Sudan University of Science and Technology

College of Graduate Studies

HbA1c Level Compared to Fasting and Random Blood Glucose Level in Sudanese Type 2 Diabetic Patients

مستوى السكر التراكمي مقارنة بمستوى السكر في الدم في حالة الصيام والعينة العشوائية لدى المرضى السودانيين المصابين بالنوع الثاني من السكري

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بسم الله الرحمن الرحيم

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صدق الله العظيم

(سورة طه الاية 114)

Dedication

To my husband Altahir Abdulmajid …..

To Dr: Mohammed Abdulrahim …..

To my father……..

To my mother……...

To my son Mazin Altahir……

To my brothers……..

To my sisters……

To my colleagues……

I dedicate this work with my best wishes to all

Acknowledgement

- I would like to thank God for giving me strength and patience to finish this study successfully.
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Abstract

This is a cross sectional and case control study during the period from February to June 2013 to evaluate the level of HbA1c in type2 diabetic patients compared to fasting and random blood glucose. HbA1c test was determined by using Nyco Card Reader II (automated analyzer) with Nyco Card kits and glucose test (fasting and random plasma glucose) were determined by spectrophotometric semi-automated analyzer. With bio system kits reagents. Statistical analysis of the data was performed using the SPSS computer system with (p. value≤ 0.05) considered significant and ANOVA – One way was used for comparison between groups of diabetes mellitus patients with (p. value ≤ 0.05).

The study was done in one hundred and twenty Sudanese patients with type2 Diabetes Mellitus (67 males and 53 females) as case groups and forty Sudanese apparently healthy individuals (21 males and 19 females) as control groups were enrolled in this study. The mean of the age (31 ± 14) range (31-75 years). Case and control groups ware matched for age.

Statistical results showed they are high significant of the mean of HbA1c levels in Sudanese patients with type2 Diabetes Mellitus the (mean ± SD) (9.852 ± 2.6433) compared with control groups the (mean ± SD) (4.478 ± 0.4902) with (P. value= 0.00). Fasting and Random Plasma Glucose also increased (mean ± SD) of Fasting Plasma Glucose (204.48 ± 53.503) compared with control groups (86.00 ± 6.214) with (P. value= 0.00) and random plasma glucose (333.64 ± 93.302) when it compared with control groups (112.78 ± 5.535) with (P. value= 0.00).

Statistical analysis explained they are high significant comparison of the HbA1c levels in reference to age between groups in Sudanese patients with

type2 Diabetes Mellitus the (mean ± SD) (7.913 ± 1.6474)(11.089 ± 1.7803)(12.917 ± 1.6375) with (p. value =0.00). The results also showed high significant comparison of the Fasting Plasma Glucose levels in reference to age between groups in Sudanese patients with type2 Diabetes Mellitus the (mean ± SD) (173.30 ± 36.525)(226.43 ± 48.366)(250.48 ± 49.033) with (p. value =0.00) . Beside that showed high significant comparison of the Random Plasma Glucose levels in reference to age between groups in Sudanese patients with type2 Diabetes Mellitus the (mean ± SD) (271.97 ± 72.038) (372.86 ± 64.641)(431.43 ± 58.076) with (p. value =0.00).

The study result told us they are significant high positive correlation coefficient between HbA1c and duration of diabetes mellitus with (r=0.828 and P. value=0.01). The study also shows significant positive correlation between duration of diabetes mellitus and plasma levels of Fasting Blood Glucose with (r=0.727 and P. value=0.01). The study also shows significant positive correlation between duration of diabetes mellitus and plasma levels of Random Blood Glucose with (r=0.786 and P. value=0.01).

The study observed that the patients with Diabetes Mellitus type 2 increase the level of HbA1c ,Fasting and Random Plasma Glucose also increased in age and duration of Diabetes Mellitus. HbA1c test was significantly can be used as biochemical panel during a clinic visit to identify poorly controlled and monitoring and to avoid long-term complications of diabetes mellitus. But HbA1c test cannot replacement the Fasting and Random Plasma Glucose test for monitoring the levels of glucose in the blood.

المستخلص

ان ارتفاع معدل اختبار السكر التراكمي في الدم لدي مرضي السكري من النوع الثاني يعطي اشاره لعدم التحكم في تنظيم نسبه الجلكوز لديهم .

الدراسه تمت في الفتره من شهر فبراير لسنه 2013م الي شهر يونيو 2013م وقد اجريت في مركز صحي جابر ابو العز للسكري ومستشفي الرباط بالخرطوم. عدد الذين شاركوا في هذه الدراسه 120 شخص من المرضي السودانين الذين يعانون من داء السكري النوع الثاني وتم تشخيصهم من قبل استشاري الباطنيه (76 من رجال و53 من النساء) وايضا عدد 40 سوداني متطوع سليم ظاهريا من داء السكري (21 من الرجال و19 من النساء) كمجموعه ضابطة . وتمت مطابقه العمر لدي جميع عينات الدراسه (31–75 سنه).

تم قياس السكر التراكمي بواسطه جهاز Nyco card Reader II باستخدام كروت ومحاليل من نفس الشركه. اما بالنسبه لعينات قياس السكر في حاله الصيام والعينه العشوائه خلال اليوم فتمت بواسطه جهاز اسبكتروفتوميتر . والمحاليل التي استخدمت في التفاعل كانت من شركه بايوسستم. وتم وضع النتائج واجريت لها تحليل احصائئ للبيانات باستخدام تي تست ومقارنه بي

مستوي المعنويه 0,05≤. وايضا تم التحليل بواسطه الانوفا ون وي للمقارنه بين اعمار المصابين بالسكري ومقارنه مستوي المعنويه0,05 ≤ .

واظهرت هذه الدراسه ان هنالك ارتفاع كبير في مستوي السكر التراكمي بين المرضي السودانين المصابين بداء السكري من النوع الثاني مقارنه بالاصحاء ظاهريا المتوسط الحسابي ± الانحراف المعياري (2.6433 ± 9.852) مقارنه بالقيمه المطلقه (0,00) وكانت نتيجة الاصحاء (0.4902 ± 4.478) . وايضا ارتفاع معدل السكر في حاله الصيام (53.503 ± 204.48) مقارنه بمجموعه الاصحاء ظاهريا (6.214 ± 86.00) القيمة المطلقة (0,00).

والعينه العشوائه خلال اليوم (93.302 ± 333.64) مقارنه بمجموعه الاصحاء ظاهريا (5.535 ± 112.78) القيمة المطلقة (0,00).

وبجانب ذالك اوضحت لنا الدراسة ان هنالك علاقة ايجابيه بين ذيادة اعمار المصابين بداء السكري من النوع الثاني وارتفاع معدل السكر التراكمي لديهم (المتوسط الحسابي± الانحراف المعياري)

(12.917 ± 1.6375) (11.089 ± 1.7803) (7.913 ± 1.6474)

والقيمه المطلقه (0,00). وايضا ارتفاع مستوي السكر في حاله الصيام مقارنه بالقيمه المطلقة (0,00) (173.30 ± 36.525) (226.43 ± 48.366)

(250.48 ± 49.033) وايضا ارتفاع السكر في الدم في حاله العينة العشوائة مقارنة بالقيمة المطلقة (0,00) (271.97±72.038) (372.86 ± 64.641) (431.43 ± 58.076).

وتبين من هذه الدراسه ان هناك علاقه قويه بين عدد سنوات الاصابه بداء السكري ومعدل ارتفاع السكر التراكمي(r=0.828 and P. value=0.01) والسكر في الدم في حاله الصيام (r=0.727 and P. value=0.01) والسكر في الدم في حاله العينه العشوائه خلال اليوم لديهم r=0.786 and P. value=0.01).

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Abbreviation

2h PG: 2hours Plasma Glucose.

A.D.A: American Diabetes Association.

ACTH: Adrenal Glucocorticoid Hormones.

ADAMC: American Diabetes Association Standard of Medical care.

AS: Absorbance of Sample.

ASTD: Absorbance of Stander.

CHD: Coronary Heart Disease.

D.M: Diabetes Mellitus.

DCCT: Diabetes Control and Complication Trial.

