**Lipid ratios and atherogenic index as surrogate biomarkers of subclinical atherosclerosis in patients with type 2 diabetes mellitus**

M.M.Suchitraa\*, T.Sasikalab

aDept of Biochemistry, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh

bDept of Biochemistry, Government Medical College, Eluru, Andhra Pradesh

**\*Corresponding author:**

**Dr.M.M.Suchitra**

**Professor**

**Dept of Biochemistry,**

**Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh**

**Email: mmsuchitra73@gmail.com**

**Abstract**

**Background:** Type 2 diabetes mellitus (T2D) associated atherosclerosis increases the risk for cardiovascular disease (CVD). Considering the cost for some important atherosclerotic plasma markers and imaging techniques, lipid ratios and atherogenic index (AI) may give better information for early prediction of atherosclerotic disease and can contribute to the CVD risk assessment. Hence the atherogenic index (AI), Castelli risk indices (CRI-1 & II), and atherogenic coefficient (AC) were evaluated as potential indicators of subclinical atherosclerosis and their association with anthropometric measurements were assessed.

**Methods:** Based on carotid intima media thickness (CIMT) measured by carotid Doppler ultra sonography, subjects were grouped as healthy controls with CIMT <0.57 mm (n=60;Group 1), Type 2 DM subjects with CIMT <0.57 mm (n=60; Group 2), CIMT >0.57 mm (n=60; Group 3).

**Results:** Waist and hip circumference (WC, HC), triglycerides (TGL), very low density lipoprotein (VLDL), AI, CRI-I and AC were found to be significantly elevated with high density lipoprotein (HDL) levels significantly lowered in T2DM subjects compared to controls. WC, HC, BMI, TGL,VLDL, AI, CRI-I and AC had significant positive correlation and HDL had significant negative correlation with CIMT. The diagnostic performance of AI in predicting subclinical atherosclerosis was significant with higher sensitivity (85%) and specificity (80.0%) at a cut off value of 0.38.

**Conclusion:** AI was found to be a strong indicator of presence of subclinical atherosclerosis in patients with T2DM. Hence AI at a cut off value of >0.38 can serve as a surrogate marker of subclinical atherosclerosis detected at a CIMT cut off value of >0.57 mm.

**Key words:** Lipid ratios**,** Atherogenic index, surrogate biomarker, carotid intima media thickness, subclinical atherosclerosis, T2DM

**Introduction**

Type 2 diabetes mellitus (T2DM) is considered as a most dreaded non-communicable disease due to the metabolic changes leading to chronic hyperglycemia and associated risk such as cardiovascular disease (CVD). Presence of diabetes heightens the risk of cardiovascular mortality by 2-4 times than in those without diabetes and accounts for about 70% of mortality in diabetes (1). Atherosclerosis which has its beginning early on in life, is an inflammatory disease and the chronicity of inflammation leads to progressive atherosclerotic changes. Dyslipidemia with a decrease in high-density lipoprotein cholesterol (HDL) and increase in total cholesterol (TC), low-density lipoprotein cholesterol (LDL), and triglycerides (TGL) is known to contribute to the progression of atherosclerosis (1).Compared with single lipid parameters, the lipid indexes, such as non-HDL, atherogenic Index (AI), Castelli Risk Index (CRI) and atherogenic coefficient (AC) are reported to predict the risk of coronary artery disease (CAD) in patients with hypertension, diabetes or dyslipidemia (2,3). Hence these ratios were proposed to be included in the new CVD risk equations to improve the therapeutic decision-making (3,4). AI has been shown to be a strong marker for predicting the risk of CAD which might reflect the balance between atherogenic and anti-atherogenic factors(5). AI was found to be positively associated with waist circumference (WC) body mass index (BMI) and inversely associated with physical activity (6). In a prospective cohort study, patients were divided into a low AI group (AI <0.24) and high AI group (AI≥0.24) and the relationship between the AI and major adverse cardiovascular events during intensive hospitalization in patients with acute myocardial infarction (AMI) was studied. It was found that a low AI value, in contrast with a high AI value, was an independent predictor for all-cause mortality in patients with AMI who were undergoing intensive hospitalization (7).

