Research Article

Lipid ratios, atherogenic coefficient and atherogenic index of plasma as parameters in assessing cardiovascular risk in type 2 diabetes mellitus

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ABSTRACT

Background: Cardiovascular disease (CVD) is responsible for morbidity and mortality in type 2 diabetes mellitus (T2DM) patients. Diabetes alters the utilization of lipids and lipoproteins which lead to diabetes induced atherogenic dyslipidemia, one of the most important risk factor for the development of atherosclerosis. The relationship between elevation of serum lipids and vascular complications of diabetes has long been of interest. The use of LDL-c alone for assessment of cardiovascular risk would ignore the TG-rich lipoproteins. Lipid ratios, atherogenic coefficient and atherogenic index of plasma have been found to indicate an atherogenic risk and are better predictors of cardiovascular risk than lipids alone. Hence the present study is taken up to evaluate the lipid ratios, atherogenic coefficient, atherogenic index of plasma in assessing the CV risk in type 2 diabetes mellitus.
Methods: This case-control prospective study included three groups. (Group 1: control, group II: T2DM without complications, group III: T2DM with complications, n=25). Total cholesterol, triglycerides and HDL-c were analysed using commercially available kits on spectrophotometer. Nitric oxide was estimated spectrophotometrically by Griess method. VLDL, LDL, Lipid ratios, non-HDL cholesterol, AC and AIP were calculated in all the three groups. Statistical analysis was performed using SPSS version 22.0.
Results: All of the atherogenic indices were found to be significantly different upon comparing these indices in both patients and control groups.
Conclusions: The ratios contribute significantly to the estimation of CVD risk in type 2 diabetes mellitus especially, when the absolute values of lipid profile seem normal or not markedly deranged or in centres with insufficient resources.

Keywords: Atherogenic dyslipidemia, Lipid ratios, Atherogenic index of plasma, Atherogenic coefficient, Cardiovascular disease

INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition characterized by high blood glucose levels and further progress to microvascular and macrovascular complications that considerably increase the morbidity and mortality. Atherosclerotic coronary artery disease (CAD) and other forms of cardiovascular disease (CVD) are the major causes of morbidity and mortality in type II DM (T2DM). Alterations in lipid and lipoprotein profile

