1992). Public health significance of upper body adiposity for non-insulin dependent diabetes in Mexican Americans. Int. J. Obes. 16(3): 177-184.
Hagander B, Asp N-G, Efendic S, Nihlsson-Ehle P, Schersten P (1988). Dietary fiber decreases fasting blood sugar levels and plasma LDL concentrations in noninsulin-dependent diabetes mellitus patients. Am. J. Clin. Nutr., 47: 852-8.
Hall JE, Guyton AC (2006). Role of the Kidneys in Long-Term Control of Arterial Pressure and in Hypertension: The Integrated System for Arterial Pressure Regulation. In:Textbook of medical physiology. Gruliow R (Ed.). Elsevier Saunders. USA. 213-228.
Hallé, J-P (2001). The Management and Treatment of Type 2 Diabetes. Can. J. CME. 13(6): 65-77.
Harris EH (2005). Elevated Liver Function Tests in Type 2 Diabetes. Clin. Diabetes. 23(3): 115-119.
Hermann LS, Melander A (1992). Biguanides: basic aspects and clinical use. In: International Textbook of Diabetes Mellitus. Zimmet PZ, Alberti KGMM, DeFronzo RA, Keen H, (Eds.). New York. John Wiley and Sons.: 77-95.
Hirst JA, Farmer AJ, Ali R, Roberts NW, Stevens RJ (2012).