Diagnosing atherosclerosis often occurs at an advanced stage or is an incidental finding when investigating a cardiovascular event, which may sometimes have fatal outcomes. Hence it becomes more meaningful to look at the early atherosclerotic changes characterized by a subclinical phase which is mostly asymptomatic. Various imaging techniques such as vascular echography, assessment of coronary calcium, techniques used to study the structural changes in the blood vessel wall such as increase in the intima-media thickness are able to diagnose presence of atherosclerosis at an early stage. Carotid intima media thickness (CIMT) is considered as an indicator of progressive atherosclerosis (8). The addition of the CIMT measure to the traditional Framingham Risk Score utilizing the presence of identifiable risk factors and the levels of some biochemical markers in the cardiovascular risk stratification in 13145 individuals reportedly reclassified 23% of all subjects and 13.5% of those initially considered to be at intermediate risk to be at high risk. Similarly in diabetic individuals with CKD, cardiovascular risk stratification using multi territorial ultrasonography was found to be an authentic non invasive tool to help predict cardiovascular events (9). In this scenario of search for biomarkers of subclinical atherosclerosis, estimated lipid ratios even in the absence of a markedly deranged lipid profile were suggestive of atherogenicity, that helped identifying risk of CVD and could serve as more sensitive risk predictors (3). A strong correlation was reported between Cardiac Risk Ratio (CRR) calculated as TC/HDL, atherogenic coefficient (AC) and atherogenic index of plasma (AIP) with CIMT values in prediabetes (10). Lipid ratios as atherogenic indices have been found to add significant value to the assessment of CAD risk and have been recommended to be considered in addition to routine lipid parameters for the better detection of subclinical atherosclerosis. Hence the present work was taken to assess the association of lipid ratios and AI with CIMT and their performance as surrogate markers of subclinical atherosclerosis.

**Material and methods**

A total of 1545 T2DM patients attending the Endocrinology outpatient department of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati were screened with a questionnaire. From these subjects 60 subjects who fulfilled the inclusion criteria with duration of diabetes of not more than five years and not less than one year and in the age group of 30-60 years, with no history of CVD, CKD, thyroid diseases and chronic inflammatory disease, were included into the study. Subjects who were on treatment with oral hypoglycemic agents were included and subjects on insulin therapy, pregnancy, other forms of diabetes other than T2DM, patients on vitamin-supplementation and those who refused to give written informed consent were excluded from study. From among a total of 68 control subjects screened, 60 subjects without T2DM who fulfilled the exclusion criteria were included. All the subjects were age and gender matched. After obtaining a written informed consent, all subjects were screened for evidence of subclinical atherosclerosis by measurement of carotid intima media thickness (CIMT) by carotid Doppler ultrasonography. This research work was approved by the Institutional Ethics committee.

**Carotid Doppler for measurement of CIMT**

Sonoline G40 Diagnostic ultrasound system (Siemens Medical Solutions USA Inc., USA) by the same radiologist who was blinded to the clinical biochemical information. Measurements were made bilaterally at the carotid bulb, distal one cm of common carotid artery far wall proximal to the bulb and in the proximal most portion of the internal carotid artery near its origin. The mean of the six readings so obtained was used to calculate the CIMT.

The study subjects were classified based on CIMT value (11):

Group 1: Healthy controls with CIMT <0.57 mm: n=60

Group 2: T2DM patients with CIMT <0.57 mm (without subclinical atherosclerosis):n=60

Group 3: T2DM patients with CIMT >0.57 mm (with subclinical atherosclerosis) :n=60

**Sample collection:** Venous blood was drawn from medial cubital vein from subjects after an overnight fast of 8-10 hours and transferred to additive free tubeand sodium fluoride and potassium oxalate anticoagulant tube. Additive free tubes were allowed to stand for 30 minutes and then centrifuged at 2000 rotation per minute for 15 minutes to obtain serum. Anticoagulant containing tubes were centrifuged immediately to obtain plasma for blood glucose estimation.

Plasma glucose was measured by glucose oxidase peroxidase method (Pathozyme Dianostics, Kagal Dist, Kolhapur, India), urea by urease method (Crest Biosystem, a division of Coral clinical systems, Goa, India), creatinine by Jaffe’s rate method (Beckman system pack), total cholesterol by cholesterol oxidase peroxidase method, Triglycerides by enzymatic colorimetric method (Agappe diagnostics Ltd, Ernakulum, Kerala, India), HDL by selective inhibition method (Beckman system pack). LDL and VLDL was calculated by Friedwald’s formula (12) and non HDL cholesterol was calculated as total cholesterol minus HDL. All the parameters were analyzed by using Synchron Unicel DxC600 auto analyzer (Beckman coulter, USA).