DRCP: Diabetes Research and clinical practice.

EDIC: Epidemiology of Diabetes Intervention and Complication.

FPG: Fasting Plasma Glucose

G.H: Growth Hormones.

GDM: Gestational Diabetes Mellitus.

HbA1c: Hemoglobin A1c.

I.E.C: International Expert Committee.

IDDM: Insulin Dependent Diabetes Mellitus.

IFCC: International Federation and Clinical Chemistry.

J.D.S.T: Journal of Diabetes Science and Technology.

JDC: Journal of Diabetes Care.

Mg/dl: Milligram/deciliter.

N.D.D.G: National Diabetes Data Group.

Nacl: Sodium Chloride.

NGSP: National Glycohemoglobin Standardization Program.

NIDDM: Non-Insulin Dependent Diabetes Mellitus.

OGIT: Oral Glucose Tolerance Test

P: Probability.

RBC: Red Blood Cells.

RBG: Random Blood Glucose.

RPG: Random Plasma Glucose.

S.I: System International.

TD: Test Device.

TSH: Thyroid Stimulating Hormone.

Chapter One

Introduction and literature review

1. Introduction and literature review

1.1. Introduction:-

Diabetes mellitus is a heterogeneous group of diseases characterized by chronic elevation of glucose in the blood also Diabetes Mellitus is a group of metabolic diseases in which there are high blood sugar levels in a prolonged period. Normally your body breaks down the sugar and carbohydrates your eat in to special sugar called glucose. Glucose fuels the cells in your body, the cells need insulin and a hormone in your blood stream in order to take in the glucose and use it for energy. Diabetes Mellitus is due to either the pancreas unable to produce enough insulin for its own needs or the cells of the body are not responding properly to the insulin produced. Blood glucose in the body was regulated by insulin and glucagon (major hormones) and also others endocrine glands hormone. (World Health Organization. 2012).

Diabetes Mellitus classifications in to three main groups according to many criteria. These groups involving (1) Primary (have two main forms (A) Type 1 and (B) Type 2 Diabetes Mellitus, (2) Secondary Diabetes Mellitus (have sub groups (A) Genetic defects of pancreas or beta cells (B) Endocrinopathies (C) Immunological disease (D) Some drugs (E) Infections (F) Chromosomal abnormalities), (3) Gestational Diabetes Mellitus. (World Health Organization. 1999, National Diabetes Data Group. 1979 and Michael, et al, 2010).

Diabetes mellitus it is called a self –managed disease meaning that take responsibility for patients own day to day care to avoid the any risk of complication of Diabetes Mellitus, if left uncontrolled and untreated can cause many complications. This complication either acute complications or chronic complication. Gestational diabetes usually gates risk for both mothers and the baby's. (Michael, et al. 2000).

Prevention and treatment of Diabetes Mellitus involves a healthy diet, physical exercise, treated with insulin injections or some oral medications. (American Diabetes Association. 2010).

Diagnosis and monitoring of Diabetes Mellitus based on plasma glucose criteria, Fasting Plasma Glucose (FPG), 2 hours Plasma Glucose after meal (2HPG), Random Plasma Glucose (RPG), Oral Glucose Tolerance Test (OGTT) and Hemoglobin A1c test (HbA1c). (American Diabetes Association. 2013).

1.2 Literature review

1.2.1 Diabetes mellitus:-

Diabetes Mellitus describes groups of metabolic disorders characterized by chronic hyper glycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from detects in insulin secretion, insulin action or both of them. Normal blood glucose regulation by insulin and glucagon hormone, if beta - cells in the pancreas (site of secretion insulin) do not produce enough insulin or the body do not respond to the insulin that is present glucose increased in the blood leading to diabetes mellitus (D.M) or pre diabetes which is a condition in which blood glucose levels reflect average blood glucose levels are higher than normal but not high enough to be diagnosed as diabetes most cases have no symptoms but they are considered to be at high risk for developing heart disease , stroke and type 2 D.M. Diabetes mellitus may present with characteristic symptoms such as classical symptoms like thirst (polydipsia) , polyuria , blurring of vision , weight loss , polyphagia , stress and very high level of blood glucose (hyper glycaemia) . In a healthy person's the body keeps blood glucose level in normal rang through several complex mechanisms (glycogenesis and glycolysis) in the liver and also if the body need more glucose the insulin stimulates the liver to make glucose from the amino acid. (World Health Organization. 1980. Michael, et al. 2010).

1.2.2 Path physiology:-

Type 2 Diabetes Mellitus is characterized by a combination of peripheral insulin resistance and inadequate insulin secretion by pancreatic beta cells. Insulin resistance, which has been attributed to elevated levels of free fatty acids and proinflammatory cytokines in plasma, leads to decreased glucose transport into muscle cells, elevated hepatic glucose production, and increased

breakdown of fat. A role for excess glucagon cannot be under estimated indeed , type 2 diabetes is an islet paracrinopathy in which the reciprocal relationship between the glucagon-secreting alpha cell and the insulin-secreting beta cell is lost, leading to hyper glucagonemia and hence the consequent hyperglycemia. For type 2 Diabetes Mellitus to occur, both insulin resistance and inadequate insulin secretion must exist. All over weight individuals have insulin resistance, but diabetes develops only in those who cannot increase insulin secretion sufficiently to compensate for their insulin resistance. Their insulin concentrations may be high, yet inappropriately low for the level of glycemia. With prolonged diabetes, atrophy of the pancreas may occur. (Tierney, at al. 2002).

1.2.3 Regulation of blood glucose:-

Hormones that regulations of blood glucose in the body are Insulin and (antagonized hormones) glucagon which made by alpha-cells of pancreas and raises low blood glucose levels by break down of glycogen in to glucose. Are major of intermediary metabolism. Also blood glucose can regulation by important respect hormones such as Growth hormone, Cortisol, Adrenal glucocorticoids (ACTH). Any deficiency in any of these hormones will be lead to any type of diabetes mellitus. (World Health Organization. 1985, National Diabetes Data Group. 1979).

Insulin is the primary hormone responsible for the entry of glucose in to the cell, it is synthesized by the beta-cells of islets of Langerhans in the pancreas, insulin receptors is a glycoprotein of approximate molecular weight (400KD) present on many target cells. When these cells detect an increase in body glucose they release insulin the action of insulin is lowers elevated blood glucose levels , by reducing glucose production in the liver (in habiting break down of glycogen in to glucose (Glycogenolysis) and stimulates the liver and

muscle and adipose tissue to store excess of glucose as glycogen in the muscles (Glycogenesis) and increase the conversion of carbohydrates to fatty acids (Lipogenesis) increase the metabolism of glucose molecule to pyruvate or lactate for production of energy (Glycolysis) any process that diffusely injures the pancreas can destroy the beta- cells then lead to lack of insulin secretion and then high blood glucose is present. Insulin can be referred to as hypoglycemic agent. (Watford. 1988, Michael, et al. 2000).

Glucagon is primary hormones responsible for increasing blood glucose levels. It is synthesized by the alpha-cells of islets of Langerhans in the pancreas. And released during stress and fasting states. When these cells detect decrease in body glucose they release glucagon, glucagon acts by increasing plasma glucose levels by increase glycogenolysis. Glucagon can be referred as hyperglycemic agent. (Michael, et al. 2000).

Two hormones produced by the adrenal gland affect carbohydrate metabolism. Epinephrine produced by the adrenal medulla, increases plasma inhibiting insulin action, increasing glycogenolysis and promoting lipolysis. Epinephrine is released during times of stress. Glucocorticoids primarily cortisol are released from the adrenal cortex on stimulation by adrenal glucocorticoids hormone (ACTH) increases plasma glucose by decreasing intestinal entry glucose in to the cells and increasing gluconeogenesis, glycogenolysis and lipolysis.(Michael, et al . 2000, Macfarlane, et al .1997).

