Sudan University of Science and Technology

College of Graduate Studies

Assessment of Plasma Lipid Levels in Sudanese Patients with Rheumatoid Arthritis in Khartoum State

تقييم مستويات الدهون في بلازما الدم لدى مرضى التهاب المفاصل الروماتويدي السودانيين في ولاية الخرطوم

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By

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الآيـــة

قَالَ تَعَالَىٰ:

﴿ ٱقۡرَأۡ وَرَبُّكَ ٱلۡأَكۡرَمُ ۝٣ ٱلَّذِي عَلَّمَ بِٱلۡقَلَمِ ۝٤ عَلَّمَ ٱلۡإِنسَٰنَ مَا لَمۡ يَعۡلَمۡ ۝٥ ﴾

صدق الله العظيم

سورة العلق الآيات 3-5

Dedication

This research is dedicate to my beloved parent. Without them endless love and encouragement .I would never have been able to complete my graduate studies. I love them both and I appreciate everything that they have done for me.

This research is also dedicated to my supervisor Dr. Nuha Eljaili who was there for me through this process and gave me lots of support. It is also dedicated to my brothers, sisters, friends and teachers.

Acknowledgment

All and first thanks to the almighty ALLAH.

I am heavily indebted to my supervisor Dr. Nuha Eljaili Abubaker for her support, valuable inputs, encouragement and patience during my research time.

I would like to thank all staff members of Clinical Chemistry Department, Sudan university of Sciences and Technology. I am also grateful to all people from whom samples were taken.

Abstract

Background: Rheumatoid arthritis (RA) is an autoimmune and inflammatory disease, which means that the immune system is attacks ,causing inflammation (painful, swelling) in the affected parts of the body. **Objectives**: This study conducted to assess serum lipid profile in rheumatoid arthritis patients.

Material and Methods: This was case control study include 50 rheumatoid arthritis patients as cases, and 50 apparently healthy individuals as controls (age and gender were matched between two groups). The serum lipid profile (TC,TG, LDL-c and HDL-c) was measured using auto-chemistry analyzer Mindray BS-200. The data obtained was subjected to analysis using statistical packaged for social science computer program (SPSS version 16).

Results: The results revealed a significant increase in total cholesterol , triglycerides and LDL-c in rheumatoid arthritis patients compared to control group.(mean ± SD:179.2 ± 33.8 mg/dl versus 166.3 ± 25.9 mg/dl P .value=0.036), (217.1 ± 36.5 mg/dl versus 132.5 ± 33.9 mg/dl P. value=0.00),(108.8+24.9 mg/dl versus 90.3+29.7 mg/dl P. value=0.001) respectively. And significant decreased in HDL-c (33.7 ± 11.1 mg/dl versus 38.7+9.8 mg/dl ,P .value=0.018).

The result showed, there were no correlation between cholesterol, triglycerides,LDL-c,HDL-c and age of rheumatoid arthritis patients (r=0.101,P-value=0.486),(r=-0.161,P-value=0.263),(r=-0.213,P-value=0.137),(r=0.070,P-value=0.627) respectively. Also there were no correlation between triglycerides, HDL-c and duration of disease (r=-0.003, P-value=0.983),(r=0.109,P-value=0.452) respectively. that there were positive correlation between cholesterol, LDL-c and duration of disease (r=0.513, P-value=0.000), (r=0.333, P-value=0.018) respectively.

Conclusion: The study concluded that, there was significant increase in cholesterol, triglycerides, LDL-c levels and significant decreased in HDL-c level in rheumatoid arthritis patients and there were positive correlation between cholesterol, LDL-c level and duration of rheumatoid arthritis.

مستخلص الدراسة

الخلفية: التهاب المفاصل الروماتويدي ،هو احد امراض المناعه الداتية والالتهابات،مما يعني مهاجمة الجهاز المناعي للخلايا السليمه في الجسم عن طريق ، ممايتسبب في التهاب(تورم مؤلم) في الاجزاء المصابة من الجسم.

الهدف: اجريت هده الدراسه لتقييم مستويات الدهون لدى مرضى التهاب المفاصل الروماتويدي.

المواد والطرق: تم جمع 50 عينه من مرضى التهاب المفاصل الروماتويدي ، و50 عينه تم جمعها من افراد اصحاء كمجموعه ضابطةواستخدم جهاز التحليل الداتي مندراي بي اس 200 لعمل التحاليل الخاصه لمستوي الدهون في المصل(الكوليسترول الكلي،ثلاثي الجليسرايد،والبروتينات الدهنيه دات الكثافة العالية والمنخفضه) وتم تحليل النتائج بواسطة برنامج الحزمة الاحصائية للعلوم الاجتماعية (16).

النتائج :اظهرت الدراسة ان نسبة الكوليسترول الكلي والدهون الثلاثية والبروتينات الدهنية منخفضه الكثافه ازدادت معنويا عند مقارنه المرضى مع المجموعة الضابطه (المتوسط +الانحراف المعياري:l33.8+179.2ملجم/دل مقابل166.3+25.9ملجم/دل،الاحتمال الاحصائي للمقارنة=0.036) ،(المتوسط+الانحراف المعياري217.1+36.5ملجم/دل مقابل 132.5+33.9ملجم/دل،الاحتمال الاحصائي للمقارنة=0.00)،(المتوسط+الانحراف المعياري108.8+ 24.9 ملجم/دل مقابل90.3 +29.7ملجم/دل ،الاحتمال الاحصائي للمقارنة=0.001) على التوالي. كما ان نسبة البروتينات عالية الكثافة نقصت معنويا (المتوسط+الانحراف المعياري33.6 +11.06ملجم/دل مقابل 38.7+9.8،الاحتمال الاحصائي للمقارنة=0.018) .

اظهرت النتائج عدم وجود علاقة ارتباط بيين الكوليسترول والدهون الثلاثية والبروتينات منخفضة الكثافة والبروتينات عالية الكثافة واعمار المرضى(معدل ارتباط بيرسون=0.101،القيمة المعنوية=0.486)،(معدل ارتباط بيرسون=-0.161،القيمة المعنوية=0.263)،(معدل ارتباط بيرسون=-0.213،القيمة المعنوية=0.137)،(معدل ارتباط بيرسون=0.070،القيمة المعنوية=0.627) على التوالي.كما لم تكن هناك ارتباطات بين الدهون الثلاثية والبروتينات عالية الكثافة ومدة المرض(معدل ارتباط بيرسون=-0.003،القيمة المعنوية=0.983)،وكان هنالك ارتباط بين الكوليسترول والبروتينات منخفضة الكثافة ومدة المرض(معدل ارتباط بيرسون=0.513،القيمة المعنوية=0.000)،(معدل ارتباط بيرسون=0.333،القيمة المعنوية=0.018) على التوالي.

الخاتمه: خلصت الدراسة الى ان هناك زيادة كبيرة في مستويات الكوليسترول والدهون الثلاثيه والبروتينات منخفضة الكثافة وان هناك نقصان في مستوى البروتينات عالية الكثافة لدى مرضى التهاب المفاصل الروماتويدي ووجود ارتباط بين مستوى الكوليسترول والبروتينات منخفضة الكثافة ومدة المرض.

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Abbreviations

BMP: basic metabolic panel

CAD: coronary artery disease

CBC: complete blood count

CHD: coronary heart disease

CVD: cardiovascular disease

HDL: high-density lipoprotein

IDL: Intermediate-density lipoprotein

LDL: low-density lipoprotein

LP: lipoprotein

RA: rheumatoid arthritis

SLE: systemic lupus erythromatosus

TC: total cholesterol

VLDL: very –low-density lipoprotein

Chapter One

Introduction-Rationale-Objectives

Introduction

1-1 Introduction

Rheumatoid arthritis is a very common health problem which is cause activity of daily living (ADL), related and instrumental activity of daily living (IADL)–related functional disability restriction of work and social participation, and fulfilling their life roles (Loyola-Sanchez, A,et al.,2015).

The genetic and environmental factors are contributory T cells B cells and the orchestrated interaction of pro-inflammatory cytokines play key roles in the pathophysiology of RA (Ernest Choy, 2012).

The mortality rate is higher among patients with rheumatoid arthritis than among healthy persons, and cardiovascular and other systemic complications remain a major challenge. Molecular remission and the capacity to reestablish immunologic tolerance remain elusive. Elucidation of the pathogenic mechanisms that initiate and perpetuate rheumatoid arthritis offers the promise of progress in each of these domains. Rheumatoid arthritis is predominantly classified on the basis of the clinical phenotype (Aletaha , *et al.*, 2010).

A Lipid panel is a blood test that measures lipids-fats and fatty substances used as a source of energy by your body. Lipids include cholesterol ,triglycerides, high density lipoprotein(HDL),and low density lipoprotein(LDL).This panel measures Total cholesterol level, triglycerides level ,HDL cholesterol level(good cholesterol) and LDL cholesterol level(bad cholesterol).Other measurement that may be done for a lipid panel include very low density lipoprotein (VLDL)cholesterol level, the ratio of total cholesterol to HDL and the ratio of LDL to HDL.Lipids are found in your blood and are stored in tissues.They are an important part of cells and they help keep your body working normally.Lipid disorders such as high cholesterol may lead to life

threatening illnesses such as coronary artery diseases(CAD), heart attack or strok(Stone, NJ , et al ,2013).

Accordingly this study aimed to measure lipid profile among rheumatoid arthritis patients and help in early detection of cardiovascular disease in this patients.

1-2 Rationale:

Rheumatoid arthritis is long term auto immune disorder that primarily affect joint.

Cardiovascular morbidity and mortality are enhanced in rheumatoid arthritis which might be due to an increased prevalence of cardiovascular risk factors such as dyslipidemia. (Nurmohamed Michael, 2007). This increased cardiovascular risk in RA patients could have several causes. Firstly, the prevalence of new or established cardiovascular risk factors, such as dyslipidemia diabetes mellitus, hypertension, higher body mass index (BMI), higher waist to hip-ratio or impaired physical fitness, might be increased (Goodson and Solomon, 2006).

In Sudan only few information's are available about the subject under study and most previous studies are focused on the assessment of lipid profile among other groups like patients with diabetes or hypertension therefore this study aimed to assess the lipid profile among Sudanese patients with RA result from this study may help the physician to place measure in order to reduce morbidity and mortality due to RA or it is medications

1-3 Objectives:

General objective

To assess plasma lipid profile among rheumatoid arthritis patients

Specific objectives:

1. To measure and compare mean concentration of cholesterol triglyceride, LDL-C, and HDL-C in study group (case and control).
2. To correlate between serum lipids profile and study variables (ages and durations of disease).

