Sudan University of Science and Technology
College of Graduate Studies

Assessment of Hemoglobin A1c, Triglyceride and Uric Acid in Plasma in Sudanese patient with Type 2 Diabetes Mellitus in Omdurman city

تقييم السكر التراكمي، وبلازما الدهون الثلاثية، وحمض اليوريك في البلازما لدى المرضى السودانيين بالنوع الثاني من السكر في مدينة امدرمان

A dissertation Submitted in the Partial Fulfillment for the requirement of
M.Sc. degree in Medical Laboratory Sciences-Clinical Chemistry

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الاية

بسم الله الرحمن الرحيم

قال تعالى: **(**أقرأ باسم ربك الذي خلق (1) خلق الانسان من علق (2) اقرأ وربك الاكرم (3) الذي علم بالقلم (4) علم الانسان ما لم يعلم)

سورة العلق-الايه1-5

Dedication

This study is whole heartedly dedicated to my beloved parents, who have been my source of inspiration and gave me strength when I thought of giving up, who continually provide their moral, spiritual, emotional, and financial support.

To my parents, brothers, aunts, husband, friends, and classmates who shared their words of advice and encouragement to finish this study.

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Abstract

Background and Aim:

Increased lipids level lead to several disease such as cardiovascular disease (CVD), and other heart diseases .

To assess HbA1c, plasma Triglyceride and Uric acid among type2 diabetic patients

Materials and methods: This was a cross sectional hospital based study, conducted in Omdurman city, the period from March to August 2022.

The study included 300 Sudanese diabetic patients with type 2 from both sex.

Five ml of blood sample was collected, 2 ml in EDTA container for HbA1c estimation and other 3 ml in Heparin container for Triglyceride and uric acid estimations. The HbA1c was measured by using fine care instrument, plasma triglyceride and plasma uric acid were measured by enzymatic methods using spectrophotometer DIRUI DR-7000D ; The data obtained was analyzed by used SPSS version 26 program.

Results: The study showed 60% (n =181) was female and 40% (n =119) was male.

The study showed 27% (n=81) of patients were good glycemic control and 73% (n=219) were poor glycemic control based on HbA1c values. Plasma triglycerides was significantly increased in Poor glycemic when compared with good glycemic patients with P-value (0.000). More over; 86.8% (190/300) of poor glycemia type 2 DM had hypertriglyceridaemia. However; plasma uric acid was insignificant increased in poor glycemic when compared with good glycemic control P- value >0.05.in addition 30.6% (67/300) of poor glycemic type 2DM had hyperuricemia. HbA1c had positive correlation with plasma triglycerides (r=0.517, p value=0.000); and insignificant correlation with plasma uric acid (r =0.069, P- value= 0.729).

Conclusion: The Sudanese patients with poor glycemic control type 2 DM had increased in plasma triglyceride and normal plasma uric acid.

المستخلص

الخلفية و الهدف من الدراسة : زيادة مستوى الدهون تؤدي الى عدة امراض مثل امراض اوعية القلب وامراض قلب اخرى.

تهدف الدراسة الي تقييم السكر التراكمي وكل من الدهون الثلاثية وحمض اليوريك.

المواد والطرق : كانت هذه دراسة مقارنة مقطعية اساسها المستشفى ، اجريت خلال في مدينة امدرمان في الفترة من مارس الى اغسطس .

الدراسة تضمنت 300 عينه من السودانيين المصابين بالنوع الثاني من السكر من كلا الجنسين.

5مل من عينة الدم تم سحبها. 2مل منها في ايدتا كونتينر لقياس السكر التراكمي وال3مل الاخرى في هيبارين كونتينر لقياس الدهون الثلاثية وحمض اليوريك تم قياس السكر التراكمي بجهاز فاين كير و قياس كل من الدهون الثلاثية و حمض اليوريك بطريقة الانزيمات بجهاز اسبيكتروفومتوميتر

تم تحليل البيانات التي تم الحصول عليها باستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية للمحوسبة اصدار26.

النتائج : الدراسة اوضحت ان 60% (ن=181) اناث و40% (ن=119) ذكور واوضحت الدراسة ان27% (ن=81) من المرضى لديهم نسبة جلوكوز في الدم متحكم بها و 73% (ن=219) من المرضى لديهم نسبة جلوكوز في الدم غير متحكم بها اعتمادا على السكر التراكمي.
بلازما الدهون الثلاثية لديها زيادة مؤثرة في مرضى السكر غير المتحكم به عند مقارنته مع السكر المتحكم به مع (قيمة ب = 0.000)، اضافة الى 86.6% (300/190) من مرضى النوع الثاني من السكر غير المتحكم به لديهم زيادة في مستوى الدهون الثلاثية في الدم، بلازما حمض اليوريك لديه زيادة غير مؤثرة في مرضى السكر غير المتحكم به عند مقارنته مع السكر المتحكم به مع (قيمة ب <0.05)، بالاضافه الى 30.6%(300/67) من مرضى النوع الثاني من السكر غير المتحكم به لديهم زيادة في مستوى حمض اليوريك في الدم، السكر التراكمي لديه علاقة موجبة مع بلازما الدهون الثلاثية (ر=0.517 وقيمة ب=0.000)، ولديه علاقة غير مؤثرة مع بلازما حمض اليوريك (ر=0.069 و قيمة ب=0.729).
الخلاصة : السودانيون من مرضى النوع الثاني من السكر الغير متحكم به لديهم زيادة في يلازما الدهون الثلاثية وبلازما حمض اليوريك لديهم طبيعي.

