بسم الله الرحمن الرحيم

**Sudan University of Science &Technology**

**College of Graduate Studies**

# Assessment of Plasma Total Cholesterol, Low Density Lipoprotein Cholesterol and Uric Acid among Sudanese patients with Type 2 Diabetes Mellitus in Khartoum state

تقييم مستويات الكولسترول الكلي ، كوليسترول البروتين الدهني منخفض الكثافة وحمض اليوريك لدى السودانيين المصابين بمرض السكري من النوع الثاني في ولاية الخرطوم

A dissertation Submitted in Partial Fulfillment for Requirement of M.Sc. degree in Medical Laboratory Science (Clinical Chemistry)

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صدق الله العظيم

**سورة طه الآية (114)**

# Dedication

To those who were very caring, helping? And
Encourage me e for Advancement and success
(My parent, my husband, my family)
To those who enlighten our way with
Knowledge since our first steps of education.
(My Teachers)
To those whom spent with them all the moments,
Happy times, and been touch with all meaning of Friendship.
(My friends and colleagues)

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# Abstract

**Background and aim of study:** Lipid abnormalities in patients with type 2 diabetes are a major problem and associated with the increased risk of cardiovascular disease (CVD). The study aims to estimate total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), uric acid (UA) and HbA1c among type 2 DM.

**Materials and Methods:** This was cross sectional hospital-based study conducted from March to July 2022, at different diabetic centers(Sad Roshwan and Gabber Abo aleis )in Khartoum state among 300 Sudanese patients with type 2 Diabetes mellitus. 5 ml of blood samples were collected from type 2 Diabetes mellitus patients, 2ml put in EDTA container to estimate HbA1c level, and 3ml in heparin in order to estimate TC, LDL-C, UA level which measured by using enzymatic method and spectrophotometer device, and commercial reagent kits from Bio system company. The data obtained were analyzed by using SPSS version 26 computer program.

**Results:** study showed 73% (n=219/300) of study group were poor glycaemic patients (HbA1c >7%). TC and LDL-C were significantly increased in poor glycemic type 2 DM, TC (245.1 ± 48.7 mg/dl vs 189.1± 23.9 mg/dl); LDL-C (158.4± 38mg/dl vs (110.5±21.5 mg/dl) when compared with good glycaemic. Moreover; HbA1c was significantly positive associated with TC (r=0.576; P=, 0.000). and LDL-C (r =0.552, P=0.000). Study revealed that there was insignificant difference in mean of UA in good glycemic control when compared with poor glycemic control.

**Conclusion:** Sudanese with type 2 diabetic patients poor glycaemic control have increased plasma TC and LDL-C, HbA1c was significantly positive associated with TC and LDL-C. Study revealed that there was insignificant difference in mean of UA in good glycemic control when compared with poor glycemic control.

# المستخلص

**الخلفية والهدف من الدراسة:** الخلل في مستوى الدهون في مرضى السكري من النوع2هي مشكله كبيرة ترتبط بزيادة خطر الاصابة بأمراض القلب والاوعية الدموية. والهدف من هذه الدراسة هو قياس الكوليسترول الكلي(TC)، البروتين الدهني منخفض الكثافة (LDL-C)، حمض اليوريك(UA) والسكر التراكمي(HbA1c ).

**المادة والطريقة**: تم اجراء دراسة عرضية في الفترة من مارس الى يوليو 2022 في مختلف من مراكز السكري(سعد رشوان وجابر ابو العز) في ولاية الخرطوم ضمن 300 سوداني من المصابين بداء السكري من النوع 2. تم جمع 5 مل من عينات الدم من مرضى السكري من النوع 2 ، ووضع 2 مل في حاوية EDTA لتقدير مستوى HbA1c ، و 3 مل في الهبارين لتقدير مستوى TC، LDL-C، UA باستخدام الطريقة الأنزيمية وجهاز مقياس الطيف الضوئي ، و مجموعات الكواشف التجارية من شركة Bio system. تم تحليل البيانات التي تم الحصول عليها باستخدام برنامج الكمبيوتر SPSS الإصدار 26.

**نتائج:** أظهرت الدراسة أن73% (ن=300/219) كانوا مرضى غير مضبوطين السكر (HbA1c>7%).كان كل من (TC) و(LDL-C) في مرضى السكري غير منضبطين السكر (48.7±245.1مجم/ديسيلتر) ضد (23.9±189.1 مجم/ديسيلتر)و (38±158.4مجم/ديسيلتر)ضد (21.5±110.5مجم/ديسيلتر) على التوالي عند مقارنته بمرضى السكري منضبطين السكر.

بالإضافة الى ذلك وجد ان السكر التراكمي كان مرتبطا ارتباطا ايجابيا معنويا مع كل من TC(76 P=0.000 r=5,) وLDL-C(552=r, P=0.000).وكشفت الدراسة عن وجود فرق ضئيل في متوسط حمض اليوريك في المرضى منضبطين السكر مقارنة بالمرضى غير منضبطين السكر.

**الخلاصة :**بلازما الكولسترول الكلي(TC) والبروتين الدهني منخفض الكثافة(LDL-C) كان بهما زيادة لدى السودانيين المصابين بداء السكري من النوع2 من الفئة غير منضبطة السكر. بالإضافة الى ذلك وجد ان السكر التراكمي كان مرتبطا ارتباطا ايجابيا معنويا مع كل من LDL-C, TC وكشفت الدراسة عن وجود فرق ضئيل في متوسط حمض اليوريك في المرضى منضبطين السكر مقارنة بالمرضى غير منضبطين السكر.

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# Chapter one

# 1. Introduction, Rationale and Objectives

## 1.1 Introduction

Diabetes mellitus (DM) is a systemic metabolic disorder characterized by a tendency towards chronic hyperglycemia with disturbances in carbohydrates, fats and proteins metabolism that arise from a defect in insulin secretion or action, or both. It is defined clinically from plasma glucose concentrations above which patients are at increased risk of retinopathy, nephropathy, neuropathy and cardiovascular disease. It is a common condition, with a prevalence rate of ~8% in the developed World (Marshal,2021).

The global prevalence of diabetes mellitus has risen in the adult population from 4.7% in 1980 to 8.5% in 2014. type 2 DM (T2D), encompasses patients who have relative rather than absolute insulin deficiency and have peripheral insulin resistance. These patients frequently do not initially require insulin treatment to survive and often do not require insulin treatment throughout their lifetime. The increase in the prevalence of diabetes is due to a significant increase in the population being overweight or obese and to a lack of physical activity in these people [additional risk factors for coronary artery disease in patients with diabetes are increased concentrations of low density lipoprotein Cholesterol, (LDL-C), decreased concentrations of high density lipoprotein(HDL),Total cholesterol, hypertension, smoking and physical inactivity . Inactive adults with diabetes have a 2.81 increased risk of cardiovascular mortality as compared with inactive adults without diabetes (Henning,2018).

## 1.2 Rationale

Lipid abnormalities in patients with type 2 diabetes are a major problem and associated with the increased risk of cardiovascular disease (CVD), In 2012 among the world population, diabetes caused 1.5 million deaths and caused an additional 2.2 million deaths in patients with higher-than-optimal blood glucose concentrations. 43% of these 3.7 million deaths occurred before the age of 70 years

and the majority of these deaths were due to cardiovascular disease. The rate of cardiovascular disease in adults with diabetes is two- to three-times greater than adults without diabetes. In addition, cardiovascular disease is the leading cause of premature death in adults with diabetes. patients with diabetes without a previous history of myocardial infarction have the same level of risk for acute coronary syndromes as no diabetic patients with previous myocardial infarctions. In addition, the risk of macro vascular disease, such as myocardial infarctions or transient ischemic attacks/strokes, increases in pre diabetic individuals well before the clinical diagnosis of diabetes. The 5-year mortality rate for diabetic patients after myocardial infarction is twice that of no diabetic individuals and can be as high as 50% (Henning,2018).

# 1.3 Objectives

## 1.3.1 General Objectives:

To assess the levels of plasma total cholesterol, low-density lipoprotein cholesterol and uric acid among T2 DM.

## 1.3.2 Specific Objectives:

1-To measure the concentrations of total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), uric acid (UA) and percentage of glycated hemoglobin in study group.

2- To measure the weight and Height and calculate BMI among T2 DM.

3-To compare between TC, LDL-C and UA based on glycemic control, sex, family history, BMI, educational levels and smoking.

4- To correlate between the glycated hemoglobin and TC, LDL-C, UA and duration of disease.

# Chapter Two

# 2. Literature Review

## 2.1 Diabetes Mellitus

Diabetes mellitus was defined by the World Health Organization in 2000 as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.(White et al. 2017).

Thus, although the diagnosis of diabetes depends upon demonstration of hyperglycemia, it is important to remember that other metabolic processes are also affected. Diabetes mellitus is a chronic, non-communicable condition which, if untreated, proceeds to micro- and macro vascular disease. The trademark hyperglycemia of diabetes mellitus arises from an inability to clear glucose into insulin-sensitive tissues, particularly skeletal muscle and adipose tissue, due to Either (i) decreased insulin production by the β cells of the pancreas (type 1; juvenile-onset, insulin-dependent diabetes mellitus) or (ii) decreased sensitivity of tissues to the prevailing insulin concentration (type 2; adult-onset, noninsulin-dependent diabetes mellitus(White et al., 2017).

Since the discovery of insulin by banting and best in Canada in the early 1920s, early death from acute insulin deficiency has become avoidable, and insulin-dependent diabetic patients can lead relatively normal lives with daily injections of the hormone. However, this increase in life-expectancy is associated with long-term complications of diabetes which have significant effects on the overall morbidity and mortality of the disease. Such complications, are also associated with the later-onset type 2 form of the disease. This form accounts for about 95% of patients presenting with diabetes in Western societies and is becoming an increasing problem in developing countries. Such patients present with variable combinations of insulin resistance and β-cell dysfunction leading to defects in insulin secretion. It is now apparent that type 2 diabetes mellitus is a syndrome with many different causes including a more sedentary lifestyle, obesity, and dietary factors imposing upon an innate genetic susceptibility(White et al., 2017).

### 2.1.1 Types of Diabetes mellitus

Diabetes is classified into several types with the largest numbers of patients having either type 1 or type 2. about 90% of diabetes worldwide is type 2. diabetes of other types should not be overlooked but make up a very small fraction of cases, type1 insulin deficiency, type 2 Relative insulin deficiency/peripheral insulin resistance ,gestational diabetes Increased insulin resistance during pregnancy maturity-onset diabetes Single gene defect affecting glucose metabolism, Of the young for example, glucokinase ,secondary diabetes due to: endocrine disease Increased concentration of hormones antagonistic to insulin pancreatic disease relative to absolute insulin deficiency ,malnutrition Relative to absolute insulin deficiency drugs Impaired insulin secretion or increased insulin resistanc (White et al., 2017).

### 2.1.2 Pathophysiology and clinical features of Diabetes mellitus

there are two aspects to the clinical manifestations of DM: those related directly to the metabolic disturbance and those related to the long-term complications of the condition. The hyperglycemia of diabetes is mainly a result of increased production of glucose by the liver and, to a lesser extent, of decreased removal of glucose from the blood. In the kidneys, filtered glucose is normally completely reabsorbed in the proximal tubules, but at plasma glucose concentrations greater than ∼10 mmol/L (the renal threshold) reabsorption becomes saturated and glucose appears in the urine. There is some variation in the threshold between individuals. It is higher in the elderly and lower during pregnancy. glycosuria results in an osmotic diuresis, ( Marshal,2021).

increasing water excretion and raising the plasma osmolality, which in turn stimulates the thirst center. osmotic diuresis and thirst cause the classic symptoms of polyuria and polydipsia. other causes of these symptoms include diabetes insipidus, hypercalcaemia, chronic hypokalaemia ,and chronic kidney disease (CKD). Polyuria also follow excessive water intake for any reason. Untreated, the metabolic disturbances may become profound, with the development of life-threatening ketoacidosis or hyperosmolar hyperglycemia The long-term complications of

diabetes fall into two groups: microvascular complications (i.e. nephropathy, neuropathy and retinopathy) and macrovascular disease caused by atherosclerosis. These occur in both type1 and type 2 DM. The prevalence of all these complications increases with the duration of the disease. The risk of microvascular complications is clearly greater if glycaemic control is poor, but other factors are undoubtedly involved: some patients never develop these complications, even after many years of having diabetes; others develop the mrapidly, even with seemingly good control. The development of microvascular disease appears to be directly related to hyperglycaemia, whereas that of macrovascular diseases more closely related to insulin resistance. The results of long-term prospective studies indicate that improved glycaemic control significantly reduces the risk of microvascular complications in both type 1 and type 2 DM. For macrovascular disease, there is evidence of benefit over the long term in type 1 DM, but the evidence is contradictory and controversial in type 2 DM, even though the risk of macrovascular disease is greater in this type of diabetes. The common pathological feature in microvascular disease is narrowing of the lumens of small blood vessels, and this appears to be directly related to prolonged exposure to high glucose concentrations. The processes involved are complex and still not fully understood; two appear to be particularly important. One is increased formation of sorbitol (an alcohol derived from glucose) by the action of the enzyme aldose reductase, leading to accumulation of sorbitol in cells. This can cause osmotic damage, alter the redox state and reduce cellular my inositol concentrations. The other relates to the formation of advanced glycation end products. Glucose can react with amino groups in proteins to form glycated plasma and tissue proteins (glycated hemoglobin). These can undergo cross-linking and accumulate in vessel walls and Disorders of carbohydrate metabolism tissues, leading to structural and functional damage. Other mechanisms of tissue damage may include the generation of free radicals and activation of tissue injury responses secondary to intracellular hyperglycaemia.The increased predisposition to atherosclerosis in patients with diabetes is also multifactorial. The abnormalities of lipids that occur as a direct result of diabetes and glycation of lipoproteins leading to altered function are particularly im-

portant. Other factors that are implicated include endothelial dysfunction and increased oxidative stress. The long-term complications of diabetes are a significant source of morbidity and mortality. Their diagnosis, with the exception of nephropathy, is largely clinical, although measurement of plasma lipids is important in assessment of the risk of macrovascular disease. In contrast, the management of the acute metabolic disturbances seen in diabetes requires intensive biochemical monitoring ( Marshal, 2021).

The pathogenesis of grossly uncontrolled non-ketotic diabetes mellitus is shown in. In contrast to the situation described above for type 1 patients, hepatic function is relatively normal in non-insulin-dependent diabetic patients and this has marked consequences for the presentation and progression of the disease. For example, the synthesis of ketone bodies is extremely sensitive to insulin and even low levels of insulin in the portal circulation will inhibit ketogenesis and protect against ketoacidosis. Thus, type 2 diabetic patients do not develop ketosis. In these patients, the major metabolic consequences arise from effects on muscle and adipose tissue, particularly those resulting from insulin resistance and the increased action of catabolic hormones. Decreased glucose uptake into both tissues leads to hyperglycemia, and increased lipolysis in adipose tissue causes a raised plasma free fatty acid concentration. In the absence of ketogenesis , fatty acids arriving at the liver must be oxidizedor converted to triglyceride for export as very low density lipoprotein (VLDL) and contribute to the hyperlipidemia. The reduced peripheral response to the prevailing insulin concentration will also decrease the activity of lipoprotein lipase and slow the clearance of VLDL. The hyperglycemia in type 2 patients may go undetected for months or even years and, without intervention, plasma glucose concentrations in extremis can often reach very high levels of >40 mmol/L (>720 mg/dL). even though such patients do not develop ketoacidosis, they are still at risk from severe dehydration and increased serum osmolarity and have a marked increase in the risk of developing a major vascular (arterial and venous) thrombosis. If untreated, these patients can enter a diabetic, hyperglycemic, hyperosmolar, non-ketoticcoma and die. The clinical features of grossly uncontrolled diabetes mellitus are:  Patients are usually elderly and often not previously known to be di-

abetic, It is often precipitated by inter current illness, especially infection, It is often associated with arterial and venous thrombosis, the condition develops over several days, Features include polyuria, thirst, and dehydration; stupor progressing to coma; no air hunger High mortality related to age of patient and arterial thrombosis, It may be diagnosed from glucose in cerebral spinal fluid(CSF) and serum osmolality, There are occasionally trace of ketones in urine, The major therapeutic requirement for patients is rehydration ( Marshal, 2021).

