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Glycated apolipoprotein B—A surrogate marker of subclinical atherosclerosis

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ABSTRACT

Aims: Sustained hyperglycemia is a causative factor for glycation of proteins. Glycated low-density lipoprotein (LDL) is strongly associated with an increased risk of CAD (Coronary Artery Disease) in diabetics. Hence, we planned to evaluate the association of glycated apo B with subclinical atherosclerosis.

Method: Forty-five non obese and 45 obese diabetics were recruited. Glycated hemoglobin (HbA1c) levels were estimated by HPLC (High Pressure Liquid Chromatography) and small dense low-density lipoprotein (sdLDL) was calculated using standard formula. Plasma Insulin was done by RIA. Insulin resistance was calculated using homeostatic model assessment insulin resistance (HOMA-IR) model. Glycated apo B in serum was estimated using ELISA. Carotid intimal media thickness (CIMT) was estimated using B mode USG of carotid arteries.

Results: Glycated apo B levels were correlated significantly with fasting blood glucose (FBG) ($p = 0.001$), post prandial glucose (PPG) ($p = 0.001$), HbA1c ($p = 0.013$). The percent glycated apo B levels correlated significantly with FBG ($p = 0.032$), PPG ($p = 0.004$) in obese diabetic group.

Multivariate regression analysis of glycated apo B and percent glycated apo B, showed that glycated apo B ($p = 0.009$) and percent glycated apo B ($p = 0.006$) were significantly correlated to FPG in diabetic population. The percent glycated apo B was also significantly correlated to PPG ($p = 0.003$) and sdLDL ($p = 0.009$). CIMT levels were higher in obese diabetics with 2 plaques positive when compared to obese non diabetic controls; however levels were not statistically significant.

Conclusion: Persistent hyperglycemia and sdLDL are independently associated with glycation of apo B. Presence of plaques and increased thickness of intima indicates that glycated apo B predisposes diabetics to atherosclerosis.

1. Introduction

Diabetes is one of the leading cause of mortality affecting at least 143 million people worldwide. The prevalence of type 2 diabetes is projected to rise from the current estimated 240 million affected (6% of adults) to some 380 million (7%) by 2025 [1]. Most of this growth is expected to be in developing countries such as India and China [2]. Macrovascular complications are the leading cause of mortality and morbidity among diabetics. The most common life threatening complication being coronary heart disease (CHD). Irrespective of the ethnic background, the risk for CHD among diabetic subjects is greater by 2 to 4 folds as compared to non-diabetic subjects [3]. Diabetes is associated with subclinical atherosclerosis and CIMT (carotid intimal media thickness) is an important marker of subclinical atherosclerosis.

Low-density lipoprotein (LDL) plays a key role in the pathogenesis of atherothrombotic processes. LDLs modify the antithrombotic properties of the vascular endothelium and change vessel contractility by reducing the availability of endothelial nitric oxide and activating proinflammatory signaling pathways. LDL entering affected vessels undergo modifications including glycation and conversion to small dense form. These modifications potentiate its atherogenic properties [4]. Once modified, these intravascular small dense LDL (sdLDL) promote the formation of foam cells derived from smooth muscle cells and macrophages, thereby increasing the vulnerability of atherosclerotic plaque [4].

In diabetics, sustained hyperglycemia is important factor for glycation of various proteins. Many studies from different ethnic

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populations have reported that glycated LDL is strongly associated with an increased risk of cardiovascular disease [5]. To the best of our knowledge, little data is available regarding evaluation of role of glycated LDL in the development of atherosclerosis in diabetics among Indian population. Thus, our study was designed to assess association of glycated apo B with glycemic index and sdLDL and its role in causation of atherosclerosis in type 2 diabetics.

2. Materials and methods

A case-control study comprising of 45 obese diabetics and 45 obese non diabetic was conducted in the department of Biochemistry and Medicine at University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi, India over 11 months period.

Patients in age group 40–60 years, irrespective of gender, diagnosed with type 2 DM (diabetes mellitus) according to ADA criteria 2013 were included in the study [6]. Obesity was defined by the international diabetic federation criteria, dyslipidemia by national cholesterol education program ATP-III criteria and hypertension was defined on the basis of Joint National Committee (JNC) VII New blood pressure (BP) classifications [7–9]. All patients on lipid lowering therapy, insulin therapy, pioglitazone therapy, corticosteroids, retinoid drugs, immune suppressive drugs, beta-blockers, and steroid hormones were excluded from the study. Ethical clearance for the study was obtained from the institutional ethical committee and an informed consent was obtained from all the participants before recruitment in the study. A careful history was taken regarding smoking, consumption of alcohol, duration of diabetes, and use of antihypertensive drugs. A detailed history and physical examination including anthropometric measurements such as height, weight, waist circumference, hip circumference, BP and waist to hip ratio was recorded. Body mass index was calculated as weight in kilograms/(height in meters^2).

