Sudan University of Science and Technology

College of Graduate Studies

Assessment of Triglycerides to High Density Lipoprotein Cholesterol Ratio and C-reactive Protein among Type 2 Diabetic Patients in Khartoum State

تقييم نسبة الدهون الثلاثية إلى نسبة كلويسترول البروتين الدهني عالي الكثافة والبروتين التفاعلي C عند مرضى السكري من النوع الثاني في ولاية الخرطوم

A Disseration Submitted in Partial Fulfillment for the Requirements of the Degree of M.Sc. in Medical Laboratory Science
(Clinical Chemistry)

Prepared By :

Manasik Adam Ahmed Zaytoun

Supervisor:

Dr. Mariam Abbas Ibrahim
Sudan University of Science and Technology
College of Medical Laboratory Science
Clinical Chemistry Department

November 2020

قال تعالى :

بسم الله الرحمن الرحيم

{قَالَ رَبِّ ٱشۡرَحۡ لِي صَدۡرِي (25) وَيَسِّرۡ لِيٓ أَمۡرِي (26) وَٱحۡلُلۡ عُقۡدَةٗ مِّن لِّسَانِي (27) }

" صدق الله العظيم "

سورة طٰـه ~الآية (25 - 27)

الإهـــــداء

اهدي هذا العمل المتواضع
الى
الوالدين الكريمين حفظهما الله
إلى
زوجي وكل افراد عائلتي الأعزاء
إلى
كل الاصدقاء
إلى
مشرفتي العزيزة الدكتورة مريم عباس

Acknowledgment

Firstly, I thank Allah…….

I would like to thank my supervisor Dr. Mariam Abass Ibrahim for this support and help. I am very fortunate and great full to head of the laboratory department of Ombada hospital and EL-emtiaz medical center. I want to thanks the entire researcher to their effort and supply us by knowledge.

English Abstract

Background: cardiovascular diseases is the most important cause of morbidity and mortality among patients with type2 diabetes mellitus and the TG:HDLc ratio recently used as predict subject at increased risk of developing metabolic and cardiovascular complications and HDLc level are inversely related to plasma TGs level and there is a dynamic interaction between high density lipoprotein and triglycerides rich lipoprotein in vivo.

Method: The study includes 52 diagnosed diabetic patients (14 male and 38 female) and 52 apparently healthy individuals (control) (14 male and 38 female) and Age was matched (32_70) years all samples were collected from Ombada Hospital and El-emtiaz medical center, from 12 November to 1 September. Statistical procedure was followed using statistic package for social science (SPSS) version 16 on programmed computer.

Result: The result showed a significant increase in TG: HDLc ratio among type2 diabetic patients in comparison with control group (9.2±6.9, 1.69±0.47mg/dl), for mean and standard deviation for case and control respectively with *P*-value 0.016, significant increase in TGs between type2 diabetic patients in comparison with non-diabetic group(169±116, 88±17.5mg/dl), for mean and standard deviation for case and control reactively with *P*-value 0.056, significant decrease in HDLc among type2 diabetic patients in comparison with control group(34±14, 50±9.5 mg/dl), for mean and standard deviation for case and control respectively with *P*-value 0.000, significant increase in percentage of CRP among type2 diabetic patients in comparison with control group (33%, 0.0%), with *P*-value 0.02 for case and control respectively and insignificant difference in BMI between type2 diabetic patients and control group

(22±4.8, 21±3.8kg/cm^2) for case and control respectively with *P*-value0.677.

Also no correlation between ratio and age, BMI, duration, among type2 diabetic patients with *P*-value (p =0.325), (p =0.800), (p =0.130) respectively, No correlation between HbA1C and Age, BMI, duration, among type2 diabetic patients with *P*-value (p= .977), (p= .208), (p = 0.604) respectively. Also no correlation between CRP and age, BMI, duration, with *P-value* (p = 0.613), (p =0.306),(p =0.588) respectively, no correlation between HDLc and Age, BMI, Duration with *P*-value(p =0.607), (p =0.825), (p =0.230) respectively, and no correlation between TG and Age, BMI, Duration with r and *P*-value (p =0.244), (p =0.561), (p =0.215) respectively in conclusion type2diabetes mellitus had increase HbA1c, TGs, CRP, TG:HDLc ratio and deteriorated HDLc compare with non-diabetic group.

Conclusion: In conclusion type 2 diabetic patients had increased triglycerides and triglyceride / high density lipoprotein cholesterol ratio, decreased high density lipoprotein cholesterol and more positively CRP results compared with control group and no difference observed in BMI among type2 diabetic patients compared with control group .No correlation between TG, HDLc, TG: HDLc, HbA1c and CRP with age, BMI, Duration among type 2 diabetic patients.

Arabic Abstract

خلفية: تعد أمراض الأوعية الدمويه من أهم الأمراض التي تؤدي إلى الوفاة عند مرضى السكري من النوع الثاني، حديثاً تم استخدام قياس نسبة الكوليسترول عالي الكثافة إلى نسبة الدهون الثلاثية كمؤشر يدل على خطورة مضاعفات الأمراض الاستقلابية والأمراض القلبية. وتناسب البروتين عالي الكثافة عكسياً مع نسبه الدهون الثلاثية في بلازما الدم.

الطريقة: احتوت الدراسة على ٥٢ من المرضى الذين تم تشخيصهم بمرض السكري من النوع الثاني(١٤من الذكور و٣٨ من الاناث) و٥٢ من الأشخاص الاصحاء كمجموعة تحكم(١٤من الذكور و٣٨ من الاناث)وطوبقت الأعمار(٣٢-٧٠سنه)،تم جمع العينات من مستشفى امبده النموذجي ومن مركز الامتياز الطبي، في الفترة من ١٢ نوفمبر الى ١ سبتمبر. تم تطبيق برنامج الحزم الاحصائيه بالنسخه١٦.

النتيجة : كانت النتيجة إرتفاع ملحوظ في نسبة الدهون الثلاثية إلى نسبة الكوليسترول عالي الكثافة عند مرضى السكري من النوع الثاني مقارنة بمجموعة التحكم (٩,٢±٦,٩, ١,٦٩±٠,٤٧) والقيمة الاحتمالية تساوي٠,٠١٦وللوسط والانحراف المعياري على التوالي. كما أظهرت النتائج إرتفاع نسبة الدهون الثلاثية عند مرضى السكري مقارنة مع مجموعةالتحكم (١٦٩±١١٦, ٨٨±١٧,٥)،للوسط والانحراف المعياري المجموعتين على التوالي والقيمة الاحتمالية تساوي0.056، انخفاض في نسبة الدهون عالية الكثافه عند مرضى السكري من النوع الثاني مقارنة بمجموعة التحكم(٣٤±١٤, ٥٠±٩,٥)، للوسط والانحراف المعياري المجموعتين على التوالي والقيمة الاحتمالية تساوي٠,٠٠٠ ارتفاع في نسبة البروتين المتفاعل(C) عند مرضى السكري من النوع الثاني مقارنة بمجموعة التحكم (٣٣%, ٠%, ٠%) والقيمة الاحتمالية تساوي٠,٠٢للنسبه المئويه المجموعتين على التوالي، عدم وجود فرق في مؤشركتلة الجسم عند مرضى السكري من النوع الثاني مقارنة بمجموعة التحكم (٢٢±٤,٨ ٢١±٣,٨)،للوسط والانحراف المعياري للمجموعتين على التوالي والقيمة الاحتمالية تساوي٠,٦٧٧. كما أنه لا توجد علاقة بين نسبة الكوليسترول عالي الكثافة إلى نسبة الدهون الثلاثية وبين العمر وكتلة الجسم والمدى الزمني لمرض السكري من النوع الثاني ، القيمة الاحتمالية تساوي

٠,٣٢٥ ،٠,٨٠٠ ،٠,١٣٠على التوالي، لاتوجد علاقة أيضا بين نسبة HbA1c وبين العمر وكتلة الجسم والمدى الزمني لمرض السكري من النوع الثاني ، القيمة الاحتمالية تساوي

٠,٩٧٧ ،٠,٢٠٨ ،٠,٦٠٤على التوالي، لاتوجد علاقة بين نسبة البروتين المتفاعل(C) وبين العمر وكتلة الجسم والمدى الزمني لمرض السكري من النوع الثاني ، القيمة الاحتمالية تساوي

٠,٦١٣، ٠,٠٣٠٦، ٠,٥٨٨لاتوجد علاقة بين نسبة الكوليسترول عالي الكثافة وبين العمر وكتلة الجسم والمدى الزمني لمرض السكري من النوع الثاني ، القيمة الاحتمالية تساوي ٠,٦٠٧، ٠,٨٢٥، ٠,٢٣٠كما أنه لاتوجد علاقه بين نسبة الدهون الثلاثية وبين العمر وكتلة الجسم والمدى الزمني لمرض السكري من النوع الثاني ، القيمة الاحتمالية تساوي ٠,٢٤٤، ٠,٥٦١، ٠,٢١٥ علي التوالي.

