بسم الله الرحمن الرحيم

Sudan University of Science and Technology (SUST)

Collage of Graduate Studies

Estimation of Fructosamine and HbA1c Levels in Type1 and Type 2 Diabetes Mellitus

(Study in El-Obied City)

تقويم مستوى الفركتوزامين والهيموقلوبين السكري في مرضى السكري النوع الأول والثاني في مدينة الأبيض

(A desertation Submitted in A partial Fulfillment of the Requirement for the Master Degree in Clinical Chemistry)

By :

Eman Mohammed Ahmed Salih Saeed

B.Sc Clinical Chemistry –Collage of Medical Laboratories Sciences (Kordofan university 2013)

Supervised by :

Dr. Nuha Eljaili Abubaker

Assistant Professor of Clinical Biochemistry

الأية

﴿ بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ ﴾

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿١﴾ خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ ﴿٢﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ﴿٣﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿٤﴾ عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ ﴿٥﴾

صدق الله العظيم

سورة العلق الآيات 1-5

Dedication

To my

Parents

Special one

Brothers

Sister

Friends

And

college

Acknowledgment

All and first thanks to the almighty ALLAH . Then I would like to express my gratitude and ever last appreciation to my supervisorDr. Nuha Eljaili Abubakerfor this guidance , helpful suggestions for solving problems , valuable supervision as well as precious advice, support continues assistance through the whole process of this research.

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Abstract

This study was conducted to estimate serum fructosamine and HbA1c in patients with type 1 and type 2 DM. 100 blood samples (50 type 1, 50 type 2) were collected from patients during period from February to April 2016, chosen randomly from health insurance – diabetic center and El-obied teaching hospital, and 50 apparently healthy individuals as control group to evaluate and estimate serum fructosamine and HbA1c in diabetic patients. Nitroblue tetrazolium method used to estimate serum fructosamine concentration, and ion exchange chromatography method used for HbA1c estimation, and results were analyzed using statistical package for social science (SPSS) computer program.

The study showed that the mean of fructosamine and HbA1c were significantly increased (P.Value 0.000)(P.Value 0.000) respectively for type 1DM.

Mean ±SD for cases versus control:

(8.19±4.31 versus 2.38± 0.4)mg/dl for fructosamine.

(9.62±2.9 versus 4.69±0.64)% for HbA1c.

Also the study showed that the mean of fructosamine and HbA1c were significantly increased (P.Value 0.000)(P.Value 0.000) respectively for type 2DM.

Mean ±SD for cases versus control:

(5.74±2.04 versus 2.38±0.4)mg/dl for fructosamine.

(7.82±1.6 versus 4.69±0.64)% for HbA1c.

The study also showed there was significant positive correlation between fructosamine and HbA1c for type 1 and type 2 DM. (P.Value 0.015, r = 0.341)(P.Value 0.001, r =457) respectively.

Also the study showed there was a negative correlation between fructosamine and duration of type1 DM. (P.value 0.384, r = - 0.235).

The study showed there was no correlation between fructosamine and duration of type 2 DM.(P.Value = 0.371, r= -0.138).

It is concluded that the serum fructosamine and HbA1c were significantly increased in type 1 and type 2 DM, also singinficant correlation between fructosamine and HbA1c.

مستخلص الدراسة

أجريت هذة الدراسة لمعرفة مستوى الهيموقلوبين السكري و الفركتوزامين في مرضى السكري النوع الأول والثاني. تم جمع ١٠٠عينة دم من المرضى في الفترة ما بين فبراير حتى أبريل ٢٠١٦م ،تم إختيارهم بطريقة عشوائية من مستشفى الأبيض التعليمي ومركز ود اليأس للتأمين الصحي (مركز السكري)، مع ٥٠ عينة من الأشخاص الأصحاء كمجموعة ضابطة أو تحكم لقياس مستوى الهيموقلوبين السكري و الفركتوزامين في النوعين الأول والثاني لمرضى السكري.

تم قياسالهيموقلوبين السكري و الفركتوزامين باستخدام جهاز الإسبكتروفوتوميتر وتم تحليل البيانات بواسطة برنامج الحزم الإحصائية للعلوم الإجتماعية .

توصلت النتائج إلي أن هناك إرتفاع في متوسط مستوى الهيموقلوبين السكري و الفركتوزامين وكان الإحتمال الإحصائي للمقارنة (0.000) و (0.000) على التوالي لمرضى السكري النوع الأول . وكانت النتائج كالآتي:-

المتوسط ± الإنحراف المعياري عن المرضى مقارنة بمجموعة التحكم:

(٨.19 ± ٣١,٤ مقابل ٢.3٨ ± ٠.41١) ملجرام /ديسيلتر للفركتوزامين

(٩.62٢ ± ٩,٢ مقابل ٦٩,٤± ٠.64٤)% للهيموقلوبين السكري

وأيضا وجد أن متوسط مستوى الهيموقلوبين السكري و الفركتوزامين يزيد زيادة ملحوظة في مرضى النوع الثاني وكان الإحتمال الإحصائي للمقارنة (0.000) و (0.000) على التوالي. وكانت النتائج كالتالي :-

المتوسط ± الإنحراف المعياري عن المرضى مقارنة بمجموعة التحكم:

(٧٤,٥±٢,٤٠ مقابل ٢.3٨±٠.41١) ملجرام/ديسيلتر للفركتوزامين

(٨٢,٧± ٦,١مقابل ٦٩,٤± ٠.64٤)% للهيموقلوبين السكري

كما أوضحت الدراسة إلي أنه يوجد ارتباط إيجابي بين الهيموقلوبين السكري و الفركتوزامين في النوع الأول والثاني لمرضى السكري (معامل بيرسون =٣٤١,٠ ومستوى المعنوية ٠١٥ ,٠) (معامل بيرسون =٤٥٧,٠ ومستوى المعنوية ٠٠١,٠) على التوالي .

ووجد أيضا أن هناك إرتباط سلبي بين الفركتوزامين وفترة الإصابة لمرضى السكري النوع الأول (معامل بيرسون = - ٢٣٥,٠ ومستوى المعنوية٣٨٤,٠) .

ولكن وجد أنه لا يوجد إرتباط بين الفركتوزامين وفترة الإصابة لمرضى السكري النوع الثاني (معامل بيرسون =- ١٣٨,٠ ومستوى المعنوية٣٧١,٠) .

وعليه خلصت الدراسة إلي أنه يوجد زيادة ملحوظة في مستوى الهيموقلوبين السكري و الفركتوزامين في النوعين الأول والثاني لمرضى السكري ، وأيضا هناك ارتباط قوي بين الهيموقلوبين السكري و الفركتوزامين.

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List of abbreviations

DM	Diabetes mellitus
DMF	Deoxmorpholi-nofructose
EDIC	Epidemiology of diabetes intervention and complications
FA	Fructosamine
GAD	Glutamic acid decarboxylase
GDM	Gestational diabetes mellitus
HbA1c	Hemoglobin A1c
HLA-DR3	Human leucocyte antigen-determinant region 3
HLA-DR4	Human leucocyte antigen-determinant region 4
HPLC	High performance liquid chromatography
ICT	Islet cell antibodies
NBT	Nitro blue terazolium

Chapter one

Introduction

Rationale

Objectives

1.1 Introduction

Diabetes mellitus is a systemic metabolic disorder characterized by a tendency to chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism that arise from a defect in insulin secretion or action or both. It is a common condition, with a prevalence of approximately 4% in western world.Diabetes can occur secondarily to other diseases, for example chronic pancreatitis, following pancreatic surgery and in conditions where there is increased secretion of hormones antagonistic to insulin (e.g. Cushing's syndrome and acromegaly). Secondary diabetes is, however, uncommon. Most cases of diabetes mellitus (DM) are primary, that is , they not associated with other conditions(Marshall,2008).

Fructosamine is the generic name for plasma protein ketamines (goldstien *et al*., 2004., Sacks *et al*., 1992). The name refers to the structure of the ketoamine rearrangement product formed by the interaction of glucose with the E-amino group on lysine residues of albumin. Like measurements of GHb, measurements of fructosamine may be used as an index of the average concentration of blood glucose over an extended (but shorter) period of time. Because all glycated serum proteins are huctosamincs and albumin is the most abundant serum protein, measurement of fructosamine is thought to be largely a measure of glycated albumin, but this has been questioned by some investigators (Carl *et al*., 2006).