2.1. Biochemical investigations

Following informed and written consent, blood samples were collected from the participants and serum was separated for biochemical analysis. All blood samples were analyzed using an automatic analyzer (Olympus-AU400 Beckman Coulter, Inc. California, United States) for total serum cholesterol [10], high-density lipoprotein (HDL) cholesterol [11], and serum triglyceride using enzymatic methods [12]. LDL cholesterol was calculated using Friedewald formula [13]. Whole blood in EDTA was used to estimate glycated hemoglobin (HbA1c) levels by high-performance liquid chromatography (Bio-Rad, D10) using commercially available kit and sdLDL was calculated using standard formula [14]. It was measured by LDL cholesterol/apolipoprotein B ratio. Serum LDL-cholesterol/Apo B ratio of <1.2 was considered as atherogenic [14]. Plasma Insulin was analyzed by commercially available kit based on RIA method according to manufacturer's protocol. Insulin resistance was calculated using homeostatic model assessment insulin resistance (HOMA-IR) model [15]. Serum was stored at $-80\,^{\circ}C$ for further analysis. Serum apo B was estimated by nephelometry using NEPHSTAR^R apolipoprotein B kit [16]. Glycated apo B in serum was estimated using competitive ELISA kit (Glycacor TM by Exocell Philadelphia, USA). Percent apo B was calculated from apo B and glycated apo B.

CIMT was estimated using B mode ultrasonography of carotid arteries by a high resolution B-mode ultrasonography system (P-700, PHILIPS) having an electronic linear array, high frequency transducer for superficial scanning with a mid-frequency of 7.5 MHz [17]. The scanning was conducted at the gain setting at Time Gain Compensation (TGC) appropriate for superficial structures. The intima-media thickness as defined by Pignoli et al. was measured as the distance from the leading edge of first echogenic line to second echogenic line [17]. The first echogenic line represents the lumen-intimal interface & second line is produced by the collagen containing upper layer of adventitia. The measurements were made in common carotid & internal carotid artery after the bulb on either side. All scans were conducted by trained a personnel who was unaware of the clinical status of the study subjects. Note was made regarding the location & echo characteristics of the plaque/thickening, presence or absence of ulceration, luminal narrowing and/or calcification if any [17]. The upper limits (97.5 percentile) of intimal media thickness (IMT) at common carotid artery (CCA) for participants age 40 to 49, 50 to 59, and 60 years or older were taken as, 0.64, 0.71, and 0.81 mm, respectively [18].

2.2. Statistical method

Data were analyzed using SPSS software version 20.0. Data in the two groups were analyzed using Students-'*t*' test. Correlation studies were performed using Pearson correlation co-efficient. Bivariate and multivariate regression analysis was also carried out. A *p*-values less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic profile

Demographic profile of obese diabetics and obese non diabetic controls are tabulated in Table 1. The confounding variables (smoking, dyslipidemia and obesity) were not significantly different among both groups. Hypertension, dyslipidemia, HbA1c and glycated apo B levels were significantly ($p < 0.000$) different in both the groups (Table 2). In our study, apo B/LDL-C ratio of <1.2 was observed in 48.9% of patients of diabetes as compared to 28.9% obese non diabetic control (Fig. 1). CIMT levels were higher in obese diabetics in comparison to obese non diabetic controls. However CIMT levels were not statistically significant (Table 3).

3.2. Correlation analysis in obese diabetic group

Glycated apo B levels were correlated significantly with fasting blood glucose (FBG) ($r = 0.544$, $p = 0.000$), postprandial glucose ($r = 0.548$, $p = 0.001$), HbA1c ($r = 0.548$, $p = 0.013$). The percent glycated apo B levels correlated significantly with FBG ($r = 0.92$, $p = 0.032$) and postprandial glucose ($r = 0.329$, $p = 0.004$) in obese diabetic group. However, similar findings were not observed in obese non diabetic group. A significant correlation was also not observed between glycated apo B and percent glycated apo B with Insulin and HOMA-IR.

3.3. Multivariate regression analysis

After adjusting sdLDL, percent glycated apo B showed significant correlation with FBG ($\beta = 0.016$, $p = 0.009$) and post prandial glucose (PPG) ($\beta = 0.012$, $p = 0.003$). Similarly percent glycated apo

Table 1
Demographic and clinical profile of diabetic obese and non diabetic obese group.

Variables	Obese non diabetic group ($n = 45$)	Obese diabetic group ($n = 45$)	*p*-Value
Mean age (years)	47.08 ± 5.84	49.68 ± 7.18	0.063
W:H ratio	0.98 ± 0.056	1.00 ± 0.057	0.134
SBP (mm of Hg)	127.04 ± 10.50	132.8 ± 14.02	0.045
DBP (mm of Hg)	81.75 ± 5.49	84.08 ± 9.66	0.167
BMI (kg/m^2)	26.56 ± 4.29	25.63 ± 3.78	0.278

Values are mean $\pm$ SD, $p < 0.05$ is significant, W:H = waist to hip ratio, SBP = Systolic blood pressure, DBP = Diastolic blood pressure; BMI = Body mass index.