### 2.1.3 Diagnosis of Diabetes mellitus

The diagnosis of diabetes depends on the demonstration of hyperglycaemia, using values defined by the world health organization (WHO). In a patient with classic symptoms and signs of thirst and polyuria, a random venous plasma glucose concentration ≥11.1 mmol/L is diagnostic of diabetes; so, too, is a fasting venous plasma glucose concentration ≥7.0 mmol/L.most patients presenting with type 1 DM, and some with type 2 DM clearly exceed these diagnostic limits and require no further tests to establish a diagnosis. In the absence of symptoms, these limits must be exceeded on more than one occasion for the diagnosis to be made. Even in symptomatic patients, diabetes is unlikely if a random venous plasma glucose concentration ≤5.5 mmol/L. individuals who have fasting plasma glucose concentrations that are elevated but not in the diabetic range have impaired fasting glycaemia (IFG). The lower threshold for IFG defined by the WHO and used in the UK and much of the rest of the world is 6.1 mmol/L, although the american diabetes association (ADA) recommends a value of5.6 mmol/L. The WHO recommends that patients found to have IFG should undergo an oral glucose tolerance test (OGTT) to determine whether they have impaired glucose tolerance (IGT) or diabetes. However, many clinicians treat IFG simply as a state of intermediate glucose intolerance, and the ADA does not endorse the use of the OGTT in this group Chronic hyperglycaemia can also be diagnosed using measurements of glycated hemoglobin (HbA1c**)**, which is a marker of average plasma glucose concentration over many weeks. haemoglobin undergoes glycation *in vivo*at a rate proportional to the plasma glucose concentration; the reaction proceeds through a reversible stage but, once the major stable product (HbA1c) is formed, it persists

in that state for the lifetime of the red cell.HbA1c therefore provides a 'time-weighted' average of plasma glucose concentrations over the previous 2–3months. More glucose concentrations contribute to a greater extent to this average than more historical ones (50% of the HbA1c concentration is accounted for by the average plasma glucose concentration during the last 30 days). HbA1c concentration is expressed as aproportion of total haemoglobin. In the UK and manyother countries worldwide, it is reported as mmol/mol haemoglobin, although in some countries it is reported as percentage (%) Since HbA1c is a marker of long-term glycaemic status, it is a convenient and valid diagnostic test for type 2 DM, which is characterized by slowly developing chronic hyperglycaemia. However, it is not a valid diagnostic test for type 1 DM ,in which hyperglycaemia develops rapidly and is therefore unlikely to have been constant over the preceding 2–3 months. HbA1c is also widely used in the monitoring of patients with all types of diabetes Since HbA1c reflects average plasma glucose during the life span of the red cells, caution with interpretations required in patients with decreased red cell life spans ,for example because of haemolytic anaemia, or increased life spans, for example in iron deficiency. Abnormal haemoglobins, for example HbS, prevent or interfere with the measurement of HbA1c or cause false results in certain analytical methods. If the abnormal haemoglobin also results in haemolysis, the HbA1c result will in any case underestimate the average plasma glucose concentration because of reduced red cell life spans .An HbA1c concentration ≥48 mmol/mol is diagnostic of diabetes. If symptoms of hyperglycaemia are not present and the HbA1c is ≥48 mmol/mol, the test should be repeated to confirm the initial result: although HbA1c concentration varies very little from day to day, a second confirmatory test is important to avoid The risk of making an incorrect lifelong diagnosis as a result, for example, of sample mislabeling or analytical error. HbA1c concentrations in the range 42–47 mmol/molsignify an intermediate state of hyperglycaemia sometimes called impaired glycaemia or prediabetes. Such patients are at increased risk of developing diabetes in the future, so the test should be repeated in 1 year The OGTT assesses the capacity for postprandial metabolism of glucose under controlled conditions. The protocol for the OGTT is given below. In the majority of

patients suspected of having diabetes, however, the simple diagnostic thresholds using either plasma glucose orHbA1c measurements as indicated earlier will establish the diagnosis, and formal glucose tolerance testing is unnecessary. The use of the OGTT is now largely limited to the investigation of possible gestational diabetes, but it is also sometimes used in the further investigation of patients who have a fasting plasma glucose concentration in the IFG range (6.1–6.9) mmol/L). A 2-h OGTT plasma glucose concentration that is below the diagnostic limit for diabetes but greater than normal is diagnostic of IGT. This is an intermediate state of hyperglycaemia where plasma glucose concentrations are higher than normal but not so high as to be associated with increased risk of diabetic microvascular disease. IGT is not are used as cutoffs for diagnosis, the existence of such variation can result in patients being misclassified if their results are close to cutoff values: this is why a confirmatory measurement is required before diabetes is diagnosed in the absence of clinical features. The results of glucose tolerance tests are additionally affected by factors such as the rate of gastric emptying and accurate adherence to the test protocol. The OGTT is an essential test for the diagnosis of gestational diabetes (diabetes with onset during pregnancy, Women who are obese, have close relatives with diabetes, belong to an ethnic group with a high prevalence of diabetes, or who have had a large baby or gestational diabetes in a previous pregnancy are at increased risk. In the UK, a 75 g OGTT is offered to all women at high risk at 24–28 weeks of gestation (earlier if gestational diabetes was diagnosed in a previous pregnancy). Different authorities stipulate different diagnostic criteria; those recommended by the International Association of the Diabetes and Pregnancy Study Groups and by the UK National Institute for Health and Care Excellence Women with gestational diabetes may revert to a normal glucose tolerance postpartum. However, they are at increased risk of developing gestational diabetes again in future pregnancies and also of developing type 2 DM. The type of diabetes is usually apparent from the history and clinical presentation. It is only occasionally necessary to perform specific diagnostic tests. The growth in childhood obesity, however, has resulted in increasing prevalence of type 2 DM in children, a group which almost exclusively developed type 1 DM in previous dec-

ades; and some 2% of paediatric diabetes patients have maturity onset diabetic of the young(MODY) Establishing the presence of early onset type 2 DM or MODY has implications for treatment and prognosis. The ongoing presence of C-peptide in the plasma or urine >3 years after the development of diabetes effectively rules out type 1 DM. Pancreatic islet cell autoantibodies (to islet antigen-2 [IA-2], GAD or zinc transporter-8 [ZnT-8]) are present in ~90% of patients with type 1 DM and hyperglycemic crises due to short-term metabolic decompensating. The chronic complications are manifestations of vascular disease and may be subdivided into microvascular and macrovascular. microvascular complications are unique to diabetes and are caused to a large extent by hyperglycemia itself. the small vessels supplying blood to the retina, nerves, and kidney tissues are affected causing retinopathy, neuropathy, and nephropathy, respectively. Diabetic nephropathy is the most common cause of renal failure. Tight control of blood glucose (as shown by low HbA1c) has been shown to reduce these complications significantly. Macrovascular complications are ischemic heart disease (angina and myocardial infarction), stroke, and peripheral vascular disease. These occur in the general population as well as in diabetic patients, but hyperglycemia is an additional risk factor and these complications often affect younger individuals ( Marshal, 2021).

## 2.2 Lipid

Fatty acids, the major component of body and dietary lipids, constitute a family of molecules having the same basic properties. all mammalian fatty acids have an even number of carbons, the common saturated fatty acids being meristic (14:0), palmitic (16:0), and stearic (18:0), where the numbers indicate carbon chain length and number of double bonds, respectively. unsaturated fatty acids contain one or more double bonds in the alkyl chain; for example, oleic acid (18:1) has one double bond while the polyunsaturated fatty acids (PUFAs) linoleic (18:2), linolenic (18:3), and arachidonic (20:4) acids have two, three, and four double bonds, respectively. In these molecules the double bonds are three carbons apart. (White et al. 2017).

## 2.2.1 Fatty acid synthesis

The synthesis of fatty acids and their incorporation into triglycerides represents a mechanism for conserving the energy of dietary fat, carbohydrate, and the carbon skeletons of some amino acids which are in excess to the immediate requirements of the body. under anabolic conditions, where the insulin–glucagon ratio is raised, the liver and adipose tissue are able to synthesize fatty acids from blood-derived glucose via acetyl-CoA and to incorporate these fatty acids into triglyceride. Under catabolic conditions, the liver can also make acetyl-CoA from non-carbohydrate sources such as amino acids and lactate and this becomes relevant in stress situations. The rate-limiting enzyme of fatty acid synthesis is acetyl-CoA carboxylase, the product of which is malonyl-CoA acyl carrier protein (ACP) is an essential co-factor. Each round of the fatty acid synthetic cycle elongates the growing fatty acyl chain by two carbons donated by the three-carbon malonyl-CoA with the release of CO2. chemically, the four reactions involved in converting the ketoacyl-ACP to acyl-ACP are the reverse malonyl-CoA itself has an inhibitory effect on β-oxidation and prevents this catabolic pathway from supplying acetyl-CoA to fatty acid synthesis, which is anabolic; it thereby prevents futile cycling of acetyl-CoA. The activities of acetyl CoA carboxylase and fatty acid synthase are high in liver, adipose tissue, and mammary gland(White et al., 2017).

## 2.2.2 Types of lipid

The three primary types of lipids are phospholipids, sterols, and triglycerides. They each play a different role in the body (White et al., 2017).

### 2.2.2.1 Phospholipids

Phospholipids make up the outermost layer of cells in the bodies of both animals and humans. They create a protective layer around the cells to help maintain them .Most people never need to think much about phospholipids. However, there is a rare autoimmune disease called anti phospholipid syndrome (APS), in which proteins on these lipids are attacked. The protective cell layers are damaged as a result. this disorder is seen more often in people with lupus, especially women. As APS attacks blood cells and vessels, it poses a risk of blood clots that can lead to

heart attacks and strokes. APS also may lead to pregnancy complications. Its common name, "sticky blood," is an apt description for what happens inside the body when things go awry with these key lipids (White et al., 2017).

### 2.2.2.2 Sterols and cholesterol

Sterols are a subset of steroids, a type of hormone. you may be familiar with plant sterol (phytosterols) found in foods that offer health benefits. They are very similar to the primary sterol in humans cholesterol, cholesterol is processed in the liver and contributes to many critical body functions. they include the making of hormones and vitamin D, as well as bile salts that work on fats so they can be absorbed by the cells. there are two types of proteins that carry cholesterol through the bloodstream: high density lipoprotein (HDL) and low density lipoprotein (LDL). HDL is considered “good” cholesterol, as it absorbs cholesterol and brings it back to the liver, whereas LDL is “bad” cholesterol that builds up in the body (White et al., 2017).

### 2.2.2.3 Triglycerides

Triglycerides are the fats and oils that you are familiar with in foods. this type of lipid can be saturated and unsaturated which is part of what makes them solid or liquid, respectively, at room temperature. omega fatty acid are essential nutrients that come from certain foods you eat. tuna and salmon are excellent sources, as are some nuts, seeds, and leafy vegetables. These fats reduce inflammation, blood pressure, and triglyceride levels. They reduce the risk of sudden death by a heart attack and prevent blood clots from forming. Trans fats are fats that have been artificially hydrogenated so that they have a texture desired for processed foods. Eating foods that contain trans fats can lead to high levels of LDL cholesterol(White et al., 2017).

### 2.2.2.4 lipoproteins

Since lipids by their very nature are hydrophobic molecules, an efficient means of transport of both exogenously and endogenously derived lipid through the aqueous environment of the circulation is required. furthermore, a mechanism for targeting circulating lipids to specific tissues must also exist. These two requirements are fulfilled by lipoproteins, which provide a vehicle both for transport and recogni-

tion by specific receptors on tissues. The generic shape of all lipoproteins is spherical and they consist of an oily lipophilic core of cholesteryl ester and varying amounts of triglyceride surrounded by a monolayer of phospholipids containing apoproteins and free cholesterol . The families of apoproteins (also called apolipoproteins) . the lipoproteins have been named according to their density, which reflects the proportion of lipid in the core of the particle. The lipoproteins in order of increasing density are: chylomicrons (CM); very-low-density lipoproteins (VLDL); low-density lipoproteins (LDL); and high-density lipoproteins (HDL) A further subgroup, the short-lived intermediate-density lipoproteins (IDL), is formed from metabolism of VLDL and may be further metabolized to LDL. The difference in density is used in the isolation of lipoproteins by sequential flotation in media of increasing density. The properties of the major lipoproteins . It should be appreciated, however, that such a classification into four major groups is something of an oversimplification and that in the circulation lipoproteins are distributed along a density continuum from a density of 1.00 g/mL (CMs) to 1.2 g/mL (high-density lipoproteins). the differences in Apo lipoprotein composition give the lipoproteins different charges and thus differing electrophilic motilities (α-HDL, pre-β-VLDL, and β-LDL), which may be of diagnostic value in qualitative analysis in patients with defects in lipoprotein metabolism. The properties of the major Apo lipoproteins, lipoproteins are highly dynamic structures whose particle size and composition are undergoing continual change, with both lipids and apolipoproteins moving within lipoprotein classes. In the following discussion of lipoprotein metabolism the following abbreviations are used. The two largest and least dense lipoproteins are responsible for the transport of exogenous diet-derived triglyceride (CM) and endogenous hepatically synthesized triglyceride (VLDL) in the circulation. The two cholesterol-rich lipoproteins are low-density and high-density lipoproteins (LDL and HDL) which perform what might be simplistically described as opposing roles; LDL transports cholesterol to extrahepatic tissues, and HDL transports cholesterol from extra hepatic tissues to liver (White et al., 2017).

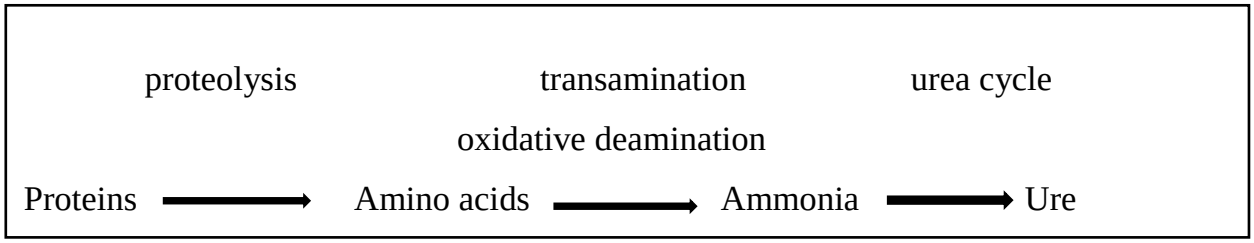
### 2.2.2.4.1 Low-density lipoproteins

Low-density lipoproteins arise from metabolism of LDL following the loss of apo E and lipid to HDL. The physical and chemical properties of LDL and its metabolism .these particles are the primary cholesterol transport particles in the circulation, with cholesteryl ester accounting for 35–45% and free cholesterol a further 6–15% of the particle weight. LDL serves as a donor of cholesterol to both peripheral tissues and the liver, with the liver being responsible for 50% of the total LDL uptake. LDL circulates with a half-life of 2–3 days. the removal of LDL by both liver and extrahepatic tissues occurs via receptor-mediated endocytosis involving the apoB100/E receptor (LDL receptor). This 839-amino-acid protein is anchored to the cell surface by a membrane-spanning region near its C-terminal. Part of the protein shows sequence homology with epidermal growth factor, and the LDL-binding region, consisting of eight repeat sequences enriched in negatively charged amino acids (especially cysteine), is located at the N-terminus on the extracellular surface. This negatively charged region binds the positively charged region of the C-terminus of apoB100. Each LDL particle has only a single molecule of apoB100 such that binding of LDL to its receptor is monovalent; that is, one particle binds to only one receptor endocytosis of LDL results in the formation of an endosome in the tissue with the LDL particle bound to its receptor inside the vesicle. acidification of the endosome causes dissociation of the lipoprotein from its receptor such that many receptors are recycled to the cell surface while the LDL is digested by lysosomal hydrolases. Amino acids derived from hydrolysis of apolipoprotein B100 enter the cellular amino acid pool and cholesteryl ester is hydrolyzed by acholesteryl ester hydrolase to free cholesterol and fatty acid. under normal conditions, the free concentration of cholesterol in the cell represents a balance between endogenous synthesis, exogenous LDL-derived cholesterol, and esterification by acyl coenzyme A:cholesterol acyl transferase(ACAT), raised concentrations of free cholesterol are toxic for the cell and the increase in free cholesterol concentration arising from endocytosis of LDL has important consequences for cellular cholesterol metabolism, each of which attempts to restore the homeostatic intracellular free cholesterol concentration:

Inhibition of HMG-CoA reductase, thereby inhibiting endogenous cholesterol synthesis, Activation of ACAT, which converts free cholesterol to cholesteryl ester, down-regulation of LDL receptor expression on the cell surface, limiting the entry into the cell of exogenous cholesterol from LDL These effects are likely mediated by an oxy-metabolite of cholesterol. Thus in the liver, a major cholesterol-synthesizing tissue, the actions of LDL-derived cholesterol have an important role in controlling endogenous (White et al., 2017).

