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Research Article

ROLE OF NOVEL LIPID INDICES AND LIPOPROTEIN (a) IN TYPE –II DIABETES MELLITUS WITH CORONARY ARTERY DISEASE

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ABSTRACT
Diabetes mellitus (DM) is a group of metabolic disorders of carbohydrate metabolism where, underutilization of glucose is associated with changes in lipid profile. Lipoprotein (a) [Lp(a)] and glycosalated hemoglobin (HbA1c) are regarded as an independent indicator of risk development of vascular disease, which is also a diabetic complication. The novel lipid indices like Non HDL Cholesterol (NHC), Atherogenic Index (AI), Lipid Tetrad Index (LTI), Castelli Risk Index 1 & 2 (CRI 1 & 2) & Atherogenic Index (AC) are being studied to find out the better alternatives for lipoprotein (a) for monitoring/ predicting CAD in DM patients with CAD. The case-control study involved 80 participants of which 40 were patients admitted with diagnosis of DM with CAD and other 40 were healthy controls. Blood samples were collected in fasting state and analyzed for FBS, PPBS, HbA1c, TG, VLDL, HDL, LDL, TC and Lp(a) and all the indices were calculated and values were tabulated for statistical evaluation. In DM with CAD patients compared to controls, significant change in following parameters was observed. FBS, PPBS, HbA1c & Lp (a) levels increased significantly increased ($P<0.001$). Also the levels of TG & VLDL were significantly increased with $P<0.008$ & $P<0.011$ respectively. All lipid indices were significantly higher in the study group whereas the Non-HDL cholesterol was not statistically significant. To conclude, the novel lipid indices may be better and cost effective tools for monitoring the CAD risk in the DM patients. Further studies are required in large population for assessing the sensitivity of the novel lipid indices.

Keywords: *Lipoprotein (a), Non HDL Cholesterol, Atherogenic Index, Lipid Tetrad Index, Castelli Risk Index, Atherogenic Index, Diabetes Mellitus, Coronary Artery Disease*

INTRODUCTION
According to WHO 71% of deaths in developing countries will be contributed by coronary artery disease (CAD) by 2020 A.D. CAD will be the major killer in coming years. There are several causes for CAD including sex, age, smoking, hypertension, diabetes etc.
Impaired glucose and Dyslipidemia has been identified as one of the most important risk factor associated with CAD by the INTERHEART- South Asia study (Yusuf, 2004)**.** Diabetes mellitus (DM), which is frequently associated with dyslipidemia becomes the major risk factor in India, as in India alone the number of diabetics is expected to rapidly increase from 40.6 million in 2006 to 79.4 million by 2030 (Lt Gen *et al.*, 2009). Despite such studies, in the absence of an abnormal lipid profile the possibility of CAD cannot be ruled out (Singh *et al.*, 2010). Individual lipoproteins have been studied in DM and CAD separately, but it would be valuable to measure the combined risk of these lipoprotein changes in predicting the CAD in Type II DM.
On the other hand, Lipoprotein (a) (Lp(a)) and Glycated hemoglobin(HbA1c) are widely accepted as standard risk factors for CAD (Bloomgarden, 2009; Fijino *et al.*, 2000). But in developing counties like India, in the absence of traditional risk factors, the risk of CAD which is reaching epidemic proportions is always missing identified due to some factors like, high cost of standard tests (Rajappa, 2006). So it is very essential to look into the combinations of these lipid parameters to measure the total burden of dyslipidaemia as it eliminates the need for various cutoff points for each individual parameter.
So the study has been undertaken on the role of varies lipid indices like Non HDL Cholesterol, Atherogenic Index (AI), Lipid Tetrad Index (LTI), Castelli Risk Index 1 & 2 (CRI 1 & 2) & Atherogenic Index (AC) in assessing the risk of CAD in DM.

Objectives

To find out the better alternatives for lipoprotein (a) for monitoring/ predicting CAD in DM patients by calculating the following lipid indices.