contribute to atherosclerosis in type 2 diabetes.[1] Although the major focus on the connection between lipids and coronary heart disease (CHD) is on LDL-cholesterol (LDL-c), the adult treatment panel III has recognized the important roles of HDL-C and TGs, calling this combination an atherogenic dyslipidemia.[2] Dyslipidaemia in type 2 diabetes and hypertension are both quantitative and qualitative.[3] Quantitative abnormalities include increased levels of total plasma cholesterol, triglyceride and low density lipoprotein cholesterol, and decreased level of high-density lipoprotein (HDL-c) cholesterol. Qualitative abnormalities include changes in the composition of LDL-cholesterol (small dense LDL-cholesterol, increased triglyceride content and increased electronegativity of LDL-cholesterol). These changes make LDL-cholesterol susceptible to oxidation and glycation, with foam cell formation, endothelial dysfunction and atherosclerosis.[4,5] Vascular endothelial dysfunction in Type II DM plays a pivotal role in the development and progression of subclinical atherosclerosis. Prostacyclin and nitric oxide (NO), produced by normal endothelium, inhibit platelet activation and relax vascular smooth muscle, promoting normal blood flow. People with DM have a reduced release of prostacyclin and NO with chronic impairment of endothelial NO synthase activity, which is a predictor of dyslipidemia and partly explains the accelerated atherosclerosis in DM.[6] Several mechanisms may account for the atherogenic lipid abnormalities in diabetic patients. Dysfunctional adipose tissue is less sensitive to insulin and has reduced hormone-sensitive lipase activity compared with normal adipose tissue. As a result, there is an increased breakdown of intracellular TG and increased release of FFAs into the circulation, leading to fatty Infiltration in the liver, muscles and pancreatic β-cells leading to predisposition to Type II DM. Increased hepatic FFAs contribute to increased hepatic TG synthesis in turn resulting in elevated concentrations of very low density lipoproteins (VLDL) particles. Various lipases contribute to remodeling of VLDL to small, dense LDL particles. In addition, cholesterol ester transfer protein (CETP) exchanges TG from VLDL to cholesterol found in HDL and LDL, leading to cholesterol-rich atherogenic VLDL particles. HDL particles that undergo these modifications are cleared more readily by the kidney, resulting in lower HDL-C levels.[7] Studies revealed that rather than the cholesterol concentration in different lipoproteins, the size and composition are found to be important in atherogenesis. Lipid ratios have also been found to indicate an atherogenic risk and are said to better predictors of coronary artery diseases than lipids alone. Hence lipid parameters can be combined into ratios that reflect the proportion of atherogenic to anti atherogenic lipids and lipoproteins. Proposed lipid ratios for CV risk assessment include TC/HDL-c (total cholesterol/high-density lipoprotein cholesterol), TG/HDL-c (triglycerides/high density lipoprotein cholesterol), LDL-c/HDL-c (low density lipoprotein cholesterol/high density lipoprotein cholesterol). The amount of LDL-c inside the lipoprotein particle varies in individuals, hence its measurement does not reflect the number of particles and therefore the true level of cardiovascular risk. A more precise way to determine risk would be to measure the number of atherogenic lipoprotein particles in the serum that is apolipoprotein-B. Non-HDL Cholesterol (TC- HDL-c) is indicator of atherogenic apolipoprotein B containing lipoproteins such as LDL, VLDL (very low density lipoproteins and intermediate density lipoproteins (IDL's) .Many reports confirm that Non-HDL Cholesterol (TC-HDL-c) is a better predictor of CVD risk than a simple measure of LDL cholesterol.[8] Atherogenic coefficient (AC) calculated as measure of cholesterol in LDL, VLDL, IDL'S fractions with respect to good cholesterol or HDL-c. Atherogenic coefficient (AC) is ratio calculated as (TC- HDL-c)/HDL-c) relying on the significance of HDL-c in predicting the risk of CAD.[9] Among the lipoprotein subclasses, disproportionate amounts of small, dense LDL particles and small HDL particles constitute atherogenic profile due to a high susceptibility to oxidation. However, as the sub fractionation of lipoproteins cannot be undertaken in all the clinical laboratories, the Atherogenic Index of Plasma (AIP) has been shown to correlate with the size and composition of lipoproteins.[10] The atherogenic index of plasma (AIP), defined as logarithm log of the ratio of plasma concentration of triglycerides to high-density lipoprotein cholesterol, has recently been proposed as a predictive marker for plasma atherogenicity and is positively correlated with cardiovascular disease risk.[11] Also, the ratio log (TG/HDL), correlates well with the size of HDL and LDL particles and with the fractional esterification rate of cholesterol by lecithin: Cholesterol acyl transferase in plasma.[12] Hence the present study is taken to evaluate lipid ratios, Atherogenic coefficient and Atherogenic index of plasma in assessing Cardiovascular risk in type 2 DM. These are the calculated fractions which can be used in the clinical setting for assessing the risk of cardiovascular disease beyond the routinely done lipid profile.

METHODS

This was a case control study done on 75 individuals of same age(35-70 years) and sex matched and were categorised into three groups (n=25). Among that 25 age and sex matched non-diabetic subjects were recruited as healthy controls (group I). Cases were divided as Group II (T2DM) and group III (T2DM with complications). The diagnosis of diabetes mellitus was based on World Health Organization (WHO) criteria, i.e. Fasting blood glucose (FBG) ≥126 mg/dL and 2-hour post- glucose blood sugar (PGBS) levels of ≥200 mg/dL after 75 g oral glucose load. The complications were based on Investigations as ECG, Fundus examination, microalbiminurea. Patients with history of hypertension, bronchial asthma, Cigarette smoking, alcoholism, active infection and malignancy were excluded from the study. A written informed consent and a detailed clinical

examination were conducted in all the participants prior to blood collection.