Two anterior pituitary hormones, Growth hormone (G.H) and (ACTH). Both promote increased plasma glucose, G.H it is release from the pituitary, is stimulated by decreased glucose levels and inhibited by increased glucose. It is increase plasma glucose by decreasing the entry of glucose in to cells by increasing glycolysis. Decreased levels of cortisol stimulate the anterior pituitary gland to release adrenal glucocorticoids hormone. In turn, stimulates

the adrenal cortex to release cortisol and increase plasma glucose levels by converting glycogen to glucose by the liver and by promoting gluconeogenesis. (Michael, et al. 2000, Macfarlane, et al. 1997).

Two other hormones affect glucose levels. Thyroxin and somatostatin , the thyroid gland is stimulated by the production of thyroid –stimulating hormone (TSH) to release thyroxin that increasing plasma glucose level by increasing glycogenolysis and gluconeogenesis and intestinal absorption of glucose. Somatostatin produced by the D cell of the islets of Langerhans of the pancreas, increase plasma glucose levels by inhibition of insulin, glucagon, and growth hormones and other endocrine hormones. (Michael, et al. 2000, Macfarlane, et al. 1997).

1.2.4. Classification of Diabetes Mellitus:-

A classification of diabetes and other categories of glucose intolerance based on contemporary knowledge of this heterogeneous syndrome were developed by an international work group sponsored by the National Diabetes Data Group of National Health. This classification and revised criteria for the diagnosis of diabetes were reviewed by the professional members of the American Diabetes Association and similar versions were circulated by the British Diabetes Association and the Australian Diabetes Society and the European Association for the study of diabetes. Are classifications diabetes mellitus according to genetic& environmental & etiological factors are involving primary & secondary & gestational diabetes mellitus (D.M) as following. (World Health Organization. 1999, National Diabetes Data Group. 1979).

(1) Primary Diabetes Mellitus: - Have two main forms:-

A) Type 1 (Insulin Dependent Diabetes Mellitus (IDDM)):-

In this type diabetes develops if the body is unable to produce insulin or a lack of insulin due to destruction of insulin producing by the beta - cells in the pancreas. Generally affects younger people and appears before the age 30 years, also called Juvenile diabetes. This type cases by autoimmune disease or genetic disorder susceptibility which done destruction of the beta – cells in the pancreas. The major goal in treatment is minimize any elevation of blood glucose by insulin injection and controls the diet (diet is contain low fat, cholesterol and with sample sugars) and regular exercise is recommended. (World Health Organization. 1985, American Diabetes Association. 2013, Garber, et al .2012-2013).

B) Type 2 (Non - Insulin Dependent Diabetes Mellitus (NIDDM)):-

This type the body can still secretion or make some insulin but it is not enough or it is properly (known as insulin resistance) and the most common form of diabetes is caused by combination of factors plus genetic susceptibility ,physical inactivity with very over weight (obese) ,abnormal glucose production by the liver ,beta –cells dysfunction , high density lipoprotein , high blood pressure (140/90) or above , family back ground D.M and patients with lipo dystrophy (is condition in which fat tissue is lost or redistributed in the body associated with insulin resistance . This type usually appears in people over age 31 years and the treatment first with weight reduction if the patient is obese, diabetic diet and exercise (self –monitoring of blood glucose). When this measures fail to control the elevated blood sugars oral medications are used, if it is still insufficient treatment with insulin is considered. (World Health Organization. 1985, American Diabetes Association .2013, Welschen, et al. 2005).

(2) Secondary diabetes mellitus: - Have sub groups includes:-

A) Genetic defects of beta-cells function:-

Several pathogenesis processes are development of diabetes mellitus. These include destroy or injures of the beta-cells of the pancreas with consequent insulin deficiency and others that result resistance of insulin action include pancreatitis, trauma, pancreatectomy, pancreatic carcinoma, pancreatic fibrosis and calcified stone in the exocrine duct are found at autopsy. (Gullo, et al. 1994, Larsen, et al. 1987).

B) Endocrinopathies:-

Diseases associated with excess secretion of endocrine hormones (antagonize hormones). Include e.g. Acromegaly, Cushing's syndrome, Glucagonoma and Phaeochromocytoma .Which lead to forms of diabetes and hyper glycaemia. (Phelps, et al. 1989).

C) Uncommon forms of immunological disease:-

Auto immune diseases with pathogenesis or an etiology different from that which leads to type1 diabetes process hypo glycaemia rather than hyper glycaemia. Include e.g. Autoantibodies Stiff man syndrome. (Hirata, et al. 1970, Solimena, et al. 1991).

D) Some Drug - or Chemical:-

Many drugs e.g. (Nicotinic acid) and treatment of HIV or treatment taken after organ transplantation and certain toxins e.g. (Rat poison) can impair insulin secretion from the beta - cells which lead to forms of diabetes and hyper glycaemia. (Yajnik, et al.1992. Macfarlane, et al. 1997).

E) Infection:-

The infections by certain viruses have been associated with beta-cells destruction. In some patients with congenital Rubella, in addition Coxsackie Cytomegalovirus, Adenovirus and Mumps. Have been implicated in inducing the diabetes mellitus. (Forrest, et al. 1971, pakcy, et al. 1988).

F) Chromosomal abnormalities:-

Other genetic syndromes are accompanied by an increased incidence of diabetes mellitus. These include the chromosomal abnormalities and autosomal recessive disorder like patient with e.g. (Down's syndrome, Turner's syndrome and Wolfram's syndrome). Characterized by insulin deficient diabetes and absences of beta- cells at autopsy. (Barrett, et al. 1995).

(3) Gestational diabetes mellitus:-

Immediately after pregnancy 5% to 10% of women with gestational diabetes (G.D.M). That means high blood glucose level appearing for the first time during pregnancy in women not previously diagnosed with other forms of diabetes mellitus. This type it is carries risk for the mother and neonate and causes due to metabolic and hormonal changes and metabolic demands of pregnancy (glucose intolerance) together with genetic and environmental factors. Women with a history of (G.D.M) found to have pre-Diabetes Mellitus should receive life style inter ventions or metformin oral medication to prevent diabetes. It is also marker of increased risk of developing of type 2 diabetes mellitus in later life for mothers if uncontrolled. Risk developing for neonate such as excess growth (macrosomia) cause by extra glucose can cross the placenta then the pancreas of baby's release extra insulin, also gets risk of low blood sugar (hypoglycemia) shortly after birth because their own insulin production is high if untreated as soon as possible they gets risk of the

death shortly after birth. G.D.M diagnosed in the second or third trimester of pregnancy that is not clearly overt diabetes. Some cases are managed with changes diet and exercise and if blood glucose still high will be used insulin injection (if patients who are pregnant or breast feeding are recommended of controlling and you should speak with doctor if you are taking this medications and considering becoming pregnant or if you have become while taking these medications. (World Health Organization (1985), American Diabetes Association. 2013, Michael, et al .2000).

1.2.5. Complications of diabetes mellitus:-

Long - term complications of Diabetes Mellitus develop gradually. The longer you have diabetes mellitus and the high blood sugar, poor metabolic control, hypertension, smoking, obesity and hyper lipidemia you most gets the higher risk of complications of diabetes mellitus (American Diabetes Association . 2010). This include:-

(i) Cardiovascular disease:-

Diabetes dramatically increases the risk of various cardiovascular problems including myocardial infarction, macro vascular, micro vascular and Coronary Heart Disease (CHD) with chest pain (angina) is 2 - 4 times greater in patient with diabetes than individuals without diabetes. Cardiovascular is the major source of mortality in patient with type2 diabetes mellitus, approximately two thirds of patients with D.M die by heart disease or a stroke and narrowing of arteries (atherosclerosis). (Wannamethee, et al. 2011)

(ii) Nerve damage (neuropathy):-

Excess sugar can injure the walls of the tiny blood vessels (capillaries) that nourish your nerves, especially in your legs. This can cause ting ling, numbness, burning or pain that usually begins at the tips of the toes or fingers

and gradually spreads upward. Left untreated you could lose all sense of feeling in the affected limbs and digestion problems with nausea, vomiting, diarrhea it may lead to erectile dysfunction. (Barret, et al. 1995).

(iii) Kidney damage (nephropathy):-

The kidneys contain millions of tiny blood vessel clusters (glomeruli) that filter waste from your blood. Diabetes can damage this delicate filtering system, severe damage can lead to kidney failure or irreversible end –stage kidney disease, which may require dialysis or kidney transplant. (Vos, et al. 2012).