Quantifying the effect of metformin treatment and dose on glycemic control. Diabetes Care. 35(2): 446-54.
Holt GI (2004). Diagnosis, epidemiology and pathogenesis of diabetes mellitus an update for Psychiatrists. Br. J. Psychiatry. 184:s55- s63.
Ibegbulem CO, Chikezie PC (2012). Serum lipid profile of rats (Rattus norvegicus) fed with palm oil and palm kernel oil-containing diets. Asian J. Biochem., 7(1): 46-53.
Idris AS, Mekky KFH, Abdalla BEE, Ali KA (2011). Liver function tests in type 2 Sudanese diabetic Patients. Int. J. Nutr. Metab. 3(2): 17-21.
Inaba M, Okuno S, Kumeda Y, Yamada S, Imanishi Y, Tabata T, Mikio Okamura, Okada S
Yamakawa T, Ishimura E, Nishizawa Y and the Osaka CKD Expert Research Group (2007). Glycated Albumin Is a Better Glycemic Indicator than Glycated Hemoglobin Values in Hemodialysis Patients with Diabetes: Effect of Anemia and Erythropoietin Injection. J. Am. Soc. Nephrol. 18: 896–903.
Javaid A, Hasan R, Zaib A, Mansoor S (2007). A comparative study of the effects of hypoglycemic agents on serum electrolytes in the diabetic patients. Pak. J. Pharm. Sci., 20(1): 67-71.
Kar P, Laight D, Rooprai HK, Shaw KM, Cummings M (2009). Effects of grape seed extract in Type 2 diabetic subjects at high cardiovascular risk: a double blind randomized placebo controlled trial examining metabolic markers, vascular tone, inflammation, oxidative stress and insulin sensitivity. Diabet. Med., 26(5): 526-31.
Katz MA (1973). Hyperglycemia-induced hyponatremia–calculation of expected serum sodium depression. N. Engl. J. Med., 289(16): 843-844.
Khan R, Ali K, Khan Z, Ahmad T (2012). Lipid profile and glycosylated hemoglobin status of gestational diabetic patients and healthy pregnant women. Indian J. Med. Sci. 66(7-8):149-54.
Kim NH, Kim DL, Choi KM, Baik SH, Choi DS (2000). Serum insulin, proinsulin and proinsulin/insulin ratio in type 2 diabetic patients: as an index of beta-cell function or insulin resistance. Korean J Intern Med. 15(3):195-201.
Klein BE, Klein R, Moss MS (1985). Prevalence of cataracts in a population-based study of persons with diabetes mellitus. Ophthalmology. 92: 1191–1196.
Kosecki SM, Rodgers PT, Adams MB (2005). Glycemic monitoring in diabetics with sickle cell plus beta-thalassemia hemoglobinopathy. Ann Pharmacother 39: 1557 –1560.
Kouzuma T (2004). Study of glycated amino acid elimination for an improved enzymatic glycated albumin measurement method. Clin. Chim. Acta., .346 : 135 – 143.
Krentz AJ, Ferner RE, Bailey CJ (1994). Comparative tolerability profiles of oral antidiabetic agents. Drug Safety. 11: 223-41.
Kwame O, Cortrell DA, Adenuwon CA, Ezenwaka EC, Akanji AO, O'Dorisio TM (1993). Serum insulin and glucose concentrations in people at risk for Type II Diabetes: A comparative study of African Americans and Africans. Diabetes care. 16(10): 1367-1375.
Kyselova Z, Stefek M, Bauer V (2004). Pharmacological prevention of diabetic cataract. J. Diabetes Complications.18: 129–140.
Larsen ML, Hørder M, Mogensen EF (1990). "Effect of long-term monitoring of glycosylated haemoglobin levels in insulin-dependent diabetes mellitus". N. Engl. J. Med. 323 (15): 1021–5.
Lobo DN (2004). Fluid, electrolytes and nutrition: physiological and clinical aspects. Proc Nutr Soc. 63(3):453-466.
Luna B, Feinglos MN (2001). Oral Agents in the Management of Type 2 Diabetes Mellitus. Am. Fam. Physician. 63(9): 1747-1757.
Lund SS, Tarnow L, Frandsen M, Smidt MU, Pedersen O, Parving H, Vaag AA (2008). Impact of metformin versus the prandial insulin secretagogue, repaglinide, on fasting and postprandial glucose and lipid responses in non-obese patients with type 2 diabetes. Eur. J. Endocrinol., 158: 35-46.
Ma J, Folsom AR, Melnick SL, Eckfeldt JH, Sharrett AR, Nabulsi AA (1995). Hutchinson RG, Metcalf PA: Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: the ARIC study. Atherosclerosis Risk in Communities Study. J. Clin. Epidemiol., 48(7): 927-940.
Manfred E (2014). All about the Hemoglobin A1C Test. Healthline. http://www.healthline.com/health/type-2-diabetes/ac1-test (08-01-15).
Manzato E, Zambon A, Lapolla A, Zambon S, Braghetto L, Crepaldi G, Fedele D (1993). Lipoprotein abnormalities in well-treated type II diabetic patients. Diabetes Care. 16(2): 469-75.
McCarty M (2005). "The low-AGE content of low-fat vegan diets could benefit diabetics – though concurrent taurine supplementation may be needed to minimize endogenous AGE production". Med Hypotheses. **64** (2): 394–398.
Mikaelian NP, Gurina AE, Terent'ev AA (2013). Dysfunction of membrane-receptor system of blood cells and kidney tissue in experimental diabetes mellitus. Bull Exp. Biol. Med. 154(5): 610-613.
Miller RA, Qingwei C, Jianxin X, Foretz Marc Viollet B, Birnbaum MJ (2013). Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. Nature. 494: 256-260.
Mithieux G, Guignot L, Bordet JC, Wiernsperger N (2002). Intrahepatic mechanisms underlying the effect of metformin in decreasing basal glucose production in rats fed a high-fat diet. Diabetes. 51(1): 139-43.
Morris B (2015). Lipid Profile (Triglycerides). Medscape. http://emedicine.medscape.com/article/2074115-overview. (19-01-15).
Nagamine Y, Mitsui K, Nakao T, Matsumoto M, Fujita C,

Doi T (2004). Evaluation of the enzymatic method for glycated albumin with liquid type reagent (Lucia GA-L) [in Japanese]. Jpn. J. Med. Pharm. Sci., 51: 737 –745,

National Kidney Foundation (NKF) (2002). K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am. J. Kidney. Dis., 39(2 Suppl 1):S1-S266.