Atherogenic Index of plasma (AIP), Castelli's Risk Index (CRI) I, Castelli's Risk Index (CRI) II, Lipid Tetrad Index (LTI), Atherogenic Coeficient (AC), Non-HDL Cholesterol (NHC).

MATERIALS AND METHODS

Methodology

40 clinically diagnosed cases of diabetes mellitus patients with CAD attending outpatient and inpatient departments of Shri Adichunchanagiri hospital and research center, Karnataka, India, were included in the study. 40 Age and sex matched healthy individuals are taken as control group. Patients suffering from obstructive jaundice, hypothyroidism, hypopituitarism, epileptic patients, psychiatric disorders, nephrotic syndrome were excluded from study.

The approval was taken by ethical and research committee of SAH&RC. After obtaining informed consent from patients and controls detailed medical history and relevant clinical examinations data were collected.

Collection of Blood Sample

7ml of fasting Blood sample was drawn under aseptic precautions from cases and controls and divided into 3 test tubes, marked as 1, 2 and 3. Using the chemicals and reagents of analytical grade the following parameters were analyzed,

1. Test tube 1 containing 2ml of blood with anticoagulant: Used for estimation of blood glucose by Glucose Oxidase method (Trinder, 1969).

2. Test tube 2 containing 3ml of blood with no anticoagulant: Serum was separated after clot formation was used for measurement of triacylglycerol (TG) by GPO-Trinder method (Trinder, 1969; McGowan *et al.,* 1983), total cholesterol (TC) by CHOD-POD method (Allain *et al.,* 1994), high density lipoprotein(HDL) by Phosphotungstic acid method (Burstein *et al.,* 1970), very low density lipoprotein(VLDL) was calculated by formula (TG/5) (Rifai, 2006), low density lipoprotein(LDL) was derived by Fredrickson-Friedwald formula [(TC-HDL)-TG/5] (Rifai, 2006) & [Lp(a)] was estimated by Immunoturbidometric method (Poulik, 1975; Levine *et al.,* 1992).

3. Test tube 3 containing whole blood: Used for estimation of HbA1c by Affinity Chromatography (Trivelli *et al.,* 1971; Gonen, 1978). And then Lipid indices were calculated by following formulas,

- AIP = log(TG / HDL) (Milada, 2004).
- CRI I = TC / HDL (Stampfer *et al.,* 1991; Ridker *et al.,* 2001; Castelli *et al.,* 1983)
- CRI II = LDL / HDL (Stampfer *et al.,* 1991; Ridker *et al.,* 2001; Castelli *et al.,* 1983)
- LTI = [TC X TG X Lp(a)] / HDL (Rajappa *et al.,* 2006)
- AC = (TC - HDL) / HDL (Brehm *et al.,* 2004)
- NHC = TC-HDL (Brehm *et al.,* 2004)

Statistical Method

Descriptive statistical analysis has been carried out in the present study. Results on continuous measurements are presented as Mean ± SD (Min-Max) and results on categorical measurements are presented in Number (%). Significance is assessed at 5 % level of significance. Student t test (two tailed, independent) has been used to find the significance of study parameters on continuous scale between two groups (Inter group analysis). Chi-square/ Fisher Exact test has been used to find the significance of study parameters on categorical scale between two groups.

RESULTS AND DISCUSSION

Table 1: Comparison of gender distribution in DM

	Controls		Cases	
	No	**%**	**No**	**%**
Age	50.10±12.86		49.50±9.66	
Male	26	65.0	24	60.0
Female	14	35.0	16	40.0
Total	40	100.0	40	100.0
BMI	24.38 ± 3.19		25.1 ± 2.83	

Samples are age and gender matched with P=0.11

Table 1 shows the demographic distribution between control group and patient group. Samples were age and gender matched with P=0.113.

