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Original article

Expression of Lipoprotein associated phospholipase A2 enzyme in medical undergraduate students with metabolic syndrome

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ABSTRACT

Aims: Metabolic syndrome (MS) and atherosclerosis are chronic inflammatory conditions. Lipoprotein-associated phospholipase A2 (LpPLA2) is a circulatory marker of systemic inflammation and a risk predictor for cardiovascular diseases. This study aims to evaluate the expression of this enzyme in an effort to understand the underlying mechanism of atherosclerosis in MS.
Methods: This study included twenty patients of MS and same number of healthy controls. Anthropometry and clinical examination were carried out in both groups. Real time PCR was performed for LpPLA2 mRNA and relative expression was calculated using ΔΔCT method keeping β2 microglobin and β-actin as internal controls.
Results: LpPLA2 mRNA expression was higher in patients of MS. Fold change was 5.7 when β2 microglobin was used as normaliser and 4.97 when β-actin was used. mRNA levels of LpPLA2 correlated significantly with waist circumference ($r = 0.462$, $p = 0.003$) and systolic blood pressure ($r = 0.392$, $p = 0.015$) as well as high density lipoprotein cholesterol ($r = -0.453$, $p = 0.003$).
Conclusions: High expression of LpPLA2 mRNA indicates that systemic inflammation has role in pathogenesis of atherosclerosis in patients of MS. This is evident from its direct correlation with blood pressure. The study also suggests that expression of LpPLA2 may be associated with obesity. Therefore, LpPLA2 mRNA expression levels may develop as an important risk predictor for vascular complications in MS.

1. Introduction

The present day world is experiencing a transition in the diseases pattern which is coinciding with the transition in the lifestyle of large-scale urban population. The global transition is such that the epicentre of the threat to our health especially in the urban areas is shifting from infectious diseases to chronic non communicable diseases like cardiovascular diseases (CVD), cancer, and diabetes mellitus (DM) [1–3]. Inappropriate diet with lack of physical work or exercise has to be blamed for this changing scenario, which is leading to conditions like obesity, high blood pressure, hyperglycemia, hypertriglyceridemia and low levels of HDL (High density lipoprotein) resulting in metabolic syndrome (MS). MS is characterised by a constellation of atherogenic risk factors and these patients are at higher risk of developing atherosclerosis and hence CVD [4]. It is estimated that 20–25% of South Asians have developed MS and many more may be prone to it [3]. By 2020, CVD will be the largest cause of disability and death in India, with 2.6 million Indians predicted to die due to CVD [5,6]. Various studies indicate that medical undergraduates in India as well as abroad are at an increased risk of DM and CVD as well as MS owing to their sedentary lifestyle and stress [7,8].

Recently various inflammatory markers have been extensively evaluated for their risk prediction in CVD and Lipoprotein-associated phospholipase A2 (LpPLA2) has attracted considerable interest in last decade [9–11]. Many prospective studies have also indicated that Lp-PLA2 is an independent predictor of CVD [9,10]. This enzyme has even emerged as a potential therapeutic target in CVD [12]. LpPLA2 is secreted by macrophages and monocytes. Since LpPLA2 acts on the oxidised phospholipids producing lysophospholipids and oxidised fatty acids, it is believed to be circulating marker of inflammation [13,14]. Although various studies have been conducted which have evaluated the mass or activity of this enzyme in MS [14–16] we have not come across any study evaluating the expression of gene for LpPLA2 in MS particularly in Indian population. We therefore carried out this

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study to determine the mRNA expression of LpPLA2 in the patients of MS. This may provide further insight into the evidence suggesting its role in pathogenesis of atherosclerosis and CVD in MS.

2. Subjects, material and methods

2.1. Subjects and setting

The study was a case control study conducted in the Department of Biochemistry, University College of Medical Sciences, Delhi. After obtaining the ethical clearance from the Institutional Ethical Committee for Human Research, twenty patients of MS and equal number of healthy controls were recruited from the undergraduate students of the college (those pursuing MBBS course). Both the groups were matched for age and gender. An informed written consent was obtained from each participant. Each participant was interviewed and underwent anthropometric analysis. Patients suffering from overt CVD, with past history of CVD, respiratory diseases and infections as assessed from history were excluded from the study. Careful history regarding smoking status was taken and smokers were excluded from the study. Individual on any kind of medication were also excluded. Venous blood was collected after an overnight fast in appropriate sample collection tubes (EDTA vials for determination of mRNA levels and plain vials for biochemical analysis) and was processed immediately.

2.2. Definition of the metabolic syndrome

Presence of MS was defined in accordance to the current National Cholesterol Education Program Adult treatment Panel III (NCEP/ATPIII) criteria [1]. Participants who had 3 or more of the following criteria were considered to have MS: abdominal obesity (>102 cm for men and >88 cm for women), hypertriglyceridemia (triglycerides level ≥150 mg/dl), low HDL (<40 mg/dl for men and <50 mg/dl for women), high blood pressure (≥130/85 mmHg or current use of blood pressure lowering medication), and elevated fasting blood glucose (≥110 mg/dl).