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List of Abbreviations

Abbreviation	Name
ASCVD	Atherosclerotic Cardiovascular Disease
BMI	Body Mass Index
CKD	Chronic Kidney Disease
CVD	Cardiovascular Disease
DM	Diabetes Mellitus
NPN	Non Protein Nitrogenous
T2DM	Type 2 Diabetes Mellitus
TG	Triglyceride
UA	Uric Acid

1. Introduction, Rationale and objectives

1.1Introduction

Diabetes mellitus (DM) is actually a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (Bishop et al., 2018). Type 2 diabetes mellitus is a major source of morbidity and mortality in south Africa, spurred by increased urbanization and unhealthy lifestyle factors (Pheiffer *et al*.,2018).

Several global estimates and projections have confirmed that diabetes mellitus is associated with increased mortality, that it has reached epidemics proportions with a high and increasing prevalence, and that its prevention and control pose a major public health challenge for this century (Saeed *et al*., 2019).

Just under half a billion people are living with diabetes worldwide and the number is projected to increase by 25% in 2030 and 51% in 2045 (Saeedi *et al*., 2019).

The prevalence of T2DM is high among the Sudanese population, especially in older people and those with a family history of DM .The high prevalence of uncontrolled DM in this setting is another hidden burden (Omar *et al*., 2019).

Hyperlipidemia is a condition that incorporates various genetic and acquired disorders that describe elevated levels within the human body. Hyperlipidemia is extremely common, especially in the western hemisphere, but also throughout the world (Hill, *et al*., 2022)

The incidence of hyperuricemia and associated gout is increasing worldwide, especially in high income countries with a western lifestyle. Growing evidence suggests that hyperuricemia is correlated with various metabolic and cardiovascular disease. For instance, elevated serum uric acid (SUA) is increasingly recognized as an important risk factor for diabetes mellitus and chronic complications of diabetes such as diabetic retinopathy (Yanan *et al*.,2021).

1.2 Rationale:

The International Diabetes Federation (IDF) estimate that worldwide, 415 million people have diabetes, 91% of whom have type 2 diabetes. Type 2 diabetes mellitus and it is consequences are a serious global public health issue. By 2030, the number of people with type 2 diabetes is predicted to reach 439 million.

Increased lipids level lead to several disease such as Cardiovascular disease (CVD) is a major cause of death and disability among people with diabetes and other heart disease which called by diabetic cardiomyopathy.

Sudan has, for a long time, suffered economic collapse, drought and civil war. Diabetes mellitus is currently emerging as an important health problem, especially in urban areas.

This study was conducted in Khartoum state, Omdurman city mainly, because diabetes mellitus is major health problem, so this study was conducted previously in eastern Sudan by Omar *et al*., 2018.

1.3 Objectives

1.3.1General objective

To assess HbA1c, plasma triglyceride and uric acid among Sudanese type 2 diabetes mellitus.

1.3.2 Specific objective

1- To estimate the means of HbA1c, plasma uric acid and Triglyceride among study group.

2- To estimate weight, height, and calculate BMI in study group

3- To compared between plasma triglycerides, uric acid, BMI in study group based on glycemic control,

4- To correlate between BMI, plasma triglycerides, plasma uric acid and HbA1c.

2. Literature review

2.1 Diabetes mellitus

Diabetes mellitus is taken from the Greek word diabetes, meaning siphon- to pass through and the Latin word mellitus meaning sweet. A review of history shows that the term “diabetes” was first used by Apollonius of Memphis around 250 to 300BC. Ancient Greek, Indian, Egyptian civilizations discovered the sweet nature of urine in this condition, and hence the propagation of the word diabetes mellitus came into being (Amit *et al*., 2022).

Mering and minkowski, in 1889, discovered the role of the pancreas in the pathogenesis of diabetes. In 1922 Banting, Best, and Collip purified the hormone insulin from the pancreas of cows at the university of tornto, leading to the availability of an effective treatment for diabetes in 1922 (Amit *et al* .,2022).

Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose level. And has several categories, including type 1, type 2, maturity-onset diabetes of young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, and steroid use (Amit *et al.*, 2022).

2.1.1 Etiology of diabetes mellitus

In the islets of Langerhans in the pancreas, there are two main subclasses of endocrine cells :insulin –producing beta cells and glucagon secreting alpha cells, without the balance between insulin and glucagon, the glucose levels become inappropriately skewed.in case of DM , insulin is either absent or impaired action(insulin resistance), and thus lead to hyperglycemia (Amit *et al*., 2022).

T1DM is characterized by the destruction of beta cells in the pancreas, typically secondary to an autoimmune process. The result is the absolute destruction of beta cells, and consequentially, insulin is absent or extremely low, T2DM involves a more insidious onset where an imbalance between insulin levels and insulin sensitivity causes a functional deficit of insulin (Amit *et al*., 2022).

The genetic background for both types is critical as a risk factor, As the human genome gets further explored, there are different loci found that confer risk for DM, Polymorphisms have been known to influence the risk for T1DM, including major histocompatibility complex (MHC) and human leukocyte antigen (HLA) (Rajaei *et al*., 2019).

T2DM involves a more complex interplay between genetics and lifestyle. There is clear evidence suggesting that T2DM is has a stronger hereditary profile as compared to T1DM, The majority of patients with the disease have at least one parent with T2DM (Klein *et al.*, 1996).

2.1.2 Epidemiology of diabetes mellitus

Globally, 1 in 11 adults has DM (90% having T2DM).The onset of T1DM gradually increases from birth and peaks at ages 4 to 6 years and then again from 10 to 14 years (Felner *et al*., 2005).

The prevalence in people under age 20 is about 2.3 per 1000.While most autoimmune disease are more common in females ,there are no apparent gander differences in the incidence of childhood T1DM. In some populations, such as in older males of European origin (over 13 years), they may be more likely to develop T1DM compared to females (3:2 male to female ratio) (Gale *et al*., 2001).