Chapter Two

Literature Review

Chapter Two

Literature Review

2-1 Rheumatoid arthritis(RA):

RA is a symmetric polyarticular arthritis that primarily affects the small arthrodial joints of the hands and feet. In addition to inflammation in the synovium, which is the joint lining, the aggressive front of tissue called pannus invades and destroys local articular structures. The synovium is normally a relatively cellular structure with a delicate intimal lining. In RA, CD4+ T cells, B cells and macrophages infiltrate the synovium and sometimes organize into discrete lymphoid aggregates with germinal centers. Hyperplasia of the intimal lining results from a marked increase in macrophage-like and fibroblast-like synoviocyte .Locally expressed degradative enzymes, including metalloproteinase, serine proteases and aggrecanases, digest the extracellular matrix and destroy the articular structures. RA occurs in 0.5–1.0% of the adult population worldwide, although the prevalence may have changed substantially in Europe since the renaissance (Firestein, GS , 2003).

2-1-1 Pathogenesis of rheumatoid arthritis:

RA the site of the initial inflammatory process is the synovial lining of diarthrodial joints, where synovial fluid provides the nutrition for the articular cartilage and lubricates the cartilage surfaces. During the inflammatory process, the synovial tissue undergoes increased vascularization and infiltration by lymphocytes, plasma cells, and activated macrophages. As the disease progresses, a pannus forms from the progressive overgrowth of this tissue as it covers the articular surface (Otero and Glodring, 2007). Although the etiology and pathogenesis of RA have yet to be completely specified, a number of

factors have been identified as contributing to the disease process. These factors include genetics, environmental sources, the interaction of genes and environment, and cellular abnormalities. Over the past two decades, our understanding of the molecular pathogenesis of RA has increased exponentially, thanks to important advances in the treatment of RA. Tumor necrosis factor (TNF), for example, has been identified as a pro inflammatory cytokine activated in the synovium of RA patients, leading to treatments such asetanercept (Enbrel), infliximab (Remicade), adalimumab (Humira), golimumab(Simponi), and certolizumabpegol (Cimzia) that directly inhibit the proinflammatory cytokines and/or interfere with their receptor binding (Smolen, JS,*et al*., 2007)

2-1-2 Signs and symptoms of rheumatoid arthritis:

primarily affects joints, but it also affects other organs in more than 15–25% of cases (Turesson, C, et al., 2003) Associated problems include cardiovascular disease, osteoporosis, interstitial lung disease, infection, cancer, feeling tired, depression, mental difficulties, and trouble working (Cutolo ,M, et al., 2014) .

Joint:

Arthritis of joints involves inflammation of the synovial membrane. Joints become swollen, tender and warm, and stiffness limits their movement. With time multiple joints are affected (polyarthritis) most commonly involved are the small joints of the hands feet and cervical spine but larger joints like the shoulder and knee can also be involved (Walker, BR ,et al., 2014) synovitis can lead to tethering of tissue with loss of movement and erosion of the joint surface causing deformity and loss of function (Majithia ,V,Geraci SA, 2007).

Skin

The rheumatoid nodule, which is sometimes in the skin, is the most common non-joint feature and occurs in 30% of people who have RA (Turesson ,C,2013)

It is a type of inflammatory reaction known to pathologists as a "necrotizing granuloma".

Lung

Lung fibrosis is a recognized complication of rheumatoid arthritis. It is also a rare but well-recognized consequence of therapy (for example with methotrexate and leflunomide). Caplan's syndrome describes lungn nodules in individuals with RA and additional exposure to coal dust. Exudative pleural effusions are also associated with RA (Kim ,EJ, et al.,2009).

Heart and blood vessels:

People with RA are more prone to atherosclerosis, and risk of myocardial infarction (heart attack) and stroke is markedly increased (Avina-Zubieta, JA, et al., 2008) Other possible complications that may arise include: pericarditis, endocarditis, left ventricular failure, valvulitis and fibrosis (Gupta ,A ,Fomberstein B, 2009).

Kidney:

Renal amyloidosis can occur as a consequence of untreated chronic inflammation (De Groot ,K, 2007). Treatment with penicillamine and gold salts are recognized causes of membranous nephropathy.

Liver:

Liver problems in people with rheumatoid arthritis may be due to the underlying disease process or as a result of the medications used to treat the disease A coexisting autoimmune liver disease, such as primary biliary cirrhosis or autoimmune hepatitis may also cause problems (Selmi , C, et al.,2011) .

Neurological:

Rheumatoid disease of the spine can lead to myelopathy. Atlanto-axial subluxation can occur, owing to erosion of the odontoid process and/or transverse ligaments in the cervical spine's connection to the skull. Such an erosion (>3mm) can give rise to vertebrae slipping over one another and compressing the spinal cord. Clumsiness is initially experienced,but without due care, this can progress to quadriplegia or even death (Wasserman ,BR, et al., 2011).

Teeth:

Periodonitits and tooth loos are common in people with rheumatoid arthritis (*De Pablo, Paola, 2009*).

2-1-3 Rheumatoid arthritis risk factor:

Older age, a family history of the disease, and female sex are associated with increased risk of RA, although the sex differential is less prominent in older patients(*Firestein GS, et al., 2009*) Both current and prior cigarette smoking increases the risk of RA (relative risk [RR] = 1.4, up to 2.2 for more than 40-pack-year smokers)(*Costenbader, et al .,2006*) Pregnancy often causes RA remission, likely because of immunologic tolerance (*Kaaja and Greer , 2005)* Parity may have long lasting impact; RA is less likely to be diagnosed in parous women than in nulliparous women (RR = 0.61)(*Guthrie etal.*,2010*)* Breastfeeding decreases the risk of RA (RR = 0.5 in women who breastfeed for at least 24 months), whereas early menarche(R R 1.3 for those with menarche at 10 years of or younger) and very irregular menstrual periods (RR 1.5) increase risk uses of oral contraceptive pills or vitamin E does not affect RA risk*(Karlson, et al., 2008).*

2-1-4 Diagnosis of rheumatoid arthritis:

Typical presentation:

Patients with RA typically present with pain and stiffness in multiple joints. The wrists, proximal interphalangeal joints, and metacarpophalangeal joints are most commonly involved. Morning stiffness lasting more than one hour suggests an inflammatory etiology. Boggy swelling due to synovitis may be visible , or subtle synovial thickening may be palpable on joint examination. Patients may also present with more indolent arthralgias before the onset of clinically apparent joint swelling. Systemic symptoms of fatigue, weight loss, and low-grade fever may occur with active disease.

Diagnostic tests:

Diseases such as RA are often characterized by the presence of autoantibodies. Rheumatoid factor is not specific for RA and may be present in patients with other diseases, such as hepatitis C, and in healthy older persons. Anti-citrullinateprotein antibody is more specific for RA and may play a role in disease pathogenesis (Balsa, A,et al.,2010) Approximately 50 to 80 percent of persons with RA have rheumatoid factor, anti-citrullinated protein antibody, or both *(Scott, DL ,et al .,2010)* Patients with RA may have a positive antinuclear antibody test result, and the test is of prognostic importance in juvenile forms of this disease (*Ravelli, et al.,2005*) C reactive protein levels and erythrocyte sedimentation rate are often increased with active RA, and these acute phase reactants are part of the new RA classification criteria (*Aletaha D, et al.*,2010*)* reactive protein levels and erythrocyte sedimentation rate may also be used to follow disease activity and response to medication Baseline complete blood count with differential and assessment of renal and hepatic function are helpful because the results may influence treatment options (e.g., a patient with renal insufficiency or significant thrombocytopenia likely would not be prescribed a

non steroidal anti-inflammatory drug [NSAID]). Radiography of hands and feet should be performed to evaluate for characteristic peri articular erosive changes which may be indicative of a more aggressive RA subtype (Scott , *et al*., 2010).

2-1-5 Prognosis of rheumatoid arthritis:

Patients with RA live three to 12 years less than the general population (*Friedwald ,et al.*, 2010*)* .Increased mortality in these patients is mainly due to accelerated cardiovascular disease, especially in those with high disease activity and chronic inflammation. The relatively new biologic therapies may reverse progression of atherosclerosis and extend life in those with RA *(Atzeni, et al.*, 2010).

2-1-6 Epidemiology of rheumatoid arthritis:

RA affects between 0.5 and 1% of adults in the developed world with between 5 and 50 per 100,000 people newly developing the condition each year (Smolen, *et al.*, 2016). In 2010 it resulted in about 49,000 deaths globall (*Lozano et al.*, 2012) Onset is uncommon under the age of 15and from then on the incidence rises with age until the age of 80. Women are affected three to five times as often as men (Shah Ankur, 2012). The age at which the disease most commonly starts is in women between 40 and 50 years of age, and for men somewhat later (Almanos ,*et al.*, 2006). RA is a chronic disease, and although rarely, a spontaneous remission may occur, the symptoms natural course is almost invariably one ofthe persistent, waxing and waning in intensity and a progressive deterioration of joint structures leading to deformations and disabili

2-2 Lipid:

in biology and biochemistry, a lipid is a biomolecule that is soluble in nonpolar solvents (IUPAC, 2006). Non-polar solvents are typically hydrocarbons used to dissolve other naturally occurring hydrocarbon lipid molecules that do not (or do not easily) dissolve in water, including fatty acids, waxes, sterols, fat-soluble

vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, and phospholipids. The functions of lipids include storing energy, signaling, and acting as structural components of cell membranes (Fahy ,*et al*., 2009). Lipids have applications in the cosmetic and food industries as well as in nanotechnology (Mashaghi, *et al* ., 2013).

2-2-1 Lipid chemistry:

Scientists sometimes define lipids as hydrophobic or amphiphilic small molecules; the amphiphilic nature some lipids allows them to form structures such as vesicles multilamellar/unilamellar liposomes, or membranes in an aqueous environment. Biological lipids originate entirely or in part from two distinct types of biochemical subunits o "buildingblocks": ketoacyl and isoprene groups Using this approach, lipids may be divided into eight categories: fatty acids, glycerolipids, glycerophospholipids, sphingolipids, saccharolipids, and polyketides (derived from condensation of ketoacyl subunits); sterol lipids and prenol lipids (derived from condensation of isoprene subunits) (Fahy, E , 2009) Although the term "lipid" is sometimes used as synonym for fats, fats are a subgroup of lipids called triglycerides. Although humans and other mammals use various biosynthetic pathways both to break down and to synthesis lipids, some essential lipids can't be made this way and must be obtained from the diet.