(iv) Eye damage (retinopathy):-

Diabetes can damage the blood vessels of the retina (diabetes retinopathy), potentially leading to blindness in adult in the world. Diabetes also increases the risk of other serious vision condition, such as cataracts and glaucoma disease. (Michael, et al. 2000).

(v) Foot damage:-

Nerve damage in the feet or poor blood flow to the feet increases the risk of various foot complications, left untreated, cuts and blisters can develop serious infections, which often heal poorly these infections may ultimately require toe, foot or leg amputation. (Michael, et al. 2000).

(vi) Skin conditions:-

Diabetes may leave you more susceptible to skin problems including bacterial and fungal infections. (Michael, et al. 2000).

(vii) Alzheimer's disease:-

Type2 diabetes may increase the risk of Alzheimer's disease the poorer your blood sugar control; the greater the risk appears to be. Although there are theories as to how these disorders might be connected, none has yet been proved. (Vos, et al. 2012).

(viii) Cancer:-

Metformin (one of oral medication for control diabetic patients) can modulate clinical outcome in cancer patient with diabetes a mange patients with prostate, pancreatic, breast colorectal. (Nelson. 2013).

1.2.6. Diagnostic and monitoring of Diabetes Mellitus:-

Diabetes mellitus may be diagnosed and monitoring based on plasma glucose criteria, either the fasting plasma glucose (FPG) or by 2houre plasma glucose after meal (2 h PG) or by oral glucose tolerance test (OGTT) value of glucose gets after 75g/kg in children and 75g for adult and 100g for pregnant women's. In last 2010 the American Diabetes Association Standards of Medical Care (ADAMC) start the hemoglobin A1c (HbA1c) criteria, is the test that used in monitoring of levels of blood glucose in diabetic patients. Criteria for the diagnosis as follow as FPG more than ≥ 126 mg/dl (7.0 mmol/L) during no caloric intake for at least 8-12 hours, 2HPG ≥ 200 mg/dl (11.1 mmol/L) during on OGTT test should be performed as described by the World Health Organization (WHO) using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water (250-300 ml of water) , HbA1c ≥ 6.5% the test should be performed in a laboratory using method standardized to the Diabetes Control and Complications Trial (DCCT). (American Diabetes Association .2013, International Expert Committee. 2009).

1.2.7. Hemoglobin A1c (HbA1c):-

Glycation is the non-enzymatic attachment of a mono saccharide to a mino group of proteins. The reaction has been recognized for many years in the food industry, where it is known as browning (also termed the Maillarel reaction) and is responsible for the formation of commonly ingested items , In patients with diabetes , glucose a accumulation results in enhanced glycation of many proteins , both in tissues (e.g. the lens) and in the blood . Of this glycated hemoglobin (GHb) is by far the most frequently measured in patient care (American Diabetes Association. 2013).

Hemoglobin (Hb) in healthy adults consists predominantly of HbA, which has 2α and 2β chains, glucose can attach to several amino acid residues in these chains. HbA1c is formed when glucose attaches specifically to the NH_2 – terminal (Valine) of the β-chain. Formation of HbA1c is essentially irreversible, and it is concentration in the blood depends on both the life span of the red blood cell (RBC), which averages 8-12 weeks (120 days) before renewal, and the blood glucose concentration. Because the rate of formation of HbA1c is directly proportional to the concentration of glucose in the blood. HbA1c represents integrated values of glucose over the preceding 8-12 weeks then the HbA1c can be used to reflect average blood glucose level over that duration. (Kunkel, et al .1955, Cohen, et al .2008).

1.2.7.1.. HbA1c test:-

The HbA1c test is simple blood test that does not required any special precautions like fasting or special time or control diet it is done at any time. It is primarily for diabetics and better management of blood sugar level then it is used as monitoring and diagnostic tool as well as for examining control of blood sugar over the last few weeks. It is more reliable test as compared to home testing records maintained by patient provides an average level where as

fasting or random blood sugar level at testing time .Which helps to gauge the trend of blood sugar control. And higher HbA1c will be given best picture of uncontrolled diabetics. And help to detect the level of blood glucose over the past three months. (American Diabetes Association. 2010).

They are certain condition affect in outcome of the test such as anemia (low hemoglobin e.g. iron deficiency anemia) , blood problems (genetic dis order like sickle cell disease) , high cholesterol , blood transfusion , blood loose (sever bleeding) , kidneys disease (kidney damage) , liver disease (G-6-P defiance) , taking high units of vitamin supplements (vitamin C - vitamin E) , alcohol taking (alcoholism) , Derek's (steroids hormones disorder) . All this condition will be raise blood glucose and HbA1c then in this case will be rapid fluctuations result and given treatment if needed . (Bry, et al .2001. Vos, et al .2012.Tarim, et al .1999).

1.2.7.2. HbA1c test done for: - 1) Earlier it was more commonly used for monitoring of diabetes rather than initial diagnosis. 2) For diagnosis purposes in people with high risk of developing diabetes mellitus. 3) People who have well controlled diabetes every six months for monitoring. 4) Peoples with poorly controlled diabetes every three months for monitoring treatment dose. 5) Also carried out to check the efficacy of any changes in diabetes mellitus medications. 6) Also for patient that not give true data for his disease and with symptoms of diabetes mellitus. (American Diabetes Association. 2010. Mbanya, et al .2011. Holman, et al. 2008) .

HbA1c has traditionally been reported as percentage (**%**) of total hemoglobin old unit. IFCC numbers are lower by 1.5 – 2.0 % HbA1c than NGSP/DCCT numbers, most likely because of the increased specificity of the IFCC method. While there is a tight linear correlation between the NGSP and the IFCC

methods, the slope and intercept differ significantly from 1 and 0 respectively. (Hoelzel, et al, International Federation of Clinical Chemistry. 2004).

1.3 Rationale:-

Diabetes mellitus complications lead to damage important biological organs such as cardiovascular, nephropathy and retinopathy some of this complication will be lead to sudden death. Then we must be prevented or reduced these risk complications of diabetes mellitus as soon as you can .The early diagnosis and good monitoring of levels of plasma glucose in the blood helps in prevention and reduction of complications of Diabetes Mellitus.

This study aimed HbA1c level in type2 diabetic Sudanese patients compared to level of fasting and random blood glucose and so for control group. This to document the HbA1c test was significantly and sensitively test done for diabetic patients with it gradually increased in uncontrolled diabetic patients so far can used for monitoring diabetic patients to avoid the risk complication of this disease .

1 .4 Objectives:-

1.4.1 General objective:-

To study the HbA1c level in type 2 diabetic compared to level of fasting and random plasma glucose.

1.4.2 Specific objective:-

1.4.2.1 To compare HbA1c level and Fasting and Random plasma glucose level in diabetic patients compared to control samples.

1.4.2.2 To compare HbA1c, Fasting and random plasma glucose level in reference to age between groups of diabetic patients.

1.4.2.3 To correlate the level of HbA1c, fasting and random plasma glucose with duration of Diabetes Mellitus in diabetic patients.

Chapter Two
Material and Methods

2. Materials and Methods

2.1. Materials:-

2.1.1. Study design: - This is a cross sectional and case control study.

2.1.2. Study duration: - In April to June 2013 in Khartoum state.

2.1.3. Study area: - The study was conducted in Hospital of Jabber Abu El-Eiz Diabetic Center and Alrebbat Hospital.

2.1.4. Study population:-The study included 120 Sudanese patients with type 2 diabetes mellitus (Inclusion criteria) and 40 Sudanese apparently healthy individuals as control (volunteers).

2.1.5. Data collection:-

Primary data was collected by using a direct interviewing questionnaire, which in clouded: personal information.

2.1.6. Collection &Transportation of samples:-

About 5 ml venous blood were collected from each diabetes patients and non-diabetes at the fasting state (2.5 ml in fluoride oxalate containers and 2.5 ml in k3EDTA blood containers) and about 2.5 ml venous blood in fluoride oxalate containers were collected also at the random state from the same study population using veinpuncture technique, and plasma obtained after centrifugation at 3000 rpm for 5 minutes then the biochemical parameters were estimated in this time.

