Estimation of Fructosamine and HbA1c Levels in Type 1 and Type 2 Diabetes Mellitus

(Study in El-Obied City)

By:

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سورة العلق الآيات 1-5

صدق الله العظيم
Dedication

Tony
Parents
Special one
Brothers
Sister
Friends
And
college
All and first thanks to the almighty ALLAH. Then I would like to express my gratitude and ever last appreciation to my supervisor Dr. Nuha Eljaili Abubaker for this guidance, helpful suggestions for solving problems, valuable supervision as well as precious advice, support continues assistance through the whole process of this research.

Also special thanks to all members of Sudan university of science and technology (SUST) Collage of graduate studies specially to staff members of clinical chemistry, i am really do not find the words that express my thanks and gratitude to him.

Finally, i am greatful to thank all patients participate in this study.
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 singinificant correlation between fructosamine and HbA1c.
مستخلص الدراسة

أجريت هذه الدراسة لمعرفة مستوى الهموميولوبين السكري والفركتوزامين في مرضى السكري النوع الأول والثاني. تم جمع 200 عينة دم من المرضى في الفترة مابين فبراير حتى أبريل 2016م، تم اختيارهم بطريقة عشوائية من مستشفى الأبيض التعليمي ومركز ود اليأس للتأمين الصحي (مركز السكري)، مع 50 عينة من الأشخاص الأصحاء كمجموعة ضابطة أو تحكم لقياس مستوى الهموميولوبين السكري والفركتوزامين في النوعين الأول والثاني لمرضى السكري.

تم قياس الهموميولوبين السكري والفركتوزامين باستخدام جهاز الإسكتروفوتوميتر وتم تحليل البيانات بواسطة برنامج الحزم الإحصائية للعلوم الاجتماعية.

توصلت النتائج إلى أن هناك ارتفاع في متوسط مستوى الهموميولوبين السكري والفركتوزامين وكان الإحتمال الإحصائي للمقارنة (0.000) و(0.000) على التوالي لمرضى السكري النوع الأول.

وكانت النتائج كالتالي:

المتوسط ± الانحراف المعياري عن المرضى مقارنة بمجموعة التحكم:

(8.19 ± 2.03 ملجرام/ديسيلتر للفركتوزامين)
(9.62 ± 0.26 ملجرام/ديسيلتر للهيوميولوبين السكري)

وأيضاً وجد أن متوسط مستوى الهموميولوبين السكري والفركتوزامين يزيد زيادة ملحوظة في مرضى النوع الثاني وكان الإحتمال الإحصائي للمقارنة (0.000) و(0.000) على التوالي. وكانت النتائج كالتالي:

المتوسط ± الانحراف المعياري عن المرضى مقارنة بمجموعة التحكم:

(5.97 ± 0.24 ملجرام/ديسيلتر للفركتوزامين)
(7.87 ± 0.26 ملجرام/ديسيلتر للهيوميولوبين السكري)
كما أوضحت الدراسة إلى أنه يوجد ارتباط إيجابي بين الهيموغلوبين السكري و الفركتوزامين في النوع الأول والثاني لمرضى السكري (معامل بيرسون = 0.415 ومستوى المعنوية 0.01) (معامل بيرسون = 0.754 ومستوى المعنوية 0.046) على التوالي.

ووجد أيضا أن هناك ارتباط سلبي بين الفركتوزامين وفترة الإصابة لمرضى السكري النوع الأول (معامل بيرسون = -0.35 ومستوى المعنوية 0.084). ولكن وجد أنه لا يوجد ارتباط بين الفركتوزامين وفترة الإصابة لمرضى السكري النوع الثاني (معامل بيرسون = -0.30 ومستوى المعنوية 0.371).