Glycosylated hemoglobin is the term used to describe the formation of a hemoglobin compound produced when glucose (a reducing sugar) reacts with the amino group of hemoglobin (a protein). The glucose molecule attaches non enzymatically to the hemoglobin molecule to form a ketoamine. The rate of formation is directly proportional to the plasma glucose concentrations. Because the average red blood cell lives approximately 120 days, the glycosylated hemoglobin level at any one time

reflects the average blood glucose level over the previous 2 to3 months. Therefore, measuring the glycosylated hemoglobin provides the clinician with a time-averaged picture of the patient‘s blood glucose concentration over the past3 months (Bishop *et al*., 2010).

1.2 Rationale

Diabetes the most common non-communicable disease in the country, is having an increasing impact on rates of morbidity and mortality in sudan.

Measurement of fructosamine may be used as index of the average concentration of blood glucose over an extended period of time, also measuring of glycosylated hemoglobin provides the clinician with a time-averaged picture of the patient's blood glucose concentration over the past 3 monthes.

Measurement of fructosamine and HbA1c are important for good control of blood glucose concentrations, and to avoid progression of diabetic complications.

1.3 Objectives:

1.3.1 General objective:

To estimate the level of fructosamine and HbA1c in type 1 and type 2 diabetes mellitus patients in El-obied city.

1.3.2 Specific objective:

1- To measure the level of fructosamine and HbA1c in study groups.

2- To compare between the mean of fructosamine and HbA1c in patients and control.

3- To correlate between fructosamine concentration and HbA1c in type 1 and type 2 DM patients.

4- To correlate between fructosamine concentration, age, and duration of diseasein type 1 and type 2 DM patients.

Chapter two

Literature review

2. Literature review

2.1 Diabetes mellitus:

Diabetes mellitusis actually a group of metabolic diseases characterized by hyperglycemia resulting fromdefects in insulin secretion, insulin action, or both. In1979, the National Diabetes Data Group developed aclassification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into twobroad categories: type1, insulin-dependent diabetes mellitus (IDDM); and type2, non–insulin-dependent diabetes mellitus (NIDDM)(Bishopetal.,2010).

Established in 1995, the International Expert Committee on the Diagnosis and Classification of DiabetesMellitus, working under the sponsorship of the American Diabetes Association, was given the task of updating the 1979 classification system. The proposed changesincluded eliminating the older terms of IDDM andNIDDM. The categories of type 1 and type 2 were retained, with the adoption of Arabic numerals instead ofRoman numerals(Bishop et al.,2010).

2.1.1 Classification of diabetes mellitus:

2.1.1.1 Type 1 diabetes mellitus:

Type 1 DM is an auto-immune disease. There is a familial incidence, though to a lesser extent than with type 2 DM (the concordance rate in monozygotic twins is approximately 40%), and there is a strong association with certain histocompatibility antigens, for example HLA-DR3, DR4 and various DQ alleles. An individual′s HLA antigens are genetically determined but it is clear

that type 1 DM is a genetically heterogeneous disorder. Environmental factors are also important and there is considerable circumstantial evidence that viral antigens (e.g. Coxsackie B) may initiate the auto-immune process in some genetically susceptible individuals. Proteins in cows/ milk have also been implicated.(William *et al .,*2008).

The pancreatic islets of newly diagnosed patients with type 1 DM show characteristic histological features of auto-immune disease. Islet cell antibodies (ICA) are frequently present in the plasma (and may be detectable long before the condition presents clinically), together with antibodies to insulin and glutamic acid decarboxylase (GAD), which like, ICA, are sensitive markers of risk of progression to clinical diabetes in the apparently healthy members of patients/ families.It is thought that β-cell destruction is initiated by activated T-lymphocytes directed against antigens on the cell surface, possibly viral antigens or other antigens that normally are either not expressed or not recognized as non-self. Clinically overt type 1 DM is thought to be a late stage of a process of gradual destruction of islet cells, and there is much interest in the possibility that it may be possible to modify this process in susceptible individuals and prevent, or at least retard, the development of clinical diabetes.(William et al 2008).

2.1.1.2 Type 2 diabetes mellitus :

The exact pathogenesis of type 2 DM is uncertain. It is undoubtedly a heterogeneous disease. In established cases, β-cell dysfunction with an adequate insulin response to hyperglycemia and insulin resistance usually coexist but it not clear which is the primary defect: hyperglycemia itself causes insulin resistance and β-cell dysfunction (glucotoxicity); so, too, does hyperlipidaemia (lipotoxicity), which is frequently present in diabetes. Immune mechanisms are thought to contribute to the loss of insulin secretion in approximately 10% of patients.(

Type 2 DM show a strong familial incidence. The concordance rate in monozygotic (identical) twins is >90% and the risk of an individual developing diabetes is >50% if both parents have the condition. Several single gene defects have been identified in specific subsets of patients with type 2 DM, notably in the dominantly inherited forms that typically develop in the young (MODY, maturity-onset diabetes of the young).

The commonest mutations responsible for MODY are in the glucokinase gene (MODY type 2: six types of MODY have been described, each due to a different mutation). Glucokinase is the rate limiting enzyme og glucose metabolism on pancreatic β-cells and through acting as a / glucose sensor/ is key to the regulation of pancreatic insulin secretion. Such specific mutations are, however, are in type 2 DM considered overall, where the tendency to develop diabetes is polygenic and there is no clear pattern of inheritance. Environmental factors are also important. Many patients with type 2 DM are obese, particularly tending to have visceral (intra-abdominal), obesity, which is known to cause insulin resistance, and have other features of the metabolic syndrome. Reduced physical

activity also causes insulin resistance, and various drugs, including corticosteroids, thiazides in high doses and some β-adrenergic antagonists, are diabetogenic.

The interaction between genetic and environmental factors in the pathogenesis of type 2 DM is exemplified by the high prevalence of the condition in certain ethnic group (e.g. Pacific islanders) following the adoption of a westernized lifestyle, with good public health facilities and ready access to an assured food supply, in comparison with the prevalence in their aboriginal state. The suggestion is that their genotype evolved to maximize the storage of ingested energy as fat, to provide protection against famine, but that a continuous food supply leads to obesity and insulin intolerance (the thrifty genotype hypothesis). There is also a thrifty phenotype hypothesis, based on the observation that low birth-weight ia associated with an increased risk of later development of type 2 DM, the putative mechanism being β-cell dysfunction induced by fetal malnutrition.

Type 2 DM is a progressive condition. Although there is evidence that it can be prevented in susceptible individuals by diet and exercise, by the time it presents clinically it will often have been present for several years. Aggressive treatment may show its progression, but the tendency is for contributing loss β-cell function and increasing insulin deficiency (William et al.,2008).

2.1.1.3 Gestational Diabetes Mellitus:

Gestational Diabetes Mellitus (GDM) is carbohydrate intolerance of variable severity with onset or first recognition during pregnancy(Metzger, et al.,1998). Note that women with diabetes who become pregnant are not included in this

category. Estimates of the frequency of abnormal glucose tolerance during pregnancy range from 1% to 14%, depending on the population studied and the diagnostic tests employed. In the United States, GDM occurs in 6% to 8% of pregnancies. Women with GDM are at a significantly increased risk of subsequent diabetes, predominantly type 2. The cumulative incidence of type 2 diabetes after GDM varies among populations, ranging from -40% to 70%. The annual incidence is markedly increased above that in the general population and rises during the first 5 years, reaching a plateau after 10 years. At 6 to 12 weeks postpartum, all patients who had GDM should be evaluated for diabetes and, if diabetes is not present, be reevaluated for diabetes at least every 3 years.(Carl et al., 2006).

2.1.1.4 Other Specific Types of Diabetes Mellitus:

This subclass includes patients in whom hyperglycemia is due to a specific underlying disorder, such as genetic defects of beta cell function; genetic defects in insulin action; disease of the exocrine pancreas; endocrinopathies (e.g., Cushing disease, acromegaly, and glucagonoma); the administration of hormones or drugs known to induce beta-cell dysfunction (e.g., dilantin and pentamidine) or impair insulin action (e.g., glucocorticoids, thiazides, and beta-adrenergics); infections; uncommon forms of immune-mediated diabetes; or other genetic conditions (e.g., Down syndrome, Klinefelter syndrome, and porphyria). This was formerly termed secondary diabetes(Carl et al.,2006).

