A STUDY ON BIOCHEMICAL CHANGES OF ATHEROSCLEROSIS IN PREDIABETICS AND DIABETICS

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ABSTRACT

BACKGROUND

Diabetic dyslipidaemias cause coronary heart disease, cerebrovascular disease and other complications of atherosclerosis thus leading to high mortality and morbidity in type 2 diabetics. But diabetic dyslipidaemia starts even before the onset of diabetes thus indicating the role of prediabetes as an atherosclerotic marker.

AIMS, SETTINGS AND DESIGN

In this study, we have tried to evaluate the dyslipidaemia associated with the type 2 diabetes mellitus and prediabetes and also the correlation between carotid artery intima media thickness (CIMT) with lipid profile in prediabetics and diabetics in a hospital based case control study consisting of 57 (42%) diabetics, 45 (33%) prediabetics and 34 (25%) healthy subjects within a span of one year.

METHODS

Fasting glucose, postprandial glucose, total serum cholesterol (TC), high density cholesterol (HDL-c) and triglyceride (TG) were measured by standard pre-validated photometric tests as applicable. HbA1c or glycated haemoglobin was measured by HPLC (High Performance Liquid Chromatography). CIMT was measured by B mode ultrasonography.

STATISTICAL ANALYSIS

The data obtained were analysed using the statistical software, SPSS version 16. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

RESULTS AND CONCLUSION

In our study, the plasma total cholesterol, triglyceride, low density cholesterol (LDL-c) and very low density cholesterol (VLDL-c) increased while plasma HDL–c decreased from Healthy to Prediabetes to Diabetes. CIMT also increased from Healthy to Prediabetes to Diabetes. Total cholesterol, triglyceride and LDL-c correlated positively, strongly and significantly with CIMT while HDL-c correlated negatively, strongly and significantly with CIMT in Healthy and Diabetics, but not in Prediabetics. Thus emphasising that though biochemical changes of atherosclerosis starts much before the onset of diabetes, in the prediabetes state only; but the insignificant correlation of lipid parameters with the Composite CIMT in the prediabetes population indicates that CIMT is not yet highly raised in the prediabetes state.

KEYWORDS

Diabetic Dyslipidaemia, Prediabetes, Atherosclerosis, Lipid Profile, Carotid Intima Media Thickness.

INTRODUCTION

The term Diabetes Mellitus (DM) describes a heterogeneous group of metabolic disorder which is characterised by chronic hyperglycaemia and disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] In 2014, according to the International Diabetes Federation, an estimated 387 million people all over the World had DM with 75 million alone in the South East Asia, while 46.3% of the population remain undiagnosed for DM.[2] Diabetic dyslipidaemias are the common cause for development of coronary heart disease, cerebrovascular disease and other complications of atherosclerosis leading to the high mortality and morbidity in Type 2 DM patients.[3]

Diabetic dyslipidaemia or atherogenic dyslipidaemia consists of moderate elevation in triglyceride (TG) levels, small dense low density lipoprotein cholesterol (LDL-c) particles and low high density lipoprotein cholesterol (HDL-c) values.[4] But, diabetic dyslipidaemia starts even before the onset of diabetes and is associated with insulin resistance.[4] Small dense LDL-c particles are highly atherogenic because of their enhanced susceptibility to oxidative modification and increased uptake by the arterial wall.[4]

Although atherosclerosis is a progressive disorder of the arterial wall involving inflammation, vascular lipid deposition and remodelling, fibrosis, and thrombosis,[5,6] it does not progress in a uniform manner, thus leading to a plethora of complications including cardiovascular disease.[7] The concept of pre-diabetes started building up since 1979 when the National Diabetes Data Group introduced the concept of "Impaired Glucose Tolerance" (IGT). Prediabetes was defined by a state midway between the normal glucose homeostasis and the diseased condition of Diabetes Mellitus.[8,9]

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Almost two decades later in 1997, the International Expert Committee (IEC) on the Diagnosis and Classification of Diabetes Mellitus introduced the concept of "Impaired Fasting Glucose" (IFG).