Sample collection

5ml of overnight fasting venous blood samples was collected from all the subjects. From that 2ml of blood was transferred into fluoride containing tubes and remaining blood transferred in plain bottle. The serum was separated after centrifuging at 2000rpm for 10-15minutes and stored at -800c until further analysis.

Laboratory analysis

The following parameters were analysed; FBS, post prandial blood sugar (PPBS), Serum total cholesterol and triglyceride were determined by enzymatic estimation while high density lipoprotein cholesterol was determined by enzymatic estimation after precipitation using commercially available kits on spectrophotometer.[13-15]

Low density lipoprotein cholesterol and very low density lipoprotein were determined using the Friedewald's formula as follows: VLDL = TG ÷ 5, LDL-c = (TC) − (VLDL +HDL).[16] Serum Nitric oxide was estimated by Griess method.[17]

Lipid ratios were calculated as TC/HDL-c, TG/HDL-c, LDL-c /HDL-c. Atherogenic Coefficient (AC) was calculated as (TC- HDL-c)/HDL-c).[9] Atherogenic index of plasma was calculated by using base 10 logarithm of the ratio TG to HDL, formula = log (TG/HDL-C).[12]

Statistical analysis

Data distribution was tested using Kolmogorov-Smirnov test. Data was expressed as mean and standard deviation (SD) for normally distributed data. Statistical comparisons of the means between the three groups were made using one way analysis of variance (ANOVA) with post hoc analysis within the groups. Pearson correlation analysis was performed to study the association between parameters. Multivariate regression analysis was performed to see the association between the dependent and independent variables. Receiver operator characteristic (ROC) curve was constructed to study the diagnostic utility of the variables in predicting CVD risk. A p value of less than 0.05 was considered statistically significant. Statistical analysis was performed using statistical software, SPSS version 22.0.

RESULTS

Mean and SD of biochemical parameters between the patients and control groups were shown in Table 1. Table 2 represents the comparison of means within the patient and control groups. One way ANOVA revealed a significant elevation in total cholesterol, TG, LDL-c, VLDL levels in group II (T2DM) and group III (diabetic complications) patients when compared to controls(p =0.001). A significant decrease in HDL-c and nitric oxide levels were found in group II and group III patients when compared to controls(p=0.001), whereas post Hoc analysis showed no significant change in the TC and LDL-c in the group II (T2DM) when compared to controls. (p=0.401, p=0.096 respectively). Among various atherogenic lipid risk factors, TC/HDL-c, TG/HDL c, LDL-c/HDL-c, AC and AIP (P < 0.01) were found to be significantly elevated in diabetic patients compared to controls.
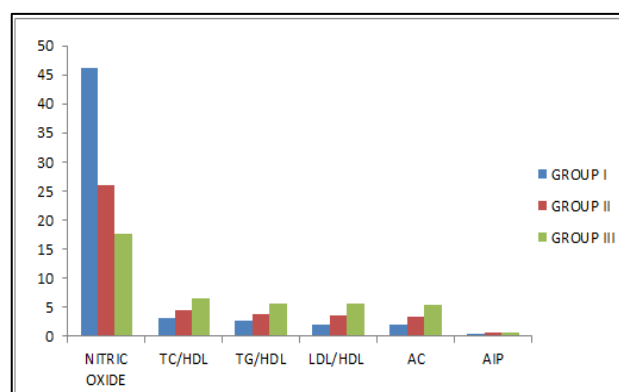