2.1.2 Metabolic complications of diabetes:

2.1.2.1 Acute complication:

A- Keto-acidosis :

Ketoacidosis may be the presenting feature of type 1 DM, or may develop in patient known tobe diabetic who omits to take his insulin or whose insulin dosage becomes inadequate because of an increased requirement, for example as a result of infection, any acute illness such as myocardial infarction, trauma or emotional disturbance. Newly diagnosed patients account for 20-25% of cases. It is a rare occurrence in patients with type 2 DM.(William et al ,.2008).

B- Non-ketotic hyperglycemia:

Not all patients with uncontrolled diabetes develop ketoacidosis. In type 2 DM, severe hyperglycemia can develop (blood glucose concentration >50 mmol/L) with extreme dehydration and a very high plasma osmolality, but with no ketosis and minimal acidosis. This complication is often referred to as hyperosmolar non-ketotic hyperglycemia, but patients with ketoacidosis usually have increased plasma osmolality, although not to the same extent.

C- Lactic acidosis:

Lactic acidosis is an uncommon complication of diabetes. It was formerly chiefly seen in patients treated with phenformin, a biguanide oral hypoglycemic drug, but is now more usually associated with severe systemic illness, for example severe shock and pancreatitis.(William .,2008).

D- Hypoglycemia :

Hypoglycemia can complicate treatment in both type 1 and type 2 DM (William et al.,2008).

2.1.2.2 Chronic complication of diabetes:

Although it had been theorized for many years that better glycemic control would decrease rates of long-term complications of diabetes mellitus, it was not until the publication of the DCCT in 1993(DCCT, 1993).

The absolute risks of retinopathy and nephropathy were proportional to the mean GHb. Intensive therapy also reduced the development of hypercholesterolemia. This landmark study has had a significant impact on therapeutic goals and comprehension of the pathogenesis of complications of diabetes. At the conclusion of the DCCT, 95% of the participants entered the long-term follow-up study, termed the Epidemiology of Diabetes Interventions and Complications (EDIC). Five years after the end of the DCCT, there was no difference in metabolic control (assessed by GHb measurements) between the former conventional and intensively treated groups. Nevertheless, the further progression of retinopathy was -70% lower in the former intensive group, demonstrating that the beneficial effects of intensive treatment persisted for at least several years beyond the period of strictest intervention. Subsequent studies indicate that intensive therapy significantly reduces the risk of cardiovascular disease (myocardial infarction and stroke)(Carl et al., 2006).

The role of hyperglycemia in the development of complications in individuals with type 2 diabetes was established in the UKPDS(UK,prospective diabetes study group,1998). Although intensive treatment decreased the rate of

occurrence of macrovascular (large blood vessel) complications, the reduction was not statistically significant. An important caveat of both the DCCT and UKPDS was that intensive therapy produced a three-fold increase in the incidence of severe hypoglycemia (Carl et al.,2006).

2.1.3 Glycemic controls:

2.1.3.1 Fructosamine:

Fructosamine is the generic name for plasma protein ketamines (Goldstien et al.,2004,.Sacks et al,. 1992). The name refers to the structure of the ketoamine rearrangement product formed by the interaction of glucose with the E-amino group on lysine residues of albumin. Like measurements of GHb, measurements of fructosamine may be used as an index of the average concentration of blood glucose over an extended (but shorter) period of time. Because all glycated serum proteins are huctosamincs and albumin is the most abundant serum protein, measurement of fructosamine is thought to be largely a measure of glycated albumin, but this has been questioned by some investigators.(Carl et al.,2006).

2.1.3.2 Glycosylated Hemoglobin/Hemoglobin A1c:

The aim of diabetic management is to maintain the blood glucose concentration within or near the non-diabetic range with a minimal number of fluctuations. Serum or plasma glucose concentrations can be measured by laboratories in addition to patient self-monitoring of whole blood glucose concentrations. Long-term blood glucose regulation can be followed by measurement of glycosylated hemoglobin.(Bishop et al., 2010).

Glycosylated hemoglobin is the term used to describe the formation of a hemoglobin compound produced when glucose (a reducing sugar) reacts with the amino group of hemoglobin (a protein). The glucose molecule attaches non enzymatically to the hemoglobin molecule to form a ketoamine. The rate of formation is directly proportional to the plasma glucose concentrations. Because the average red blood cell lives approximately 120 days, the glycosylated hemoglobin level at any one time reflects the average blood glucose level over the previous 2 to3 months. Therefore, measuring the glycosylated hemoglobin provides the clinician with a time-averaged picture of the patient‘s blood glucose concentration over the past3 months.(Bishop et al., 2010).

Hemoglobin A1c (HbA1c), the most commonly detected glycosylated hemoglobin, is a glucose molecule attached to one or both N-terminal valines of the β-polypeptide chains of normal adult hemoglobin. HbA1c is a more reliable method of monitoring long-term diabetes control than random plasma glucose. Normal values range from 4.5 to 8.0. Using a linear regression model, Rohlfing et al(Rohlfing et al,2002). determined that for every 1% change in the HbA1c value, there is a 35 mg/dL (2 mmol/L) change in the mean plasma glucose. However, this information needs to be used carefully, as a recent study has

shown that the relationship between average plasma glucose and HbA1c can differ substantially depending on the glycemic control of the population studied(Kilpatrick et al., 2007).

It is also important to remember that two factors determine the glycosylated hemoglobin levels: the average glucose concentration and the red blood cell life span. If the red blood cell life span is decreased because of another disease state such as hemoglobinopathies, the hemoglobin will have less time to become glycosylated and the glycosylated hemoglobin level will be lower.Current ADA guidelines recommend that an HbA1c test be performed at least two times a year with patients who are meeting treatment goals and who have stable glycemic control. For patients whose therapy has changed or who are not meeting glycemic goals, a quarterly HbA1c test quarterly is recommend. The use of point-of-care testing for HbA1c allows for more timely decisions on therapy changes and has been shown to result in tighter glycemic control.

Lowering HbA1c to an average of less than 7% has clearly been shown to reduce the microvascular, retinopathic, and neuropathic complications of diabetes. Therefore, the HbA1c goal for non-pregnant adults in general is less than 7%. Further studies have shown a small benefit to lowering HbA1c to less than 6%, making this a goal for selected individual patients if possible without significant hypoglycemia.(Bishop et al.,2010).

Chapter three

Materials and methods

3. Materials and methods:

3.1 Materials:

3.1.1 study approach:

A quantitative method was used to estimate fructosamine concentration and HbA1c in type 1 and type 2 diabetic patients during the period from February to May 2016.

3.1.2 Study design:

This is case control study.

3.1.3 Study area:

The study was conducted in El-obied city, the capital of Northern Kordofan state.

3.1.4 Study population:

The study included patients with diabetes mellitus type1 and type 2.

3.1.5 Sample size:

The study included one hundred and fifty volunteered to participate in this study, 50 type 1, 50 type 2, and 50 control group healthy subjects without any diseases.

3.1.6 Inclusion criteria:

Sudanese patients with type 1 and type 2 diabetes mellitus, and healthy volunteer were included in this study.

3.1.7 Exclusion criteria:

The criteria of exclusion based on excluding any patient with hemolytic diseases,auto-immune disease, and liver diseases.

3.1.8 Ethical consideration:

Consent was taken regarding acceptance to participate in the study and re-assurance of confidentiality. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

3.1.9 Data collection:

Data were collected using a structural interviewing questionnaire, which was designed to collect and maintain all valuable information concering each case examined.

3.1.10 sample collection and processing:

About 5 ml of venous blood were collected from each participant (both case and control). The sample collected under aseptic conditions and 2.5 ml placed in sterile EDTA containers for HbA1c, and 2.5 ml in sterile plain containers, the plain containers centrifuged for 5 minutes at 3000 RPM to obtain serum for fructosamine, then the obtained sample were kept at 2-8 C^0 until the time of analysis.

3.2 Methods :

3.2.1. Estimation of fructosamine by using NTB mehod:

3.2.1.1 Principle of method:

Serum glycated protein reduce nitroblue tetrazolium (NBT) salts in alkaline medium. The rate of formazan formation at a given temperature is proportional to the serum concentration of glycated proteins.(Baker et al,.1985).