IFG and IGT represent different pathophysiological and biochemical process and these were grouped together, as a common clinical entity, termed as "Prediabetes".[10,11] This marked the logical conclusion of the natural history of diabetes representing the continuum in metabolic dysregulation which kept worsening as the disease progresses or went unchecked. Various longitudinal studies showed a clear association between various macrovascular complications of prediabetes, most notable among them being the cardiovascular complications.[12] However, the role of prediabetes (IFG or IGT) as an atherosclerotic risk factor as well as its influence on lipid metabolism is still not well defined. The relationship between lipid metabolism with prediabetes is poorly defined. The relation between lipid changes with intima media thickness- all determinants of cardiovascular disease is also unsettled in prediabetes and diabetes.

Carotid intima media thickness (CIMT) is considered a recognised surrogate marker for assessing cardiovascular risk and recognising the subclinical atherosclerosis. The American Heart Association (AHA), the National Cholesterol Education Program(NCEP) expert panel and the European Third Joint Task Force recommend the use of non-invasive, quick and cost-effective screening test, CIMT by ultrasound method, for identifying abnormal arterial structure and atherosclerosis.[13] CIMT using high frequency ultrasound (10 MHz) accurately measures the arterial wall thickness[14] and is extremely accurate as compared to histologic examination.[15] So, this study aims for the lipid profile changes of atherosclerosis in diabetes and prediabetes. It also aims to see the correlation of Carotid Intima Media Thickness (CIMT) with lipid profile.

AIMS AND OBJECTIVES
- To study lipid profile in prediabetics and diabetics.
- To determine the correlation between carotid artery intima media thickness with serum total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol level in prediabetics and diabetics.

MATERIALS AND METHODS
A cross-sectional, observational, descriptive study was conducted on adult patients (>18 years of age), irrespective of sex, attending the outpatient department and admitted in the inpatient wards of the Department of Medicine of a Tertiary Care Medical College Hospital, West-Bengal, India from a period of July 2014 to June 2015 after taking Institutional ethical clearance. ICMR's Ethical Guidelines for biomedical research on human participants, (2006) and the Helsinki Declaration 1975, revised in 2000 was followed. Only those who met the study criteria as outlined in inclusion criteria and exclusion criteria were included in the study. 136 patients were included in our study and 447 patients were excluded according to the inclusion and exclusion criteria of our study.

Inclusion Criteria
Patients who met the definition of Prediabetes (IFG or IGT or HbA1C-5.7%-6.4%) and Diabetes Mellitus as outlined by the

International Expert Committee formed by the American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation were included in the study.[16] Healthy controls were also included in the study. The Criteria are:

Criteria for Diagnosis of Prediabetes
- **Impaired Fasting Glucose (IFG)**
  Fasting Plasma Glucose: 100 to 125 mg/dL (5.6-6.9 mmol/L).

  OR

- **Impaired Glucose Tolerance (IGT)**
  Fasting Plasma Glucose less than 100 mg/dL (5.6 mmol/dL).
  Two-hour plasma glucose level after 75-g oral glucose intake: 140 to 199 mg/dL.

  OR

- **HbA1c :5.7% to 6.4%**

Criteria for Diagnosis of Diabetes
- HbA1c or Glycated haemoglobin ≥ 6.5%.

  OR

- Fasting Plasma Glucose >126 mg/dL.

  OR

- 2-Hour plasma glucose >200 mg/dL (11.1 mmol/L) after oral glucose intake. The test was performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

  OR

- In a patient with classic symptoms of hyperglycaemia or hyperglycaemic crisis, a random plasma glucose> 200 mg/dL (11.1 mmol/L).

Criteria for Diagnosis of Healthy
- Fasting Plasma Glucose: <100 mg/dL (5.6 mmol/L).
- Two- hour plasma glucose level after 75 g oral glucose intake: <140 mg/dL.
- HbA1c: <5.7%.