2.1.7. Ethical Consideration:-

- Every subject was informed before collection of specimen.

2.1.8. Data analysis:-

Statistical analysis was performed using statistical package for social sciences (SPSS) computer programs . The mean and standard deviation, (T –test) were used to compare between the mean and standard deviation (SD) with (P. value less than 0.05), confidence value 95%. And correlation was use with (P. value less than 0.01) was considered significant.

2.2. Methodology:-

2.2.1 Measurement of HbA1c level using the Nyco Card Reader II method :(Appendix II)

(A) Principle of method:-

HbA1c is boronate affinity assay the kit contains test devices with a porous membrane filter, test tubes pre-filled with reagent and a washing solution. The reagent contains a gents that lyse erythrocytes and precipitate hemoglobin specifically, As well as a blue boronic acid conjugate that binds cis-diols of glycated hemoglobin .When blood is added to the reagent, the erythrocytes immediately lyse. All hemoglobin percentage precipitates **.**The boronic acid conjugate binds to the cis-diol configuration of glycated hemoglobin. An aliquot of the reaction mixture is added to the test device, and all the precipitated hemoglobin conjugate bound, and un bound remains on top of the filter. Any excess of coloured conjugate is removed with the washing solution. The precipitate is evaluated by measuring the blue (glycated hemoglobin) and the red (total hemoglobin) colour intensity with the Nyco card reader II, the ratio between them being proportional to the percentage of HbA1c in the sample**.**

(B) Quality Control:-

A quality control material with Nyco Card HbA1c specific target values was used to confirm the efficacy of the reagents and the correct performance of the test.

2.2.2. Estimation of glucose concentration using the glucose oxides and peroxides method: - (Appendix III).

(I) Principle of method:-

Glucose in the sample originates, by means of the coupled reactions described below, a coloured complex (red) that can be measured by spectrophotometry in filter (500 ± 20 nm) which the color is proportional to the amount of glucose in the sample show the principle blew.

Glucose $+½O_2$ $+H_2O$-------glucose oxides ----Gluconate + H_2O_2

$2H_2O_2$+4-Aminoantipyrine +phenol-----peroxides---Quinoneimine $+4H_2O$

(III) Quality Control:

The Biochemistry Control Serum Level (cod .18005, 18009 and 18042) and II (cod .18007, 18010 and 18043) was used to verify the performance of the measurement procedure.

Chapter three

Results

3. Results

One hundred and twenty (53 females and 67 males) Sudanese patients with type2 Diabetes mellitus and forty Sudanese apparently healthy individuals (19 females and 21 males) were enrolled in this Study, study and control groups were matched for age from (31-75 years). The results of statistical analysis were as follow:

For Level of HbA1c:-

Table (3.1): Shows a significant difference between the means of level of HbA1c of diabetic patients groups compared to non-diabetic healthy peoples groups. (Mean ± SD) (9.852 ± 2.6433) versus (4.478 ± 0.4902) and respectively (P. value = 0.00). The mean of the diabetic patients is significantly raised.

Table (3.2): Shows a significant difference between the means of level of HbA1c and the age between groups of diabetic patients. (Mean ± SD) (7.913 ± 1.6474) (11.089 ± 1.7803) (12.917 ± 1.6375) (P. value = 0.00). The mean of the age between groups is significantly raised.

Figure (3.1): A scatter plot shows significant high positive correlation between the duration of diabetes mellitus (range 1 – 27 years) and the level of HbA1c (r=0.01, p. value= 0.828).

For Plasma Fasting Blood Glucose:-

Table (3.1): Shows significant difference between the means of plasma fasting plasma glucose (FPG) of Diabetic patients groups compared to non-Diabetic healthy peoples groups. (Mean ± SD) (204.48 ± 53.503) mg/dl versus (86.00 ± 6.214) mg/dl and respectively, (P. value = 0.00). The mean of the diabetes is significantly raised.

Table (3.2): Shows a significant difference between the means of level of plasma fasting blood glucose (FBG) and the age between groups of diabetic patients. (Mean ± SD) (173.30 ± 36.525) (226.43 ± 48.366) (250.48 ± 49.033) mg/dl, (P. value = 0.00). The mean of the age between groups is significantly raised.

Figure (3.2): A scatter plot shows significant high positive correlation between the duration of diabetes mellitus (range 1 – 27 years) and the level of plasma fasting blood glucose(FBG) (r=0.01, P. value = 0.727).

For Plasma Random Blood Glucose:-

Table (3.1): Shows significant difference between the means of level of plasma random blood glucose (RBG) of diabetic patients groups compared to non - diabetes healthy peoples groups. (Mean ± SD) (333.64 ± 93.302) mg/dl and versus (112.78 ± 5.535) mg/dl respectively (p. value = 0.00). The mean of the diabetic patient is significantly raised.

Table (3.2): Shows significant difference between the means of level of plasma random blood glucose (RBG) and the age between groups of diabetic patients. (Mean ± SD) (271.97 ± 72.038) (372.86 ± 64.641) (431.43 ± 58.076) mg/dl, (P. value = 0.00). The mean of the age between groups is significantly raised.

Figure (3.3): A scatter plot shows significant positive correlation between the duration of diabetes mellitus and the plasma levels of random blood glucose (r=0.01 ,P. value = 0.786) . The mean of the age between groups is significantly raised.

Table (3.1) : Comparison of the means of the HbA1c levels and plasma levels of fasting & random blood glucose in reference to case and control (diabetes and non diabetes).

Variable	Diabetic N=120	Non-diabetic N=40	P. value
HbA1c (%)	9.852 ± 2.6433	4.478 ± 0.4902	0.00
Fasting plasma glucose (mg/dl)	204.48 ± 53.503	86.00 ± 6.214	0.00
Random plasma glucose (mg/dl)	333.64 ± 93.302	112.78 ± 5.535	0.00

The table shows the (mean ± SD), and the probability (P).

T-test was used for comparison.

P. value ≤ 0.05 is considered significant.

Table (3.2): Comparison of the means of the HbA1c levels, fasting plasma glucose levels (FPG), and random plasma glucose levels (RPG). In reference to age between groups (diabetes males and females).

Independent Variable / Age groups/ years	**31 – 45 (Years) N = 60**	**46 – 61 (Years) N = 37**	**62 – 75 (Years) N = 23**	**P. value**
HbA1c%				
Mean	7.913	11.089	12.917	
SD	1.6474	1.7803	1.6375	0.00
FBG (mg/dl)				
Mean	173.30	226.43	250.48	
SD	36.525	48.366	49.033	0.00
RBG (mg/dl)				
Mean	271.97	372.86	431.43	
SD	72.038	64.641	58.076	0.00

The table shows the mean and SD, range in brackets and the probability (P).

ANOVA –One way (post HOC tests) was used for comparison.