## 2.3 Non-protein compounds of nitrogen

Blood serum contains compounds of nitrogen other than proteins and peptides. Urea, creatinine, uric acid, ammonia and amino acids are the most important of them and have implications in clinical biochemistry. these nitrogen compounds remain dissolved even after precipitating the serum proteins with deproteinizing agents. Metabolism of some nitrogen compounds is tightly connected (Vejražka ,2020).




### 2.3.1 Creatinine

Creatinine (inner anhydride of creatine) is formed in muscles by irreversible non-enzymatic dehydration and cleavage of phosphate from creatine phosphate, which serves in muscle as a source of energy for muscle contraction Rate of creatinine production in the body is relatively constant. It reflects amount of musclemass and under condition of physical rest and meat-free diet is stable. Creatinine is excretedin the kidney by glomerular filtration; an additional secretion by renal tubules becomes significant only at elevated serum creatinine levels. (Vejražka, 2020).

#### 2.3.1.1 Creatinine in serum

Concentration of creatinine in serum is directly proportional to the function of kidney glomerules and muscle mass of the body; for this reason it is usually some what higher in men than in women. estimation of creatinine in serum is a valuable indicator of glomerular filtration and is widely used mostly for monitoring of kidney diseases, including dialysed patients. The relationship between serum creatinine and glomerular filtration is hyperbolic: creatinine excretion decreases with falling glomerular filtration, but its serum levels do not rise above reference limits until the glomerular filtration drops below 50 % . It is evident from this relationship that for an early diagnosis of an initial stage of kidney damage estimation of serum creatinine is not very sensitive; rather, measurement of creatinine clearance should be used for this purpose. On the other hand, in an advanced stage of glomerular damage serum creatinine is a good measure of glomerular filtration. Creatininemia can be elevated also as a result of release of creatinine from muscles due to acute destruction of skeletal muscles (rhabdomyolysis) (Vejražka, 2020).

#### 2.3.1.2 Creatinine in urine

When compared to other endogenous substances, creatinine excretion into urine is rather constant during the day; in persons with normal glomerular filtration it reflects amount and activity of muscles. concentration of creatinine in urine can be used as a check of 24-hour urine collection used for measurements of daily output of some substances into urine. incorrect or incomplete urine collection often makes these measurements faulty; and simultaneous estimation of creatinine output provides a simple way to check it. creatinine is estimated in a sample of urine from the 24-hour collection. The result in μmol/kg/day is compared with the reference values for urinary creatinine output in dependence on age and gender: If output of creatinine is 30 % or more below the tabular value, it is almost certain the collection of urine was incomplete. next, urinary creatinine is used for standardisation of urinary output of other substances if information on the 24-hour volume of urine is entirely missing. concentration of the substance of interest is expressed per 1 mmol of creatinine. (Vejražka, 2020).

## 2.3.2 Uric acid

Uric acid is the major excretory product arising from the catabolism of purines. The differing reference ranges for the uric acid concentration in serum in adult males and females, as well as those for children,. Since uric acid has limited solubility in water, it is important that the concentrations of uric acid do not go over these reference ranges, as whatever the cause of hyperuricemia, the net effect is the precipitation of urate and uric acid crystals. the solubility product of uric acid in physiological saline at room temperature is 570 μmol/L. however, this is increased in blood due to effects of protein and counterions on urate solubility, such that concentrations in excess of 570 μmol/L can be tolerated without precipitation occurring. These protective effects do not occur in other body fluids, such as synovial fluid and glomerular filtrate, and uric acid crystals deposit more readily in joints, kidneys, and the urinary tract when the urate concentration exceeds 570 μmol/L in these fluids. The clinical consequences of hyperuricemia range from kidney stones, which can lead to renal failure, to joint inflammation and destruction. An operational definition of hype uricemia is a serum urate concentration >420 μmol/L in adult reflects a balance between rates of production and excretion, and the major factors influencing serum uric acid concentration a. Many foodstuffs contain appreciable levels of purines . and their catabolism may contribute significantly to uric acid production uric acid is a weak acid of low water solubility; in its enol tautomeric form it will dissociate at a pH above its pKa of 5.7 to urate and a proton . Thus at plasma pH of 7.4, most of the uric acid is dissociated to urate. this is critically important, since urate is more soluble than uric acid in aqueous solution. In urine, however, particularly when it is slightly acidic, appreciable amounts of uric acid rather than urate may be present and may precipitate out of solution as crystals of the free acid in the urinary tract(White et al., 2017).

### 2.3.2.1 Purines

Purines have a number of fundamental roles in the cell as components of key molecules. The bicyclic structures of the common purine bases—adenine, guanine, and hypoxanthine, differences between these structures are evident in the substituents at C2 and C6, with further oxidation at carbon C8 in uric acid. The

structure of caffeine is included for comparison. purine nucleosides (adenosine, guanosine, and inosine) are formed by addition of a five-carbon sugar—ribose (ribonucleoside) or deoxyribose (deoxyribonucleoside) to N9. Inosine is the trivial name given to the nucleoside derived from hypoxanthine. Nucleotides are formed by phosphorylation of the sugar residues of nucleosides (White et al., 2017).

#### 2.3.2.2 Generation of uric acid

Purine nucleosides, adenosine and guanosine, are released by hydrolysis of nucleic acids derived from exogenous (dietary) or endogenous sources. Adenosine loses its amino group to form inosine in a reaction catalyzed by adenosine deaminase. purine nucleoside phosphorylase hydrolyzes the ribose moiety from both inosine and guanosine, forming hypoxanthine and guanine, respectively. Both purines are then converted to xanthine; guanine by guanase and hypoxanthine by xanthine oxidase. Xanthine oxidase catalyzes the oxidation of xanthine to uric acid (White et al., 2017).

#### 2.3.2.3 Renal handling of Uric acid

In healthy humans, most (>95%) of the blood urate is filtered through the glomerulus and all of this is reabsorbed in the early proximal tubule Approximately 50% of this fraction is secreted in the proximal tubule and 80% of this secreted urate (40% of the filtered urate) is again reabsorbed, such that only about 10% of the filtered urate is finally excreted. the reabsorption rate rarely exceeds 15 mg/min, The renal tubular excretion and reabsorptive processes are mediated by a number of membrane antiport transporter proteins (OATs, organic anion transporters) which co-transport sodium and anions. These transporters are present in the basolateral (for example OAT1, OAT3) and apical membranes (for example urate transporter (URAT1) , multi drug Resistance Protein 4 (MRP4) of the cells of the proximal tubule and are responsible for the movement of anions and urate between plasma and urine. They transport in both directions, dependent on the relative concentrations of the ligands being transported. The compensating ligand for anti-port transfer of urate is a dicarboxylic acid such as glutarate or α-ketoglutarate. Raised plasma urate enters the proximal tubular cell in exchange for a dicarboxylic acid via OAT1, for example, and exits the cell via one of the apical

membrane transporters. when the concentration of urate in the filtrate rises significantly, it is reabsorbed back into the cell via URAT1, for example, in exchange for a dicarboxylic acid anion. In conditions of excessive anion secretion, such as diabetic ketoacidosis or high alcohol intake, excretion of urate is impaired due to a lack of compensating antiport anion and cellular urate, and eventually plasma urate rises. conversely, inhibition of URAT1 by drugs such as probenecid decreases reabsorption of urate, leading to a decrease in blood uric acid. A number of drugs can affect these transport systems, thereby compromising renal handling of urate and giving rise to hyperuricemia or, in some cases, to excessive urate excretion (White et al., 2017).

#### 2.3.2.4 Uric acid in blood serum

Concentration of uric acid in blood depends on intake of purines in food, intensity of its production and on its excretion. especially increased levels of uric acid are of clinical significance. hyper uricemia results from overproduction or decreased excretion of uric acid. concentration of urates can exceed their solubility in hyperuricemia (White et al., 2017).

#### 2.3.2.5 Uric acid in urine

Majority of uric acid (75 – 80 %) is eliminated by kidneys. It is freely filtered in glomeruli (uric acid is only minimally bound to proteins) and then most of it is reabsorbed in the proximal tubuli. next, uric acid is again secreted in the distal part of proximal tubuli and once again reabsorbed. approximately 0.6 g of uric acid per day (3.6 mmol/day) is excreted under purine-free diet. normally, with a common diet, these values are higher – about 0.8 g/day (5.0 mmol/day). tubular secretion of uric acid is inhibited if other organic ions (e.g. acetoacetic acid, β-hydroxybutyric acid, lactate or some drugs) are also excretedto a high extent.

If the excretion of uric acid is increased there is a higher risk of urate urolithiasis. Especially important is this risk in individuals with permanently acidic and concentrated urine. urate concrements are usually made of pure uric acid, sometimes of sodium urate. ammonium rate may precipitate in weakly alkaline urine, typically in case of urinary infection. crystals of sodium urate can precipitate even in renal inter stitium and cause inflammatory response (chronic interstitial nephritis).

Acute renal failure is a relatively rare disorder that results from sudden increase of uric acid in blood (e.g. cytostatic therapy in patients with leukemia) in case that urine is concentrated and acidic at the same time (dehydration). crystals of uric acid are formed in distal tubuli and collecting ducts under these conditions and drainage of urine is blocked (acute urate nephropathy). examination of uric acid in urine is especially important in patients with increased blood concentration of uric acid and in patients with urolithiasis. the amount of uric acid in urine can be expressed in several ways: concentration in the morning sample of urine. According to results of this test, the amount and type of beverages is adapted in order to decrease the urate concentration, The amount of uric acid excreted in 24 hours. Urine is collected for 24 hours and a mixed sample is analyzed. this method can distinguish between hyperuricemia from increased production and decreased excretion. uric acid/creatinine ratio in a random sample of urine. results give information similar to the previous technique but it is not necessary to collect urine for 24 hours, clearance of uric acid may be calculated according to formula:

$$ClUA\ (ml/s) = \frac{UUA \times V}{PUA}$$

UUA: concentration of uric acid in urine (mmol/l)
PUA: concentration of uric acid in serum or plasma (mmol/l)
V: volume of urine in ml/s(White et al., 2017).

## 2.3.3 Urea

In quantitative terms, urea is the most significant degradation product of protein and amino acids in the body. It is produced in the liver from ammonia released by deamination reactions from amino acids. ,urea diffuses freely through cell membranes and so its concentrations in plasma and intracellular fluid are equal. Urea is excreted from the body mainly in the kidney by a combination of glomerular filtration and tubular reabsorption. the latter is variable: lower at higher diuresis while increasing when diuresis is low. concentration of urea in the blood depends on amount of protein in the diet, excretion by the kidney, and metabolic function of the liver. for instance, serum urea can increase due to high protein intake in the food. one gram of protein (dietary or endogenous) can give rise to 5.74

mmol (0.34 g) of urea. increased concentration of serum urea without changes in other non-protein nitrogen compounds (esp. creatinine) is a hallmark of an intense protein catabolism, which occurs e.g. in starvation, febrile state or malignancy. Children have lower catabolism of protein, and show demonstrably lower serum urea levels. serum urea increases in diseases of the kidney that lead to marked restriction of glomerular filtration (below 30 %); simultaneously, high levels of creatinine are found. unlike creatinine, estimation of urea is not suitable for an early detection of decrease in glomerular filtration. Sensitivity of the urea estimation for renal in sufficience is low: it starts to exceed the reference values when more than 75 % of glomerular filtration is lost. On the other hand, urea is a sensitive parameter of renal hypoperfusion – in this case not only glomerular filtration is lowered but also tubular reabsorption of urea is increased. Serum concentration of urea therefore rises much more quickly than that of creatinine. In renal failure of the prerenal type (e.g. in hypoperfusion, most frequently in dehydration) the ratio of serum concentrations of urea and creatinine (in μmol/l) is above 160. In liver function failure, synthesis of urea falls down, and so its concentration in the serum. concentration of urea in serum and urine can also be used for calculation of nitrogen balance (White et al., 2017).

## 2.4 Association between diabetes mellitus and lipid

Insulin resistance is present before the onset of prediabetes or diabetes mellitus, and increases progressively over time, whereas hyperglycemia develops in prediabetes and worsens with development of diabetes mellitus. Insulin resistance with impairment of insulin signaling, hyperinsulinemia, and hyperglycemia contribute to multiple processes including elevated free fatty acids (FFA), advanced glycation end-product (AGE) production, protein kinase C (PKC) activation, oxidative stress, mitochondrial dysfunction, and epigenetic modifications, which together contribute to endothelial dysfunction and inflammation resulting in activation of vascular smooth muscle cells (VSMC), endothelial cells (EC), and monocytes. concentrations of modified (oxidized) low-density lipoproteins (LDL) are higher in diabetes mellitus, and are retained in the sub endothelial layer of vulnerable sections of the vasculature. circulating leukocytes attach and migrate through

the endothelial wall into the VSMC layer of the intimal media. these monocytes engulf retained lipoproteins and transform into lipid-laden foam cells/macrophages producing proteinases and inflammatory mediators including tumor necrosis factor-α (TNF-α) and interleukins. Stress responses including inflammasome complex formation and endoplasmic reticulum (ER) stress result in macrophage proliferation and inflammatory activation with resultant macrophage and VSMC phenotypic switch (proliferation, migration, and dedifferentiation). In response to vascular injury, VSMC secrete collagen to form a fibrous cap, which promotes atherosclerotic plaque stability. however, when stable lesions remodel inward, progressive stenosis of arteries occurs. Plaques can become vulnerable with thinning of the fibrous cap and apoptosis of macrophages in advanced atherosclerotic lesions, where impaired effero cytosis (phagocytic clearance) of lipid laden macrophages results in formation of a necrotic core accelerating vascular inflammation, necrosis, thrombosis. The resulting unstable atherosclerotic lesion complex is prone to sudden expansion from acute thrombus formation, forming a Indus for platelet thrombosis, hemorrhage of atherosclerotic plaque microvessels, and rupture of the fibrous cap. Akt indicates protein kinase B; ERK, extracellular signal-regulated kinase; GlcNAc, N-Acetylglucosamine; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κβ, nuclear factor–kappa beta; NOS, nitric oxide synthase; PI3K, phosphoinositide 3-kinase; RNS, reactive nitrogen species; and ROS, reactive oxygen species. Over time, high blood sugar can damage blood vessels and the nerves that control your heart. People with diabetes are also more likely to have other conditions that raise the risk for cardiovascular disease ,high blood pressure increases the force of blood through your arteries and can damage artery walls. having both high blood pressure and diabetes  ides (a type of fat in your blood) and low HDL (good) cholesterol or high LDL cholesterol is thought to contribute to hardening of the arteries (Xiong , 2019 ).

None of these conditions has symptoms. your doctor can check your blood pressure and do a simple blood test to see if your LDL, HDL, and triglyceride levels are high, These factors can also raise your risk for cardio vascular disease (CVD

)Smoking ,being overweight or having obesity ,Not getting enough physical activity ,eating a diet high in saturated fat, trans fat, cholesterol, and sodium (salt) Drinking too much alcohol ,people with diabetes are also more likely to have CVD. CVD is a serious condition, but it doesn't mean the heart has stopped beating; it means your heart can't pump blood well. This can lead to swelling in your legs and fluid building up in your lungs, making it hard to breathe. CVD tends to get worse over time, but early diagnosis and treatment can help relieve symptoms and stop or delay the condition getting worse (Xiong ,2019 ).

## 2.5 association between diabetes mellitus and uric acid (pathological mechanism of uric acid on diabetes and Its chronic complications) pathological mechanisms

Inflammation increased uric acid levels in the blood promoted the expression of interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNFα), and C reactive Protein (CRP) production. In animal studies, the activation of inflammation induced by UA decreases insulin sensitivity in mice, and infusion of UA into mice can increase TNF-α levels and activate the classical inflammatory pathway. In human studies, serum UA was positively associated with TNF-α, interleukin-6 and C-reactive protein in healthy people (Xiong ,2019 ).

Oxidative Stress excessive uric acid will lead to an increase in reactive oxygen species (ROS) production, which leads to inflammation and dysfunction in the vessel. UA is a powerful antioxidant that can remove superoxide and hydroxyl radicals in plasma, and UA has prooxidant effects in vascular tissue by increasing ROS production, such as H2O2. UA-mediated oxidative stress-induced lipid peroxidation, deoxy ribo nucleic acid( DNA) damage, and activation of inflammatory factors finally lead to cellular damage. oxidative stress also can affect the expression of insulin gene, causing a decrease in insulin secretion. (Xiong Q.,2019 ).

Endothelial dysfunction is characterized by deficiencies in the synthesis and/or bioavailability of endothelium-derived NO. In addition, UA reduces endothelial NO bioavailability in humans. uric acid inhibits proliferation and migration of endothelial cells and NO secretion. UA can react with NO to form 6-aminouracil, UA dependent ROS reacts with NO to form peroxynitrite, and UA can hold back

L-arginine uptake and stimulate L-arginine degradation. As a result of the effects of hyperglycemia and neurohormonal activation, UA levels are independently associated with endothelial dysfunction in animals and humans, thereby promoting hypertension (Xiong ,2019 ).