Exclusion Criteria
- History of diabetes or history of intake of any hypoglycaemic agent.
- History of hypertension.
- History of severe renal, hepatic, pulmonary or neurological disease.
- History of heart disease.
- History of psychiatric disease.
- Patients on drugs that modify CIMT (Statins, Aspirin, Angiotensin Converting Enzyme Inhibitor, Angiotensin receptor blocker).
- Pregnant women.
- Patient or patient's caretaker unable to respond satisfactorily to verbal questions.
- Refusal to provide informed consent.

METHODOLOGY
- 10 mL of fasting venous blood was collected from each participant in the fasting state (After overnight fasting of 12 hours and 3 days of fat free diet), 5 mL dispensed in clotted vial for estimation of total cholesterol, triglyceride and HDL-c, and 2 mL in ethylenediaminetetraacetic acid (EDTA) vial for estimation of HbA1c by High Performance

Liquid Chromatography (HPLC), 3 mL in fluoride vial for estimation of fasting glucose. From the clotted blood, serum was separated and stored at -20°C.

- And 3 mL of postprandial venous blood was collected after 2 hours of having 75 g of glucose load and dispensed in the fluoride vial for estimation of postprandial glucose.

- Fasting and postprandial plasma glucose were estimated by Glucose oxidase-Peroxidase method. Total cholesterol was estimated by using cholesterol oxidase- Peroxidase method. Triglyceride was estimated by Glycerokinase Peroxidase- Peroxidase method. HDL cholesterol was estimated by direct method. Mindray kits were used for estimation of all these tests. Glycated haemoglobin or HbA1c was estimated by using High Performance Liquid Chromatography (HPLC) of Biorad D 10. LDL-c and VLDL-c was estimated by Friedewald's formula.

- Carotid artery intima media thickness (CIMT) was measured by B-mode ultrasound on both the right and left carotid artery by an experienced radiologist blinded of other study data as per the guidelines of American Society of Echocardiography by ultrasonographic machine(GE Logic P 5).[14] Three segments were measured in each side (Common Carotid Artery, Carotid Bulb Region and Internal Carotid Artery) and Composite CIMT was calculated by taking the mean of the right and left segmental mean thicknesses.

- All the participants have undergone detailed assessment of history, thorough physical examination and assessment of the biochemical parameters (as per our study protocol) and they are classified into three groups: Healthy, Prediabetes and Diabetes (newly diagnosed type 2 DM).

STATISTICS
The data obtained were analysed using the statistical software, SPSS version 16. Number of subjects and percentage of study population are compared across the groups using Pearson's Chi Square test. Mean±Standard Deviation of different parameters were compared across the 3 groups using One Way Analysis of Variance (ANOVA) Test. Association among continuous variables is captured by Pearson's Correlation coefficient. An alpha level of 5% has been taken, i.e. if any p value is less than 0.05 it has been considered as significant.

RESULTS
25% of participants are found to be apparently healthy or normoglycaemic, 33% prediabetic and 42% to be type 2 DM patients (Newly Diagnosed) according to their fasting and postprandial plasma glucose levels and glycated haemoglobin (HbA1c) levels. The mean age of Healthy, Prediabetes and Diabetes groups are 49.85±8.53, 51.56±10.62, 55.49±10.34 years respectively. One Way ANOVA test shows significant difference in the mean age of the three groups. Healthy group consists of 50% male and 50% female subjects, Prediabetes group consists of 44.44% male and 55.56% female subjects whereas Diabetes group consists of 49.12% male and 50.88% female subjects.

There is no significant difference in gender distribution between the three groups. The mean fasting glucose increased from Healthy (84.18±8.04 mg/dL) to Prediabetes (110.09±5.88 mg/dL) to Diabetes (232.3±52.38 mg/dL) with significant difference between the fasting glucose levels between the three groups. The mean post–prandial blood

glucose increased from Healthy (122.03±7.01 mg/dL) to Prediabetes (153.47±25.04 mg/dL) to Diabetes (335.56±86.79 mg/dL) with significant difference among them. The mean HbA1c increased from Healthy (5.13±0.28%) to Prediabetes (6.1±0.19%) to Diabetes (8±2.19%) with significant difference between them.

