بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Triglyceride/ HDL-c Ratio and Triglyceride/ glucose Index as Markers for Glycemic Control in Patient with Type2 Diabetes Mellitus in Khartoum State.

تقييم نسبة الدهون الثلاثية الي الدهون عالية الكثافة و نسبة الدهون الثلاثية الي سكر الجلكوز كمؤشرلرصد التحكم الأيضي لمرضي السكري من النوع الثاني في ولاية الخرطوم.

A dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science -Clinical Chemistry

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December 2020

الأية

بسم الله الرحمن الرحيم

قال تعالي:

" كُلُواْ مِن طَـيبات ما رزقناكم ولا تطغوا فيه فيحل عليكم غضبي ومن يحلل عليه غضبي فقد هوى (٨١)وَإني لغفار لمن تاب وءامن وعمل صالحا ثم اهتدي (٨٢) "

سورة طه

الإهداء

إلي من هذبت روحي جعلت الروح مسكنه

إلي من قومت خلقي جعلت القلب مأواها

أمي

إلي من غاص أبحارا ليحضر لؤلؤا خصبا

إلي من نور الدرب وكان الشمع متقدا

إلي من أفني أياما ليسعد بسمتي شغفي

أبي

إلي من سطر الدرب طريقا ننهل الصعبا

إلي من لازم العلم لنصبح بعده علما

أساتذتي

إلي من كانوا أعوانا ولم يستكثروا زمنا

إلي من كانوا روادا نصون العهد نتبعهم

إخوتي

إلي من صادفوا فكري وكانوا العون والاهل

إلي من خففو كربي وكانوا بسمتي طربي

أصدقائي

Acknowledgment

- All praise is to God for the blessing of the venerable and great peace, the almighty, by whose grace and mercy we have completed this work and blessing upon the Messenger of Allah.
- My sincere appreciation is due to my Supervisor, department of clinical chemistry, University of Sudan for science and technology who always tries to progress and set us apart and guide us to the best direction.
- Special thanks also to the Alraghi hospital, ombada hospital, saadreshoan specialist and Alemteazspecialist for facilitating the equipment for measurement the parameter and our thanks also reach to staff-member of clinical chemistry at the University of Sudan for science and technology he taught me and all of the characters were promoted in the ladder of science.

Abstract

Background and objective: Diabetes Mellitus (DM) is increasing throughout the world , glycated hemoglobin (HbA1c) has been considered as a good indicator of overall glycemic control but it had some limitation.This study aim to evaluate Triglyceride/HDL-c Ratio and Triglyceride/glucose Index as Markers for Glycemic Control.

Materials and Method: A case control study was done in (104) participants 52with type 2 DM (male and female patients) and 52 apparently health control.

Blood sample was taken from each participant to prepare the plasma, which used for measurement of fasting blood glucose, triglyceride, HDL-c by Biosystem 350 semi-automated analyzer, and glycated hemoglobin levels by I-CHROMA, the data obtained were analyzed by using statistic package for social science (SPSS).

Results: The results showed that HDL-C was significantly lower and triglyceride , fasting blood glucose, glycated hemoglobin, TG/ HDL ratio and TG/FBG index were significantly higher in type 2 diabetic patients in compare with control with p -value ($p<0.05$).

Also the result revealed that both TG/ HDL ratio and TG/FBG index were positively correlated with fasting blood glucose and glycated hemoglobinwhile HDL–c was negatively correlated with TG/ HDL ratio and TG/FBG index ($p<0.05$) and TG/FBG index , TG/HDL-C ratio were significantly higher in poor glycemic control ($p<0.05$).

Conclusion: TG/ HDL ratio and TG/FBG index can be used as alternative biomarkers of glycemic control and could be the better as surrogate markers of glycemic control, besides HbA1c.

Key words: Type 2 DM, glycatedhemoglobin, Triglyceride, HDL-c and Fasting blood glucose.

ملخص الدراسة:

خلفية :مرض السكرآخذ في الازدياد في جميع انحاء العالم ، وقد تم اعتبار السكري التراكمي مؤشر جيد للتحكم العام في نسبة السكر في الدم والهدف من هذه الدراسة هو تقييم استخدام نسبة الدهون الثلاثية الي البروتين الدهني عالي الكثافة ونسبة الدهون الثلاثية الى نسبة الجلوكوز كمؤشران للتحكم في نسبة السكر في الدم .

الطريقة :تم إجراء هذه الدراسة علي)١٠٤ (مشاركا،٥٢ مريض بالسكري من النوع الثاني)زكورا واناثا (و٥٢ شخص سليم كمجموعة ضابطة.

تم أخذ عينة الدم من كل مشارك وتم استخدامها لقياس الجلكوز والدهون الثلاثية ،والبروتين الدهني عالي الكثافة بواسطة محلل نصف آلي بايو سستم ٣٥٠ومستويات السكري التراكمي بواسطة جهاز الاي كروما .

تم تحليل البيانات التي تم الحصول عليها باستخدام الحزمة الاحصائية للعلوم الإجتماعية الإصداررقم١٦.

النتائج:

أظهرت النتائج أن البروتين الدهني عالي الكثافة كان أقل بشكل ملحوظ، بينما كانت نسبة الدهون الثلاثية،الجلوكوز، والسكري التراكمي، ونسبة الدهون الثلاثية الي البروتين الدهني عالي الكثافة ومؤشرالدهون الثلاثية الي الجلكوز أعلى بكثير عند مرضى السكر من النوع الثاني مقارنة بمجموعة التحكم بقيمة معنويةأقل من ٠,٠٥

كما اظهرت وجود ارتباط ايجابي بين كل من نسبة الدهون الثلاثية الي البروتين الدهني عالي الكثافة ومؤشر الدهون الثلاثية الى الجلكوز مع سكر الصيام والسكري التراكمي .بينما كان البروتين الدهني عالي الكثافة مرتبطًا ارتباطا سلبيًا مع نسبة الدهون الثلاثية الي البروتين الدهني عالي الكثافة ومؤشر الدهون الثلاثية الى نسبة الجلكوزبقيمة معنوية أقل من ٠,٠٥

كما ان مؤشرنسبة الدهون الثلاثية الى الجلكوز ومؤشرنسبة الدهون الثلاثية الي البروتين الدهني عالي الكثافة أعلى بشكل ملحوظ عند مرضي السكري غير المحافظين مقارنة مع المحافظين)القيمة المعنوية أقل من ٠,٠٥

الخلاصة :يمكن استخدام نسبة الدهون الثلاثية الى الجلكوز ومؤشرنسبة الدهون الثلاثية الي البروتين الدهني عالي الكثافة كمؤشرات حيوية بديلة للتحكم في نسبة السكر في الدم ويمكن ان تكون افضل كعلامات بديلة للتحكم في نسبة السكر في الدم الي جانب السكري التراكمي .

Table of contents

List of tables:

Abbreviations:

Abbreviation	Full writing
ADA	American Diabetic Association
BARI 2D	Bypass Angioplasty Revascularization Investigation 2 Diabetes
BMI	body mass index
CVD	Cardio Vascular Disease
DKA	Diabetic Ketoacidosis
DM	Diabetes mellitus
DN	Diabetic nephropathy
EDTA	EthyleneDiamineTetraacetic Acid
ESRD	End stage renal disease
FFAs	free fatty acids
FPG	Fasting Plasma Glucose
GA	Glycated Albumin
GDM	gestational diabetes mellitus
Ghb	Glycated hemoglobin
HbA1c	Hemoglobin A1c
HDL	High Density Lipoprotein
HHS	Hyperosmolar Hyperglycaemic State
HNC	Hyperosmolar nonketotic coma
HONK	Hyperosmolal non-ketotic
LDL-C	Low Density Lipoprotein Cholestrol
NDRN	Non Diabetic Tolerance Test
NGSP	National Glycohemoglabin standardization

Abbreviation	Full writing
OGTT	oral glucose tolerance test
P value	Probability value
SMBG	Self-monitoring of blood glucose
TC	Total cholesterol
TG	Triglyceride
TNF a	Tumor Necrosis Factor alpha
USD	United State Dollar
VEGF	vascular endothelial growth factor
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization

CHAPTER-ONE

Introduction ,Rationale and Objectives

1. Introduction ,Rationale and Objectives

1.1. Introduction:

Diabetes mellitus (DM) is a serious, long-term condition with a major impact on the lives and societies worldwide. It is among the top 10 causes of death in adults, and was estimated to have caused four million deaths globally in 2017(Saeedi etalic; 2019).

The WHO eastern Mediterranean region has the highest prevalence of DM in the world. Seven countries in this region have a high prevalence of DM and a further seven countries (including Sudan) have a medium prevalence (9–12%) of DM. Type 2 diabetes mellitus (T2DM) is the major type of DM, accounting for approximately 90% of all cases. The estimated prevalence of DM in Africa in 2017 was 3.3%, and Sudan was among the countries that had a prevalence of DM of more than 12 %(Omar etalic; 2019)

The spectrum of chronic complications related to DM is extended to include microvascular complications like nephropathy, neuropathy and retinopathy and chronic macrovascular complications like coronary artery disease, peripheral artery disease, stroke, diabetic encephalopathy and diabetic foot (Omar etalic; 2019).

Glycemic control is fundamental to diabetes management because a good glycemic control reduces risk of complications. Glycated hemoglobin A1c (HbA1c) is the gold standard ofglycemic control that reflects average blood glucose in patients overapproximately 2-3 months, HbA1c value less than 7% has been shown to reduce diabetic vascular complication (IDF; 2015,American Diabetes Association; 2018 and Badedi; 2016).