Figure 1: Comparison of biochemical atherogenic indices among cases and control.
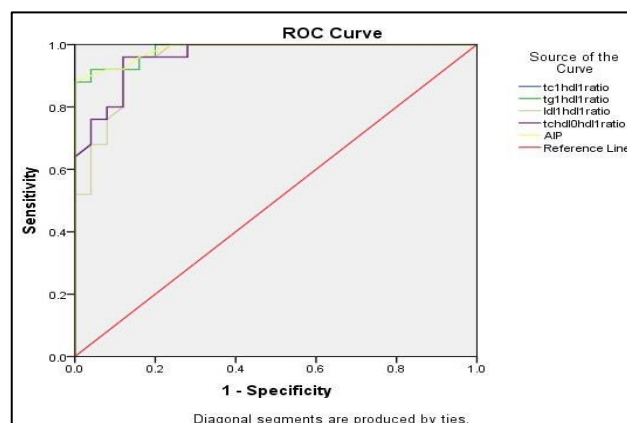

Figure 2: ROC curve analysis among atherogenic indices.

The bivariate Pearson correlations between Nitric oxide and lipid risk factors were shown in Table 3. The correlation analysis found a significant negative correlation of Nitric oxide with TC/HDL-c, TG/HDL c, LDL-c/HDL-c, AC and AIP when both the case groups were combined together, but found no significant association when nitric oxide was correlated with lipid indices independently in both the case groups.

Table 1: Mean and standard deviation for the biochemical parameters among controls (group I), t2dm (group II) and diabetic complications (group III) patients.

Parameter	Group I (n=25)	Group II (n=25)	Group III (n=25)
Fbs (mg/dl)	77±5.08[**]	142±6.69[**]	171.12±7.29[**]
Ppbs (mg/dl)	126.28±6.82	210±8.23[**]	246.3±8.14[**]
Tc (mg/dl)	171.08±20.23[†]	178.48±17.52[**]	221.32±13.25[**]
Hdl (mg/dl)	49.28±5.52[**]	40.64±4.07[**]	34.56±5.19[**]
Tgl (mg/dl)	127.28±12.86[**]	154.5±8.77[**]	189.04±9.72[**]
Vldl (mg/dl)	25.52±2.46[**]	29.76±2.75[**]	37.76±2.06[**]
Ldl (mg/dl)	96.28±21.58[†]	108.08±19.1[**]	149.0±16.13[**]
Serum no (µmol/l)	46.2±7.39[**]	26.04±3.76[**]	17.6±2.88[**]
Tc/hdl	3.51±0.60[**]	4.44±0.711[**]	6.57±1.26[**]
Tg/hdl	2.61±0.412[**]	3.84±0.46[**]	5.6±0.965[**]
Ldl/hdl	1.99±0.572[**]	2.7±0.661[**]	4.45±1.08[**]
Non – hdl cholesterol (tc-hdl)	121.8±21.02[**]	137.84±18.41[**]	186.76±16.41[**]
Ac(tc-hdl/hdl)	2.51±0.6[**]	3.44±0.711[**]	5.57±1.26[**]
AIP	0.412±0.06[**]	0.581±0.051[**]	0.742±0.073[**]

Group I- control group, Group II- T2DM, Group III - T2DM with complications, n= Sample size, FBS-fasting blood glucose, PPBS-post prandial blood glucose, TC-total cholesterol, HDL- High density Lipoprotein, LDL- Low density Lipoprotein, TG- triglycerides, VLDL-Very low density Lipoprotein, NO –Nitric oxide, AC-Atherogenic coefficient, AIP- Atherogenic index of plasma.**significant at the 0.01 probability level. NS; Non-significant at the 0.05 probability level.

Table 2: The Anova post- Hoc findings within in the study groups.

Parameter	Group I versus II	Group I versus III	Group II versus III
Fbs (mg/dl)	0.001	0.001	0.001
Ppbs (mg/dl)	0.001	0.001	0.001
Tc (mg/dl)	0.401[†]	0.001	0.001
Hdl (mg/dl)	0.001	0.001	0.001
Tgl (mg/dl)	0.001	0.001	0.001
Vldl (mg/dl)	0.001	0.001	0.001
Ldl (mg/dl)	0.096 [†]	0.001	0.001
Serum no (µmol/l)	0.001	0.001	0.001
Tc/hdl	0.001	0.001	0.001
Tg/hdl	0.001	0.001	0.001
Ldl/hdl	0.007	0.001	0.001
Non - hdl cholesterol (tc-hdl)	0.010	0.001	0.001
Ac (tc-hdl/hdl)	0.002	0.001	0.001
AIP	0.001	0.001	0.001

† NS, Non-significant at the 0.05 probability level.

