ASSOCIATION BETWEEN ATHEROGENIC RATIO TO FASTING PLASMA GLUCOSE AND HbA1c

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ABSTRACT: Hyperlipidemia is a common finding in uncontrolled Diabetes Mellitus (DM) and patients with DM generally show alteration in lipid profile, notably in triglycerides value. Research done in the past has pointed out the usefulness of Atherogenic Index of Plasma (AIP) as a useful parameter for the diagnosis of Type 2 DM. HbA1c is well established as gold standard to monitor long term diabetic control. Further elevated lipid profile markers have been linked to a host of diseases like Cardiovascular, liver and kidney. Some studies have shown reasonable association between AIP to plasma glucose and HbA1c, but this study did not find any association between AIP to HbA1c and glucose, but very good association have been observed between Atherogenic ratio to both plasma glucose and HbA1c suggesting that lipid profile test to be made as routine for the diagnostic improvement of all DM patients. The contents of this study will serve as a model for future researchers to explore more studies in this field to define a set of diagnostic tests linking cardiac, liver and kidney to be used as routine Master Health Checkup package.

INTRODUCTION: Although hypertension, hypercholesterolemia and Diabetes Mellitus (DM) are recognized as major cardio-metabolic risk factors in primary Acute Coronary Syndrome (ACS) prevention, studies focusing on secondary ACS incidence are scarce.

DM was the only clinical factor that aggravates ACS prognosis, whereas abnormal lipids profile and blood pressure did not seem to determine prognosis. Thus, glycaemic control may play a critical role in the secondary Cardiovascular Disease (CVD) prevention management of ACS patients ¹. Type 2 diabetes mellitus (T2DM) leads to the typical known form of dyslipidaemia among the patients.

This dyslipidaemia type re-presents prognostically important type of atherogenic dyslipidaemia that significantly increases the risk of atherothrombosis.
The course of the disease later leads to the change of dyslipidaemia, characterized by an increase of LDL and triglyceride levels and the persistence of the lower levels of HDL cholesterol. Hypolipidemic treatment leads to the significant lowering of cardiovascular risk, however despite treatment with statin or fibrate residual cardiovascular risk remains still very high.

Non-Alcoholic Fatty Liver Disease (NAFLD) increasingly affects children (paediatric prevalence is 4.2%-9.6%). T2DM, insulin resistance (IR), obesity, metabolic syndrome and NAFLD are particularly closely related. Increased hepatic lipid storage is an early abnormality in IR women with a history of gestational diabetes mellitus. The accumulation of triacylglycerols in hepatocytes is predominantly derived from the plasma nonesterified fatty acid pool supplied largely by the adipose tissue. A few NAFLD susceptibility gene variants are associated with progressive liver disease, IR, T2DM and a higher risk for hepatocellular carcinoma. Although not approved, pharmacological approaches might be considered in Non-alcoholic Steato Hepatitis (NASH) patients.

Several epidemiological studies demonstrated that total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C) ratios or low-density lipoprotein cholesterol/high-density lipoprotein cholesterol (LDL-C/HDL-C) ratios could be better predictors of atherosclerosis than any single lipid parameter.

Intima-Media Thickness (IMT), a well-established marker of early atherosclerosis, is associated with Hypertriglyceridaemia (HTG) / low HDL-C. In Impaired Glucose Tolerance (IGT) study for risk factors of Atherosclerosis and Diabetes and total and HDL-C were independent determinants of IMT in subjects at risk for T2DM. Postprandial HTG was also shown to be correlated with increased IMT in T2DM.