Inhibiting insulin pathway UA directly inhibits the trigger of insulin signaling pathway by an ectonucleotide pyro phosphatase/phosphor diesterase 1 (ENPP1) recruitment at the receptor level. All factors interference with glucose homeostasis and insulin sensitivity promotes the development of diabetes. (Xiong, 2019 ).

### 2.5.1 Uric Acid and diabetic chronic complications.

Eafore mentioned changes to diabetes are also directly related to the metabolic disorder: desulfation of glycosaminoglycans (GAGs) and formation of advanced glycation end products (AGE) and receptors (RAGE). It is widely believed that polyol bypass, protein kinase C, hexosamine activation, advanced glycosylation products (AGEs), increased hyperglycemia-induced mitochondria production of reactive oxygen species (ROS), inflammation, and endothelial dysfunction are the common pathogenic characteristics of chronic complications of diabetes mellitus , which mainly include macroangiopathy, microangiopathy, and neuropathy. Two other mechanisms are associated with chronic complications as: Activation of RAAS , uric acid can lead to the activation of the renin-angiotensin-aldosterone System (RAAS), through increasing the production of juxtaglomerular renin. UA-induced ROS stimulated the increase of plasma angiotensin II which induced aldosterone release, leading to activation of RAAS. RAAS activation induced afferent renal arteriolopathy and tubulointerstitial fibrosis in rodent models. In diabetes, RAAS activation causes a range of pathological changes including vascular dysfunction, high intraglomerular pressure, inflammation, and so on, leading to cardiovascular and renal complications. thrombus Uric acid seems to trigger platelet adhesion and aggregation, thus favoring vascular thrombosis. (Xiong, 2019 )

# Chapter Three

# 3. Materials and Methods

## 3.1. Materials:

### 3.1.1. Study design, area and period:

this was cross sectional hospital-based study conducted, in different diabetic center (Sad Roshwan and and Gabber Abo aleis ) in Khartoum state among 300 Sudanese patients with type 2 Diabetes mellitus from March to July 2022.

### 3.1.2 Inclusion criteria:

Sudanese patients with type 2 diabetes mellitus included.

### 3.1.3 Exclusion criteria:

patients took any treatment to lowering lipid and patients with kidney dieses, rheumatoid arthritis, malignant diseases, pregnant women, and patients apparently with any type of inflammations were excluded from this study.

### 3.1.4 Ethical consideration:

Approval from university ,Khartoum state ministry of health research department , from the center, statistical unit of the center and verbal consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donors knew that this specimen was collected for research purpose.

### 3.1.5 Data collection:

Data were collected using structural interviewing questionnaire (see appendix I), which was designed to collect and maintain all valuable information concerning each case examined.

### 3.1.6 Sampling:

Five ml of venous blood sample was collected from each participant, the blood was drawn in heparin containers, and then centrifuge at 4000 rpm for three minutes to get plasma. the plasma prepared was collected into 1.5 ml eppendorf tubes and kept frozen at (-20c) until analysis

# 3.2 Methods:

## 3.2.1 Estimation of plasma total cholesterol level:

### 3.2.1.1. Principle for TC:

Cholesterol ester will be converted to cholesterol and fatty acid by using cholesterol esterase enzyme. Then cholesterol will be converted to cholestenone and hydrogen peroxide in the presence of cholesterol oxidase enzyme. Hydrogen peroxide, aminoantipyrine and phenol in the presence of peroxidase enzyme will be converted to colored complex (Quinoneimine) that will be measured by colorimetric assay ( Marshal., 2021).

### 3.2.1.2 Reagent composition, storage and reagent preparation for TC

(See Appendix II).

**3.2.1.3 Procedure and calculation of TC:** (see Appendix II).

## 3.2.2 Estimation of low-density lipoprotein (LDL-C):

### 3.2.2.1. Principle of LDL-C:

LDL in the sample precipitate with polyvinyl sulphate. Their concentration will be calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation. Then, by using pervious serial reactions in principle of total cholesterol estimation, the cholesterol will be converted to colored complex (Quinoneimine)which will be measured by colorimetric assay ( Marshal., 2021).

### 3.2.2.2 Reagent composition, storage and reagent preparation for LDL-C:( see Appendix III).

**3.2.2.3 Procedure and calculation of LDL-C:** (Appendix III).

## 3.2.3 Estimation of Uric acid:

### 3.2.3.1 Principle of UA:

Uric acid is determined after enzymatic oxidation in the presence of uricase (based on modified Trinder peroxidase method ) the formed hydrogen peroxide react under catalysis of peroxidase (PAP) with 3,5-dichloro-2-hydroxybenzenessulfonic

acid (DCHB) 4-aminoantipyrine to form a red violet quinonemine dye .where its absorbance is proportional to the concentration of uric acid in sample ( Marshal., 2021).

### 3.2.3.2 Reagent composition, storage and reagent preparation for UA

( see Appendix IV).

### 3.2.3.3 Procedure and calculation of UA: (Appendix IV).

### 3.2.4 Estimation of HbA1c:

### 3.2.4.1 Principle of HbA1c:

The test uses a sandwich immune detection 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 ( Marshal., 2021).

### 3.2.4.2 Reagent composition,storage and reagent preparation for HbA1c:

( see Appendix V).

### 3.2.4.3 Procedure and calculation of HbA1c: (Appendix V).

### 3.3 Quality control:

The precision and accuracy of all methods used in this study were checked each batch was analyzed by including commercially prepared control level 1 as normal rang and control level 2 as pathological sample before it is application for the measurement of the test samples.

## 3.4 Statistical analysis:

The Data obtained from this study was analyzed using statistical package for the social science (SPSS version 26). Independent t test was used for comparison ,0.5% was taken as cut of limit for 95% statistical significance frequency and percentage testes were used and then the data were presented in tables ,and chi-square

test was used ,P. value $\leq 0.05$ was considered as the level of significance, and person correlation was used for correlation.

# Chapter Four

# 4-Result:

In this study of three hundred Sudanese with T2DM their age between (25-90) years, from different diabetic center (Khartoum state).