The onset of T2DM is usually later in life, though obesity in adolescents has led to an increase in T2DM in younger populations. T2DM has a prevalence of about 9% in the total population of United State, but approximately 25% in those over 65 years, The International Diabetes Federation estimates that 1 in 11 adults between 20-79 years had DM globally in 2015. Experts expect the prevalence of DM to increase from 415 to 642 million by 2040, with the most significant increase in populations transitioning from low to middle-income levels (Zheng *et al*., 2018).

2.1.3 Pathophysiology of diabetes mellitus

A patient with DM has the potential for hyperglycemia.The pathology of DM can be unclear since several factors can often contribute to the disease. Hyperglycemia alone can impair pancreatic beta-cell function and contributes to impaired insulin secretion. Consequentially, there is vicious cycle of hyperglycemia leading to an impaired metabolic state.Blood glucose levels above 180 mg/dl are often considered hyperglycemic. Chronic hyperglycemia causes nonenzymatic glycation of protein and lipids. The extent of this measurable via the glycation hemoglobin (HbA1c) test. Glycation leads to damage in small blood vessels in the retina, kidney, and peripheral nerves.This damage leads to classic complications of diabetic retinopathy, nephropathy, and neuropathy and the preventable outcomes of blindness, dialysis, and amputation, respectively (Amit *et al*., 2022).

2.2 Obesity-body mass index:

Obesity and type 2 diabetes mellitus (T2DM) are closely linked and are increasing in prevalence worldwide. Both chronic conditions have multisystem impact and are associated with increased mortality and cardiovascular risk.1 Individuals from non-White communities and those living in deprived areas are disproportionately affected. These associations were clearly highlighted during the recent COVID-19 pandemic (Taher *et al*., 2020).

Obesity is a key modifiable risk factor for the development of diabetes, with 90% of adults with T2DM classified as overweight or obese. There is an estimated threefold increase in the development of diabetes associated with being overweight and a 7-fold increase in those with obesity. Current models predict 9.5% of the adult population will have diabetes by 2030 and a third of this increase can be directly attributable to obesity (Grant *et al*., 2021).

2.3 Plasma lipid

Lipids, commonly referred to as fats, are ubiquitous constituents of all living cells and have a dual role. First because they are composed of mostly carbon –hydrogen bonds,

they are rich source of energy and an efficient way for the body to store excess calories. Because of their unique physical properties, lipids are also integral part of cell membranes and, therefore, also play an important structural role in cells. Lipids are also precursors for the steroid hormones (Bishop *et al*., 2018).

Plasma lipids include lipoproteins, cholesterol, and triglycerides. General lipoprotein structure include very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and lipoprotein a (Bishop *et al.*, 2018).

2.3.1 Lipoproteins

Constitute the body "petroleum industry" Like the great oil tankers that travel the oceans of the world transporting petroleum for fuel needs, chylomicrons are large, lipid-rich transport vessles that ferry their cargo dietary triglycerides, the main oil in the body, throughout the circulatory system to peripheral tissues, finally docking when nearly empty at the liver as chylomicron remnants. The very-low-density lipoprotein (VLDL) are like tanker trucks, redistributing dietary and hepatic synthesized triglycerides to peripheral cells mostly during fasting for energy needs or storage as fat. The low-density lipoprotein (LDL), rich in cholesterol, which start out as VLDL, are like nearly empty tankers that just deliver cholesterol to peripheral cells and return to the liver after their main cargo triglycerides have been off-loaded. The high-density lipoprotein (HDL) are the cleanup crew, gathering up excess cholesterol for transport back to the liver (Bishop *et al.*, 2018).

Lipid and lipoproteins, which are central to energy metabolism of the body, have become increasingly important in clinical practice, primarily because of their association with coronary heart disease (CHD). Numerous epidemiologic studies have demonstrated that, especially in affluent countries with high fat consumption, there is a clear association between the blood lipid levels and the development of atherosclerosis.

The accurate measurement of the various lipid and lipoprotein parameters is critical in the diagnosis and treatment of patients with dyslipidemia (Bishop *et al*., 2018).

2.3.2 Cholesterol

Is used by the body for such useful functions as facilitating triglyceride transport by lipoproteins and maintaining the normal structure and integrity of cell membranes and as a precursor for steroid hormone synthesis, but when in excess, it can lead to cardiovascular disease. Is an saturated steroid alcohol containing four rings (A, B, C, and D), and it has a single C-H side chain tail similar to a fatty acid in its physical properties. Cholesterol is almost exclusively synthesized by animals, but plants do contain other sterols similar in structure to cholesterol. Is also unique in that, unlike other lipids, it is not readily catabolized by most cell and therefore, does not serve as a source of fuel (Bishop *et al*., 2018).

2.3.3 Serum Triglyceride levels

As can be inferred from the name, triglyceride contains three fatty acid molecules attached to one molecule of glycerol by ester bond. Each fatty acid in the triglyceride molecule can potentially be different in structure, thus producing many possible types of triglycerides (Bishop *et al*., 2018).

The importance of triglyceride (TG) level as a risk factor for cardiovascular disease (CVD) has been extensively investigated in the general population; however, their relationship in patients with type 2 diabetes mellitus is uncertain (Xiaofeng *et al*., 2019).

In T2DM patients, an elevated triglyceride level cannot serve as an independent marker for an increased risk of cardiovascular events, but still the higher serum TG levels tend to be associated with increased risks of CVD (Xiaofeng *et al* ., 2019).