الخلاصة: خلصت الدراسة الى ان مرضى السكري من النوع الثاني لديهم ارتفاع في نسبة الدهون الثلاثية ونسبة الدهون الثلاثية الى نسبة الكوليسترول عالي الكثافة ، انخفاض في نسبة الكوليسترول عالي الكثافة وعدد اكبر من النتائج الايجابية للبروتين المتفاعل (C) مقارنة مع مجموعة التحكم، كما أنه لا يوجد فرق في مؤشر كتلة الجسم عند مرضى السكري مقارنة مع مجموعة التحكم .كما لا توجد علاقه بين الدهون الثلاثية ،الكوليسترول عالي الكثافة ،نسبة الكوليسترول عالي الكثافه الى الدهون الثلاثيه،الهيموقلوبين السكري ،البروتين المتفاعل C وبين العمر وكتلة الجسم والمدى الزمني للمرض عند مرضى السكري من النوع الثاني.

List of Contents

List of Tables

List of abbreviations

Abbreviation	Full terms
ADA	American Diabetic Association
APO	Apo lipo protein
ATP	Adenosine Tri Phosphate
BMT	Body Mass Index
CHD	Coronary Heart Disease
CRP	C Reactive Protein
CVD	Cardio Vascular Disease
D M	Diabetes Mellitus
DKA	Diabetic Keto Acidosis
DPP-4	Dipeptidyl Peptidase – 4
DR	Death Receptor
EDTA	Ethylen Diamine Tetra Acetic Acid
GAD	Glutamic Acid Decarboxylase
GLP-1	Glucagon – like Peptide
HMG- COA	Hydroxy methyle Glutamyl Co enzyme A
H S CRP	High Sensitive CRP
HbAIC	Glycated Hemoglobin
HDLC	High Density lipoProtein
HNF	Hepatocyte Nuclear Factor
IFG	Impire fasting Glucose
IA	Islet cell Auto Antibody
IGT	Impire Glucose Tolerance
LDL	Low Density Lipoprotein
LADA	Latent Auto Immune Diabetes in Adult
MODY	Maturity Onset Diabetes of the Young
OGTT	Oral Glucose Tolerance Test
SGLT2	Sodium Glucose Co Transporter
SPSS	Statistical Package For the Social Science
TG	Triglycerides
VLDL	Very Low Density LipoProtein

Chapter one

Introduction, Rationale, objectives

Introduction, Rationale, objectives

1.1 Introduction

Diabetes is an increasingly important problem, worldwide estimated to be 9.3% in 2019, prevalence higher in urban 10.8% than rural 7.2% and in high income 10.4% than low income countries 4.0% one in two (50.1)% people living with diabetes do not know that they have diabetes (Yuen *et al*.,2019).

Seven countries in eastern mediterranean region have seven high prevalence of DM and seven have medium prevalence 9-12% of DM, the Sudan is one of the medium prevalence. Type 2 DM is the major type of DM accounting for approximately 90% of all cases, the estimate prevalence of DM in Africa in 2017 was 3.3% and the Sudan was among the country that had a prevalence of DM of more than 12%(Omar *et al*.,2019).

With increase in prevalence of type2 DM, arise in incidence of secondary co morbidity including hypertension, nephropathy and retinopathy (Hamiel and Zeitler, 2007).

The importance of protecting the body from hyperglycemia cannot be overstating the direct and indirect worldwide effect on the human vascular tree (are the major source of morbidity and mortality in both type 1 and type 2 DM) (Fawler, 2008).

1.2 Rationale

Cardiovascular disease is the most important cause of morbidity and mortality among patients with type2 DM.Many recent studies demonstrates that there is strong relationship between abnormal TG:HDLc ratio and positively CRP result with atherosclerosis, and therefore we will try to demonstrate this correlation among Sudanese diabetic patients due to lack of researches that clarify this relationship,

and to give an entrance for other researches that joining TG:HDLc ratio with high sensitive CRP and other factors among Sudanese diabetic patients.

1.3 Objective

1.3.1 General objective

To assess triglycerides: HDLc ratio and CRP in type2 DM as a marker for atherogenic index.

1.3.2 Specific objective

1. To measure and triglycerides, HDLc, CRP among type2diabetic patients and control group.
2. To measure HbA1c to classify diabetic patients according to their glycemic control.
3. To calculate triglycerides: HDLc ratio among the two study groups.
4. To correlate triglycerides: HDLc ratio with CRP as indicator for the atherogenic process.
5. To correlate triglycerides,TG: HDLc , HDLc ,CRP with Age, Duration and BMI among the diabetic group.

Chapter Two

Literature Review

Literature Review

2.1 Diabetes mellitus

Clinical diabetes mellitus is a syndrome of disordered metabolism with inappropriate hyperglycemia due either to an absolute deficiency of insulin secretion or a reduction in the biologic effectiveness of insulin or both (Gardner and Shoback, 2007).

2.1.1 Type 1 Diabetes mellitus

Type 1 diabetes is a chronic auto immune disease characterize by insulin deficiency and resultant hyperglycemia (Dimeglio *et al.,* 2018). Most common chronic chilhood illnesses, affecting 18 to20 per 100000 children ayear in United kingdom(Devendra *et al.,*2004).The causes of type 1diabetes is unknown (WHO, 2013) however it is believed to involve a combination of genetic and environmental factor (Yoon,1990),it is consider as a result of cellular mediated auto immune destruction of beta cell of the pancreas causing an absolute deficiency of insulin secretion (Islet cell auto antibodies) or due antibodies against insulin (insulin auto antibodies), glutamic acid decarboxylase auto antibodies, tyrosine phosphatase IA -2 and IA-2B *(*Bishop *et al.,*2018).The largest genetic component of the risk for type1 diabetes, is the major histocompatibility complex in chromosome 6, including DR3-DQ2 and DR4-DQ8 alleles that increase risk, and DR2-DQ6 alleles which is protective(Karuranga *et al.,*2008).Environmental factor despite decades of research, the relation between viruses and islets cell are circumstantial, the most robust association with viruses and type 1 diabetes involve enterovirus species, of which some strain have the ability to induce or accelerate disease in animal models (Coppieters *et al.,*2012). Congenital rubella, coxakie B, and viruses such as cytomegalovirus, adenovirus and mumps are also involved (Who, 2019).

Family members who expressed auto antibodies to insulin, GAD 65 and IA-2 have 75% 5-yrs risk of diabetes compared with a 25% 5-yrs risk in relative who expressed one of those auto antibodies (Barker,2006).

2.1.1.1 Latent Autoimmune Diabetes in Adult (LADA):

LADA is slowly progressive form of autoimmune diabetes that develops in adult and does not require insulin therapy for some time after diagnosis (Redondo,2013), it is occur in 10% of individual older than 35 years and in 25% below that age. Prospective studies of β cell function show that LADA patients with multiple islets antibodies develop β cell failure within 5 years (*S*tenstrom *et al.*,2005).The diagnosis of LADA is currently based on three criteria.A.adult age at onset of diabetes. B. The presence of circulating Islet autoantibodies (Distinguish LADA from classic type2 diabetes). C. Insulin independence at diagnosis (Distinguish LADA from classic type 1diabetes) (Fourlanos *et al.,* 2005).

2.1.2 Typ2 diabetes mellitus:

Previously called noninsulin diabetes mellitus, this is the most common variety worldwide (90% of all diabetes causes), (Crook,2012).More than 90% of type2 diabetes is insulin resistant and at least 25% of subject with normal glucose tolerance may be insulin resistant, but secrete sufficient insulin to overcome resistance (Melmed and Coon,2005).Most patients in this type are obese or have an increase percentage of body fat distribution in the abdominal region.an increase in age, obesity and lack of exercise (Bishop, 2018), ethnicity and family history, smoking, in adequate intake of fruit and vegetables, whole grains and dietary fibers and high intake of energy as saturated fat. Consider as risk factor for type2 D.M (Karuranga *et al.*,2017).The causes of type2 diabetes mellitus remain unclear. Insulin resistance and abnormal insulin secretions are central to the development of type2 D.M.

A- Insulin resistance syndrome (syndrome x): is defined as decrease biological response to normal concentration of circulating insulin.

B- Loss of B cell function: the increase of β cell demand induced by insulin resistance is ultimately associated with progressive loss of B cell function that is necessary for the development of fasting hyperglycemia (Burtis *et al*.,2008).

Type2 diabetes has strong genetic component, The familial clustering of type2 diabetes suggest strong genetic component .monozygotic twins have a60% to90% concordance for type2 diabetes. The risk for type 2 in siblings of diabetic patients is 10% to33% versus5% for the general population. Off spring of women with type2 diabetes have two fold to three fold greater risk for diabetes, than offspring of men with the disease (Dermmott, 2013).

2.1.3 Gestational diabetes mellitus:

Gestational diabetes is defined as any degree of glucose in tolerance with onset or first recognition during pregnancy.

Usually occur in Women who are overweight or women with family members who have had gestational diabetes or have type2 diabetes (African American Indian and Hispanic)(Buchanan *et al* ,2007).