Table 4 shows multivariate linear regression analysis results with Nitric oxide as dependent variable and atherogenic ratios as predictors indicating significant association (p=0.001). Table 5 shows the ROC curve analysis for the lipid ratios, AC and AIP which found a significant area under the curve (AUC) for TG/HDL-c, TC/HDL-c and LDL-c/HDL-c ratios, AC and AIP (p<0.001). The present study findings revealed that at best cut off value, AIP is better marker in identifying cardiovascular risk with high sensitivity 92% and specificity 96% than other parameters.

Table 3: The correlation of nitric oxide with lipid ratios, AC, AIP.

Parameter	R	P
Tc/hdl-c	-0.513	0.001[**]
Tg/hdl-c	-0.586	0.001[**]
Ldl-c/hdl-c	-0.491	0.001[**]
Ac(tc-hdl-c/hdl-c)	-0.513	0.001[**]
Aip	-0.616	0.001[**]

TC-Total cholesterol, HDL- High density Lipoprotein, LDL-Low density Lipoprotein, TG- triglycerides, VLDL-Very low density Lipoprotein, AIP- Atherogenic index of plasma, r = correlation coefficient, **significant at the 0.01 probability level.

Table 4: Multivariate regression analysis showing the association of nitric oxide with lipid ratios, AC, AIP.

Parameter	B	P
tc/hdl-c	-0.555	0.001[**]
tg/hdl-c	-0.575	0.001[**]
ldl-c/hdl-c	-0.544	0.001[**]
Ac(tc-hdl-c/hdl-c)	-0.644	0.001[**]
Aip	-0.644	0.001[**]

TC-Total cholesterol, HDL- High density Lipoprotein, LDL-Low density Lipoprotein, TG- triglycerides, VLDL-Very low density Lipoprotein, AIP- Atherogenic index of plasma, β = regression coefficient, **significant at the 0.01 probability level.

Table 5: ROC curve analysis.

Parameter	AUC	95% C I	P value	Sensitivity	Specificity
Tc/hdl	0.835	0.722-0.949	0.001[**]	92%	64%
Tg/hdl	0.981	0.952-1.000	0.001[**]	92%	96%
Ldl/hdl	0.785	0.654-0.916	0.001[**]	84%	76%
Ac (tc-hdl/hdl)	0.835	0.722-0.949	0.001[**]	88%	76%
AIP	0.981	0.952-1.000	0.001[**]	92%	96%

*Statistically significant, TC-Total cholesterol, HDL- High density Lipoprotein, LDL- Low density Lipoprotein, TG- triglycerides, VLDL-Very low density Lipoprotein, AC- Atherogenic coefficient, AIP- Atherogenic index of plasma, AUC: Area under the curve; CI: Confidence interval.

DISCUSSION

It is well known that diabetes patients have a high incidence of CVD, the risk of which becomes substantially elevated when complications develop. Though the pathogenesis of CVD in diabetes is multifactorial, dyslipidemia is found to be a powerful risk factor. In diabetes, long-term hyperglycaemia causes generalized vascular endothelial damage, which reduces functional lipoprotein lipase, leading to increase in triglycerides and a decrease in HDL. Increased levels of and triglycerides, cholesterol, LDL mediate the progression of atherosclerosis.[18] Lipid ratios and AIP have been reported to indicate atherogenic dyslipidemia. In the present study we observed significant elevation in the lipid ratios, non - HDL cholesterol, AC and AIP in both T2DM patients with and without complications when compared to controls. Similar findings were reported by Suchitra MM et al in their study to assess atherogenic dyslipidemia in T2DM and diabetic nephropathy patients.[19] Various lipid and lipoprotein fractions were shown to be associated in diabetes with and without complications.[20] In the present study we found a significantly higher concentration of total cholesterol, TG, VLDL, and lower HDL-c in patients with and without complications. However LDL-c and total Cholesterol were not significantly different in diabetes patients without complications when compared to controls. These findings are in concordance with a study by Imran Ahmed Siddiqui who estimated lipid indices in type II diabetes mellitus and their association with macro and micro vascular complications. Their findings suggest atherogenic index and TC/HDL-c levels were significantly higher in diabetics than in controls and both the indices were found to be lowered in patients on treatment with insulin.[21] These findings suggest the role of lipid ratios in identifying the CV risk rather than the individual lipids alone.