**The calculated lipid parameters** (12):

VLD = TG/5

LDL= TC - (VLDL + HDL)

Non HDLc = TC-HDL

**The Atherogenic indices were calculated as**:

Atherogenic Index (AI) = log (TGL/HDL)(5)

Castelli’s Risk Index (CRI-I) = TC/HDL (13)

Castelli’s Risk Index (CRI-II) = LDL/HDL (13)

Atherogenic Coefficient (AC) = (TC– HDL)/HDL (14)

**Statistical Analysis:**

Data distribution was studied by using Kolmogrov Smirnov test. Data obtained was expressed as mean ± SD for normal distributed data and median inter quartile range for data not having normal distribution. The comparisons of parameters across the groups were tested using analysis of variance (ANOVA) and post hoc for pair wise comparisons in data with normal distribution or Kruskal Wallis test for data that was not having normal distribution. Differences in means among the groups were tested using unpaired two tailed t test. Pearson’s correlation or Spearman rank correlation analysis was done to study the correlations among the parameters with and without normal distribution respectively. Receiver operative characteristic (ROC) curve analysis was performed to study diagnostic utility of parameters. A ‘p’ value of < 0.05 was considered as statistically significant. Analyses were performed using Microsoft Excel spread sheets (Microsoft Redmond USA) and IBM, SPSS statistics (version 22.0).

**Results**

**Table 1** shows the clinical, biochemical characteristics and anthropometric indices of the study population. The anthropometric indices, WC and HC were found to be significantly elevated across the groups.

**Table 2** shows lipids, lipid ratios and AI among the study groups. Significant elevated levels of TGL and VLD accompanied by elevated AI, CR-I and AC and a significant lowered HDL levels were found across the groups.

**Table 3** shows the changes in the anthropometric indices, lipids and AI, lipid ratios between the study groups. Both groups of T2DM with and without subclinical atherosclerosis were found to have significant higher WC with significant elevated levels of TGL, VLDL, significant lowered HDL levels, with significantly elevated AI, CRI-I and AC when compared to controls. The HC was found to be significantly higher in T2DM with subclinical atherosclerosis when compared to controls.

**Table 4** shows the correlation among lipids, lipid ratios and AI. Total cholesterol was found to have significant positive correlation with TGL, VLDL and LDL. TGL had a significant positive correlation with VLDL. TGL, VLDL and LDL were found to have significant negative correlation with HDL. Association of lipids with lipid ratios and AI found significant positive correlation of total cholesterol, TGL and VLDL with AI, CRI-I and AC, LDL had significant positive correlation with CRI-I, CRI-II and AC and HDL was found to have a significant negative correlation with AI, CRI-I, CRI-II and AC.

**Table 5** shows that correlation analysis of anthropometric indices, lipids, lipid ratios and AI with the CIMT of subclinical atherosclerosis. WC, HC, BMI, TGL, VLDL, AI, CRI-I, AC were found to be significantly positively correlated with CIMT. A significant negative correlation of HDL with CIMTwas observed.

**Table 6** shows the diagnostic utility of lipid ratios and AI in subclinical atherosclerosis.

AIP, CRI-1 and AC had significant area under the curve (AUC 0.887, 0.675, 0.675 respectively; p<0.001). AI at a cut off value of 0.38 was found to have a higher sensitivity and specificity (85% and 80% respectively) for diagnosis of subclinical atherosclerosis in T2DM at a CIMT cut off of ≥ 0.57 mm.