Mean total cholesterol, triglyceride, LDL-c and VLDL-c increased while, HDL-c decreased from Healthy to Prediabetes to Diabetes (Table 1 and Figure 1). One way ANOVA test shows significant difference in all of these between the Healthy, Prediabetes and Diabetes group.

The intima-media thickness of right common carotid artery (CCA RT), left common carotid artery (CCA LT), right carotid bulb region (BULB RT), left carotid bulb region (BULB LT) and the Composite value increased from Healthy to Prediabetes to Diabetes. One way ANOVA test shows significant difference of all the carotid intima media thickness (CIMT) values in the three groups. (Table 2 and Figure 2).

There is significant, strong and positive correlation noted between composite carotid intima-media thickness (CIMT) and Total Cholesterol in the Healthy and Diabetes group (p value <0.001) whereas, there is hardly any significant correlation noted in the Prediabetes group (Table 3).

There is significant, strong and positive correlation noted between triglyceride cholesterol level and Composite Carotid Intima Media Thickness (CIMT) in Healthy and Diabetes group (p value <0.001) whereas, there is hardly any significant correlation noted in Prediabetes group (Table 4). There is significant, strong and negative correlation noted between composite carotid intima media thickness (CIMT) and HDL cholesterol in the Healthy and Diabetes group (p value<0.001) whereas, there is hardly any significant correlation noted in Prediabetes group (Table 5). There is significant, strong and positive correlation noted between Composite carotid intima media thickness (CIMT) and LDL cholesterol in the Healthy and Diabetes group (p value <0.001) whereas, there is hardly any significant correlation noted in Prediabetes group (Table 6).

DISCUSSION
In our study, the mean fasting plasma glucose, mean postprandial plasma glucose, and mean glycated haemoglobin (HbA1c) levels have increased from Healthy to Prediabetes to Diabetes and this increase has been found statistically significant and our results are consistent with the studies done by Dullart et al.[17] Ahsaan et al.[18]

Diabetic dyslipidaemia consists of increased hepatic secretion and impaired clearance of VLDL-c thus causing increased production of precursors of small dense LDL-c particles.[19,20] In Type 2 DM, there is increased transfer of cholesterol from HDL-c to triglyceride rich lipoproteins, with reciprocal transfer of triglyceride to HDL-c leading to reductions in HDL-c levels.[21] In our study, mean HDL-c levels have decreased from Healthy to Prediabetes to Diabetes, with results being consistent with that of by Samatha et al.[22]Lalitha et al.[23] Uttra et al.[24]

Hyperglycaemia leads to lesion initiation by causing infiltration of monocytes into the subendothelial space, and subsequent accumulation of lipid loaded macrophages.[25,6] and recruitment of monocytes is regulated by endothelial adhesion molecules and their corresponding monocyte ligands mostly E-selectin, P-selectin, vascular cell adhesion

molecule-1 (VCAM-1), and intracellular cell adhesion molecule-1 (ICAM-1).[26] Progression from the fatty streak lesion to an advanced, clinically significant complex lesion encompasses encapsulation of the macrophage-rich mass by smooth muscle cells (SMCs) to form a fibrous cap, and the death of macrophages to form one or more necrotic cores, calcification of advanced plaque and intraplaque haemorrhage and rupture of thin-cap fibroatheromas and its thrombosis causes most of the clinical events in humans.[27] The long-chain fatty acids (such as palmitate, oleate, and linoleate), and the oxidised phospholipids, which are present in modified LDL-c exerts an effect similar to that of glucose by inducing vascular cell adhesion molecule (VCAM-1) expression in macrovascular cells.[28]

Elevated glucose levels in human aortic endothelial cells also results in increased release of thromboxane B2.[29] and prostaglandin E2.[30] 12(*S*)HETE (Hydroxyeicosatetraenoic acids).[31] thus stimulating monocyte adhesion to cultured endothelial cells.[32] Elevated glucose also results in increased activity of aldose reductase dependent sorbitol pathway, thus causing increased levels of Advanced Glycated End products (AGE).[33] Moreover, there is increased production of reactive oxygen free radicals from mitochondrial oxidative phosphorylation in hyperglycaemia, causing cell damage.[33] In our study, the mean CIMT of right common carotid artery, left common carotid artery, right bulb region, left bulb region and composite CIMT have increased from Healthy to Prediabetes to Diabetes, the difference being statistically significant. Results are consistent with the studies done by Kota et al[34], Ahmed et al.[35]