In diabetic patients, for each 1% increase in absolute HbA1c value estimated risk ofcardiovascular diseases (CVD) increases by 18% (Selvin etalic; 2004 and Patel etalic; 2014). Another risk factor for CVD in patients with DM2 is diabetic

dyslipidemia. Study have shown that TG/HDL-C ratio and TyG index are positively associated with HbA1c levels in DM2 patients and both were higher in patients with poor glycemic control .Triglyceride-to-HDL-Cholesterol ratio is a good predictive marker for insulin resistance and for early assessment of CVD. In some studies, TG-to-HDLC ratio less than 0.87 are considered ideal, value above >1.74 is too high and high risk for coronary artery disease. (Kim-Dorner etalic; 2010)

Recently, the TyG index, a product from the fasting levels of triglycerides and glucose, presented promising results as a surrogate marker for the assessment of insulin resistance (IR) (Lee etalic; 2016) with a good correlation with gold standard hyperglycemic clamp according to a study in Brazilian (Vasques etalic; 2011) and Mexician (Guerrero-Romero etalic; 2010).In addition, it has been used as a tool to recognize metabolically obese and normal weight individuals (Lee etalic; 2015) and as a predictor of coronary artery calcification and subclinical atherosclerosis (Kim-Dorner etalic; 2017).

1.2. Rationale

To reduce the risk of progressing complication of diabetes, effective methods will be required, the current biomarkers (HbA1c, Fructose amine and glycated albumin) have limitation including moderate sensitivity, specifity and are inaccurate in certain clinical conditions .Non-glycemic factors affecting HbA1c levels include erythropoiesis, hemoglobinsynthesis and conditions influencing red blood cell survival. Deficiency anemia generally elicit falsely increased HbA1c levels due to the increased levels of aged erythrocytes that are found in patients with this disease(include iron deficiency anemia, B12 deficiency anemia, folate deficiency anemia, chronic alcohol use, and asplenia), whereas falsely decreasedHbA1c levels can be observed in hemolytic anemia of any cause(acute and chronic blood loss, splenomegaly) . Hemoglobinopathies can affect HbA1c by altering glycation (thalassemia) interfering with the assay, or causing erythrocytes to be more prone to hemolysis (as sickle cell hemoglobin). therefore combining several biomarkers may were precisely identify glycemic status, all of this necessitates the determination of Triglyceride: HDL ratio and Triglyceride: glucose index as glycemic control in order to play role in control of the disease and delay it is complications in type 2 diabetes mellitus.

There are limited studies that aimed to assess association between TG/HDL-C ratio and TyG index with glycemic control and measuring serum TG level as part of TYG index or alone can be a useful and cost effective marker and represent the glycemic and cardiovascular status of an individual simultaneously.

1.3. Objective:

1.3.1 General objective:

To assess the use of triglyceride/ HDL ratio and triglyceride/ glucose index as markers for glycemic control in patient with type2 diabetes mellitus in Khartoum state.

1.3.2 Specific objectives:

- To measure and compare blood glucose, triglyceride, HDL-c, and HbA1c levels in type2 diabetic patients and the control group.
- To calculate and compare triglyceride: HDL Ratio and triglyceride/glucose index in type 2 diabetes mellitus and the control group.
- To correlate fasting blood glucose, triglyceride, HDL-c, triglyceride /HDL-c ratio and triglyceride/ Glucose index with glycosylated HbA1c in type 2 DM.
- To correlate age, BMI, duration of diabetes, fasting blood glucose, triglyceride, HDL-c, triglyceride/HDL-c ratio and triglyceride/ Glucose index with glycosylated HbA1c in type 2 DM.
- To compare triglyceride /HDL-c ratio and triglyceride/ Glucose index in type 2 DM those with poor glycemic control (HbA1c≥7%) and patients with good glycemic control (HbA1c<7%).

CHAPTER-TWO

Literature review

2. Literature review

2.1. Diabetes mellitus:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. (Freeman; 2018).

The name of this disease is derived from the fact that glucose "spills over" into the urine when the blood glucose concentration is too high (mellitus is derived from a Latin word meaning "honeyed" or "sweet"). The general term diabetes comes from a Greek word meaning "siphon;" it refers to the frequent urination associated with this condition. (Fox; 2016)

2.1.1. Type of diabetes mellitus:

There are two major forms of diabetes mellitus. Type 1 (or insulin-dependent) diabetes, it was once known as juvenile-onset diabetes because this condition is usually diagnosed in people under the age of 20. Type2 (non-insulin-dependent) diabetes has also been called maturity-onset diabetes, because it is usually diagnosed in people over the age of 40. The incidence of type 2 diabetes in children is rising (due to an increase in the frequency of obesity), however, so these terms are no longer preferred. (Fox; 2016)

2.1.1.1 Type 1 diabetes mellitus:

Type1 diabetes is a result of cellular-mediated autoimmune destruction of the β-cells of the pancreas, causing an absolute deficiency of insulin secretion. Type 1 constitutes only 10% to 20% of all cases of diabetes. This disease is usually initiated by an environmental factor or infection (usually a virus) in individuals with a genetic predisposition and causes the immune destruction of the β-cells of the pancreas and, therefore, a decreased production of insulin. Characteristics of type 1 diabetes include abrupt onset, insulin dependence, and ketosis tendency.

This diabetic type is genetically related. One or more of the following markers are found in 85% to 90% of individuals with fasting hyperglycemia: islet cell autoantibodies, insulin autoantibodies, glutamic acid decarboxylase autoantibodies, and tyrosine phosphatase IA-2 and IA-2B autoantibodies. Type 1 diabetes accounts for 10-20% of all diagnosed cases of diabetes. Signs and symptoms include polydipsia (excessive thirst), polyphagia (increased food intake), polyuria (excessive urine production), rapid weight loss, mental confusion, and possible loss of consciousness (due to increased glucose to brain). (Freeman; 2018)

2.1.1.2 Type 2 diabetes mellitus:

This form of diabetes, which accounts for 90–95% of those with diabetes, previously referred to as non-insulin dependent diabetes, type II diabetes, or adult-onset diabetes, encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency at least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes. Although the specific etiologies are not known, autoimmune destruction of βcells does not occur. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes. Nevertheless, such patients are at increased risk of

developing macrovascular and microvascular complications. (ADA; 2004)

Whereas patients with this form of diabetes may have insulin levels that appearnormal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal. Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal. The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity. It occurs more frequently in women with prior GDM and in individuals with hypertension or dyslipidemia, and its frequency varies in different racial/ ethnic subgroups. It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type1 diabetes. However, the genetics of this form of diabetes are complex and not clearly defined. (ADA; 2004)

2.1.1.2.1. Metabolic disturbances in type 2 DM:

Insulin resistance can be associated with hypertension, dyslipidemia (and thus with increased risk of cardiovascular diseases). Metabolic syndrome in obesity may be caused by inflammation; the number of macrophages in adipose tissue increases in proportion to the obesity, as do inflammation markers in the blood such as C-reactive protein. In obesity, adipose tissue (including adipocytes and macrophages) secretes several pro-inflammatory adipokines, including tumor necrosis factor alpha (TNF a), interleukin-1, and resistin, that also reduce the insulin sensitivity of target tissues (adipose tissue, liver, and muscles). By contrast, the adipose tissue of lean people releases an anti-inflammatory adipokine—adiponectin— that increases insulin sensitivity and protects against metabolic syndrome. (Fox; 2016)

The insulin resistance syndrome (also known as syndrome X or the metabolic syndrome, http://www.americanheart.org) is a constellation of associated clinical and laboratory findings, consisting of (I) insulin resistance, (2) hyperinsulinemia, (3) obesity, (4) dyslipidemia (high triglyceride and low HDL cholesterol), and (5) hypertension. The metabolic syndrome is diagnosed if an individual meets three or more of the following criteria:

- Abdominal obesity: waist circumference greater than 35 inches (women) or 40 inches (men)
- Triglycerides greater than 150 mg/dL
- HDL cholesterol less than 50 mg/dL (women) or less than 40 mg/dL (men)
- Blood pressure greater than or equal to 130/ 85 mm He
- Fasting plasma glucose (FPG) greater than or equal to 110 mg/dL

Individuals with this syndrome are at increased risk for cardiovascular disease. Several rare clinical syndromes are also associated with insulin resistance. The prototype is the type A insulin resistance syndrome, which is characterized by (1) hyperinsulinemia, (2) acanthosisnigricans, and (3) ovarian hyperandrogenism. (Sacks; 2008)

Obesity:

Obesity is often diagnosed using a measurement called the body mass index (BMI). This measurement is calculated using the following formula: $BMI = \frac{w}{h^2}$

Where

W = weight in kilograms (pounds divided by 2.2)

h = height in meters (inches divided by 39.4). (Fox; 2016)

The risk of developing type 2 diabetes increases tenfold in people with a body mass index (BMI) > 30 kg/m. (Frier and Fisher; 2010)

Dyslipidemia:

The dyslipidemia of obesity and type 2 diabetes usually features increased VLDL-TG. The production of VLDL-TG is increased by insulin, and this effect appears to persist when other actions of insulin are reduced by insulin resistance. Small dense LDL-C, which is the more atherogenic subclass of LDL-C, often is increased in association with insulin resistance and hyperinsulinemia together with a reduction in HDL-C. (Tripathi and Srivastava; 2006)

2.1.1.2.2 Type2DM management:

The aim of diabetes treatment is to maintain blood glucose levels within the normal range, which is between 3.5 and 6.0 mmol/ l (63mg/dl) before meals and 3.5 and 8 mmol/l(144mg/dl) two hours after meals. This will help prevent possible long term problems that can affect the heart, blood vessels, eyes, kidneys and nerves. Keeping the blood pressure and cholesterol within their recommended range, is very important to prevent long term problems. Healthy eating, achieving and maintaining a healthy weight, and doing regular physical activity can help to prevent long term problems. Sometimes tablets and then insulin may also been needed. (Frier and Fisher; M. 2010)

2.1.1.3 Gestational diabetes:

It is a form of glucose intolerance diagnosed in some women during pregnancy. Causes of GDM include metabolic and hormonal changes. Patients with GDM frequently return to normal postpartum. (Freeman; 2018)

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. (Sacks; 2008)

2.1.1.4 Other specific types of diabetes mellitus:

Other specific types of diabetes are associated with secondary conditions including genetic defect of β-cells function or insulin action, pancreatic disease, diseases of endocrine origin, drug or chemical induced insulin receptor abnormalities, and certain genetic Syndrome. The characteristics and prognosis of this form of diabetes depend on the primary disorder. Maturity-onset diabetes of youth is a rare form of diabetes that is inherited in an autosomal dominant fashion. (Freeman; 2018)

2.1.2 .Diagnosis of Diabetes mellitus:

The standards criteria for diagnosing diabetes mellitus depend on:

(1) Symptoms of diabetes plus a random plasma glucose level of ≥200mg/dl.