In the present study we found a significant increase in the levels of Non-HDL cholesterol and Atherogenic Coefficient (AC) in both T2DM patients with and without complications when compared to controls. Non-HDL cholesterol serves as an index of cardiovascular risk in diabetic patients in whom LDL-c may not be elevated.[8] Studies have shown Non-HDL-c being analogous to Apo-

B in assessing atherogenic cholesterol and lipoprotein burden.[22] The apolipoprotein B assay is not routinely available because of its cost and general unfamiliarity with its interpretation outside of the research setting. Because of its simple calculation, the Non-HDL Cholesterol level is easily available with every lipid profile ordered and eliminating any additional costs.[23] In a case-control study conducted with 60 angiographically confirmed patients and 60 healthy volunteers by Shilpa Bhardwaj et al found high Atherogenic Coefficient (AC), calculated as (Non-HDL-c)/HDL-c) or (TC-HDL-c)/HDL-c) is a measure of cholesterol in LDL, VLDL, IDL fractions with respect to good cholesterol or HDL-c.[24] All the findings suggest that AC reflects atherogenic potential of the entire spectrum of lipoprotein fractions and hence indicates the CV risk. The present study showed a significant correlation and association of lipid indices with nitric oxide, marker of endothelial dysfunction. As in insulin resistance state there is an increase in the release of free fatty acids from adipose tissue and stimulates the protein kinase -C pathway, which directly and indirectly inhibits eNOS activity through increased Reactive oxygen species generation and inhibits NO production. All these further impair the vasodilatory response in diabetes and hence predict the cardiovascular risk.[25] AIP is a ratio calculated as (log TG)/HDL-c. In the present study, ROC curve analysis revealed significant areas under the curve for the lipid ratios of TG/HDL-c, TC/HDL-c and AIP. The present study findings found to have high sensitivity and specificity for AIP. Hence, AIP would be applied as an additional cardiovascular risk assessor even in the presence of insignificant changes in the individual lipids.[26] In a study by Karbala et al also showed that AIP had highest sensitivity and specificity when compared with the other three atherogenic indices where its value of sensitivity was 84% versus 68%, 73%, and 76% for TC/HDL-c, HDL-c/LDL-c and LDL-c/HDL-c ratios.[27] Dobiasova and Frohlich suggested that people with high AIP have a higher risk for coronary artery disease than those with low AIP and that AIP is positively correlated with the fractional esterification rate of HDL (FERHDL), and is inversely correlated with LDL particle size. In view of this, calculating AIP can be more reliable in predicting the risk for development of atherosclerosis in diabetes mellitus patients.[12]

CONCLUSION

Lipid ratios, AC and AIP are found to have a good implication prospect in daily practice to assess cardiovascular risk in type 2 diabetes mellitus. These indices are calculated from the routinely done lipid profile parameters especially in centres where new tests are not possible due to cost factor. AIP is found to have better predictability to assess cardiovascular risk in type 2 diabetes mellitus. However there is need for more data to help the clinician to decide which indices to be measured and when to be measured. Our study has some limitations as inability to follow up the subjects with complications, due to paucity of time and limited resources. The other limitation is the small sample size, because of which the correlation of nitric oxide with the atherogenic indices in independent groups was not obtained.

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