Results are also consistent with studies done on prediabetics by Karbek et al.[36] Parildar et al.[37] In our study, the Composite CIMT strongly, positively and significantly correlates with the total cholesterol, triglyceride and LDL-c

and negatively with HDL-c levels in the Healthy and Diabetic (Type 2 DM) subjects. But, there is hardly any significant correlation noted between composite CIMT and any of the lipid parameters in the Prediabetes group. Results are consistent with a study by Kota et al [34] and also with the Kora F4 study.[38]

Although early atherosclerotic lesions provide precursors for the formation of advanced complex plaques, these early lesions are not clinically significant and also sometimes regresses.[39] Intermediate lesions and atheroma involve increased focal accumulation of lipid within the neo intima due to increased levels of foam cells. Complicated lesion forms when the plaque becomes disrupted due to fissure, haemorrhage or thrombus formation.[6] Thus atherogenesis progresses in a chain reaction but, not in an uniform manner and it takes time to develop a lipid rich fibrous plaque core from proatherogenic dyslipidaemia. This may explain the insignificant correlation of lipid parameters with the Composite CIMT in the prediabetes population where, there is evidence of chronic hyperglycaemia and proatherogenic dyslipidaemia but the CIMT is not yet raised to that level.

In conclusion, findings of our study indicates that the biochemical changes of atherosclerosis start even before the onset of diabetes, in the prediabetes state only; thus emphasising the immense importance of prediabetes state as an atherosclerotic marker, in which, the precautionary measures can be taken for prevention of cardiovascular complications of diabetes in the future. So, we propose the need for further research in larger sample sizes in different population with different genetic factors owing to the small sample size of our study.

	Status				
	Healthy	Prediabetes	Diabetes		
	Mean±Std. Deviation	Mean±Std. Deviation	Mean±Std. Deviation	p Value	Significance
Total Cholesterol	126.97±11.24	181.29±31.6	230.89±26.13	<0.001	Significant
Triglyceride	69.5±9.43	132.29±50.28	187.02±22.73	<0.001	Significant
LDL Cholesterol	70.81±13.07	114.05±26.64	161.75±22.84	<0.001	Significant
HDL Cholesterol	42.26±3.9	40.78±6.09	31.74±4.48	<0.001	Significant
VLDL Cholesterol	13.9±1.89	26.46±10.06	37.40±4.55	<0.001	Significant
Table 1					

Table 1: Shows the Mean Total Cholesterol, Triglyceride, Low Density Lipoprotein-Cholesterol (LDL-c), High Density Lipoprotein-Cholesterol (HDL-c) and very low Density Lipoprotein- Cholesterol (VLDL-c)In Healthy, Prediabetes and Diabetes
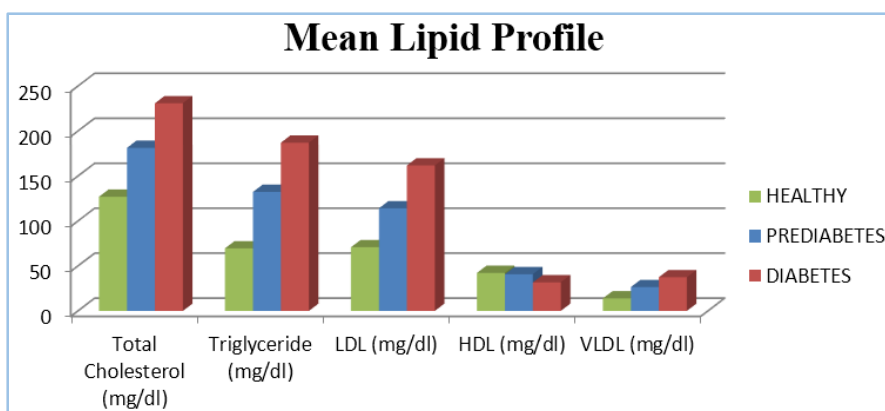