(2) Fasting plasma glucose of ≥126 mg/dl.

(3) Oral glucose tolerance test (OGTT) with a 2-hour post load (75g glucose load) level ≥200 mg/dl.

An intermediate group of individuals who did not meet the criteria of diabetes mellitus but who have glucose levels above normal be placed into three categories for the risk of developing diabetes. First, those individuals with fasting glucose levels greater than or equal to 100 mg/dL but less than 126 mg/dL are placed in the impaired fasting glucose category. Another set of individuals who have 2-hour OGTT levels greater than or equal to 140 mg/Dl but less than 200 mg/dL are placed in the impaired glucose tolerance category. Additionally, individuals with an HbA1c of 5.7% to 6.4% are placed in the third at-risk category. Individuals in these three categories are referred to as having “prediabetes” indicating the relatively high risk for the development of diabetes in these patients. Each of previous tests must be confirmed on a subsequent day by any one of the three

methods. The preferred test for diagnosing diabetes is measurement of the fasting plasma glucose level. (Freeman; 2018)

2.1.3 Complications of diabetes mellitus:

The complications of diabetes mellitus can be divided into acute and chronic categories.

2.1.3.1 Acute Complications:

Patients with diabetes mellitus may develop various metabolic complications that require emergency treatment, including coma, and these include the following.

I. **Diabetic Ketoacidosis:**

Diabetic ketoacidosis may be precipitated by infection, acute myocardial infarction or vomiting. The patient who reasons 'no food, therefore no insulin' could mistakenly withhold insulin. In the absence of insulin, there is increased lipid and protein breakdown, enhanced hepatic gluconeogenesis and impaired glucose entry into cells. The clinical consequences of diabetic ketoacidosis are due to:

- Hyperglycaemia causing plasma hyperosmolality,
- Metabolic acidosis,
- Glucosuria.

II. **Hyperosmolar non-ketotic coma (HNC):**

In diabetic ketoacidosis there is always plasma hyperosmolality due to the hyperglycaemia, and many of the symptoms, including those of confusion and coma, are related to it. However, the term 'hyperosmolal' coma or 'pre-coma' is usually confined to a condition in which there is marked hyperglycaemia but no detectable ketoacidosis. The reason for these different presentations is not clear. It has been suggested that insulin activity is sufficient to suppress lipolysis but insufficient to suppress hepatic gluconeogenesis or to facilitate glucose transport

into cells. Hyperosmolal non-ketotic (HONK) coma now may be referred to as hyperosmolar hyperglycaemic state (HHS) and may be of sudden onset. It is more common in older patients. Plasma glucose concentrations may exceed 50mmol/L (900mg/dl). The effects of glycosuria are as described above, but hypernatraemia due to predominant water loss is more commonly found than in ketoacidosis and aggravates the plasma hyperosmolality. Cerebral cellular dehydration, which contributes to the coma, may also cause hyperventilation, and a respiratory alkalosis, although sometimes plasma lactic acid may rise, evoking a metabolic acidosis and thus a mixed acid–base disturbance may occur. There may also be an increased risk of thrombosis. (Crook; 2012)

III**. Hypoglycemia:**

This is probably the most common cause of coma seen in diabetic patients. Hypoglycaemia is most commonly caused by accidental over administration of insulin or sulphonylureas or meglitinides. Precipitating causes include too high a dose of insulin or hypoglycaemic drug; conversely, the patient may have missed a meal or taken excessive exercise after the usual dose of insulin or oral hypoglycaemic drugs. Hypoglycaemia is particularly dangerous, and some patients lack awareness of this; that is to say, they lose warning signs such as sweating, dizziness and headaches. Patients should monitor their own blood glucose closely, carry glucose preparations to abort severe hypoglycaemia and avoid high-risk activities during which hypoglycaemic attacks could be dangerous. (Crook; 2012)

2.1.3.2 Chronic complications: (Long-term effects of diabetes mellitus)

The chronic complications of diabetes mellitus affect many organ systems and are responsible for the majority of morbidity and mortality. Chronic complications can be divided into vascular and nonvascular complications.

1-The vascular complications are further subdivided into

- Microvascular complications

It includes retinopathy, Neuropathy, and nephropathy.

- Macrovascular complications

It includes coronary artery disease, peripheral vascular disease, and cerebrovascular disease.

2-Nonvascular complications

It includes problems such as gastroporesis, sexual dysfunction, and skin changes. As a consequence of its chronic complications, DM is the most common cause of adult blindness, a variety of debilitating neuropathies, and cardiac and cerebral disorders. (Tripathi and Srivastava; 2006)

I. Diabetic nephropathy (DN):

It caused by diabetes mellitus and it is one of the major causes of end-stage renal failure worldwide. Clinically, microalbuminuria is an important index to assess the progression of DN. However, it is not accurate to evaluate the severity or prognosis simply based on the degree of proteinuria. It is now well recognized that not all diabetic patients who develop renal function failure have massive albuminuria. Therefore, nephrologists and endocrinologists should be aware of the significance of pathological changes of DN in their clinical practice. Specifically, non-diabetic renal disease (NDRD), which might commonly be superimposed with diabetic renal lesions in some patients with type2 diabetes, could only be confirmed and excluded by biopsy. (Qi etalic; 2017)

II. Diabetic retinopathy:

Diabetic retinopathy is one of the commonest causes of blindness in adults between 30 and 65 years of age in developed countries. Hyperglycemia increases

retinal blood flow and metabolism and has direct effects on retinal endothelial cells and pericyte loss, which impairs vascular auto regulation.

The resulting uncontrolled blood flow initially dilates capillaries but also increases production of vasoactive substances and endothelial cell proliferation, resulting in capillary closure. This causes chronic retinal hypoxia and stimulates production of growth factors, including vascular endothelial growth factor (VEGF), which plays a major role in stimulating the deleterious changes of endothelial cell growth (causing new vessel formation) and increased vascular permeability (causing retinal leakage and exudation). (Frier and Fisher; 2010)

III. Diabetic neuropathy:

It is the most common and debilitating complication of diabetes and results in pain, decreased motility, and amputation. Diabetic neuropathy encompasses a variety of forms whose impact ranges from discomfort to death. Hyperglycemia induces oxidative stress in diabetic neurons and results in activation of multiple biochemical pathways. These activated pathways are a major source of damage and are potential therapeutic targets in diabetic neuropathy. Though therapies are available to alleviate the symptoms of diabetic neuropathy, few options are available to eliminate the root causes. The immense physical, psychological, and economic cost of diabetic neuropathy underscore the need for causally targeted therapies. (Edwards etalic; 2008)

2.1.4 Control of DM:

Glycemic control is fundamental to diabetes management because a good glycemic control reduces risk of complications. (Babic etalic; 2019)

Diabetes monitoring at the present time is currently managed by a combination of daily self-monitoring of blood glucose (SMBG) and regular assessments of HbA1c. (ADA; 2010)

And recent study assess the association of triglyceride - to - HDL cholesterol (TG/HDL-C) ratio and triglyceride glucose (TyG) index with HbA1c as predictors of glycemic control in patients with diabetes mellitus type 2 (DM2). (Babic etalic; 2019)

2.1.4.1Glycated hemoglobin A1C (HbA1c):

Hemoglobin A1C reflects average glycemia over approximately 3 months. The performance of the test is generally excellent for National Glycohemoglobin Standardization Program (NGSP)-certified assays .The test is the major tool for assessing glycemic control and has strong predictive value for diabetes complications. Thus, HbA1C testing should be performed routinely in all patients with diabetes as initial assessment and as part of continuing care. Measurement approximately every 3 months determines whether patients' glycemic targets have been reached and maintained. The frequency of A1C testing should depend on the clinical situation, the treatment regimen, and the clinician's judgment. The use of point-of-care HbA1C testing may provide an opportunity for more timely treatment changes during encounters between patients and providers. Patients with type 2 diabetes with stable glycemia well within target may do well with A1C testing only twice per year. Unstable or intensively managed patients or people not at goal with treatment adjustments may require testing more frequently (every 3 months). (ADA; 2020)

2.1.4.2. Fructosamine&Glycated albumin:

In selected patients with diabetes mellitus (e.g., GDM or change in therapy), there may be a need for assays that are more sensitive than GHb to shorter-term alterations in average blood glucose levels. Non-enzymatic attachment of glucose to amino groups of proteins other than hemoglobin (e.g., serum proteins, membrane proteins, and lens crystallins) to form ketoamines also occurs. Because

serum proteins turn over more rapidly than erythrocytes (the circulating half-life for albumin is about 20 days), the concentration of glycated serum albumin reflects glucose control over a period of 2 to 3 weeks. Therefore evidence of both deterioration of control and improvement with therapy is evident earlier than with GHb. Fructosamine is the generic name for plasma protein ketoamines."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.Although the fructosamine assay has been automated and is cheaper and faster than GHb, there is a lack of consensus on its clinical utility.(Sacks; 2008)

2.1.4.3. (TG/HDL-C) ratio:

Diabetic dyslipidemia it consists of increased triglycerides (TG), reduced high density lipoprotein cholesterol (HDL-C), and postprandial lipemia. In addition, low density lipoprotein cholesterol (LDL-C) is converted to small,dens LDL that is more atherogenic.The serum triglyceride to high-density lipoprotein cholesterol (TG/HDL-C) ratio, known as atherogenic index of plasma, is one of the major risk factors for CVD and metabolic syndrome. Higher TG/HDL C ratio has been associated with the presence of endothelial dysfunction. Furthermore, TG/HDL-C ratio has been proposed as a marker of insulin resistance. The TG/HDL-C ratio was calculated by dividing the serum concentration of TG by HDL-C measured in mg/dl (Babic etalic; 2019)

2.1.4.4. Triglyceride to glucose (TyG) index:

Additional marker associated with insulin resistance is triglyceride to glucose (TyG) index. It helps in identification of subjects at high risk of CVD in asymptomatic subjects with DM2. (lee etalic; 2016).