**Figure (4-1)** Distribution of sex among study group according to age groups.

**Figure (4-2)** Distribution of body mass index groups among type 2 DM based on sex.

**Figure (4-3** Distribution of education levels in the study group based on sex.

**Figure (4-4)** distribution of family history in study group based on sex.

**Figure (4-5)** Distribution of the habitual smoking in the study group based on sex.

**Table (4-1) ):** Comparisons between age, BMI, WC, HbA1c, TC, LDL-C & UA in Diabetic males and females.

**Table (4-2**). Comparisons between age, BMI, WC HbA1c, TC, LDL-C &UA to glycemic control intype 2 DM patients.

**Table (4-3)** Cross tabulation between TC groups, LDL-C groups and UA groups based on glycemic control.

**Figure (4-6)** scatter plot showed correlation between HbA1c and TC (r =0.576, p = 0.000) among diabetic patients.

**Figure (4-7)** scatter plot showed correlation between HbA1c and LDL-C (r =0.552, p = 0.000) among diabetic patients.

**Figure (4-8)** scatter plot showed correlation between HbA1c and UA (r =0.069, p = 0.232) among diabetic patients.

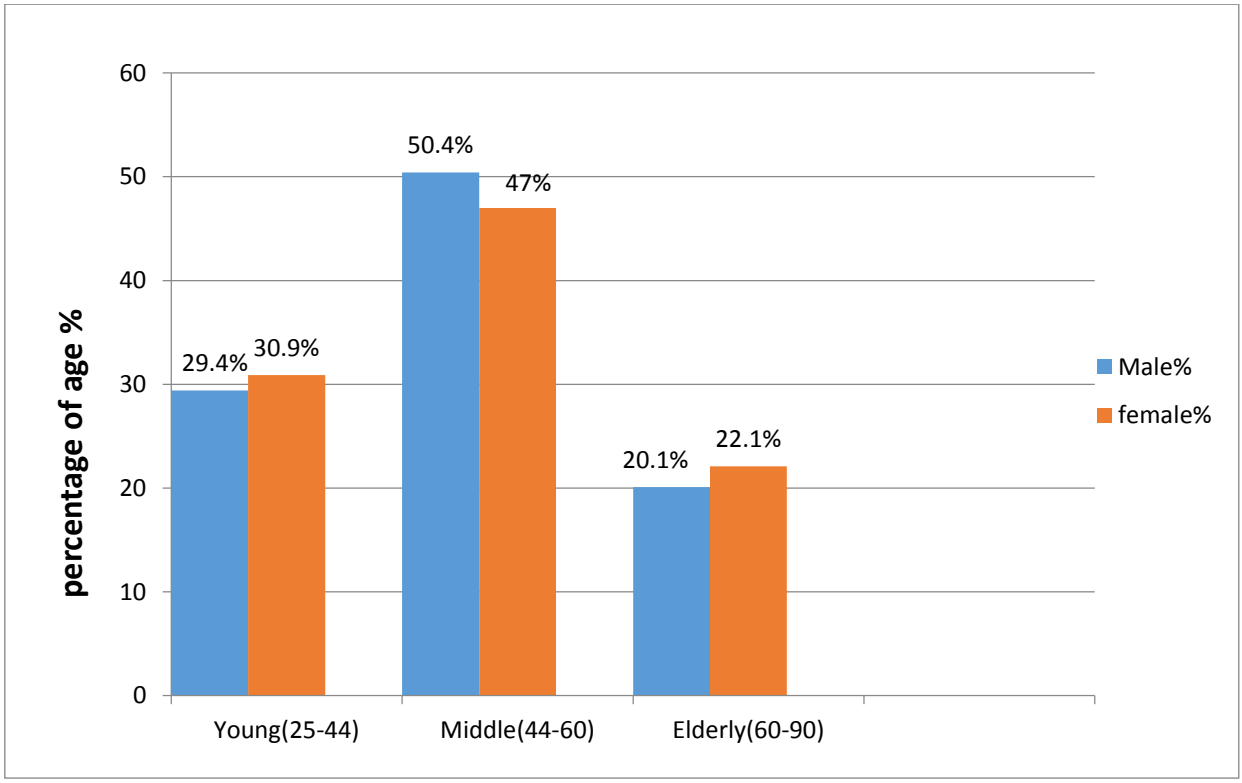



**Figure (4-1)**: Distribution of sex among study group according to age groups.

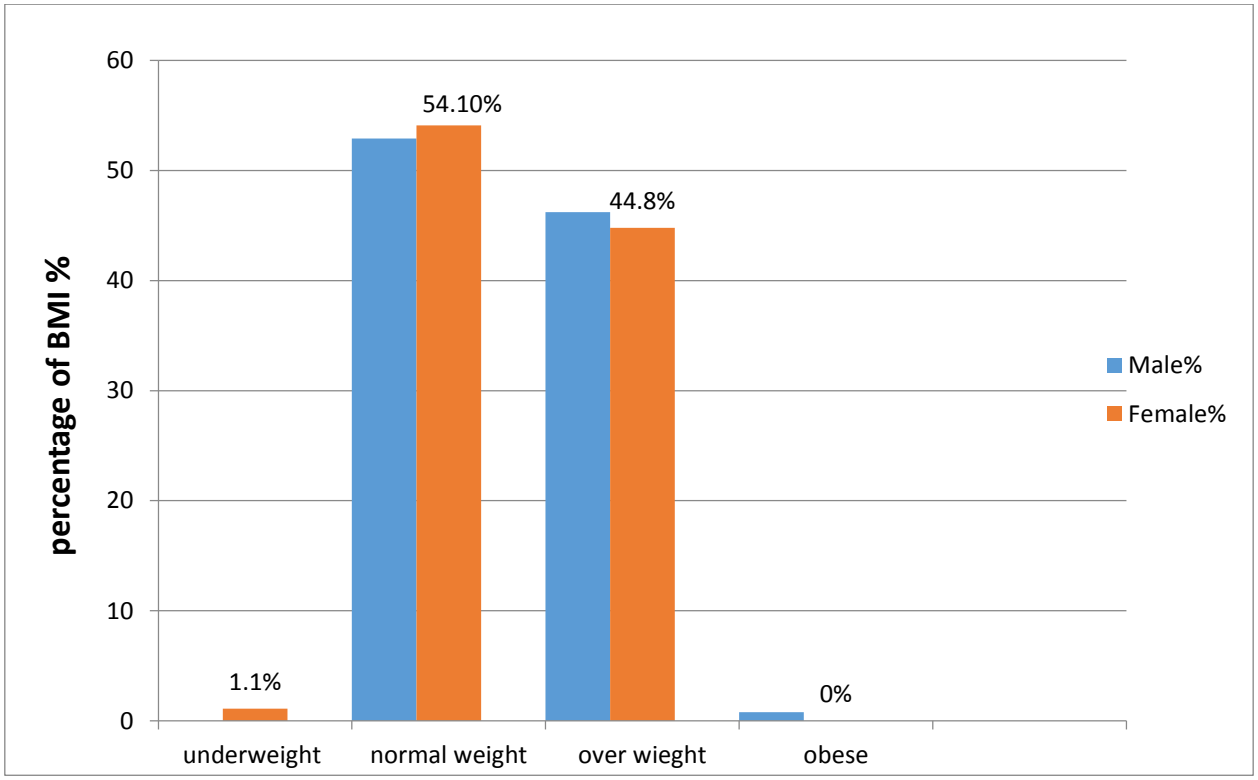



**Figure (4-2)**: Distribution of body mass index groups among type 2 DM based on sex.

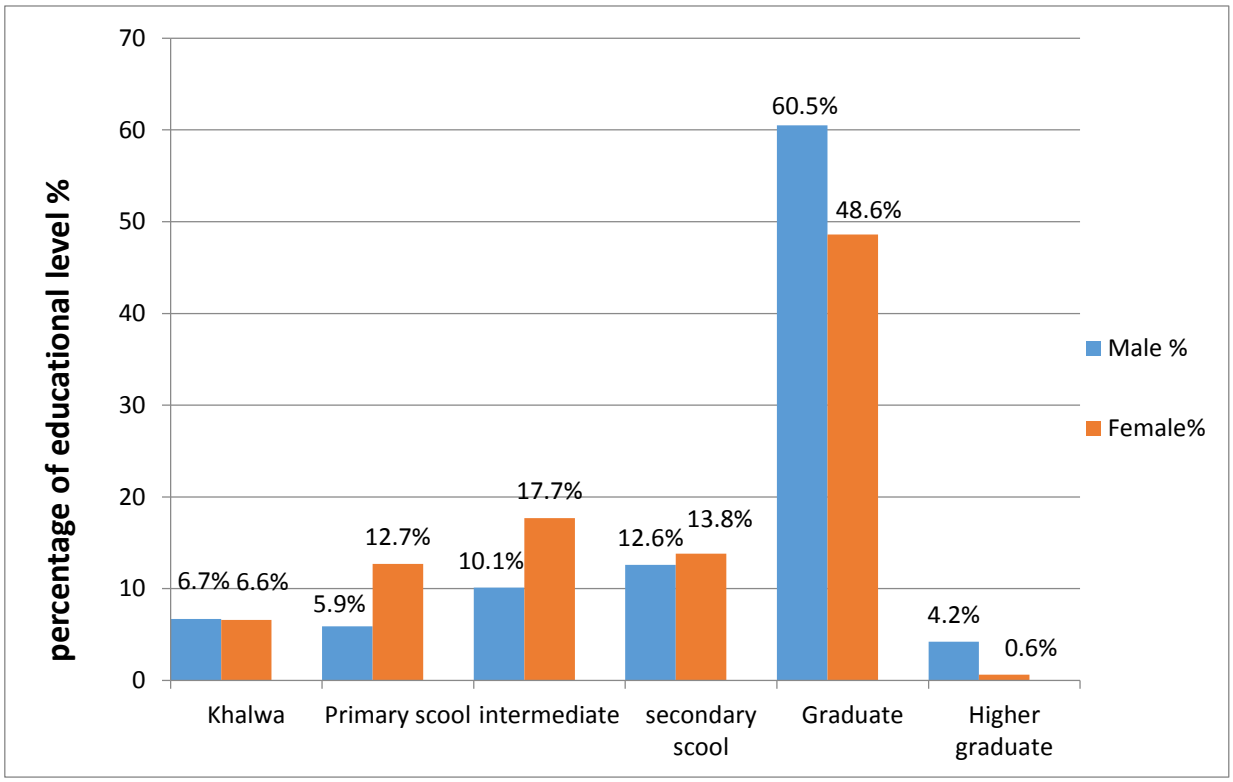



**Figure (4-3)**: Distribution of education levels in the study group based on sex.

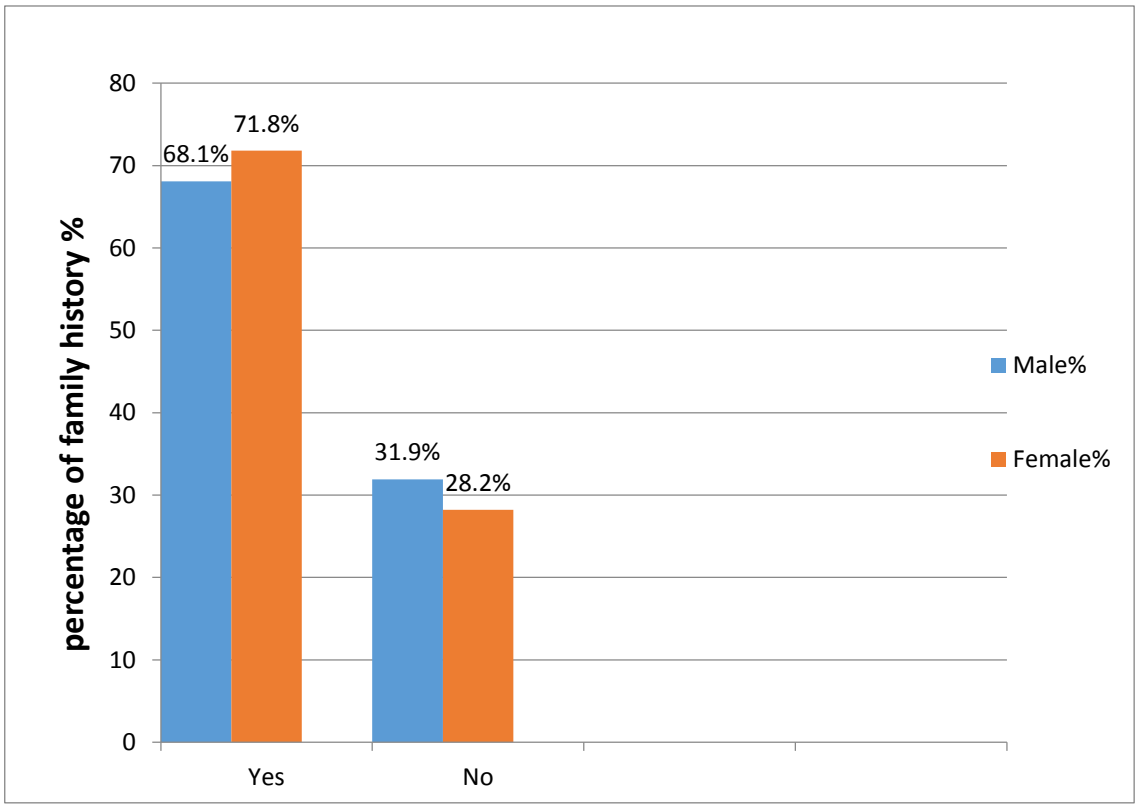



**Figure (4-4)** : distribution of family history in study group based on sex.

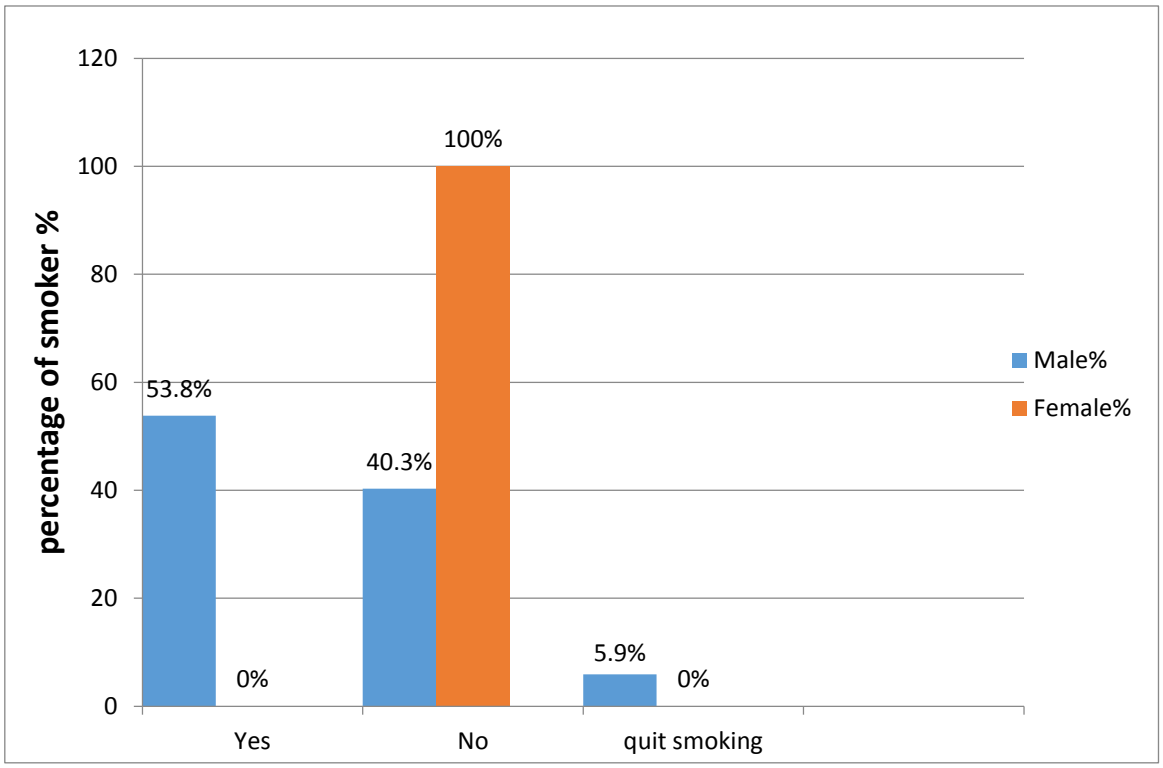



**Figure (4-5):** Distribution of the habitual smoking in the study group based on sex.

**Table (4-1):** Comparisons between age, BMI, WC, HbA1c, TC, LDL-C & UA in Diabetic males and females.

| Variables | Males n=119 (39.7%) Mean ± SD | Females n=181 (60.3%) Mean ± SD | P. value |
|---|---|---|---|
| **Age** (years) | 51.0 ± 11.4 | 50.7 ± 11.8 | 0.813 |
| **BMI** ($Kg/m^2$) | 25.0 ± 3.1 | 24.7 ± 3.4 | 0.378 |
| **WC** (Cm) | 88.5 ± 9.5 | 85.7 ± 9.0 | 0.014* |
| **HbA1c** % | 8.7 ± 2.4 | 9.0 ± 2.4 | 0.235 |
| **TC** mg/dl | 221.1 ± 47.7 | 235.8± 50.8 | 0.012* |
| **LDL-C** mg/dl | 137.2 ± 39.7 | 150.9 ±40.6 | 0.004* |
| **UA** mg/dl | 5.4 ± 1.9 | 5.7 ± 3.4 | 0.332 |

Independent T-Test was used to compare between the means.

P.value was significant <=0.05 (5%). BMI: body mass Index, WC: waist circumference, HbA1c: hemoglobin A1c (glycated hemoglobin), TC: total cholesterol, LDL-C: low density lipoprotein cholesterol, UA: uric acid.

**Table (4-2):** Comparisons between age, BMI, WC HbA1c, TC, LDL-C &UA to glycemic control intype 2 DM patients.

| **Variables** | **Good glycemic. (HbA1c≤ 7%); n= 81 (27%) Mean ± SD** | **poor glycemic (HbA1c>7%); n=219 (73%) Mean ± SD** | **P. value** |
|---|---|---|---|
| **Age** (years) | 50.9 ± 12.8 | 50.8 ± 11.1 | 0.922 |
| **BMI** ($Kg/m^2$) | 24.7 ± 3.0 | 24.8 ± 3.4 | 0.77 |
| **WC** (Cm) | 86.2 ± 9.2 | 87.1 ± 9.3 | 0.485 |
| **HbA1c** % | 6.1 ± 0.7 | 9.9 ± 1.9 | 0.000 |
| **TC** mg/dL | 189.1 ± 23.9 | 245.1 ± 48.7 | 0.000 |
| **LDL-C** mg/dL | 110.5 ± 21.5 | 158.4 ± 38.5 | 0.000 |
| **UA** mg/dL | 5.5 ± 4.4 | 5.7 ± 2.2 | 0.729 |

Independent T-Test was used to compare between the means.

P. value was significant ≤ 0.05 (5%).

BMI: body mass Index , WC: waist circumference ,HbA1c:hemoglobin A1c (glycated hemoglobin), TC: total cholesterol, LDL-C: low density lipoprotein cholesterol, UA: uric acid.

**Table (4-3)**: Cross tabulation between TC groups, LDL-C groups and UA groups based on glycemic control.

| **Variables** | **Good glycemic (HbA1c ≤ 7%) Frequency (%)** | **poor glycemic (HbA1c >7%) Frequency (%)** | **P. value** |
|---|---|---|---|
| **TC group** | | | |
| **≤200** mg/dl | 67 (82.7%) | 42 (19.2%) | 0.000* |
| **>200** mg/dl | 14 (17.3%) | 177 (80.8%) | |
| **LDL-C group** | | | |
| **≤130** mg/dl | 69 (85.2%) | 54 (24.7%) | 0.000* |
| **>130** mg/dl | 12 (14.8%) | 165 (75.3%) | |
| **UA group** | | | |
| **Normal** †mg/dl | 70 (86.4%) | 152 (69.4%) | 0.003* |
| **High**§ mg/dl | 11 (13.6%) | 67 (30.6%) | |

Chi-square test was used. P=Value was significant at level <=0.05.

HbA1C: hemoglobin A1c (glycated hemoglobin), TC: total cholesterol, LDL-C: low density lipoprotein cholesterol, UA: uric acid. † normal UA for male up to 7.5 mg/dl and female up to 5.5 mg/dl; § High: UA more than 7.5 for male and more than 5.5 for female.

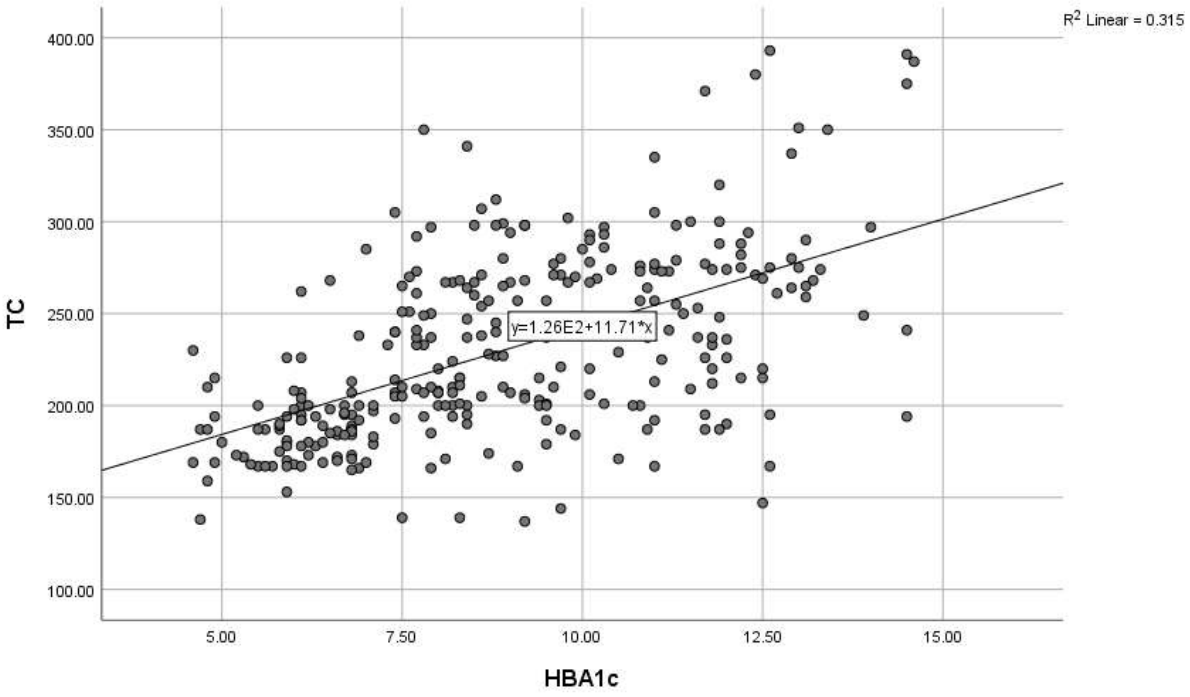



**Figure (4-6):** Scatter plot shows significant positive correlation between HbA1c and total cholesterol (r = 0.576; P = 0.000) among diabetic patients.

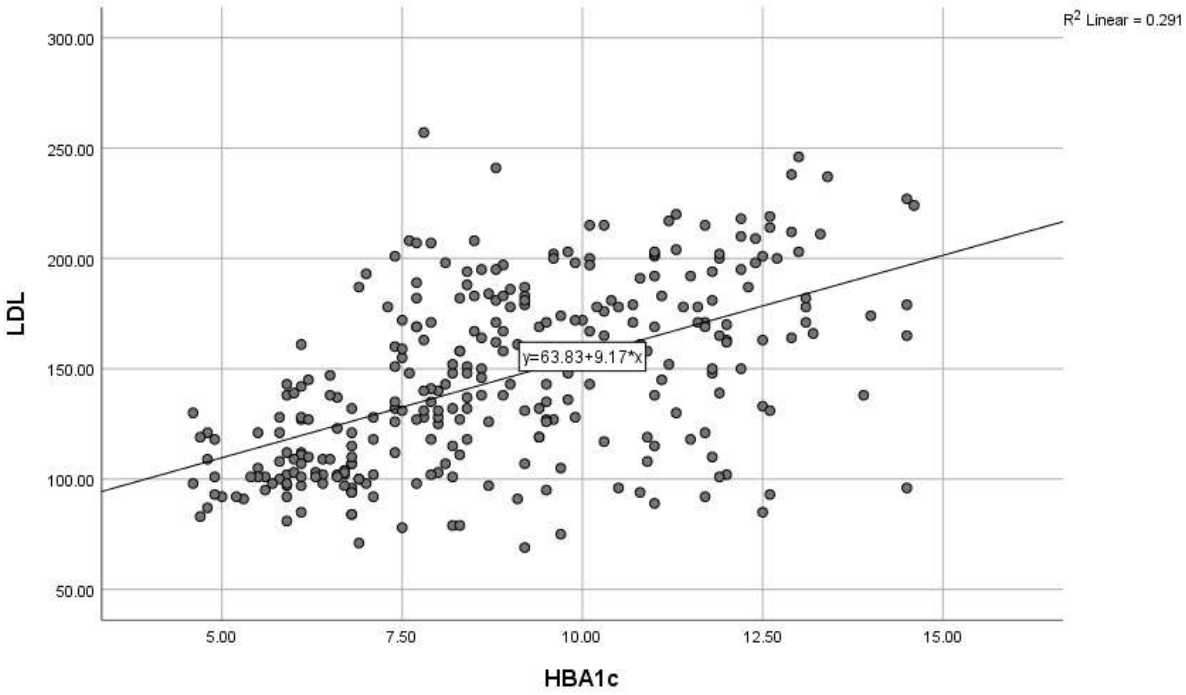



**Figure (4-7):** scatter plot showed significant Positive correlation between HbA1c and LDL-C (r = 0.552, P = 0.000) among diabetic patients.