HDL-Cholesterolin DM:
T2DM and the cluster of pathologies including IR, central obesity, high blood pressure, and HTG that constitute the metabolic syndrome are associated with low levels of HDL cholesterol and the presence of dysfunctional HDLs. There is well-established association of T2DM to IR with alterations of lipid metabolism and how these alterations may lead to low levels of HDL and the occurrence of dysfunctional HDLs. There is evidence showing that HDL modulates insulin sensitivity, insulin-independent glucose uptake, insulin secretion, and beta cell survival. A dysfunction in these actions could play a direct role in the pathogenesis of T2DM. HDL exerts a series of potentially beneficial effects on many cell types including anti-atherogenic actions on the endothelium and macrophage foam cells. HDLs may also exert anti-diabetogenic functions on the beta cells of the endocrine pancreas, notably by potently inhibiting stress-induced cell death and enhancing glucose-stimulated insulin secretion. HDLs have also been found to stimulate insulin-dependent glucose uptake into skeletal muscle, adipose tissue and liver.

HDLs protect β-cells against Endoplasmic Reticulum (ER) stress induced by thapsigargin, cyclopiazonic acid, palmitate, insulin overexpression and high glucose concentrations. ER stress marker induction and ER morphology disruption mediated by these stimuli were inhibited by HDLs. Using a temperature-sensitive viral glycoprotein folding mutant has been shown that HDLs correctly impair protein trafficking and folding induced by thapsigargin and palmitate. The ability of HDLs to protect β-cells against ER stress was inhibited by brefeldin A, an ER to Golgi trafficking blocker. These results indicate that HDLs restore ER homeostasis in response to ER stress, which is required for their ability to promote β-cell survival, indicating that there is a cellular mechanism mediating the beneficial effect of HDLs on β-cells against ER stress-inducing factors. IR and T2DM are generally accompanied by low HDL cholesterol and high plasma triglycerides, which are major cardiovascular risk factors. A decreased post heparin plasma Lipoprotein Lipase (LPL)/Hepatic Lipase (HL) ratio is a determinant of low HDL-cholesterol in IR.

The esterification of free cholesterol by Lecithin: Cholesterolacyltransferase (LCAT) increases HDL particle size. Plasma cholesterol esterification is unaltered or increased in T2DM, probably depending on the extent of triglyceride elevation. Phospholipid transfer protein (PLTP) generates...
small pre beta-HDL particles that are initial acceptors of cell-derived cholesterol. Its activity in plasma is elevated in insulin resistance and T2DM in association with high plasma triglycerides and obesity. CVD is the major cause of morbidity and mortality in T2DM. Among the established risk factors, the lipid triad (elevated triglycerides, decreased HDL cholesterol and increased small dense LDL cholesterol) is a powerful risk factor for atherosclerosis in T2DM. The prevalence of HTG in T2DM is two to three times higher than in non-diabetics. The Copenhagen Male study, the AMORIS study, and several other trials showed hypertriglyceridaemia to be an independent predictor of Coronary Heart Disease (CHD). T2DM is characterized by low HDL-C and HDL dysfunction. Persistent HDL dysfunction despite improved HbA1c and HDL-C can contribute to residual cardiovascular risk in T2DM. In subjects with impaired glucose regulation, HDL-C levels are associated with indices of beta-cell dysfunction linking to HDL-C concentrations in IFG/IGT patients due to their potential conversion to DM2. Low levels of HDL-C have been associated with an increased risk of CHD in prospective population studies and clinical trials of high-risk patients treated with a low to moderate intensity statin. Therefore, strategies that increase HDL-C without expanding the pool of HDL-P with its rich proteome/lipidome do not seem to be an effective strategy. There are potential mechanisms of action for the anti-atherogenic effect of HDL and the impact of current and emerging therapies on the functional capacity of HDL-P.