In UK about 4-5% of pregnancies are complicated by gestational diabetes mellitus it is associated with increased fetal abnormalities for example high birth weight, cardiac defect and poly hydrmnios. In addition, birth complication, maternal hypertension and need for cesarean section may occur (Crook, 2012).

2.1.3.1Diagnosis of Gestational diabetes mellitus:

It diagnosed if two or more of the value (venous serum or plasma glucose level are met or exceeded (Seshiah *et al*., 2004).

2.1.3.2 Treatment of gestational diabetes:

Excellent glycemic control to prevent complication in the newborn monitoring and insulin some cases with high dosage, >75% of these women will go to develop type2 DM) in later life (Greensteinand Wood, 2011).

Also stay at a healthy weight, eat healthy diet, get active and tested regularly can reduce the percentage (Davis*et al.*, 2009).

2.1.4 Other types of diabetes mellitus:

Diabetes may cause by:

1. **Disease of exocrine pancreas:**

Fibro calclouspancreatopathy, pancreatitis, trauma, pancreatectomy, Neoplasia, heamochromatosis and others (Who,2020).

2. **Disease of endocrine pancreas:**

Cushing Syndrom, Acromegaly, Pheaochromocytom, Glycogenom, Hyperthyrodism,Polyglandular Syndrom1, 2 and others (Wass and Owen ,2014).

3. **Drugs of chemical during therapeutic use:**

Pantamidine, Asperginase, Dizoxide (by interfering with insulin production and secretion).Steroid, Betagonists, growth hormone (reducing effectiveness of insulin). Thiazide diuretics, cyclosporine, Fr-506 (act on both insulin secretion and action). Nicotinic acid, total parenteral nutrition, miscellaneous drugs (act independent of insulin) (Mohan and Mohan,1997).

4. **Infections**:

Cytomegalovirus (Hjelmeasaeth *et al.,* 2005), Coxackivirus, mumps, rubella, and others (Who,2019).

2.1.5 Genetic defect of β cell function:

Monogenic diabetes:

Monogenic diabetes is account for approximately 1-2% of all cases of diabetes with overlapping clinical features with common form of Diabetes, make diagnosis challenging (Carroll and Murphy,2013). Its Diabetes result from mutations in a single gene. the main form of monogenic diabetes is:

Neonatal Diabetes: condition occur in the first 6 month of life, infants do not grow well before birth and born small for their age.

Permanent Neonatal Diabetes (lifelong condition).

Transient Neonatal Diabetes which goes away during infancy but can return later in life(Anhalt *et al.*,2017).

Maturity Onset Diabetes of the Young (MODY):

MODY1: Mutation of hepatocyte nuclear factor (HNF4A).

MODY2: Mutation of glucokinase gene.

MODY3: Mutation of the HNFIA gene (Crook,2012).

2.1.6 Genetic defect in insulin action:

1) Lipo dystrophies.
2) Insulin receptor mutation.
3) Rare Down Stream insulin signaling defect(Wass and Owen,2014).

2.1.7 Other genetic syndrom like:

Down syndrome, Walfer syndrome

,Turner syndrome, Prades-willi syndrome (Baco,2019).

2.1.8 Uncommon forms of immune mediated diabetes:

Stiff person syndrome, anti-insulin receptor antibody.(Azam,2013).

Impair glucose tolerance(also known as prediabetes):

prediabetes is an intermediate state of hyperglycemia with glycemic parameter above normal but below the diabetes threshold. It remains a state of high risk of developing diabetes with yearly conversion rate of 5-10 % prediabetes commonly **.**

The mechanism whereby obesity predispose to prediabetes and metabolic syndrome are incompletely understood but likely have a common metabolic soil(Grundy,2012).Subject with IFG /IGT have an increased risk of diabetes and higher prevalence of cardiovascular disease than norm glycemic (Colagiuri,2011).

Metabolic syndrome: metabolic syndrome is multiplex risk factor that consists of several risk correlates of metabolic origin.in addition to dyslipidemia, hypertension, and hyperglycemia (Grundy,2008).the pathophysiology seems to be largely attributable to insulin resistance with excessive flux of fatty acids implicated (Eckel*et al*.,2005).

2.1.1.1 The Pathogenesis of type1 diabetes mellitus:

It depends on destruction of β cell by host T cell which causes complete absence of insulin. The auto immune process include Auto antibodies to Islet cell, insulin and glutamic acid decarboxylase, and also auto immune process may related to genetic predisposition in combination with environmental factor (Dermmott,2013). The contribution of T cell in pathogenesis is beyond doubt. Therapies directed against T cell have been shown to halt the disease and prevent recurrent β cell destruction after Islet transplantation (Roep,2003).Various inflammatory cy tokines and oxidative stress produced by Islet-infiltrating immune cells have been proposed to play an important role in mediating the destruction of β cell (anti-oxidant and redox signaling) (Kanto *et al*.,2007).

2.1.2.1 The Pathogenesis of type2 diabetes mellitus:

When glucose hemostasis defect by reduce β cell mass (either through genetic and or cytotoxic factor) predispose for glucose in tolerance raise in blood glucose make further defect in βcell responsiveness to meal by impairing the first phase Insulin response, also adipocyte play very important role in maintenance total body glucose homeostasis by regulating the release of free fatty acid and production of adipocytokinase that influence insulin sensitivity in muscle and liver (Leahy, 2005).

Also Raise in blood glucose perhaps in concert with fatty acid (feature for obesity and insulin resistance) cause additional deterioration in β cell function along with further Insulin resistance(Antonio and Drive,2004).

Signs and symptoms of diabetes mellitus:

Increased thirst, Frequent urination, Extreme hunger, Fatigue, Irritability, Blurred vision, Slow healing sores, Frequent infec toin, Unexplained weight loss (Myoclinic,2019), Numbness or tingling in hands and feet (*Martin et al ;2003*), Bedwetting (type1 DM) (Cho *et al.*,2017).

The Complications of diabetes mellitus:

People with type2 diabetes are at increased risk of many complications, which are mainly due to complex and interconnected mechanisms such as hyperglycemia, insulin-resistance, low grade inflammation and accelerated atherogenesis (Schlienger,2013).

1. Acute complications of diabetes mellitus:

It include hyper osmolar non ketotic coma. (HNC)(Result from insulin deficiency), Lactic acidosis (Associate with other factors that may be related to diabetes such as cardiovascular disease), hypoglycemia (from the treatment of diabetes). (Fishbin and Palumbo, 1995) and diabetic ketoasidosis.

Diabetic ketoacidosis: (DKA) is life threatening condition in which sever deficiency lead to hyperglycemia. Excessive lipolysis, and

unrestrained fatty acid oxidation producting the ketone bodies acetone (Wiliams, 2003).

2. Chronic complications of diabetes mellitus:

The Micro vascular complications of diabetes mellitus:

A. Diabetic retinopathy:

Diabetic retinopathy it is involves anatomic changes in retinal vessels and neurologia. The pathogenetic mechanisms is imperfectly understood (Engerman, 1989), the molecular and physiologic abnormalities that have been found to develop in the retina in diabetes are consistent with inflammation (Tang and Kern,2011).

B. Diabetic nephropathy:

Diabetic nephropathy is the leading cause of renal failure in the United States.it is defined by proteinuria >500mg in 24hours in the setting of diabetes, but is preceded by lower degree of proteinuria (**micro albuminuria** is defined as albumin excretion of 30-299mg⁄24 hours (Fawler,2008).

It occur in 15-40% of patients with type1and 5-20% in patients with type2, the pathogenic mechanism involve generation of reactive oxygen species (ROS) accumulation of adrenal glycation end product (AGE), and activation of intracellular signaling molecules like protein kinase C (Chawla *et al.*,2016).

C. Diabetic neuropathy:

Diabetic neuropathy is the most common neuropathy in industrialized countries, responsible for most limb Amputations and considerable morbidity in diabetic patients (Said, 2007).

It is exact pathogenetic mechanism remains unclear (Greene*et al.*,1990).

The Diagnosis of diabetes mellitus:

- Symptoms plus random plasma glucose concentration ≥11.1 mmol/l (200 mg/dl),

Or fasting plasma glucose ≥7.0 mmol/l (126 mg/dl)(Silink M *et al* ; 2004).

- 2-h plasma glucose ≥11.1 mmol/l (200 mg/dl) during an oral glucose tolerance test (OGTT).

The test should be performed in the morning, after an overnight fast of 8–14 h, using glucose load containing the equivalent of 1.75 g/kg anhydrous glucose up to a maximum of 75 g (i.e., 1.75 g/kg for those weighing <43 kg and 75 g for those weighing > 43 kg). The glucose load should be dissolved in 250–300 ml water and drunk over the course of 5 min. The test is timed from the beginning of the drink. Before the OGTT, there should have been at least 3 days of an unrestricted diet containing at least 150 g of carbohydrate daily.