2.4 Diabetes mellitus, lipids, and cardiovascular disease

Type 2 DM is a common metabolic disorder predisposing to diabetic cardiomyopathy and atherosclerotic cardiovascular disease (CVD), which could lead to heart failure through a variety of mechanisms, including myocardial infarction and chronic pressure overload. Pathogenic mechanisms, mainly linked to hyperglycemia and chronic sustained hyperinsulinemia, cardiovascular disease represent a leading health problem worldwide

and risk factors, such as dyslipidemia, hypertension and obesity can also raise the risk of type 2 diabetes mellitus (Rosa *et al*., 2018).

2.5 Non protein nitrogenous (NPN)

The term non protein nitrogenous originated in the early days of clinical chemistry when analytic methodology required removal of protein from specimen before analysis. Numerous compounds of clinical interest are include in the NPN fraction of plasma and urine .The most abundant of these are urea, amino acids, uric acid, creatinine, creatine, ammonia (Bishop *et al*., 2018).

2.5.1 Serum Uric acid

Uric acid is the product of catabolism of the purine nucleic acid .Although it is filtered by the glomerulus and secreted by the distal tubules into the urine, most uric acid is reabsorbed in the proximal tubules and reused. Uric acid is relatively insoluble in plasma and, at high concentrations, can be deposited in the joints and tissue, causing painful inflammation (Bishop *et al*., 2018).

Purines, such as adenine and guanine from the breakdown of ingested nucleic acids or from tissue destruction, are converted into uric acid. Primarily in the liver. Uric acid is transported in the plasma from the liver to the kidney, where it is filtered by the glomerulus .Reabsorption of 98% to 100% of the uric acid from the glomerular filtrate occurs in the proximal tubules. Small amount of uric acid are secreted by the distal tubules into the urine .Renal excretion accounts for about 70% of uric acid elimination; the reminder passes into the GI tract and is degraded by bacterial enzymes (Bishop *et al*., 2018).

2.5.2Uric acid and cardiovascular disease

Uric acid, the end product of purine metabolism in humans, is not only a cause of gout, but also may play roles in developing cardiovascular disease such as hypertension, atrial fibrillation, chronic kidney disease, heart failure, coronary artery disease, and

cardiovascular death. Several clinical investigations have reported serum uric acid as a predictive marker for cardiovascular outcomes (Saito *et al*., 2021).

2.5.3 Hyperuricemia and diabetes mellitus

Hyperuricemia was relatively common among type 2 diabetic patients, The prevalence of hyperuricemia was common among patients with obesity, a long duration of DM and increased diastolic blood pressure, and alcohol drinkers (Arersa *et al*., 2019).

Hyperuricemia is linked to a variety of non-communicable disease such as atherosclerotic cardiovascular disease (ASCVD), chronic kidney disease (CKD), and hypertension, with evidence showing its role in the development of diabetes mellitus (DM) (Gaita *et al*., 2019).

3. Materials and methods

3.1 Materials

3.1.1 Study design:

This was cross sectional hospital based study.

3.1.2 Study area:

The study was conducted in Sudanese diabetic patients type 2 in Saad Rshowan center (Omdurman).

3.1.3 Study period:

The study was conducted during a period from March to August 2022.

3.1.4 Ethical consideration:

The study was approved by the Committee of Clinical Chemistry Department at College of Medical Laboratory Science of the Sudan University of Science and Technology.

3.1.5 Study population:

This study included 300 Sudanese diabetic patients with type 2 in saad rushowan center with age 23- 80 year.

3.1.6 Inclusion Criteria:

Sudanese males and females who were diagnosed with type 2 diabetes mellitus.

3.1.7 Exclusion Criteria:

Females and males with type 2 diabetes mellitus were excluded from this study if they had taken medication lowering lipid or allopurinol. Patients with malignancy or any others disease affecting lipid or uric acid were excluded.

3.1.8 Sampling:

Five ml of blood sample taken from patients to measured HbA1c put part of whole blood in EDTA container and measured by using fine care instrument, and to measure plasma TGs and plasma UA put the other quantity of blood in Heparin container to centrifuged and obtained plasma.

- Data was collected by using questionnaire: See Appendix 1.

3.2 Methods:

3.2.1 HbA1C estimation:

Principle of the method

The Finecare HbA1c rapid quantitative test is based on fluorescence immunoassay technology. The Finecare 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 detectorHbA1c 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 inflorescent signals of HbA1c and Hb is the ratio between HbA1c and Hb (Bunn HF., 1981).

Procedure and reagent of the method:

See appendix 2.

Instrument:

HbA1c has been estimated by rapid quantitative test using finecare.

3.2.2 Plasma Triglyceride estimation:

Principle of the method:

Triglyceride in the sample originates , by means of the coupled reactions described below, a coloured complex that can be measured by colorimeter (Bucolou, *et al*., 1973) (Fossati, *et al*., 1982).

Procedure and reagent of the method:

See Appendix 3.

Instrument:

TGs has been estimated by colorimetric method from biosystem company using spectrophotometer device (DIRUI DR-7000D).

3.2.3 Plasma Uric Acid estimation:

Principle of the method:

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 3,5dichloro-2 hydroxybenzenesulfonic acid (DCHB) 4-aminoantipytrine to form red violet quinoneimine dye . where it is absorbance is proportional to the concentration of uric acid in sample (Barham, *et al*., 1972).

Procedure and reagent of the method:

See Appendix 4.

Instruments:

UA has been estimated by colorimetric method from biosystem company using spectrophotometer device (DIRUI DR-7000D).

3.2.4 Quality control:

All devices and method were compared with control samples (normal and pathological) from biosystem company give same result as external control

3.2.5 Statistical analysis:

Using statistical analysis rules to obtain this result such as mean, standard deviation, cross tabulation, chi square, independent sample t-test, and also using figures to show the correlations.