Atherogenic Index of Plasma in DM:
AIP values increase with increasing CV risk. Thus umbilical cord, young children, healthy women have values below 0.1 while men and subjects with CV risk factors such as hypertension, diabetes, dyslipidemia have increasing values up to 0.4. Based on these data it is suggested that AIP values of ~0.3 to 0.1 are associated with low, 0.1 to 0.24 with medium and above 0.24 with high CV risk. In the population study men had higher AIP values than women. In a cohort undergoing coronary angiography AIP, in model that included age, BMI, waist circumference, T2DM, blood pressure, smoking, TG, TC, LDL-C, apoB, HDL-C, and TC/HDL-C, AIP was the best predictor of positive findings. AIP was also a highly sensitive marker of differences of lipoprotein profiles in families of patients with premature myocardial infarction and control families. Treatment with ciprofibrate, and combination of statin and niacin dramatically decreased AIP. Combination with hypoglycemic therapy that included pioglitazone decreased AIP in patients with T2DM.

LDL-Cholesterol in DM:
After multivariate adjustment for age, gender, ethnicity, hypertension, smoking, statin use, duration of diabetes, and HbA1c showed an increased CHD risk in women and men with metabolic dyslipidemia compared to those with normal HDL and TG. Even in subjects with an LDL-C <100 mg/dL, presence of metabolic dyslipidemia in adults with diabetes is associated with an increased risk of CHD. The effective CHD prevention strategies are needed for adults with diabetes and metabolic dyslipidemia. The importance of an intervention by means of Proprotein Convertase Subtilisin Kexin 9 (PCSK9) inhibitors for the cardiovascular risk reduction in patients with diabetes mellitus is the subject of study. Dyslipidemia contribute to an excess of CHD risk observed in women with T2DM. LDL-C is the major target for CHD prevention, and T2DM women seem to reach LDL-C targets less frequently than men.

To explore age- and gender-related differences in LDL-C management in a large sample of outpatients with T2DM. LDL-C management is worst in women with T2DM, who are monitored and reach targets less frequently than T2DM men. Similarly to men, they do not receive medications despite high LDL-C. These gender discrepancies increase with age and diabetes duration, exposing older women to higher CHD risk.

Triglycerides in DM:
All DM group suffered from heart diseases including coronary artery diseases and many subjects had multi-vessel disease. Coronary arterial TG contents were significantly higher in DM group compared with non-Diabetes. Spatial distribution of
TG in transverse sections of coronary arteries showed TG deposition mainly in smooth muscle cells by Imaging Mass Spectrometry. Abundant TG deposition in coronary artery might be associated with advanced DM \(^{17}\). Cholesterol is a vital causal factor and focus of research for heart diseases; however the involvement of triglycerides remains unclear. It has been shown that massive accumulation of triglycerides was noted in coronary atherosclerotic lesions as well as in the myocardium and named as phenotype "triglyceride deposit cardio myovasculopathy (TGCV)" and they are identified as homozygous for a genetic mutation in the adipose triglyceride lipase (ATGL), an essential molecule for hydrolysis of intracellular triglycerides \(^{18}\).

Multifactorial intervention reduces the risk even further, but significant danger remains. Current guidelines call for an aggressive treatment strategy to reduce LDL cholesterol, blood pressure, and glucose levels in diabetic patients, but data concerning the management of high TG levels and low HDL cholesterol levels remains inconclusive \(^{19}\).

**MATERIALS AND METHODS:**
100 patients consisting of males and females in the age group of 40-70 years were selected for this study. All 100 patients & 50 control groups were selected from those attending the Master Health Checkup. The main aim of this study is to find out the association between Atherogenic ratio to fasting plasma glucose and HbA1c.

Dirui CS 1300B analyser & Dialab reagents were used to measure plasma glucose and Lipid profile and BioRad D10 analyser was used to measure HbA1c using HPLC method. The accuracy of all the analytes were validated by the use of Bio-Rad accuracy controls at 2 levels.

**Inclusion criteria:**
Patients who attended the MHC & whose HbA1c values were > 6.5% and FPG values > 150 mg/dL were included. 50 patients whose diabetic profile tests were normal were used as controls.

**Exclusion criteria:**
Patients whose HbA1c values were < 6.5% and FPG values < 150 mg/dL were excluded. For statistical analysis of data, a software downloaded from the website http://www.vassarstats.com was used to calculate correlation coefficient (r), students’ t’ distribution (t) and probability (p) between Diabetic patients and controls.