Njolstad PR, Sagen JV, Bjorkhaug L, Odili S, Shehadeh N, Bakry D, Sarici SU, Alpay F, Molnes J, Molven A, Sovik O, Matschinsky FM (2003). Permanent neonatal diabetes caused by glucokinase deficiency: inborn error of the glucose-insulin signaling pathway. Diabetes. 52(11): 2854-60.

Ovuakporaye SI (2011). Effect of malaria parasite on some haematological parameters: red blood cell count, packed cell volume and haemoglobin concentration. J. Applied Biosciences 3: 45- 51.

Ozougwu JC, Obimba KC, Belonwu CD, Unakalamba CB (2013). The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. J. Physiol. Pathophysiol., 4(4): 46-57.

Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, Willett WC, Hu FB (2011). —Red meat consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis‖. Am. J. Clin. Nutr. 94(4): 1088-96.

Pham PC, Pham PM, Pham SV, Miller JM, Pham PT (2007). Hypomagnesemia in patients with type 2 diabetes. Clin. J. Am. Soc. Nephrol., 2(2): 366-373.

Quinn BJ, Kitagawa H, Memmott RM, Gills JJ, Dennis PA (2013). Repositioning Metformin for cancer prevention and treatment. Trends Endocrinol. Metab., 24(9): 469-80.

Raju SM, Raju B (2010). Regulation of Blood Glucose and Diabetes. In: Illustrated medical biochemistry. 2nd Edition. Jaypee Brothers Medical Publishers Ltd. New Delhi. India. pp. 445-456.

Rehman A, Zamir S, Bhatti A, Jan SS, Ali S, Wazir F (2012). Evaluation of albuminuria,

total plasma proteins and serum albumin in diabetics. Gomal J Med Sci. 10: 198-200.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetate and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28: 56-62.

Rendell (2004). "Advances in diabetes for the millennium: drug therapy of type 2 diabetes". Med. Gen. Med., 6(3): 9.

Robinson AC, Burke J, Robinson S, Johnston DG, Elkeles RS (1998). The effects of metformin on glycemic control and serum lipids in insulin-treated NIDDM patients with suboptimal metabolic control. Diabetes Care. 21(5):701-5.

Rodriguez-Moran M, Guerrero-Romero F (2001). Low serum magnesium levels and foot ulcers in subjects with type 2 diabetes. Arch Med Res. 32(4):300-303.

Roohk HV, Zaidi AR (2008). A review of glycated albumin as an intermediate glycation index for controlling diabetes. J Diabetes Sci. Technol., 2: 1114.

Rosenblit J, Abreu RC, Szterling NL, Kutner JM, Hamerschlak N, Frutuoso P, Stracieri de Paiva TRS, Orlando da Costa FJ (1999). Evaluation of three methods for hemoglobin measurement in a blood donor setting. Sao Paulo Med., J. 117 (3):108-12.

Rowley WR, Bezold C (2012). "Creating public awareness: state 2025 diabetes forecasts." Population Health Management. p. 15.

Sezik E, Aslan M, Yesilada E, Ito S (2005). Hypoglycaemic activity of Gentiana olivieri and isolation of the active constituent through bioassay- directed fractionation techniques. Life Sci. 76(11): 1223–1238.

Shavakhi A, Minakari M, Firouzian H, Assali R, Hekmatdoost A, Ferns G (2013). Effect of a Probiotic and Metformin on Liver Aminotransferases in Non-alcoholic Steatohepatitis: A Double Blind Randomized Clinical Trial. Int. J. Prev. Med., 4(5): 531-7.

Shenqi W, Xuhong H, Yu L, Huijuan L, Li W, Yuqian B, Weiping J (2013). Serum electrolyte levels in relation to macrovascular complications in Chinese patients with diabetes mellitus. Cardiovasc Diabetol. 12:146.