Prediabetes:

- Impaired glucose tolerance (IGT) = 2-h plasma glucose ≥7.8 to

<11.1 mmol/l (≥140 to <200 mg/dl)

- Impaired fasting glucose (IFG) = ≥ 6.1 to < 7.0 mmol/l (≥110 to <126 mg/dl) (Silink *et al.*,2003).

Treatment of diabetes mellitus:

For type1 diabetes administration of Basel, exogenous insulin is essential for regulating glycogen break down, gluconeogenesis, lipolysis and ketogenesis, and it is appropriate for carbohydrate intake and promotes normal glucose utilization and storage (Jameson.,2010). For type2 diabetes: metformin (Glucophage, glumetza, others), sulfonylureas, meglitinides Thiazolidinediones, DPP -4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, insulin (Myoclinic ,2019).

2.2Triglycerides:

Triglyceride classified as neutral lipid, its contain three fatty acid molecules attached to one molecule of glycerol by ester bond(Bishop,2018).Dietary s are digested in the duodenum and absorbed in the proximal ileum through pancreatic and intestinal lipase and bile acids, first hydrolyzed to glycerol monoglycerides and fatty acids, these component are ressembled as triglyceridess in the intestinal epithelial cells and then package with cholesterol and apo B—48 to form chylomicrons(Burtis *et al.*,2008).

although four databases were searched without language restriction for relevant studies(PubMed, Science Direct, EMBASE, and Google Scholar), All prospective cohort studies reporting an association between TG and CVDs or all-cause mortality published before July 2013 were include The relationship of triglycerides to (CVD) remains unclear(Liu *et al.*,2013). Different study like (Vega *et al.*,2014), (Khan *et al.*,2008),(Awadalla *etal.*,2018) and others were proved The elevated triglycerides in patients with type2 diabetes mellitus, the mechanism is still unclear. Triglycerides can be measured in the non-fasting or fasting states, with concentrations of 2–10 mmol/L conferring increased risk of cardiovascular disease, and concentrations greater than 10 mmol/L conferring increased risk of acute pancreatitis and possibly cardiovascular disease (Nordestegaard,2014).

2.3High Density Lipoprotein(HDLc):

Its smallest and most dense lipoprotein particle, synthesized by liver and intestine. Discoidal or spherical shape, Discoidal represent recent and most active form in removing excess cholesterol from peripheral cell (Bishop *et al.*,2018).Plasma levels of high-density lipoprotein (HDLc) cholesterol are strongly inversely associated with atherosclerotic CVD.

The molecular regulation of HDL metabolism is not fully under stood, but it is influenced by several extracellular lipase (Jin *et al.*,2002).

there are many scientific studies prove the relationship between the Low density HDLC and the cardiovascular disease in patients with diabetes mellitus like (Golay *et al.*,1987), (Awadalla *et al*.,2018), (Elnasri and Ahmed.,2008) and others. Alterations of HDLc in diabetes are probably based on the presence of insulin deficiency or, more commonly, on hyperinsulinemia and insulin resistance. HDLc (particularly the subfractionHDLc2) concentration is regulated by two endothelial lipolytic enzymes, lipoprotein lipase and hepatic lipase, both of which are insulin sensitive (Nikkila,1981).

2.4 The Triglycerides: high density lipoprotein ratio:

The triglycerides: HDLc ratio recently used as predict subject at increased risk of developing metabolic and cardiovascular complications. (tommaso *et al.*,2013).Also its prdictive for the severity of CHD. It could predict in hospital_new onset heart failure incidents of CHD patients (Yunke *et al*., 2014).

HDLc levels are inversely related to plasma triglycerides levels and there is a dynamic interaction between HDLc and triglycerides (TGs) rich lipoproteins in vivo (Lamarche, 1999).

The atherogenic link between high Triglycerides and HDLc-cholesterol is due to higher plasma concentration of Triglycerides-rich; very low density lipoprotein, That generates small, dense LDL, during lipid exchange and lipolysis. This LDL particle accumulate in the circulation and form small dense HDLc particles, which undergo accelerated catabolism, this dosing the atherogenic circle (Protasio *et al.*, 2008**).**

The treatment of lipids disordered include statin, one of the most powerful classes of agents for the treatment of cardiovascular diseases the

reductions in circulating serum lipid levels that were mediated by inhibition of liver 3-hydroxy 3-methyl glutaryl coenzyme A (HMG-CoA) reductase (Lefer, 2002).

Dietary supplementation with soluble fiber, such as psyllium husk, oat bran, guar gum and pectin, and fruit and vegetable fibers, lowers serum LDL cholesterol concentrations by 5 to 10 percent (Knop,1999).

The Macro vascular complication of diabetes:

The major cause of death in people with type2 diabetes according for 50% of death in this group, the relative risk for cardiovascular disease is 2 to 3 times higher in men and 3 to 4 times higher in women (Greenstein and Wood, 2011).

2.5 Dyslipidemia:

Usual risk factor for coronary artery disease account for 25-50%increased atherosclerotic risk in diabetes mellitus. Insulin resistant diabetes course affects virtually all lipids and lipoprotein (Kreisberg, 1998).

There is strong evidence that cholesterol lowering improve cardiovascular outcome (Schofield *et al.,* 2016).

A significant component of the risk association with type2 because of itis characteristic lipid (triad) profile of raised small dense, low density lipoprotein, and elevated triglyceridess (TGs) (Carmena, 2005).

The Pathogeneses of atherosclerosis:

Atherosclerosis develops over the course of 50 years, beginning in the early teenage years. The causes of this process appear to be lipid retention, oxidation, and modification, which provoke chronic inflammation at susceptible sites in the walls of all major conduit arteries. Initial fatty streaks evolve into fibrous plaques, some of which develop into forms that are vulnerable to rupture, causing thrombosis or stenosis. Erosion of the surfaces of some plaques and rupture of a plaque's calcific nodule into the artery lumen also may trigger thrombosis. The process of

plaque development is the same regardless of race/ethnicity, sex, or geographic location, apparently worldwide. However, the rate of development is faster in patients with risk factors such as hypertension, tobacco smoking, diabetes mellitus, obesity, and genetic predisposition. Clinical trial data demonstrate that treatment with 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) favorably alters plaque size, cellular composition, chemical composition, and biological activities centered on inflammation and cholesterol metabolism, as well as the risk of clinical events due to atherosclerosis.(Insull. 2009)

2.6 C-reactive protein (CRP):

CRP is an ancient highly conserved molecule and member of pentraxin family of proteins, secretes by liver in response to trauma, infection, inflammation (Do clos, 2000).

Recent evidence implicates inflammation in the pathogenesis of coronary heart disease; C reactive protein, a plasma marker of inflammation, is a marker of CHD (Folsom *et al*.,2002), and others multiple prospective studies now demonstrate that high sensitivity C reactive protein is a potent predictor of future cardiovascular events at all level of low density lipoprotein cholesterol (Ridker,2003).

Elevated HS-CRP was significantly correlate with electrocardiogram; defined coronary artery disease (Thakur *et al.*, 2011).

Chapter three

Materials and Methods

Materials and Methods

3.1Materials

3.1.1 Study Design:

This study is an analytical cross sectional study.

3.1.2 Study area and period:

The study was conducted in Ombada Hospital and EL-emtiaz medical center from 12 November to 1 September.

3.1.3 Ethical consideration:

The study was approved from scientific committee of Clinical Chemistry department in college of medical laboratory science at Sudan University of Science and Technology.an informed consent was obtained from all participants (Appendix I).Demographic data was collected by questionnaire (Appendix II).

3.1.4 Study Population:

The study was consisted of 52 Patients as case group already diagnosed as type 2 D.M (according to American Diabetes Association, 2004) (14 male and 38 female) and 52 apparently healthy individuals as control group (14 male and 38 female), patients with familial hypercholesterolemia, familial hyper triglyceridemia were excluded, and Age was matched ranged from (32_70) years.

3.2 Methods

3.2.1 Sampling:

After obtaining an informed consent, samples were collected by using dry plastic syringes and tourniquet, venous blood (5ml) was collected in to plain, and EDTA container under aseptic conditions. EDTA containers

were storage at 4◦c until analysis Plain container samples were allowed to clot at room temperature and then were centrifuged at 4000 rpm for 5 minutes to obtain serum for triglycerides, HDL and CRP. The samples were stored for -20◦c until analysis. The sample with interference substance like hemolysis and icteric sample was excluded.

3.2.2 Estimation of triglycerides:

Lipoprotein lipase hydrolyzed triglycerides to glycerol and free fatty acid, the glycerol formed with ATP in the presence of glycerol kinase formed glycerol 3 phosphate which is oxidised by the enzymes glycerol phosphate oxidase and formed hydrogen peroxide. The hydrogen peroxide further reacted with phenolic Compound and 4amino antipyrine by catalytic action of peroxidase and formed a red color quinonamine dye complex, the color was directly proportional to the amount of triglycerides presented in the sample (Appendix III).

