

# LIPID RATIOS AND ATHEROGENIC INDEX AS SURROGATE BIOMARKERS OF SUBCLINICAL ATHEROSCLEROSIS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

## Abstract

**Background:** Type 2 diabetes mellitus (T2DM) increases the risk for cardiovascular disease (CVD). Considering the cost factor for some important biomarkers for atherosclerosis and imaging techniques, it is proposed that 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 atherogenic index (AI), Castelli risk indices (CRI-I & II), and atherogenic coefficient (AC) were evaluated as potential indicators of subclinical atherosclerosis and their association with anthropometric measurements were assessed.

**Methods:** Subjects were categorized in groups using the measurement of carotid intima media thickness (CIMT) assessed by carotid Doppler ultra sonography, as non diabetic controls with CIMT less than 0.57 mm (n=60; Group 1), T2DM subjects with CIMT less than 0.57 mm (n=60; Group 2), CIMT greater than or equal to 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 were found to have significant positive correlation with CIMT. HDL was found to have significant negative correlation with CIMT. The diagnostic performance of AI in predicting

## Authors

### Dr. M. M. Suchitra

Department of Biochemistry  
Sri Venkateswara Institute of Medical  
Sciences, Tirupati  
Andhra Pradesh, India.  
mmsuchitra73@gmail.com

### T. Sasikala

Department of Biochemistry  
Government Medical College, Eluru  
Andhra Pradesh, India.

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subclinical atherosclerosis was significant with higher sensitivity (85%) and specificity (80.0%) at a cut off value of 0.38.

**Conclusion:** In subjects with T2DM having CIMT of greater than or equal to 0.57 mm, the AI cut off value of greater than or equal to 0.38 was found to strongly indicate the presence of subclinical atherosclerosis. Hence AI can serve as a surrogate biomarker of subclinical atherosclerosis.

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

## I. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is considered as one of the most dreaded non-communicable disease with metabolic changes encompassing chronic hyperglycemia and dyslipidemia which in turn are associated with risk of morbidity and mortality due to cardiovascular disease (CVD). Presence of diabetes doubles or quadruples the risk of cardiovascular mortality compared to in those without diabetes. A mortality rate of about 70% due to CVD is reported 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 presenting with a lowered high-density lipoprotein cholesterol (HDL) levels, with elevated total cholesterol (TC) levels, low-density lipoprotein cholesterol (LDL), and triglycerides (TGL) levels is known to lead to the progression of atherosclerosis (1). It was found that compared with measurement of individual lipid parameters, the lipid ratios, which include non-HDL cholesterol, atherogenic Index (AI), Castelli Risk Index (CRI) and atherogenic coefficient (AC) are reported to predict risk of occurrence of coronary artery disease (CAD) in hypertensive subjects, subjects with diabetes or in the presence of dyslipidemia (2,3). Hence it was proposed to include these lipid ratios in the equations used in the assessment of CVD risk to improve the therapeutic decision-making (3,4). AI is considered to reflect the shift in equation between the atherogenic and anti-atherogenic factors and has been shown to strongly predict the risk of CAD (5). A positive association of AI with waist circumference (WC), body mass index (BMI) and negative association with physical activity has been reported (6).

In a prospective cohort study, patients with acute myocardial infarction (AMI), were categorized into two groups based on an AI cut off value, as those with AI less than 0.24 (low AI) and those with AI greater than or equal to 0.24 (high AI). The relationship between AI and major adverse cardiovascular events during intensive hospitalization in patients was studied. It was found that a subjects with 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). As acute phase of MI alters lipid levels, it has been suggested that in patients with myocardial infarction the lipid profile should be measured within 24 hours of the acute episode.