4. Result

A total of 300 sample (119 males, 181 females) Sudanese patients with type 2 diabetes mellitus their age between (23-80) years old, from Saad rushwan center in Omdurman city,

Table (4-1):

Shows demographic data of study group as frequency and percentage, the variables, sex, age group, BMI, family history and HbA1c poor and good glycemic.

Table (4-2):

Shows comparison between BMI, TG and UA in type 2DM based on poor glycemic and good glycemic control using means, standard deviation and P. value.

Table (4-3):

Shows cross tabulation between TG (normal and high) and UA (normal and high), based on poor and good glycemic control among type 2DM patients, using frequency, percentage and p. value.

Figure (4-1):

A scatter plot shows insignificant correlation between BMI and HbA1c in study group with (r =0.042).

Figure (4-2):

A scatter plot shows positive significant correlation between TG and HbA1c in study group with (r =0.517) and p. value 0.000.

Figure (4-3):

A scatter plot shows positive insignificant correlation between UA level and HbA1c in study group with (r =0.069) and p. value 0.729.

Table (4-1): Demographic data of study group

Variables	Frequency (%)
Male	119 (40%)
Female	181 (60%)
Age / year	
≤44	35 (29.5%)
45-59	60 (50.4%)
≥60	24 (20.1%)
BMI kg/m^2	
Normal weight	163 (54.6%)
Over weight	137 (45.6%)
Family History	
Yes	89 (29.7%)
No	211 (70.3%)
HbA1c	
Good glycemic control (≤7%)	81 (27%)
Poor glycemic control (>7%)	219 (73%)

Data was shown as frequency and percentage.

Table (4-2): Comparison between means of BMI, Plasma triglyceride and Plasma uric acid in good glycemic and poor glycemic type 2 diabetes mellitus.

Variable	Good glycemic (HbA1c ≤ 7%; n =81)	Poor glycemic (HbA1c >7% ;n=219)	P.Value
BMI kg/m^2	24.7 ± 3.0	24.8 ± 3.0	0.700
TG mg/dl	149.3 ± 26.3	195.9 ± 55.0	0.000
UA mg/dl	5.4 ± 4.3	5.6 ± 2.1	0.729

-Independent sample t-test was used to compare between means.
-p.value considered significant at level ≤ 0.05.

Table (4-3): Cross tabulation between Plasma triglyceride and Plasma uric acid based on control among type 2 diabetes mellitus patients.

HbA1c

Variables	≤ 7% Good glycemic control	>7% Poor glycemic control	p.value
TG group			
Normal ≤150 mg/dl	39 (48.1 %)	29 (13.2 %)	0.000
High >150 mg/dl	42 (51.9 %)	190 (86.8 %)	
UA group			
Normal ≤7 mg/dl	70 (86.4 %)	152 (69.4 %)	0.003
High >7 mg/dl	11 (13.6 %)	67 (30.6 %)	

-Chi-square test was used to compare between frequency.

-p.value significant at level ≤ 0.05.