وعلى مدار الدراسة إلى أنه يوجد زيادة ملحوظة في مستوى الهيموغلوبين السكري والفركتوزامين في النوعين الأول والثاني لمرضى السكري، وأيضا هناك ارتباط قوي بين الهيموغلوبين السكري والفركتوزامين.
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<td>DMF</td>
<td>Deoxmorpholi-nofructose</td>
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<td>EDIC</td>
<td>Epidemiology of diabetes intervention and complications</td>
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<td>FA</td>
<td>Fructosamine</td>
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<td>GAD</td>
<td>Glutamic acid decarboxylase</td>
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<td>GDM</td>
<td>Gestational diabetes mellitus</td>
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<td>HbA1c</td>
<td>Hemoglobin A1c</td>
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<td>HLA-DR3</td>
<td>Human leucocyte antigen-determinant region 3</td>
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<td>HPLC</td>
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<td>ICT</td>
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<td>NBT</td>
<td>Nitro blue terazolium</td>
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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 to 3 months. Therefore, measuring the glycosylated hemoglobin provides the clinician with a time-averaged picture of the patient’s blood glucose concentration over the past 3 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 months.

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 disease in type 1 and type 2 DM patients.
Chapter two

Literature review
2. Literature review

2.1 Diabetes mellitus:

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. In 1979, the National Diabetes Data Group developed a classification and diagnosis scheme for diabetes mellitus. This scheme included dividing diabetes into two broad categories: type 1, insulin-dependent diabetes mellitus (IDDM); and type 2, non-insulin-dependent diabetes mellitus (NIDDM) (Bishop et al., 2010).

Established in 1995, the International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, working under the sponsorship of the American Diabetes Association, was given the task of updating the 1979 classification system. The proposed changes included eliminating the older terms of IDDM and NIDDM. The categories of type 1 and type 2 were retained, with the adoption of Arabic numerals instead of Roman 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 of 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 to be 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 et al., 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 fructosamins 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 to 3 months. Therefore, measuring the glycosylated hemoglobin provides the clinician with a time-averaged picture of the patient’s blood glucose concentration over the past 3 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 type 1 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 concerning 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 until the time of analysis.
3.2 Methods:

3.2.1. Estimation of fructosamine by using NTB method:

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 Principle 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 A1c+b fractions (HbA1c+b), and is quantified by direct photometric reading at 415 nm. The estimation of the relative concentration of HbA1c is made by the measure of total hemoglobin concentration by direct photometric reading at 415 nm.(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 1 DM 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 type 1 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. \( r = 0.341, P.\text{Value} = 0.015 \).

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. \( r = 0.457, P.\text{Value} = 0.001 \).

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. \( r = -0.235, P.\text{value} = 0.384 \).

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. \( r = -0.138, P.\text{Value} = 0.371 \).
Table (4-1): Comparison of mean concentration of fructosamine in patients and control group.

<table>
<thead>
<tr>
<th>variable</th>
<th>Group</th>
<th>mean±SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructosamine (mg/dl)</td>
<td>Control N=50</td>
<td>2.38±0.4</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Type 1 DM N=50</td>
<td>8.19±4.31</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Type 2 DM N=50</td>
<td>5.74±2.04</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Results given in mean ± SD.

P.Value ≥ 0.05 consider significant.
Table (4-2): Comparison of mean of HbA1c in patients and control group.

<table>
<thead>
<tr>
<th>variable</th>
<th>Group</th>
<th>mean±SD</th>
<th>P.Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HbA1c( %)</td>
<td>Control N=50</td>
<td>4.69±0.64</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Type 1 DM N=50</td>
<td>9.62±2.9</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Type 2 DM N=50</td>
<td>7.82±1.6</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Results given in mean ± SD.

P.Value ≥ 0.05 consider significant.
Figure (4-1): Correlation between fructosamine and Hb A1c in type 1 DM.

$R = 0.341^*$

$P = 0.015$
Figure (4-2): Correlation between fructosamine and Hb A1c in type 2 DM.

\[ R = 0.457^{**} \]
\[ P = 0.001 \]
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 1 and 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 patients 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 months.

- Fructosamine should be done as routine investigation in the laboratories of diabetic centers.
References
References


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 : ........%
FRUCTOSAMINE

CONTENTS AND COMPOSITION
A. Reagent: 2 x 50 mL, NSI 0.25 mmol/L, carbonate buffer 0.2 mol/L, pH 10.55.
B. Fructosamine Standard: 2 x 1 mL. Human serum. Concentration is given on the label in mmol/L of DMF (deoxymethylfructosone) and in µmol/L of glycated albumin.