**TABLE 1: MEAN & SD FOR THE ANALYTES STUDIED (CONTROLS VS PATIENTS)**

<table>
<thead>
<tr>
<th>S.No</th>
<th>Analyte</th>
<th>Sex</th>
<th>Controls n</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>TG/HDL Ratio</td>
<td>All</td>
<td>50</td>
<td>2.75</td>
<td>1.64</td>
<td>5.3</td>
<td>9.18</td>
</tr>
<tr>
<td>2</td>
<td>AIP</td>
<td></td>
<td>0.21</td>
<td>0.15</td>
<td>0.18</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>HbA1c</td>
<td></td>
<td>5.5</td>
<td>0.36</td>
<td>5.5</td>
<td>9.5</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>FPG</td>
<td></td>
<td>93</td>
<td>7.56</td>
<td>207</td>
<td>57.56</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>LDL/HDL Ratio</td>
<td></td>
<td>2.61</td>
<td>0.79</td>
<td>2.61</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>TG/HDL Ratio</td>
<td>Females</td>
<td>25</td>
<td>2.21</td>
<td>1.18</td>
<td>5.7</td>
<td>11.07</td>
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<tr>
<td>7</td>
<td>AIP</td>
<td></td>
<td>0.2</td>
<td>0.16</td>
<td>0.17</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>HbA1c</td>
<td></td>
<td>5.4</td>
<td>0.36</td>
<td>9.3</td>
<td>1.66</td>
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<tr>
<td>9</td>
<td>FPG</td>
<td></td>
<td>95</td>
<td>5.69</td>
<td>200</td>
<td>53.32</td>
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<td>10</td>
<td>LDL/HDL Ratio</td>
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<td>0.79</td>
<td>2.5</td>
<td>1.03</td>
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<tr>
<td>11</td>
<td>TG/HDL Ratio</td>
<td>Males</td>
<td>25</td>
<td>2.17</td>
<td>1.95</td>
<td>4.8</td>
<td>6.89</td>
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<tr>
<td>12</td>
<td>AIP</td>
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<td>0.21</td>
<td>0.16</td>
<td>0.18</td>
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<tr>
<td>13</td>
<td>HbA1c</td>
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<td>6.5</td>
<td>0.34</td>
<td>9.8</td>
<td>1.77</td>
<td></td>
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<tr>
<td>14</td>
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<td>90</td>
<td>8.61</td>
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<tr>
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<td>LDL/HDL Ratio</td>
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<td>2.66</td>
<td>0.75</td>
<td>2.73</td>
<td>0.89</td>
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</table>
Table 1 shows the Mean & SD values for TG/HDL ratio, AIP, HbA1c, FPG and LDL/HDL ratio for controls & patients. A visual inspection of Table 1 shows that TG/HDL ratios are elevated in all patient groups compared to controls. Except HbA1c and glucose, there is no elevation observed in AIP and LDL/HDL ratio suggesting that Atherogenic ratio is indeed an useful Index to differentiate Diabetic from non-diabetic groups.

Table 2 presents statistical parameters (CR & p-value) to find out the association between controls and patients for the analytes TG/HDL ratio, HbA1c and FPG. From this Table it is clear that HbA1c and FPG shows the highest significant correlation when calculated AIP was used for correlation. The two Diabetic profile tests HbA1c & FPG are generally used for the diagnosis of DM. Few studies done in the past have shown correlation of AIP to Diabetic markers but not with individual lipid profile tests. The outcome of this study has strongly established associations of TG/HDL ratio, HbA1c & FPG between controls and patients. Further researches are required to confirm the findings of this study on a large number of patients and to recommend TG/HDL ratio as routine test along with diabetic profile tests to find out if improvement in Diabetic status correlates with normalization of TG/HDL ratio.

CONFLICT OF INTEREST: None

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REFERENCES:
