ADDIS ABABA UNIVERSITY

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DEPARTMENT OF BIOCHEMISTRY

Assessment of Serum lipid profiles and high sensitivity C-reactive protein among patients suffering from Rheumatoid arthritis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

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A thesis submitted to School of Graduate Studies, Addis Ababa University in partial fulfillment of the requirement for the degree of master of sciences in Medical Biochemistry.

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School of Graduate Studies

This is to certify that the dissertation prepared by Gashaw Dessie, entitled "**Assessment of Serum lipid profiles and high sensitivity C-reactive protein among patients suffering from Rheumatoid Arthritis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia"** and Submitted in partial fulfillment of the requirements for the degree "Master of Science in Biochemistry" in the department of biochemistry complies with regulations of the university and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this research paper entitled **"Assessment of Serum lipid profiles and high sensitivity C-reactive protein among patients suffering from Rheumatoid Arthritis at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia"** is my original work and has not been presented for any degree in any other university, and that all sources of materials used for the research have duly been acknowledged.

Gashaw Dessie

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Date_____

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ABBERAVIATION/ ACRONYMS

HDL-C	High density lipoprotein cholesterol
LDL-C	low density lipoprotein cholesterol
TC	Total cholesterol
RA	Rheumatoid arthritis
CVD	Cardiovascular diseases
ATP	Adenosine triphosphate
LPL	lipoprotein lipase
EC	Endothelial cells
BMI	Body Mass index
DHAP	Dihydroxyacetone phosphate
ACR	American College of Radiology
EULAR	European League Against Rheumatism
GSO	Glycerophosphate Oxidase
SOP	Standard operation procedures
POD	Peroxidase
G-3-P	glycerol-3-phosphate
ERA	Early Rheumatoid Arthritis
DMARD	Disease-Modifying Anti-rheumatic Drugs
Apo E	Apo lipoprotein E
HsCRP	high sensitivity C-reactive proteins

ABSTRACT

Background: Rheumatoid arthritis is a chronic inflammatory disease characterized by severe pain of joints and swelling, joint damage and disability which leads to joint destruction and loss of function with unknown etiology. According to global burden of 2010 study, prevalence of 0.24%, rheumatoid arthritis is ranked among the top 15 of diseases causing disability worldwide. The complication of RA is related to cardiovascular diseases. The morbidity and mortality is related to systemic inflammation and dyslipidaemia.

Objective: The general objective of this project is to assess serum lipid profiles and high sensitivity C-reactive proteins among patients suffering from rheumatoid arthritis and attending in Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia.

Material and Methods: Hospital based cross sectional comparative study was conducted to assess serum lipid profiles and high sensitivity C-reactive protein on rheumatoid arthritis patients in RA clinic at TASH. Patients who came to TASH during the study period were selected by convenience sampling method until the required sample size was achieved. Data was cleaned manually, coded, entered and analyzed by SPSS version 20. Written consent was obtained for willingness of patients to participate after getting official permission letter from Addis Ababa university and then from respective selected rheumatoid arthritis clinic.

Result: The result of this study demonstrated that there was significant elevation of mean ± SD of TC, TC/HDL, LDL/HDL and lowered value of HDL-C was seen among RA patients than controls (P-value < 0.05). The mean ± SD of inflammatory marker, hsCRP was significantly higher among rheumatoid arthritis patients compared to controls (P value < 0.05). HDL-C had significant negative correlation with high sensitive C-reactive protein where as TC/HDL-C and LDL/HDL-C had significant positive correlation with hsCRP (P < 0.05).

Conclusion: In this study rheumatoid arthritis patients had lipid abnormalities and elevated systemic inflammation than controls. An increase in hsCRP and dyslipidaemia status among rheumatoid arthritis patients indicates the possible development of atherosclerotic event which is the underlying cause of cardiovascular risk. So, it is possible to conclude that RA patients are more likely to develop future cardiovascular coronary artery diseases compared to controls.

Key words: Rheumatoid arthritis, serum lipid profiles, cardiovascular risk, high sensitivity C-reactive protein

1. INTRODUCTION

1.1 BACKGROUND

Rheumatoid arthritis is a heterogeneous and progressive autoimmune disease which affects all ethnic groups throughout the world. The World Health Organization considers it as one of the diseases with the greatest impact on society. According to Global burden of 2010 study, Clinical and epidemiological research, prevalence of RA was 0.24% and continues without change from 1990 to 2010 (Cross *et al*., 2014).

Rheumatoid arthritis is a chronic systemic inflammatory disease characterized by chronic inflammation of the joints with severe pain, swelling, joint damage and disability which leads to joint destruction and loss of function. It is characterized by a high systemic inflammatory condition which leads to reduced life expectancy and increased mortality (Ungurianu *et al*., 2017). It also causes significant morbidity due to synovial inflammation, joint destruction and associated disability. Epidemiological studies have shown that mortality is increased in patients with rheumatoid arthritis compared to the general population (Georgiadis *et al*., 2006).

The etiological factor of rheumatoid arthritis is basically unknown, but several studies have implicated a combination of a genetic background and environmental triggers, such as infections and smoking, leading to defects in immune regulation and a host of inflammatory mechanisms involved in joint tissue damage, including a role for oxidative stress (McInnes and Schett, 2011). It is an inflammatory disease characterized by chronic inflammation of the synovial joints associated with proliferation of synovial cells and infiltration of activated immune inflammatory cells including memory T cells, macrophages and plasma cells leading to progressive destruction of cartilage and bone (Filippin *et al*., 2008).

Rheumatoid arthritis is characterized by evidence of disordered innate immunity including immune complex-mediated complement activation, adaptive immune responses against self-antigens comprising predominantly post-transitionally modified proteins and dysregulated cytokine networks (Firestein and McInnes, 2017). This inflammatory process is considered to be mediated by a number of cytokines, such as TNF-a, IL-1, IL-6, IL-8, IL-12, IL-17, IL-18, IL-23 and IFN-g. A network of cytokines involve during inflammation serve as a positive feedback loop in order to respond to stimuli.

The immune complexes produced during the inflammatory process causes for the production of auto-antibodies (Chimenti *et al*., 2015). Immune complexes are products of reactions that involve non covalent interactions between foreign antigens and antibody molecules. Circulating ICs are possibly pathogenic unless they are removed by phagocytosis. CICs and ICs in synovial fluid are likely to contribute to the pathogenesis of rheumatoid arthritis through the activation of the complement cascade, direct destruction of cartilage and production of tumor necrosis factor in synovial tissues (Ohyama *et al*., 2011).

The formation of immune complexes during infection may trigger the induction of rheumatoid factor, a high-affinity auto antibody against the Fc portion of immunoglobulin which serves as a diagnostic marker of rheumatoid arthritis. The auto antibodies, rheumatoid factors and antibodies reactive with citrullinated peptides are produced (McInnes and Schett , 2011).

Endothelial cells play a major role in development of inflammation and atherosclerosis (Packard and Libby, 2008). Infectious agents, autoimmune dysregulation and oxidative stress cause endothelial dysfunction. Injury to the vascular endothelium plays major role during acute inflammatory disease process. The endothelial dysfunction results from different factors that cause inflammatory diseases. LDL-C enters the vessel wall and bind to gluxosaminoglucanes, part of the extracellular matrix of the intima. This binding is facilitated by apolipoprotein B-100 (ApoB–100). The accumulation of LDL in the vessel wall contributes to the formation of fatty strikes. Following LDL adhesion in the vessel wall, it undergoes oxidation by free radicals produced locally.

Macrophages express scavenger receptors for oxidized LDL-C to internalize lipoprotein particles. Macrophages changes to foam cells. Foam cells contain lipid droplet within their cytoplasm. They secrete pro-inflammatory cytokines. This process amplifies local inflammation and reactive oxygen species. T-cells are activated by Ox-LDL to produce cytokines. T-lymphocytes enter the intima facilitated by VCAM-1. Cytokines produced by T-cells influence behavior of other cells present in the atheroma (Packard and Libby, 2008).Growth factors produced by foam cells together with oxidized LDL causes the attraction of Smooth muscle cells. Then they differentiated to fibroblasts and start to produce collagen. This collagen covers foamy cells which become destroyed or forced apoptosis. The final result is the formation of a

pool of extracellular cholesterol trapped under a fibrous capsid. As the plaque extents to the inner layers of the vessel wall, the point of foamy cell formation becomes in stable and may cause rapture of the plaque. The rupture of atherosclerotic plaque leads to hypercholesterolemia (Packard and Libby, 2008).

Rheumatoid arthritis shares some common pathways with atherosclerosis including endothelial dysfunction which is the underlying cause of chronic inflammation (Chimenti *et al*., 2015). The chronic inflammatory condition of rheumatoid arthritis is related to atherosclerotic coronary artery diseases. The chronic inflammatory condition causes abnormality in serum lipid profiles which in turn leads to atherosclerosis condition. Inflammation induces a variety of alterations in lipid metabolism. Abnormality in lipid metabolism causes for the development of atherosclerosis (Munro *et al*., 1997). Atherosclerosis is not a passive event like accumulation of lipids in the vessel walls. It represents an active inflammation of the vessels. Inflammatory cells such as macrophages, monocytes and T-cells play important role in the development of both rheumatoid arthritis and atherosclerosis (Ozbalkan *et al*., 2010).

The complication of rheumatoid arthritis is related to cardiovascular diseases. The morbidity and mortality of cardiovascular diseases increases in patients with rheumatoid arthritis. The morbidity and mortality is related to dyslipidaemia or effect of adverse lipid profiles. Several studies show that an increase in serum cholesterol level increases cardiovascular risk. Systemic inflammation in rheumatoid arthritis is a factor for abnormality in serum lipid profiles. Untreated rheumatoid arthritis leads to elevated levels of TC, LDC-C and decreased concentration of HDL-C (Myasoedova *et al*., 2011).

The purpose of conducting this study was to investigate the concentration of serum lipid profiles and high sensitivity C-reactive proteins in patients with rheumatoid arthritis. Even though the risk factors for cardiovascular diseases are assessed by different studies, the study related to biochemical analysis has not yet been investigated in Ethiopia.

The impact of systemic inflammation and dyslipidaemia on cardiovascular risk in RA is not fully understood. As I mentioned earlier, systemic inflammation in RA leads to abnormality in lipid metabolism. This abnormal lipid profiles is related to atherosclerotic plaque rupture condition. The atherosclerotic plaque rupture causes atherosclerosis which in turn leads to acute coronary

syndrome. So, the purpose of this study was to investigate complication of dyslipidaemia and inflammatory level among RA patients. During the study serum lipid profiles such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C), Triglycerides and inflammatory marker (high sensitivity C-reactive protein) was measured.

1.2 Literature review

1.2.1 Lipid profiles in RA patients

The lipid metabolism is altered in rheumatoid arthritis patient due to the presence of chronic inflammation. Inflammation is highly complicated process. Systemic inflammation is associated with metabolic risk factors, including insulin resistance and decreased high density lipoprotein (HDL-C) cholesterol concentrations in rheumatoid arthritis (Dessein *et al*., 2013). According to this cross sectional study, systemic inflammation causes the change in HDL-C and insulin resistance. The change in HDL-C and insulin resistance also leads to metabolic disturbance of lipid profiles. Inflammation occurs through the involvement of innate and adaptive immune system to respond to endothelial dysfunction. So, during this complicated process the lipid metabolism become altered.

A recent research which was published in 2017 showed that higher serum levels of total cholesterol (TC), higher low density lipoprotein cholesterol patients(LDL-C) and lower serum high density lipoprotein cholesterol (HDL-C) levels were seen in early untreated RA patients compared to controls (Parveen *et al*., 2017). According to this published research the atherogenic lipid ratio increases until the patients got treatment with anti-rheumatoid drug. The ratio of atherogenic indices such as TC/HDL-C and LDLC/HDL-C were significantly higher in early untreated rheumatoid arthritis patients compared to controls.

The other monthly edited journal called ‘journal of Rheumatology’ published different clinical investigation done by scientists. On August 27, 2017 this journal published clinical investigation on inflammation, insulin resistance and aberrant lipid metabolism as cardiovascular risk factors in RA. According to this paper, cytokine production increased, adhesion molecule production

increased and acute phase response elevated and fibrinolysis become reduced. The level of HDL cholesterol Reduced where as small dense LDL particles become increased (Rifai *et al*., 2003).

The report of Tejera-Segura *et al*. also explained that dyslipidaemia happen both in early stages and advanced disease condition. According to this study high-density lipoprotein cholesterol will be lost. So, the cholesterol released from macrophage will not return to liver (Tejera-Segura *et al*., 2017). According to this published research, high density lipoprotein plays a key role in reverse cholesterol transport. The research explains about high-density lipoprotein cholesterol efflux capacity (CEC). HDL efflux capacity is the ability of HDL-C to accept cholesterol from macrophages. The lower level of HDL-C causes higher incidence of cardiovascular events. They concluded that both in early rheumatoid arthritis and advanced condition the concentration of HDL-C decreases.

The other prospective study which was published in 2006 reported on atherogenic lipid indices in early RA patients. Early rheumatoid arthritis patients exhibited higher serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides whereas their high-density lipoprotein cholesterol (HDL-C) levels were significantly lower compared to controls. According to this research the atherogenic ratio of TC/HDL-C as well as that of LDL-C/HDL-C was significantly higher in early rheumatoid arthritis patients compared to controls. The significant reduction of atherogenic ratio occur after treatment as compared to control groups (Georgiadis *et al*., 2006).

Finally, the study conducted by Tejera-Segura *et al* (2017), Georgia, *et al*(2006) and Dessein *et al* (2013) both reported on the presence of change in atherogenic indices in rheumatoid arthritis patients.