**Discussion**

The use of biomarkers as surrogate endpoints has gained importance especially in the approach to management and treatment of cardiovascular disease due to the associated high mortality and morbidity. The criteria of a biomarker being considered as a surrogate biomarker were based on the ease of measurement, the association between the surrogate marker and the clinical endpoint and the ability of the surrogate biomarker to produce an estimate of the risk and benefits related to the disease processes. In this context CIMT, LDL cholesterol and CRP are being used as surrogate endpoints (15). A number of lipid parameters have been explored at subclinical atherosclerosis level to predict the risk of CAD. The present study observed that subjects with T2DM without subclinical atherosclerosis had higher WC compared to controls and T2DM subjects with subclinical atherosclerosis defined by a CIMT of ≥ 0.57mm had higher WC and HC compared to controls (Table 3). with higher TGL lower HDL levels (p=0.001). AI, CRI-I, higher AC and CIMT (p=0.0001, p=0.0001, p=0.0001, p=0.0001 respectively) which were found to be significant. Recently, AI assessment was shown to be a predictor for myocardial infarction and atherosclerotic heart diseases (16). The AI value has been correlated with serum small, dense LDL levels and lecithin cholesterol acyl transferase activity, an index of cholesterol fraction esterification rate. Increased TG and/or reduced HDL levels increase AI values, and these effects are associated with a high cardiac risk (16). The CIMT measurement is a powerful marker of cardiac risk and atherosclerotic heart diseases and has been widely used for the detection of subclinical atherosclerosis (17).

The present study explored the relationship between lipid ratios and AI with subclinical atherosclerosis in patients with type 2 diabetes mellitus and found a strong positive correlation between CIMT with AI. The elevated TGL levels in T2DM cases when compared to controls could contribute to the positive association with the WHR, indicating that occurrence of dyslipidemia will tip the balance towards atherogenesis even in the absence of changes in BMI and anthropometric measurements. It is reported that the early indication of atherosclerosis visualized as an increase in intima thickness is due to increased expression of adhesion molecules which is in turn promoted by TGL. A direct association of TGL with CIMT in a population of healthy young adults with family history of CAD has been reported (18).

In a study, a mean CIMT value of 0.79 mm in young males and 0.72 mm in females aged 30 to 40 years were reported to be associated with increased coronary artery calcification (19). Death from cardiovascular causes was found to be significantly higher in patients with moderately increased CIMT (1–2 mm). Increased CIMT is correlated with atherosclerosis in the coronary and large arteries and a linear correlation was demonstrated between increased CIMT and CAD (19-21). Epidemiological studies demonstrated correlations between CIMT and classical cardiovascular risk factors, including age, smoking, high blood pressure, levels of cholesterol and triglyceride, body mass index, American Heart Association reported that CIMT measurement can be used as a cardiovascular risk marker in asymptomatic persons aged over 45 years. CIMT can identify the persons under a high risk for CAD (22). CIMT was shown to be an independent predictor in all-cause and cardiovascular mortality (17).It was knownthat cardiovascular risk increases with high serum cholesteroland LDL-c levels and that small-dense LDL particlesare strong risk factor for atherogenesis. Again, a strong correlation was found between high TG and low HDL cholesterol levels and small-dense LDL-c levels. The mostimportant factor regarding development and progressionof CAD was found as small-dense LDL particles. Infact, LDL cholesterol is at the normal level in more than half of CAD cases. This is explained by excessive small dense LDL content. Key point in formation of the small dense LDL is elevated plasma levels of triacylglycerol (23). In the present study in spite of levels of total cholesterol and LDL being lower in the T2DM groups compared with the controls, a significant positive correlation was found between AI and CIMT, which may indicate that size of the particles rather than levels of LDL is important in progressive atherosclerosis. Due to the variable degree of chronic inflammation, the individual lipid concentrations may frequently fluctuate during the course of disease making the impact ofsuch changes on CVD risk less clear. In line with this, it has been suggested that the AI is less susceptible to disease activity fluctuations in rheumatoid arthritis (24,25). The AI measure is said to reflect the balance between protective and atherogenic lipoproteins (5). Therefore, one can hypothesize that AI may be more a better tool to assess the relative contribution of lipids to the CVD risk than individual cholesterol fractions measurements. It is also reported that inflammation may not only modulate the levels but also the composition of lipoproteins (26). Previous studies were done in advanced atherosclerotic diseases and the need was felt to study subclinical atherosclerosis and lipid indices. Hence in the present study ROC curve analysis was performed to assess the diagnostic ability of lipid ratios and AI in predicting the presence of subclinical atherosclerosis among the T2DM patients. The present study found that AI, CRI-1 and AC had significant diagnostic performance (AUC 0.887, 0.713, 0.713 respectively). Among the lipid ratios and AI, the performance of AI was much more significant for the detection of atherosclerotic changes in subclinical atherosclerosis at an AI cut off value of 0.38. Hence AI can be proposed to be used to indicate presence of subclinical atherosclerosis and hence used in CAD risk assessment in patients with type 2 diabetes mellitus.