3.2.3 Estimation of HDLc:

Very low density lipoproteins (VLDLc), and low density lipoprotein (LDLc) in the sample precipitated with phosphotungestate and magnesium ions. The supernatant contained high density lipoproteins (HDLc). The HDLc cholesterol was then spectro -photo metrically measured (Appendix IV).

3.2.4 Detection of CRP:

The test was an immunologic reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles (Appendix V).

3.2.5 Estimation of HbA1c:

HbA1c estimation is based on the fluorescence immunoassay technology, specifically the competition immune detection method. Whole blood added to the mixture of hemolysis buffer and detection

buffer, which resulted in hemolysis of red blood cells. The mixture containing HbA1c from the hemolyzed red blood cells and fluorescence-labeled HbA1c peptides from detection buffer was loaded on to the sample well of the cartridge. The mixture then migrated 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 produced a lower fluorescence signal from HbA1c-peptides. The signal was interpreted and the result displayed. on i-CHROMA reader in units of percentage (Appendix VI).

3.3 Quality control:

Pathological and normal control sera were measured to verify the performance of the measurement procedure, for TG use control serum level I (cod.18040) and II (cod. 18041) and for HDLc use control serum level I (cod.18005,18009 and 18042).

3.4 Statistical analysis:

Statistical procedure was followed using statistic package for social science (SPSS) version 16 on programmed computer. Independent sample T test was applied to compare biochemical quantitative data between case and control groups and Person's correlation test was applied for the correlations. The level of significant was expressed as $P<0.05$.

Chapter Four

Results

Results

The study induced 52 patients with type2 diabetes and 52without diabetes, SPSS was done and the results were as follow:

Table 4.1 shows: significant increase in TG: HDLc ratio among type2 diabetic patients in comparison with control group, with p-value 0.000, significant increase in TG between type2 diabetic patients in comparison with non-diabetic group, with p-value 0.000,also significant decrease in HDLc among type2 diabetic patients in comparison with control group, with p-value 0.000,and significant increase in percentage of CRP among type2 diabetic patients in comparison with control group, with p-value 0.02,in significant difference in Age between type2 diabetic patients and control group with p-value .616, and in significant difference in BMI between type2 diabetic patients and control group with p-value 0.677.

Table 4.2 shows: No correlation between ratio and age, BMI, duration, among type2 diabetic patients with r and p-values (r .120, p .325),(r - .036 p .800), (r - .211 p .130) respectively, No correlation between HbA1C and Age, BMI, duration, among type2 diabetic patients with r and p-values(r .000 p .977),(r - .177 p .208),(r .074 p .604 respectively. Also no correlation between CRP and age, BMI, duration, with r and p-values (r - .072 p .613),(r .145 p .306),(r - .077 p .588) respectively, no correlation between HDL and Age, BMI, Duration with r and p-values(r .73 p .607),(r - .031 p .825),(r . 314 p .23) respectively, and no correlation between TG and Age, BMI, Duration with r and p-values (r .165, p .244),(r - .082 p .561),(r - .175 p .215) respectively.

Table 4.3 shows: No correlation between CRP and ratio, HbA1c among type2 diabetic patients with r and p-values (r .246 p .078), (r .254 p .064) respectively.

Table 4-1 Comparison of the mean and standard deviation of Age, BMI, TG,HDL,TG:HDL Ratio and CRP between cases (diabetic patients HbA1c >7) and control (HbA1c≤7%) with p-values.

Variable	**Case (n=52) Mean ± SD**	**Control (n=52) Mean ± SD**	**P value**
Age (years)	51±9.8	48±9.8	0.616
BMI (Kg/m^2)	22±4.8	21±3.8	0.677
Triglycerides (mg/dl)	169±116	88±17.5	0.000
HDLc (mg/dl)	34±14	50±9.5	0.000
TG: HDLc ratio	9.2±6.9	1.69±0.47	0.000
CRP Positive Test (%)	33.0%	0.0%	0.02

Independent T-test was used for comparison Diabetics and Control groups, P-value ≤ 0.05 considered significant.

Table 4-2Correlation between HDL, TG, TG:HDL ratio, HbA1c, CRP and Age, BMI, Duration among type 2 DM.

	Age		BMI		Duration	
	P.value	**r**	**P.value**	**R**	**P.value**	**r**
HDLc (mg/dl)	.607	.073	.825	-.031	0.230	.314
TG (mg/dl)	.244	.165	.561	-.082	.215	-.175
TG/HDLc ratio	.395	.120	.800	-.036	0.133	-.211
HbA1c (%)	.997	.000	.208	-.177	.604	.074
CRP Positive Test (%)	0.613	.072	.306	.145	.588	-.077

Person's Correlation was used, P value ≤ 0.05 considered significant.

r: is coefficient and strength.

Table 4:3Correlation between CRP, TG/HDL ratio and HbA1c in type2 diabetic patients.

Variable	CRP	
	P.value	**r**
TG/HDLc ratio	0.078	0.246
HbA1c (%)	0.064	0.259

Person's Correlation was used, P value ≤ 0.05 considered significant.

r: is coefficient and strength.

Chapter five

Discussion, Conclusion and Recommendations

Chapter five

Discussion, Conclusion and Recommendations

5.1 Disccussion

The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (Diabetic care,2005).

The results of this study revealed that, there is a significant increase in the mean of TG among type2 diabetic patients versus control group. The result is in agreement with some studies like (Smith *et al*.,2008;Elnasri and Ahmed, 2008). the elevation of TG among type2 diabetic patients although its stated in many studies like(Khan *et al.*,2008;Liu *etal*,.2013;Vega *et al.*, 2014).The mechanism by which TG increase among type2 diabetic patients its still unclear but may be caused by increased free fatty acid flux secondary to insulin resistance and aggravated by increased inflammatory adipokines (Chehade *et al*.,2013).There is a significant decrease in the mean of HDL among type2 diabetic patients versus control group came in agreement with other studies like(Gloay *et al*.,1987;Awadalla *et al*.,2018),and a good explanation of that result is (the presence of insulin deficiency or more commonly on hyperinsulinemia and insulin resistance, HDL particularly the subfraction HDL2 concentration is regulated by two endothelial lipolytic enzymes, lipoprotein lipase and hepatic lipase, both of which are insulin sensitive(Nikkila,1981).

There is a significant increase in the mean of TG:HDL ratio among type2 diabetic patients versus control group. This result came in agreement with some studies like (Khan *et al*.,2008;Vega *et al*.,2014;Mohieldein *et al*., 2014).that insure the relationship between type2 diabetes and high TG:HDL ratio and The result can be interpreted by the abnormal results of TG and HDL and the theory of Protasio (higher plasma concentration of Triglycerides-rich,

very low density lipoprotein, That generates small, dense LDL, during lipid exchange and lipolysis. This LDL particle accumulate in the circulation and form small dense HDL particles, which undergo accelerated catabolism, this dosing the atherogenic circle (Protasio,2008).there is a significant increase in the percentage of CRP among type2 diabetic patients versus control group, the results is in agreement with some studies like (Erbacci *et al*.,2002;Ojiminiyi *et al*,2002).The elevated percentage of CRP among type2 diabetic patients versus control group may due to elevation in lipid which may cause Inflammation and then presence of CRP as immunological response (Allin and Nordestgaard,2011).There is no significant differences in BMI among type2 diabetic patients versus control group . although many studies insure the relationship between diabetic patients and increased BMI like (Elnasri and Ahmed, 2008;Gray *et al.*,2008), although the pathogenesis of obesity induced-insulin resistance is still obscure in many aspects(Zeyda and Stulnig ,2009), many diligence to know the mechanism like (adipocytes secrete a unique signaling molecule, which we have named resistin (for resistance to insulin).Circulating resistin levels are decreased by the anti-diabetic drug rosiglitazone, and increased in diet-induced and genetic forms of obesity(Steppan *et al*., 2001). So type 2 diabetes mellitus can happen by many causes like genetic or βcell destruction and viruses and many reasons and not required just increased BMI to occur. There is no correlation between TG and Age, BMI, Duration. The relation between TG and Age is variable (Okecka *et al*.,2011; Elnasri and Ahmed,2008) and the cause may due to (Age related decrease in the capacity of respiring tissue to oxidize fat rather than decrease free fatty acid release is more likely determinant of lipid implance and the Age related increase in adiposity)(Toth and Tchernof,2000) and absence of relationship between TG and BMI, Duration may due to the using of lipid reducing Drugs like statin.

There is no correlation between HDLc and Age, BMI, Duration among type2 diabetic patients. We find that HDLc has no relation with Duration and Age and itis Impairment is due to other causes mention by (Rogres G and Cheney K(2019); Holland K (2019). like Smoking, inheritance factor, lack of some hormones, obesity and lastly due to the therapeutic used in reducing cholesterol which may Interpret absence of the relationship between HDLc and those factors mentioned above.

There is no correlation between TG: HDLc ratio and Age, BMI, Duration among type2 diabetic patients. Actually there is a relationship between abnormal lipid profile and BMI (Sandoval *et al*.,2014) and here can interpreted by the closed value of BMI among type2 diabetic patients and the absence of the correlation between HDLc and Age, Duration have interpreted by that (the Age and duration are variable and their relation is depending on the life style which mainly effect on BMI).