1.2.2 Mechanism of lipid profile alteration in RA patients

Inflammation and atherosclerosis play a major role in modification of serum lipid profiles in rheumatoid arthritis patients. The inflammatory cytokines which is released into the circulation in rheumatoid arthritis patients cause modification of the lipid metabolism (Holmdahl *et al*., 2014). The modification of lipid metabolism relates to the release of free fatty acid (FFA) from the adipose tissue. Free fatty acid and triglyceride synthesis increases in the liver and a reduced lipoprotein lipase activity which results in dyslipidaemia. The other published article explained

that even though the beginning of accelerated atherosclerosis is unclear, there is clear association between inflammation, accelerated atherosclerosis and cardiovascular clinical outcomes in RA patients (Hannawi *et al*., 2007).

Another review published in 2002 discussed about the mechanism of pathogenesis of inflammation and atherosclerosis. Endothelial cells play a major role in the development of atherosclerosis (Packard and Libby, 2008). Endothelial cells are in a constant contact with low density lipoprotein (LDL-C). Infectious agents, autoimmune dysregulation and oxidative stress cause endothelial dysfunction. Injury to the vascular endothelium also a critical event in acute inflammatory disease processes. The endothelial dysfunction is the outcome of different factors which results in chronic inflammatory diseases. LDL-C enters the vessel wall and binds to gluxosaminoglucanes which are part of the extracellular matrix of the intima. This binding is facilitated by apolipoprotein B-100 (ApoB–100). The accumulation of LDL in the vessel wall contributes to the formation of fatty strikes. Following LDL adhesion in the vessel wall, it undergoes oxidation by free radicals produced locally.

Macrophages express scavenger receptors for oxidized LDL to internalized lipoprotein particles. Macrophages get altered to foam cells. Foam cells contain lipid droplets within their cytoplasm. They secrete pro-inflammatory cytokines. This process amplifies local inflammation and reactive oxygen species. T-cells are activated by Ox-LDL to produce cytokines. T-lymphocytes enter the intima facilitated by VCAM-1. Cytokines produced by T-cells influence behavior of other cells present in the atheroma (Packard and Libby, 2008). The author finally explains about atherosclerotic plaque formation and its effect on lipid profiles. Growth factors produced by foam cells together with oxidized LDL cause the attraction of smooth muscle cells. Then they differentiated to fibroblasts and start to produce collagen. This collagen covers foamy cells which become destroyed or forced apoptosis. The final result is the formation of a pool of extracellular cholesterol trapped under a fibrous capsid. As the plaque extents to the inner layers of the vessel wall, the point of foamy cell formation becomes unstable and causes rapture of the plaque. The rupture of atherosclerotic plaque leads to hypercholesterolemia (Packard and Libby, 2008).

The other research which was done in university of Hawaii explained about cholesterol crystal formation. Endothelial cell takes up and metabolize LDL-C. Endothelial cells over burdened

with intracellular cholesterol to produce cholesterol crystal. The deposition of cholesterol on the endothelial wall compromises endothelial function. The research paper showed that endothelial cells contribute to atherogenesis under hyperlipidemic conditions (Baumer *et al*., 2017). According to this article, the change in endothelium is characterized by accumulation of lipoprotein particles within the sub-endothelial space. Under pathological conditions, the endothelial mono layer becomes inflamed which results in uncontrolled transport of cholesterol.

Monocytes infiltration and increased endothelial apoptosis with reduced regenerative capacity leading to atherosclerotic plaque formation over time. EC take up LDL through either receptor dependent pathways using either caveolae or clathrin-coated. It is generally believed that LDL taken up by endothelial cell is transcytosed through the cell and deposited in the intima. During this process,someof the lipoprotein particles become oxidatively modified. The oxidative modification of lipoprotein particles in sub endothelial space result in expression of cell adhesion molecules and synthesis of pro-inflammatory cytokines and mediators (Klingenberg and Lüscher, 2015).According to this article the activation of the endothelial cells, subsequent expression of selectins and adhesion molecules is mainly caused by oxidation of lipoprotein.

Emerging studies indicate that uptake of LDL-C by endothelial cell under hypercholesterolemic conditions is activated by activin-like kinase1. Most researches indicate that cholesterol crystal is originated from cholesterol-rich cells in necroticcore (Tejera-Segura *et al*.,2017). The transcytosis process of LDL-C across endothelial cell is mediated by scavenger receptor B1. Recent studies have indicates that cholesterol crystals may be an important factor in the pathogenesis of atherosclerosis. Studies concerning on the biological effects of cholesterol crystal within the plaque focused mainly on the contribution of macrophage foam cells.

Different types of study demonstrate that endothelial cells play critical roles to process LDL particles. After a short period of hyperlipidemic condition, cholesterols are produced and deposited on endothelial space. The presence of cholesterol formation in sub endothelial space indicates the pathogenesis of atherosclerosis. The formation of cholesterol happens mostly in advanced atherosclerotic plaques condition. The modification of cholesterol metabolism affects cholesterol formation. The research determine that the storage form of cholesterol, cholesteryl ester or free cholesterol contributed to much cholesterol formation in endothelial cell (Baumer *et al*., 2017).

The other updated work published at 2012 in Los Angeles by Charles- Schoeman explained that the modification of function and structure of HDL-C is due to systemic inflammation. The research was done on animal models. The modification of HDL-C occurs in an inflammatory condition including rheumatoid arthritis. The major function of HDL-C mentioned in this paper is cholesterol efflux capacity from artery wall cells. The other additional function mentioned is protection of LDL-C from oxidation. The study has been show that the level of HDL-C depends on disease status. Patients with high disease status had significantly decreased ability to promote cholesterol efflux compared to HDL-C from patients with low disease activity. So, higher rheumatoid arthritis disease activity was associated with decreased efflux capacity of HDL-C (Charles-Schoeman, 2012).

The recent finding which has been tried on mice in china shows that lipid peroxidation is caused by systemic inflammation and development of atherosclerosis. It has been reported that lack of apolipoprotein-E results in marked increase in cholesterol-rich materials in blood circulation and subsequent atherosclerotic lesion formation (Wu, Zheng *et al*. 2015). According to this paper, oxidative stress and inflammation play the central role in the development and progression of atherosclerosis. Reactive oxygen species such as superoxide mediates a wide range of pathological processes including lipid per oxidation, reduction of nitric oxide bio activity and induction of inflammatory genes. An important mechanism for reactive oxygen species-mediated atherosclerosis appears to be through stimulation of pro inflammatory events. Previous study showed the critical role of inflammation in the atherosclerotic lesion formation.

Finally, Chinese medicinal trial that reduce atherosclerotic condition (2015), cardiovascular disease and rheumatoid arthritis done by Charles-Schoeman (2012), HDL cholesterol efflux capacity in RA patients by Tejera-Segura *et al*., 2017, inflammation in atherosclerosis by Libby P(2002) and other research articles mentioned that systemic inflammation and atherosclerosis cause for modification of serum lipid profiles.

1.2.3 cardiovascular outcome of patients with rheumatoid arthritis

Rheumatoid arthritis is highly associated with accelerated atherosclerosis. The accelerated atherosclerosis also linked to an increase in cardiovascular complication. The influence of age

and traditional CV risk factors causes plaque formation. The atherosclerotic plaque formation serve as a risk factor for cardiovascular events (González-Gay and González-Juanatey, 2012). According the author, early detection of atherosclerosis is important to reduce the complication of cardiovascular events.

The other research done on the assessment of atherosclerosis by measuring intima media thickness indicates that the occurrence of rheumatoid arthritis is likely to be associated with a higher effect of atherosclerosis (Mahajan *et al*., 2008). The article mainly correlates the complication of atherosclerosis with acute coronary artery diseases. The paper explains about association of mortality and morbidity with cardiovascular events in rheumatoid arthritis patients. So, chronic inflammation, the higher swollen joint counts and higher average C-reactive protein levels were both associated with incident or progressive plaque. Therefore, it seems to be evident that active treatment of the disease may be required to reduce the inflammatory burden. This leads to a reduction in the progression of sub clinical atherosclerosis and reduce the risk of CV events in RA patients. RA is a major risk factor for the occurrence cardiovascular disease.

The patient with RA is 1.5 fold increases to develop cardiovascular diseases compared to general population (Zampeli *et al*., 2012).The author mentioned about the traditional cardiovascular risk factor in his conducted research paper. The traditional cardiovascular risk factors mentioned in the paper are hypertension, dyslipidaemia, smoking, insulin resistance, obesity and altered body composition and physical inactivity.

The effect of RA on cardiovascular diseases is explained by different researchers. The research done by vaghef *et al* explained that CVD are prevalent among rheumatoid arthritis patients and causes for approximately half of the deaths in RA (Vaghef-Mehrabany *et al*., 2017). According to this conducted clinical trial lower levels of high-density lipoprotein-cholesterol (HDL-C) have been seen in RA patients. Due to their greater limitations to exercise their physical activity and painful joints, RA patients increases their risk of developing CVD.

Another paper also discussed on the risk of developing cardiovascular diseases among patients with RA. Increased cardiovascular risk in rheumatoid arthritis patients is due to the presence of inflammation (Lazaros and Tousoulis, 2015). It explained that patients with RA have 2 or 3 fold were more exposed to cardiovascular diseases who lives with disease for more than 10 years.

The cardiovascular complications that result from severity of RA are ischemic heart disease, heart failure and cerebrovascular disease. The article gives emphasis on cardiovascular risk disproportionally increased when traditional risk factors are present. The author also has described about the effect of the traditional risk factor on cardiovascular conditions. Atherogenic lipid profiles are among the traditional risk factor that has been explained. According to the author low levels of high-density lipoprotein cholesterol and high levels of low-density lipoprotein cholesterol, triglycerides and free fatty acids are factor that increases cardiovascular risk.

A cross sectional study done in 2013 by Sandoo *et al*. also explained that rheumatoid arthritis patients have higher risk probability to develop cardiovascular disease. It is possible due to the inflammatory process and atherosclerosis in RA. Vascular functionality is improved after anti rheumatic treatment (Sandoo *et al*., 2013). The presence of both functional and morphological abnormalities in the vasculature in rheumatoid arthritis is due to inflammatory condition. Even though there is unclear evidence, the paper gave suggestion on vascular morphology and function distinctly affected by rheumatoid arthritis.

The research done by Khan *et al* (2013) on lipid profile of patients with acute myocardial infarction, in Tokyo on early Statin treatment in patients with acute coronary syndrome (2004), by Sandoo *et al* (2013) on the association between functional and morphological assessments of endothelial function in patients with rheumatoid arthritis and other published researches commonly discussed that there is higher risk of developing CVD with RA patients.

1.2.4 The inflammatory marker, C-reactive protein among RA patients

C-reactive protein is the most sensitive acute phase reactant. Its level increases in response to acute or chronic infectious and inflammatory process. Its level increases when tissue damage occurs. Since the increase in CRP levels and its duration are closely related to the severity and activity of the inflammatory disease, CRP determination is useful for the diagnosis and treatment of inflammatory condition (Peltola *et al*., 1984).

C-reactive protein is a sensitive marker of systemic inflammation and elevated in patients with RA. C-reactive protein is a marker of inflammation and may be more closely linked with tissue

inflammation in symptomatic RA. C-reactive protein is synthesized by hepatocytes in response to pro-inflammatory cytokines particularly to IL-6. It has been shown to be of great value as an inflammatory marker in RA and has been suggested to mediate part of the complement activation in RA (Molenaar *et al*., 2001).

C-reactive protein usually appears in the sera of patients in the acute stages of a number of inflammatory conditions such as most bacterial, some viral infections, acute rheumatoid fever with or without carditis, rheumatoid arthritis and most other collagen diseases. C-reactive protein is considered to be a sensitive indicator of inflammation. Changes in the serum level of C-reactive protein with time from the same patient can be used as an index of recovery.

C-reactive protein (CRP) serves as an early marker of inflammation or infection. The protein normally found at concentrations of less than 10 mg/L in the blood. During infectious or inflammatory disease states, CRP levels rise rapidly within the first 6 to 8 hours and peak at levels of up to 350–400 mg/L after 48 hours. CRP binds to phosphocholine expressed on the surface of damaged cells, as well as to polysaccharides and peptosaccharides present on bacteria, parasites and fungi. This binding activates the classical complement cascade of the immune system and modulates the activity of phagocytic cells, supporting the role of CRP in the opsonization of infectious agents and dead or dying cells. When the inflammation or tissue destruction is resolved, CRP levels fall, making it a useful marker for monitoring disease activity (Duncan *et al*., 2011).

1.2.5 STATEMENT OF THE PROBLEM

Rheumatoid arthritis is an autoimmune disease which causes mortality and morbidity of population throughout the world. The World Health Organization considers it as one of the disease with the greatest impact on society. According to global burden of 2010 study, clinical and epidemiological research, prevalence of RA was 0.24% and continues without change from 1990 to 2010. According to this research there was an increment from 3.3 million to 4.8 million between1990 to 2010. This was due to a growth in population and increase in aging. Globally, by considering 291 studied conditions, RA was ranked as the 42nd highest contributor to global disability, just below malaria and just above iodine deficiency (Cross *et al*., 2014).

The epidemiological research conducted in 1991 indicates that sub-Saharan African countries are affected by RA. This epidemiological study compared the prevalence of rheumatoid arthritis in these countries. By using uniform criteria of rheumatoid arthritis definition, the study tried to compare and contrast different results. The study included adults among Uganda, Kenya, Nigeria, West Africa, central and southern Africa countries (McGill, 1991)**.** The study were tried to show different factors for variation of rheumatoid arthritis in different sub-Saharan African countries including age structure of the population and Genetic factors in different races. However, the environmental factors are major factors for variation of prevalence of rheumatoid arthritis in these countries (McGill, 1991).