**Conclusion:** Findings indicated AI and lipid ratios, CRI-I and AC as biomarkers of subclinical atherosclerosis as they were found to correlate with CIMT and were associated with the changes in CIMT. When compared to lipid ratios, AI was found to be a stronger predictor of subclinical atherosclerosis at a cut of value of 0.38 at the CIMT cut off value of ≥ 0.57 mm. Hence AI can be considered as a simple and cost effective surrogate biomarker of subclinical atherosclerosis in patients with T2DM, especially in remote and resource limited centres. The diagnosis of subclinical atherosclerosis is of prime importance as the preventive therapeutic measures can be initiated along with life style modifications to slow down, prevent or even revert the early atherosclerotic changes. Early diagnosis and treatment of subclinical atherosclerosis can attenuate the cardiovascular risk in patients with T2DM.

**REFERENCES**

1. Baynest HW. Classification, Pathophysiology, Diagnosis and Management of Diabetes Mellitus. J Diabetes Metab 2015;6:541 doi:10.4172/2155- 6156.1000541
2. Bhardwaj, S., Bhattacharjee, J., Bhatnagar, M. K., Tyagi, S. Atherogenic index of plasma, castelli risk index and atherogenic coefficient - new parameters in assessing cardiovascular risk. Int. J.Pharm. Biol. Sci 2013: 3;359–64.
3. Adedokun AK, Olisekodiaka MJ, Adeyeye DA, Muhibi AM, Ojokuku OH, Adepeju AA, et al. Castelli Risk Index, Atherogenic Index of Plasma, and Atherogenic Coefficient: Emerging Risk Predictors of Cardiovascular Disease in HIV-Treated Patients. Saudi J. Med. Pharm. Sci. 2017; 3: 1101-10.
4. Orozco-Beltran D, Gil-Guillen VF, Redon J, Martin-Moreno JM, Pallares-Carratala V, NavarroPerez J, et al. Lipid profile, cardiovascular disease and mortality in a Mediterranean high-risk population: The ESCARVAL-RISK study. 2017; PLoS ONE 12(10): e0186196. doi:10.1371/journal.pone.0186196.
5. Dobiasova M, Frohlich J, Sedova M, Cheung M C, Brown B G. Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography. J. Lipid Res 2011; 52:566–71.
6. Shabnam Niroumand, Mohammad Khajedaluee, MajidKhadem-Rezaiyan, Maryam Abrishami, MohammadrezaJuya, Gholamhasan Khodaee, et al. Atherogenic Index of Plasma (AIP): A marker of cardiovascular disease. Med J Islam Repub Iran 2015; 29: 240.
7. Hartopo AB, Arso IA, Setianto BY. Low Plasma Atherogenic Index Associated with Poor Prognosis in Hospitalized Patients with Acute Myocardial Infarction. Acta Med Indones 2016;48(2):106-13.
8. Elko Randrianarisoa, Angela Lehn‑Stefan, AnjaHieronimus, RobertWagner, JakobMaucher, KilianRittig, et al. Reduced insulin clearance is linked to subclinical atherosclerosis in individuals at risk for type 2 diabetes mellitus. Scientifc Reports 2020; 10:22453.
9. Ana Palanca, Esmeralda Castelblanco, ÀngelsBetriu, HèctorPerpiñán, Berta Soldevila, José Manuel Valdivielso, et al. Subclinical atherosclerosis burden predicts cardiovascular events in individuals with diabetes and chronic kidney disease. Cardiovasc Diabetol 2019; 18:93.
10. Roshan Kumar Mahat, NeelimaSingh, VedikaRathore, Akshara Gupta, Rakesh Kumar Shah. Relationship between Atherogenic Indices and Carotid Intima-Media Thickness in Prediabetes: A Cross-Sectional Study from Central India. Med Sci 2018; 6(3): 55.
11. Mohan A, Sujay Sada, Siddhartha Kumar B, Sarma K V S, Vijayalakshmi Devi B, SrinivasaRao P V L N et al. Subclinical atherosclerosis in patients with rheumatoid arthritis by utilizing carotid intima-media thickness as a surrogate marker. Indian J Med Res 2014;140:379-86.
12. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972, 18;499-502.