(Appendix II).

3.2.2 Estimation of HbA1c by Ion exchange chromatography method:

3.2.2.1 Prinpciple of method:

After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Hemoglobin A1c (HbA1c) is specifically eluted after washing away the hemoglobin A_{1c+b} fractions (HbA_{1c+b}), and is quantified by direct photometric reading at 415 nm. The estimation of the relative concentration of HbA_{1c} is made by the measure of total hemoglobin concentration by direct photometric reading at 415nm.(Bisse. et al,. 1985).

(Appendix III)

3.3 Quality control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of test and control samples.

3.4 Statistical analysis:

Data obtained from this study was analyzed using statistical package for the social science (SPSS).

Chapter four

Results

4. Results:

The result of the biochemical determinant of serum fructosamine and HbA1c in patients with type 1 and type 2 diabetes mellitus are given in tables and figures:

Table (4-1): Illustrate the mean of fructosamine concentration in type 1 DM, type 2 patients and control group.

The mean of fructosamine was significantly increased in type 1DM and type 2 patients compared with control group (P.Value =0.000).

Mean ±SD for cases versus controls:

(8.19±4.31 versus 2.38 ± 0.41) mg/dl for type 1 DM.

(5.74 ± 2.04 versus 2.38±0.41) % for type 2 DM.

Table (4-2): Show the mean concentration of HbA1c in patients and control.

The mean of HbA1c was significantly increased in type1 and type 2 DM compared with control group (P.Value =0.000).

Mean ±SD for cases versus controls:

(9.62± 2.9 versus 4.69 ± 0.64) mg/dl for type 1 DM.

(7.82± 1.6 versus 4.69 ±0.64)% for type 2 DM.

Figure (4-1):Show correlation between fructosamine concentration and HbA1c.

The scatter showed there was a significant positive correlation between HbA1c and fructosamine concentration in type 1 DM. (r =0.341,P.Value = 0.015).

Figure (4-2): Correlation between fructosamine concentration and HbA1c in type 2 DM.

The scatter showed there was a significant positive correlation between HbA1c and fructosamine in type 2 DM . (r = 0.457,P.Value = 0.001).

Figure (4-3): Correlation between fructosamine concentration and duration of type 1 DM.

The scatter showed there was a negative correlation between fructosamine concentration and duration of disease of type 1 DM. (r=- 0.235 , P.value = 0.384).

Figure (4-4):Correlation between fructosamine concentration and duration of type 2 DM.

The scatter showed there was no correlation between fructosamine concentration and duration of type 2 DM. (r= - 0.138,P.Value=0.371).

Table (4-1): Comparison of mean concentration of fructosamine in patients and control group.

variable	Group	mean±SD	P.Value
Fructosamine (mg/dl)	Control N=50	2.38±0.4	0.000
	Type 1 DM N=50	8.19±4.31	0.000
	Type 2 DM N=50	5.74±2.04	0.000

Results given in mean ± SD.

P.Value ≥ 0.05 consider significant.

Table (4-2): Comparison of mean of HbA1c in patients and control group.

variable	Group	mean±SD	P.Value
HbA1c(%)	Control N=50	4.69±0.64	0.000
	Type 1 DM N=50	9.62±2.9	0.000
	Type 2 DM N=50	7.82±1.6	0.000

Results given in mean ± SD.

P.Value ≥ 0.05 consider significant.

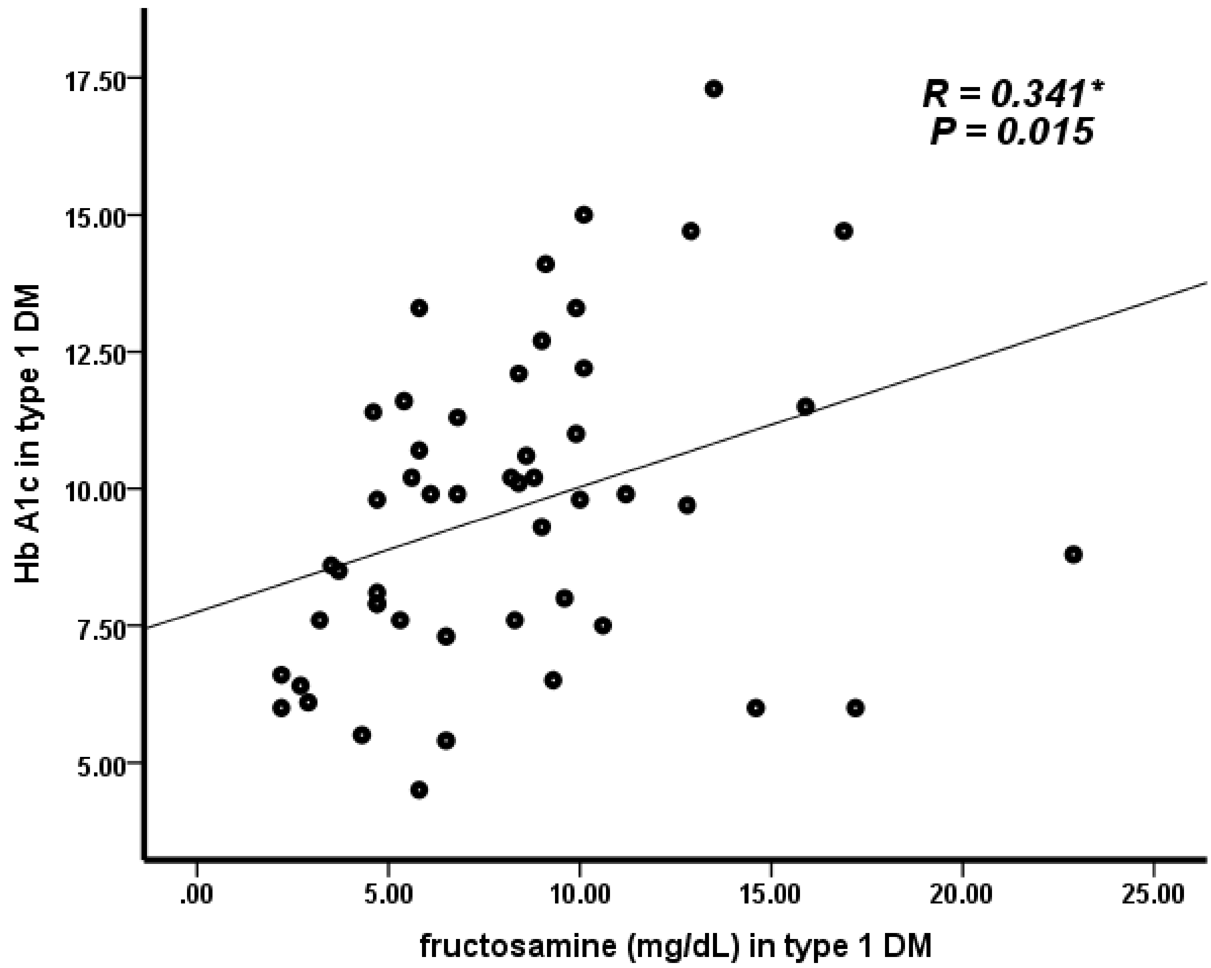


Figure (4-1): Correlation between fructosaimne and Hb A1c in type 1 DM.