Emphasis given to RA is low compared to its risk on the society in Africa. The disease causes morbidity and mortality related to cardiovascular complications. Ethiopia is one of the African countries which give low emphasis to the disease in case of research and resource allocations. So, awareness creation on the disease burden and disease process is necessary (Jima, 2016). Hypertriglycerdemia, elevated TC, high level of LDL-C level, low HDL-C seen in RA patients contribute to increase risk of CVD. Rheumatoid arthritis is a disease associated with accelerated atherosclerosis. Early detection of atherosclerosis has a major importance to reduce the increased incidence of cardiovascular complications observed in patients with rheumatoid arthritis (González-Gay and González-Juanatey, 2012).

Rheumatoid arthritis is a chronic inflammatory disease characterized by joints with severe pain and swelling, joint damage and disability which leads to joint destruction and loss of function

with unknown etiology (Kumar *et al*., 2013).RA may be caused by genetic and environmental factors as well as an abnormal activation of the innate and adaptive immune system. It is an autoimmune disorders manifested by increased level of inflammatory cytokines produced by activated B cells, T cells and other cell populations (Klein *et al*., 2012).

Rheumatoid arthritis is related to systemic inflammatory condition. The genetic or environmental factors that damage endothelial dysfunction also lead to inflammation at different parts of the body. The chronic inflammation which occurs at the area of joints leads to rheumatoid arthritis. The inflammatory disorders disrupt the lipid metabolism. An aberrant in lipid metabolism related to innate and adaptive immune response. The greater the severity of the underlying inflammatory disease, the more abnormalities are observed in lipid metabolism (Feingold and Grunfeld, 2015).

In Ethiopia, there is no clear clinical investigation done on rheumatoid arthritis. The progress and complication of the disease related to abnormal levels of lipid profiles have not been assessed. Prevalence and risk factors of renal impairment among rheumatoid arthritis were assessed in Tikur anbessa specialized hospital conducted in a period of 6 months from July 2015 to January 2016. The study didn't investigate about lipid profiles related to the disease. The study describes that low emphasis is given to the diseases in terms of research and resource allocations. So, awareness creation on the disease burden and disease process is necessary (Jima 2016).

Clinical investigation and reports related to RA are not enough and complete. The risk of CVD related to dyslipidaemia among RA patients is not well studied. The prevention and management of rheumatoid arthritis could help to reduce other related disease by reducing shared risk factors and prevalence of systemic manifestations. So, management of the problem is important to reduce risk of cardiovascular diseases. This study assessed lipid profiles and high sensitivity C-reactive protein in rheumatoid arthritis patients in the study area in order to give recommendation to minimize CVD related morbidities and mortalities.

1.2.6 Significance of the study

This study is planned to assess the level of serum lipid profiles and high sensitivity C-reactive protein among rheumatoid arthritis patients. Since there is no adequate research done related to this topic in Ethiopia, it will serve as a baseline for future researches. It helps policy makers to give much attention to serum lipid profiles and high sensitivity C-reactive protein of rheumatoid arthritis patients to control dyslipidaemia and inflammation. The management of dyslipidaemia and inflammatory status are important to reduce risk of development of cardiovascular diseases at early stages in RA patients.

2. OBJECTIVES

2.1 General objective

To assess serum lipid profiles and high sensitivity C-reactive protein among patients suffering from Rheumatoid arthritis in Tikur anbessa specialized hospital, Addis Ababa, Ethiopia.

2.2 Specific objectives

- To assess the level of serum lipid profiles among RA patients and control groups.
- To measure the inflammatory marker (hsCRP) among RA patients and control groups and evaluate their inflammatory status.
- To assess the risk factors for RA.
- To make correlation analysis of serum lipid profiles with high sensitivity C-reactive protein (hsCRP).

3. MATERIALS AND METHODS

3.1 Study area and period

The study was conducted at rheumatology clinic of Tikur anbessa specialized hospital. Tikur Anbessa specialized hospital is a large referral teaching hospital, under the administration of Addis Ababa University, located in Addis Ababa, Ethiopia. The hospital is the country's top referral hospital. It has 800 beds and gives diagnostic and treatment service for about 370,000-400,000 patients per year.

3.2 Study design

Hospital based cross sectional comparative study was conducted. The purpose of conducting this study was to assess both risk factors (dyslipidaemia and inflammatory status) and cardiovascular disease status simultaneously. Both exposure risk factor and disease status were assessed simultaneously. This study design method is important to assess outcomes of risk factor. The study made comparison of risk factors (dyslipidaemia and inflammatory status) between individuals having rheumatoid arthritis as "**case group**" with apparently healthy individuals as "**control group**". The study compared simultaneously both exposure risk factors and risk of cardiovascular complication in both groups.

3.3 Source population

All rheumatoid patients seen at the rheumatology clinic at Tikur Anbessa specialized Hospital during the study period served as source population.

3.4 Study population

Patients who fulfill the ACR (American College of Radiology) / EULAR (European League Against Rheumatism) rheumatoid arthritis criteria were utilized for selection of patients during the study (Hochberg *et al*.,1992). The ACR/EULAR criteria used by physician were the number of joints and type of joints (small or large) affected by disease. In addition to joint involvement both serological and acute phase response were examined. But, rheumatoid factor and C-reactive protein measurements were not complete in patient's medical chart. Patients who fulfilled the inclusion criteria and available during the time of data collection period were included in the study.

3.5 Eligibility Criteria for patients

3.5.1 Inclusion criteria

Study participants were included in the study

- If he or she was volunteer to participate.
- If he or she was 18 years old and above.
- If they were at different progression of the disease.

3.5.2 Exclusion criteria

- Study participants with mental health problems, hearing impairment or any other serious health problems and those patients who were not able to provide the appropriate information were excluded.
- RA patients with known diabetes mellitus, pregnancy and anti TB drugs were excluded.
- Patients with other chronic diseases like cancer were also excluded.

Eligibility criteria for control group

Inclusion and exclusion of healthy control groups were done carefully by professional nurses.

Inclusion criteria

Study participants were included as control group

- If he or she was volunteer to participate
- If he or she was 18 years old and above
- Apparently healthy individuals who take care of rheumatoid arthritis patients and other patients who were in other outpatient class other than rheumatology clinic. Few staff members of TASH (n = 5) also selected as control group.

Exclusion criteria

- Study participants with mental health problems, hearing impairment or any other serious health problems and those patients who were not able to provide the appropriate information were excluded.
- Individuals with chronic disease like rheumatoid arthritis, diabetes mellitus, hypertension, cancer, tuberculosis and other diseases were excluded.
- Pregnant women also excluded from the study.

3.6 Sample size determination

Many international researches regarding serum lipid profiles and hsCRP measurement in RA patient were based on relatively small sample size due to practical constraints. Practical constrains such as time, subject availability, finance and sensitivity of the measurement used.

Prevalence of rheumatoid arthritis hasn't yet investigated in Ethiopia. Previous investigation done by Georgiadis *et al*., 2006 and Parveen *et al*., 2017 were with similar context and study design to this study. The study measured lipid profiles and C-reactive protein in RA patients. Many studies done on RA patients to assess lipid profiles and hsCRP level, sample size varies from 30 to 80. By using the sample size of those comparative studies carried out internationally, 73 RA patients were recruited.

The previous investigations didn't use similar proportion of cases and controls. The expense of hsCRP laboratory investigation was high and it was available only in Ethiopian public health institute clinical chemistry laboratory. So, 40 apparently healthy subjects were selected as control in the study.

3.7 Sampling procedure and techniques

Convenience sampling method was applied.

3.8 Study Variables

3.8.1 Independent variables

- Socio-demographic characteristics
- Age
- BMI
- Smoking
- Types of occupation
- Physical exercise
- Alcohol usage
- Rheumatoid Arthritis

3.8.2 Dependent variables

- Serum TC concentration
- Serum HDL-C concentration
- Serum TG concentration

- Serum LDL-C concentration
- High sensitivity C-reactive proteins (hsCRP)

3.9 Operational definition

Dyslipidaemia- Abnormal lipid profile is defined in accordance with the US National cholesterol Education programme, Adult treatment panel III (NCEP-ATP III) guidelines as TC ≥ 200mg/dl, HDL-C < 40mg/dl, LDL-C > 130mg/dl, TG ≥ 150 mg/dl and TC/HDL-C ratio > 5.

3.10 Data collection Procedure

The data was collected from the study hospital in two months by two BSC nurses. The study was controlled by one supervisor who is BSC in nursing. The data collectors were introduced to patients to communicate easily with patients. In this study quantitative data was collected using clinical data and patient history (from rheumatoid arthritis patient's card). The data collectors mainly focused on the objective of the study. The primary data and information about each patient such as physical examination (height and weight) and relevant previous medical histories were recorded using data abstraction format from patient's medical chart.

3.10.1 Anthropometric Measurement

The height and weight of all study participants were recorded without shoes using standard apparatus. Weight was measured to the nearest 0.1kg and height was measured to the nearest 0.5cm. Body Mass index (BMI) was calculated by dividing weight (Kg) by height (m^2). BMI (kg / m^2) is recognized as the measure of overall obesity (Seidell, 2000). According to WHO classification underweight is defined as BMI <18 , normal weight as BMI between 18-25, overweight as BMI between 25.0-29.9 and obese as BMI ≥ 30 (Seidell , 2000).

3.10.2 Blood sample collection

Blood was collected from both RA patients and control after overnight fasting state immediately before the procedure. After overnight fasting 5ml venous blood sample was collected using a BD Vacutainer containing EDTA by nurses under aseptic condition. 70% alcohol was used as disinfectant during venous blood collection. Serum was isolated by centrifugation at 3000 rpm for 5 minutes. After separation, serum was stored at -20 degree centigrade. Then serum lipid

profiles and high sensitive C-reactive protein were measured by calibrated fully automated mind array, clinical chemistry analyzer according to reagent manufacturer's instruction in central laboratory of Zewditu referral hospital and Ethiopian public health institute (EPHI).

3.10.3 Determination of serum Triglyceride level

Principle: The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by Adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP).G-3P is then oxidized by glycerophosphate oxidase (GSO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide. A red colored product is formed by peroxidase (POD) catalyzed coupling of 4-amnioantipyrine (4-AA) and phenol with hydrogen peroxide(H2O2),the optical density was read at 540 nm of which is proportional to the concentration of triglyceride in the sample (Klotzsch and McNamara, 1990). Triglyceride + $3H_2O$ $\xrightarrow{LPL}$ Glycerol + 3FFA

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

Glycerol-3-p +O_2 $\xrightarrow{GPO}$ DHAP +H_2O_2

4-AA + 4phenol $\xrightarrow{H_2O_2}$ Quinoeimine + H_2O

Reagent composition

R1- mono reagent pipes buffer 50mmol/L, PH 6.8, LPL ≥ 12KU/L, GK ≥ 1KU/L, POD ≥ 2.5KU / L4-AA 0.5 mmol /L, non-ionictensioactives 2g/l Biocides.

Triglyceride Standard: Glycerol 2.25mmol/L, equivalent to 200mg/dl of glycerol trioleate.

Procedure: Ten micro liter of serum was mixed in a cuvvate with 1ml of triglyceride mono-reagent R1 then incubated at room temperature for 10 minutes. Then the optical density was read at 540nm against blank and compared with standard triglyceride concentration used as samples.

3.10.4 Serum Total Cholesterol Determination

A commercial kit developed by coxon and Schaffer was used to estimate serum total cholesterol concentration (Coxon and Schaffer, 1971).Cholesterol was measured enzymatically in serum or plasma in a series of coupled reactions that hydrolyzed cholesterol esters and oxidize the 3-OH group of cholesterol. One of reaction products, H_2O_2 is measured quantitatively in per oxidase

catalyzed reaction that produces color. Absorbance was measured at 500nm. The color intensity is proportional to cholesterol concentration. Desirable or normal cholesterol levels were considered to be those below 200mg/dl. The reaction sequence is as follows.

$$\text{Cholesterol Easter} + H_2O \xrightarrow{\text{cholesterol Ester Hydrollase}} \text{cholesterol} + \text{Fatty acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2H_2O_2 + \text{4-amminophenazone} \xrightarrow{\text{phenol peroxidase}} \text{4-(pbenzoquinonemonoimino)} + 4\,H_2O$$

Serum total cholesterol concentration calculation and serum total cholesterol concentration were calculated as follows.

Total cholesterol (mg/dl) = (A sample /A standard) X (concentration of standard)

Whereas A is the Absorbance at 546nm of solutions after reaction is completed.

3.10.5 HDL - Cholesterol

Principle: This technique is a separation method based on the selective precipitation of Apo lipoprotein B containing lipoprotein by phospotungstic acid/ Mgcl2, sedimentation of precipitate by centrifugation and subsequent enzymatic analysis of high density lipoprotein (HDL-C) as residual cholesterol remaining in clear supernatant (Wilson and Spiger, 1973).

Reagent composition

Precipitating Reagent, phospotungstic acid 0.63mmol, magnesium chlorides 25 mmol/L stabilizers will be used. Cholesterol standard, 50mg/dl also used.

Procedure

I, Precipitation

0.2ml of serum was mixed with 0.4ml of precipitating Reagent in a test tube then allowed to stand for 10 min at room temperature. The sample then centrifuged for 10min at 10,000 rpm and supernatant containing HDL-C removed and further processed for cholesterol determination.

II, Colorimetry

50 micro liter of supernatant containing HDL-C was mixed in cuvvate with 1ml of cholesterol MR mono reagent R1and then incubated at room temperature for 10 minutes. Then the optical density was read at 540nm against reagent blank as described under the method for total cholesterol determination.

3.10.6 LDL-Cholesterol

The Frieda Wald formula calculation was actually proposed to calculate LDL concentration. Even though there is certain variability among different laboratories, epidemiologic studies and clinical laboratory was later rapidly adopted it. The formula became the method of choice by routine clinical laboratories (Nauck *et al*., 2002).