Patients with diabetes mellitus had higher levels of lipid profiles (LDL-C, total cholesterol (TC), TG) and lipid ratios (TG/HDL, LDL/HDL) compared to healthy people and a significant positive correlation between lipid profiles and HBA1c level. These demonstrate there is a link between lipid profiles on the glycemic index and vice versa. This provides correlation between glycemic control and dyslipidemias in patients with diabetes mellitus. (Lin, D et al 2018) (. Longo-Mbenza B et al 2011).

TyG index was calculated based on formula: Ln [TG (mg/dL) x FG (mg/dL)/2]. (lee etalic; 2016)

CHAPTER-THREE

Material and Methods

3. Materials and Methods

3.1Materials:

3.1.1Study Design

The study was analytical comparative case control study.

3.1.2Study area and period:

The study was conducted in Ombada Hospital and alemteaz medical center located in Omdurman from November 2019 to February 2020.

3.1.3 Study Population and sample size:

The target population of this study was individual who had type 2 DM (52 patients) and apparently healthy individual (number52) as a control group.

3.1.4 Inclusion criteria:

Patients already diagnosed as type 2 DM (according to American Diabetes Association; 2004) and apparently healthy individual age match were included in the study.

3.1.5 Exclusion criteria:

Diabetic patients with hypercholesterolemia, hypertriglyceridemia, hypertension and cardiovascular diseases were excluded.

3.1.6 Ethical consideration:

The study was approved from research committee in collage of medical laboratory sciences in Sudan University of Science and Technology, all patients and controls were informed about the aim of the study and accepted. Their participation in this study was fully voluntary and a verbal consent was taken from all participants included in the study.

3.1.7 Data collection.

Questionnaire interview:

Interview used for filling in the questionnaire which designated for matching the

study need appendix (1).

3.1.8 Sample collection:

After informed consent a sample were collected by using sterile syringes and tourniquet, venous blood (5ml) were collected and distributed into plain, EDTA and floride oxalate container under a septic condition. Plain container samples were allowed to clot at room temperature and then the tubes were centrifuged at 4000 rpm for 5 minutes. The samples was stored for -20◦ until analysis.

The sample with interference substance like hemolysis and icteric sample was excluded.

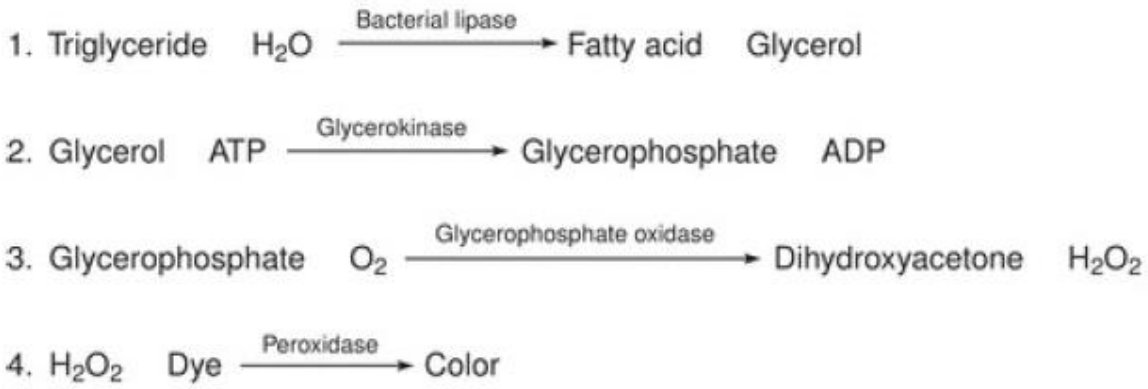
3.2 Methodology:

Biosystem 350 Photo electric semi-automated analyzer operates in the visible portion of the electromagnetic spectrum where the absorbance of the specific colors measures and gives the concentration by beer´s-Limber law was used.

3.2.1 Estimation of triglyceride:

Principle:

The sequences of reactions in the determination of triglyceride were as follows.



Reagent, procedure and calculations appendix (2)

3.2.2 Estimation of HDL-c:

Principle:

Very low density lipoproteins (VLDL), and low density lipoprotein (LDL) in the sample precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL cholesterol is then spectrophotometrically measured.

Reagent, procedure, calculations appendix (3)

3.2.3 Estimation of glucose:

Principle:

The enzymatic method uses Glucose oxidase (GOD) to catalyse the oxidation of glucose to hydrogen peroxide and gluconic acid. The hydrogen peroxide, when combined with 4-aminoantipyrine and aderivative from phenol, forms a red dye compound. The intensity of the red colour produced is directly proportional to the glucose quantity in the sample which can be measured colorimetrically.

Reagent, procedure, calculations appendix (4)

3.2.4 Estimation of HbA1c:

I-CHROMA TM HbA1c is an immunoassay system for quantitative measurement of hemoglobinA1c in Human blood with i-CHROMATM reader. The test is used for routine monitoring of the long-term glycemic status in patient with diabetes mellitus.

Principle

HbA1c estimation is based on the fluorescence immunoassay technology, specifically the competition immune detection method.

Whole blood is added to the mixture of hemolysis buffer and detection buffer,

which results in hemolysis of red blood cells. The mixture containing HbA1c from the hemolyzed red blood cells and fluorescence-labeled HbA1c peptides from detection buffer is loaded onto the sample well of the cartridge. The mixture then migrates through the nitrocellulose matrix of the test strip by capillary action.

HbA1c from the blood competes with fluorescence-labeled HbA1c peptides for binding sites on HbA1c antibodies fixed on the nitrocellulose matrix. As a result, the higher concentration of HbA1c produces a lower fluorescence signal from HbA1c-peptides.The signal is interpreted and the result displayed on i-CHROMA reader in units of percentage.

Reagent, procedure, calculations appendix (5)

3.3 Quality control:

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

3.4 Statistic analysis:

Data was analyzed by using statistic package for social science (SPSS) version 16 for Windows. T-test and spearman correlation was used to analysis the results obtained.

CHAPTER-FOUR

Results

Results

This study was include 104 participants, 52with T2DM with duration of disease range (1-40years) as case and 52healthy apparent individual age match as a control group. The results were illustrated in tables as follows:

Table(4.1) : The age, gender, body mass index(BMI)showed no significant differences (p>0.05), HDL-C were significantly lower, triglyceride, FG, HbA1c, Tg/ HDL ratio and Tg/FBG index was significant increase (p<0.05)between case and control groups.

Table (4.٢): Showed that DM2 HbA1c<7% (15.4%), DM2 HbA1c≥7% (84.6%).Lipid HDL-C was significantly lower in patients with poor glycemic control (p=0.020). However, there was no significant difference in triglyceride, FG, between poor and good glycemic control (p>0.05). Lipid ratio parameter Tg/HDL-C ratio were significantly higher in poor glycemic control compare to good glycemic control [8.9 (5.9-11.8) vs. 3.4 (2.0-5.6); p=0.012] FG/Tg index also were significantly higher in poor glycemic control (9.5±0.06vs. 8.7±0.05; significant at 0.010).

Table (4.٣): Age, Gender, BMI, Duration of disease showed no significant correlation (P-value >0.05). FG, Triglyceride, HbA1clevels, Tg/FBG index showed significant positive correlation (P-0.00) (r=0.64), (r=0.626), (r=0.78)(r=0.77)respectively while HDL-c showed significantly negatively associated with (r=-0.827 – P 0, 00) when correlation was done with Tg/HDL-c ratio.

Table (4.٤): Age, Gender, BMI, Duration of disease showed no significant correlation (P-value >0.05). FG, Triglyceride, HbA1clevels, showed significant positive correlation (P-0.00)(r=0.84), (r=0.77),(r=0.78),(r=0.68)respectively while HDL-c showed significantly negatively association (r=-0.65 – P 0, 00) when

correlation was done with TG/FBG index.

Table (4.1): Comparison of Age, gender, BMI, FBG, HDL-c, HbA1c, Triglyceride, Tg/ HDL ratio and Tg/G index between the type2 diabetic patients and the control group.