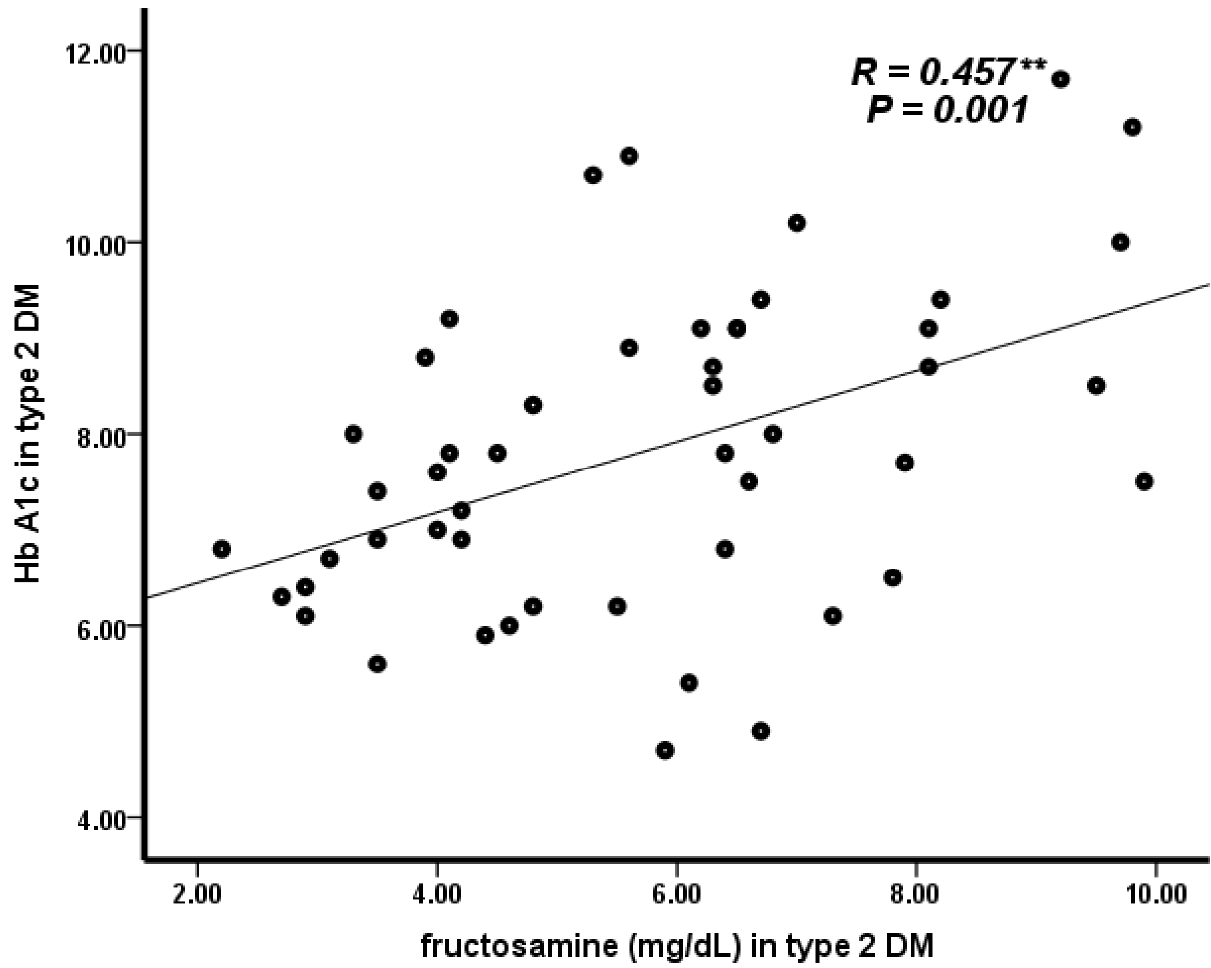
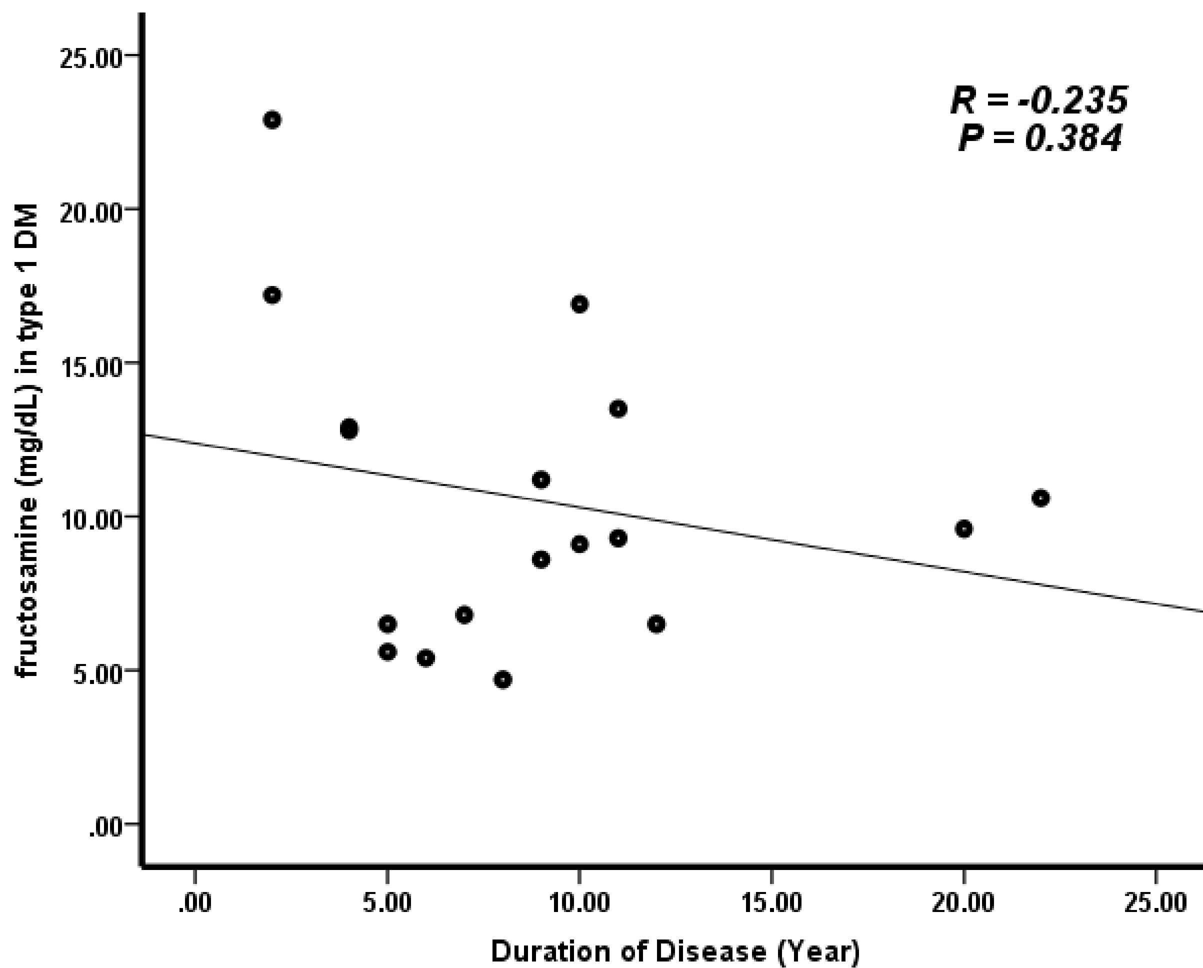


Figure (4-2): Correlation between fructosamine and Hb A1c in type 2 DM.



Figure(4-3): Correlation between fructosamine and duration of disease (year) in type 1 DM.