Fig. 1: Shows the Mean Total Cholesterol, Triglyceride, LDL-c, HDL-c and VLDL-c in Healthy, Prediabetes and Diabetes

	Status				
	HEALTHY	**PREDIABETES**	**DIABETES**		
	Mean±Std. Deviation	Mean±Std. Deviation	Mean±Std. Deviation	p Value	Significance
CCA RT CIMT(mm)	0.57±0.08	0.66±0.11	1.17±0.12	<0.001	Significant
CCA LT CIMT(mm)	0.56±0.07	0.7±0.08	1.14±0.13	<0.001	Significant
BULB RT CIMT (mm)	0.58±0.07	0.78±0.1	1.16±0.12	<0.001	Significant
BULB LT CIMT(mm)	0.56±0.06	0.66±0.22	1.13±0.14	<0.001	Significant
COMPOSITE CIMT(mm)	0.56±0.06	0.69±0.05	1.14±0.1	<0.001	Significant
Table 2					

Table 2: Shows mean Carotid Intima Media Thicknesses (CIMT) of Right Common Carotid Artery (CCA RT), Left Common Carotid Artery (CCA LT), Right Carotid Bulb Region (BULB RT), Left Carotid Bulb Region (BULB LT), and of the Composite Value in Healthy, Prediabetes and Diabetes
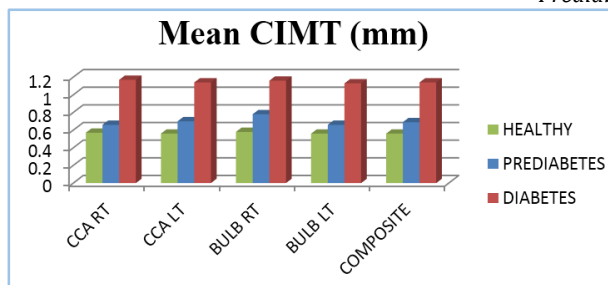

Fig. 2: Shows the Mean Carotid Intima Media Thickness (CIMT) of Right Common Carotid Artery (CCA RT), Left Common Carotid Artery (CCA LT), Right Carotid Bulb Region (BULB RT), Left Carotid Bulb Region (BULB LT) and of the Composite Value

Status			Total CHOLESTEROL
Healthy	Composite CIMT	Pearson Correlation	0.752
Prediabetes	Composite CIMT	Pearson Correlation	-0.375
Diabetes	Composite CIMT	Pearson Correlation	0.618

Table 3 : Shows Correlation of Composite Carotid Intima Media Thickness (CIMT) with Total Cholesterol in Healthy, Prediabetes and Diabetes

Status			Triglyceride
Healthy	Composite CIMT	Pearson Correlation	0.683
Prediabetes	Composite CIMT	Pearson Correlation	-0.493
Diabetes	Composite CIMT	Pearson Correlation	0.538

Table 4: Shows Correlation of Composite Carotid Intima Media Thickness (CIMT)with Triglyceride Level in Healthy, Prediabetes and Diabetes

Status			HDL
Healthy	Composite CIMT	Pearson Correlation	-0.653
Prediabetes	Composite CIMT	Pearson Correlation	0.222
Diabetes	Composite CIMT	Pearson Correlation	-0.455

Table 5: Shows Correlation of Composite Carotid Intima Media Thickness (CIMT)with the HDL Cholesterol Level in Healthy, Prediabetes and Diabetes

Status			LDL
Healthy	Composite CIMT	Pearson Correlation	0.743
Prediabetes	Composite CIMT	Pearson Correlation	-0.309
Diabetes	Composite	Pearson	0.690

	CIMT	Correlation	

Table 6: Shows the Correlation of Composite Carotid Intima Media Thickness (CIMT) with LDL Cholesterol in Healthy, Prediabetes and Diabetes

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