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 which include vascular echography, assessment of coronary calcium score, the study of the structural changes in the blood vessel wall, detecting an increase in the intima-media thickness are able to detect presence of early atherosclerotic changes. Changes in carotid intima media thickness (CIMT) is considered as an indicator of progressive atherosclerosis (8). A study found that in the cardiovascular risk stratification in 13145 individuals carried out by applying the CIMT measure along with the traditional Framingham Risk Score which utilizes the presence of identifiable risk factors and the levels of some biochemical markers reportedly reclassified 23% of all subjects and also 13.5% of those initially considered to be at intermediate risk as those 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 in identifying risk of CVD and could serve as more sensitive risk predictors (3). A strong correlation was reported between the lipid ratio, 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 contribute significantly to the assessment of CAD risk and have hence been recommended to be considered in addition to routine lipid parameters. Hence the present work was taken up to assess the association of lipid ratios and AI with CIMT and to evaluate the performance of lipid ratios and AI in predicting subclinical atherosclerosis.

## II. MATERIAL AND METHODS

T2DM patients attending the Endocrinology outpatient clinic of Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati were screened with a questionnaire. From a total of 1545 subjects, 60 subjects were included into the study, who fulfilled the inclusion criteria, with duration of diabetes of not more than five years and not less than one year, with ages ranging between 30-60 years, with no history of CVD, CKD, thyroid diseases and chronic inflammatory disease. Patients with T2DM on treatment with oral hypoglycemic agents were taken into the study. Exclusion criteria were subjects on insulin therapy, pregnant females, subjects with other forms of diabetes other than T2DM, patients on vitamin-supplementation and those who refused to give written informed consent. From the 68 control subjects screened, 60 subjects without T2DM who fulfilled the exclusion criteria were included. All the subjects were age and gender matched. A written informed consent was obtained from all the study subjects. Anthropometric measurements such as height and weight were recorded and body mass index (BMI) was calculated as weight measured in kilograms divided by square of the height measured in meters, waist circumference (WC) was measured at the level of the navel and hip circumference (HC) was measured at the level of the buttocks. All the subjects underwent carotid Doppler ultrasonography for measurement of carotid intima media thickness (CIMT). The present research work was approved by the Institutional Ethics committee.

## III. CAROTID DOPPLER FOR MEASUREMENT OF CIMT

CIMT measurement was carried out by Sonoline G40 Diagnostic ultrasound system (Siemens Medical Solutions USA Inc., USA) by a single radiologist who was blinded to the clinical and biochemical information of the study participants. Bilateral CIMT measurements were made 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.

Based on CIMT measurement(11), the study subjects were grouped as:

- **Group 1:** Healthy controls with CIMT less than 0.57 mm: n=60
- **Group 2:** Patients with T2DM having CIMT less than 0.57 mm (without subclinical atherosclerosis):n=60

- **Group 3:** Patients with T2DM having CIMT greater than or equal to 0.57 mm (with subclinical atherosclerosis) :n=60

- 1. Sample collection:** Subjects after an overnight fast of 8-10 hours were made to sit comfortably at the blood collection centre and were explained the phlebotomy procedure. About four ml of blood sample was drawn from the medial cubital vein, two ml of blood was transferred into additive free tube and two ml of blood was transferred into tube containing sodium fluoride and potassium oxalate anticoagulant. The additive free tubes was made to stand for 30 minutes to allow clotting to take place and then centrifuged at 2000 rotation per minute for 15 minutes to obtain serum. Anticoagulant containing tubes were centrifuged immediately to obtain plasma for glucose estimation which was done immediately.

Plasma glucose concentration was measured by glucose oxidase peroxidase method (Pathozyme Dianostics, Kagal Dist, Kolhapur, India), serum urea by urease method (Crest Biosystem, a division of Coral clinical systems, Goa, India), serum creatinine by Jaffe's rate method (Beckman system pack), serum total cholesterol by cholesterol oxidase peroxidase method, serum triglycerides by enzymatic colorimetric method (Agappe diagnostics Ltd, Ernakulum, Kerala, India), serum HDL by selective inhibition method (Beckman system pack). All the parameters were analyzed by using Synchron Unicel Dx C600 auto analyzer (Beckman coulter, USA).