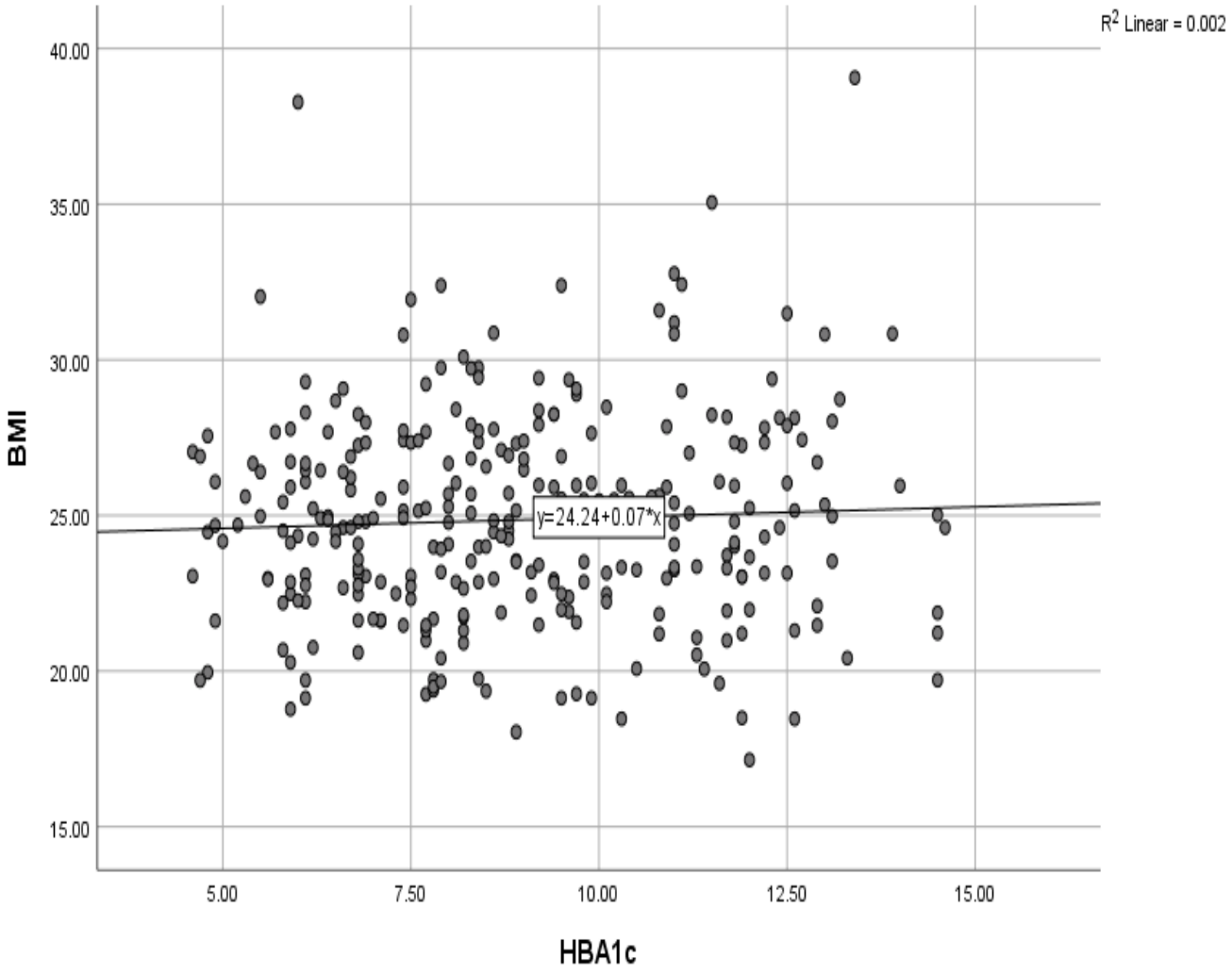

Figure (4-1): A scatter plot shows insignificant correlation between BMI (kg/m^2) and HbA1c (%) , (r=0.042; p.value 0.470).

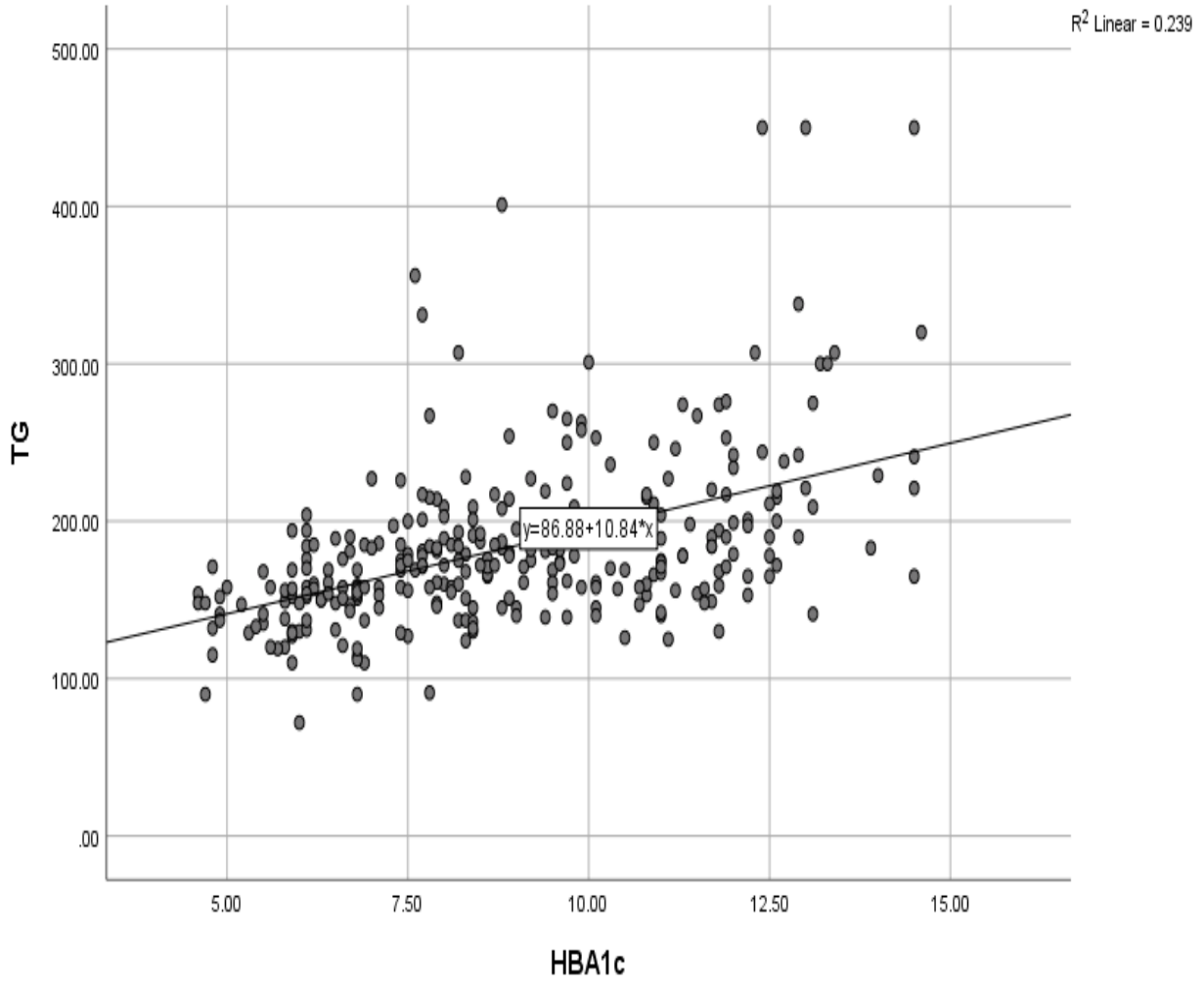

Figure (4-2): A Scattered plot shows positive significant correlation between Plasma triglyceride level (mg/dl) and HbA1c (%) in study group r = 0.517 ; p.value (0.000).