Sidorenkov G, Haaijer-Ruskamp FM, de Zeeuw D, Denig P (2011). "A longitudinal study examining adherence to guidelines in diabetes care according to different definitions of adequacy and timeliness.". PLoS ONE 6 (9): e24278.

Sievenpiper JL, Kendall CW, Esfahani A, Wong JM, Carleton AJ, Jiang HY, Bazinet RP, Vidgen E, Jenkins DJ (2009). Effect of non-oil-seed pulses on glycaemic control: a systematic review and meta-analysis of randomised controlled experimental trials in people with and without diabetes. Diabetologia 52: 1479-1495.

Simpson HC, Simpson RW, Lousley S, Carter RD, Geekie M, Hockaday TD, Mann JI (1981). A high carbohydrate leguminous fiber diet improves all aspects of diabetic control. Lancet. 1:1–5.

Song Y, He K, Levitan E, Manson J, Liu S (2006). Effects of oral magnesium supplementation on glycaemic control in Type 2 diabetes: a meta–analysis of randomized double–blind controlled trials. Diabetic med., 23(10): 1050-1056.

Sumitani S, Morita S, Deguchi R, Hirai K, Mukai K, Utsu Y, Miki S, Sato B, Nakamura H, Kasayama S, Koga M (2015). Metformin decreases glycated albumin to glycated haemoglobin ratio in patients with newly diagnosed type 2 diabetes. Ann. Clin. Biochem., 52(Pt 1): 76-81.

Takhelmayum R, Thanpari C, Singh TP (2014). Liver Dysfunction In Diabetic Patients Admitted In Referral Hospital. Bali Med. J. 3 (3): 122-124.

Thavasu PW, Longhurst S, Joel SP, Slevin ML, Balkwill FR (1992). Measuring cytokine levels in blood. Importance of anticoagulants, processing, and storage conditions. J. Immunol. Methods 153:115-124.

Tzamaloukas AH, Hsi KC, Quintana BJ, Merlin TL, Avasthi PS (1989). Glycosylated hemoglobin measured by affinity chromatography in diabetic and non-diabetic

patients on long-term dialysis therapy. West J. Med., 150(4): 415–419.
Ugwuja E, Eze N (2006). A Comparative Study of Serum Protein, Calcium and Electrolytes, Total Phosphate Among Diabetic and HIV/AIDS Patients in Abakaliki, Southeastern, Nigeria. The Internet J. of Laboratory Medicine. 2(1). https://ispub.com/IJLM/2/1/8631#
Vajo Z, Fawcett J, Duckworth WC (2001). Recombinant DNA technology in the treatment of diabetes: insulin analogs. Endocr. Rev. 22(5): 706-17.
Verdonck Sangster B, Van Heijst AN, De Groot G, Maes RA (1981). "Buformin concentrations in a case of fatal lactic acidosis". Diabetologi. 20(1): 45–6.
Viollet B, Guigas B, Garcia NS, Leclerc J, Foretz M, Andreelli F (2012). Cellular and molecular mechanisms of metformin: an overview. Clin. Sci. (Lond). 122(6): 253–270.
WMA. (2000). World Medical Association declaration of Helsinki ethical principles for medical research involving human subjects. 52nd WMA General Assembly, Edinburgh, Scotland.
Wulffelé MG, Kooy A, Lehert P, Bets D, Ogterop JC, van der Burg BB, Donker AJM, Stehouwer CDA (2002). Combination of Insulin and Metformin in the Treatment of Type 2 Diabetes. Diabetes Care. 25(12): 2133-2140.
Yajnik C, Smith R, Hockaday T, Ward N (1984). Fasting plasma magnesium concentrations and glucose disposal in diabetes. British med. J. (Clin res ed). 288(6423):1032-1034.
Yeh GY, Eisenberg DM, Kaptchuk TJ, Phillips RS (2003). "Systematic review of herbs and dietary supplements for glycemic control in diabetes". Diabetes Care 26(4): 1277–94.