P. value$\leq$ 0.05 is considered significant.

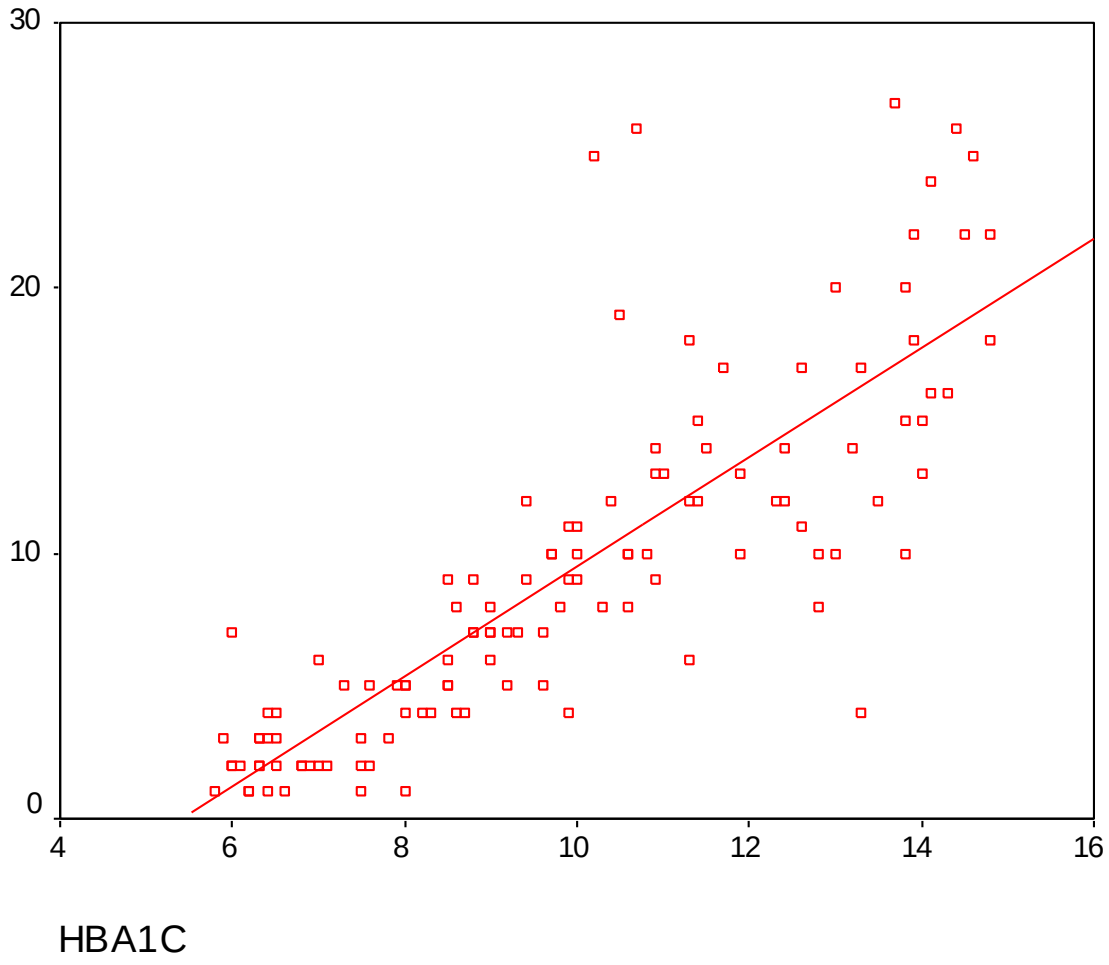

Figure (3.1): A scatter plot shows significant high positive correlation between the duration (rang 1 – 27years) of diabetes mellitus and the levels of HbA1c(r=0.828, P .value = 0.01).

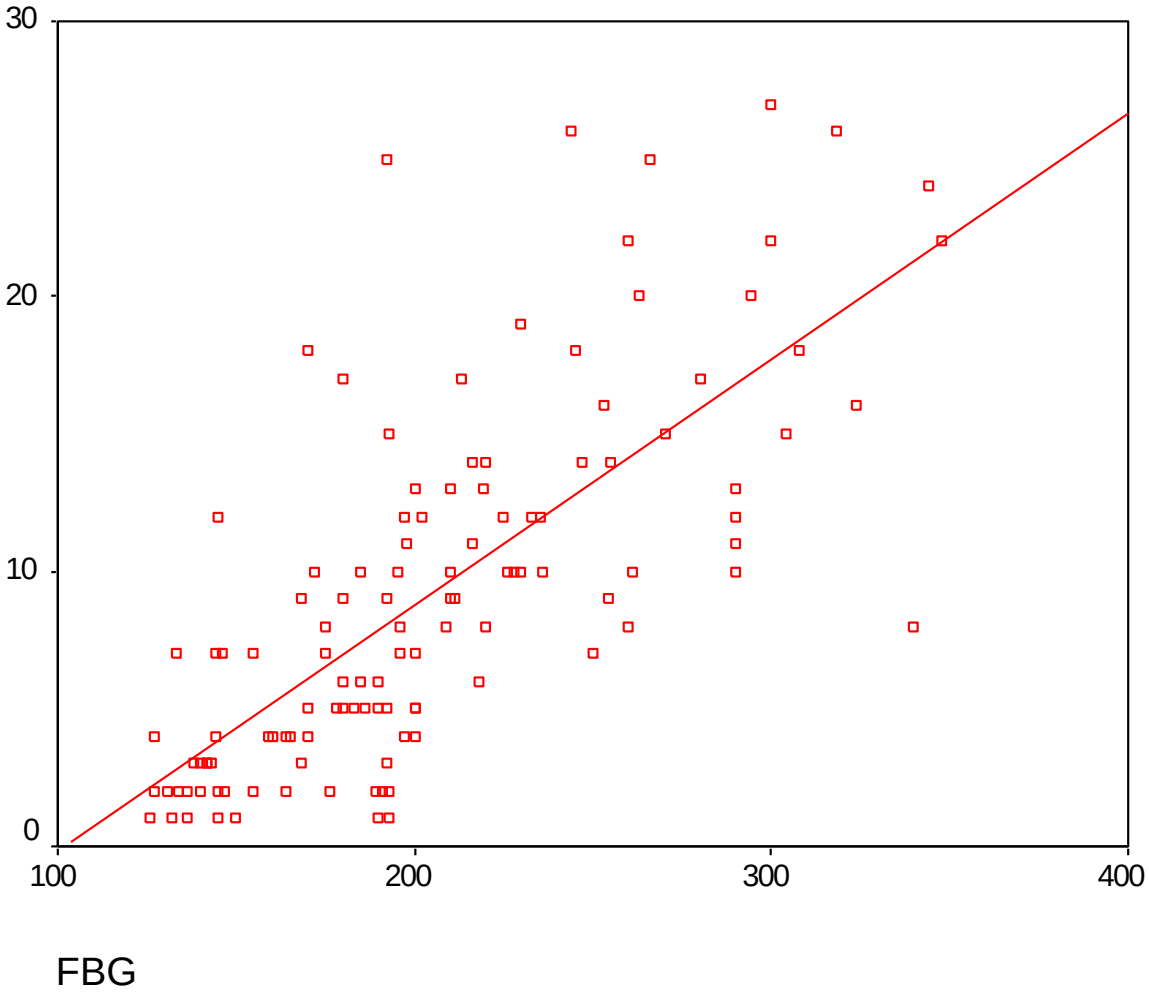

Figure (3.2) : A scatter plot shows significant positive correlation between the duration of diabetes mellitus and the plasma levels of fasting blood glucose (r=0.727 , P value=0.01) .