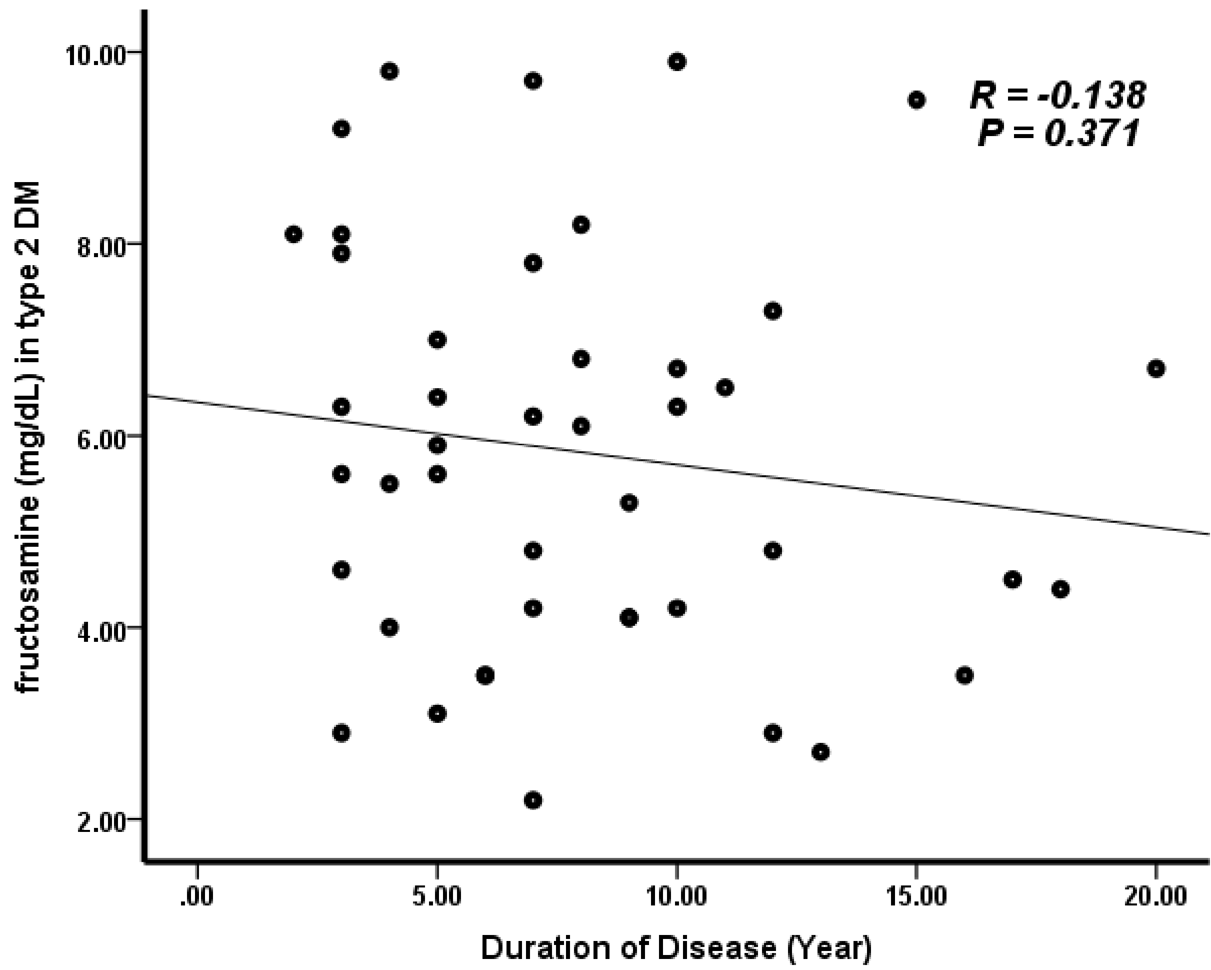


Figure (4-4): Correlation between fructosamine and duration of disease (year) in type 2 DM..

Chapter five

Discussion, conclusion and recommendations

5.1 Discussion

Fructosamine, glycosylated albumin, have been drawing attention for use in populations whose HbA1c levels may be difficult to interpret, such as those with anemia, hemolysis, or renal disease.

Serum fructosamine, nonenzymatic glycosylated substance in the blood, reflects the mean glucose levels over the recent two to three weeks. It can be obtained very quickly and is inexpensive to perform.

From the finding of this study it appears that serum fructosamine concentration and HbA1c were significantly increased at (P.Value 0.000, P.Value 0.000) respectively in type 1and type 2 DM patients group compared to control group. This results agreed with another results of study carried by (Santiago et al., 2012), showed a significantly increased in serum fructosamine and HbA1c in patints compared to control group.

The finding of this study showed there was a significant positive correlation between fructosamine and HbA1c for type 1 DM (r = 0.381,P.Value =0.015), the serum fructosamine was directly proportional to HbA1c. This result agree with result of study done by (Robert et al., 2003), showed a significantly correlation of fructosamine and HbA1c in type 1 DM.

The finding of this study showed there was a significant positive correlation between fructosamine and HbA1c (r= 0.457,P.Value 0.001) for type 2 DM. This agreed with study carried by (Santiago et al., 2012), showed significant correlation between fructosamine and HbA1c in type 2 DM.

The finding of this study showed there was a negative correlation between fructosamine concentration and duration of disease of type 1DM (r = -0.235,P.Value = 0.384) .

The finding of this study showed there were no correlation between fructosamine concentration and duration of type 2 DM.(r = - 0.138,P.Value =0.371) , this result agreed with result carried by (Van Wersch et al., 1991).

5.2 Conclusion

According to the results of this study it is concluded that:

1- Serum fructosamine and HbA1c were increased in type 1 and type 2 DM.

2- Fructosamine positively correlated to HbA1c, negatively correlated with age and duration of type 1 DM, and not correlated with age and duration of type 2 DM.

5.3 Recommendations

It is recommended that:

- HbA1c should be measured in diabetic patients at least every 3 monthes.
- Fructosamine should be done as routine investigation in the laboratories of diabetic centers.

References

References

Baker R J, Metcalf A P, Johnson N R, Newman D and Rietz P.(1985). Use of protein-based standards in automated colorimetric determinations of fructosamine in serum. Clin Chem; 31: 1550-1554.

Bisse E and Abraham EC.(1985). New less temperature-sensitive microchromatographic method for the separation and quantitation of glycosylated hemoglobins using a non-cynide buffersystem. J Chromatog; 344:81-91.

Boden G, Master RW, Gordon SS, Shuman CR, Owen OE.(1980). Monitoring metabolic control in diabetic out patients with glycosylated hemoglobin. Ann Intern Med;92:357-60.

Carl A, Bart's Edward R , Ashood R , David E.Bruns,(2006). Tietz fundamental of clinical chemistry. 6 th edition:397-398.

Chen HS, Wu TE, Lin HD, Jap TS, Hsiao LC, Lee SH,(2010). Hemoglobin A(1c) and fructosamine for assessing glycemic control in diabetic patients with CKD stages 3 and 4. Am J Kidney Dis ;55:867-74.

Diabetes control and complication trial.(1993). The effect if intensive treatment of diabetes on the development and progression of long-term complications in insulin- dependent diabetes mellitus. NEJM ;329:977-86.

Dong Soo Kang,and Jiyun Park.(2015). Clinical usefulness of the measurement of serum fructosamine in childhood diabetes mellitus. Ann pediatr Endocrinol Metab;20:21-26.

Eckfeldt JH, and Bruns DE.(1997). Another step towards standardization of methods for measuring hemoglobin A1c. clin Chem;43:1811-1813.

Elbagir M,and Eltom MA.(1996). A population based study on prevalence of diabetes in northern sudan. Diabetes care;24:1126-8.

Goldstien DE, Little RR, LorenzRA, Malone JI , Narhan D, Peterson CM (2004). Test of glycemia in diabetes. Diabetes Care; 27:1761-73.

Kilpatrick ES, Rigby AS, Atkin SL.(2007). Variability in the relationship mean plasma glucose and HbA1c . implications for the assessment of glycemic control. Clin Chem;53:897-901.

Metzger BE,and Coustan DR.(1998). Summary and recommendations of the fourth international workshop - conference on Gestational Diabetes Mellitus. Diabetes care;21:13161-7.

Micheal L. Bishop, Edward P. Fady , Larry ES.(2010). Clinical chemistry principles, procedures, correlations, fifth edition, Lippincott Williams and Wikins ed:324- 325.

Robert M,Cohen, Thomas C. Ehener,Yeracy R.Holmes, Clinton H.J.(2003). Discordance between HbA1c and fructosamine – evidence for a glycosylation gap and its relation to diabetic nephropathy. Diabetes Care;26:163-167.

Rohlfing CC, Wied meyer HM, Little RR.(2002). Defining the relationship between plasma glucose and HbA1c. Diabetes Care;25:275-278.

Sacks DB, Bruns DE, Goldstien DE, Maclaren NK, MC Donald JM, Parott M. (1992). Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem;48:436-72.

Shafi T, Sozio SM, Plantinga LC, Jaar BG, Kim ET, Parekh RS.(2013). Serum fructosamine and glycated albumin and risk of mortality and clinical outcomes in hemodialysis patients. Diabetes Care ;36:1522-33.

Santiago R, Segade J R, Jose M G, Felipe FC, Feleix C.(2012). Estimation of the glycation gap in diabetic patients with stable glycemic control. Diabetes Care;35:2447-2450.

U.K. Prospective Diabetes Study (UKPDS) Group.(1998). Intensive blood- glucose control with sulphonylareas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes . Lancet;352:837- 53.