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

$$\text{VLD} = \text{TG}/5$$

$$\text{LDL} = \text{TC} - (\text{VLDL} + \text{HDL})$$

$$\text{Non HDLc} = \text{TC} - \text{HDL}$$

#### **The Lipid ratios were calculated as:**

$$\text{Atherogenic Index (AI)} = \log (\text{TGL}/\text{HDL}) \quad (5)$$

$$\text{Castelli's Risk Index (CRI-I)} = \text{TC}/\text{HDL} \quad (13)$$

$$\text{Castelli's Risk Index (CRI-II)} = \text{LDL}/\text{HDL} \quad (13)$$

$$\text{Atherogenic Coefficient (AC)} = (\text{TC} - \text{HDL})/\text{HDL} \quad (14)$$

- 2. Statistical Analysis:** The data distribution was analyzed using Kolmogrov Smirnov test. Data obtained was expressed as mean  $\pm$  standard deviation (SD) for data with normal distribution, and median inter quartile range for data not having normal distribution. The comparisons of means of parameters across the three groups were tested using analysis of variance (ANOVA) and post hoc for pair wise comparisons for data with normal distribution or Kruskal Wallis test for data that was not having normal distribution. Differences in means between the groups were tested using unpaired two tailed t test. The correlations among the parameters with data having normal distribution was done by using Pearson's correlation. For data not having normal distribution, Spearman rank correlation analysis was done. Receiver operating characteristic (ROC) curve analysis was performed to study the diagnostic utility of lipid ratios and AI. A 'p' value of  $< 0.05$  was considered as statistically significant. Statistical analysis were performed using Microsoft Excel spread sheets (Microsoft Redmond USA) and IBM, SPSS statistics (version 22.0).

#### IV. RESULTS

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

**Table 2** shows the lipid profile, lipid ratios and AI among the study groups. Significant elevated levels of TGL and VLDL 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 anthropometric changes such as higher WC, significant biochemical changes such as elevated levels of TGL, VLDL with lowered HDL levels, significant changes in lipid ratios such as elevated AI, CRI-I and AC when compared to controls. The anthropometric measurement, HC was found to be significantly higher only in T2DM subjects with subclinical atherosclerosis when compared to controls.

**Table 4** shows the correlation among lipids, lipid ratios and AI. Significant positive correlations were found between Total cholesterol with TGL, VLDL and LDL and between TGL with VLDL. A significant negative correlation between HDL with TGL, VLDL and LDL were found. Association of lipids with lipid ratios and AI found significant positive correlations between total cholesterol, TGL and VLDL with AI, CRI-I and AC, between LDL with CRI-I, CRI-II and AC. A significant negative correlation between HDL with AI, CRI-I, CRI-II and AC was observed.

**Table 5** shows the 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 CIMT was observed.

**Table 6** shows the diagnostic utility of lipid ratios and AI in subclinical atherosclerosis. AIP, CRI-I 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 the diagnosis of subclinical atherosclerosis in T2DM at a CIMT cut off of greater than or equal to 0.57 mm.

**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 $\geq$ 0.57mm ) n=(60)	p value
Age (years)	48.5	45.0	50.0	0.025*

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	(39.2-52.75)	(41.0-48.75)	(44.0-52.0)	
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/m <sup>2</sup> )	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 index 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-I	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-I and CRI-II- Castelli Risk Index I and II, AC-Atherogenic coefficient

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

Parameter	Group 1 vs 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-I	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: Correlation 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

## V. DISCUSSION

The use of biomarkers as surrogate endpoints has gained importance especially in the approach to management and treatment of CVD as it is associated with high mortality and morbidity. The requirement of a biomarker to be considered as a surrogate biomarker is based on the ease of measurement, the association of the surrogate biomarker with the clinical endpoint and the ability of the surrogate biomarker to be able to produce an estimation of the risk and its utility 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 CAD risk. When the anthropometric measurements were compared between subjects with T2DM and controls, higher WC was observed in T2DM without subclinical atherosclerosis compared to controls and in T2DM subjects with subclinical atherosclerosis, higher WC and HC compared to controls was observed (Table 3). With regard to lipid profile both groups of T2DM were found to have significant higher TGL and VLDL levels with lower HDL levels compared to controls. The AI and lipid ratios, CRI-I, CRI-II and AC were significantly higher in both groups of T2DM compared to controls. Recently, the AI was found to be a