Variable	Control (n=52) (normal individual)	cases(n=52) (diabetic patients)	P value
Age	48.7±9.8	51±9.8	0.240
Gender (n; %) M	26.9%	26.9%	1.000
BMI	21.3±3.81	22±4.8	0.309
FG	86.9±13.9	177.9±65.2	0.000
HDL-c	77.1±37.6	58.7±26	0.019
HbA1c%	5.5±0.52	9.1±2.1	0.000
Triglyceride	88.8±17.5	169±116	0.000
TG/ HDL ratio	1.26±0.50	3.18±1.9	0.000
TG/FBG index	8.2±0.3	9.3±0.6	0.000

The table shows the mean ± standard deviation (STD) and probability (P)

t-test was used for comparison.

P value ≤ 0.05 considered significant.

Table (4.٢): Comparison of age , gender, BMI, FBG, HDL-c, HbA1c ,Triglyceride , Tg/ HDL ratio and Tg/G index between the type2 diabetic patients with poor glycemic control and type2 diabetic patients with good glycemic control.

Variable	HbA1c<7% - DM2 patients with good glycemic control15.4%	HbA1c≥7% DM2 patients with poor glycemic control. 84.6%	P value
Age	52.8±11.9	50.7± 9.5	0.588
Gender (n; %) M	25%	27%	0.897
BMI	24.9±3.7	21.6±4.8	0.078
FG	149.6±64	183±64.7	0.185
HDL-c	35.1±16.75	28.48±12.35	0.001
HbA1c%	6.0±0.8	9.7±1.7	0.000
Triglyceride	123±79	177.6±119.9	0.224
TG: HDL ratio	2.0±1.7	3.42±1.9	0.006
TG/FBG index	8.7±0.5	9.5±0.59	0.002

The tables show the mean ± standard deviation (STD) and probability (P).

t-test was used for comparison.

P value ≤ 0.05 considered significant.

Table (4.٣): Correlation between TG/ HDL-C ratio with age , gender,BMI, Duration of disease ,FBG , HDL-c, Triglyceride , HbA1c and Tg/G index in type2 diabetic patients.

Variable	TG/HDL-c ratio	
	p. value	R
Age	0.310	0.101
Gender	0.309	0.101
BMI	0.919	-0.010
Duration of disease	0.385	-0.123
FBG	0.000	0.649
HDL-c	0.000	-0.827
HbA1c	0.000	0.783
TG	0.000	0.626
TG/FBG index	0.000	0.773

The table shows (R and P values).

P value ≤ 0.05 considered significant.

Spearman correlation was used for comparison.

Table (4.٤) : Correlation between TG/FBG index with age , gender,BMI, Duration of disease ,FBG , HDL-c, Triglyceride , HbA1c in type2 diabetic patients.

Variable	TG/FBG index	
	p. value	R
Age	0.628	0.048
Gender	0.310	-0.101
BMI	0.310	-0.118
Duration of disease	0.709	0.053
FG	0.000	0.844
HDL-c	0.000	-0.656
HbA1c	0.000	0.681
TG	0.000	0.768

The tables show (R and P values).

P value ≤ 0.05 considered significant.

Spearman correlation was used for comparison.

CHAPTER FIVE

Discussion, conclusion and recommendation

Discussion, conclusion and recommendation

5. 1. Discussion:

Maintaining of good glycemic control is primary in diabetes care to reduce the risk of diabetic complications, this study was done to evaluate the using of triglyceride/HDL-c ratio and triglyceride/glucose index as glycemic control and its correlation with HbA1C in a sample of Sudanese subjects with type 2 DM.

The main results of this study was shown that TG/HDL-C ratio and TyG index are positively associated with HbA1c and had showed significant correlations with the cardiometabolic risk factor (HDL-C).

Several possible mechanisms have been suggested to explain the correlation between TyG index and glycemic control. Increased triglyceride levels can lead to increased free fatty acids and, thus, increased flux of free fatty acids from adipose to nonadipose tissue, which may affect the glycemic control. (Parhofer KG; 2015)

Common feature of insulin resistance and DM2 is dyslipidemia; the characteristic features of diabetic dyslipidemia are a high plasma triglyceride concentration, low HDL cholesterol concentration and increased concentration of small dense LDL-cholesterol particles which agree with study which showed high triglyceride and low HDL-c in the diabetic patients. The lipid changes associated with diabetes mellitus are attributed to increased free fatty acid flux secondary to insulin resistance. (Mooradian; 2009).

The result was in concordance with the results of the study that included 113 patients with DM2 classified according to their HbA1c values in two groups: DM2 HbA1c <7% - DM2 patients with good glycemic control (n=39) and DM2 HbA1c ≥7% - DM2 patients with poor glycemic control (n=74) had found thatTG/HDL-C ratio might be a useful predictor of glycemic control in normal weight, and TyG index in overweight and obese patients with DM2 (Babic *etaic;l* 2019).

This result were also in concordance with the results of the retrospective study recruiting 140 patients with T2DM during a one-year period, 2018–2019, at the Diabetic Center Sanglah General Hospital and Internal Medicine Polyclinic PuriRaharja General Hospital. It was found that lipid HDL-C was significantly lower in patients with poor glycemic control (p=0.001). Meanwhile, a negative correlation was observed on HDL-C with the HBA1c level. (Artha *etaic;l* 2019).

Joel Zonszein and *etalic* in A Post Hoc Analysis of the BARI 2D were found that TG/HDL-C ratio a useful marker in individuals who achieved better glycemic control.(Zonszein *etaic;l* 2015)

Previous study have shown that high levels of the triglycerides to high-density lipoprotein cholesterol (TG/HDL-C) ratio are associated with obesity, metabolic syndrome, and insulin resistance. (Meigs etalic; 2004)(Grundy etalic; 2004)

Our results are in concordance with the results of the study that shown the effects of glycemic control upon serum lipids and lipid transfers to HDL in patients with type 2 diabetes mellitus (novel findings in unesterified cholesterol status) which found that patients with HbA1c more than 6.5% had higher triglyceride and lower HDL-C that served as markers of poor glycemic control in mentioned study (Laverdy; 2015).

Shantha *etalic* (2013) were found that TG/HDL-C ratio is an effective screening tool to predict success with dose reductions of anti-diabetic medications in obese patients who successfully lose weight.

In identifying insulin-resistant individuals and found that optimal cut-points of TG/HDL ratio were (3.0 mg/dl) is a better marker of insulin resistance than triglycerides or insulin levels (McLaughlin T*et al* 2003). In present study, average value of TG/HDL-C ratio was 1.6 mg/dL in healthy control group and 9.2 mg/dL

in diabetic group suggesting that patients in cases groups were also insulin resistant.

Study that included apparently healthy individuals also showed that the fasting TG/HDL-C ratio was a better predictor of insulin resistance than triglycerides (McLaughlin T et al 2005). Moreover, in our study TG/HDL-C ratio was significantly associated with cardiometabolic markers such as HDL-C levels and triglycerides.

Results of the present study have shown that TyG index was positively associated with fasting glucose, HbA1c. triglycerides, and negatively associated with HDL-c in patients with DM2.Hameed EK (2019) he found that TyG indices were significantly correlated with HbA1c and were significantly increased in the diabetics with poor glycemic control.

5.2. Conclusion

This study concluded that there was association between TG/HDLC ratio and TyGindex with HbA1c and this index has the advantage of being applicable into clinical practice since both glucose and triglyceride determination are inexpensive and routinely measured.

5.3. Recommendations

1- Type2 diabetic patient periodically measure biomarkers of glycemic control to investigate the risk of diabetes complications.

2- TG/HDLC ratio and TyG index can be useful as alternative biomarkers of glycemic control.

3- HbA1c is relatively expensive; TyG was an alternative test that is inexpensive for follow up of patients with confirmed DM2 and for screening of patients with poor glycemic control.

4- Further studies with large sample size is required to verify the use of these alternative new biomarkers for glycemic control in diabetes mellitus.

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Appendices

Appendix I

الموافقة المستنيرة

Informed consent

أنا الباحثة أمل ابكر سليمان حبيب من كلية علوم المختبرات الطبية قسم الكيمياء السريرية، جامعة السودان للعلوم والتكنولوجيا ،أقوم بدراسة علمية بحثية لنيل درجة الماجستير بعنوان : (تقييم نسبة الدهون الثلاثية الي الدهون عالية الكثافة و نسبة الدهون الثلاثية الي سكر الجلكوز كمؤشرلرصدالتحكم الأيضي لمرضي السكري من النوع الثاني في ولاية الخرطوم.).

لقد قمنا بإختيارك على أن تشاركنا هذا البحث العلمي لأنك تنطبق عليك شروط الشخص الذي يمكن أن يكون من المشمولين في الدراسة (شخص يعاني من مرض السكري من النوع الثاني حضر إلى المركز للمتابعة ، ليس لديه أي من أمراض الدهون الوراثية، زيادة ضغط الدم و امراض القلب).
خلال هذه الدراسة سوف أقوم بأخذ الطول والوزن واخذ (٥مل) من الدم لإجراء فحص الجلكوز، الكوليسترول عالي الكثافة ، الدهون الثلاثية والسكر المرتبط بجزئ الهيموقلوبين وهذا يتطلب الصيام من(٩ ـ ١٢) ساعات للحصول على نتائج سليمة .
علماً بأن سحب العينة قد يؤدي إلى إحداث بعض الألم وقد يؤدي إلى ظهور ورم في منطقة الحقن قد يزول بمرور ساعات ،او ظهور كدمات زرقاء وسوف نعمل على تفادي كل هذه المضاعفات .
بعد الموافقة منك واخذ العينة والإجابة على جميع الأسئلة المكتوبة في البحث والتي سوف تكون في سرية تامة ولن يطلع عليها غير العاملين في البحث ، سوف يتم أخذ العينة والعمل على تحليلها وسيتم إخبارك بالنتائج التي تحصلنا عليها خلال شهر من زمن سحب العينة .
علماً مسبقاً بأن الفحص لن يتطلب منك أي تكلفة مادية ولن يعود عليك اي عائد مادي أيضا ؛ وعلما بأن اشتراكك في البحث عن طواعية؛ ويمكنك الانسحاب منه متى أردت ذلك.