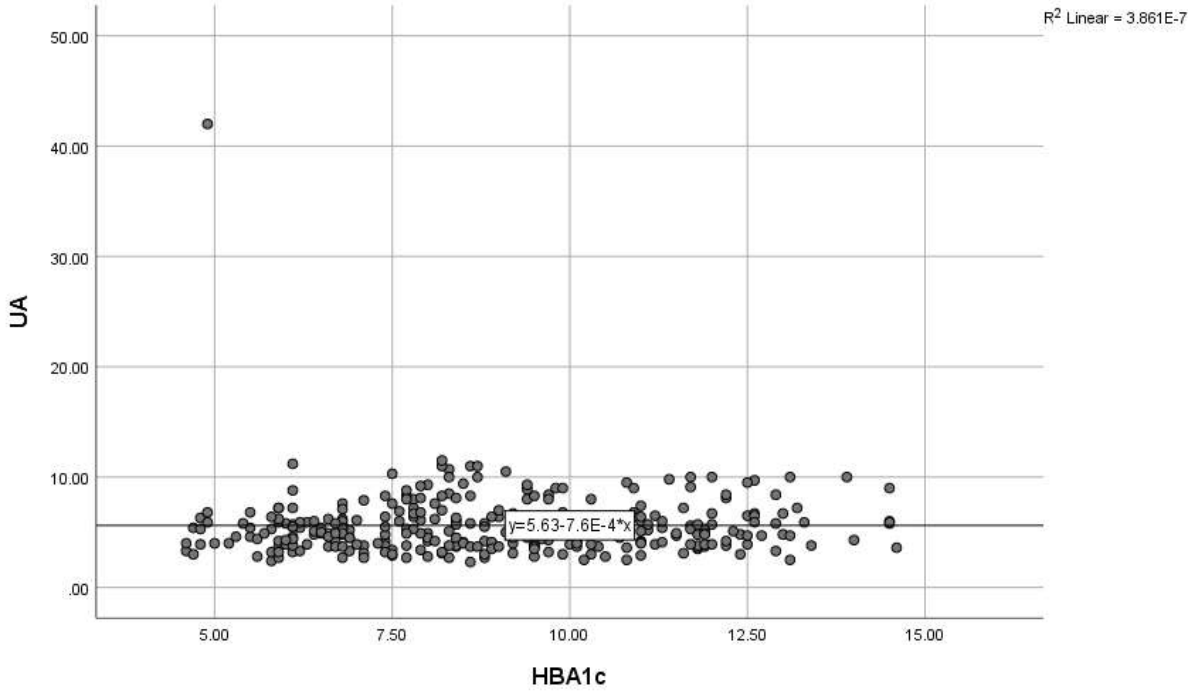



**Figure (4-8):** Scatter plot showed insignificant Positive correlation between HbA1c and UA (r = 0.069, p = 0.232) among diabetic patients.

# Chapter Five

# 5. Discussion, Conclusion and Recommendations

## 5.1 Discussion:

Diabetes mellitus is a metabolic disorder of multiple etiology, characterized by chronic hyperglycemia with disturbances of carbohydrates, fats, and proteins metabolism resulting from defects in insulin secretion, insulin action, or both. The main complication of uncontrolled diabetic is hyperlipidemia which causes more serious disease as cardiovascular disease (Marshal, 2021).

A Study showed that high frequency type 2 diabetes Mellitus among Sudanese females. This disagree with study done by Xing et al, whom revealed that men were more affected than females (Xing et al., 2020). This may be due to difference in ethnicity or dietary habits ..etc.

the study showed the most of type 2 diabetes Mellitus their age between 44-60 years; about 70% of patients had positive family history to diabetes, and about 50% of patients were overweight to obese. These were in agreement with Miah and et al., whom found that in developing countries, the majority of people with diabetes their age between 45–64 age and the most affected group is those whom have postive family history and overweight (Miah et al .,2018). Study showed that most Sudanese patients with type 2 had higher educational level and habitual smoker. Bellou and et al revealed that low educational level and smoker were most affected by DM (Ballou et al., 2018).

In this study 73% of Sudanese with type2 DM patients had poor glycaemic control based on HbA1C level; this agree with study done by Fox and et la…,whom revealed that poor control of blood sugar in patients with type2 diabetes is still common in the United Kingdom (Fox et al., 2006). Moreover; our study found that poor glycaemic control had significant increase in TC and LDL-C level. Additionally, 80.0% of poor glycaemic had hypercholesterolemia (TC> 200mg/dL) and 75.0% had LDL-C > 130mg /dL).This agrees with study done by Artha and et al revealed poor control glycaemic of type 2 diabetic patients had increase in lipid profile (Artha et al., 2019).

However, study found there was insignificant difference in plasma uric acid between poor and good glycaemic control, although 30.0% of poor glycaemic had hyperuricemia. This was in agreement with study done by Suijk and et al, whom found all diabetic patients had average mean of UA (Suijk et al., 2020).

Study showed significant positive association between HbA1c & TC (r= 0.576, P = 0.000); LDL-C (r =0.552, p = 0.000), insignificant positive association between HbA1c & UA (r= 0.069, p = 0.232). Artha and et al found positive association between HbA1c &TC, LDL-C (Artha et al., 2019). Hu and et al.. revealed insignificant negative association between HbA1c and UA (Hu et al.,2021).

# 5.conclusion:

Poor glycaemic type 2DM had increased plasma TC and LDL-C. HbA1c was associated positively with TC and LDL-C. hypercholesterolemia (80%) , hyper-LDL-C (75%) and hyperuricemia (30%) were found in poor glycemic control type 2DM.

# 5.3Recommendations:

1- Awareness of diabetic patients about the importance of monitoring the level of sugar and lipid profile periodically to prevent complications of cardiovascular diseases.
2- Awareness of diabetic patients to stop bad habits such as smoking and move towards good habits such as proper food and exercise.
3- Change the type of study design as cohort study.
4- Further hospitals or centers are specialized for monitoring of cardiovascular disease in diabetes mellitus type2 are required.
5- great importance to establish strategies for combatting T2DM and its associated chronic conditions.

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# Appendices

# Appendices (I)

## Sudan University of Science &Technology

## Assessment of Plasma Total Cholesterol ,Low Density Lipoprotein Cholesterol and Uric Acid among Sudanese with Type 2 Diabetes in Khartoum state

## Questionnaire

**General information:**

NO .....

Age .....

Gender .....

Addressee .....

Contact phone number .....

Nationality .....

Educational level .....

**Clinical information:**

Having a chronic disease .....

Duration of Diabetes Mellitus .....

type of treatment .....

family history .....

Do you smoke .....

**Body measurements:**

Weight ..... mg/dl , Height ..... cm ,Waist ..... cm

**Biochemical Finding:**

TC ..... mg/dl , LDL ..... mg/dl

HBA1c ..... %UA ..... mg/dl

(Appendix II)

| COD 11805<br>1 x 50 mL | COD 11505<br>1 x 200 mL | COD 11506<br>1 x 500 mL | COD 11539<br>1 x 1 L |
|---|---|---|---|
| STORE AT 2-8ºC | | | |
| Reagents for measurement of cholesterol concentration<br>Only for *in vitro* use in the clinical laboratory | | | |

# CHOLESTEROL

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## CHOLESTEROL
CHOLESTEROL OXIDASE/PEROXIDASE

## PRINCIPLE OF THE METHOD

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[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

| | COD 11805 | COD 11505 | COD 11506 | COD 11539 |
|---|---|---|---|---|
| A. Reagent<br>S. Standard | 1 x 50 mL<br>1 x 5 mL | 1 x 200 mL<br>1 x 5 mL | 1 x 500 mL<br>1 x 5 mL | 1 x 1 L<br>1 x 5 mL |

## COMPOSITION

A. Reagent: Pipes 35 mmol/L, sodium cholate 0.5 mmol/L, phenol 28 mmol/L, cholesterol esterase > 0.2 U/mL, cholesterol oxidase > 0.1 U/mL, peroxidase > 0.8 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.0.

S. Cholesterol Standard. Cholesterol 200 mg/dL (5,18 mmol/L). Aqueous primary standard.

## STORAGE

Store at 2-8ºC.

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

Indications of deterioration:

- Reagent: Presence of particulate material, turbidity, absorbance of the blank over 0.200 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

## REAGENT PREPARATION

Reagent and Standard are provided ready to use.

## ADDITIONAL EQUIPMENT

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

## SAMPLES

Serum or plasma collected by standard procedures.

Cholesterol is stable for 7 days at 2-8ºC. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

## PROCEDURE

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

| | Blank | Standard | Sample |
|---|---|---|---|
| Cholesterol Standard (S) | — | 10 µL | — |
| Sample | — | — | 10 µL |
| Reagent (A) | 1.0 mL | 1.0 mL | 1.0 mL |

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

## CALCULATIONS

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

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

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

| | |
|---|---|
| $\frac{A_{Sample}}{A_{Standard}}$ | x 200 = mg/dL cholesterol<br>x 5.18 = mmol/L cholesterol |

## REFERENCE VALUES

The following uniform cut-off points have been established by the US National Cholesterol Education Program and have also been adopted in many other countries for the evaluation of coronary artery disease risk[3].

| | |
|---|---|
| Up to 200 mg/dL = 5.2 mmol/L<br>200-239 mg/dL = 5.2-6.21 mmol/L<br>> 240 mg/dL = > 6.24 mmol/L | Desirable<br>Borderline High<br>High |

## QUALITY CONTROL

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

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

## METROLOGICAL CHARACTERISTICS

- Detection limit: 0.3 mg/dL = 0.008 mmol/L.
- Linearity limit: 1000 mg/dL = 26 mmol/L. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

| Mean Concentration | CV | n |
|---|---|---|
| 121 mg/dL = 3.13 mmol/L | 1.1 % | 20 |
| 257 mg/dL = 6.66 mmol/L | 0.9 % | 20 |

- Reproducibility (run to run):

| Mean Concentration | CV | n |
|---|---|---|
| 121 mg/dL = 3.13 mmol/L | 1.8 % | 25 |
| 257 mg/dL = 6.66 mmol/L | 1.0 % | 25 |

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemolysis (hemoglobin up to 500 mg/dL), bilirubin (up to 10 mg/dL) and lipemia (triglycerides up to 1000 mg/dL) do not interfere. Ascorbic acid (up to 6.25 mg/dL) does not interfere. Other drugs and substances may interfere[4].

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

## DIAGNOSTIC CHARACTERISTICS

Cholesterol is a steroid of high molecular weight and possesses the cyclopentanophenanthrene skeleton. Dietary cholesterol is partially absorbed and it is also synthesized by the liver and other tissues. Cholesterol is transported in plasma by lipoproteins. It is excreted unchanged into bile or after transformation to bile acids.

Increased total cholesterol values are associated with a progressively escalating risk of atherosclerosis and coronary artery disease[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. This reagent may be used in several automatic analysers. Instructions for many of them are available on request.
2. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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| КОД 11805<br>1 x 50 мл | КОД 11505<br>1 x 200 мл | КОД 11506<br>1 x 500 мл | КОД 11539<br>1 x 1 n |
|---|---|---|---|
| Хранить при 2-8°C | | | |
| Реагенты для измерения концентрации холестерина Использовать только для работы «in vitro» в клинической лаборатории | | | |

CHOLESTEROL

BioSystems

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# ХОЛЕСТЕРИН
ХОЛЕСТЕРОЛОКСИДАЗА/ПЕРОКСИДАЗА

## ПРИНЦИП МЕТОДА

Свободный и этерифицированный холестерин образца образует в результате сопряженных реакций, описанных ниже, цветной комплекс, который может быть измерен спектрофотометрически[1,2].

$$\text{Эфиры холестерина} + H_2O \xrightarrow{\text{холестеразa}} \text{Холестерин} + \text{Жирные кислоты}$$

$$\text{Холестерин} + S\,O_2 + H_2O \xrightarrow{\text{холестеролоксидаза}} \text{Холестенон} + H_2O_2$$

$$2\,H_2O_2 + \text{4-Аминоантипирин} + \text{Фенол} \xrightarrow{\text{пероксидаза}} \text{Хинонимин} + 4\,H_2O$$

## НАБОРЫ

| | КОД 11805 | КОД 11505 | КОД 11506 | КОД 11539 |
|---|---|---|---|---|
| A. Реагент<br>S. Стандарт | 1 x 50 мл<br>1 x 3 мл | 1 x 200 мл<br>1 x 3 мл | 1 x 500 мл<br>1 x 3 мл | 1 x 1n<br>1 x 3 мл |

## СОСТАВ

A. Реагент: PIPES 35 ммоль/л, холат натрия 0,5 ммоль/л, фенол 28 ммоль/л, холестеролэстераза > 0.2 Ед/мл, холестеролоксидаза > 0.1 Ед/мл, пероксидаза > 0.8 Ед/мл, 4-Аминоантипирин 0.5 ммоль/л, pH 7.0.

S. Стандарт Холестерина: Холестерин 200 мг/дл (5.18 ммоль/л). Первичный водный стандарт.

## ХРАНЕНИЕ

Хранить при 2 - 8°C.

Реагенты и стандарт стабильны до окончания срока годности, указанного на этикетке, при хранении в плотно закрытом сосуде и предотвращении загрязнения во время использования.

Показатели загрязнения:

– Реагент: присутствие взвешенных частиц, мутность, абсорбция свыше 0.200 при 500 нм (1 см кювета).

– Стандарт: присутствие взвешенных частиц, мутность.

## ПРИГОТОВЛЕНИЕ РЕАГЕНТА

Реагент и стандарт поставляются готовыми к использованию.

## НЕОБХОДИМОЕ ОБОРУДОВАНИЕ

– Термостатируемая водяная баня на 37°C.

– Анализатор, спектрофотометр или фотометр с фильтром 500 ± 20 нм.

## ОБРАЗЦЫ

Сыворотка или плазма, полученные с помощью стандартных процедур.

Стабильность составляет 7 дней при 2-8°C. Гепарин, ЭДТА, оксалат и фторид могут использоваться в качестве антикоагулянтов.

## ПРОЦЕДУРА

1. Нагреть рабочий реагент до комнатной температуры.
2. Разлить в промаркированные пробирки (примечание 1):

| | Холостая проба | Стандарт | Образец |
|---|---|---|---|
| Стандарт Холестерина (S) | — | 10 мкл | — |
| Образец | — | — | 10 µL |
| Реагент (A) | 1.0 мл | 1.0 мл | 1.0 мл |

3. Тщательно перемешать и инкубировать 10 минут при комнатной температуре (16-25°C) или 5 минут при 37°C (примечание 2).
4. Измерить абсорбцию (A) Стандарта и Образца при 500 нм против Холостой пробы. Окраска раствора стабильна не менее 2 часов.

## РАСЧЕТ

Концентрация холестерина в образце вычисляется по следующей формуле:

$$\frac{A_{\text{образца}}}{A_{\text{стандарта}}} \times C_{\text{стандарта}} = C_{\text{образца}}$$

Если для калибровки используется поставляемый стандарт холестерина (прим.2):

| | |
|---|---|
| $\frac{A_{\text{проба}}}{A_{\text{стандарт}}}$ | x 200 = мг/дл холестерина<br>x 5.18 = ммоль/л холестерина |

## НОРМАЛЬНЫЕ ЗНАЧЕНИЯ

Следующие пограничные значения были установлены Национальной Образовательной Программой по Холестеролу (США) и были приняты во многих других странах для оценки риска заболевания коронарной артерии[3].

| | |
|---|---|
| До 200 мг/дл = 5.2 ммоль/л | Допустимый |
| 200-239 мг/дл = 5.2 - 6.21 ммоль/л | Пограничные |
| >240мг/дл = 6.24 ммоль/л | Высокие |

## КОНТРОЛЬ КАЧЕСТВА

Для проведения контроля качества теста и процедуры исследования рекомендуется использовать Контрольную сыворотку Уровень I (код 18005, 18009 и 18042) и уровень II (код 18007, 18010 и 18043). Каждая лаборатория должна выработать собственную схему внутреннего контроля качества и процедуры для коррекции действий в случае, если контроль качества не укладывается в приемлемые диапазоны.

## МЕТРОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ

– Предел обнаружения: 0,3 мг/дл = 0,008 ммоль/л

– Предел линейности: 1000 мг/дл = 26 ммоль/л. Для более высоких значений разведите образец в два раза дистиллированной водой и повторите измерение.

– Сходимость (внутри серии)

| Средняя концентрация | CV | n |
|---|---|---|
| 121 мг/дл = 3.13 ммоль/л | 1.1 % | 20 |
| 257 мг/дл = 6.66 ммоль/л | 0.9 % | 20 |

– Воспроизводимость (между сериями)

| Средняя концентрация | CV | n |
|---|---|---|
| 121 мг/дл = 3.13 ммоль/л | 1.9 % | 25 |
| 257 мг/дл = 6.66 ммоль/л | 1.0 % | 25 |

– Достоверность: Результаты, полученные при использовании данных реагентов, не показывают систематической ошибки при сравнении с референсными реагентами (примечание 2). Детали сравнительных экспериментов доступны по требованию.

– Влияние: Гемолиз (гемоглобин до 500 мг/дл), Билирубин (до 10 мг/дл) и липемия (триглицериды до 1000 мг/дл) не мешают определению. Аскорбиновая кислота (до 6.25 мг/дл) не мешает определению[4].

Данные метрологические характеристики были получены при использовании анализатора, при использовании другого оборудования или ручных методов результаты могут варьировать.

## ДИАГНОСТИЧЕСКИЕ ХАРАКТЕРИСТИКИ

Холестерин – стероид с высокой молекулярной массой и циклопентанофенантреновым скелетом. Холестерин пищи частично абсорбируется, также холестерин синтезируется печенью и другими тканями. Холестерин транспортируется в плазму липопротеинами. Холестерин экскретируется неизменным в желчь или после трансформации в желчные кислоты.