Van wersch JW.(1991). Hba1c and serum fructosamine in diabetic patients: relationship of age, collting and fibrinolysis parameters and urinary microalbumin excretion. Clin Chim ;210:99-104.

William J Marchall, Stephen K, Bangert A.(2008). Clinical chemistry, sixth edition,ed 209.

Appendices

Appendix I

Questionnaire

Sudan University of science and Technology

College of graduate studies

Estimation of fructosamine and HbA1c in type 1 and type 2 DM in El-Obied city

Number (   )

A. General information:-

1- Name :…………………………   2- Age:……………

B-Gender : ……………………….

C- Duration of disease:(in years)…………………….

D- Type of DM:………………………………………

E- History of other disease:

1- Hypertention ( )

2- Renal disease ( )

3- Heart disease ( )

4- Other ( )

G- Investigation:-

1- S.fructosamine : ……. Mg/dl :……..mmol/l

2- HbA1c :………%

COD 11046 2 x 50 mL
STORE AT 2-8°C
Reagents for measurement of fructosamine concentration Only for *in vitro* use in the clinical laboratory

Appendix II

FRUCTOSAMINE

CE

BioSystems
REAGENTS & INSTRUMENTS

FRUCTOSAMINE
NBT

PRINCIPLE OF THE METHOD

Serum glycated proteins reduce nitroblue tetrazolium (NBT) salts in alkaline medium. The rate of formazan formation at a given temperature is proportional to the serum concentration of glycated proteins[1].

CONTENTS AND COMPOSITION

A. Reagent. 2 x 50 mL. NBT 0.25 mmol/L, carbonate buffer 0.2 mol/L, pH 10.35.

S. Fructosamine Standard. 2 for 1 mL. Human serum. Concentration is given on the label expressed in mmol/L of DMF (deoximorpholi-nofructose) and in µmol/L of glycated albumin.

Human serum used in the preparation of the standard has been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the standard should be handled cautiously as potentially infectious.

STORAGE

Store at 2-8°C. Keep Reagent in the darkness.

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

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance over 0.065 at 530 nm (1 cm cuvette).
- Standard: Absence of lyophilised material. Presence of moisture.

REAGENT PREPARATION

Reagent (A) is provided ready to use.

Standard (S): Reconstitute with 1.00 mL of distilled water. Mix gently and let stand for 30 minutes before using. The solution is stable for 15 days at 2-8°C if contaminations are prevented during their use, or for 45 days at -20°C when frozen in aliquots.

ADDITIONAL EQUIPMENT

- Analyzer, spectrophotometer or photometer with cell holder thermostatable at 37°C and able to read at 530 ± 20 nm.

SAMPLES

Serum collected by standard procedures. Hemolysed samples are not suitable for testing.

Fructosamine in serum is stable for 7days at 2-8°C.

PROCEDURE

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

	Sample	Standard
Reagent (A)	1.0 mL	1.0 mL
Sample	50 µL	—
Standard (S)	—	50 µL

3. Mix thoroughly and incubate immediately at 37°C. Start the stopwatch.
4. Read the absorbance (A) of the Sample and Standard at 530 nm after exactly 10 minutes (A_1) and 15 minutes (A_2) of incubation against distilled water.

CALCULATIONS

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

$$\frac{(A2 - A1)_{Sample}}{(A2 - A1)_{Standard}} \times C_{Standard} = C_{Sample}$$

REFERENCE VALUES

Serum[1,2]: 1.9-2.9 mmol/L (DMF), 205-285 µmol/L (glycated albumin). Concentrations are slightly lower (5%) in child. Fructosamine reference values depends on albumin concentration. Plasmas give lower results than serum[4].

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

QUALITY CONTROL

It is recommended to use the Fructosamine Control Serum (cod. 18057) to verify the performance of the measurement procedure. Concentration is given on the label, expressed in mmol/L of DMF (deoximorpholi-nofructose) and in µmol/L of glycated albumin.

Components from human origin have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for Hbs antigen. However, they should be handled cautiously as potentially infectious.

Reconstitute the serum with the volume of distilled water indicated in the label. The solution is stable for 20 days at 2-8°C if contaminations are prevented during their use, or for 3 months at -20°C when frozen in aliquots. Treat the Control in the analytical procedure as patient samples.

The intervals of suggested acceptable values have been calculated from previous experience in interlaboratory variability and are given for orientation only; each laboratory should establish its own precision parameters.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.14 mmol/L (DMF), 16 µmol/L (glycated albumin).
- Linearity limit: 7 mmol/L (DMF), 800 µmol/L (glycated albumin). For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatability (within run):

Mean Concentration	CV	n
3.9 mmol/L = 446 µmol/L	2.7 %	20
5.7 mmol/L = 651 µmol/L	2.5 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
3.9 mmol/L = 446 µmol/L	4.3 %	25
5.7 mmol/L = 651 µmol/L	4.0 %	25

- Sensitivity: 21.2 mA·L/mmol (DMF), 0.17mA·L/µmol (glycated albumin).
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (10 g/L), bilirubin (20 mg/dL) and lipemia (triglycerides 10 g/L) do not interfere. Other drugs and substances may interfere[5].

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

DIAGNOSTIC CHARACTERISTICS

Fructosamine is the generic name for plasma protein ketoamines formed by non-enzymatic attachment of glucose to amino groups of proteins (largely albumin).

The measurement of fructosamine is useful to monitor the average concentration of blood glucose for an extended period of time (2-3 weeks) in individuals with diabetes mellitus. Because fructosamine determination monitors short-term glycemic changes different from glycated hemoglobin, it is recommended to be used in conjuction with glycated hemoglobin rather than instead of it[2].

Glycated protein levels are a valuable adjunt to blood glucose determinations in the assessment of glycemic control. However, these proteins are not reliable for the diagnosis of diabetes mellitus[2,6].

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

NOTES

1. The reagent may be used in several automated analyzers. Instructions for many of them are available on request.

BIBLIOGRAPHY

1. Baker R John, Metcalf A Patricia, Johnson N Roger, Newman David and Rietz Peter. Use of protein-based standards in automated colorimetric determinations of fructosamine in serum. Clin Chem 1985; 31: 1550-1554.
2. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
3. Van Dieijen-Visser MP, Seynaeve C and Brombacher PJ. Influence of variations in albumin or total-protein concentration on serum fructosamine concentration. Clin Chem 1986; 32: 1610.
4. Hurst L Paul. Effect of anticoagulants on fructosamine determination. Clin Chem 1987; 33: 1947.
5. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.

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Appendix III

HEMOGLOBIN A1C

COD 11044 20 tests	COD 11045 100 tests
STORE AT 15-30ºC	
Reagents for measurement of hemoglobin A_{1c} concentration Only for *in vitro* use in the clinical laboratory	

HEMOGLOBIN A1C

Chromatographic - spectrophotometric
ION EXCHANGE

PRINCIPLE OF THE METHOD

After preparing the hemolysate, where the labile fraction is eliminated, hemoglobins are retained by a cationic exchange resin. Hemoglobin A_{1c} (HbA_{1c}) is specifically eluted after washing away the hemoglobin A_{1a+b} fraction[1] (HbA_{1a+b}), and is quantified by direct photometric reading at 415 nm. The estimation of the relative concentration of HbA_{1c} is made by the measure of total hemoglobin concentration by direct photometric reading at 415 nm.

CONTENTS

	COD 11044	COD 11045
1. Reagent	1 x 30 mL	1 x 30 mL
2. Reagent	1 x 50 mL	1 x 240 mL
3. Reagent	1 x 450 mL	4 x 450 mL
4. Microcolumns	1 x 20	1 x 100

COMPOSITION

1. **Reagent.** Potassium phtalate 50 mmol/L, detergent 5 g/L, pH 5.0, sodium azide 0.95 g/L.
2. **Reagent.** Phosphate buffer 30 mmol/L, pH 6.5, sodium azide 0.95 g/L.
3. **Reagent.** Phosphate buffer 72 mmol/L, pH 6.5, sodium azide 0.95 g/L.
4. **Microcolumns.** Contain a pre-weighted amount of resin equilibrated with phosphate buffer 72 mmol/L, pH 6.5, sodium azide 0.95 g/L.

Use only microcolumns (4) and reagents 2 and 3 of the same kit.

STORAGE

Store at 15-30ºC.