2.2.2. Categories of lipids:

2-2-2-1 Fatty acids:

Fatty acids, both free and as part of complex lipids, play a number of key roles in metabolism – major metabolic fue (storage and transport of energy), as essential component of all membranes, and as gene regulators. In addition dietary lipids provide polyunsaturated fatty acid (PUFAs) that are precursors of powerful locally actin metabolites, i.e. the eicosanoids. As part of complex lipids fatty acids are also important for thermal and electrical insulation, and for

mechanical protection. Moreover, free fatty acids and their salts may function as detergents and soaps owing to their amphipathic properties and the formation of micelles (Muller, H, *et al.*, 2001).

2-2-2-2 Phospholipid:

Phospholipids are a class of lipids that are a major component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic "head" consisting of phosphate group. The two components are usually joined together by a glycerol molecule. The phosphate groups can be modified with simple organic molecules such as choline, ethanolamine or serine. The first phospholipid identified in 1847 as such in biological tissues was lecithin, or phosphatidylcholine, in the egg yolk of chickens by the French chemist and pharmacist Theodore Nicolas Gobley. Biological membranes in eukaryotes also contain another class of lipid, sterol, interspersed among the phospholipid and together they provide membrane fluidity and mechanical strength. Purified phospholipids are produced commercially and have found applications in nano technology and materials science (Mashaghi , *et al*., 2013).

2-2-2-3 Triglycerides:

Triglycerides (TGs) are nonpolar lipid molecules composed of a glycerol molecule associated with three fatty acid (FA) molecules, and they represent the main form of lipid storage and energy in the human organismThey are synthesized primarily through the glycerol phosphate pathway and the traffic of TGs in specific tissues, such as muscle, liver, and adipose tissue, depends on the nutritional state of the individual, and is biological process that is essential for life. An imbalance in this process may lead to various metabolic disorders, such as obesity, lipotoxicity, or hypertriglyceridemia. The elucidation of this process,

at molecular and cellular levels, has profound implications for the understanding of disease related to TGs, as well as for the development of new therapies (R.Zhang,2016).

2-2-2-4 Cholesterol:

Cholesterol, the major sterol in animals, is both a structural component of membranes and precursor to a wide variety of steroids. Cholesterol is one of the lipids. It is an essential component of the cellular membrane determining the fluidity and biophysical properties by lowering the permeability and increasing the compactness. The distribution of this lipid in the membrane is not uniform but it is enriched in micro-domains, the so-called rafts (Ledesma, MD, *et al.*, 2003). Cholesterol is required for embryonic and fetal development (Woollett, LA, 2011). Cholesterol is also a source of bioactive molecules such as steroid hormones, vitamin D and bile acids, which in turn can regulate cellular metabolism and both intracellular and extracellular communication. It is also important for signal transduction (Liu, JP, 2009). It forms a vital part of the membranes of the spinal cord, nervous system, peripheral nerves and the brain. It is the main constituent of myelin sheath that functions as an insulation layer. Cholesterol is also a forerunner of important hormones such as testosterone, estradiol. Cholesterol homeostasis is a matter of vital importance in animal physiology, and perturbations in its normal levels have been associated with diseases such as atherosclerosis, diabetes and Alzheimer's disease (Coreta-Gomes , FM, et al.,2012). Disorders in lipid (e.g., cholesterol and triglycerides) and lipoprotein metabolism are major established independent risk factors in the development and progression of atherosclerotic CHD.

2-2-2-5 Lipoprotein :

Since the triglycerides and cholesterol esters are both non polar and thus insoluble, the only way they can be transported in blood is in association with

polar proteins called apo proteins. These complexes of lipids and proteins are called Lipoproteins, and the amounts of these in serum are what determined in order to estimate the amount of cholesterol in blood Lipoproteins are mainly of five types, depending upon the specific apo proteins they contain as also their size. These are: Chylomicrons (CM), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) in order of size, the largest being CM, and the smallest HDL. Density of particles is a function of the relative amounts of lipids and proteins which they contain - since lipids are less dense than water and float on the surface of water, it is obvious that the more lipids particle contains the less dense it would be. The reverse holds true for proteins, in this case apo proteins. Hence, VLDL will have greater proportion of lipids and lesser apo proteins than HDL (Bali S, Maneshwar Singh Utaal , *et al*.,2019).

2-2-2-6 Classification of lipoproteins:

1- Chylomicrons are the largest lipoproteins found in circulation the formation of the chylomicron is complex. In healthy humans virtually all fat is absorbed, but cholesterol is tightly regulated depending on body needs. A very flexible mechanism has evolved to keep serum cholesterol in a very narrow range. The chylomicron is responsible for the transport of medium- and long-chain fatty acids, together with cholesterol into the lymph. Apo B 48, the solubilizing protein for the chylomicron, is secreted by the entrecote. Unused protein is degraded, a mechanism that ensures that there is sufficient apo B48 for even the largest fat meal. Fat feeding increases apo AIV expression, and apo A1V serves as a surface component for apo B48 particles in the entrecote (I.Neeli, SA, *et al* .,2007).

2- Very-low-density lipoprotein(VLDL),density relative to extracellular water, is a type of lipoprotein made by the liver (Gibbons, *et al.*, 2004) VLDL is one of the five major groups of lipoproteins (chylomicrons, VLDL, intermediate-

density lipoprotein, low density lipoprotein , high-density lipoprotein) that enable fats and cholesterol to move within the water based solution of the bloodstream. VLDL is assembled in the liver from triglycerides, cholesterol, and Apo lipoproteins. VLDL is converted in the bloodstream to low-density lipoprotein (LDL) and intermediate-density lipoprotein (IDL). VLDL particles have a diameter of 30–80 nm. VLDL transports endogenous products, whereas chylomicrons transport exogenous (dietary) products.

3- Intermediate –density lipoprotein (IDL) belong to the lipoprotein particle family and are formed from the degradation of very low-density lipoproteins as well as high-density lipoproteins(IDL is one of the five major groups of lipoproteins (chylomicrons, VLDL, IDL, LDL, HDL) that enable fats and cholesterol to move within the water-based solution of the blood stream. Each native IDL particle consists of protein that encircles various lipids, enabling, as a water-soluble particle, these lipids to travel in the aqueous blood environment as part of the fat transport system within the body. Their size is, in general, 25 to 35 nm in diameter, and they contain primarily arrange o triacylglycerol's and cholesterol esters. They are cleared from the plasma into the liver by receptor-mediated endocytosis, or further degraded by hepatic lipase to form LDL particles.

4- Low-density lipoprotein (LDL) is one of the five major groups of lipoprotein which transport all fat molecules around the body in the extracellular water (centers of diseases control and prevention 2017) These groups, from least dense to most dense, are chylomicrons (aka ULDL by the overall density naming convention),very low-density lipoprotein (VLDL),intermediate-density lipoprotein (IDL),low-density lipoprotein and high-density- lipoprotein (HDL). LDL delivers fat molecules to cells. LDL can contribute to atherosclerosis if it is oxidized within the walls of arteries. It is important to note that while in the popular press LDL may be called "bad cholesterol".

5- High –density- lipoprotein (HDL) is one of the five major groups of lipoproteins(centers for diseases control and prevention 2017) Lipoproteins are complex particles composed of multiple proteins which transport all fat molecules (lipids) around the body within the water outside cells. They are typically composed of 80–100 proteins per particle (organized by one, two or three Apo A; more as the particles enlarge picking up and carrying more fat molecules) and transporting up to hundreds of fat molecules per particle. HDL particles are sometimes referred to as "good cholesterol" because they can transport fat molecules out of artery walls, reduce macrophage accumulation, and thus help prevent or even regress atherosclerosis

2-2-2-7 Lipid profile:

Serum lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test. The test includes four basic parameters: total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides. It is usually done in fasting blood specimen. Fasting refers to 12–14 h overnight complete dietary restriction with the exception of water and medication. This may hold true due to two main reasons: (1) post prandial triglycerides remain elevated for several hours (Campose ,H,et al.,2005). Most reference values for serum lipids are established on fasting blood specimen. NCEP and European guidelines (De Backer ,G ,et al.,2003) .also recommend doing lipid profile in fasting blood specimen for assessment of cardiovascular risk. However, these guidelines allow total and HDL cholesterol in the non-fasting specimen as these lipids are not much different in fasting and non-fasting specimens. In addition, non-HDL cholesterol (total cholesterol - HDL cholesterol), a secondary target of therapy in adult treatment panel III, may also be used in the nonfasting state (NCEP,2002). In many situations, the concentrations of these lipids and/or lipoproteins are not in normal amounts in the human body, in what is known in the scientific literature as dyslipidemia. Studying the lipid profile (total

cholesterol biochemical determinations – TC, HDL-c, TG and LDL-c) after fasting for 12 to 14 hours, has been an activity of great value, considering that the research already carried out, and correlation between the morphology of the arteries obtained from autopsies and cardiovascular risk factors, has allowed it to be demonstrated that dyslipidemia is a factor of great importance for the development of atherosclerosis in later life (Carreras,G,Ordonez,J ,2007).

2-2-2-8 Lipids and rheumatoid arthritis:

Rheumatoid arthritis (RA) is a chronic systemic disease affecting primarily the synovium, leading to joint damage and bone destruction (Gravalles ,2002). RA causes significant morbidity as a result of synovial inflammation, joint destruction and associate disability (Gabriel , *et al.*, 2003). Several investigators reported an excess of cardiovascular morbidity and mortality among RA patients. In active RA, the majority of cardiovascular deaths result from accelerated atherosclerosis (Goodson, 2002). Risk factors for atherosclerotic events and cardiovascular disease include male sex, increased age, elevated plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C), decreased high-density lipoprotein cholesterol (HDL-C), high blood pressure, smoking and diabetes mellitus (Cui , *et al.*, 2001)|.

Patients with RA face an increased risk of developing premature cardiovascular disease and limited ability to modify risk factors, age through exercise. RA is associated with an abnormal lipoprotein patterns principally low level of high density lipoprotein (HDL) cholesterol. Most treatment for RA tend to improve the a therogenic index (total/HDL cholesterol ratio) whit more evidence for biologics in this regard. The improvement in the lipoprotein profile in RA appears to be associated with suppression of inflammation (George Steiner,Murray B Urowitz ,2009).

An increasing number of studies worldwide provide compelling evidence for the excess cardiovascular (CV) risk in patients with rheumatoid arthritis (RA) compared to the general population.(Solomon ,DH, et al., 2006) .The interplay of two major contributors (traditional CV risk factors and inflammation) is being investigated to better understand the mechanisms underlying the high CV morbidity and mortality in RA. The contribution of inflammation to atherogenesis is supported by epidemiological evidence on the independent predictive value of inflammatory markers for subclinical and clinical atherosclerosis and for associated CV events(Rizzo, M ,et al.,2009).Recent research has shown that systemic inflammation plays apivotal role in development of atherosclerosis.Hence inflammation might explain the increase cardiovascular risk in RA pateints(Sattar,et al., 2003).

Chapter Three

Materials and Methods

Chapter Three

Materials and Methods

3-1 Materials:

3-1-1 Study approach:

A quantitative method was used to measure the levels of plasma lipid profile in rheumatoid arthritis patients during the period from June to October 2019

3-1-2 Study design:

This is case control study

3-1-3 Study area:

The study was conducted in Alrayyan center in Khartoum state

3-1-4 Study population and sample size:

The study included 50 patients with rheumatoid arthritis and 50 apparently healthy subjects as control (ages and gender were matched between two groups).

3-1-5 Inclusion criteria:

Patients with rheumatoid arthritis and healthy individual as control were included in this study.

3-1-6 Exclusion criteria:

RA patients with hypertension- smoking - liver - renal -heart diseases- alcoholism - bone diseases and SLE were excluded.

3-1-7 Ethical considerations:

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

3-1-8 Data collection:

Data were collected using structural questionnaire which was designed to collect all valuable information concerning each case examined**: (Appendix1).**

3-2 Methods:

3-2-1 Sample collection and processing:

About 2.5 ml of venous blood were collected by safe aseptic procedures .Sample were collected by using dry plastic syringes tourniquet was used to make for veins more prominent then they were centrifuged at 4000 rpm to obtain the serum or plasma samples and stored in -20 until the analyzed

3-2-2 Estimation of total cholesterol:

3-2-2-1 Principle of total cholesterol method:

By the catalysis of CHE and CHO cholesterol ester is catalyzed to yield H2O2 which oxidates 4-Aminoantipyrine with phenol to form acolored dye of quinonemine .The absorbency increase is directly proportional to the concentration of cholesterol

$$\text{Cholesterol ester} + H_2O \xrightleftharpoons{CHE} \text{cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightleftharpoons{CHO} \text{4-cholestenone} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipyrine} + \text{phenol} \xrightleftharpoons{POD} \text{Quinoneamine} + 4H_2O$$

3-2-2-2 Procedure of total cholesterol: (Appendix 11)

3-2-3 Estimation of triglyceride:

3-2-3-1 Principle of triglycerides method:

Through a sequence of enzymatic catalysis steps by lipase GK and GPD triglycerides is catalyzed to yield H2O2 which oxidize 4-Aminoantipyrine to yield a colored dye of Quinone mine .The absorbance increase is directly proportional to the concentration of triglycerides

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{Lipase}} \text{Glycerol} + \text{Fatty acid}$$

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

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

$$H_2O_2 + \text{4-Aminoantipyrine} + \text{4-ChlorophenoL} \xrightarrow{POD} \text{Quinoneamine} + HCL + 2H_2O$$

3-2-3-2 Procedure of triglycerides: (Appendix 111)

3-2-4 Estimation of high density lipoprotein (HDL-c):

3-2-4-1 Principle of Method:

The system monitors the change in absorbance at 600.This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the system to calculate and express the HDL-cholesterol concentration

$$\text{(1) LDL, VLDL, Chylomicrons} \xrightarrow{\text{CHE+CHO}} \text{Cholestenone} + H_2O_2$$

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

$$\text{(2) HDL} \xrightarrow{\text{CHE+CHO}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{HDAOS} + \text{4-Aminoantipyrine} \xrightarrow{POD} \text{Quinone amine}$$

3-2-4-2 Procedure of high density lipoprotein: (Appendix 1V)

3-2-5 Estimation of low density lipoprotein (LDL-c):

3-2-5-1 Principle of method:

The system monitors the change in absorbance at 600 nm. This change in absorbance is directly proportional to the concentration of cholesterol in the sample and is used by the system to calculate and express the LDL-cholesterol

$$\text{(1) HDL.VLDL .Chylomicrons} \xrightleftharpoons{\text{CHE+CHO}} \text{Cholestenone+H2O2}$$

$$2\text{H2O2} \xrightleftharpoons{\text{Catalase}} 2\ \text{H2O+O2}$$

$$\text{(2) LDL} \xrightleftharpoons{\text{CHE+CHO}} \text{Cholestenone+H2O2}$$

$$\text{H2O2+TOOS+4-Aminoantipyrine} \xrightleftharpoons{\text{POD}} \text{Quinoeamine}$$

3-2-5-2 Procedure of low density lipoprotein :(Appendix V)

3-3 Quality Control:

The precision and accuracy of all methods used in this study were checked by commercially prepared control (control serum normal 1 and control serum abnormal 2) sample before application for the measurement of test and control sample.

3-4 Statistical analysis:

Data were analyzed using statistical package for social science (SPSS) version 16 in computer program, Independent sample t test and person correlation were applied for correlation between variables.

Chapter Four

Result

Chapter Four

Result

4-Results

The result of biochemical determinant of serum total cholesterol, triglycerides, HDL-C and LDL-C in rheumatoid arthritis patients (cases, the mean of ages was 45.86±18.21) and healthy subjects (control, the mean of ages was 47.56±19.05, p- value =0.649) are given in tables and figures:

Table (4-1): Represent the mean of levels of serum cholesterol triglyceride ,HDL-C and LDL-C in both study groups.

The levels of cholesterol, triglycerides and LDL-C were significantly increased, while HDL-C was significantly decreased in rheumatoid arthritis patients compared to control group. (P-value=0.036-0.000-0.001-0.018) respectively.

Figure(4-1): illustrate 52% of patients <45 years,and 48% of patients>45 years

Figure (4-2): illustrate 62% of patients were females, while 38% of patients were males

Figure (4-3): show correlation between cholesterol level and age of rheumatoid arthritis patients. The scatter showed no correlation between cholesterol level and age of rheumatoid arthritis patients (r=0.101, p-value 0.486).

Figure (4-4): show correlation between triglycerides level and age of rheumatoid arthritis patients .The scatter showed no correlation between triglycerides level and age of rheumatoid arthritis patients(r=-0.161,p-value=0.263).

Figure (4-5): show correlation between LDL-C level and age of rheumatoid arthritis patients. The scatter showed no correlation between LDL-C level and age of rheumatoid arthritis patients(r=-0.213, p-value=0.137).

Figure (4-6): show correlation between HDL-C level and age of rheumatoid arthritis patients. The scatter showed no correlation between HDL-C level and age of rheumatoid arthritis patients(r=0.070, p- value=0.627) .

Figure (4-7): show correlation between cholesterol level and duration of rheumatoid arthritis. The scatter showed moderate positive correlation between cholesterol level and duration of rheumatoid arthritis(r=0.513, p-value=0.000).

Figure (4-8): show correlation between triglycerides level and duration of rheumatoid arthritis. The scatter showed no correlation between triglycerides level and duration of rheumatoid arthritis(r=-0.003, p-value=0.983).

Figure (4-9): show correlation between LDL-C level and duration of rheumatoid arthritis. The scatter showed correlation between LDL-C level and duration of rheumatoid arthritis(r=0.333,p -value=0.018).

Figure (4-10): show correlation between HDL-C level and duration of rheumatoid arthritis .The scatter showed no correlation between HDL-C level and duration of rheumatoid arthritis(r=0.109, p-value=0.452).

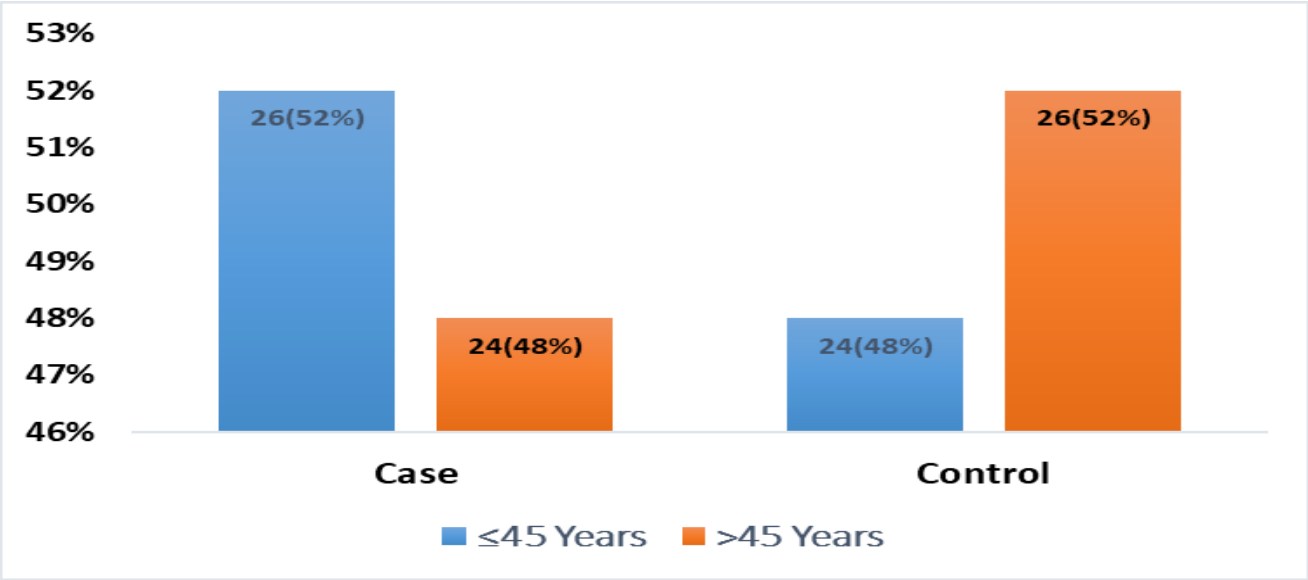


Figure (1) distribution of study group according to age group

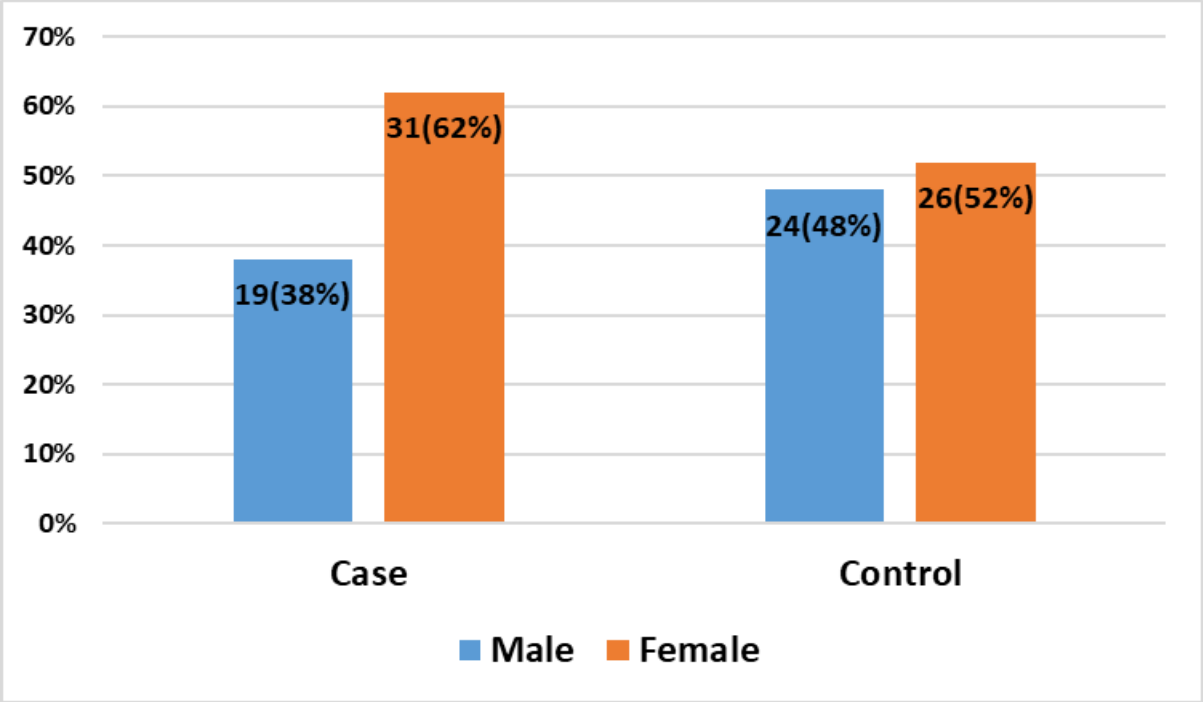


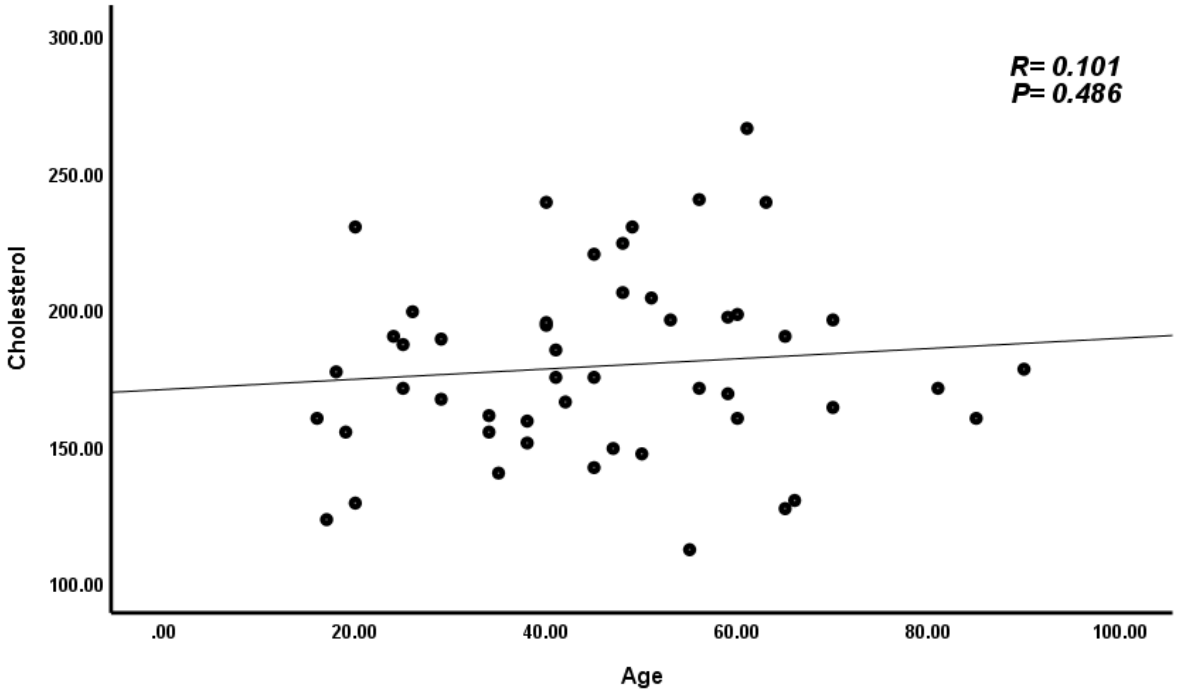
Figure (2) distribution of study group according to gender

Table (4-1) comparison of cholesterol, triglycerides, LDL-C and HDL-C in case versus and control group

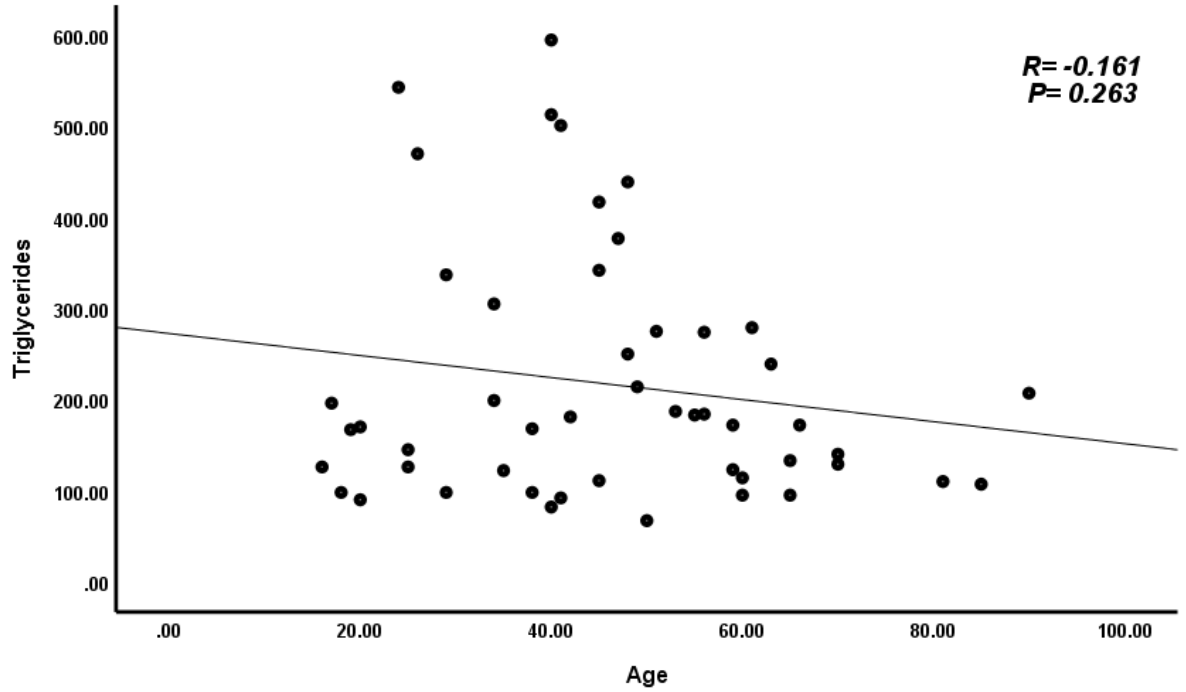
Parameters	Case (Mean ± SD) n=50	Control (Mean ± SD) n=50	*P-value*
Cholesterol (mg/dl)	179.16±33.80	166.32±25.92	0.036
Triglycerides(mg/dl)	217.1±36.5	132.5±33.92	0.000
LDL-C (mg/dl)	108.8±24.94	90.32±29.79	0.001
HDL-C (mg/dl)	33.66±11.06	38.70±9.82	0.018

Result given in mean ± SD, P. value ≤0.05 consider significant.

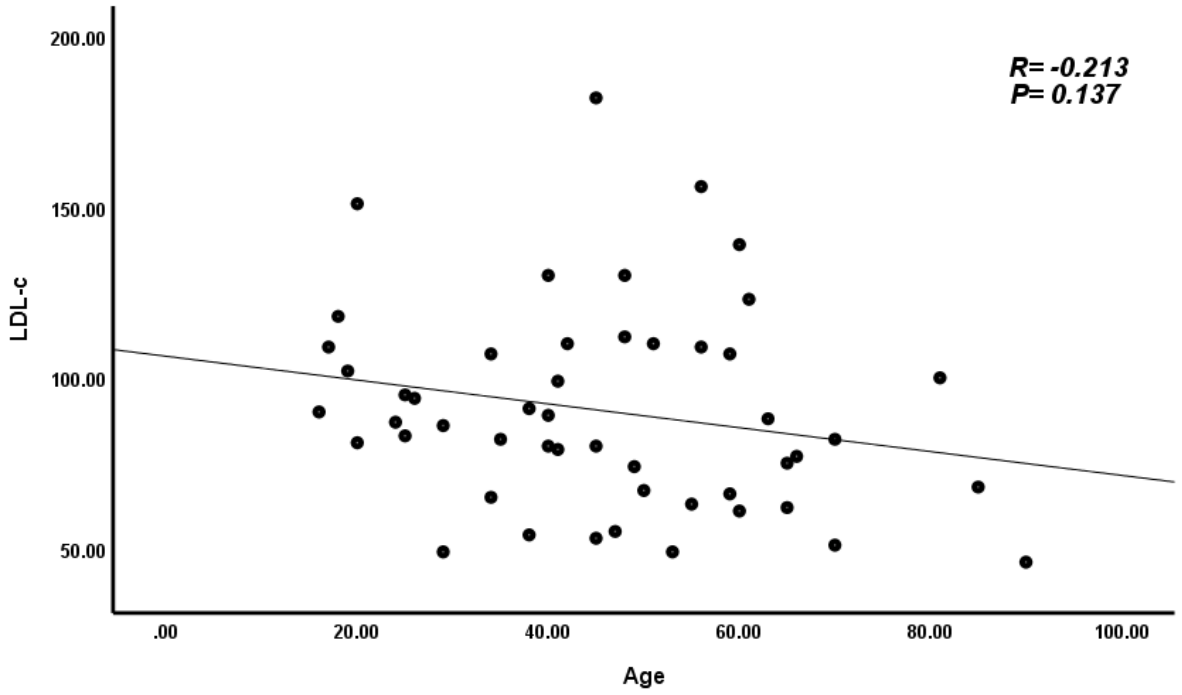
Independent sample T test was used for comparison.



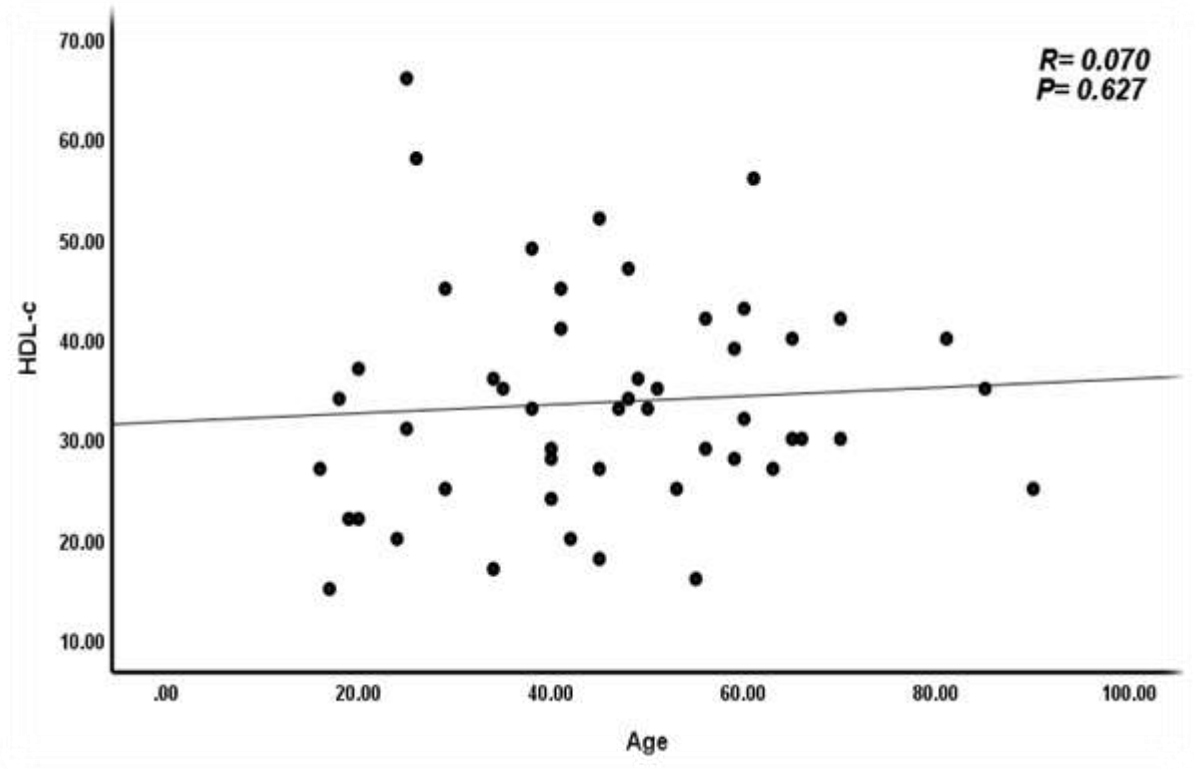
Figure(4-3)Correlation between cholesterol level and age of rheumatoid arthritis pateints (r=0.101,P-value=0.486)



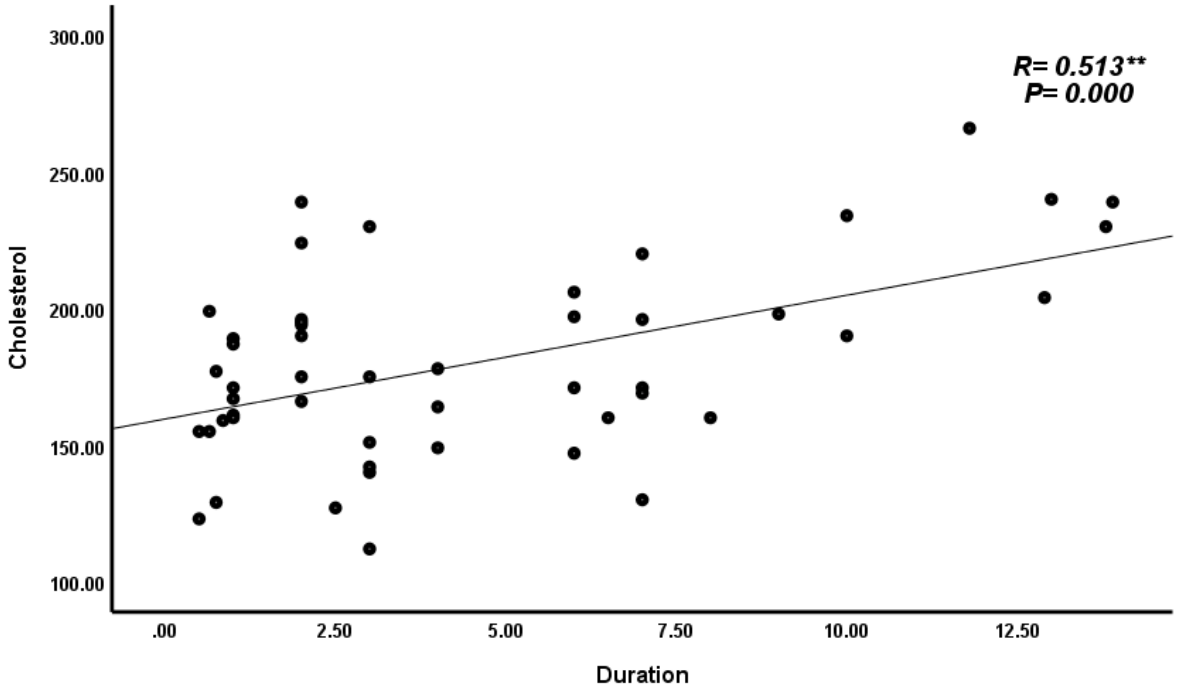
Figure(4-4)Correlation between triglycerides level and age of rheumatoid arthritis pateints (r=-0.161, P-value=0.263)



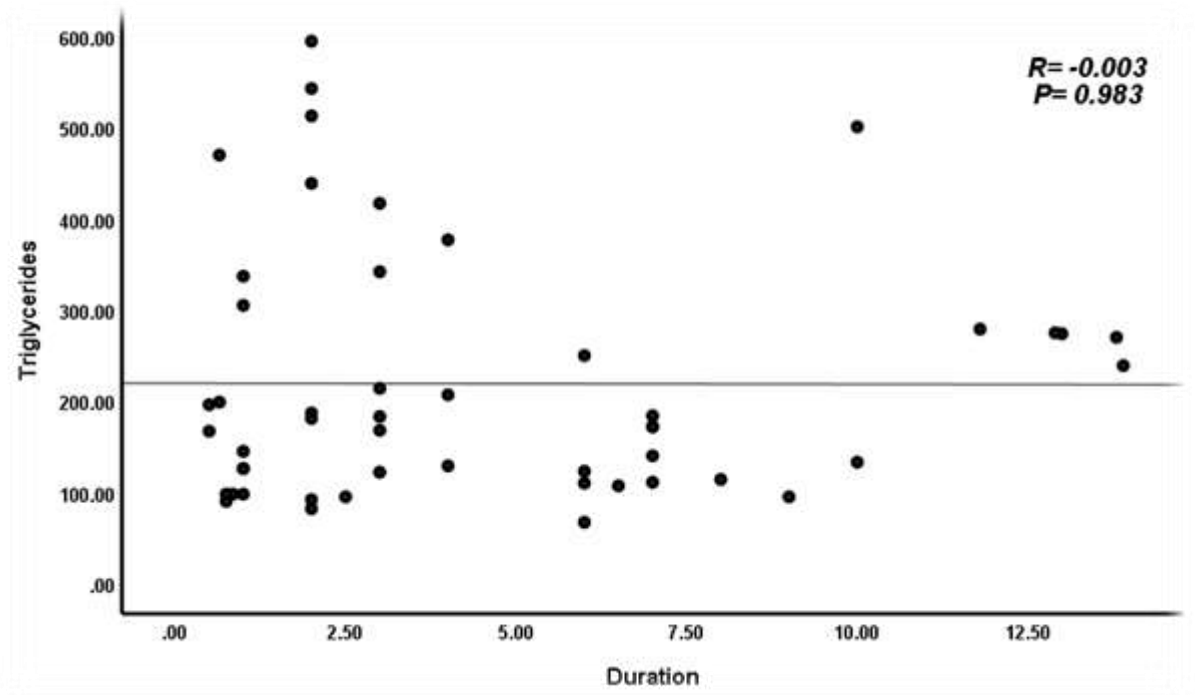
Figure(4-5) Correlation between LDL-C level and age of rheumatoid arthritis pateints(r=-0.213,P-value=0.137)



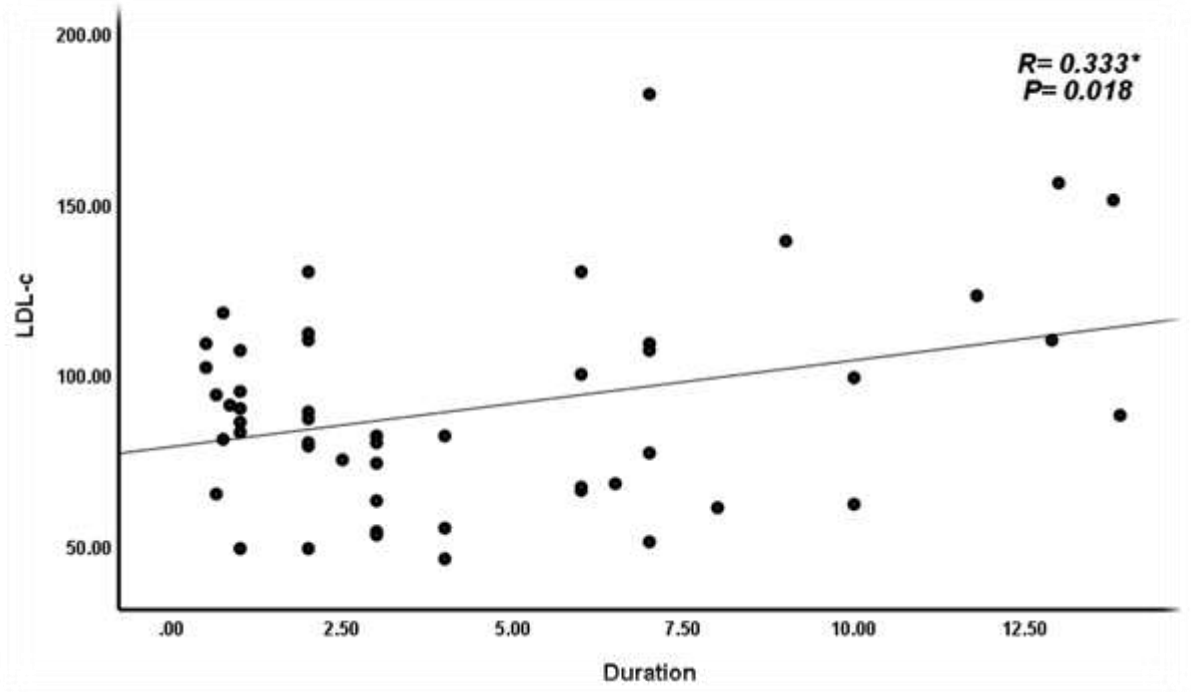
Figure(4-6)Correlation between HDL-C level and age of rheumatoid arthritis pateints(r=0.070,P-value=0.627)



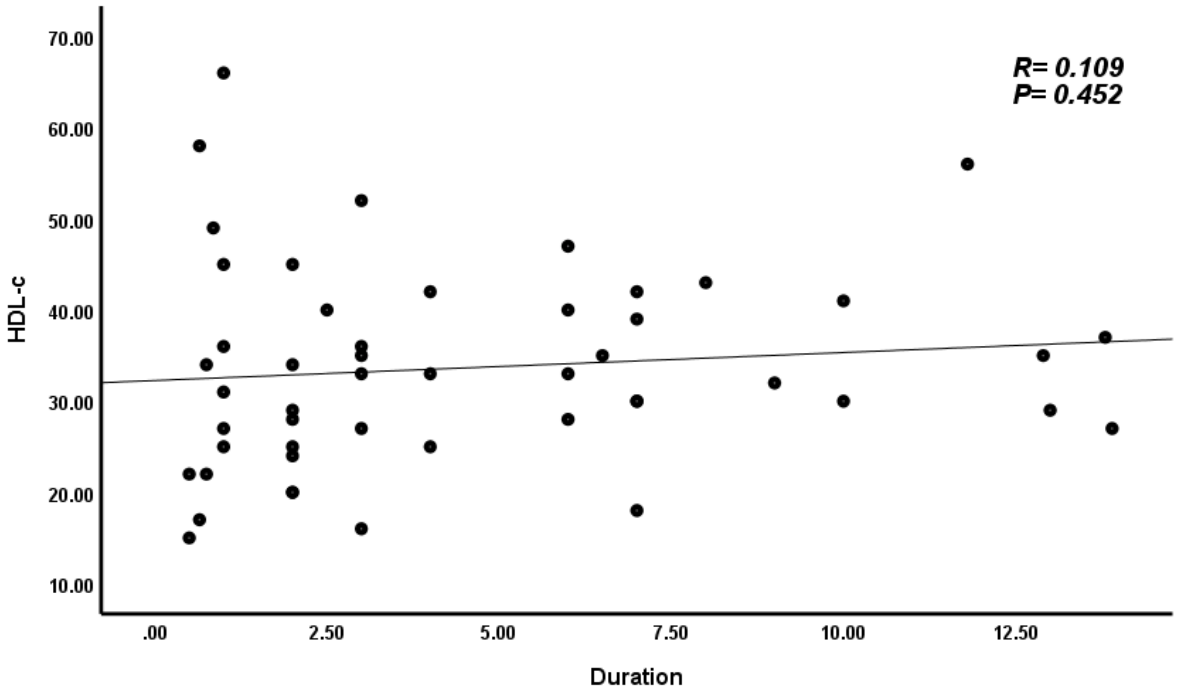
Figure(4-7) Correlation between cholesterol level and duration of rheumatoid arthritis(r=0.513,P-value=0.000)



Figure(4-8) Correlation between triglycerides level and duration of rheumatoid arthritis(r=-0.003,P-value=0.983)



Figure(4-9) Correlation between LDL - C level and duration of rheumatoid arthritis(r=0.333,P-value=0.018)



Figure(4-10) Correlation between HDL-C level and duration of rheumatoid arthritis(r=0.109,P-value=0.452)

Chapter Five

Dissussion , Conclusions Recommendations

Chapter Five

Dissussion , Conclusions & Recommendations

5-1 Discussion:

Patients with RA have higher rates of morbidity and mortality than the general population, which is highly attributed to an increased risk of CVD among RA patients (Maradit-Kremers, *et al*., 2005). The increased risk of CVD appears to be linked to coronary atherosclerosis (Gabriel, 2010). and may be directly caused by chronic inflammation or secondarily caused by physical inactivity and medications used to treat RA (Turesson, *et al*., 2008)

This study conducted to estimate lipid profile in rheumatoid arthritis patients. preliminary investigated and findings obtain from specially designed questionnaire.The result of lipid profile revealed that serum level of cholesterol ,triglycerides and LDL-C were significantly increased in RA patients compared to control groups (p-value =0.036 , 0.000, 0.001) respectively, while HDL –C was significantly decrease (p- value=0.018). This result agreed with study carried by(Van Halm ,*et al.,* 2007),which demonstrate that elevated serum lipid profile levels are associated with increased risk of cardiovascular disease in person with RA .Also this result similar to another result carried by (Cui ,*et al.,* 2001) ,which showed significantly increased in cholesterol, LDL-C levels and significantly decreased in HDL-C level (p- value =0.000), also this result in agreement with another result , carried by (Mullic, *et al*., 2014),this result disagreed with study which showed , there were no significant differences in lipid profile in RA compared to control group.

(Dessein , *et al.*, 2002).The findings of this study showed, there were no correlation between cholesterol , HDL-C, triglyceride , LDL-C levels and ages of RA patients. (r=0.101 ,p-value=0.486)(r=0.070,p-value=0.627)(r=-0.161,p-value=0.263) (r=-0.213,p-value=0.137) respectively. There were significant positive correlation between cholesterol, LDL-C and duration of disease (r=0.513,p-value=0.000),(r=0.333,p-value=0.018) respectively. Also there were no correlation between triglycerides, HDL-C and duration of disease (r=-0.003,p-value=0.983), (r=0.109,p-value=0.452) respectively.

The high level of TC,LDL-C correlate with long term rheumatoid barthritis patients that ocurr because the rheumatoid arthritis is persistant chronic inflammation that lead to dyslipidemic pattern in thes patients for long time (Migule ,A, et al .,2006).

5-2 Conclusion:

According to the results of this study it is concluded that:

Serum cholesterol, triglycerides and LDL-C levels are increased, while HDL-C level is decreased in RA patients. There were positive correlations between cholesterol LDL-C levels and duration of RA.

5-3 Recommendations:

From the finding of this study it is recommended that:

-1 Patient with rheumatoid arthritis should be monitoring of lipid profile to prevent cardiovascular disease.

2-Management of dyslipidemia should be considered as a part of cardiovascular risk management in RA patie

3-future researches is highly needed targeting role of lipid-lowering drugs to be given as adjuvant therapy in the treatment of rheumatoid arthritis patients with hyperlipidemia.

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Appendices

Sudan University of Sciences and Technology

College of Graduate Studies

Appendix (I)

Questionnaire

General Information:

-Pt. No: …….

-Age: ……years

-Sex: …….Male{ } Female{ }

-Duration of disease: ……years

Lab investigations:

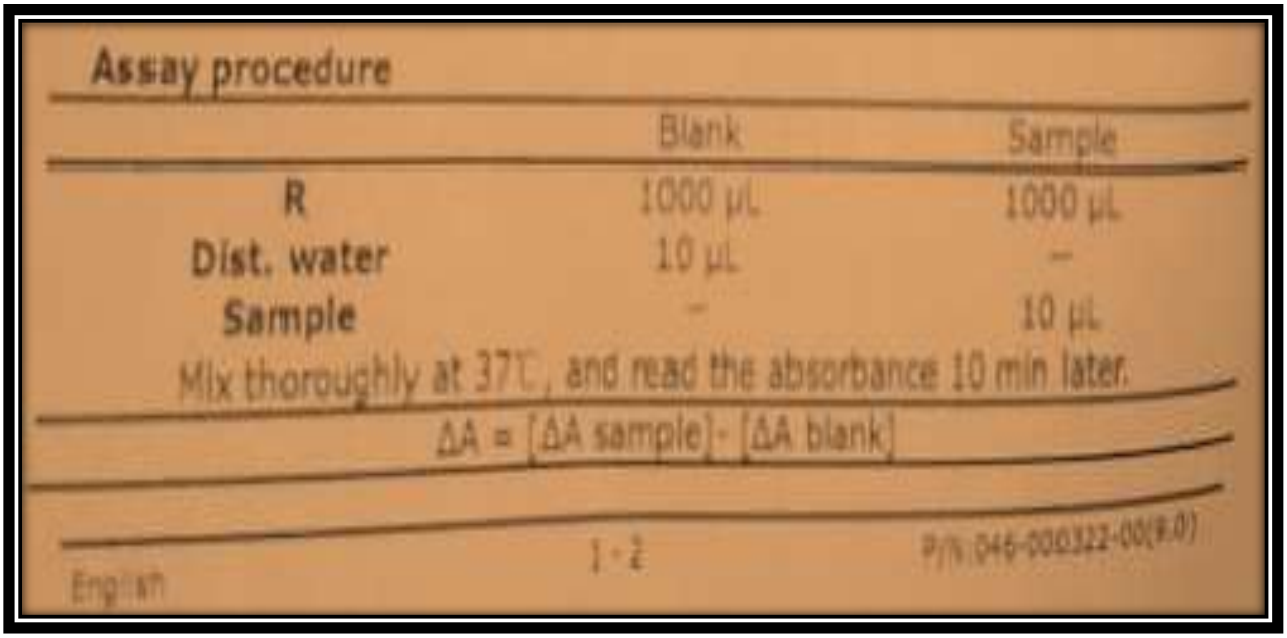
-Serum Total Cholesterol…….mg/dl

-Serum Triglycerides……mg/dl

-Serum HDL-cholesterol……..mg/dl

-Serum LDL-cholesterol……..mg/dl

Appendix (II)