There is no correlation between CRP and Age, BMI, Duration among type2 diabetic patients. There is no previous data interpreted absence of the relationship.

5.2 Conclusion

Type2 diabetic Sudanese patients had increase HbA1c, TGs, CRP, TG: HDLc ratio and deteriorated HDLc compared with non-diabetic control group.

5.3 Recommendations

. From the results of this study it's recommended that:

.TGs: HDLc ratio and CRP can be used routinely for type2 diabetic patients to assess atherogenic index to avoid the complications that may suddenly happen.

. Cohort study have recommended for these variables to clarify the changes will happen at successive periods of time.

References

Allin.K.H and Nordestgaard B.G.(2011).elevated c-reactive protein in the diagnosis,prognosis and cause cancer, critical reviews in clinical laboratory sciences;**48**(4):155-170.

Awadalla.H .,Noor S.K ;Elmadhoun W.M;Sarra.O; Ahmed B Almobarak O; Mohamed A.A ;and Ahmed.H.(2018). Comparison of serum lipid profile in type 2 diabetes with and without adequate diabetes control in Sudanese population in north of Sudan,Diabetes & Metabolic Syndrome: Clinical Research & Review**; 12**(6) :961-964

Azam.(2013**).**uncommon form of immune mediated diabetes,azams note in anethesthesiology;**3**:75.

Baco F.B.(2019).other genetic synndrom, ACP, Diabetes Care; **42**(1): 13-28.

Barker.JM.(2006).Type 1 Diabetes-Associated Autoimmunity: Natural History, Genetic Associations, and Screening, *The Journal of Clinical Endocrinology & Metabolism*; **91** (4):1210–1217.

Bishop M.J., Fody E.P;and Schoeff L.E.(2018).type1 diabetes mellitus,triglycerides,clinical chemistry;**8**: 757-758.

Buchanan T.A., Kjos; Anny Xiang; Siri L. Richard Watanabe**(2007).** What Is Gestational Diabetes,Diabetes Care (2):105-111

Burtis C.(2008) Insulin, triglycereides, tietz fundamentals of clinical chemistry;**6**(891):407-408.

Carmena.R. (2005).Type 2 diabetes, dyslipidemia, and vascular risk Rationale and evidence for correcting the lipid imbalance, American Heart Journal ;**150**(5): 859-870.

Carrrol RW and Murphy R .(2013).monogenic diabetes;Adiagnostic Alogarithm for clinicians ,genes2013,**4**: 522-535.

Chawla.A., Chawla.R; and Jagg.S.(2016). Microvasular and macrovascular complications in diabetes mellitus: Distinct or continuum, Indian J Endocrinol Metab.; **20**(4): 546–551.

Chehade J.M., Gladysz M ;& Mooradian A.D.(2013).Dyslipidemia in Type 2 Diabetes: Prevalence, Pathophysiology, and Management,Springer link ;**73** : 327–339.

Coppieters,KT ; tobias Boettler.T; and Herrath.M.(2011).Virus Infections in Type 1 Diabetes **,** Diabetes Research Center for Type 1, La Jolla Institute for Allergy and Immunology La Jolla California, medicine**;2**(1)

Coppieters K., Boettler T and Herrath M. (2012). virus infection in type 1 diabetes, cold spring**;2**(1)

Crock. M.A.(2012).genetic defect of βcell function,gestational diabetes mellitus,clincal biochemistry and metabolic medicine;**8**: 164, 184-185

Da Luz.PL.,Desiderio Favarato ; Faria.RJ-Ne to Junior;Pedro Lemos; Carlos.A.; ChagasI.P.(2017). High ratio of triglycerides to hdl cholesterol predict extensive goronary disease,I, clinics2008;**64** (427-32): 197-204.

Dermmott M.T.(2013).Desripe the pathogenesis of typ1diabetes,endocrine secret;**6**: 9

Devendra D., Eisen barth G and Liu E (2004). Type 1 Diabetes, BMJ;328(7442) :750-754.

DIABETES CARE. (2005).diabetes mellitus,difinition of diabetes mellitus; **28** (1).

Dimeglio.M;Molina.CE;Aoram,**.(2018).**Type 1 diabetes; **391** (10138): 2449-2462**.**

Do clos T.W.(2000).function of CRP protein, Med;**32(**4):8-|274.

Eckl.M ., Scott.H; GrundyS M ;and Zimmet.(2003).The metabolic syndrome**;365** (9468): 1415-1428.

Elnasri, H.A. & Ahmed, A.M. (2008). Patterns of lipid changes among type 2 diabetes patients in Sudan. EMHJ - Eastern Mediterranean Health Journal, **14** (2): 314-324.

Engerman R.L.(1989).Pathogenesis of Diabetic Retinopathy, Diabetes 1989; **38**(10): 1203-1206.

Erbačci .AB.(2002). Diagnostic value of CRP and Lp(a) in coronary heart disease,JournalActa Cardiologica ; **57** (3)

Fishbein.H and Palumbo P.J. (1995). Acute complications in diabetes , Diabetes in America1995, books. Google .com .

Folsom A.R., S.Juneja HS;and Kenneth K.(2002).C-reactive protein and incident coronary heart disease in the Atherosclerosis Risk In Communities(ARIC**),**The American Journal of Cardiology;**144**(2):233-238**.**

Fourlanos.S., Dotta.F, Greenbaum.C.J, Palmer.J.P, Rolandsson.O, Colman. P. G. & Harrison. L. C. (2005).Latent au toimmune diabetes in adults (LADA) ,Diabe tologia **48**, (2206–2212).

Fowler .MJ.(2008**).** Microvascular and Macrovascular Complications of Diabetes ,Clinical Diabetes ; **26**(2): 77-82.

Fowler.M.G.(2008).Microvascular and Macrovascular Complications of Diabetes,Clinical Diabetes 2008 ;**26**(2): 77-82.

GarrdnerD.G and shoback D.(2007).diabetes mellitus ,green span;**8**: 672.

Golay.A., Zech.L,SHI M. Z; Chiouy A. M ;Reaven G. M; and Cheny.-D.(1987). High Density Lipoprotein (HDL) Metabolism in Noninsulin-Dependent Diabetes Mellitus, *The Journal of Clinical Endocrinology & Metabolism*; **65**, (3): 512–518.

Greene D.A., Sima **A** A F; Pfeifer M.A;and Albers J W.(1990).Diabetic Neuropathy,Annual Review of Medicine : 303-31.

Greenstein B and Wood D.(2011).Gestational diabetes, macrovascular complications ,AT Aglance ;**3**:89.

Grundy S.M.(2011).Metabolic Syndrome Pandemic**,** arterioloclrosis,thrombosis and vascular biology 2008;**28**:629–636.

Hamiel and Zeitler, (2007). Acute and Chronic Complication of Type 2 Diabetes in Children and Adolescence, the lancet;**369**(9575) :1823-1831

Holland K.(2019). 11 foods to increase your HDL,healthline.com 27March 2019.

Insull W. (2009). The Pathology of Atherosclerosis: Plaque Development and Plaque Responses to Medical Treatment, The American Journal of Medicine;**122**(1):3-14.

Yoon J W 1990,Type 1 diabetes: recent developments; *BMJ* ; **328** (164): 95-123.

Karuranga S ., Fernandes J.R;Huang Y;and Malanda B. (2017).type2 diabetes mellitus, diabetes atlas;**8**:18-19.

Khan S.R., Ayub N;Nawab S ;and Shamsi T.S.(2008).Triglyceride Profile in Dyslipidaemia of Type 2 Diabetes Mellitus,Journal of the College of Physicians and Surgeons Pakistan ;**18** (5): 270-273.

Kreisberg.R.A.(1998). Diabetic dyslipidemia,The American Journal of Cardiology; **82**(12): 67-73.

Laakso.M.(1999). Hyperglycemia and cardiovascular disease in type 2 diabetes. **,** Diabetes ; **48**(5): 937-942.

Lamarche B.,Rashid S.;and FLewis G.(1999). HDL metabolism in hypertriglyceridemic states, Clinica Chimica Acta; 286(1–2): 145-161.

LeahyJ.L.(2005). Pathogenesis of Type 2 Diabetes Mellitus, Archives of Medical Research;**36** (3): 197-209.

Lefer D.J.(2002).statin as Potent Anti inflamma tory Drug ,circulation ;**106** (16).

Liu.J., Zeng.F; Liu.Z; Zhang.C; Ling.W ;& Chen.Y.(2013).Effects of blood triglycerides on cardiovascular and all-cause mortality: a systematic review and meta-analysis of 61 prospective studies,Lipids in Health and Disease;**12**(159).

Melmed S and conn M.(2005).Insulin resistance,Endocrinology basic and clinical principle;**2**: 318-319.