LDL cholesterol was determined using the Frieda Wald formula:

Total cholesterol = HDL+ LDL + TG/5

3.10.7 Determination of C- reactive protein

The detection of CRP is a more reliable and sensitive indicator of the inflammatory process than the erythrocyte sedimentation rate, which may also be influenced by physiological changes not associated with an inflammatory process. Current testing methods including latex agglutination, nephelometry, and radial immune-diffusion (RID) have the general disadvantages of low sensitivity whereas enzyme-linked immune-sorbent assays provide the highest sensitivity and specificity (Wilkins *et al*., 1998).

Since the discovery of rabbit's precipitating forms of antibodies against CRP1, various immune-precipitation techniques have been applied for its detection. The Cortex CRP TEST is based on the latex-agglutination method introduced by Singer et al in 1957. The principle of this test is based on the immunological reaction between CRP as an antigen and the corresponding antibody coated on the surface of biologically inert latex particles (Al-khafaji, 2017).

3.10.8 Principles for quantitative determination of hsCRP

High sensitivity C-reactive protein levels were measured by turbid-metric or immune-nephelometry using Co-bas Integra 400 Plus (Roche Diagnostics GmbH, Mannheim, Germany) clinical chemistry analyzer) is continuous and random-access analyzer with a consolidated test menu for routine clinical chemistry, specific proteins, drugs of abuse screening and therapeutic drug monitoring (TDM) and different measuring technologies. Turbidimteric assay was used in which 6µl of serum was incubated with Reagent 1 (TRIS buffer with bovine serum albumin and immunoglobulin (mouse) and Reagent 2 (SR Latex particles coated with anti-CRP (mouse) in glycine buffer) for 98 seconds. The precipitate was determined turbidimetrically at 552 nm.

The analyzer automatically calculates the analyte concentration (in mg/l) of each sample against standards. The measuring range of hsCRP is 0.1-20 mg/l (defined by the lower detection limit and the maximum of the master curve). The precision was determined using Cobas-intgra 400 plus reagent, samples and Controls. 3.5and 2.2 was found both control level 1 and 2 respectively. The analytical sensitivity of the assay is 0.1mg/l. The normal value for hsCRP in a healthy individuals are expected to be <1.0mg/l.

Product characteristics of hsCRP

Assay	hsCRP
Sample material	Serum
Sample volume	2ul
Assay time	10 min
Measuring range	0.3 – 350 mg/L
Analytical sensitivity	0.3 mg/L
Traceability	WHO Standard

3.11 Data collection Tool

Interviewer administered and structured questionnaire data collection tool was used.

3.12 Data quality assurance

Both the data collectors and supervisors were trained for half day on the objective, methodology of the research and data collection approach. The questionnaire was translated to Amharic language and back translated into English by another person to check for consistency. Pretest was conducted in 5% (4) of the samples to see the completeness, consistency and applicability of the instruments and ratified accordingly. This also gives a feedback on whether the intended study objectives were captured well, any omission and any need for additional items so appropriate modification could be made after viewing the pre-test result.

The blood samples for biochemical assay were collected by adherence with standard operation procedures (SOP) and measurement of analysis was carried out after running quality control samples by the investigator.

3.13 Data Processing and analysis

Data was checked, cleaned and entered into Epi data software and then it was imported to SPSS version-20 software for analysis. Simple descriptive statistics was used to present the Socio-

demographic and clinical characteristics of the study subjects. One way ANOVA was used to compare the three level of hsCRP in terms of other continuous variables. Independent sample t-test was used to compare case and control groups based on clinical and demographic data. The Other association was performed using Pearson's correlation coefficients. Adjusted odd ratio was calculated for multivariate analysis to assess risk factor for rheumatoid arthritis. P-value of < 0.05 at 95 % confidence level was considered statistically significant.

3.14 Ethical consideration

Ethical clearance and officials letter was obtained from the research and ethics committee of Department of Medical biochemistry of AAU. Permission was obtained from RA unit. Written consent was obtained for willingness of patients to participate. They were informed that there is no any incentive or harm for their participation in this study.

3.15 Result dissemination plan

The final result of this research will be presented to the department of Medical Biochemistry of AAU and disseminated to the school library of AAU, respective RA unit. Finally, it will be published in peer reviewed journals for further utilization.

4. Results

4.1 Socio-demographic characteristics

A total of 113 subjects were involved in this study. Among the total study participants, 73 were RA patients and 40 were controls. This study enrolled 64(87.6%) females and 9(12.4%) male rheumatoid arthritis patients. The majority of the rheumatoid arthritis patients were found within the age group of 40-59 years (Figure 2). Most of the patients in the study were married (54.7%), urban residents (89%), and had below secondary school literacy (56%) as far as educational status is concerned. In addition, 45% of patients were categorized as low income.

Table1. Socio- demographic characteristics of the study participants at Tikur Anbessa specialized referral hospital, from July to August 2018 (n=113)

Characteristics		Cases (n = 73) N (%)	Control (n=40) N (%)
Age	20 - 39	25 (34.4 %)	15 (37.5%)
	40 – 59	38 (52 %)	20 (50 %)
	60 – 79	10 (13.6%)	5 (12.5%)
Sex	Male	9 (12.4 %)	13 (32.5 %)
	Female	64 (87.6 %)	27 (67.5 %)
Residence area	Urban	65 (89 %)	26 (65 %)
	Rural	8 (11 %)	14 (35 %)
Marital status	Single	18 (24.6 %)	07 (17.5 %)
	Married	40 (54.7 %)	32 (80 %)
	Divorced	07 (9.5 %)	01 (2.5 %)
	Death	03 (4.2 %)	-
Educational status	Illiterate	19 (26 %)	16 (40 %)
	Complete primary school	22 (30 %)	05 (12.5 %)
	Complete secondary school	20 (27 %)	12 (30 %)
	Complete college or university	12 (17 %)	17 (42.5 %)
Monthly income (ETB)	Low (< 500)	33 (45 %)	9 (12 %)
	Middle (500 – 1000)	23 (31.5 %)	12 (16 %)
	High (> 1000)	17 (23.5 %)	19 (72 %)

Smoking habit	Yes	0	0
	No	73 (100 %)	40 (100%)
Type of oil used for food preparation	Palm cooking oil	29 (39.7 %)	18 (45 %)
	Olive oil	44 (60.3)	22 (55 %)
Types of occupation	Industry worker	04 (5 %)	01 (2.5 %)
	House wife	34 (46.5 %)	04 (10 %)
	Farmer	08 (10.9 %)	07 (17.5 %)
	Government civil servant	09 (12.3 %)	18 (45 %)
	Private worker	11 (16.3 %)	12 (30 %)
Alcohol drinking	No	65 (89 %)	35 (87.5 %)
	Sometimes	08 (11 %)	05 (12.5 %)
	Always	0	0
Physical exercise	Yes	08 (10.9 %)	02 (5 %)
	No	65 (89.1 %)	38 (95 %)

(N = number of categorical demographic data, % = percent value of frequency of data, Monthly income was assessed by Ethiopian birr)

In this study, 73 rheumatoid arthritis patients were included who fulfill the appropriate clinical definition for RA. Of total cases 64(87%) were females and 9(13%) were males and among 40 control subjects, 13(32.5%) were males and 27(67.5%) were females respectively. Figure 1 shows comparison of gender variation among case and control groups.

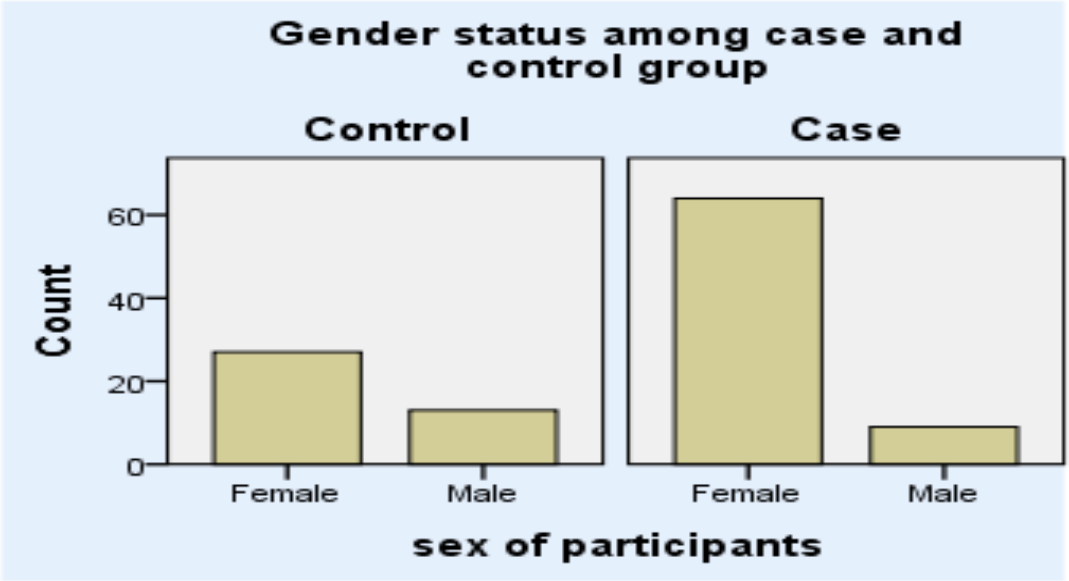

Figure 1.Sex of participants among case and control groups.

The study showed that 25(34.4%), 38(52%) and 10(13.6%) of total case group fall under the age group of 20–39, 40 - 59 and 60 -79 years respectively. The control group showed that 15(37.5%), 20(50%) and 5(12.5%) fall in 20 -39, 40-59 and 60-79 years respectively.

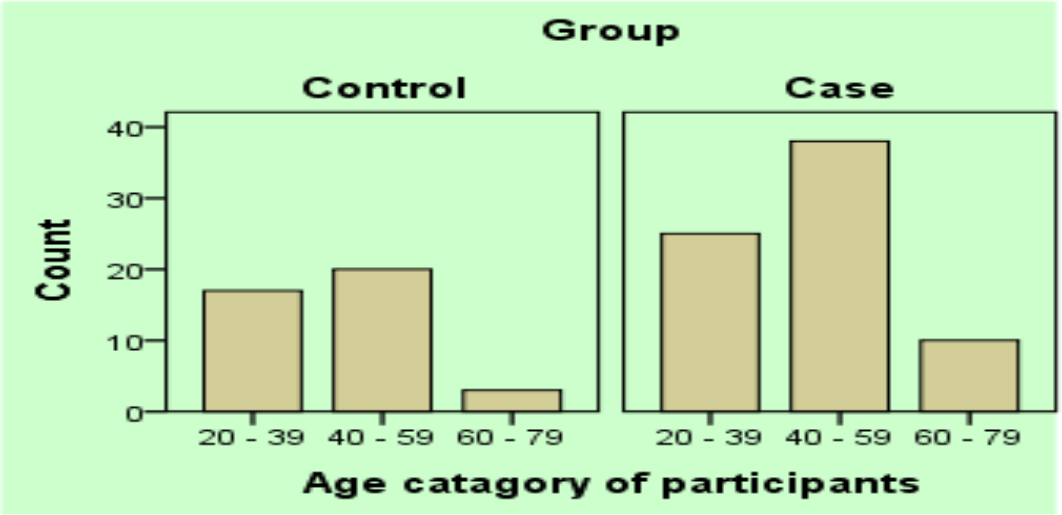

Figure 2.Age category of case and control groups.

The levels of high sensitivity C-reactive protein were categorized as low, moderate and high risk to evaluate the inflammatory status of study participants. Among 73 rheumatoid arthritis patients 14(19.1%), 16(22%) and 43(58.9%) fall in < 1mg/l, 1–3mg/l and >3mg/l levels of hsCRP respectively. The control group shows that 19(47.5%), 14(35%) and 7(17.5%) found in < 1mg/l, 1– 3mg/l and > 3mg/l levels of hsCRP respectively.

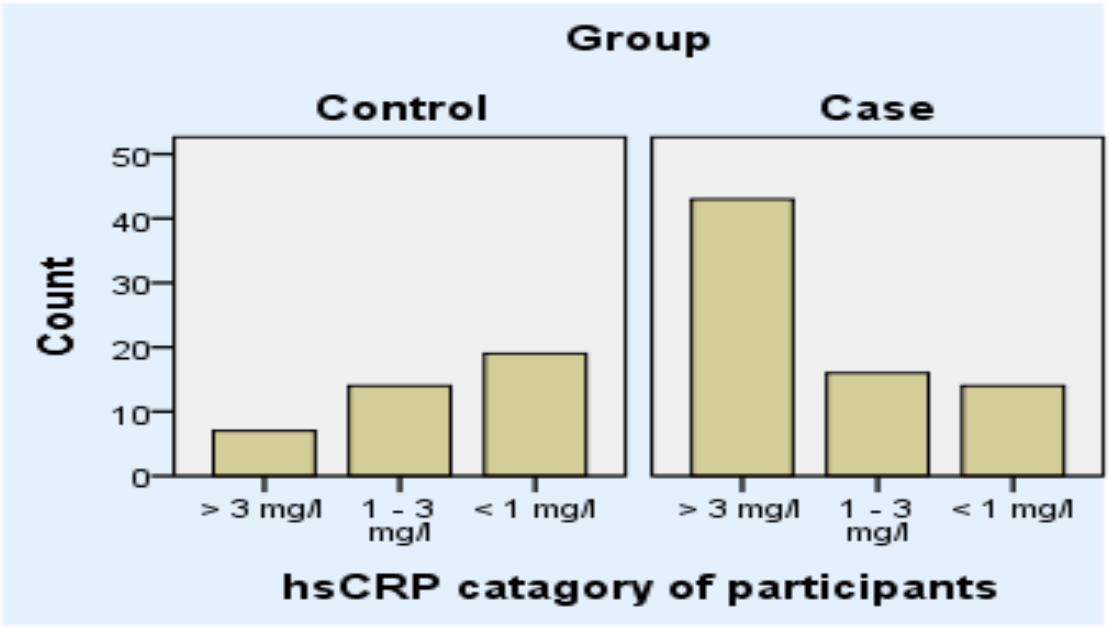

Figure 3.Category of high sensitivity C-reactive protein for both case and control groups

4.2 Estimated levels of biochemical parameters

4.2.1 Serum levels of lipid profiles and hsCRP in RA patients and control groups

The Mean ± SD of ages among patients and controls were 44.41 ± 11.53 and 41.93 ±12.84 respectively and it was not statistically significant. Out of 73 total RA patients, 52 were positive for IgG Rheumatoid factor test. The sensitivity was 71%. The body mass index of both cases and controls were measured and the difference was not statistically significant. High sensitivity C-reactive protein and TG were statistically higher among patients than control where as the level of HDL-C were statistically lowered compared to controls. But the value of LDL-C was not statistically significant.