13. Castelli W.P, Abbott R.D., McNamara P.M., Summary estimates of cholesterol used to predict coronary heart disease. Circulation 1983; 67(4): 730-4.
14. National Cholesterol Education Program (NCEP) ExpertPanel on Detection,Evaluation, and Treatment of HighBlood Cholesterol in Adults (Adult Treatment Panel III).Third Report of the National Cholesterol EducationProgram (NCEP)Expert Panel on Detection, Evaluation,and Treatment of High Blood Cholesterol in Adults(Adult Treatment Panel III) final report. Circulation 2002; 106:3143–421.
15. Maheshwar Pauriah, Allan D. Struthers, Chim C. Lang. Biomarkers and Surrogate Endpoints in Cardiovascular Therapeutics Research: Under Scrutiny Following Results of the ENHANCE Study. Cardiovascular Therapeutics 2008; 26: 85–8.
16. UI Nwagha, EJ Ikekpeazu, FE Ejezie, EE Neboh, IC Maduka. Atherogenic index of plasma as useful predictor of cardiovascular risk among postmenopausal women in Enugu, Nigeria. Afr Health Sci. 2010; 10(3): 248–52.
17. Roumeliotis A, Roumeliotis S, Panagoutsos S, Theodoridis M, Argyriou C, Tavridou A, et al. Carotid intima-media thickness is an independent predictor of all-cause mortality and cardiovascular morbidity in patients with diabetes mellitus type 2 and chronic kidney disease. Ren Fail. 2019;41(1):131-8.
18. Meng-Yu Wu , Chia-Jung Li, Ming-FengHou, Pei-Yi Chu. New Insights into the Role of Inflammation in the Pathogenesis of Atherosclerosis. Int J MolSci 2017; 18:2034-6.
19. Rampersaud E, Bielak LF, Parsa A, Shen H, Post W, Ryan KA, et al. The association of coronary artery calcification and carotid artery intima-media thickness with distinct, traditional coronary artery disease risk factors in asymptomatic adults. Am J Epidemiol. 2008;168(9):1016-23.
20. Greenland P, LaBree L, Azen SP, Doherty TM, Detrano RC. Coronary artery calcium score combined with Framingham score for risk prediction in asymptomatic individuals Coronary artery calcium score combined with Framingham score for risk prediction in asymptomatic individuals. JAMA. 2004;291(2): 210–5.
21. P C Keelan, L F Bielak, K Ashai, L S Jamjoum, A E Denktas, J A Rumberger et al. Long-term prognostic value of coronary calcification detected by electron-beam computed tomography in patients undergoing coronary angiography. Circulation. 2001;104(4):412–7.
22. Mosepele M, Hemphill LC, Palai T, Nkele I, Bennett K, Lockman S, et al. Cardiovascular disease risk prediction by the American College of Cardiology (ACC)/American Heart Association (AHA) Atherosclerotic Cardiovascular Disease (ASCVD) risk score among HIV-infected patients in sub-Saharan Africa. 2017; PLoS ONE 12(2): e0172897. doi:10.1371/journal. pone.0172897.
23. Hoogeveen RC, Gaubatz JW, Sun W, Dodge RC, Crosby JR, Jiang J, et al. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk In Communities (ARIC) study. Arterioscler Thromb Vasc Biol. 2014:34(5):1069-77.
24. Maureen McMahon, Jennifer Grossman, John FitzGerald, Erika Dahlin-Lee, Daniel J. Wallace, Bernard Y, et al. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. Arthritis & Rheumatism. 2006;54(8):2541–9.
25. Popa C, van Tits LJH, Barrera P, et al. Anti-inflammatory therapy with tumour necrosis factor alpha inhibitors improves high-density lipoprotein cholesterol antioxidative capacity in rheumatoid arthritis patients. Annals of the Rheumatic Diseases. 2009;68(6):868–872.
26. Valerio Chiurchiù, Alessandro Leuti, Mauro Maccarrone. Bioactive Lipids and Chronic Inflammation: Managing the Fire Within. Front. Immunol. 2018; 9. https://doi.org/10.3389/fimmu.2018.00038.