Assay procedure

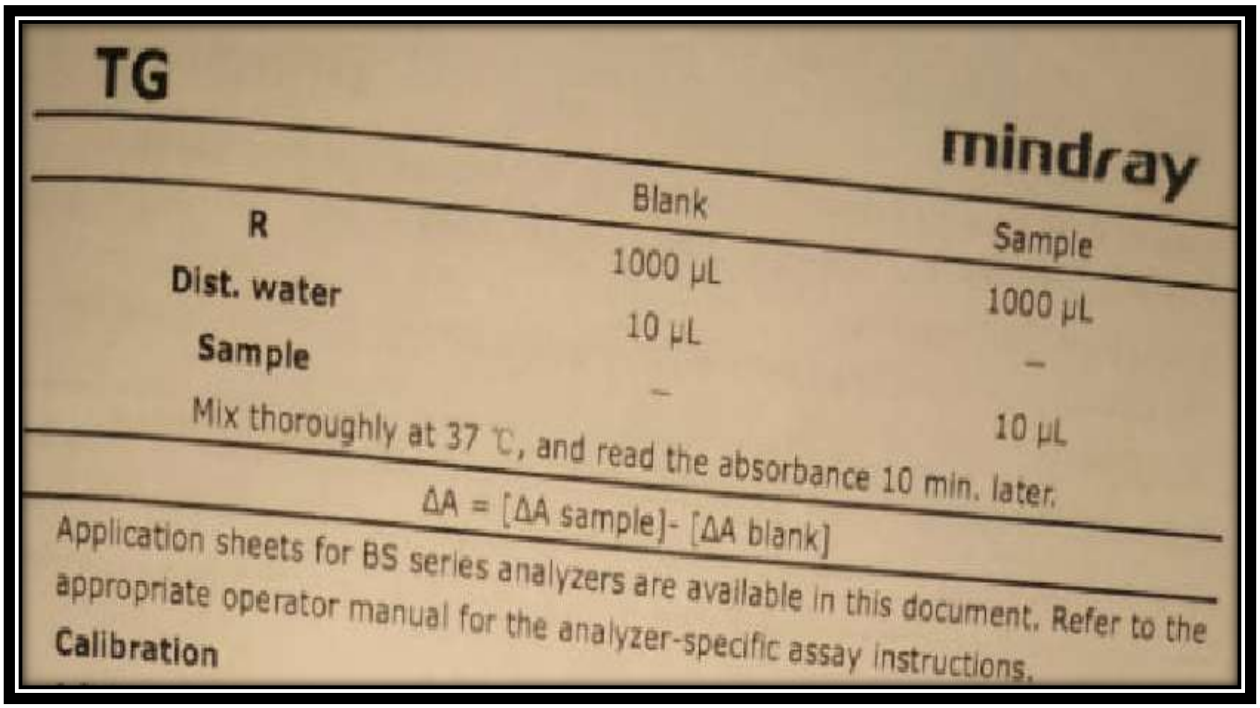
	Blank	Sample
R	1000 µL	1000 µL
Dist. water	10 µL	–
Sample	–	10 µL

Mix thoroughly at 37℃, and read the absorbance 10 min later.

ΔA = [ΔA sample]- [ΔA blank]

English

Appendix (III)



TG

mindray

	Blank	Sample
R	1000 µL	1000 µL
Dist. water	10 µL	–
Sample	–	10 µL

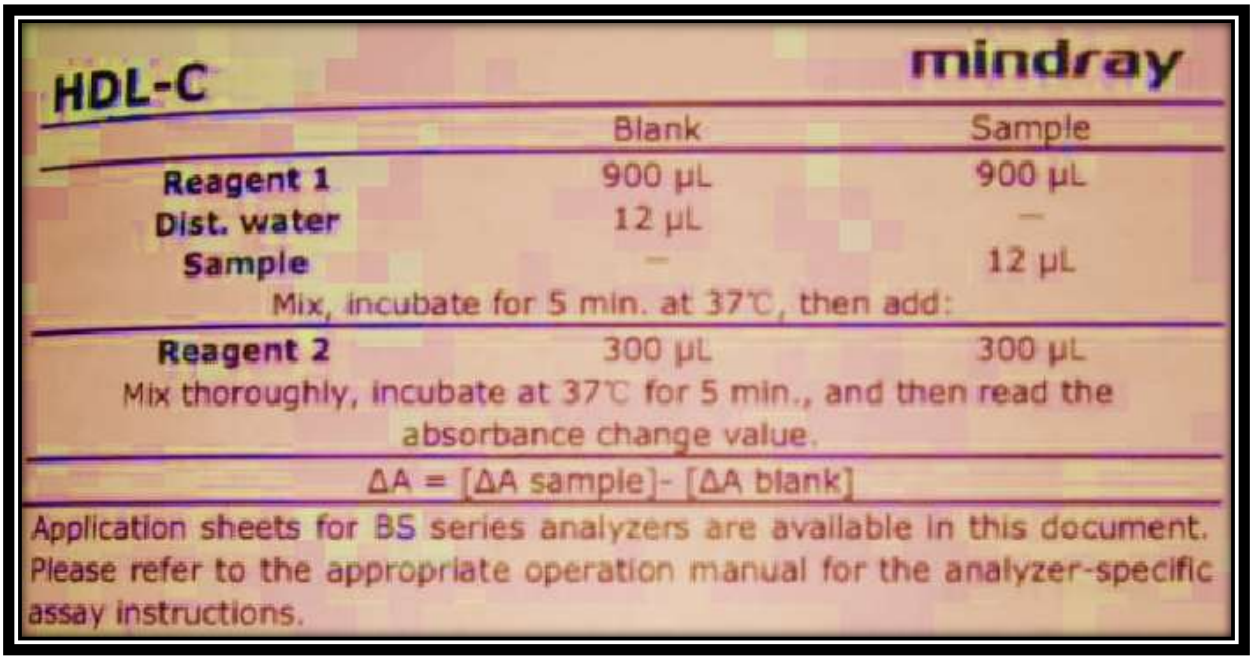
Mix thoroughly at 37 ℃, and read the absorbance 10 min. later.

ΔA = [ΔA sample]- [ΔA blank]

Application sheets for BS series analyzers are available in this document. Refer to the appropriate operator manual for the analyzer-specific assay instructions.

Calibration

Appendix (IV)



HDL-C — mindray

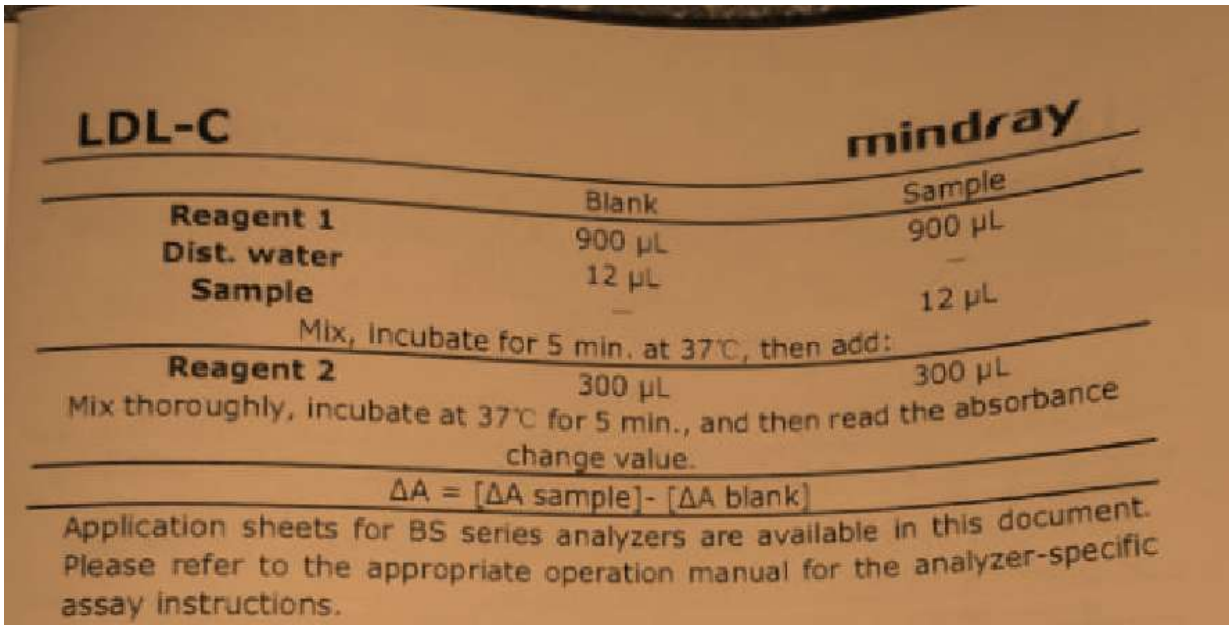
	Blank	Sample
Reagent 1	900 µL	900 µL
Dist. water	12 µL	–
Sample	–	12 µL
Mix, incubate for 5 min. at 37℃, then add:		
Reagent 2	300 µL	300 µL

Mix thoroughly, incubate at 37℃ for 5 min., and then read the absorbance change value.

ΔA = [ΔA sample]- [ΔA blank]

Application sheets for BS series analyzers are available in this document. Please refer to the appropriate operation manual for the analyzer-specific assay instructions.

Appendix (V)



LDL-C — mindray

	Blank	Sample
Reagent 1	900 µL	900 µL
Dist. water	12 µL	–
Sample	–	12 µL
Mix, incubate for 5 min. at 37℃, then add:		
Reagent 2	300 µL	300 µL

Mix thoroughly, incubate at 37℃ for 5 min., and then read the absorbance change value.

ΔA = [ΔA sample]- [ΔA blank]

Application sheets for BS series analyzers are available in this document. Please refer to the appropriate operation manual for the analyzer-specific assay instructions.