Table 2: Levels of FBS, PPBS, and HbA1c in controls and cases

Parameters	Controls	Cases	Significance
FBS (mg/dl)	86.20±8.28	**167.70±59.80**	t=6.037;p<0.001**
PPBS (mg/dl)	107.20±19.11	**238.30±81.31**	t=7.019;p<0.001**
HbA1c	5.41±0.30	**9.44±2.31**	t=7.742;p<0.001**

Results are presented in Mean ± SD
*+ Suggestive significance (P value: 0.05<P<0.10); * Moderately significant (P value: 0.01<P ≤ 0.05); ** Strongly significant (P value: P≤0.01)*

Table 2 shows levels of fasting blood sugar(FBS), post prandial blood sugar(PPBS), and HbA1c in controls and cases presented as Mean±SD and it observed that all the parameters of cases were statistically strongly significant (P value: P≤0.01) as compared to controls.

Table 3: Levels of Lipid parameters in controls and cases

Lipid profile	Controls	Cases	Significance
Triglycerides (mg/dl)	128.60±28.25	**195.25±102.65**	t=2.800;p=0.008**
Total cholesterol (mg/dl)	169.20±24.57	174.25±42.86	t=0.457;p=0.650
HDL (mg/dl)	45.05±6.64	**37.25±8.03**	t=3.348;p=0.002**
LDL (mg/dl)	100.95±21.22	100.40±34.83	t=0.060;p=0.952
VLDL (mg/dl)	24.95±5.98	36.60±18.59	t=2.667;p=0.011*
Lipoprotein(a) (mg/dl)	20.16±6.26	**46.20±22.92**	t=4.783;p<0.001**

*+ Suggestive significance (P value: 0.05<P<0.10); * Moderately significant (P value: 0.01<P ≤ 0.05); ** Strongly significant (P value: P≤0.01)*

Table 3 depicts the levels of lipid parameters in controls and cases, where TG, HDL, Lp(a) were strongly significant (P value: P≤0.01) for cases whereas, TC & LDL were not statistically significant as compared to controls.

Table 4: Levels of Lipid Indices in controls and cases

Lipid Indices	Controls	Cases	Significance
AC	2.80±0.68	3.7±1.32	t=2.71;p<0.01**
AIP	0.088±0.126	**0.31±0.26**	t=3.43;p<0.00**
CRI I	3.80±0.68	**4.79±1.20**	t=3.21;p<0.00**
CRI II	2.26±0.59	2.74±0.88	t=2.02;p<0.05*
LTI	9269.00±4375.10	**43642.14±33006.01**	t=4.61;p<0.00**
NHC	124.15±23.74	136.7±40.95	t=1.18;p<0.24

*+ Suggestive significance (P value: 0.05<P<0.10); * Moderately significant (P value: 0.01<P ≤ 0.05); ** Strongly significant (P value: P≤0.01)*

Table 4 represents the levels of various lipid indices in controls and cases. AIP, CRI I, LTI & AC show statistically strongly significant results in patients when compared to controls, whereas CRI II shows suggestive significance and NHC show no significance.

Table 5: Sensitivity and specificity of risk factors

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Accuracy (%)
AC > 3.25	65	60	63	61	62.5
AIP > 0.21	**60**	**80**	**75**	**66**	**70**
CRI I > 4	**70**	**65**	**66**	**68**	**67.5**
CRI II > 3.4	20	100	100	55	60
LTI > 20000	**75**	**100**	**100**	**80**	**87.5**
TG > 150	55	85	79	65	70
HDL< 35	45	100	100	65	72.5
HbA1c >7	95	100	100	95	82.5
Lp(a) > 30	70	95	93	76	97.5

Table 5 gives the sensitivity and specificity of risk factors. Overall accuracy was found to be high for Lp(a) (97.5%) followed by LTI(87.5%), HbA1c(82.5%), HDL(72%), AIP(70%) and TG(70%) and accuracy was very low for AC(62.5%) and CRI II (60%).