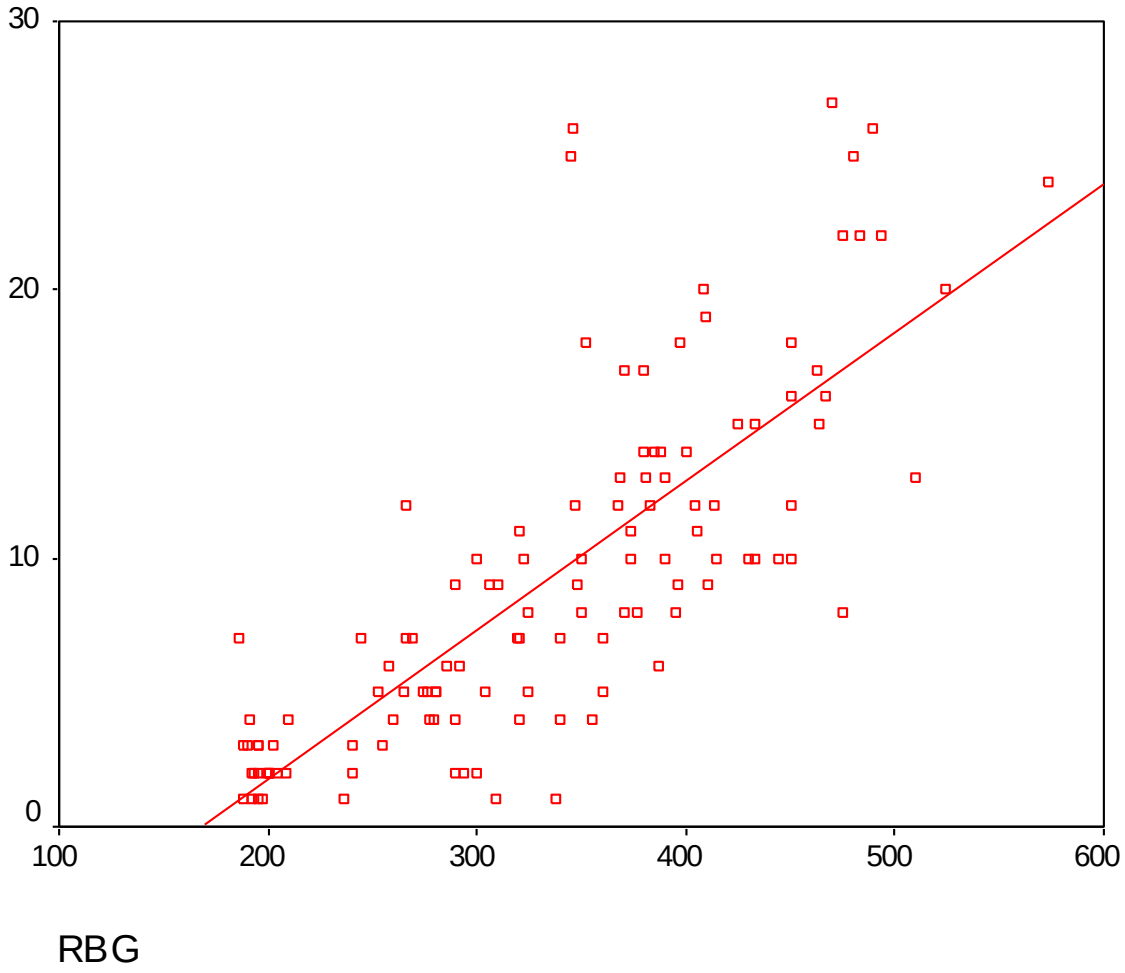

Figure (3.3) : A scatter plot shows significant positive correlation between the duration of diabetes mellitus and the plasma levels of random blood glucose (r=0.786 , P .value=0.01) .

Chapter four

Discussion, Conclusion and Recommendation

4. Discussion, Conclusion and Recommendation

4.1 Discussion:-

Diabetes mellitus is the term of groups of metabolic disease characterized by high elevation of glucose in the blood. Diabetes Mellitus should be controlled and monitored to decrease and a void it is complications (coronary artery, peripheral arterial disease and stroke (macro vascular complications) and diabetic nephropathy, neuropathy and retinopathy (micro vascular complications). (American Diabetes Association .2013, Michael, et al. 2000).

The aim of this study was evaluate HbA1c level in type2 diabetics compared to level of Fasting and Random Blood Glucose. One hundred and twenty (53 females and 67 males) Sudanese patients with type 2 Diabetes Mellitus and forty Sudanese apparently healthy individuals (19 females and 21 males) were enrolled in this Study, study and control groups were matched for age.

Statistical analysis results explained a significant increase in the (mean ± stander deviation) of HbA1c (9.852 ± 2.6433) in diabetic patients compared to the (mean ± stander deviation (SD)) of HbA1c (4.478 ± 0.4902) in control groups with (P. value is 0.00). This results agreed with study done by Louis Monnier and his groups with mean of HbA1c (8.0) and (P. value < 0.001), (Louis Monnier, et al. 2003) and agreed by Laura and her team with mean of HbA1c (6.8) and (P. value < 0.002) (Laura, et al. 2000). Also agreed by Hans Woerle, and his groups with mean of HbA1c (9.0) and (P. value 0.0001) (Hans Woeele, et al. 2006).

Beside that showed a significant different in the (mean ± SD) of Fasting Plasma Glucose (204.48 ± 35.503) in diabetic patients compared to the (mean

± SD) of fasting plasma glucose (86.00 ± 6.214) in control groups with (P. value 0.00), this findings were agreed by Louis Monnier and his groups with (mean 210 mg/dl) and (P. value < 0.001), (Louis Monnier, et al. 2003) and agreed by Laura and her team with (mean 165 mg/dl) and cutoff > 9.2 mmol/L and (P. value < 0.002) (Laura ,et al. 2000). Also agreed by Hans Woerle and his groups with (mean 174 mg/dl) and (P. value 0.0001) (Hans Woerle, et al. 2006).

The study told us a significant increase in the (mean ± stander deviation) of Random Plasma Glucose (333.64 ± 93.302) in patients groups (diabetic patients) compared to the (mean ± stander deviation) of Random Plasma Glucose (112.78 ± 5.535) in control groups (non diabetic) and (P. value is 0.00). The results agreed by Louis Monnier and his groups with (mean 250 mg/dl) and (P. value < 0.001), (Louis Monnier, et al. 2003) and agreed by Laura and her team with (mean 200 mg/dl) and cutoff >11.1 mmol/L and (P. value <0.002), (Laura, et al. 2000). Also agreed by Hans Woerle and his groups with (mean 224 mg/dl) and (P. value 0.0001), (Hans Woerle, et al. 2006).

The analysis also explained positive significant correlation between the HbA1c levels and duration of Diabetic Mellitus in case groups (r = 828, p.value = 0.01) .This results agreed by Hans Woerle and his group with the (r = 0.87, p. value = 0.001) (Hans Woerle, et al. 2006) and agreed by Louis Monnier and his groups with the (r = 0.79, p. value = 0.02) (Louis Monnier, et al. 2003). Also agreed by Laura and her team with the (r = 0.88, p. value = 0.002) (Laura, et al. 2000).

The results showed a significant positive correlation between the Fasting Plasma Gucose levels and duration of disease in patients groups (r = 0.727, p = 0.01). This results agreed by Hans Woerle, and his groups with the (r = 0.74,

p = 0.001) (Hans Woerle , et al .2006). Also agreed with results done by Louis Monnier and his groups with the (r = 0.85, p. value = 0.02) (Louis Monnier, et al. 2003). Also agreed by Laura and her team with the (r = 0.81, p. value = 0.002) (Laura, et al. 2000).

In addition to that explained positive a significant correlation between the Random Plasma Glucose levels and duration of disease in diabetic patients (r=0.786, p.value= 0.01) .This results agreed with results done by Hans Woerle and his groups with the (r=0.91, p. value = 0.001) (Hans Woerle, et al. 2006) and agreed don by Louis Monnier and his groups with the (r = 0.9, p. value = 0.02) (Louis Monnier, et al. 2003). Also agreed by Laura and her team with the (r = 0.93, p. value = 0.002) (Laura, et al. 2000).

Statistical analysis told us that there are high significant comparison of the HbA1c levels in reference to age between groups of Sudanese patients with type2 Diabetes Mellitus the (mean ± SD) (7.913 ± 1.6474)(11.089 ± 1.7803)(12.917 ± 1.6375) with (p. value =0.00). Also high significant comparison of the Fasting Plasma Glucose levels in reference to age between groups of Sudanese patients with type2 Diabetes Mellitus the (mean ± SD) (173.30 ± 36.525)(226.43 ± 48.366)(250.48 ± 49.033) with (p. value =0.00) . Beside that showed high significant comparison of the Random Plasma Glucose levels in reference to age between groups of Sudanese patients with type2 Diabetes Mellitus the (mean ± SD) (271.97 ± 72.038)(372.86 ± 64.641)(431.43 ± 58.076) with (p. value =0.00).

4.2 Conclusion:-

This study concluded that:-

(1) There were a significant relationship between increasing of HbA1c level and increasing of fasting and random plasma glucose level in type 2 diabetics.

(2) Fasting Plasma Glucose, Random Plasma Glucose and HbA1c level are significantly increased in uncontrolled diabetic patients to control groups.

(3) HbA1c, Fasting and Random Plasma Glucose level increasing in reference to age between groups of diabetic patients.

(4) HbA1c, Fasting and Random Plasma Glucose level increasing correlated with duration of Diabetes Mellitus in diabetic patients.

4.3 Recommendations:-

- A further study need to be done on serum 1,5anhydroglucitol and level compared to HbA1c level in diabetics to assess a new test for monitoring the Diabetes Mellitus.
- A study should be done in evaluation of HbA1c level compared to microalbuminuria in diabetics to assess the Renal Disease.

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Appendices

Appendix (1)

College of Graduate Studies

Questionnaire

HbA1c level compared to level of fasting and random plasma glucose in Sudanese diabetic patients

- **Name: -** ----------.
- **Age: -** ----------.
- **Duration of disease: -Month:** ----------.
- **Years** ----------.
- **Treatment: -** ----------.
- **Gender: - Male ---------- Female ----------.**
- **Diet control: - Yes ---------- (or) NO----------.**
- **Complication: - Yes ---------- (or) NO----------.**

Appendix (II)

COD 11803	COD 11503	COD 11504	COD 11538
1 x 50 mL	1 x 200 mL	1 x 500 mL	1 x 1 L
STORE AT 2-8ºC			
Reagents for measurement of glucose concentration Only for *in vitro* use in the clinical laboratory			

GLUCOSE

BioSystems
REAGENTS & INSTRUMENTS

CE

GLUCOSE
GLUCOSE OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Glucose in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1].

$$\text{Glucose} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{Gluconate} + H_2O_2$$

$$2\,H_2O_2 + 4\text{-Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4\,H_2O$$

CONTENTS

	COD 11803	COD 11503	COD 11504	COD 11538
A. Reagent	1 x 50 mL	1 x 200 mL	1 x 500 mL	1 x 1 L
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent: Phosphate 100 mmol/L, phenol 5 mmol/L, glucose oxidase > 10 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine 0.4 mmol/L, pH 7.5

S. Glucose/Urea/Creatinine Standard. Glucose 100 mg/dL (5.55 mmol/L), urea 50 mg/dL, creatinine 2 mg/dL. Aqueous primary standard.

STORAGE

Store at 2-8ºC.

Reagent and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.150 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm

SAMPLES

Serum or plasma collected by standard procedures. Serum or plasma must be separated from the red cells promptly to prevent glycolysis. The addition of sodium fluoride to the blood sample prevent glycolysis.

Glucose in serum or plasma is stable for 5 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes: (Note 1)

	Blank	Standard	Sample
Glucose Standard (S)	—	10 µL	—
Sample	—	—	10 µL
Reagent (A)	1.0 mL	1.0 mL	1.0 mL

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25ºC) or for 5 minutes at 37ºC.
4. Measure the absorbance (A) of the Standard and the Sample at 500 nm against the Blank. The colour is stable for at least 2 hours.

CALCULATIONS

The glucose concentration in the sample is calculated using the following general formula:

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = C_{Sample}$$

If the Glucose Standard provided has been used to calibrate (Note 2):

$\frac{A_{Sample}}{A_{Standard}}$	x 100 = mg/dL glucose x 5.55 = mmol/L glucose

REFERENCE VALUES

Serum and plasma[2]:

Newborn, premature	25-80 mg/dL = 1.39-4.44 mmol/L
Newborn, term	30-90 mg/dL = 1.67-5.00 mmol/L
Children, adult	70-105 mg/dL = 3.89-5.83 mmol/L

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

According to the National Diabetes Data Group (US)[3], elevation of fasting plasma glucose values over 140 mg/dL (7.77 mmol/L) on more than one occasion is diagnostic of diabetes mellitus.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.23 mg/dL = 0.0126 mmol/L.
- Linearity limit: 500 mg/dL = 27.5 mmol/L. For higher values dilute sample 1/4 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
88 mg/dL = 4.84 mmol/L	1.2 %	20
326 mg/dL = 17.93 mmol/L	0.9 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
88 mg/dL = 4.84 mmol/L	2.7 %	25
326 mg/dL = 17.93 mmol/L	1.9 %	25

- Sensitivity: 4 mA·dL/mg = 0.22 mA·L/mmol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (> 3 g/L), lipemia (triglycerides > 1.25 g/L) and bilirubin (10 mg/dL) may interfere. Other drugs and substances may interfere[4].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Glucose is the major source of energy in the body. Insulin, produced by islet cells in the pancreas, facilitates glucose entry into the tissue cells. A deficiency of insulin or a decrease of its effectiveness increases blood glucose.

Elevated serum or plasma glucose concentration is found in diabetes mellitus (insulin dependent, non-insulin dependent) and in other conditions and syndromes[2,3].

Hypoglycemia can occur in response to fasting, or it may be due to drugs, poisons, inborn errors of metabolism or previous gastrectomy[2,3].

Clinical dianosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Specific instructions for application in many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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4. Young DS, Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
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Quality System certified according to
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Appendix (III)

Reference range[2-4]

For methods reporting DCCT traceable values, the upper limit of non-diabetic reference range is approximately 6% HbA1c.

Precision

In professional use, a coefficient of variation (CV) < 5% is usually obtained.

Limitations of the test

- Elevated amounts of glucose, bilirubin, lipids and fructosamine were added to blood samples with normal and elevated HbA1c values. No interference was obtained.
- Pre-glycated hemoglobin does not interfere with the test.
- Hemolysed samples with plasma Hb >3 g/100 mL will interfere with the test system.
- NycoCard® READER II corrects for Hb-concentrations in the range 6-18 g/100 mL.

STABILITY AND STORAGE

Unopened kits

The expiry date of the kit applies to storage at 2-8°C in the original container. Exposure to temperatures above 25°C or humidity above 70% should be avoided. Do not freeze.

Opened kits

R1/Reagent must be stored dark at 2-8°C. Equilibrate the R1/Reagent to room temperature (20-25°C) before use. Equilibration to room temperature can be achieved by holding the tube in a closed hand for 30 seconds. The reagent tube can be stored for maximum 6 hours at room temperature before use. Avoid direct sunlight.

TD/Test Device can be stored at room temperature (15-25°C). Store the test devices in the original bag and avoid humidity below 20% and above 70%. The TD/Test Device should have room temperature when used.

R2/Washing Solution can be stored at room temperature (15-25°C). The R2/Washing Solution should have room temperature when used.

Sample material

Blood samples (with anticoagulant) can be stored up to 10 days at 2-8°C before analysis. Avoid measuring hemolysed samples (see "Limitations of the test"). Do not freeze.

TEST PROCEDURE

Important procedural notes!

- Do not interchange components from different kits or kit lots.
- Bring the R1/Reagent to room temperature (20-25°C) before use.
- Do not touch the membrane with the pipette tip.
- Change the pipette tip between each pipetting step.

Sample material

Capillary blood and venous blood with or without anticoagulant (EDTA, heparin and NaF) can be used.

Internal quality control

A quality control material with NycoCard® HbA1c specific target values should be used to confirm the efficacy of the reagents and the correct performance of the test. Lyophilised control materials should not be used with the NycoCard® HbA1c assay

Test procedure (illustrations on page 67)

1 Precipitate hemoglobin
Add 5 µL whole blood to the test tube with R1/Reagent. Mix well. Incubate the tube for minimum 2 minutes, maximum 3 minutes. Use a timer.
Note! Equilibrate the R1/Reagent to room temperature (20-25°C) before use.

2 Apply sample
Remix to obtain a homogenous suspension. Apply 25 µL of the reaction mixture to a TD/Test Device by holding the pipette approx. 0.5 cm above the test well. Empty the pipette quickly in the middle of the test well. Allow the reaction mixture to soak completely into the membrane (approx. 10 seconds).
Note! Avoid air bubbles.

3 Apply R2/Washing Solution
Apply 25 µL R2/Washing Solution to the TD/Test Device. Allow the washing solution to soak completely into the membrane. Wait for minimum 10 seconds.
Note! Avoid air bubbles.

4 Read the test result
Read the test result within 5 minutes using the NycoCard® READER II.
Note! Follow the NycoCard® READER II instruction manual.

INTERPRETATION OF RESULT

Interpret the NycoCard® HbA1c test results with careful consideration to the patient's medical history, clinical examinations and other laboratory results. If the test result is questionable or if clinical signs and symptoms appear inconsistent with the test result, re-test the sample or confirm the result using another method. Analyse control materials frequently to verify the performance of the NycoCard® READER II test system.