Dilute the solution with distilled water so the concentration is 0.19 mmol/L of DMF and 985 µmol/L of glycated albumin. 

ADDITIONAL EQUIPMENT
- Analyser, spectrophotometer or photometer with cell holder thermostated at 37°C and 560±20 nm.

PROCEDURE
1. Bring the Reagent to room temperature.
2. Pipette into labelled test tubes (Note 1):
   - Reagent (A) 1.0 mL
   - Sample 50 µL
   - Standard 1.0 mL
   - 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 560 nm exactly 10 minutes (A1) and 15 minutes (A0) of incubation against distilled water.

CALCULATIONS

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

\[ \text{Sample (mmol/L)} = \frac{A0 - A1}{A0 - A1} \times C \text{Standard} \times C \text{Sample} \]

REFERENCE VALUES

Serum 0.01-1.8 mmol/L (20-255 µmol/L, glycated albumin). Concentrations are slightly lower (5%) in chl.

QUALITY CONTROL

It is recommended to use the Fructosamine Control Serum (cod. 10507) to verify this performance of the measurement procedure. Concentration is given on the label, expressed in mmol/L of DMF (deoxymethylfructosone) 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 HLA antigen. However, they should be handled cautiously as potentially infectious.

METROLOGICAL CHARACTERISTICS

- Detection limit: 3.14 mmol/L (DMF), 3110 µmol/L (glycated albumin).
- Linearity limit: 7 mmol/L, 780 µmol/L (glycated albumin). For higher values dilute sample 1:10 with distilled water and repeat measurement.
- Reproducibility (within run):
  - Mean Concentration 1.36 mmol/L (246 µmol/L)
  - Coefficient of variation 2.1%
  - Repeatability (not to be used):
  - Mean Concentration 1.36 mmol/L (246 µmol/L)
  - Coefficient of variation 4.3%
  - Sensitivity: 21.2 mg/mL (DMF), 217 mg/mL (glycated albumin).
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiment are available on request. 

- Interferences: Hemoglobin (10 g/L), bilirubin (20 mg/dL) and Iopamin (glycerol esters 10 g/L) do not interfere. Other drugs and substances may interfere.

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 ketonatins 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 used in conjunction with glycated hemoglobin, rather than instead of it.

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

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

5. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, XXI.
HEMOGLOBIN A1C

CONTENTS

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>CCD 11044</th>
<th>CCD 11045</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Reagent</td>
<td>1 x 30 mL</td>
<td>1 x 30 mL</td>
</tr>
<tr>
<td>2. Reagent</td>
<td>1 x 50 mL</td>
<td>1 x 240 mL</td>
</tr>
<tr>
<td>3. Reagent</td>
<td>1 x 450 mL</td>
<td>4 x 450 mL</td>
</tr>
<tr>
<td>4. Microcolumns</td>
<td>1 x 20 mL</td>
<td>1 x 100 mL</td>
</tr>
</tbody>
</table>

COMPOSITION

1. Reagent. Potassium phosphate 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 15-30°C.

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 A1C is stable for 7 days at 2-8°C. Heparin or EDTA may be used as anticoagulants.

PROCEDURE

Hemolysate Preparation and Labile Fraction Elimination

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

<table>
<thead>
<tr>
<th></th>
<th>50 µL</th>
<th>200 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reagent 1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 HbA1c fraction

6. Carefully pipette on the upper filter:

<table>
<thead>
<tr>
<th></th>
<th>50 µL</th>
<th>Let the column drain to waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolysate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>200 µL</th>
<th>Let the column drain to waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

8. Pipette:

<table>
<thead>
<tr>
<th></th>
<th>2.0 mL</th>
<th>Let the column drain to waste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>4.0 mL</th>
<th>Collect the eluate (HbA1c Fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

10. Shake thoroughly and read the absorbance (A) of the HbA1c fraction at 415 nm against distilled water (A0 TOTAL). The absorbance is stable for at least one hour.