Table2. Comparison of serum lipid profiles and hsCRP among case and control group

Characteristics	Case (n=73)	Controls (n=40)	P-value
Sex (M/F)	9/64	13/27	0.102
Age (years)	44.41 ± 11.53	41.93 ± 12.84	0.188
BMI (kg/m^2)	23.26 ± 3.67	23.87 ± 3.77	0.842
IgG RF (+/-)	52/21	0/0	-
Total Cholesterol (mg/dl)	179.64 ± 40.39	163.35 ± 30.62	**0.033***
Triglyceride (mg/dl)	132.94 ± 62.15	101.02 ± 38.64	**0.026***
HDL-C (mg/dl)	54.04 ± 16.12	50.10 ± 11.86	**0.023***
LDL-C(mg/dl)	99.04 ± 33.65	93.5 ± 28.28	0.105
hsCRP (mg/l)	10.54 ± 17.26	3.54 ± 7.60	**0.010***
TC / HDL-C	3.61 ± 1.52	3.40 ± 0.84	**0.011***
LDL / HDL-C	2.07 ± 1.18	2.98 ± 0.73	**0.043***

Independent t-test was done to compare compute (mean ± SD) of groups. Values are expressed as mean ± standard deviation. (* = statistically significant at p-value < 0.05) hsCRP= high-sensitivity C-reactive protein; TC= total cholesterol; HDL-C= HDL cholesterol; LDL-C= LDL cholesterol.

4.2.2 Categorical values of serum lipid profiles and hsCRP in rheumatoid arthritis patients and control groups

Out of the total 73 RA patients, 19 (26.1%), 18(24.6 %), 24(32.8 %), 11(15 %) and 42(57.5 %) were above baseline value of NCETPATP-III guideline for TC, HDL-C, TG, LDL-C and hsCRP respectively. These patients had serious dyslipidaemia. On the contrary, out of 40 controls 6(15%), 9(22.5%), 4(10%), 4(10%) and 7(17.5%) were above baseline value of NCETP ATP- III guideline for TC, HDL, TG, LDL and hsCRP respectively.

We compared the level of dyslipidaemia between case and control groups according to baseline value. The patients had 22.8%, 11% and 5%, for TG, TC and LDL-C higher values than controls respectively. The RA patients showed 2% lowered HDL-C value compared to control group.

Table 3.Categorical values of clinical and demographic data for case and control groups

Variables		Cases (n= 73) N (%)	Control (n= 40) N (%)
Age (years)	20 – 39	25 (34.4)	15 (37.5)
	40 – 59	38 (52)	20 (50)
	60 – 79	10(13.6)	5 (12.5)
BMI (Kg/m^2)	< 18.5	8 (10.9)	2 (5)
	18.5 -24.9	43 (58.9)	25 (62.5)
	25 – 29.9	18 (24.6)	7 (17.5)
	>29.9	4 (5.4)	6 (15)
TC (mg/dl)	≥ 200	19(26.02)	6 (15)
	< 200	54 (73.9)	34 (85)
HDL-C (mg/dl)	< 40	18 (24.6)	9 (22.5)
	≥ 40	55 (75.4)	31 (77.5)
TG (mg/dl)	≥ 150	24 (32.8)	4 (10)
	< 150	53 (72.2)	36 (90)
LDL-C (mg/dl)	< 130	62 (84.9)	36 (90)
	≥ 130	11(15.1)	4 (10)
TC / HDL	≤ 5	51 (84)	40 (100)
	> 5	12 (16)	0
hsCRP (mg/l)	< 1	14 (19.1)	19 (47.5)
	1 – 3	16 (22)	14 (35)
	> 3	43 (58.9)	7 (17.5)

hsCRP = high-sensitivity C-reactive protein; TC= total cholesterol; HDL-C= HDL cholesterol; LDL-C= LDL cholesterol

4.2.3 Atherogenic indices among case and control groups

The atherogenic indices are important to investigate atherosclerosis. The logarismic value of TG/HDL-C was not statistically significant, hence the result showed that no significant variation between groups. The mean values of both TC/HDL-C and LDL/HDL-C for RA patients were 3.64 and 2.07 respectively. The mean values of both TC/HDL-C and LDL-C/HDL-C for controls were 3.40 and 1.98 respectively. The calculated atherogenic ratios for both TC/HDL-C and LDL-C/HDL-C were statistically significant and higher in cases compared to controls. From the total enrolled 73 RA patients, TC/HDL-C value of 12 patients had beyond the baseline value (> 5). But controls had value lower than the baseline values. See results in Table 4.

Table 4.Comparison of atherogenic indices between case and control group

Parameters	Case (n= 73)	Control (n=40)	P-value
log (TG/HDL-C)	0.36 ± 0.25	0.28 ± 0.19	0.152
TC/HDL-C	3.61 ± 1.52	3.40 ± 0.84	**0.011***
LDL-C/HDL-C	2.07 ± 1.18	1.98 ± 0.73	**0.043***

Independent t-test was done to compute Mean ± SD values of groups. Values are expressed as mean ± standard deviation, (* = mean difference is significant at $P < 0.05$).

4.3 Correlation analysis of serum lipid profiles with hsCRP in RA patients

4.3.1 Correlations analysis of HDL-C with hsCRP in RA patients

The level of HDL-C was negatively or inversely correlated with hsCRP value of rheumatoid arthritis patients (r = -0.328). There were a significant association between variables (P = 0.005)

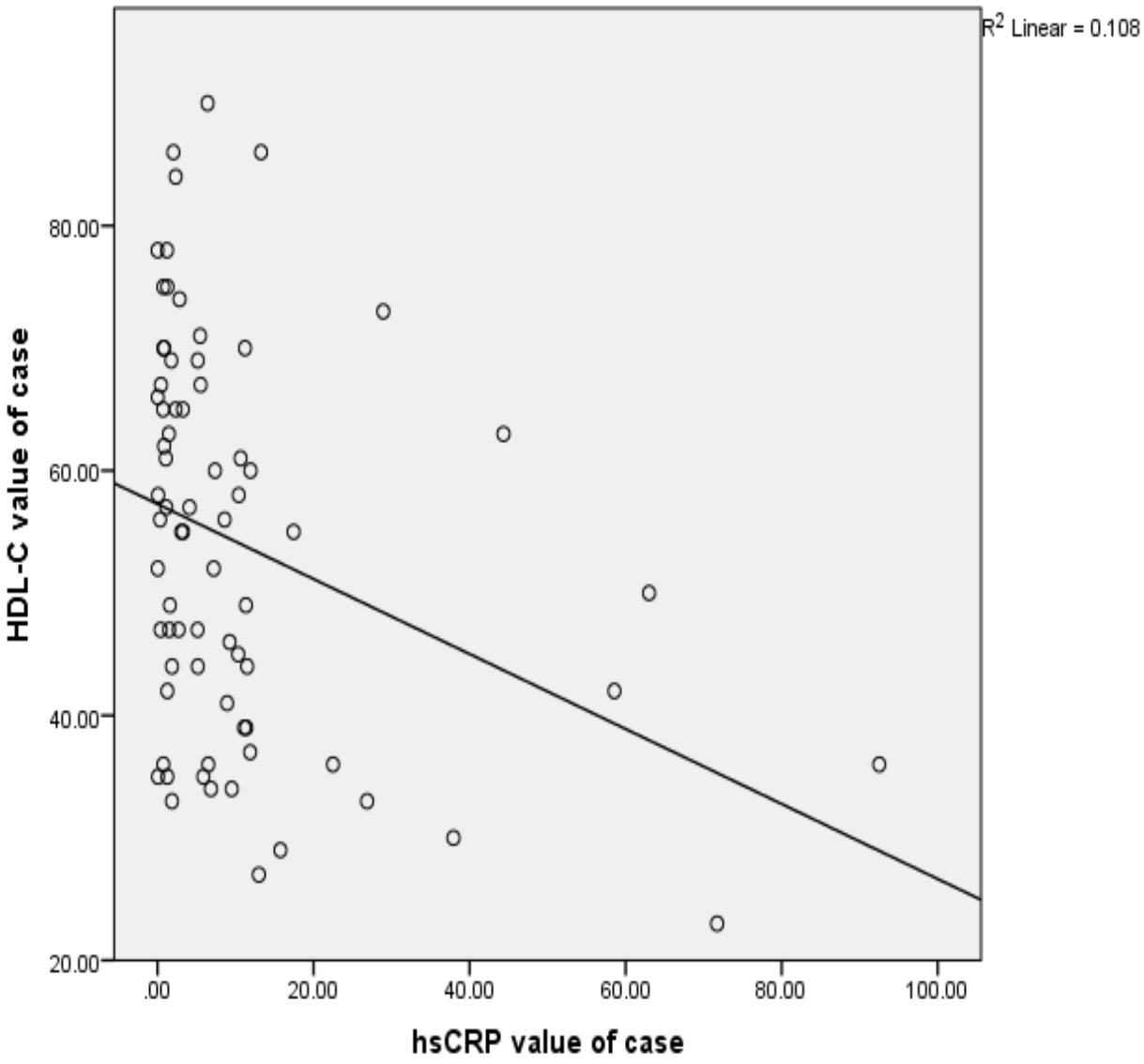

Figure 4.Correlation analysis of HDL-C with hsCRP value of patient

4.3.2 Correlation analysis of atherogenic indices with hsCRP of RA patients

The level of hsCRP showed a positive correlation with TC/HDL-C (r = 0.318). The association between the two variable was statistically significant (P = 0.006)

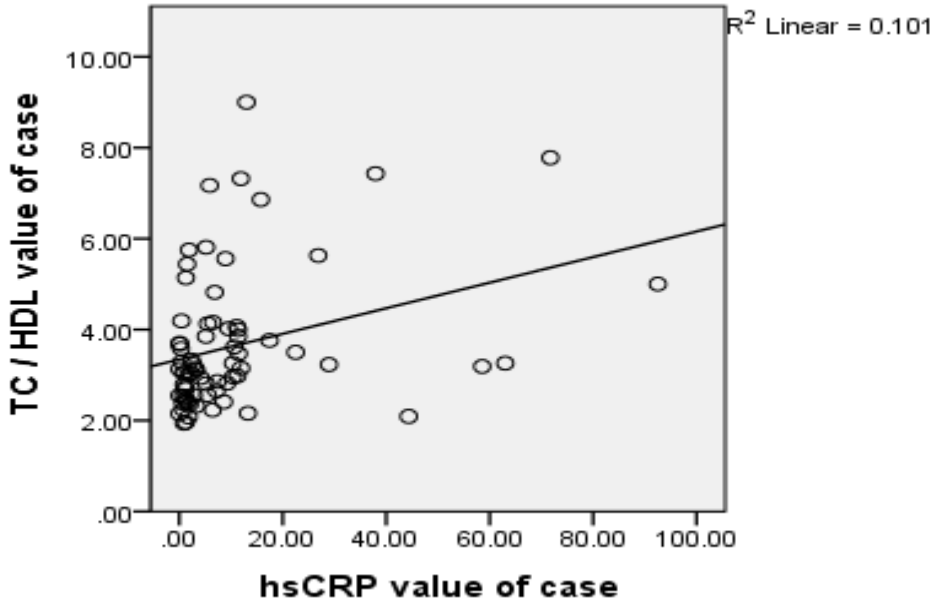

Figure 5.Correlation analysis of TC/HDL-C with hsCRP of patients

The level of high sensitivity C-reactive protein positively correlated with LDL/HDL-C value (r = 0.327). The association between variables was statistically significant (P = 0.005)

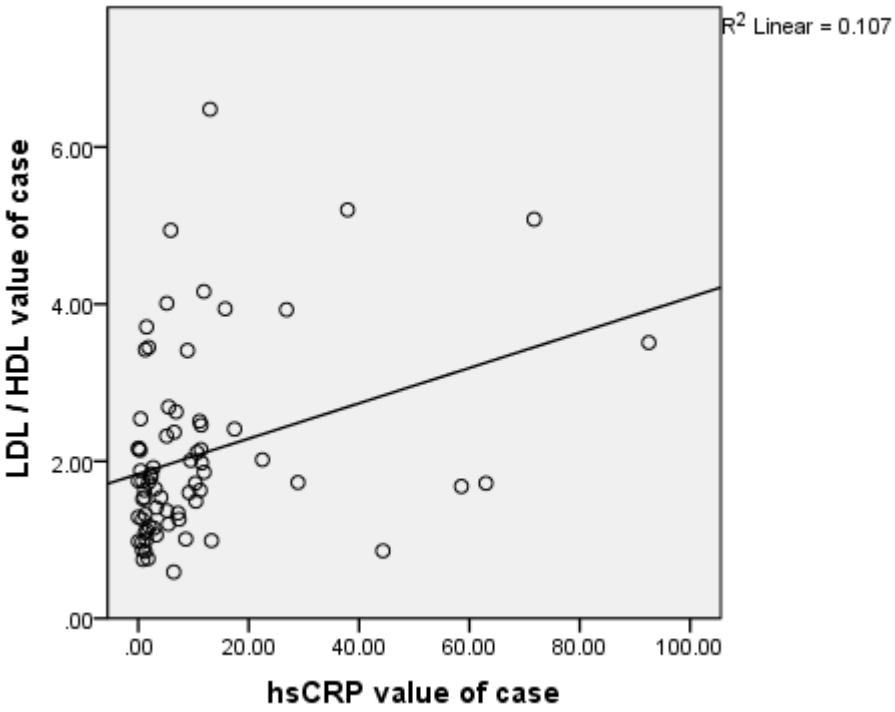

Figure 6.Correlation analysis of LDL-C/HDL-C with hsCRP

4.4 Comparison of serum lipid profiles among different levels of hsCRP in RA patients

Serum lipid profiles and demographic data of RA patients were compared according to different levels of hsCRP. The three levels of hsCRP indicate the severity status of disease. Patient's having < 1mg/l, 1-3 mg/l and > 3mg/l were categorized as low, moderate and high inflammatory risk respectively. The value of TC/HDL-C and LDL/HDL-C significantly varied across three levels of hsCRP.