إقرار المشارك :

لقد إطلعت على الملعومات الحالية والتي تم شرحها لي واتيح لي طرح الأسئلة عنها كيفما شئت ، ولقد تلقيت الإجابات الوافية على كل الأسئلة ، وانا أقرشفهيا بالموافقة على المشاركة طواعية في هذه الدراسة ، وأعلم بحقي في التوقف عن المشاركة في أي وقت دون أن يؤثر ذلك على حقوقي الأخرى او الإستفادة من هذه النتائج .

Appendix II

Questionnaire:

Code No Date

Personal data:

Name

Age years

Gender male ( ) female ( )

Height m

Weight kg

BMI kg/m2

Duration of DM years

Laboratory Investigation:

FBG mg/dl

Triglyceride mg/dl

HDL mg/dl

Hba1c %

BioMed-Triglycerides L.S

BIOMED DIAGNOSTICS

Enzymatic, Colorimetric GPO-PAP

REF:

TG117090 (3×30 ml)
TG117120 (2×60 ml)
TG117100 (2×50 ml)
TG117040 (2 × 20 ml)
TG117200 (10×20 ml)
TG117240 (2×120 ml)

INTENDED FOR USE:

For the quantitative determination of Triglycerides in serum and plasma

PRINCIPLE :

For the enzymatic determination of Triglycerides according to the following reaction:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{Dihydroxyacetone phosphate} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AAP} + \text{4-CHLOROPHENIL} \xrightarrow{\text{POD}} \text{colored compound} + H_2O$$

The intensity of the red colour produced is directly proportional to Triglycerides in the sample

SPECIMEN COLLECTION :

Fresh serum or plasma (heparin or EDTA) obtained from fasting individuals.
Do not use grossly hemolized or highly lipemic specimens.
Triglycerides in serum or plasma is stable for 2 days when stored in the refrigerator at 2-8°C.
Do not store samples at room temperature as phospholipids may hydrolyze, releasing free glycerol and falsely elevating Triglycerides value
Shake and bring the samples at room temperature (+15-25°C) before using.

REAGENTS COMPOSITION:

R1 Standard	Triglycerides Standard	200mg/dl (2.28mmol/l)
R2	Good's Buffer	100mmol/L
	Magnesium Chloride	15mmol/L
	ATP (Adenosina-5-Triphosphate)	4mmol/L
	4-AAP (4-Aminoantipyrine)	1mmol/L
	4-CHLOROPHENOL	0.1mmol/L
	LPL (Lipoprotein Lipase)	2500U/L
	GK (Glycerol Chinasi)	1000U/L
	GPO (Glycerol-3-phosphate oxidase)	5500U/L
	POD (Peroxidase)	1800U/L

PACKAGE : Collection & Storage .

Store at +2-8°C.
Stable until the expiration date reported upon the package.

After the unsealing and the taking of the reagent , it is advised to close up the bottle immediately in order to avoid evaporation , direct light exposure and bacterial contamination .

PRECAUTION & WARNINGS

Avoid pipette by mouth .
The preparation , according to current regulation . is classified as not dangerous.
The total concentration of non active components (preservatives , detergents ,stabilizers) is below the minimum required for citation .
Anyway handle with care , avoid ingestion , avoid contact with eyes , skin and mucous membranes
The samples must be handle as potentially infected from HIV or Hepatitis .

REAGENT PREPATION & STABILITY :

Before using reagents must be at room temperature.(+15-25°C.)
The reagent is limpid and rose-coloured.
A light reagent coloration (less than 0.050 O.D.) due to air or direct light exposure, will not impair its functioning.
Stable until the date indicated on the label.

REQUIRED MATERIALS NOT PROVIDED :

General Laboratory Equipment and instrumentations.

PROCEDURE :

Wavelength	500-550 nm
Optical path :	1 cm light path
Temperature :	+20-25/37°C
Reading :	Against blank reagent
Assay tipe :	Endpoint

Procedure : Pipetting in tubes :

	BLANK	STANDARD	SAMPLE
Reagent (R2)	1000 µL	1000 µL	1000 µL
Distilled Water	10 µL		
Standard (R1)		10 µL	
Sample			10 µL

Mix, incubate for 5 min at 37°C or 10 min at room temperature (+15-25°C) and read sample and standard extinction.
Color is stable for 60 min at room temperature.
Volumes can be proportionally modified.
This methodology describes the manual procedure to use the kit. For automated procedure, ask for specific application.

CALCULATION :

$$\textbf{Triglycerides mg/dl} = \frac{\text{(A) sample}}{\text{(A) Standard}} \times 200$$

Unit conversion:
mg/dl x 0.0114 = mmol/l

PERFORMANCE :

In comparing CRP tests, it must be remembered that the different techniques vary in sensitivity. The latex agglutination technique is more sensitive than precipitation in capillary tubes or in agar gel and gives positive results at lower CRP concentrations. For this reason the latex agglutination test usually gives a higher percentage of positive results than the other methods.
Expressed in absolute terms.
The amount of C-reactive protein in serum from patients with strongly positive CRP reactions is given by different workers as 33 mg/dl or 14 mg/dl while the content of normal serum is less than 6 mg/dl.

LIMITATIONS :

The strength of the agglutination reaction is not indicative of the CRP concentration. Weak reactions may occur with slightly elevated or markedly elevated concentrations. A prozone phenomenon (antigen excess) may cause false negatives. It is advisable, therefore, to check all negative seras-by retesting at a 1:10 dilution. Reaction times longer than specified (4 minutes) may produce apparent false reactions due to a drying effect. Strongly lipemic or, contaminated sera can cause false positive reactions.
Normal adult levels of CRP are reported to be less than 6 mg/dl when they can be detected. Recent refined techniques, however, have shown the routine appearance of trace amounts of the protein in the sera of apparently normal children and healthy adults.

	Consult Instructions for Use
	Caution, Consult accompanying
IVD	In Vitro Diagnostic Medical Device
2C 8C	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
	Use by

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Appendix IV

Appendix IV

COD 11648 50 mL
STORE AT 2-8ºC
Reagents for measurement of HDL cholesterol concentration Only for *in vitro* use in the clinical laboratory

CHOLESTEROL HDL
PRECIPITATING REAGENT

BioSystems
REAGENTS & INSTRUMENTS

CE

CHOLESTEROL HDL
PRECIPITATING REAGENT

PRINCIPLE OF THE METHOD

Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in the sample precipitate with phosphotungstate and magnesium ions. The supernatant contains high density lipoproteins (HDL). The HDL cholesterol is then spectrophotometrically measured by means of the coupled reactions described below[1,2].

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{chol. esterase}} \text{Cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{chol. oxidase}} \text{Cholestenone} + H_2O_2$$

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

CONTENTS AND COMPOSITION

A. Reagent: 1 x 50 mL. Phosphotungstate 0.4 mmol/L, magnesium chloride 20 mmol/L.

S. HDL Cholesterol Standard: 1 x 5 mL. Cholesterol 15 mg/dL. Aqueous primary standard.

STORAGE

Store at 2-8ºC.

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

Indications of deterioration:
- Reagent: Presence of particulate material, turbidity.
- Standard: Presence of particulate material, turbidity.

ADDITIONAL REAGENTS

These auxiliary reagents are to be used together with the Cholesterol Reagent contained in any of the BioSystems Cholesterol kits (cod. 11805, 11505, 11506, 11539).

REAGENT PREPARATION

Reagent and Standard are provided ready to use.

ADDITIONAL EQUIPMENT

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

SAMPLES

Serum or plasma collected by standard procedures.

HDL cholesterol in serum or plasma is stable for 7 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

PROCEDURE

Precipitation

1. Pipette into labelled centrifuge tubes (Note 1):

Sample	0.2 mL
Reagent (A) (Cholesterol HDL kit)	0.5 mL

2. Mix thoroughly and let stand for 10 minutes at room temperature.
3. Centrifuge at a minimum of 4000 r.p.m. for 10 minutes.
4. Carefully collect the supernatant (Note 2).

Colorimetry

5. Bring the Reagent (Cholesterol kit) to room temperature.
6. Pipette into labelled test tubes: (Note 3)

	Blank	Standard	Sample
Distilled water	100 µL	—	—
HDL Cholesterol Standard (S)	—	100 µL	—
Sample supernatant	—	—	100 µL
Reagent (A) (Cholesterol kit)	1.0 mL	1.0 mL	1.0 mL

7. Mix thoroughly and incubate the tubes for 30 minutes at room temperature (16-25ºC) or for 10 minutes at 37ºC.
8. Measure the absorbance (A) of the Standard and Sample at 500 nm against the Blank. The colour is stable for at least 30 minutes.

CALCULATIONS

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

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} \times \text{Sample dilution factor} = C_{Sample}$$

If the HDL Cholesterol Standard provided has been used to calibrate (Note 4):

$\frac{A_{Sample}}{A_{Standard}}$	x 52.5 = mg/dL HDL cholesterol x 1.36 = mmol/L HDL cholesterol

REFERENCE VALUES

HDL cholesterol concentrations vary considerably with age and sex. The following cut-off point has been recommended for identifiying individuals at high risk of coronary artery disease[3].

Up to 35 mg/dL = 0.91 mmol/L	High
> 60 mg/dL = > 1.56 mmol/L	Low

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005 and 18009) 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: 3.0 mg/dL = 0.078 mmol/L.
- Linearity limit: 150 mg/dL = 3.9 mmol/L.
- Repeatibility (within run):

Mean Concentration	CV	n
30 mg/dL = 0.78 mmol/L	3.3 %	20
55 mg/dL = 1.42 mmol/L	2.0 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
30 mg/dL = 0.78 mmol/L	4.2 %	20
55 mg/dL = 1.42 mmol/L	3.2 %	20

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 4). Details of the comparison experiments are available on request.
- Interferences: Lipemia (triglycerides 10 g/L) does not interfere. Bilirubin (10 mg/dL) and hemoglobin (5 g/L) may intefere. Other drugs and substances may interfere[4].