Повышенные значения общего холестерина связаны с постепенно возрастающим риском атеросклероза и заболеваниями коронарных артерий [3,5].

Клинический диагноз не должен основываться на результатах отдельного теста, он должен согласовываться с результатами клинических и лабораторных данных.

## ПРИМЕЧАНИЯ

1. Данные реагенты могут быть использованы в различных автоматических анализаторах. Инструкции предъявляются по запросу.
2. Использование водного стандарта, особенно в некоторых анализаторах, может вызывать отклонения калибровочного графика, в этом случае рекомендуется использовать для калибровки стандарт на основе сыворотки (Сыворотка-Калибратор код 18011 и 18044).

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(Appendix III)

| COD 11579 20 mL |
|---|
| STORE AT 2-8ºC |
| Reagents for measurement of LDL cholesterol concentration<br>Only for *in vitro* use in the clinical laboratory |

# CHOLESTEROL LDL PRECIPITATING REAGENT

BioSystems

CE

CHOLESTEROL LDL
PRECIPITATING REAGENT
POLYVINYL SULPHATE / POLYETHYLENEGLYCOL

## PRINCIPLE OF THE METHOD

Low density lipoproteins (LDL) in the sample precipitate with polyvinyl sulphate. Their concentration is calculated from the difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation[1]. The cholesterol is spectrophotometrically measured by means of the coupled reactions described below.

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

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

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

## CONTENTS AND COMPOSITION

A. Reagent. 1 x 20 mL. Polyvinyl sulphate 3 g/L, polyethyleneglycol 327 g/L.

## STORAGE

Store at 2-8ºC.

Reagent is 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.

## ADDITIONAL REAGENTS

This precipitating reagent is to be used together with the Cholesterol Reagent contained in any of the BioSystems Cholesterol kits (cod. 11505, 11506, 11539).

## REAGENT PREPARATION

Reagent is 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 collected by standard procedures.

LDL cholesterol in serum is stable for 24 hours at 2-8ºC.

## PROCEDURE

Precipitation

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

| | |
|---|---|
| Sample | 0.2 mL |
| Reagent (A) (Cholesterol LDL kit) | 0.2 mL |

2. Mix thoroughly and let stand for 15 minutes at room temperature.
3. Centrifuge at a minimum of 4000 r.p.m. for 15 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 | 20 µL | — | — |
| Cholesterol Standard (S) | — | 20 µL | — |
| Sample supernatant | — | — | 20 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 cholesterol concentration in the supernatant is calculated using the following general formula:

$$\frac{A\ \text{Sample}}{A\ \text{Standard}} \times C\ \text{Standard} \times \text{Sample dilution factor} = C\ \text{Supernatant}$$

If the Cholesterol Standard, provided in the Cholesterol kit, has been used to calibrate (Note 4):

| | |
|---|---|
| $\frac{A\ \text{Sample}}{A\ \text{Standard}}$ | x 200 x 2 = mg/dL cholesterol in supernatant<br>x 5.18 x 2 = mmol/L cholesterol in supernatant |

The LDL cholesterol concentration in the sample is calculated as follows:

LDL cholesterol = total cholesterol – cholesterol in supernatant

## REFERENCE VALUES

The following uniform cut-off points have been established by the US National Cholesterol Education Program and have also been adopted in many other countries for the evaluation of coronary artery disease risk[2].

| | |
|---|---|
| Up to 100 mg/dL = 2.59 mmol/L | Optimal |
| 100-129 mg/dL = 2.59-3.34 mmol/L | Near optimal/above optimal |
| 130-159 mg/dL = 3.37-4.12 mmol/L | Borderline High |
| 160-189 mg/dL = 4.14-4.90 mmol/L | High |
| > 190 mg/dL = 4.92 mmol/L | Very High |

## QUALITY CONTROL

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

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

## METROLOGICAL CHARACTERISTICS

– Detection limit: 0.45 mg/dL = 0.01 mmol/L.

– Linearity limit: 1000 mg/dL = 26 mmol/L.

– Repeatability (within run):

| Mean Concentration | CV | n |
|---|---|---|
| 120 mg/dL = 3.11 mmol/L | 1.5 % | 20 |
| 200 mg/dL = 5.18 mmol/L | 1.4 % | 20 |

– Reproducibility (run to run):

| Mean Concentration | CV | n |
|---|---|---|
| 120 mg/dL = 3.11 mmol/L | 2.8 % | 25 |
| 200 mg/dL = 5.18 mmol/L | 1.5 % | 25 |

– 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 interfere. Other drugs and substances may interfere[3].

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

## DIAGNOSTIC CHARACTERISTICS

LDL is the main lipoprotein transporting cholesterol from liver to tissues.

Increased plasma LDL-cholesterol concentrations are positively correlated with the incidence of atherosclerosic diseases, basis of myocardial infarction and cerebrovascular accidents[4,5].

There are several disease states or environmental influences associated with increased levels of LDL cholesterol: nephrosis, diabetes, obesity, some drugs and smoking[4,5].

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 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 0.2 mL of Reagent, mix thoroughly and centrifuge. Multiply the obtained concentration by 1.5 (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).

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www.biosystems.es

| COD 11579 20 mL |
|---|
| CONSERVAR A 2-8ºC |
| Reactivos para medir la concentración de colesterol LDL<br>Sólo para uso in vitro en el laboratorio clínico |

# CHOLESTEROL LDL PRECIPITATING REAGENT

BioSystems

CE

# COLESTEROL LDL REACTIVO PRECIPITANTE

POLIVINIL SULFATO / POLIETILENGLICOL

## FUNDAMENTO DEL MÉTODO

Las lipoproteínas de baja densidad (LDL) presentes en la muestra, precipitan en presencia de polivinil sulfato. La concentración de colesterol LDL se calcula por diferencia entre los valores de colesterol en el suero y en el sobrenadante obtenido tras la precipitación[1]. El colesterol se cuantifica espectrofotométricamente mediante las reacciones acopladas descritas a continuación.

$$\text{Colesterol esterificado} + H_2O \xrightarrow{\text{col. esterasa}} \text{Colesterol} + \text{Ácido graso}$$

$$\text{Colesterol} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{col. oxidasa}} \text{Colestenona} + H_2O_2$$

$$2\,H_2O_2 + 4\text{-Aminoantipirina} + \text{Fenol} \xrightarrow{\text{peroxidasa}} \text{Quinoneimina} + 4\,H_2O$$

## CONTENIDO Y COMPOSICIÓN

A. Reactivo. 1 x 20 mL Polivinil sulfato 3 g/L, polietilenglicol 327 g/L.

## CONSERVACIÓN

Conservar a 2-8ºC.

El Reactivo es estables hasta la fecha de caducidad indicada en la etiqueta, siempre que se conserven bien cerrados y se evite la contaminación durante su uso.

Indicaciones de deterioro:

– Reactivo: Presencia de partículas, turbidez.

## REACTIVOS ADICIONALES

Este reactivo precipitante debe ser utilizado junto con el Reactivo de Colesterol contenido en cualquiera de los kits de Colesterol BioSystems (cod. 11505, 11505, 11506, 11539).

## PREPARACIÓN DE LOS REACTIVOS

El Reactivo está listo para su uso.

## EQUIPO ADICIONAL

– Centrífuga de sobremesa.

– Baño de agua a 37ºC.

– Analizador, espectrofotómetro o fotómetro para lecturas a 500 ± 20 nm.

## MUESTRAS

Suero recogido mediante procedimientos estándar.

El Colesterol LDL en suero es estable 24 horas a 2-8ºC.

## PROCEDIMIENTO

Precipitación

1. Pipetear en un tubo de centrífuga (Nota 1):

| | |
|---|---|
| Muestra<br>Reactivo (A) (kit de Colesterol LDL) | 0,2 mL<br>0,2 mL |

2. Agitar bien y dejar durante 15 minutos a temperatura ambiente.
3. Centrifugar durante 15 minutos a un mínimo de 4.000 r.p.m.
4. Recoger con cuidado el sobrenadante (Nota 2).

Colorimetría

5. Atemperar el Reactivo (kit de Colesterol) a temperatura ambiente.
6. Pipetear en tubos de ensayo: (Nota 3)

| | Blanco | Patrón | Muestra |
|---|---|---|---|
| Agua destilada | 20 µL | — | — |
| Patrón Colesterol (S) | — | 20 µL | — |
| Sobrenadante muestra | — | — | 20 µL |
| Reactivo (A) (kit de Colesterol) | 1,0 mL | 1,0 mL | 1,0 mL |

7. Agitar bien e incubar los tubos durante 30 minutos a temperatura ambiente (16-25ºC) o durante 10 minutos a 37ºC.
8. Leer la absorbancia (A) del Patrón y de la Muestra a 500 nm frente al Blanco. El color es estable durante al menos 30 minutos.

## CÁLCULOS

La concentración de colesterol en el sobrenadante se calcula a partir de la siguiente fórmula general:

$$\frac{A_{Muestra}}{A_{Patrón}} \times C_{Patrón} \times \text{Factor de dilución de muestra} = C_{Sobrenadante}$$

Si se utiliza para calibrar el Patrón de Colesterol, incluido en el Kit de Colesterol (Nota 4):

| | |
|---|---|
| $\frac{A_{Muestra}}{A_{Patrón}}$ | x 200 x 2 = mg/dL colesterol en sobrenadante<br>x 5,18 x 2 = mmol/L colesterol en sobrenadante |

La concentración de colesterol LDL en la muestra se calcula:

colesterol LDL = colesterol total – colesterol en sobrenadante

## VALORES DE REFERENCIA

Los siguientes valores discriminantes universales han sido establecidos por el US National Cholesterol Education Program y también aceptados en otros países para la evaluación del riesgo de enfermedad de las arterias coronarias[2].

| | |
|---|---|
| Hasta 100 mg/dL = 2,59 mmol/L | Óptimo |
| 100-129 mg/dL = 2,59-3,34 mmol/L | Casi óptimo |
| 130-159 mg/dL = 3,37-4,12 mmol/L | Moderado |
| 160-189 mg/dL = 4,14-4,90 mmol/L | Elevado |
| > 190 mg/dL = 4,92 mmol/L | Muy elevado |

## CONTROL DE CALIDAD

Se recomienda el uso de los Sueros Control Bioquímica nivel I (cod. 18005, 18009 y 18042) y nivel II (cod. 18007, 18010 y 18043) para verificar la funcionalidad de la colorimetría con el reactivo de colesterol.

Cada laboratorio debe establecer su propio programa de Control de Calidad interno, así como procedimientos de corrección en el caso de que los controles no cumplan con las tolerancias aceptables.

## CARACTERÍSTICAS METROLÓGICAS

– Límite de detección: 0,45 mg/dL = 0,01 mmol/L.

– Límite de linealidad: 1000 mg/dL = 26 mmol/L.

– Repetibilidad (intraserie):

| Concentración media | CV | n |
|---|---|---|
| 120 mg/dL = 3,11 mmol/L | 1,6 % | 20 |
| 200 mg/dL = 5,18 mmol/L | 1,4 % | 20 |

– Reproducibilidad (interserie):

| Concentración media | CV | n |
|---|---|---|
| 120 mg/dL = 3,11 mmol/L | 2,8 % | 25 |
| 200 mg/dL = 5,18 mmol/L | 1,5 % | 25 |

– Veracidad: Los resultados obtenidos con estos reactivos no muestran diferencias sistemáticas significativas al ser comparados con reactivos de referencia (Nota 4). Los detalles del estudio comparativo están disponibles bajo solicitud.

– Interferencias: La lipemia (Hyntralípicos 10 g/L) no interfiere. La hemólisis (hemoglobina 5 g/L) y la bilirrubina (10 mg/dL) pueden interferir. Otros medicamentos y sustancias pueden interferir[3].

Estos datos han sido obtenidos utilizando un analizador. Los resultados pueden variar al cambiar de instrumento o realizar el procedimiento manualmente.

## CARACTERÍSTICAS DIAGNÓSTICAS

Las LDL son las principales lipoproteínas que transportan colesterol hepático hacia los tejidos.

Existe una correlación positiva entre concentraciones elevadas de LDL-colesterol en plasma y la incidencia de aterosclerosis, base del infarto de miocardio y accidentes cerebrovasculares[4,5].

Existen diversos estados patológicos o influencias ambientales asociados con niveles elevados de LDL: nefrosis, diabetes, obesidad, algunos medicamentos y el tabaco[4,5].

El diagnóstico clínico no debe realizarse teniendo en cuenta el resultado de un único ensayo, sino que debe integrar los datos clínicos y de laboratorio.

## NOTAS

1. Se pueden modificar los volúmenes de muestra y Reactivo, manteniendo la misma proporción.
2. El sobrenadante debe ser completamente claro. En caso de persistir la turbidez o de no obtener una buena sedimentación del precipitado, adicionar otros 0,2 mL de Reactivo, mezclar bien y centrifugar de nuevo. Multiplicar el resultado obtenido por 1,5 para corregir la dilución efectuada.
3. Estos reactivos pueden utilizarse en la mayoría de analizadores automáticos. Solicite información a su distribuidor.
4. La calibración con el patrón acuoso suministrado puede causar sesgos, especialmente en algunos analizadores. En estos casos, se recomienda calibrar usando un patrón de base sérica (Calibrador Bioquímica, cod. 18011 y 18044).

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Appendix (IV)

ARENA BioScien

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# Uric Acid – PAP

Diagnostics single reagent for the in-vitro quantitative determination of uric acid in human serum, plasma or urine on both manual and automated systems.

| | | | |
|---|---|---|---|
| REF: BS.1/ UA02.025.0050 | 50 test | REF: BS.1/ UA02.050.0100 | 100 test |
| REF: BS.1/ UA04.025.0100 | 100 test | REF: BS.1/ UA02.100.0200 | 200 test |

## CLINICAL SIGNIFICANCE [1]

Uric acid is the end product of purine metabolism. Nearly half of the uric acid is eliminated and replaced daily by way of urinary excretion and through microbial degradation in the intestinal tract. Increased uric acid level may be observed in renal dysfunction, gout, leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, or in some genetic diseases. Decreased levels are present in patients with severe hepatocellular disease, Wilson's disease, bronchogenic carcinoma and Hodgkin's disease.

## METHOD PRINCIPLE [2]

Uric acid is determined after enzymatic oxidation in the presence of Uricase (based on modified Trinder peroxidase method). The formed hydrogen peroxide reacts under catalysis of peroxidase (PAP) with 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHB) 4-aminoantipyrine to form a red violet quinoneimine dye. Where its absorbance is proportional to the concentration of uric acid in sample.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + 4AAP + DCHB \xrightarrow{\text{POD}} \text{Quinoneimine Dye} + 2H_2O$$

## REAGENT COMPOSITION

| | |
|---|---|
| **R1: Uric acid standard** | 6 mg/dl (0.357mmol/L) |
| **R2: Reagent** | |
| Phosphate Buffer | 100 mmol/L |
| DCHB | 5.0 mmol/L |
| Potassium hexacyanoferrate | 80.0 mmol/L |
| 4-amino-antipyrine | 0.6 mmol/L |
| Peroxidase | > 3000 U /L |
| Uricase | > 500 U/L |
| Sodium Azide | 8 mmol/L |

## PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  ***S56:*** dispose of this material and its container at hazardous or special waste collection point.
  ***S57:*** use appropriate container to avoid environmental contamination.
  ***S61:*** avoid release in environment.

For further information, refer to the Uric Acid reagent material safety data sheet.

## REAGENT PREPARATION, STORAGE AND STABILITY

*Bioscien* Uric acid reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C. Once opened, the opened vial is stable for 3 months at the specified temperature.

***Deterioration***

The *Bioscien* Uric acid reagent is normally clear or pale pink. Do not use Uric acid reagent if it is turbid or if the absorbance is greater than 0.15AU at 546 nm.

## SPECIMEN COLLECTION AND PRESERVATION [3,6]

***Serum or plasma***

Uric acid in serum and EDTA or heparinized plasma samples are stable for 3 days at 25°C or up to 5 days at 4°C, and for 6 months if stored at -20°C.

***Urine***

Urine samples once received should be tested for pH value. In order to prevent urate precipitation, it is recommended to adjusted the urine pH to over 8.0 (alkaline) by adding 15 ml of sodium hydroxide 2mol/l. Urine samples should be diluted 1:10 before assay with physiological saline.