Mojiminiyi.A., Abdella.N.M ; MoussaA.A;OAkanji.HAl Mohammedi;Zaki.M .(2002).Association of C-reactive protein with coronary heart disease risk fac tors in patients with type 2 diabetes mellitus, Diabetes Research and Clinical Practice; **58** (1): 37-44.

Myo Cinic.org Diabetes medication and insulin therapy,2019.

Myo Cinic.org Diabetes-symp toms and causes,2018.

Nikkilä E.A.(1981). High Density Lipoproteins in Diabetes,Diabetes.(1981); **2**: 82-87.

Nordestgaard .BG.(2014**).** Triglycerides and cardiovascular disease**,** The lancet; **384** (9943): 626-635.

Okęcka.J.S., Hübner.E.H; Piątkowska.I; and Malara.M.(2011). Effects of age, gender and physical activity on plasma lipid profile Biomedical Human Kinetics;**3**.

Omar S M., Musa I.R, Elsouli Aand Adam I. (2019).prevalence, risk factor and glycemic control of type 2 diabetes mellitus in eastern Sudan, Therapeutic advances in endocrinology and metabolism;**5**

Protásio.DL.,Mesquita ;and Tinoco.C. High ratio of triglycerides to hdl-cholesterol predicts extensive coronary disease**.** *Clinics* [online]. 2008, **63**; n.4, 427-432. ISSN 1980-5322.

Redondo.MJ .(2013). Latent Au toimmune Diabetes in Adults,LADA: Time for a New Definition, Diabetes 2013**; 62(**2**):** 339-340.

Ridker P.M.(2003).High-sensitivity C-reactive protein and cardiovascular risk: rationale for screening and primary prevention ,The American Journal of Cardiology ;**92**(4):17-22

Robert KH.(1999). Antiinflammatory Drug,N Engl J Med 1999; **341**:498-511.

Roep B.A (2003).The role of T-cells in the pathogenesis of Type 1 diabetes,Diabe tologia ;**46**: 305–321.

Rogres G and Cheney K.(2019).can hdl level be too high ,11 foods to increase your HDL ,healthline.com 29 March 2019.

Saeed M., Omar; Imad R. Musa; ElSouli A;and Ishag Adam.(2019).Prevalence, risk factors, and glycaemic control of type 2 diabetes mellitus in eastern Sudan: a community-based study,saga journals Acute and chronic complications of type 2 diabetes mellitus in children and adolescents; **369** (9575) : 1823-1831.

Said.G.(2007).Diabetic neuropathy—a review , Nature Clinical Practice Neurology ; **3**: 331–340.

Sandoval G., Burke.D;Ruiz M; Díaz M;and Morales JA. Prevalence of obesity and altered lipid profile in university students Nutricion Hospitalaria; **29**(2):315-321.

Schlienger J.L(2013).Type 2 diabetes complications,Presse Medicale (Paris, France); **42**(5):839-848.

Schofield j.D., Liu.Y;Rao-Balakrishna.P; Rayaz A. Malik; & Soran.H.(2016). Diabetes Dyslipidemia,springer link;**7**:203–219.

Seshiah.V.,Das.A, Balaji V, Shashank R Joshi;and Parikh.M.N.(2006). Gestational Diabetes Mellitus – Guidelines ,Sunil Gupta For Diabetes In Pregnancy Study Group (DIPSI) JAPI ; **54** .

SilinkM.,Kida K;and Rosenbloom A.L.(2003). Criteria for the diagnosis of diabetes,type 2 Diabetes in childhood(2003): 11.

Stenström. G., Gottsäter.A**,** Bakhtadze**.E,** Bo Berger and Göran Sundkvist.(2005). Definition, Prevalence, β-Cell Function, and Treatment **,** American Diabetes Association Diabetes 2005 c; **54**(2): 68-72**.**

Steppan.C.M., Shannon S.T; Bailey; Bhat.S; Elizabeth J; Brown; Ronadip R; Banerjee; Chris topher M; Wright;Hiralben R; Patel; Rexford S; Ahima & Mitchell A; Lazar .(2001). The hormone resistin links obesity to diabetes,Nature ; **409**: 307–312.

Tang.J and Kern.TS.(2011). Inflammation in diabetic retinopathy Progress in Retinal and Eye Research;**30**(5), September 2011: 343-358.

Toth.M.J & Tchernof.A.(2000). Triglyceride- to-HDL cholesterol ratio, Lipid metabolism in the elderly European Journal of Clinical Nutrition ; **54**: 121–1250.

Vega G.L.,Carolyn E. Barlow, MS†; Scott G.M;Leonard D; Laura F. ;and Defina L.F.(2014). Triglyceride– to–High-Density-Lipoprotein-Cholesterol Ratio Is an Index of Heart Disease Mortality and of Incidence of Type 2 Diabetes Mellitus in Men, BMJ journal; **62** (2).

WassJ.,Owen K ;and Helen T.(2014).Aetiological classification of diabetes mellitus,Oxford;**3**: 685-686.

WHO.(2013).Type 1 Diabetes mellitus, media center.

WHO.(2019). Disease of exocrine pancreas ,infections ,Diabetes mellitus

William.(2003).Diabetic keto acidosis,William text book in endocrinology; **10**

Yuen.L; Saeedi.P; Riaz.M; aruranga.K; Divakar.H;and Levitt.N.(2019|).Proj ections of the prevalence of hyperglycaemia in pregnancy ,the International Diabetes Federation Diabetes Atlas, Diabetes Research and Clinical Practice 157, 107841.

Yunke Z., Guoping L ;and Zhenyue C.(2014).Triglyceride- to-HDL cholesterol ratio**,** springer link ; **39**: 105–110.

Zeyda M. and Stulnig T.M.(2009). Obesity, Inflammation, and Insulin Resistance – A Mini-Review ,Gerontology;**55**:379–386.

Appendices

Appendix I

الموافقة المستنيرة

Informed consent

أنا الباحثة مناسك آدم أحمد زيتون من كلية علوم المختبرات الطبية قسم الكيمياء السريرية، جامعة السودان للعلوم والتكنولوجيا ،أقوم بدراسة علمية بحثية لنيل درجة الماجستير بعنوان : (حساب نسبة الدهون الثلاثية إلى نسبة الكوليسترول عالي الكثافة وربط نتائجها مع تفاعل البروتين المتفاعل **C** لدى نفس الأشخاص المصابين بمرض السكري من النوع الثاني).

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 ، حصاوي الكبد ، أمراض الكلى ، نقص هرمون الغدة الدرقية، ولا يتعاطى الكحول أو أي أدوية تؤثر على نسبة الدهون ولا يعاني من اي التهاب في هذه الفتره).

خلال هذه الدراسة سوف أقوم بأخذ الطول والوزن واخذ (٥مل) من الدم لإجراء فحص الكوليسترول عالي الكثافة والدهون الثلاثية والسكر المرتبط بجزئ الهيموقلوبين وهذا يتطلب الصيام من(٩_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 ،اوظهور 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 أقر كتابياً 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 .

توقيع المشارك .....

توقيع الباحث .....

Appendix II

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Triglycerides to High density lipoprotein Cholesterol ratio and C-reactive protein among type 2 diabetic patients In Khartoum State

Code No Date

Personal data:

Name

Age years

Gender male female

High m

Weight kg

BMI kg/m2

Smoking Yes No

Type of DM type 1 type 2

Duration of DM years

Treatment used

Investigation:

CRP mg/dl

Triglyceride mg/dl

HDL mg/dl

% HbA1c

Appendix III

BioMed-Triglycerides L.S

BIOMED DIAGNOSTICS

Enzymatic, Colorimetric GPO-PAP

REF:

TG117090 (3×30 ml)
TG117120 (2×60 ml)
TG117100 (2×50 ml)

TG117044) (2 × 20 ml)
TG117200 (10×20 ml)
TG117240 (2×125 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:

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

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

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

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

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 icteric 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)
	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
R2	LPL (Lipoprotein Lipase)	2500U/L
	GK (Glycerol Chirasi)	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

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:	3 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	500 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
2°C 8°C	Temperature Limitation
	Manufacturer
EC REP	Authorized Representative in the European Community
REF	Catalogue Number
LOT	Batch Code
	Use by

Egy Chem for lab technology

EGY- CHEM for lab technology
Bader City, Industrial Area Piece 170
250 Fadan In East of Elrubaki, EGYPT
Tel: +202 26236727 / +202 26236598
Fax: +202 26240986
www.egy-chem.com

CE
EC REP
MOSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

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.
3. National Cholesterol Educarion Program Expert Panel. Third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III). NIH Publication. Bethesda: National Heart, Lung, and Blood Institute; 2001.
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-CRP

C-Reactive Protein

BIOMED DIAGNOSTICS

REF: CRP301040 (40 test)
CRP301050 (50 test)
CRP301100 (100 test)

INTENDED FOR USE :

Rapid latex agglutination test for the qualitative screening and semi-quantitative determination of C-reactive protein (CRP) in serum.

PRINCIPLE :

The principle of the test is an immunologic reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles.