**Table 1: Clinical, biochemical characteristics and anthropometric indices among the study groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Group 1**  (Controls)  (CIMT <0.57mm) n=(60) | **Group 2**  T2DM without subclinical atherosclerosis (CIMT <0.57mm) n=(60) | **Group 3**  T2DM with subclinical atherosclerosis  (CIMT > 0.57mm ) n=(60) | **p value** |
| Age (years) | 48.5  (39.2-52.75) | 45.0  (41.0-48.75) | 50.0  (44.0-52.0) | 0.025\* |
| WC(cm) | 81.7±7.3 | 86.6±9.7 | 87.4±10.4 | 0.002\* |
| HC (cm) | 92.16±6.07 | 95.7±10.39 | 96.5±10.29 | 0.024\* |
| WHR | 0.90  (0.73-1.04) | 0.91  (0.77-0.99) | 0.91  (0.81-0.99) | 0.081 |
| BMI(kg/m2) | 26.65  (24.97-28.44) | 28.17  (25.70-30.46) | 27.83  (25.59-30.45) | 0.046 |
| FPG (mg/dL) | 89 ± 9.62 | 130 ± 26.8 | 126±24.2 | <0.001\* |
| Urea (mg/dL) | 21.0 (17.25-27.0) | 23.50 (19.25-29.0) | 24.0(20.0-28.0) | 0.08 |
| Creatinine (mg/dL) | 0.71±0.12 | 0.66 ±0.17 | 0.72±0.17 | 0.149 |

\*p value- Statistically significant, WC-Waist circumference, HC-Hip circumference, WHR-waist hip ratio, BMI- Body mass index, FPG- fasting plasma glucose

**Table 2: Lipids, lipid ratios and atherogenic indexs among the study groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Parameter** | **Group1**  (Controls)  (CIMT <0.57mm) n=(60) | **Group2**  T2DM without subclinical atherosclerosis (CIMT <0.57mm) n=(60) | **Group3**  T2DM with subclinical atherosclerosis  (CIMT > 0.57mm ) n=(60) | **p** |
| Total cholesterol (mg/dL) | 184.5(155.2-200.0) | 166.0(139.25-200.75) | 175.0(141.0-200.0) | 0.514 |
| TGL (mg/dL) | 101.0(86.2-126.7) | 164.0(116.7-228.0) | 165.0(118.5-200.75) | <0.001\* |
| VLDL (mg/dL) | 20 (12-40) | 30 (15-78) | 33(16-79) | <0.001\* |
| HDL (mg/dL) | 52.0(48.0-59.75) | 42.0(38.0-48.0) | 40.50(37.25-45.50) | <0.001\* |
| LDL (mg/dL) | 102.0±27.03 | 91.7±40.30 | 94.9±32.25 | 0.232 |
| Non-HDL(mg/dL) | 124.0±27.3 | 127.7±39.1 | 130.0±40.3 | 0.714 |
| AI | 0.30±0.13 | 0.59±0.18 | 0.58±0.19 | <0.001\* |
| CRI-1 | 3.4±0.74 | 4.1±1.23 | 4.22±1.24 | <0.001\* |
| CRI-II | 1.99±0.70 | 2.24±1.16 | 2.37±0.96 | 0.94 |
| AC | 2.41±0.7 | 3.10±1.23 | 3.29±1.24 | <0.001\* |

\*p value- Statistically significant, TGL-triglycerides,VLDL- Very low density lipoprotein, HDL- High density lipoprotein, LDL- Low density lipoprotein, AI- atherogenic index, CRI-1 and CRI-II- Castelli Risk Index I and II, AC-Atherogenic coefficient

**Table 3: Changes in anthropometric indices, lipids, lipid ratios and atherogenic index between the study groups**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Group1vs Group 2**  p value | **Group 1 vs Group 3**  p value | **Group 2 vs Group 3**  p value |
| WC | 0.014\* | 0.003\* | 0.964 |
| HC | 0.107 | 0.031\* | 0.965 |
| WHR | 0.139 | 0.178 | 0.191 |
| BMI | 0.97 | 0.90 | 1.000 |
| Total cholesterol | 0.607 | 0.675 | 1.000 |
| TGL | <0.001\* | <0.001\* | 1.000 |
| VLDL | <0.001\* | <0.001\* | 1.000 |
| HDL | <0.001\* | <0.001\* | 0.380 |
| LDL | 0.288 | 0.738 | 1.000 |
| Non-HDL | 0.911 | 0.786 | 0.994 |
| AI | <0.001\* | <0.001\* | 1.000 |
| CRI-1 | 0.002\* | <0.001\* | 1.000 |
| CRI-II | 0.447 | 0.099 | 1.000 |
| AC | 0.002\* | <0.001\* | 1.000 |