## SYSTEM PARAMETERS

| | |
|---|---|
| Wavelength | 546 nm (500 – 550 nm) |
| Optical path | 1 cm |
| Assay type | End-point |
| Direction | Increase |
| Sample Reagent Ratio | 1:50 |
| e.g: Reagent volume | 1 ml |
| Sample volume | 20 µl |
| Temperature | 37 °C or 15– 25°C |
| Incubation time | 10 min. at 15–25°C<br>5 min. at 37°C |
| Zero adjustment | Reagent Blank |
| Reagent Blank Limits | Low 0.00 AU<br>High 0.15 AU |
| Sensitivity | 1 mg/dL (0.06 mmol/L) |
| Linearity | 20 mg/dL ( 1.19 mmol/L) |

## EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

Appendix (IV)

ARENA BioScien

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# Uric Acid – PAP

Diagnostics single reagent for the in-vitro quantitative determination of uric acid in human serum, plasma or urine on both manual and automated systems.

| | | | |
|---|---|---|---|
| REF: BS.1/ UA02.025.0050 | 50 test | REF: BS.1/ UA02.050.0100 | 100 test |
| REF: BS.1/ UA04.025.0100 | 100 test | REF: BS.1/ UA02.100.0200 | 200 test |

## CLINICAL SIGNIFICANCE [1]

Uric acid is the end product of purine metabolism. Nearly half of the uric acid is eliminated and replaced daily by way of urinary excretion and through microbial degradation in the intestinal tract. Increased uric acid level may be observed in renal dysfunction, gout, leukemia, polycythaemia, atherosclerosis, diabetes, hypothyroidism, or in some genetic diseases. Decreased levels are present in patients with severe hepatocellular disease, Wilson's disease, bronchogenic carcinoma and Hodgkin's disease.

## METHOD PRINCIPLE [2]

Uric acid is determined after enzymatic oxidation in the presence of Uricase (based on modified Trinder peroxidase method). The formed hydrogen peroxide reacts under catalysis of peroxidase (PAP) with 3,5-dichloro-2-hydroxybenzenesulfonic acid (DCHB) 4-aminoantipyrine to form a red violet quinoneimine dye. Where its absorbance is proportional to the concentration of uric acid in sample.

$$\text{Uric acid} + O_2 + H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + 4AAP + DCHB \xrightarrow{\text{POD}} \text{Quinoneimine Dye} + 2H_2O$$

## REAGENT COMPOSITION

| | |
|---|---|
| **R1: Uric acid standard** | 6 mg/dl (0.357mmol/L) |
| **R2: Reagent** | |
| Phosphate Buffer | 100 mmol/L |
| DCHB | 5.0 mmol/L |
| Potassium hexacyanoferrate | 80.0 mmol/L |
| 4-amino-antipyrine | 0.6 mmol/L |
| Peroxidase | > 3000 U /L |
| Uricase | > 500 U/L |
| Sodium Azide | 8 mmol/L |

## PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person. Do not ingest or inhale as reagent (R) contains sodium azide which is classified as dangerous substance for environment.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,..).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  ***S56:*** dispose of this material and its container at hazardous or special waste collection point.
  ***S57:*** use appropriate container to avoid environmental contamination.
  ***S61:*** avoid release in environment.

For further information, refer to the Uric Acid reagent material safety data sheet

## REAGENT PREPARATION, STORAGE AND STABILITY

*Bioscien* Uric acid reagents are supplied ready-to-use and stable up to the expiry date labeled on the bottles when properly stored refrigerated at 2–8°C. Once opened, the opened vial is stable for 3 months at the specified temperature.

***Deterioration***

The *Bioscien* Uric acid reagent is normally clear or pale pink. Do not use Uric acid reagent if it is turbid or if the absorbance is greater than 0.15AU at 546 nm.

## SPECIMEN COLLECTION AND PRESERVATION [5,6]

***Serum or plasma***

Uric acid in serum and EDTA or heparinized plasma samples are stable for 3 days at 25°C or up to 5 days at 4°C, and for 6 months if stored at -20°C.

***Urine***

Urine samples once received should be tested for pH value. In order to prevent urate precipitation, it is recommended to adjusted the urine pH to over 8.0 (alkaline) by adding 15 ml of sodium hydroxide 2mol/l. Urine samples should be diluted 1:10 before assay with physiological saline.

## SYSTEM PARAMETERS

| | |
|---|---|
| Wavelength | 546 nm (500 – 550 nm) |
| Optical path | 1 cm |
| Assay type | End-point |
| Direction | Increase |
| Sample Reagent Ratio | 1:50 |
| e.g: Reagent volume | 1 ml |
| Sample volume | 20 µl |
| Temperature | 37 °C or 15– 25°C |
| Incubation time | 10 min. at 15–25°C<br>5 min. at 37°C |
| Zero adjustment | Reagent Blank |
| Reagent Blank Limits | Low 0.00 AU<br>High 0.15 AU |
| Sensitivity | 1 mg/dL (0.06 mmol/L) |
| Linearity | 20 mg/dL ( 1.19 mmol/L) |

## EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes and automatic pipet
- Centrifuge and spectrophotometer

## ASSAY PROCEDURE

| | Blank | Standard | Specimen |
|---|---|---|---|
| Reagent (R) | 1.0 ml | 1.0 ml | 1.0 ml |
| Standard | | 20 µl | |
| Specimen | | | 20 µl |

Mix and incubate for 5 minutes at 37°C or 10 minutes at 15-25°C. Measure absorbance of specimen "A" and standard "A" against reagent blank within 30 minutes.

## CALCULATION

***Serum or Plasma:***

Uric acid concentration (mg/dl) = $\frac{\text{(A specimen)}}{\text{(A standard)}} \times 6$

***Urine:***

Uric acid concentration (mg/dl) = $\frac{\text{(A specimen)} \times 6 \times 10}{\text{(A standard)}}$

**N.B.:** Extremely lipemic samples may give falsely elevated results and a serum blank must be run. Add 20 µl serum to 1 ml water. Zero the spectrophotometer with water. Read and record absorbance and subtract reading from test absorbance.

## QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact *Bioscien* technical support.

## PERFORMANCE CHARACTERISTICS

| ***Precision*** | Within run (Repeatability) | | Run to run (Reproducibility) | |
|---|---|---|---|---|
| | Normal level | High level | Normal level | High level |
| n | 20 | 20 | 20 | 20 |
| Mean mg/dl | 4.46 | 11.42 | 4.51 | 11.59 |
| SD. mg/dl | 0.15 | 0.21 | 0.23 | 1.32 |
| CV. % | 3.38 | 1.88 | 3.46 | 1.97 |

The results of the performance characteristics depend on the analyzer used.

***Accuracy (Methods Comparison)***

Result obtained from *Bioscien* Uric acid reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.979.

***Sensitivity***

When run as recommended, the minimum detection limit of the assay is 1 mg/dL (0.06 mmol/L).

***Linearity***

The reaction is linear up to uric acid concentration of 20 mg/dl; specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

## INTERFERING SUBSTANCES [3,4]

***Haemolysis***

No significant interference from haemoglobin up to 200 mg/dl.

***Icterus***

No significant interference from free and conjugated bilirubin up to levels of 12 mg/dl.

***lipemia***

No significant interference with mild to moderate lipemia.

***Drugs***

Of the drugs tested in vitro, methyldopa and noramidopyrine cause artificially Low uric acid values at the tested drug Level.

***Others***

Physiological ascorbic acid concentration does not interfere with the test. Ascorbic Acid levels higher than 170 mmol/l (3.0 mg/dl) decreases the apparent uric acid concentration significantly.

## EXPECTED VALUES [6]

| ***Serum and plasma*** | ***mg/dl*** | ***[mmol/L]*** |
|---|---|---|
| Children | 2.0-5.5 | [0.119-0.327] |
| Adults Male | 3.5-7.2 | [0.208- 0.428] |
| Adults Female | 2.6-6.0 | [0.155-0.357] |
| ***Urine*** | ***g/24hrs*** | ***[mmol/day]*** |
| 24 hours | 250-750 | [14.8-44.6] |

## DYNAMIC RANGE

1.0 - 20 mg/dl (0.06 - 1.19 mmol/L).

## REFERENCES


1. Barham D.and Trinder P., Analyst 97,142-145 (1972).
2. Fossati P.,Prencipe L.,and Berti G., Clin. Chem . 26/2,227-273 (1980).
3. Young D.S., Effects of drugs on clinical laboratory tests. 4th Ed. (1995), p.3-274 to 3-294.
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6. Tietz NW, ED. Clinical guide to laboratory tests. 2nd ED. philadelphia: WB Sauners; 1990: 566.


| SYMBOLS IN PRODUCT LABELLING | | | |
|---|---|---|---|
| IVD | For in-vitro diagnostic use | Σ | Number of <n> test in the pack |
| LOT | Batch Code/Lot number | | Caution |
| REF | Catalogue Number | | Do not use if package is damaged |
| | Temperature Limitation | | Consult Instruction for use |
| | Expiration Date | | |
| | Manufactured by | | |

EC REP


**Medical Device Safety Service**
**MDSS GmbH**
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CE

(Appendix V)

# Finecare™ HbA1c Rapid Quantitative Test

Catalog No. W207

## INTENDED USE

The Finecare™ HbA1c Rapid Quantitative Test along with Finecare™ FIA Meter is intended for vitro quantitative determination of HbA1c in human blood.
-Fluorescence immunoassay
-Diabetics monitoring

For in vitro diagnostic use only.For professional use only.

## SUMMARY

Glycated hemoglobin(HbA1c) is a glycated form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed by the attachment of glucose residue in blood to hemoglobin molecule. The level of glucose is proportional to the amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous months prior to the measurement.

Normal Reference Value: < 6.5%
Note: Individual reference range is suggested to be established for each laboratory.

## PRINCIPLE

The Finecare™ HbA1c Rapid Quantitative Test is based on fluorescence immunoassay technology. The Finecare™ HbA1c Rapid Quantitative Test uses a sandwich immunodetection method to measure percentage of HbA1c in human blood. After mixing with sample and buffer, sample mixture is added to the sample well of the Test Cartridge, the fluorescence-labeled detector HbA1c antibody binds to HbA1c in blood specimen. As the sample mixture migrates on the nitrocellulose matrix of test strip by capillary action, the complexes of detector antibody and HbA1c are captured to HbA1c antibody that has been immobilized on test strip. The fluorescence-labeled detector Hb antibody binds to Hb in blood specimen, the complexes are captured to Hb antibody that has been immobilized on test strip. Signal intensity of fluorescence is proportional to concentrations of HbA1c and Hb in blood specimen. The ratio between influorescent signals of HbA1c and Hb is the ratio between HbA1c and Hb.

## PRECAUTIONS

1. Whole blood is only applicable for this test kit. Do not repeat using test kit, do not use test kit beyond the expiration date.
2. Appropriate protective measures should be applied during the process of collection, disposal, storage and sample mixing.
3. Do not mix components(buffer, ID chip and Test Cartridge) from different kit lots. Their lot numbers must match each other.
4. The Finecare™ HbA1c Rapid Quantitative Test kit is only operational in the Finecare™ FIA Meter.
5. Do not use the Test Cartridge if the pouch is punctured or not well sealed.
6. Test Cartridge contaminated by blood or other liquid must not be inserted into FIA meter, or the meter might be contaminated or damage. Please appropriate dispose used Test Cartridge.
7. High working temperature should be avoided, Test Cartridge stored in low temperature should be recovered to room temperature.
8. The Test Cartridge and Meter should be used away from vibration and magnetic field. During normal usage, the Meter itself may cause vibration, which should be regarded as normal. Please do not pull out ID chip during testing.
9. Use of fresh blood specimen is recommended, please do not use sample with obvious appearance of hemolysis or blood clot, which might interfere test causing wrong result.
10. The results should be interpreted by the physician along with clinical findings and other laboratory test results.
11. If there is any problem or suggestion please contact manufacture.

## MATERIAL

### Material Provided

| | |
|---|---|
| Test Cartridge | 25 |
| Test Cartridge ID Chip | 1 |
| Detector buffer | 25 |

Leaflet with instructions for use

### Material Required But Not Provided

1. Finecare™ FIA Meter
2. Transfer Pipette Set (10μL, 100μL size)
3. Specimen Collection Containers
4. Sterile Lancets (for Fingerstick Whole Blood only)
5. Alcohol Pads
6. Timer

## STORAGE AND STABILITY

1. Store the detector buffer at 4 - 30℃. The buffer is stable up to 24 months.
2. Store Finecare™ HbA1c Rapid Quantitative Test Cartridge at 4 - 30℃, shelf life is up to 24 months.
3. Test Cartridge should be used within 1 hour after opening the pack.

## SPECIMEN COLLECTION AND PREPARATION

The test can be performed with whole blood only.

For Whole Blood Collected by Fingerstick:
1. Usually the lateral side of the ring finger is used to puncture. Clean the area to be lancet with an alcohol pad. Allow the finger to dry thoroughly.
2. Using a sterile lancet to puncture the skin just off the centre of the finger pad. Hold the finger downward. Apply gentle pressure beside the point of the puncture. Avoid squeezing the finger to make it bleed. Wipe away the first drop of blood with a sterile swab. Allow a new drop of blood to form. If blood flow is inadequate, the subject's finger may have to be gently massaged at the finger base to produce a droplet of sufficient volume. Avoid 'milking' the finger.
3. Blood is collected into the transfer pipette following the standard procedure.

Whole Blood samples collected by fingerstick should be used immediately after collection.

For Whole Blood Collected by Venipuncture:
1. All blood specimens must be restored to room temperature (20℃), and ensured integrated components and proportion (proportion of serum and red blood cell).
2. Fresh blood must be performing test within 24 hours after collection.

## TEST PROCEDURE

Refer to Finecare™ FIA Meter Operation Manual for the complete instructions on use of the Test. The test should be in room temperature.

**Step1: Preparation**
Check/Insert ID Chip into the instrument.

**Step2: Sampling**
Draw 10 μL of whole blood with a transfer pipette and add it to the buffer tube.

**Step3: Mixing**
Mix well the specimen with buffer for 1minute by tapping or inverting the tube.

**Step4: Loading**
Take 75 μL of sample mixture and load it onto the sample well of the Test Cartridge.

**Step5:Testing**
1. Finecare™ FIA meter:
Standard test: Insert the Test Cartridge onto the Test Cartridge Holder and click "Test". 5 minutes later, the result will show in the display and print out when click "Print".
Quick test: Put the Test Cartridge on the operation platform, 5 minutes later, insert the Test Cartridge onto the Test Cartridge Holder and click "Test". The result will show in the display and print out when click "Print".
2. Finecare™ multi-channel FIA meter:
Insert the Test Cartridge onto the Test Cartridge Holder. 5 minutes later, the result will show in the display and print out when click "Print".

Please refer to the Operation in user manual of Finecare™ FIA Meter for details.

## QUALITY CONTROL

Each Finecare™ HbA1c Rapid Quantitative Test Cartridge contains internal control that satisfies routing quality control requirements. This internal control is

performed each time a patient sample is tested. This control indicates that the Test Cartridge was inserted and read properly by Finecare™ FIA Meter. An invalid result from the internal control causes an error message on Finecare™ FIA Meter indicating that the test should be repeated.

## LIMITATIONS OF PROCEDURE

1. This test has been developed for testing human whole blood only.
2. The results of Finecare™ HbA1c Rapid Quantitative Test should be evaluated with all clinical and laboratory data available. If HbA1c test results do not agree with the clinical evaluation, additional tests should be performed.
3. The false positive results may come from cross-reactions with some similar antibodies in blood, and similar epitopes from non-specific components in blood capturing fluorescent labeled antibodies.
4. The false negative results may from some unknown substances blocking epitope adhering antibodies, unstable or degenerated HbA1c that cannot be identified due to prolonged time and temperature and storage condition of sample and reagent.
5. Other factors may interfere with Finecare™ HbA1c Rapid Quantitative Test and may cause erroneous results. These include technical or procedural errors, as well as additional substances in blood specimens.

## PERFORMANCE CHARACTERISTICS

### Accuracy

Two Cartridges from same batch were tested with HbA1c control of 5%, 13% and 14%, mean and Bias% were calculated, Bias% was within 10%.

### Assay Range and Detection Limit

•Assay Range: 4.0~14.5%
•Detection Limit: 4%

### Linearity

A serial concentration of HbA1c controls of 5%, 8%, 10%, 12% and 14% were tested respectively, the Correlation Coefficient (R) is ≥ 0.99.

### Precision

Intra-Run

Within-run precision has been determined by using 10 replicates from same batch to test with 8% HbA1c control, C.V. is ≤ 5%.

Inter-Run

Between-run precision has been determined by using 3 replicates from random 3 continuous batches to test with 8% HbA1c control, C.V. is ≤ 10%.

## BIBLIOGRAPHY OF SUGGESTED READING


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