Present study shows strong significant change in TG & HDL levels. As increased triacylglycerol levels are directly associated with high plasma levels of tissue plasminogen activator inhibitor, the hypertriglyceridaemia observed in our study becomes an important risk factor for CAD (Mehta *et al.*, 1987; Krishnaswami *et al.*, 1996). Also the lower HDL levels in the study are well known risk factors for CAD (Von, 2001). Our study found statistically strong significant increase in the levels of HbA1c and Lp(a) which are proven standard risk factors for CAD (Mehdi, 2006).

But in our contrary 80% of patients with proven CAD had a serum TC < 200 mg/dl & LDL < 130 mg/dl which was statistically insignificant as compared to controls. This is because the well-known fact that Indians develop CAD at much lower TC and LDL cholesterol levels (Rani, 2005; Verschuren, 1995). Also the small, dense, undesirable type of LDL, which is a component of "Atherogenic lipoprotein phenotype" of diabetic dyslipideamia (high TG with low HDL and small, dense, undesirable type of LDL), should be considered which is more atherogenic than regular LDL (Musunuru, 2010).

In the absence of traditional risk factors like in the present study, statistically insignificant TC and LDL levels chances of none identifying the risk of CAD will be more. This is due to some factors like, lack of the facilities, insufficient resources and high cost of standard tests like Lp(a) and small, dense, undesirable type of LDL. In such conditions lipid indices play a major role.

In the present study among the lipid indices, the LTI (Lipid Tetrad Index) of the cases was significantly higher with higher positive predictive value (PPV), negative predictive value (NPV) & accuracy, as

shown by studies of Enas *et al.*, (1993, 1996) and becomes the single predictor of CAD in Asian region (Yeolekar, 1998).

Studies have shown that the ratio of TG to HDL (AIP) is a strong predictor of infarction (Gaziano *et al.*, 1997; Dobiasova, 2006). Studies have also shown that in situations where TG and HDL appear normal, AIP may be the diagnostic alternative (Nwagha, 2010). In the current study, we not only observed that AIP was significantly higher in cases as compared to controls but also increased sensitivity of AIP compared to individual parameters of TG & HDL.

We observed significant change in CRI-I& CRI-II in our case group as compared to controls. We observed many individual values of CRI-I > 4 & CRI-II >3 in our case group as observed in other studies and the above cut-off values has been shown to be one of the risk factors for coronary plaques formation (Subia, 2012; Nair *et al.*, 2009).

In our study, there was no significant difference in TC and LDL levels amongst cases and controls whereas, the ratio, CRI-I& CRI-II, showed a significant difference. This clearly suggests the relevance of ratios over individual lipid parameters.

NHC is the second target of therapy after LDL as per ATPIII guidelines especially in individuals with hyper-triglyceridemia (NCEP, 2002). Studies have shown NHC being similar to Apo-B in assessing atherogenic cholesterol and lipoprotein burden (Hermans, 2011). As shown in study done by Mannangi *et al.*, in our study also even though we found increased NHC levels in cases compared to controls, the values are not statistically significant (Mannangi *et al.*, 2015). But the AC based on NHC was found to be significantly higher in cases. This shows that indices have an edge over individual lipid parameters.

We found that LTI has maximum accuracy among the indices, approx. 80% to the risk of developing CAD followed by AIP, CRI II, AC & CRI I with 70%, 67%, 62% & 60% respectively.

To conclude, the lipid indices are related to atherogenesis and may be a key link between lipid and CAD. LTI could be a novel predictor of premature CAD. AIP have the potential to emerge as the best cost effective marker of risk for CAD especially when the absolute values of individual lipoproteins seem normal.

Thus, the use of these indices should be encouraged to complement the existing profile of tests for screening and identifying high risk individuals for CAD and effective drug management as the target of the lipid lowering therapies instead of individual lipid subsets.

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