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

DIAGNOSTIC CHARACTERISTICS

HDL play an important part in the removal of cholesterol from tissues and its transportation to the liver for removal as bile acids.

Decreased plasma HDL-cholesterol concentrations are positively correlated with the incidence of atherosclerosic diseases, basis of myocardial infarction and cerebrovascular accidents[5,6].

There are several disease states or environmental influences associated with reduced levels of HDL: acute or chronic hepatocellular diseases, intravenous hyperalimentation, severe malnutrition, diabetes, chronic anemia, myeloproliferative disorders, Tangier disease, analphalipopro-teinemia, acute stress, some drugs and smoking[5,6].

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

NOTES

1. Sample and Reagent A volumes may be varied as long as the same ratio is maintained.
2. Supernatant must be clear. When supernatant is turbid or the pellet floats, add again 0.5 mL of Reagent A, mix thoroughly and centrifuge. Multiply the obtained concentration by 1.7 (dilution).
3. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
4. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

BIBLIOGRAPHY

1. Grove TH. Effect of reagent pH on determination of high-density lipoprotein cholesterol by precipitation with sodium phosphotungstate-magnesium. *Clin Chem* 1979; 25: 560-564.
2. Burstein M, Scholnick HR and Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *Scand J Clin Lab Invest* 1980; 40: 583-595.
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4. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
5. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. Burtis CA, Ashwood ER, Bruns DE. WB Saunders Co, 2005.
6. Friedman and Young. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.

M11648i-21

BioSystems S.A. Costa Brava 30, 08030 Barcelona (Spain)
Quality System certified according to
EN ISO 13485 and EN ISO 9001 standards

10/2013

Appendix V

BioMed Glucose L.S

BIOMED DIAGNOSTICS

Enzymatic colorimetric method (GOD- POD)

REF:

GLU109480	(4 x 120 ml)	GLU 109130 (2 x 65 ml)
GLU1091000	(2 x 500 ml)	GLU109250 (1 x 250 ml)
GLU 109100	(2 x 50 ml)	GLU109240 (2 x 120 ml)
	GLU1091001 (4 x 250 ml)	

INTENDED FOR USE :

For the quantitative determination of Glucose in serum, plasma and CSF.

PRINCIPLE :

The enzymatic method uses Glucose oxidase (GOD) to catalyze the oxidation of glucose to hydrogen peroxide and gluconic acid.
Hydrogen peroxide, when combined with 4-aminoantipyrine and aderivative from phenol , forms a red dye compound.
The intensity of the red colour produced is directly proportional to the glucose quantity in the sample.

SPECIMEN COLLECTION :

Non-hemolyzed serum or heparinized plasma and liquor (CSF).
Serum must be separated from the clot promptly. Glucose in serum is stable for 24 hours at +2/8°C., and 8 hours at room temperature.
Dilute 24h-Urine 1:10 with physiologic solution.
Shake and bring the samples at room temperature (+15-25°C) before using.

REAGENT COMPOSITION :

READY TO USE LIQUID REAGENT:			
R1	Glucose Standard	100 mg/dl (5.56 mmol/L)	
	Phosphate Buffer	100 mmol/L	
	Glucose oxidase	10000 U/L	
R2	Peroxidase	2000 U/L	
	4-AAP	1 mmol/L	
	Phenol	10 mmol/L	

PACKAGE : Collection & Storage .

Store at +2/8°C.
Stable till the expiration date reported upon the package.
After the unsealing and the taking of the reagent it is advised to close up the bottle immediately in order to avoid evaporation, direct light exposure and bacterial contamination.

PRECAUTIONS & WARNING :

Avoid pipetting with mouth.
The preparation, according to current regulation, is classified as not dangerous.
The total concentration of non active components (preservatives, detergents, stabilizers) is below the minimum required for citation.
Anyway handle with care, avoid ingestion, avoid contact with eyes, skin and mucous membranes. The samples must be handle aspotentially infected from HIV or Hepatitis.

REAGENT PREPARATION & STABILITY :

Liquid reagents must be at room temperature (+15/25 C) before using.
The reagent is limpid and rose-colored.
Pale coloring of the reagent (< 0.050 O.D.) due to air-light exposure doesn't compromise the working. Stable until the expiration date reported upon the label.

REQUIRED MATERIALS NOT PROVIDED :

General Laboratory Equipment and instrumentations.

PROCEDURE :

Wavelength: 546 nm
Optical path: 1 cm light path
Temperature: +37°C
Reading: Against blank reagent
Assay Type: End point
Sample/Reagent Ratio: 1/100

Pipetting in tubes:

	BLANK	STANDARD	SAMPLE
Reagent (R2)	1000 µL	1000 µL	1000 µL
Distilled water	10 µL		
Standard		10 µL	
Sample			10 µL

Mix, incubate for 10 min at 37°C and read sample and standard extinction.
Volumes can be proportionally modified.
This methodology describes the manual procedure to use the kit.
For automated procedure, ask for specific application.

CALCULATION :

Serum, plasma and liquor:

$$\textbf{Glucose } mg/dL = \frac{(A)\ Sample}{(A)\ Standard} \times 100\ (\text{standard value})$$

Urine:

$$\textbf{Glucose } mg/24h = \frac{(A)\ Sample}{(A)\ Standard} \times 10 \times L/24h$$

Standard 100 mg/dL = 5.56mmol/L.
To convert mg/dl in mmol/L, multiply by 0.0556.

EXPECTED VALUE :

serum , plasma:	60-110 mg/dL
	3.33-6.11 mmol/L
(CSF)	50- 70 mg/dL
	2.78-3.89 mmol/L
Urine	< 0.5 g/24h
	< 28 mmol/24h

The above mentioned values are to be considered as a reference. It is strongly recommended that each laboratory establish its own normal range

EXPECTED VALUES :

Men	40 – 160 mg/dl	0.45 – 1.82 mmol/l
Women	35 – 135 mg/dl	0.4 - 1.54 mmol/l

The above mentioned values are to be considered as a reference. It is strongly recommended that each laboratory establish its own normal range according to its geographic area , according to IFCC protocol .

WASTE DISPOSAL :

The disposal of the product must be in accordance with local regulation concerning waste disposal .

QUALITY CONTROL :

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology.

REFERENCES :

Fossati,P.,Principe, et al.Clin.Chem.28,2077-80(1982).
Vassault,A. et al. Ann.Biol.Clin.,44,686,(1986).

PERFORMANCE :

MEASURE INTERVAL L INEARITY :	4.78 - 900 mg/dl	
DETECTION LIMIT :	4.78 mg/dl	
SENSITIVITY:	5 mg/dl=0.00173A at 546 nm	
INTER-ASSAY PRECISION: n=20		
LOW LEVEL	M = 59.98 mg/dl	C.V.=2.85%
MEDIUM LEVEL	M = 120.64 mg/dl	C.V.=2.08%
HIGH LEVEL	M = 687.40 mg/dl	C.V.=2.43%
INTRA-ASSAY PRECISION: n=20		
LOW LEVEL	M = 57.03 mg/dl	C.V.2.3%
MEDIUM LEVEL	M = 123.08 mg/dl	C.V.1.2%
HIGH LEVEL	M = 686.23 mg/dl	C.V.2.4%
ANALIZED INTERVAL	42.5 – 383.9 mg/dl	
CORRELATION	r = 0.9981	n=53
LIN. REGRESSION	y = 1.054× +0.50915	n=53

INTERFERENCE:

Interferences are negligible up to :			
Glucose	5.0 mg/dl	Bilirubin	10 mg/dl
Ascorbic Acid	3.0 mg/dl	Hemoglobin	0.5 g/l
Uric Acid	20 mg/dl		

METHOD LIMITATIONS:

Glycerol (free glycerol and glycerol release upon hydrolysis of Triglicerides) is measured by this procedure.
Free glycerol levels in serum are generally low in fresh samples (<9,6 mg/dl), but elevation may be caused by improper storage or sample contamination.
For a thorough evaluation of the interfering substances , consult : Young, D.S.,et al.,Clin.Chem. 21:1D (1975).

	Consult Instructions for Use
	Caution, Consult accompanying Documents
IVD	In Vitro Diagnostic Medical Device
8°C / 2°C	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
	Use by

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Fax: +202 26240986
www.egy-chem.com

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EC REP
MOSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

EXPECTED VALUES :

Men	40 – 160 mg/dl	0.45 – 1.82 mmol/l
Women	35 – 135 mg/dl	0.4 - 1.54 mmol/l

The above mentioned values are to be considered as a reference. It is strongly recommended that each laboratory establish its own normal range according to its geographic area , according to IFCC protocol .

WASTE DISPOSAL :

The disposal of the product must be in accordance with local regulation concerning waste disposal .

QUALITY CONTROL :

It is recommended to execute the quality control at every kit utilization to verify that values are within the reference range indicated by the methodology.

REFERENCES :

Fossati,P.,Principe, et al.Clin.Chem.28,2077-80(1982).
Vassault,A. et al. Ann.Biol.Clin.,44,686,(1986).

PERFORMANCE :

MEASURE INTERVAL I. INEARITY :	4.78 - 900 mg/dl	
DETECTION LIMIT :	4.78 mg/dl	
SENSITIVITY:	5 mg/dl=0.00173A at 546 nm	
INTER-ASSAY PRECISION: n=20		
LOW LEVEL	M = 59.98 mg/dl	C.V.=2.85%
MEDIUM LEVEL	M = 120.64 mg/dl	C.V.=2.08%
HIGH LEVEL	M = 687.40 mg/dl	C.V.=2.43%
INTRA-ASSAY PRECISION: n=20		
LOW LEVEL	M = 57.03 mg/dl	C.V.2.3%
MEDIUM LEVEL	M = 123.08 mg/dl	C.V.1.2%
HIGH LEVEL	M = 686.23 mg/dl	C.V.2.4%
ANALIZED INTERVAL	42.5 – 383.9 mg/dl	
CORRELATION	r = 0.99811	n=53
LIN. REGRESSION	y = 1.054× +0.50915	n=53

INTERFERENCE:

Interferences are negligible up to :			
Glucose	5.0 mg/dl	Bilirubin	10 mg/dl
Ascorbic Acid	3.0 mg/dl	Hemoglobin	0.5 g/l
Uric Acid	20 mg/dl		

METHOD LIMITATIONS:

Glycerol (free glycerol and glycerol release upon hydrolysis of Triglicerides) is measured by this procedure.

Free glycerol levels in serum are generally low in fresh samples (<9,6 mg/dl), but elevation may be caused by improper storage or sample contamination.

For a thorough evaluation of the interfering substances , consult : Young, D.S.,et al.,Clin.Chem. 21:1D (1975).

	Consult Instructions for Use
	Caution, Consult accompanying Documents
IVD	In Vitro Diagnostic Medical Device
8°C / 2°C	Temperature Limitation
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Appendix VI

Document No. : INS-AA-EN (Rev. 14)
Revision date : February 8, 2017

Diabetes

ichroma™
HbA1c

INTENDED USE

ichroma™ HbA1c is a fluorescence Immunoassay (FIA) for the quantitative determination of HbA1c(Hemoglobin A1c) in human whole blood. It is useful as an aid in management and monitoring of the long-term glycemic status in patients with diabetes mellitus.
For *in vitro* diagnostic use only.

INTRODUCTION

Glycated protein is formed post-translationally through the slow, nonenzymatic reaction between glucose and amino groups on proteins. HbA1c is a clinically useful index of mean glycemia during the preceding 120 days, the average life span of erythrocytes. Carefully controlled studies have documented a close relationship between the concentrations of HbA1c and mean glycemia. HbA1c is considered as a more reliable parameter in monitoring glycemia over the glycemic reading with the conventional glucometer.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.
The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody. Instrument for ichroma™ tests displays the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood.

COMPONENTS

ichroma™ HbA1c consists of 'Cartridges', 'Detection Buffer Tubes', 'Hemolysis Buffer Vial' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human HbA1c at the test line; while rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human HbA1c-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is pre-dispensed in a separate tube.
- The hemolysis Buffer contains nonionic detergent and sodium azide as preservative in PBS.
- 25 detection buffer tubes and hemolysis buffer vial are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this 'Instruction for use'.
- It is recommended to use fresh samples.
- It is possible to use frozen samples. Please refer to "SAMPLE COLLECTION AND PROCESSING".
- Do not expose ichroma™ HbA1c test kit to direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip, detection buffer and hemolysis buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. HbA1c sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature about 30 minutes.
- **ichroma™ HbA1c** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The mixture of Detection Buffer and Hemolysis buffer must be used within 1 hour after mixing.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ HbA1c** will provide accurate and reliable results subject to the following conditions.
  - **ichroma™ HbA1c** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, sodium heparin, sodium citr**ate should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer pre-dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- The hemolysis buffer dispensed in a vial is stable for 20 months if stored at 4-30 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.
- The test environment conditions for **ichroma™ HbA1c** are as follow.
  - Temperature: 20-30 °C
  - Humidity: 10-70 %
  - i-chamber target temperature: 30 °C

MATERIALS SUPPLIED

REF CFPC-38

Components of **ichroma™ HbA1c**

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
- Detection Buffer Box
  - Detection Buffer Tubes 25
  - Hemolysis Buffer Vial (3 mL) 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ HbA1c**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ D** REF 13303
- **i-Chamber** REF FPRR009

양식-GE02-15 (Rev. 03)

- Ichroma™ Printer — REF FPRR007
- Boditech HbA1c Control — REF CFPO-96
- Boditech HbA1c Calibrator — REF CFPO-108
- 5 μL Capillary tube — REF CFPO-19

SAMPLE COLLECTION AND PROCESSING

The sample type for ichroma™ HbA1c is human whole blood.

- It is recommended to test the sample within 12 hours after collection.
- Samples may be stored for up to a week at 2-8 °C prior to being tested.
- If testing will be delayed more than a week, samples should be frozen at -70 °C or below. Samples stored frozen at -70 °C or below for 3 months showed no performance difference.
- Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in erroneous results.

TEST SETUP

- Check the components of the ichroma™ HbA1c as described below. : Cartridge, ID chip, instruction for use, detection buffer tube and hemolysis buffer vial
- Ensure that the lot number of the test cartridge matches that of ID chip, detection buffer as well as hemolysis buffer.
- Keep the sealed cartridge (if stored in refrigerator), detection buffer and hemolysis buffer at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for ichroma™ test.
- Insert the ID chip into the "ID chip port".
- Press the 'Select' button on the instrument for ichroma™ test. (Please refer to the 'Instrument for ichroma™ tests Operation manual' for complete information and operating instructions.)
- Insert a cartridge into I Chamber slot. Temperature of i-chamber should be 30 °C.

TEST PROCEDURE

1) Draw 100 μL of hemolysis buffer and transfer it into detection buffer tube.
2) Draw 5 μL of fingertip blood or tube blood using 5 μL capillary tube and put the capillary tube into the detection buffer tube.
3) Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 15 times.
4) Take out the cartridge half from i-Chamber slot.
5) Pipette out 75 μL of the sample mixture and load it into a sample well in the test cartridge.
6) Wait till the sample mixture flow appears in the windows. (about 10 seconds)
7) Insert the cartridge into i-Chamber slot (30 °C).
8) Leave the cartridge in i-Chamber for 12 minutes before removing.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If no', it will cause inexact test result.*
9) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
10) Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
11) Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
12) Read the test result on the display screen of the instrument for ichroma™ tests

INTERPRETATION OF TEST RESULT

- Instrument for ichroma™ tests calculates the test result automatically and displays HbA1c concentration of the test sample in terms of % (NGSP), mmol/mol (IFCC), mg/dL (eAG).
- The cut-off (reference range)
  - NGSP (%): 4.5-6.5 %
  - IFCC (mmol/mol): 26-48 mmol/mol
- Working range
  - NGSP (%): 4-15 %
  - IFCC (mmol/mol): 20.2-140.4 mmol/mol
  - eAG (mg/dL): 68.1-383.8 mg/dL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with ichroma™ HbA1c. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical Specificity**
  - Cross-reactivity

There was no significant cross-reactivity from these materials with the ichroma™ HbA1c test measurements.

Cross-reactivity material	Standard material conc		
	5.2 %	6.5 %	10.5 %
	Recovery (%)		
HbA0 (20 mg/mL)	99.9	96.3	99.0
HbA1a,b (20 mg/mL)	100.9	96.8	101.0
Acetylated hemoglobin (100 mg/mL)	101.0	98.4	99.7
Carbamylated hemoglobin (100 mg/mL)	100.5	97.8	100.0
Glycated human Albumin (100 mg/mL)	100.3	97.4	100.8
HbA1d (100 mg/mL)	100.9	97.0	100.3
Acetylated hemoglobin (100 mg/mL)	100.8	95.8	99.1

  - Interference

There was no significant interference from these materials with the ichroma™ HbA1c test measurements.

Interference material	Standard material conc		
	5.2 %	6.5 %	10.5 %
	Recovery (%)		
Non-interference	101.0	96.2	98.7
Acetaminophen (20 mg/dL)	100.4	97.4	100.9
L-ascorbic acid (500 mg/dL)	101.0	97.8	99.8
Bilirubin (2 g/dL)	100.8	97.8	100.4
D-glucose (1,000 mg/dL)	100.9	97.6	99.8
Intralipid (800 U/L)	100.8	96.2	100.6
Triglyceride (827 M)	100.9	98.1	99.6
Urea (125 g/dL)	100.1	98.1	99.2

- **Precision**

The intra-assay precision was calculated by one evaluator, who tested different concentration of control standard five times each with three different lots of ichroma™ HbA1c.

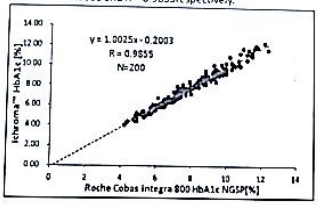
HbA1c (%)	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)	Accuracy (%)
5.2	5.28	5.18	5.24	5.23	0.12	2.36	100.6
6.5	6.46	6.48	6.38	6.44	0.16	1.99	98.9
10.5	10.4	10.56	10.38	10.51	0.19	1.83	100.1

The inter-assay precision was confirmed by 3 different evaluators with 3 different lots, testing five times each different concentration.

HbA1c (%)	Between-person			Between-lot		
	AVG	SD	CV (%)	AVG	SD	CV (%)
5.2	5.19	0.03	0.61	5.23	0.05	0.96
6.5	6.51	0.02	0.36	6.49	0.07	1.12
10.5	10.50	0.01	0.10	10.51	0.19	0.92

- **Comparability:**

HbA1c concentrations of 200 clinical samples were quantified independently with ichroma™ HbA1c and Roche Cobas integra800 as per prescribed test procedures. Test results were compared and their comparability was investigated with Linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=1.0025X - 0.2003 and R = 0.9855respectively.



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Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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1 / 1