Table5. Characteristics of serum lipid profiles and demographic data of RA patient according to three levels of hsCRP.

Variables	hsCRP			ANOVA P-value
	< 1mg/l (n =14)	1 – 3mg/l (n= 16)	>3mg/l (n = 43)	
Age (years)	40.00 ± 10.74	42.75 ± 13.35	46.46 ± 10.79	0.15
BMI (kg/m^2)	22.47 ± 3.79	23.15 ± 3.10	23.55± 3.86	0.63
HDL-C (mg/dl)	59.78 ± 13.34	57.81± 16.04	50.76± 16.46	0.10
TC (mg/dl)	171.64 ± 38.36	169.43±36.39	186.04± 42.02	0.26
LDL-C (mg/dl)	90.44 ± 28.38	90.17± 31.59	105.14± 35.25	0.18
TC/HDL	2.94 ± 0.68	3.14± 1.19	4.05± 1.71	**0.01***
LDL/HDL	1.57 ± 0.56	1.72 ± 0.95	2.36 ± 1.33	**0.03***

Continuous variables were compared interms of three levels of hsCRP by one way ANOVA using Tukey post hoc test for specific comparison. hsCRP = high-sensitivity C-reactive protein; TC = total cholesterol; HDL-C = HDL cholesterol; LDL-C = LDL cholesterol; TC/HDL = ratio of total cholesterol to high density lipoprotein; LDL/HDL= ratio of total cholesterol to high density lipoprotein. (* = statistically significant at $P < 0.05$)

Mean plots of different serum parameters with three levels of hsCRP

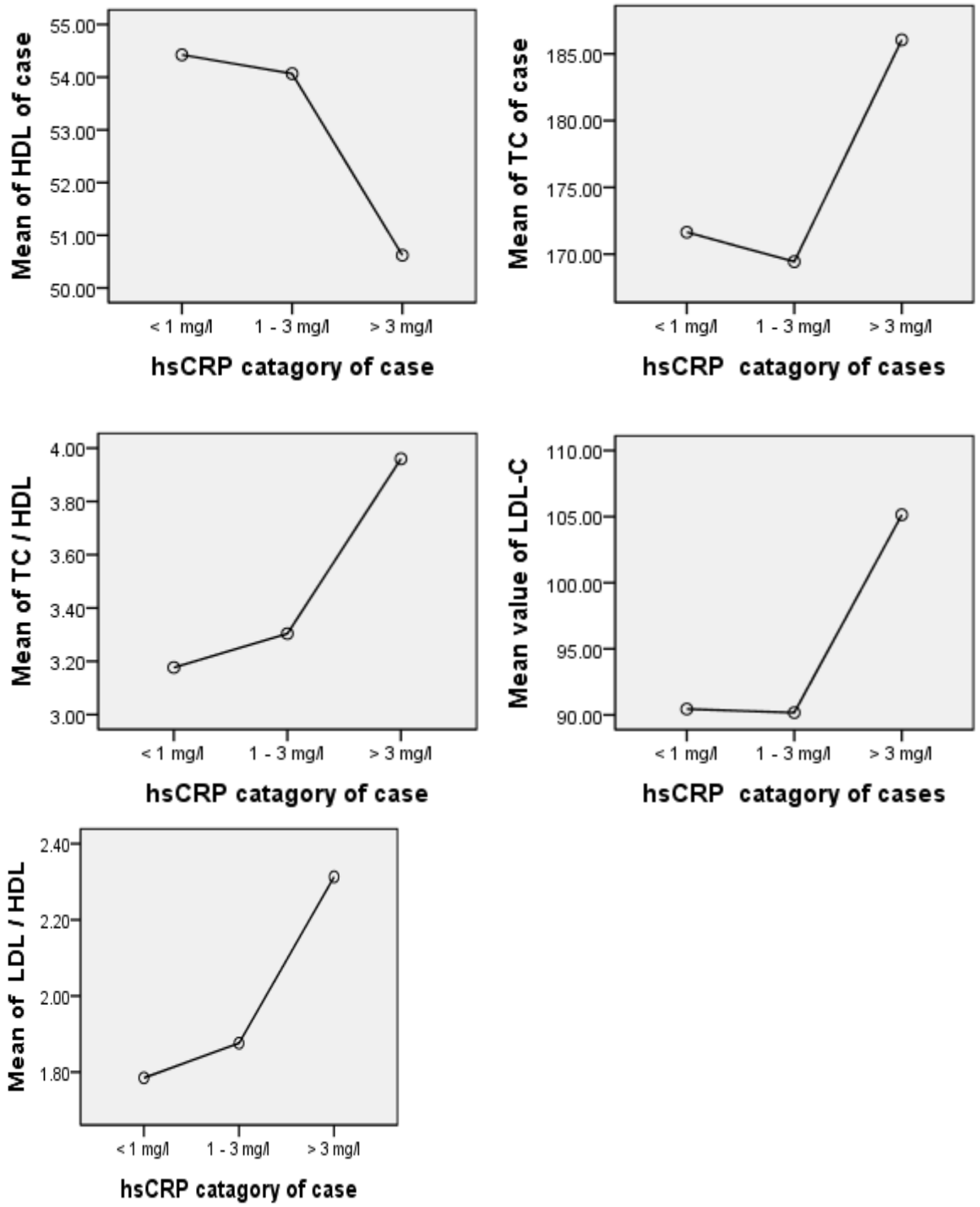

Figure 4.Mean plots of different serum parameters with three levels of hsCRP

Table 6.Multivariate analysis of demographic and clinical risk factors for RA

	Rheumatoid arthritis		
		Adjusted odd ratio (95%CI)	P-value
Sex (M/F)	Male	1	
	Female	8.15(2.14-30.96)	**0.002***
Age (Years)	20 – 39	1	
	40 – 59	1.21(0.53 – 2.78)	0.64
	60 – 79	1.60(0.42 – 5.98)	0.48
Educational status	Complete college or university	1	
	Illiterate	9.23(2.25 – 37.5)	**0.002***
	Complete primary school	6.92(1.90 - 25.12)	**0.003***
	Complete secondary school	2.76(0.97 – 7.84)	0.055
Monthly income (ETB)	> 1000	1	
	500 – 1000	7.52 (0.84 – 67.14)	0.071
	< 500	45.17 (5.56 – 366.9)	< **0.001**
BMI (Kg/m^2)	< 25	1	
	25.9 – 29.9	0.82(0.25 – 26.23)	0.74
	> 29.9	0.093(0.13 – 0.695)	0.65
HDL-C (mg/dl)	≥ 40	1	
	< 40	1.23(0.47 – 3.19)	0.668
hsCRP (mg/l)	< 1	1	
	1 – 3	1.60(0.53- 4.86)	0.40
	>3	20.67(0.53 – 84.2)	< **0.001**
TC (mg/dl)	< 200	1	
	≥ 200	2.20(0.58 – 8.21)	0.241
LDL-C (mg /dl)	< 130	1	
	≥ 130	0.85(0.172 – 4.24)	0.848

(***** = Statistically significant at P < 0.05, AOR = Adjusted odd ratio, ETB = Ethiopian birr)

5. Discussion

Rheumatoid arthritis is a chronic inflammatory disease which causes mortality and morbidity related to cardiovascular risk complication. Although the causative factor for early accelerated atherosclerosis is unclear, but systemic inflammation is related to it (Hannawi *et al*., 2007). RA is a chronic inflammatory autoimmune disease characterized by inflammation of synovial tissue of joints with unknown etiological factor (Nielen *et al*., 2004).The causative factor for RA is not clearly known (Choy *et al*., 2012). This review discussed on the interaction of T-cells, B-cells and proinflammatory cytokines in the pathophysiology of rheumatoid arthritis.

In this study, 73 identified rheumatoid arthritis patients were involved. From the total enrolled RA patients 87.6% and 13.4% were females and males respectively whose age range from 26 to 73 years. The assessment of this study shows that females were more affected by RA than males. Being female was significantly associated as a risk factor for the development of disease ($P < 0.05$, as shown in Table-6). Women whose age ranges from 40-60 showed three-fold increase of risk of development of RA than men (Liu *et al*., 2013).This is in line with our study. Rheumatoid arthritis is seen among middle aged women (McGill, 1991). This review paper discusses on the effect of residence area to develop RA. According to this review women who are living in less facility rural area are prone to develop RA. We collected data about residence area of study participants by questionnaires. We found that there were patients who came from rural areas, especially farmers who were with the disease.

The risk of developing RA is associated with socioeconomic status of individuals (Bengtsson *et al*., 2005). According this extended report socioeconomic status is related with education and occupation. They stated that manual women workers were more exposed to RA compared to men. This agrees with our study which shows people with low economic and educational status are more vulnerable to the disease. In this study, majority of rheumatoid arthritis patients were feels under low educational status. Patients who are illiterate and completed primary school were more exposed for RA than others who completed college or university. This is due to the fact that low educational status leads to low accessibility to medical care service and life style modification. Rheumatoid arthritis occurs more frequently in individuals with lower educational attainment (Callahan and Pincus, 1998).

The risk of developing RA is age dependent (Weyand *et al*., 1998)**.**According to this article, the incidence rates of RA among women increased as their age increased. This idea fitted with our study which shows that disease incidence correlates with increase in age. Age exerts an exponentially increasing effect on cardiovascular risk in seropositive rheumatoid arthritis patient, but no increased effect among seronegative patients. The causes of accelerated aging in patients with seropositive RA deserves further investigation (Crowson *et al*., 2012).

Occupational exposure is a risk factor to develop rheumatoid arthritis (Olsson *et al*., 2004). According to this original research work, occupational exposure to vibration and mineral dust is associated with risk of developing RA. Physically strenuous work and organic dust were also mentioned as exposure in the paper. This agrees with our research. According to our study, there were farmer patients who might have been exposed to organic dust around their farm land. Exposure to pesticides at farming land is associated with risk of development of RA (Parks *et al*., 2016). The reseason why exposure to pesticides causes RA is due their complex immune dysregulation effect. So, farming as an occupation is a risk factor for development of RA.

Studies explain that rheumatoid arthritis is an autoimmune disease. So, we performed serological test called rheumatoid factor for all RA patients. When we see rheumatoid factor value on their clinical data chart, patients don't have complete clinical data on this serological agglutination reaction test. Due to seronegativity effect of test, we conducted tests for all of 73 RA patients. From the total of 73 rheumatoid arthritis patients, 52 patients were positive for IgG rheumatoid factor test whereas the other 21 patients were negative for the test.

The result of our study shows that the sensitivity of IgG rheumatoid factor test is 71%. Different types of studies explain about the sensitivity and specificity of rheumatoid factor test for diagnosis of RA. According to different studies, the level of detection for rheumatoid factor is within range of 50-80% of RA sera. The sensitivity of the test done by our study agrees with one work (Schellekens *et al*., 2000). Due to lowered sensitivity and specificity of rheumatoid factor, RA is diagnosed primarily according to clinical disease manifestations. The result of this study also agrees with another previous research (Song and Kang, 2009). The study mentioned sensitivity of IgG rheumatoid factor and ACPA within range of 60-80%, 70–90% respectively. As we have seen from clinical chart of patients, diagnosis of RA mainly depends on clinical aspects.

It may be due to low sensitivity and specificity of test. Emerging data strongly suggest that anti-CAP antibodies have the power to predict the development of RA in patients.

The systemic inflammation in rheumatoid arthritis leads to cardiovascular risk complication (Sandoo *et al*., 2012).The immune dysregulation and inflammatory condition play a major role in the development of cardiovascular complication (Kahlenberg and Kaplan, 2013).The presence of both inflammatory and immune dysregulation leads to aberrant lipid metabolism. The modified lipid metabolism contributes to the development of accelerated atherosclerosis, which also leads to cardiovascular complication (Kahlenberg and Kaplan, 2013). The main purpose of this study was to evaluate the level of serum lipid profiles and the inflammatory marker hsCRP among RA patients. During this study,73 rheumatoid arthritis patients and 40 age and sex matched apparently healthy controls were included as case and control group respectively. The study showed that there were statistically significant elevation of TC, hsCRP and TG among cases than controls. LDL-C was not statistically significant compared to controls whereas HDL-C was lowered in patients compared to controls.

The inflammatory marker hsCRP was significantly ($P<0.05$) higher among rheumatoid arthritis patients compared to controls (as shown in Table 2). The significant elevation of hsCRP level among cases indicates that the presence systemic inflammation. The result of our study agreed with previous finding (Pearle *et al*., 2007). This research article explained about elevated level of hsCRP associated to cardiovascular risk among RA and osteoarthritis patients. The elevation of inflammatory biomarker (hsCRP) seen in our result indicates the higher systemic inflammatory status among RA patients compared to controls. The higher level of hsCRP among rheumatoid arthritis patients show the elevation of systemic inflammation which is the underlying cause of future cardiovascular risk (Choy *et al*., 2014). This research paper also explained that hsCRP serves as independent predictor of cardiovascular risk especially for MI.