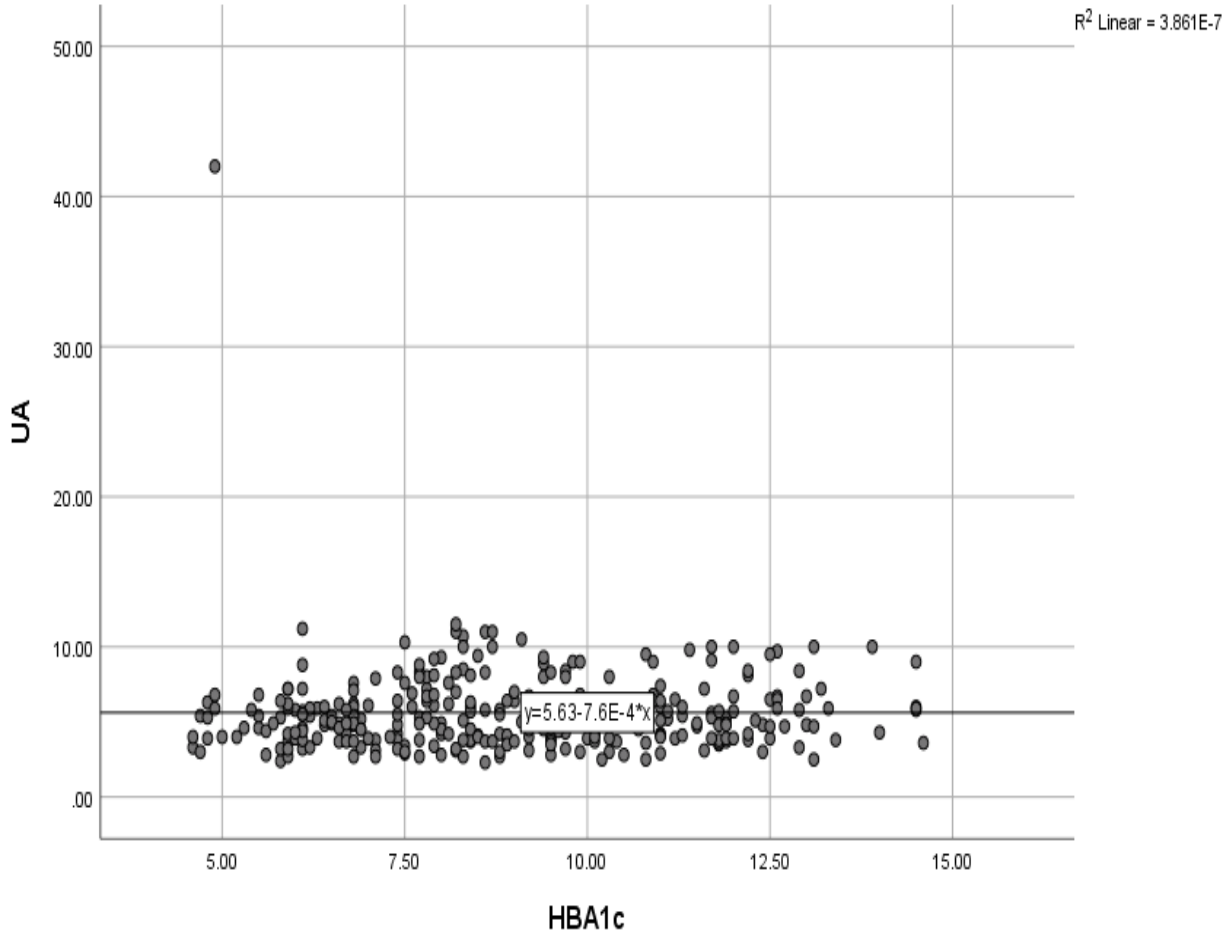

Figure (4-3):

A Scatter plot shows insignificant correlation between Plasma uric acid level (mg/dl) and HbA1c (%) in study group , r = 0.069 ; p.value(0.729).

5. Discussion, Conclusion and Recommendations

5.1 Discussion:

Diabetes mellitus was a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbance of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. Main complication of uncontrolled diabetic is hyperlipidemia which cause more serious disease as cardiovascular disease (Marshall *et al.,* 2020).

In this cross sectional hospital-based study the frequency of females with type 2 diabetes mellitus more than males, this result disagree with result of Naveed study at 2013 showed that adult men have higher risk for type 2 diabetes mellitus, an observation which has important clinical implication.

Study showed that poor glycemic control was (73.0%) more common among Sudanese with type 2DM. This is agree with study done by Omar *et al*., at 2018, whom find high rate (71.9%) of poor glycemic control. Some researcher reports that diabetes it is related complication were major health problem in Sudan and cause morbidity and mortality (Awadalla, *et al*., 2017). It has been shown that poor glycemic control was associated with diabetes complications, and these complications could be avoided by good diabetic control (Omar *et al*., 2018).

Study revealed that poor glycemic type 2 DM patients were significant increase in mean TG compared with control glycemic type 2 DM. Moreover; (86.6%) of poor glycemic had hypertriglyceridemia (TG>150 mg/dl). Additionally, HbA1c correlate positively with TG. This result agree with Irie *et al., at 2019,* study showed that very severe hypertriglyceridemia, which seemed to be caused by disturbed life style and poorly controlled T2DM. And also agree with Naqvi *et al., at 2017,* study showed that glycated hemoglobin was positively correlated with triglyceride.

Study revealed that insignificant correlation between plasma uric acid and HbA1c with p.value (0.729), this result disagree with Babikr *et al*., at 2016 study showed that in

diabetic patients, serum uric acid level was found to correlate positively with HbA1c p.value (0.000).

5.2 Conclusion:

Sudanese patients with poor glycemic type 2 Diabetes mellitus had increased in plasma triglyceride and normal plasma uric Acid.