SPECIMEN COLLECTION :

The test should be performed on serum. Specimens can be drawn by venipuncture or convenient fingertip method. Plasma should not be used because fibrinogen may cause nonspecific agglutination of the latex particles.

Strongly lipemic sera and/or bacterial contamination may cause false positive agglutination.

The serum specimen should be stored refrigerated. If testing is to be prolonged in excess of 24 hours, serum should be frozen. Bacterial contamination may cause protein denaturation

REAGENT COMPOSITION :

Latex Reagent: a suspension of polystyrene latex particles in glycine-saline buffer pH: 8.6 ± 0.2. The latex particles are coated with monospecific anti-human **CRP**, produced in laboratory animals
Positive Control Serum: Stabilized human serum containing **CRP** as an antigen.
All components contain 0.1% sodium azide as preservative.

PACKAGE : Collection & Storage .

All reagents are stable up to the expiration date specified when stored at 2 - 8°C. Do Not Freeze. Avoid extended exposure of reagents to elevated temperatures.

PRECAUTIONS & WARNING :

All human blood components used to prepare controls have been tested for Hepatitis B surface antigen (HbsAg) and HTLV-lll antibodies by an FDA approved procedure and found to be non-reactive. No known test method for HbsAg or HTLV-lll antibodies offers total assurance that a human derived product will not transmit hepatitis or HTLV-lll virus. The user is therefore cautioned to handle reagents as if being capable of transmitting these diseases.
The reagents in each kit are matched. Reagents from different kits must not be interchanged or pooled. If the kit does not yield expected results when controls are tested, the kit should be discarded. Mix the reagents well before use. Use clean equipment. Traces of detergent to dried reactants on the test slide may adversely affect test performance and results.

REAGENT PREPARATION & STABILITY :

Expiration date is specified on the kit label. Biological indication of product instability is evidence by inappropriate reaction of the latex reagent with the corresponding positive control serum.

REQUIRED MATERIALS NOT PROVIDED :

Materials supplied with CRP kit,
- CRP latex reagent.
- Positive control serum.
- Negative control serum.
- 3-cell glass slide.
- Dispensing pipettes.

Materials required, but not provided,
- Pipettes (serological).
- Lab rotator.
- Laboratory timer.

PROCEDURE :

1. Bring all reagents and specimens to room temperature.
2. Shake the **CRP** test reagent gently, expel contents of dropper and refill, then place one drop (50 µl) onto glass slide. Using pipette, add one drop of the patient serum (50 µl) onto the glass slide, and mix both together with the flat end of the dispensing pipettes.
3. Continue to mix for about 2 minutes with rotator or by hand and observe for macroscopic clumping using the indirect oblique light source.
4. Positive control should be run with each series to test sera. The positive control supplied is to be used exactly as outlined in steps 1 through 3 above.
5. The reaction of the test serum is compared to the CRP positive control serum and negative control.

WASTE DISPOSAL :

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

QUALITY CONTROL :

A positive control will produce, usually within 1 minute, coarse agglutinated flocs against a clear background, as demonstrated by the positive control.
If the indicated results, using the positive controls not obtained, the CRP kit should not be used.
Result
Negative result: No agglutination of the latex particle suspension will occur within two minutes.
Positive result: An agglutination of the latex particle suspension will occur within two minutes, showing a CRP level of more than 6 mg/dl. Since negative results may be caused by antigen excess, the test should be repeated using a diluted serum.

Same as described in screening test.
Results
The serum CRP concentration can then be calculated approximately by multiplying the dilution factor (i.e. 2, 4, etc) by the detection limit (6 mg/L).
e.g. if the agglutination titer appears at 1:4 the approximate serum CRP level is 4 x 6 = 24 mg/L

REFERENCES :

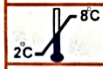
1. Tillet, W.S. & T. Francis: J. Exper.Med. 52, 561, 1930.
2. Fischel, E:E., in: Cohen. A.A. (Editor) Laboratory Diagnostic Procedures in Rheumatic Disease. Little Brown & C. Boston, P. 70, 1967.
3. MacLeod, C.M., & O.T. AVERY: J. Exper.Med. 73, 191, 1950.

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**
	**Temperature Limitation**
	**Manufacturer**
EC REP	**Authorized Representative in the European Community**
REF	**Catalogue Number**
LOT	**Batch Code**
	**Use by**

Egy Chem for lab technology

EGY-CHEM for lab technology
Badr City, Industrial Area Piece 170
250 Faddan in East of Elrubaki, EGYPT.
Factory Tel.: +202 23108170/+202 23108171
Office Tel.: +202 26236727/+202 26236598
Fax: +202 26240986
www.egy-chem.com

CE

EC REP

MDSS Gmbh
Schiffgraben 41
30175 Hannover,
Germany

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 citrate** 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

- Ichroma™ Printer
- Boditech HbA1c Control — REF FPRR007
- Boditech HbA1c Calibrator — REF CFPO-96
- 5 µL Capillary tube — REF CFPO-108, 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*t, *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	96.1	99.0
HbA1a,A1b (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
HbA2d (100 mg/mL)	100.9	97.0	100.3
Acetylsalicylate 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	97.8	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	96.1	99.6
Urea (35 g/dL)	100.1	98.1	99.7

- **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.

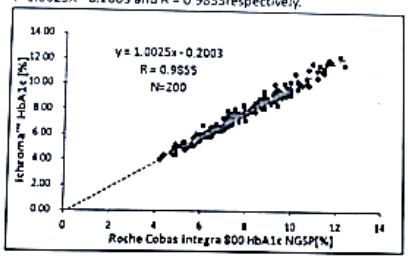
HbA1c (%)	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)	Accuracy (%)
5.2	5.28	5.18	5.24	5.23	0.12	2.36	100.6
6.5	6.46	6.48	6.38	6.43	0.13	1.99	98.9
10.5	10.4	10.56	10.58	10.51	0.19	1.83	100.1

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

HbA1c (%)	Between-person AVG	SD	CV (%)	Between-lot 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	6.43	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.



REFERENCES

1. Goldstein DE,Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM. Tests of glycemia in diabetes. Diabetes Care 1995; 18:896-909.
2. Bunn HF. Nonenzymatic glycosylation of protein: relevance to diabetes. Am J Med 1981; 70:325-30.
3. Jovanovic L, Peterson CM. The clinical utility of glycosylated hemoglobin. Am J Med 1981; 70:331-8.
4. Nathan DM, Singer DE, Hurathal K, Goodson JD. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med 1984; 310:341
5. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycated hemoglobin: methodologies and clinical applications. Clin Cher 1986; 32:864-70.
6. Goldstein DE, Little RR, England JD, Wiedemeyer H-M, McKenzie E. Methods of glycosylated hemoglobins: high performance liquid chromatography and thiobarbituric acid colorimetric methods. In: Clari WL, Larner J, Pohl SL, eds. Methods in diabetes research, Vol. 2. New Yo John Wiley, 1986:475-504.
7. Tahara Y, Shima K. The response of GHb to stepwise plasma glucose change over time in diabetic patients. Diabetes Care 1993; 16:1313-4.
8. Svendsen PA, Lauritzen T, Soegaard U, Nerup J. Glycosylated haemoglob and steady-state mean blood glucose concentration in type 1 (insulin-dependent) diabetes. Diabetologia 1982; 23:403-5.
9. Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD, Izlar C. Glycohemoglobin measured by automated affinity HPLC correlates with both short-term a long-term antecedent glycemia. Clin Chem 1994; 40:1317-21.
10. Singer DE, Coley CM, Samet JH, Nathan DM. Tests of glycemia in diabete mellitus. Their use in establishing a diagnosis and in treatment. Ann Inte Med 1989; 110:125-37.
11. Molnar GD. Clinical evaluation of metabolic control in diabetes. Diabete 1978; 27:216-25.
12. UK Prospective Diabetes Study. Reduction in HbA1c with basal insulin supplement, sulfonylurea or biguanide therapy in maturity-onset diabet Diabetes 1985; 34:793-8.
13. Baker JR, Johnson RN, Scott DJ. Serum fructosamine concentrations in patients with type II (non-insulin-dependent) diabetes mellitus during changes in management. BMJ [Clin Res Ed) 1984; 288:1484-6.
14. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamin and analysis of their weight functions against preceding plasma glucose level. Diabetes Care 1995; 18:440-7.
15. Brooks DE, Devine DV, Harris PC, et al. RAMP(TM): A rapid, quantitative whole blood immunochromatographic platform for point of care testing Clin Chem 1999; 45:1676-1678.

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

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
Tel: +82 33 243-1400
E-mail: sales@boditech.co.kr

Boditech Med Incorporated
43, Geodudanji 1-gil, Dongnae-myeon,
Chuncheon-si, Gang-won-do, 24398
Republic of Korea
Tel: +(82) -33-243-1400
Fax: +(82) -33-243-9373
www.boditech.co.kr

Obelis s.a
Bd. Général Wahis 53,
1030 Brussels, BELGIUM
Tel: +(32) -2-732-59-54
Fax: +(32) -2-732-60-03
E-Mail: mail@obelis.net

CE