Reading of Hb TOTAL

11. Pipette into a test tube:

<table>
<thead>
<tr>
<th></th>
<th>12.0 mL</th>
<th>50 µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemolysate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

CALCULATIONS

The HbA1c relative concentration in the sample is calculated using the following general formula:

\[
\text{HbA1c} = \frac{A_{\text{HbA1c}}}{A_{\text{Hb TOTAL}}} \times 100 = \% \text{HbA1c}
\]

The volume of HbA1c (VHbA1c) is 4 mL, the volume of Hb total (V TOTAL) is 12 mL. The following formula is deduced for the calculation of the concentration:

\[
\frac{A_{\text{HbA1c}}}{A_{\text{Hb TOTAL}}} \times \frac{12}{4} = \% \text{HbA1c}
\]

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 equations:

\[
\text{HbA1c-IFCC (mmol/mol)} = 10.93 \times \text{HbA1c-NGSP-DDCT (\%)} - 23.5
\]
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.

<table>
<thead>
<tr>
<th>NGSP-DCCT (%)</th>
<th>IFCC (mmol/mol)</th>
<th>Reference values / Degree of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.0 - 6.5</td>
<td>20 - 48</td>
<td>Normal</td>
</tr>
<tr>
<td>6.0 - 7.0</td>
<td>42 - 53</td>
<td>Good</td>
</tr>
<tr>
<td>7.0 - 8.0</td>
<td>53 - 64</td>
<td>Good Control</td>
</tr>
<tr>
<td>&gt; 8.0</td>
<td>&gt; 64</td>
<td>Action suggested</td>
</tr>
</tbody>
</table>

QUALITY CONTROL
It is recommended to use the Hemoglobin A1C 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 % = 182 mmol/mol.
- Repeatability (within run):
  - Mean Concentration: CV 6.4 % 25
  - 7.2 % = 55 mmol/mol
  - 9.3 % = 65 mmol/mol
- Reproducibility (run to run):
  - Mean Concentration: CV 6.9 % 25
  - 7.2 % = 55 mmol/mol
  - 9.9 % = 65 mmol/mol
- 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.

In the isoelectric exchange chromatographic methods, the presence of hemoglobin C, S, or in the sample may slightly alter results, but differences are not clinically significant. Other hemoglobin variants like HbE, HbF, carbamyl-Hb, and acetyl-Hb can interfere. The incubation with Reagent (II) eliminates the interference due to HbA1C.

In hemolytic anemia, iron deficiency anemia and transfusion, the average age of erythrocytes is altered. Caution should be used when interpreting the HbA1C results from patients with these conditions.

DIAGNOSTIC CHARACTERISTICS
HbA1C is the product of the irreversible condensation of glucose with the C-terminal residue of the β-chain of hemoglobin A.

The HbA1C 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, and the estimated average glucose (eAG) during this period can be calculated with the formulas below:
- eAG (mg/dL) = 28.7 x HbA1C - NGSP-DCCT (%) - 46.7
- eAG (mmol/L) = 1.59 x HbA1C - IFCC (mmol/mol) - 2.59
- eAG (mg/dL) = 2.64 x HbA1C - IFCC (mmol/mol) - 15.0
- eAG (mmol/L) = 0.146 x HbA1C - IFCC (mmol/mol) - 8.43

HbA1C levels are a valuable adjunct to glucose determinations in the assessment and follow up of individuals with diabetes mellitus, providing much more reliable information than glycemic monitoring when no 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 HbA1C concentration may also be a useful tool in the diagnosis of diabetes.

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 range of 18-20°C. If working temperature is out of range, multiply the obtained value by the corresponding factor shown in the following table:
   - Working temperature: 18-20°C Factor 1.00
   - 27°C Factor 1.00
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 opening the columns.
3. Some air bubbles may occasionally appear inside the main bed. Their presence does not alter the test performance.

BIBLIOGRAPHY