5.3 Recommendation:

1-Periodic monitoring lipid profile among type 2 diabetic patients to detect elevations.

2-To do this study with other design like cohort study.

3- Advice the poor glycemic diabetic patients take care about their diet, medications, and life style.

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Appendix

Sudan University of Science and Technology
College of Graduate Studies

علاقة السكر التراكمي، بلازما الدهون الثلاثية، وبلازما حمض اليوريك لدى المرضى السودانيين بالنوع الثاني من السكر في ولاية الخرطوم

الجزء الأول: البيانات:

الاسم:		
الجنس	ذكر	أنثى
العمر بالسنين		
المستوى التعليمي خلاوي	ابتدائي متوسط	ثانوي جامعي فوق جامعي

الجزء الثاني: القياسات الجسمية:

الوزن (كجم)	الطول (سم)	محيط الخصر (سم)

الجزء الثالث: القياسات في المعمل:

HbA1c	TG	UA

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 substance 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

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

AssayRange 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 ≤ 6%.

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%.

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IVD	In Vitro Diagnostic Use
	See Instruction for Use
	Expiry Date
Σ	Tests per Kit
	Manufacturing Date
	Keep Dry
LOT	Batch Number
EC REP	Authorized Representative
	Keep away from Sunlight
30°C 4°C	Store between 4～30℃

Guangzhou Wondfo Biotech Co., Ltd.
No.8 Lizhishan Road, Science City, Luogang District, 510663,
Guangzhou, P.R.China

CE EC REP
Qarad b.v.b.a.
Cipalstraat 3
B-2440 Geel, Belgium

Version: 15/06/2015

COD 11828 1 x 50 mL	COD 11526 4 x 50 mL	COD 11529 2 x 250 mL
STORE AT 2-8°C		
Reagents for measurement of triglycerides concentration Only for *in vitro* use in the clinical laboratory		

TRIGLYCERIDES

CE

BioSystems
REAGENTS & INSTRUMENTS

TRIGLYCERIDES
GLYCEROL PHOSPHATE OXIDASE/PEROXIDASE

PRINCIPLE OF THE METHOD

Triglycerides in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry[1,2].

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

$$\text{Glycerol} + ATP \xrightarrow{\text{glycerol kinase}} \text{Glycerol} - 3 - P + ADP$$

$$\text{Glycerol} - 3 - P + O_2 \xrightarrow{\text{G-3-P-oxidase}} \text{Dihidroxyacetone} - P + H_2O_2$$

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

CONTENTS

	COD 11828	COD 11526	COD 11529
A. Reagent S. Standard	1 x 50 mL 1 x 5 mL	4 x 50 mL 1 x 5 mL	2 x 250 mL 1 x 5 mL

COMPOSITION

A. Reagent: Pipes 45 mmol/L, magnesium acetate 5 mmol/L, 4-chlorophenol 6 mmol/L, lipase > 100 U/mL, glycerol kinase > 1.5 U/mL, glycerol-3-phosphate oxidase > 4 U/mL, peroxidase > 0.8 U/mL,4-aminoantipyrine 0.75 mmol/L, ATP 0.9 mmol/L, pH 7.0.

S. Triglycerides Standard: Glycerol equivalent to 200 mg/dL (2.26 mmol/L) triolein. Aqueous primary standard.

STORAGE

Store at 2-8°C.

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 0.150 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 37°C.
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum or plasma collected by standard procedures.

Triglycerides in serum or plasma are stable for 5 days at 2-8°C. 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
Triglycerides 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 15 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
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 triglycerides concentration in the sample is calculated using the following general formula:

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

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

$\frac{A_{Sample}}{A_{Standard}}$	x 200 = mg/dL triglycerides x 2.26 = mmol/L triglycerides

REFERENCE VALUES

The following uniform cut-off points have been established by the US National Institutes of Health and have also been adopted in many other countries for the evaluation of risk[3].

Up to 150 mg/dL = 1.7 mmol/L	Normal
150-199 mg/dL = 1.70-2.25 mmol/L	Borderline-high
200-499 mg/dL = 2.26-5.64 mmol/L	High
> 500 mg/dL = > 5.65 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 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: 1.6 mg/dL = 0.018 mmol/L
- Linearity limit: 600 mg/dL = 6.78 mmol/L. For higher values dilute sample 1/ 4 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean Concentration	CV	n
100 mg/dL = 1.13 mmol/L	1.7 %	20
245 mg/dL = 2.77 mmol/L	0.7 %	20

- Reproducibility (run to run):

Mean Concentration	CV	n
100 mg/dL = 1.13 mmol/L	2.6 %	25
245 mg/dL = 2.77 mmol/L	1.7 %	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 1000 mg/dL), bilirubin (up to 2.5 mg/dL) do not interfere. Ascorbic acid (up to 5 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

Triglycerides are esters of glycerol and fatty acids coming from the diet or obtained by synthesis mainly in the liver. Triglycerides are transported in plasma by lipoproteins and used by adipose tissue, muscle and other. Their primary function is to provide energy to the cell.

Elevated serum triglycerides levels can be caused by liver disease, diabetes mellitus, nephrosis, hypothyroidism, alcoholism, familial hyperlipoproteinemia IV and V, and other[3,5].

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

NOTES

1. 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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M: 15281-22

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02/2019

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

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 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 then 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/ specimens 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 5 min. at 37°C
Zero adjustment	Reagent Blank
Reagent Blank Limits	Low 0.00 AU 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

Fits your perfection

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) = $\frac{\text{(A specimen)} \times 6}{\text{(A standard)}}$

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 [illegible] 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 [5,6]

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).

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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
Schiffgr aben 41
30175 Hannover, Germany

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