Three levels of hsCRP concentration were used to evaluate inflammatory status. The levels of hsCRP used in our study are <1mg/l, 1 – 3mg/l and >3mg/l. Many other workers used those baseline values for comparison such as Koenig *et al*., 2008, Rifai and Ridker, 2001 and *Graf et al.*, 2009. The level beyond 3mg/l indicates higher inflammatory status whereas hsCRP with 1 – 3mg/l and < 1mg/l levels indicate as moderate and lower inflammatory risk respectively. So, we

evaluated the inflammatory status of rheumatoid arthritis patients using those three levels of hsCRP.

Different researchers discussed about the relationship between hsCRP and cardiovascular risk. Researches explained that premature atherosclerosis causes cardiovascular risk. Elevated level of hsCRP predict subsequent atherosclerotic cardiovascular disease (Rho *et al*., 2009). hsCRP is used as the main biomarker of inflammation (Mora *et al*., 2009). In line with the above work our study focused on hsCRP to evaluate future cardiovascular risk. Our study shows the presence of statistically significant increase in hsCRP among RA patients than controls. 57.5% of total RA study patients show elevation of hsCRP when compared to baseline. The possible reseason for elevation of hsCRP is due to systemic inflammation seen in RA patients. Systemic inflammation leads to accelerated atherosclerosis which is the underlying cause of CVD (Hannawi *et al*., 2007). It is also an early preclinical marker of atherosclerosis and is commonly found in rheumatoid arthritis patients (Yang *et al*., 2016).

Our study evaluated the dyslipidaemia status of both RA patients and control subjects. The study showed that significant ($P< 0.05$) elevation of TC and TG was seen among RA patients than controls (Shown in Table 2). In this study there was statistically significant lower value of HDL-C among RA patients compared to controls. This research paper shows that the presence of systemic inflammation causes adverse lipid profiles in RA patients. So, the result of our study showed the presence of dyslipidaemia among rheumatoid arthritis patients than controls. This is due to elevation of systemic inflammation in patients. Our result fitted in line with previous work (Kumar *et al*., 2013).

In this study we found that 26.1 % of RA patients showed higher total cholesterol (≥ 200 mg/dl) values compared to baseline value of NCETP-ATP III guidelines. The elevated level of TC among cases is higher by 5% than controls (shown in Table 3). According to Framingham heart study, there is an association of total cholesterol with cardiovascular risk. Elevated level of TC among cases compared to control was observed and this is supported by other finding (Chavan *et al*., 2015). The possible reason for elevation of TC among rheumatoid arthritis patients is chronic inflammation and immune dysregulation. Another laboratory test done in this study was measurement of LDL-Cholesterol. We applied the Friedewald calculation to determine the level

LDL-C among cases and controls. Friedewald determination of LDL-C is most commonly used in different studies. The study uses this calculation because the value of TG was below 400mg/dl. This is supported by a literature (Nauck *et al*., 2002).The Friedewald calculation also used in (Park *et al*., 2002).

From the total of 73 enrolled RA patients (15%) showed higher LDL-C compared to baseline value of NCETP –ATP III guideline and 5% higher as compared to control subjects (shown in Table 3). Our study showed that the concentration of serum LDL-C was not significantly increased compared to controls. The result was similar with some literatures which indicates paradoxical characteristics of LDL-C (Myasoedova *et al*., 2011). It has been reported that cardiovascular risk complication could be elevated although LDL-C concentration is within normal range (Boulman *et al*., 2004). Others also explained the importance of consideration of other related factor when LDL-C is within normal range (Mohty *et al*., 2008).

There was no significant difference in LDL-C concentration among cases and controls. But, there was a variation between groups in reference to baseline. When we compared with baseline, there was a slight elevation among cases compared to control groups. There is no clear statement on the correlation between systemic inflammation and LDL-C. Even though there is no adequate data, some literatures correlate decreased concentration of LDL-C with cardiovascular risk. It may be due to the fact that small LDL particles are more atherogenic because they have an increased ability to infiltrate tissues. Small dense LDL-C is more vulnerable to oxidation than normal LDL-C particles (Mohty *et al*., 2008).

The possible reseason for cardiovascular risk complication related to LDL-C concentration is due to release of inflammatory cytokines that activates the transport of LDL-C towards damaged tissue. The uptake of LDL-C by macrophage becomes enhanced. So, the deposition of this LDL-C will be increased. This is clearly stated by researchers (Choy *et al*., 2014). The lower value of LDL-C with elevated level of hsCRP indicates the presence of higher cardiovascular risk (Ridker *et al*., 2004). This research paper was in line with our study.

Our result showed statistically significant ($P< 0.05$) lowered value of HDL-c among RA patients compared to controls. Decrease in HDL-C value was seen among rheumatoid arthritis patients

compared to controls agreed with similar work (Van Halm *et al*., 2007). However, the actual value of HDL showed slight elevation among patients than controls. When we evaluated the level of HDL-C with reference value, 24.6% of RA patients had lowered HDL-C value. The concentration of HDL-C among patients lowered by 2% compared to healthy control subjects. Even if there were no higher variation in both groups, the lowered value of HDL with significant elevation of hsCRP seen in patients was clinically significant. The significant lowering value of HDL is due to reverse effect of atherosclerosis and inflammatory burden. This is agreed with a previous report (Lin *et al*., 1998). The concentration of HDL-C was decreased as inflammatory status increased.

The significant elevation of hsCRP seen in our result inversely correlated with HDL-C. The same type of result was obtained previously (McMahon *et al*., 2006). The other laboratory test done during the study was measurement of Triglycerides (TG). 32.8% of total cases showed higher TG value compared to baseline value of NCETP –ATP III guideline. The TG value of RA patients was higher by 22.8% compared to controls. Statistically significant elevation of TG among cases was observed. Our study fitted in line with another research report (Ansari and Jaiswal, 2011).

The major complication among rheumatoid arthritis patients is elevation of cardiovascular risk compared to the general population. Different scholars associate rheumatoid arthritis with CVD such as MI, heart failure and ischemic heart diseases. Previous finding (Liao, 2017)indicated that cardiovascular risk increases by 1.5– 2 fold higher in rheumatoid arthritis patients compared to age and sex matched control subjects. The cardiovascular risk complication among rheumatoid arthritis patients is caused by the presence of risk factors and inflammatory process (Boers *et al*., 2003). Boers and co-workers correlate the cardiovascular risk complication with systemic chronic inflammation. Elevation of cardiovascular risk among RA patients was also previously reported (Rho *et al*., 2009). The reason mentioned for elevation of cardiovascular risk was chronic inflammation associated with RA.

According to this study, the concentration of hsCRP and TC were statistically increased among patients compared to controls. On the contrary, the concentration of HDL-C was lowered among RA patients than age-sex matched control subjects. Elevated serum hsCRP level serve as

independent predictor of CVD (Galarraga *et al*., 2008). Higher concentration of hsCRP among RA patients causes the development of atherosclerotic vascular disease. This scientific view is also supported by other researchers (Gotto Jr and Moon, 2012). In line with those work, our study also tried to correlate future cardiovascular risk complication with increased levels of hsCRP.

We found that the elevated level of hsCRP among RA patients was 40% higher than age-sex matched control subjects. The higher the concentration of hsCRP indicates higher inflammatory status among RA patients. Our study suggests that there might be development of atherosclerosis among cases than controls. The result of our study agreed with another same work (Park *et al*., 2002). According to park *et al* chronic inflammation serve as independent risk factor for pathogenesis of atherosclerosis. Moreover, high sensitivity C-reactive protein plays a major role in the development of atherosclerotic plaque. The presence of systemic inflammation indicates the progression of atherosclerosis (del Rincón *et al*., 2015). In line with our study, this paper indicates the development of atherosclerosis which causes cardiovascular diseases among RA patients than controls.

It was noted that hsCRP negatively correlated with HDL-C concentration in RA patient. The inverse correlation is associated with disease progression. This implies that as inflammation increases, progress of disease also increases. This is agreed with one report (Georgiadis *et al*., 2006). The inverse correlation of HDL with the disease progress was also mentioned by others (Lin *et al*., 1998). So, in our study the disease condition negatively correlated with HDL-C concentration. In a study done on mortality and morbidity of RA, patients having >3mg/l hsCRP value have a chance 2-4 fold more chance of future cardiovascular risk development (Koenig *et al*., 2008).

The elevation of high sensitivity C-reactive protein among rheumatoid arthritis patients as shown in our study indicates diminished anti-inflammatory activity of HDL-C. HDL-C serves as independent predictor of future cardiovascular risk complication (Jahangiri *et al*., 2010). Our result agreed with this work. So, statistically significant lowering of HDL-C among rheumatoid arthritis patients indicates the possible development of future cardiovascular risk. The possible mechanism for the development of cardiovascular risk in relation to HDL-C level was stated by

different researchers. The presence of higher inflammation among active RA patients leads to modification of structure of HDL-C (Gonzalez-Gay *et al*., 2014).Paraoxonase-1 is an enzymatic component of HDL-C which has anti-oxidant effect. Therefore, oxidation of LDL-C will be continued and causes progression of atherosclerosis. According to our result higher inflammatory status among RA patients may cause lose of antioxidant activity of HDL-C. This leads to the progress of pathogenesis of atherosclerosis which is the underlying cause of cardiovascular risk.

In this study, the concentration of HDL-C among patients is significantly lowered compared to control group. The result indicates lower anti-atherogenic feature of HDL-C among rheumatoid arthritis patients compared to controls. The possible reseason for low anti-atherogenic feature is aberrant HDL-C efflux capacity.HDL efflux capacity correlates with cardiovascular risk assessment (Liao *et al*., 2015). The HDL efflux capacity is a mechanism of reverse cholesterol transport from peripheral tissue to liver for excretion through bile (Tall *et al*., 2008). Diminished anti-atherosclerotic properties of HDL-C causes decreased stimulation of nitric oxide (O'neill *et al*., 2017). Decreased concentration of HDL-C causes decreased stimulation of nitric oxide which leads to endothelial dysfunction. Endothelial dysfunction also a typical features of early stages of atherosclerosis (O'neill *et al*., 2017).In line with the above similar work our study showed that the risk of development of cardiovascular risk increases among rheumatoid arthritis patients than age-sex matched controls.

The rheumatoid arthritis patients showed higher dyslipidaemia condition compared to controls. The research article written by Georgiadis et al. mentioned it as risk factor for CVD and development of atherosclerotic events (Georgiadis *et al*., 2006). This research article stated that increased level of TC and lowered value of HDL-C was seen among early treated RA patients. The change in lipid profile contributes to cardiovascular complication. Our study shows that dyslipidaemia condition (elevated TC and decreased HDL-C) among RA patients may contribute to future cardiovascular events. It may be due to adverse effect of lipid profiles in the pathogenesis of atherosclerosis. This is in line with another study (Montagna *et al*., 2007). The variable in disease activity causes fluctuation of serum lipid profiles (Popa *et al*., 2012). The paper explained about variability of atherogenic ratio as the disease activity changes. According to this review, atherogenic index ratios are more appropriate to assess cardiovascular risk than actual concentration of lipids.

The ratio of TC/HDL-C and LDL/HDL-C was statistically elevated among patients compared to controls. The Mean ± SD ratio of TC/HDL-C among cases and controls was 3.64 ± 1.52 and 3.40 ± 0.84 respectively. The mean value of TC/HDL-C was 3.64 and 3.40 for patients and controls respectively (shown in Table 4). This study showed that there was a significant ($P<0.05$) elevation of atherogenic indices among rheumatoid arthritis patients compared to controls. From the total enrolled 73 RA patients, TC/HDL-C value of 12 patients had beyond the baseline value (> 5). But controls had value lower than the baseline values. It was in line with Georgiadis *et al*., 2006. The risk of myocardial infarction increases considerably when the ratio of TC/HDL-C is higher than five (Georgiadis *et al*., 2006). The significant elevation among cases agreed with previous research work (Parveen *et al*., 2017).

One previous research work agreed with our study (Georgiadis *et al*., 2006). The study indicated significant elevation of atherogenic indices among cases than age-sex matched healthy subjects. The significant elevation of atherogenic indices among rheumatoid arthritis patients showed the possible development of atherosclerosis. Atherogenic indices are important prognostic markers for cardiovascular disease because of their higher atherosclerotic risk seen when their level increases. A review article written by Rifai and Ridker explained that evaluation of combined effect of hsCRP and TC/HDL-C has stronger predictive value of cardiovascular events (Rifai and Ridker, 2001). It was in line with our study.

The value of TC/HDL-C strongly predicates the presence of future cardiovascular risk than other lipid parameters (Smith Jr *et al*., 2004). This previous work explained that both TC/HDL-C and hsCRP are the strongest predictors of cardiovascular risk and this was in agreement with our study. According to our study both atherogenic ratio and hsCRP were significantly elevated. So, higher probability of cardiovascular risk was seen among rheumatoid arthritis patients compared to age-sex matched controls.

6. Conclusion

The result of this study showed that significant elevation of TC, TC/HDL, LDL/HDL and lowered value of HDL-C was seen among rheumatoid arthritis patients than controls. So, it is possible to conclude that dyslipidaemia was seen among RA patient than controls.

The inflammatory marker hsCRP was significantly higher among rheumatoid arthritis patients compared to controls. The higher hsCRP shows high grade of systemic inflammation. Elevated value of hsCRP and atherogenic indices indicate elevation of atherosclerotic events which is the underlying cause of cardiovascular risk among RA patients.

Dyslipidaemia and systemic inflammation status were higher among RA patients than controls. Both of them indicate the presence of atherosclerotic event which is the underlying cause of cardiovascular risk. So, it is possible to conclude that rheumatoid arthritis patients are more risky (exposed) to develop future cardiovascular coronary artery diseases compared to controls.