\*p value- Statistically significant

**Table 4: Correlation among lipids, lipid ratios and atherogenic index**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | TC | TGL | HDL | VLDL | LDL | AI | CRI-I | CRI-II | AC |
| TChol | r | 1 | .284 | -.131 | .283 | .925 | .263 | .775 | .802 | .775 |
|  | p | . | .000\* | .080 | .000\* | .000\* | .000\* | .000\* | .000\* | .000\* |
| TGL | r | .284 | 1.000 | -.326 | .999 | .022 | .938 | .374 | .127 | .374 |
|  | p | .000\* | . | .000\* | .000\* | .765 | .000\* | .000\* | .090 | .000\* |
| HDL | r | -.131 | -.326 | 1.000 | -.330 | -.259 | -.612 | -.684 | -.578 | -.684 |
|  | p | .080 | .000\* | . | .000\* | .000\* | .000\* | .000\* | .000\* | .000\* |
| VLDL | r | .283 | .999 | -.330 | 1.000 | .022 | .940 | .375 | .128 | .375 |
|  | p | .000\* | .000\* | .000\* | . | .774 | .000\* | .000\* | .088 | .000\* |
| LDL | r | .925 | .022 | -.259 | .022 | 1.000 | .093 | .822 | .920 | .822 |
|  | p | .000\* | .765 | .000\* | .774 | . | .214 | .000\* | .000\* | .000\* |
| AI | r | .263 | .938 | -.612 | .940 | .093 | 1.000 | .545 | .297 | .545 |
|  | p | .000\* | .000\* | .000\* | .000\* | .214 | . | .000\* | .000\* | .000\* |
| CRI-I | r | .775 | .374 | -.684 | .375 | .822 | .545 | 1.000 | .951 | 1.000 |
|  | p | .000\* | .000\* | .000\* | .000\* | .000\* | .000\* | . | .000\* | 0.000\* |
| CRI-II | r | .802 | .127 | -.578 | .128 | .920 | .297\*\* | .951 | 1.000 | .951 |
|  | p | .000\* | .090 | .000\* | .088 | .000\* | .000 | .000\* | . | .000\* |
| AC | r | .775 | .374 | -.684 | .375 | .822 | .545 | 1.000 | .951 | 1.000 |
|  | p | .000\* | .000\* | .000\* | .000\* | .000\* | .000\* | .000\* | .000\* | . |

\*p value- statistically significant

Table 5: C**orrelation of CIMT with anthropometric indices, lipids, lipid ratios and atherogenic index**

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameter** | **Parameter** | **r value** | **p value\*** |
| CIMT | WC | 0.230 | 0.002 |
| HC | 0.188 | 0.012 |
| BMI | 0.173 | 0.020 |
| TGL | 0.221 | 0.003 |
| VLDL | 0.223 | 0.003 |
| HDL | -0.413 | <0.001 |
| AI | 0.333 | <0.001 |
| CRI-1 | 0.177 | 0.007 |
| AC | 0.200\*\* | 0.007 |

r - correlation coefficient, \*p value- statistically significant

**Table:6 Receiver operating characteristics curve analysis for diagnostic utility of lipid ratios and atherogenic index in subclinical atherosclerosis**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Parameter | AUC | Cut off value | Sensitivity  (%) | Specificity  (%) | p value | 95% CI |
| AI | 0.887 | 0.38 | 85.0 | 80.0 | <0.001\* | 0.839-0.935 |
| CRI-1 | 0.675 | 3.58 | 61 | 60 | <0.001\* | 0.597-0.753 |
| CRI-II | 0.580 | 1.97 | 60 | 51 | 0.080 | 0.497-0.663 |
| AC | 0.675 | 2.58 | 62 | 60 | <0.001\* | 0.597-0.753 |

\*p value- Statistically significant, AUC-area under the curve, CI- confidence interval