Reagents are stable until the expiry date shown on the label when stored tightly closed and if contamination is prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity.
- Microcolumns (4): Absence of buffer over the resin bed.

ADDITIONAL EQUIPMENT

- Spectrophotometer or photometer with a 415 nm filter (405-425)

SAMPLES

Whole blood collected by standard procedures.

Hemoglobin A_{1c} is stable for 7 days at 2-8ºC. Heparin or EDTA may be used as anticoagulants.

PROCEDURE

Hemolysate Preparation and Labile Fraction Elimination

1. Bring the columns and reagents to room temperature (21-26ºC) (Note 1).
2. Pipette into a test tube:

Blood	50 µL
Reagent 1	200 µL

3. Shake thoroughly and let it stand at room temperature for 10-15 minutes. This hemolysate will be used in steps 6 and 11.

Column Preparation (Notes 2 and 3)

4. Remove the upper cap of the column and then snap the tip off the bottom.
5. Using the flat end of a pipette, push the upper disc down to the resin surface taking care not to compress it. Let the column drain completely to waste.

Separation and Reading of HbA_{1c} fraction

6. Carefully pipette on the upper filter:

Hemolysate	50 µL	Let the column drain to waste

7. In order to drain any sample residue left above the upper disc, pipette:

Reagent 2	200 µL	Let the column drain to waste

8. Pipette:

Reagent 2	2.0 mL	Let the column drain to waste

9. Place the column over a test tube and add:

Reagent 3	4.0 mL	Collect the eluate (HbA_{1c} Fraction)

10. Shake thoroughly and read the absorbance (A) of the HbA_{1c} fraction at 415 nm against distilled water (A_{HbA1c}). The absorbance is stable for at least one hour.

Reading of Hb_{TOTAL}

11. Pipette into a test tube:

Reagent 3	12.0 mL
Hemolysate	50 µL

12. Shake thoroughly and read the absorbance (A) at 415 nm against distilled water ($A_{HbTOTAL}$). The absorbance is stable for at least one hour.

CALCULATIONS

The HbA_{1c} relative concentration in the sample is calculated using the following general formula:

$$\frac{A_{HbA1c} \times V_{HbA1c}}{A_{HbTOTAL} \times V_{HbTOTAL}} \times 100 = \%HbA_{1c}$$

The volume of HbA_{1c} (V_{HbA1c}) is 4 mL, the volume of Hb total ($V_{HbTOTAL}$) is 12 mL. The following formula is deduced for the calculation of the concentration:

$$\frac{A_{HbA1c}}{A_{HbTOTAL}} \times \frac{100}{3} = \%HbA_{1c}$$

The results obtained with the present method are equivalent to a US National Glycohemoglobin Standardization Program certified method (NGSP) and can be converted into equivalent to the International Federation of Clinical Chemistry standardized method (IFCC), using the internationally recommended master equation[2,3]:

HbA_{1c}-IFCC (mmol/mol) = 10.93 x HbA_{1c}-NGSP-DCCT (%) – 23.5

REFERENCE VALUES

The following cut-off points have been established by the Diabetes Control and Complications Trial Research Group (DCCT) and have been adopted by many countries for a reference population (Non diabetic) and for the evaluation of the degree blood glucose control in diabetic patients[4,5].

NGSP-DCCT (%)	IFCC (mmol/mol)	Reference values / Degree of control
4.0 - 6.5	20 - 48	Non Diabetic
6.0 - 7.0	42 - 53	Goal
7.0 - 8.0	53 - 64	Good Control
> 8.0	> 64	Action suggested

QUALITY CONTROL

It is recommended to use the Hemoglobin A_{1c} Controls, Normal (cod. 18001) and Elevated (cod. 18002), 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: Lower than 4.0 % = 20 mmol/mol.
- Linearity limit: At least 17.0 % = 162 mmol/mol.
- Repeatibility (within run):

Mean Concentration	CV	n
7.2 % = 55 mmol/mol	5.4 %	25
9.9 % = 85 mmol/mol	6.3 %	25

- Reproducibility (run to run):

Mean Concentration	CV	n
7.2 % = 55 mmol/mol	7.3 %	25
9.9 % = 85 mmol/mol	5.9 %	25

- Trueness: Results obtained with this method did not show systematic differences when compared with reference methods. Details of the comparison experiments are available on request.
- Interferences: Bilirubin (20 mg/dL) and lipemia (triglycerides 10 g/L) do not interfere. Some drugs and other substances may interfere[6].

  In the ionic exchange chromatographic methods, the presence of hemoglobin C or S in the sample may slightly alter results, but differences are not clinically significant[7]. Other hemoglobin variants like HbE, HbF, carbamyl-Hb and acetyl-Hb can interfere[7,8]. The incubation with Reagent (1) eliminates the interference due to HbA_{1c}-labile.

  In hemolytic anemia, iron deficiency anemia and transfusion, the average age of erythrocytes is altered. Caution should be used when interpreting the HbA_{1c} results from patients with these conditions.

DIAGNOSTIC CHARACTERISTICS

HbA_{1c} is the product of the irreversible condensation of glucose with the N-terminal residue of the β-chain of hemoglobin A.

The HbA_{1c} concentration in blood is directly proportional to the mean concentration of glucose prevailing in the previous 6-8 weeks, equivalent to the lifetime of the erythrocytes[4], and the estimated average glucose (eAG) during this period can be calculated with the formulas below[9].

$$eAG\ (mg/dL) = 28.7 \times HbA_{1c}\text{-NGSP-DCCT}\ (\%) - 46.7$$

$$eAG\ (mmol/L) = 1.59 \times HbA_{1c}\text{-NGSP-DCCT}\ (\%) - 2.59$$

$$eAG\ (mg/dL) = 2.64 \times HbA_{1c}\text{-IFCC}\ (mmol/mol) + 15.0$$

$$eAG\ (mmol/L) = 0.146 \times HbA_{1c}\text{-IFCC}\ (mmol/mol) + 0.843$$

HbA_{1c} levels are a valuable adjunct to glucose determinations in the assessment and follow up of individuals with diabetes mellitus, providing much more reliable information for glycemia monitoring than do determinations of glucose. Numerous studies have shown that diabetes related complications may be reduced by the long term monitoring and tight control of blood glucose levels.

The HbA_{1c} concentration may also be a useful tool in the diagnosis of diabetes[10].

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

NOTES

1. The obtained values are temperature-independent when working in the recommended interval (21-26°C). If working temperature is out of range, multiply the obtained value by the corresponding factor showed in the following table:

Working temperature	Factor
18-20ºC	1,15
27-30ºC	0.90

2. The storage of the columns may lead to an excessive packing of the resin, diminishing the flow rate and lengthening the elution. To avoid it, invert the column, do a gentle spin movement, let it stand upside down for 10 minutes, then place it back to its upright position and let the resin settle for a few minutes before of opening the column.
3. Some air bubbles may occasionally appear inside the resin bed. Their presence does not alter the test performance.

BIBLIOGRAPHY

1. Bissé E, Abraham EC. New less temperature-sensitive microchromatographic method for the separation and quantitation of glycosylated hemoglobins using a non-cyanide buffer system. *J Chromatog* 1985; 344: 81-91.
2. Hoelzel W, et al. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* 2004; 50: 166-174.
3. Hanas R, et al. 2010 Consensus statement on the worldwide standardization of the hemoglobin A_{1c} measurement. *Clin Chem Lab Med* 2010; 48: 775-776.
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
5. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 1993; 329: 977-986.
6. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
7. Roberts WL et al. Effects of hemoglobin C and S traits on eight glycohemoglobin methods. *Clin Chem* 2002: 48: 383-385.
8. Bry L, Chen PC, Sacks DB. Effects of hemoglobin variants and chemically modified derivatives on assays for glycohemoglobin. *Clin Chem* 2001; 47: 153-163.
9. Nathan DM, et al. Translating the A1C assay into estimated average glucose values. *Diabetes Care* 2008; 31: 1473-1478.
10. Nathan DM, et al. International Expert Committee report on the role of the HbA1C assay in the diagnosis of diabetes. *Diabetes care* 2009: 32: 1327-1334.

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BioSystems S.A. Costa Brava 30, 08030 Barcelona (Spain)

Quality System certified according to EN ISO 13485 and EN ISO 9001 standards

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