Measuring of lipid profiles and hsCRP in early RA patients may help to prevent coronary artery diseases.

7. Recommendation

- It is recommended that rheumatoid arthritis patients, especially women have to empower their economical and educational status to prevent and manage RA.
- Monitoring or evaluation of serum lipid profiles and hsCRP is important to assess risk of CVD in RA patient.
- Management of systemic inflammation in RA patients should be given more emphasis to control the further complication of disease.
- This study should be conducted by measuring additional serum parameters in addition to lipid profiles and hsCRP to evaluate CVD complication.
- This study should be conducted in wider scale to confirm that dyslipidaemia and systemic inflammation are risk factor for cardiovascular diseases.
- Multi-marker approach may be an effective strategy to improve evaluation of cardiovascular risk in RA patients.

8. STRENGTH AND LIMITATION OF THE STUDY

8.1 Strength of the Study

Measurement of serum lipid profiles and high sensitivity C-reactive protein in rheumatoid arthritis patients and correlations of those variables to evaluate or assess atherosclerosis is attempted for the first time in Ethiopia.

Therefore, this study is expected to offer the baseline information for further studies on RA in Ethiopia, in relation to lipid profiles and high sensitivity C-reactive protein.

8.2 Limitation of Study

- The study didn't include additional biomarkers that are important to investigate cardiovascular risk assessment.
- The major limitation of this study was effect of DMARDs, Statin and Steroids on the levels of lipid profiles and hsCRP were not assessed.
- Baseline data of serum lipid profiles and hsCRP of patients was not collected from their medical chart.
- Ultrasound imaging was not assessed to check atherosclerotic plaque formation to enhance further evaluation of CVD.
- Financial and time constraints were limitations in this work, affecting the sample size used which could have been affect the effectiveness of the study.

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10. Annex

10.1 Annex I. Subject information Sheet (English Version)

Principal investigator: Gashaw Dessie

Addis Ababa University

College of Health sciences

Department of medical Medical Biochemistry

Dear Participant! Here I the undersigned , at Addis Ababa university college of Health Sciences , Department of Medical Biochemistry Graduate study Programme , currently I will be undertaking research on topic entitled as Serum lipid profiles among Rheumatoid arthritis patients at TASH. For this study you will be selected as a participant and before getting your consent, you need to know all necessary information related to the study which will be detailed as follows.

Introduction

Privacy is the state of being free from intrusion and in the context of health care. It concerns the responsibility of a care provider to protect a clients from any disclosure (i.e. discovery by others), even unintentional of personal health data by providing security to the patients records. Confidentiality , in contrast is the limiting of information to only those for whom it is appropriate. Therefore this information sheet briefly provides the necessary guide to be considered during the study.

Objective of this study is to assess serum lipid profiles among rheumatoid arthritis patients in TASH.

Participants to be included: All rheumatoid arthritis patients who meet the inclusion criteria will be included in the study.

Risk and discomfort: There is no risk in participating in this study. However there might be some discomfort in answering questions which will take a few minute. (About 30 minutes)

Incentive: There is no financial or material incentive in participating in this study.

Benefits: There is no immediate benefit in participating in this study. However your participation will contribute in improving the health delivery system service.

Confidentiality: The information that we will collect from this research project will be kept confidential. Information about you that will be collected from the study will be stored in a file. It will not be revealed to anyone except the principal investigator.

Participant Rights: Your participation is entirely voluntary and up to you to decide. There is no penalty if you don't agree to participate. Also you have the right not to answer any questions you don't want to. You may also withdraw from the study at any time. If in the middle you decide to stop filling questions and no longer participate, you can stop without worry.

Persons to contact: If you have any question, you can ask at any time. If you have additional questions about the study, you can contact the principal investigator: **Gashaw Dessie**, cell phone-0975152796, E-mail - dessiegashaw@yahoo.com

Thank you for your cooperation

If you are voluntary to participate in the study we kindly request you to provide your response for the questionnaire in next page.

Instruction:-The questionnaire contains closed ended questions so circle the letter of your choice (you can answer more than one choice)

10.2 Annex 2. Subject information Sheet (Amharic version)

የመረጃና የስምምነት ቅፅ

ለጥናቱ ተሳታፊዎች በሙሉ፤ እኔ ጋሻው ደሴ በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሜዲካል ባዮኬሚስትሪ ትምህርት ክፍል የድህረ ምረቃ ተማሪ ስሆን የመመረቂያ ፅሁፌን በጥቁር አንበሳ ስፔሻላይዝድ ሆስፒታል በአዋቂዎች ተመላላሽ የቁርጥማት ታካሚዎችን የቅባት መጠንና ሌሎች ግንኙነት ያላቸውን ተያያዥ ነገሮች መጠንና ሁኔታ መለካት ነው።ለዘዚህ ጥናት ደግሞ እርስዎ የተመረጡ ስለሆነ አስፈላጊውን መረጃ እንዲሰጡን በትህትና እንጠይቃለን።

የመረጃ ሰብሳቢው ስም ---------- የአባት ስም ----------የተጠያቂው መለያ ቁጥር ----------

መግቢያ ፤ የጥናቱ ሚስጥራዊነት ሙሉ ለሙሉ የተጠበቀ ነው። እንዲሁም ጥናቱ በሙሉ ፈቃደኝነት የሚሰራ መሆኑ ፤ በፈለጉት ሰዓት ጥናቱን የማቋረጥ ወይም ያለመሳተፍ መብትዎ የተጠበቀ ነው። የጥናቱ ዓላማ ፤የቁርጥማት ህመማንን በደማቸው ውስጥ ያለውን የቅባት መጠንና ሌሎች ተያያዥ ነገሮች መጠንና ሁኔታ መለካት ነው። ጥናቱ የሚያካትታቸው ተሳታፊዎች፤ መረጃ በሚሰበሰቡበት ወቅት ለህክምና የሚመጡ ህመማን ይካተታሉ።

ሀ. **የጥናቱ መረጃ**፦ ጤና ይስጥልኝ ፤እንደምን አደሩ፤ እንደምን ዋሉ፤ እንደምን አመሹ፤(እንደ አስፈላጊነቱ በመረጃ ሰብሳቢው)

ስሜ ---------- ይባላል። የመጣሁት በአዲስ አበባ ዩኒቨርሲቲ ህክምናና ጤና ሳይንስ ኮሌጅ የሁለተኛ ዲግሪ ተማሪ በሆነው በተማሪ ጋሻው ደሴ እየተሰራ ባለው ጥናታዊ ፅሁፍ ዙሪያ በመረጃ ሰብሳቢነት ሲሆን በዛሬው ዕለት እዚህ የተገኙት ከቁርጥማት ህመም ጋር ተያይዞ በደማቸው ውስጥ ያለውን የቅባት መጠንና ሌሎች ተያያዥ ነገሮች በሚመለከት በሚደረገው ጥናት ዙሪያ መረጃ ለመሰብሰብ ነው። ይህ ጥያቄ የተዘጋጀው ለምርምር ስራ ሲሆን በአዲስ አበባ ዩኒቨርሲቲ በጥናትና ምርምር ኮሜቴ ተገምግሞ እንደ አስፈላጊነቱ ግድፈት ካለበት እርማት ይደረግበታል። በዚህ ጥናት በመሳተፍዎ የሚያገኙት ጥቅም የለም ቢሆንም ከዚህ ጥናት የሚገኘው ውጤት በቀጥታ ማህበረሰቡን የሚጠቅም ሲሆን ለእርስዎ ደግሞ እርካታን እንደሚሰጥዎት ተስፋ አደርጋለሁ።

ከጥናቱ የሚገኘው መረጃ ከላይ ከተጠቀሰው ዓላማ ውጭ ለሌላ ተግባር የማይውል ሲሆን መረጃው በሙሉ በሚስጥር የሚጠበቅ መሆኑን ቃል እየገባሁ ለወደፊቱም ለሚፈልጉት የጤና አገልግሎት በእርስዎም ሆነ በቤተሰብዎ ላይ ምንም ዓይነት ተፅዕኖ እንደሌለ ልገልፅልቆት እንወዳለን። በማንኛውም ጉዳይ መረጃ ማግኘት ከፈለጉ ዋናውን የጥናት ባለቤት ጋሻው ደሴን በሚከተሉት አድራሻዎች ማግኘት ይችላሉ። ስ.ቁ- 0975152796 ኢሜል- dessiegashaw@yahoo.com

10.3 Annex 3. Consent form (English version)

In undersigning this document , I am giving my consent to participate in the study entitled as serum lipid profiles and hsCRP among patients suffering from Rheumatoid arthritis at TASH, Addis Ababa, Ethiopia. I have been informed that purpose of this study is to assess serum lipid profiles among patients suffering from Rheumatoid arthritis at TASH, Addis Ababa , Ethiopia. I have been told that my answers to the questions will not be given to anyone else and no reports of this study ever identify me any way. I have also been informed that my participation or non participation or my refusal to answer questions will have no effect on me. I understand that participation in this study doesn't involve risks. I understand that Gashaw Dessie is the contact person if I have questions about the study or about my rights as a study participants.

Respondent's signature _____

Interviewer Name _____ signature _____ Date _____

10.4 Annex 4. Informed consent form (Amharic version)

የፈቃደኝነት ማረጋገጫ ቅፅ

የምርምር ጥናቱ ክፍል የሆኑ መረጃዎችና ሂደቶች ከተብራሩልኝ በኃላ ለተመላላሽ የቁርጥማት ህመም ታካሚዎች የቅባት መጠን እና ተዛማጅነት ያላቸውን ጉዳዮች ለማጥናት በተዘጋጀው ጥናታዊ ፅሁፍ ለመሳተፍ ሙሉ ፍቃደኝነቴን አሳይቻለሁ፡፡ እኔም በተብራራልኝ መንገድ ተረድቻለሁ፡፡ ምርምሩ ምንም የተለየ የገንዘብ ጥቅማ ጥቅም የሌለው ፤አደጋ የማያስከትል መሆኑን እንዲሁም የሚደረገው ተሳትፎ እና መረጃ በሚስጥር የሚያዝና ለማንም ተላልፎ የማይሰጥ መሆኑን ተረድቻለሁ፡፡ ስለዚህ በዚህ የምርምር ጥናት ላይ ለመሳተፍ ፍቃደኛ መሆኔን በፊርማየ አረጋግጣለሁ፡፡

የመረጃ ሰብሳቢው ስም ----------

የተሳታው ስም ----------

ፊርማ ----------

ቀን ----------

10.5 Annex 5. Questionnaire (English version)

Part1. Socio-demographic, Anthropometric and clinical information

1. Age ---------- years
2. Sex A. male B. female
3. What is your ethnicity? A. Amhara B. Oromo C. Tigre D. South E. others
4. What is your marital status? A. Single B. Married C. Divorced D. Widowed
5. From which residence you came? A. urban B. Rural
6. What is your educational status? A. Illiterate B. Reading and writing C. Primary school D. Secondary School E. College / University completed
7. Income _____ birr / year
8. Regular physical exercise? A. Yes B. No
9. Height (m) _____
10. Weight (in Kg) _____
11. Body Mass Index (Kg/m^2) _____
12. Do you drink Alcohol? A. Non drinker B. Non habitual C. Habitual drinker
13. Do you Smoke? A. Yes B. No
14. Which type of oil is used for your food preparation? A. Palm cooking oil B. Liquid olive oil C. others
15. What is your occupation? A. industry Workers B. House wife C. Farmer D. Other

10.6 Annex 6. Questionnaires (Amharic version)

1. እድሜዎት ስንት ነው? _____ ዓመት
2. ጾታ? ሀ. ወንድ ለ. ሴት
3. መኖሪያ ቦታ የት ነው? ሀ. ከተማ ለ. ገጠር
4. ብሔርዎ ምንድን ነው? ሀ. አማራ ለ. ትግሬ ሐ. ኦሮሞ መ. ደቡብ ሠ. ሌላ ካለ ይገለጹ _____
5. የጋብቻ ሁኔታወስ? ሀ. ያላገባ/ች ለ. ያገባ/ች ሐ.የፈታ/ች መ. የሞተችበት/ የሞተባት ሠ. ተለያይተው የሚኖሩ
6. መደበኛ የትምህርት ደረጃዎ ስንት ነው? ሀ. ማንበብም ሆነ መጻፍ አልችልም ለ. ማንበብ መጻፍ እችላለሁ ሐ. አንደኛ ደረጃ አጠናቅቄያለሁ (1-8) መ. ሁለተኛ ደረጃ አጠናቅቄያለሁ (9-12) ሠ. ኮሌጅ ወይም ዩኒቨርሲቲ አጠናቅቄያለሁ
7. አማካይ የወር ገቢዎ ስንት ነው? /በኢትዮጵያ ብር / _____ ብር
8. መደበኛ የአካል ብቃት እንቅስቃሴ ያደርጋሉ ሀ. አዎ ለ. አላደርግም
9. የሰውነት ክብደት ልኬት (ኪ.ግ/ሜ^2) _____
10. ክብደት (በኪ.ግ) _____
11. ቁመት (በሜትር) _____
12. አልኮል ይጠጣሉ ሀ. አልጠጣም ለ. አልፎ አልፎ እጠጣለሁ ሐ. ሁሌ እጠጣለሁ
13. ሲጋራ ያጨሳሉ ሀ. አዎ ለ. አላጨስም
14. ምግብ ለማዘጋጀት የሚጠቀሙት የዘይት ዓይነትስ? ሀ. ፈሳሽ ለ. ሃያት ሐ. ___
15. የሚተዳደሩበት የስራ ዓይነት ምንድን ነው; ሀ. ፋብሪካ ሰራተኛ ለ. የቤት እመቤት ሐ. አርሶ አደር መ. ሌላ ካለ ይጠቀስ