indicator for atherosclerotic changes in CVD and hence could be considered as a predictor for myocardial infarction (16). The AI value is said to correlate with the levels of small, dense LDL levels and the lecithin cholesterol acyl transferase activity, an indicator of cholesterol esterification. The factors affecting AI values are an increased TG and/or reduced HDL levels which cause an increase AI values. It is reported that both hypertriglyceridemia and lowered HDL levels are associated with a high cardiac risk. The atheroprotective effect of HDL is attributed to its property of reverse cholesterol transport (16). However between the T2DM groups no significant change in lipid profile and AI or lipid ratios was observed. In the present study the interaction between the lipid fractions and between the lipid fractions with AI and lipid ratios found a significant positive correlation of TGL with cholesterol, VLDL, AI, CRI-I and AC and a negative correlation of TGL with HDL levels. Similarly lowered HDL levels were negatively associated with TGL, VLDL, LDL, AI, CRI-I, CRI-II and AC, establishing the fact that hyper triglyceridemia along with lowering of HDL levels is associated with atherogenic changes. Compared to TGL, HDL was negatively associated with all the lipid ratios and AI indicating the importance of a lowered HDL levels as a vital forerunner for the propagation of atherogenesis. Cholesterol was found to be positively associated with the TGL, VLDL, and LDL and AI and all the lipid ratios. The calculated lipid fractions, VLDL was found to have a positive correlation with AI, CRI-I and AC similar to TGL and LDL was found to have significant positive correlation with CRI-I, CRI-II and AC. All the lipid ratios and AI were found to have significant positive correlation among themselves.

The CIMT measurement has been widely used for the detection of subclinical atherosclerosis and changes in CIMT is considered as a powerful predictor of cardiac risk due to atherosclerotic heart diseases (17). The objective of the present study was to explore the relationship between lipid ratios and AI with subclinical atherosclerosis in patients with type 2 diabetes mellitus in order to find promising alternate biomarkers of subclinical atherosclerosis. In line with these objectives, the changes in anthropometric measurements, WC, HC, BMI were found to be associated with changes in CIMT. Though BMI was not significantly elevated in T2DM groups compared to controls, the subtle changes in BMI was found to have a significant positive correlation with CIMT. As CIMT is presently considered to indicate presence of subclinical atherosclerosis, the changes in CIMT in the present study were found to be significantly associated with the elevated TGL, VLDL and lowered HDL levels. When the changes in CIMT were analyzed with relation with the AI and lipid ratios, CIMT was found to be significantly associated with AI, CRI-I and AC. It is reported that the early indication of atherosclerosis visualized as an increase in CIMT is due to increased expression of adhesion molecules which is in turn promoted by high TGL levels. 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, triglyceride and BMI. 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 an all-cause cardiovascular mortality (17). It was known that cardiovascular risk increases with high serum cholesterol and LDL-c levels and that small-dense LDL particles are strong risk factor for atherogenesis. A strong correlation was reported between high TG and low HDL cholesterol levels and small-dense LDL-c levels. The most important factor regarding development and progression of CAD was found as an increase in small-dense LDL particles. In fact, it was found that LDL cholesterol was at the normal level in more than half of CAD cases. This is explained by the presence of excessive small dense LDL content. The primary stimulus in the formation of the small dense LDL is elevated plasma levels of triglycerides (23). Similarly 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 of such 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 the protective and atherogenic lipoproteins (5). Therefore, one can hypothesize that AI may be a better tool to assess the relative contribution of lipids to the CVD risk than individual lipid 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-I 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 can be introduced as a tool in the CAD risk assessment in patients with type 2 diabetes mellitus.

## VI. 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 greater than or equal to 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.

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