2.3. Anthropometric measurement

Waist circumference was measured at midpoint between the lower margin of the last palpable rib and the top of the iliac crest after expiration. Hip circumference was taken around the widest portion of the buttocks [17]. Weight was measured using electric weighing machine and height using wall mounted scale and these parameters were further used in deriving the Body mass index (BMI) which was calculated as weight (kg) divided by squared height (m^2). Systolic and diastolic blood pressure was obtained with a mercury sphygmomanometer using auscultatory method.

2.4. Expression analysis of LpPLA2 mRNA

Whole blood was collected in EDTA and used for gene expression analysis. Total RNA was isolated from whole blood using Tri-reagent BD from Sigma Chemicals, USA as per the instructions of manufacturer. The quality of RNA was checked by taking the optical density ratio at 260/280; a ratio of 1.8–2.0 was considered adequate. The reaction for cDNA synthesis was carried out using 200 U reverse transcriptase (Revert Aid from Thermo Scientific, Inc), 10 mM dNTPs, 20 U RNase inhibitor (Thermo Fisher Scientific, Inc), 50 mM random hexamer (Sigma Aldrich, India),and 500 ng of RNA, and the final volume made to 20 μl with DEPC treated water. First strand cDNA synthesis was carried out using a thermocycler (Eppendorf Master cycler Gradient-5331). The reaction was allowed to proceed at 25° for 10 min, 42 °C for 1 h followed by 75 °C for 10 min.

Expression of the genes was analyzed by real time PCR (Roto-Gene Q, Qiagens) using specific primers (Forward-5′-CCACC-CAAATTGCATGTGC-3′ and reverse-5′-GCCAGTCAAAAGGA-TAAACCACAG-3′-) for LpPLA2 using dye based method. β2 microglobin and β actin were used as normaliser. cDNA was amplified using specific primers, PCR master mix (Thermo Fischer Scientific) and Syto 9 dye (Life technologies Inc). All the reactions were run in duplicates. To account for between-sample differences, mRNA levels were normalised to β2 microglobin and β-actin for each sample and relative expression of the gene was analysed by ΔΔCT and fold change was calculated using $2^{-\Delta\Delta Ct}$ method.

2.5. Biochemical analysis

Blood samples were collected by venipuncture after an overnight fast for 12–14 h. Serum samples were used for routine biochemical parameters to rule out any liver and renal abnormalities. Plasma glucose, serum cholesterol and serum triglycerides were estimated using standard colorimetric enzymatic methods (Olympus AU 400, Japan). Plasma glucose was measured using glucose oxidase enzyme [18]. Serum cholesterol estimation was carried out by using cholesterol esterase and cholesterol oxidase enzymes [19]. Serum triglyceride levels were determined by glycerol kinase and glycerol phosphate oxidase method after enzymatic hydrolysis with lipase [20]. HDL-C levels was analysed by enzymatic method [21] and LDL-C was calculated by Freidwald formula [22].

2.6. Statistical analysis

Data was analyzed using SPSS software version 20.0. The data in the two groups were compared using unpaired Student *t* test. Correlation studies were carried out using Pearson Coefficient Analysis taking ΔCt as one of the variables. Multiple linear regression was also carried out and $p < 0.05$ was considered statistically significant. Collinearity and Variance Inflation Factor (VIF) was calculated wherever required.

3. Results

In the present observational case control study, all participants were aged 18–26 years and the mean age was not significantly different in the two groups. There were 4 females in control group and 3 females in patient group. Table 1 depicts the anthropometric, clinical and biochemical parameters. Abdominal circumference,

Table 1
Anthropometric, clinical and biochemical parameters.

	Controls ($n=20$)	Patients ($n=20$)	*p* Value
Age (years)	19.73 ± 0.88	20.69 ± 2.43	0.198
Hip circumference (cm)	95.13 ± 5.42	103.62 ± 7.79	0.003
Abdominal circumference (cm)	82.53 ± 10.95	105.77 ± 9.23	0.000
Waist hip ratio	0.87 ± 0.08	1.02 ± 0.08	0.000
Weight (kg)	64.27 ± 13.28	81.77 ± 14.21	0.003
BMI (kg/m^2)	22.45 ± 5.13	27.36 ± 3.88	0.008
Triglycerides (mg/dl)	0.88 ± 0.24	1.42 ± 0.52	0.003
HDL cholesterol(mg/dl)	1.29 ± 0.33	0.87 ± 0.29	0.001
LDL cholesterol(mg/dl)	2.67 ± 0.77	3.06 ± 1.18	0.285
Total Cholesterol(mg/dl)	4.38 ± 0.75	4.84 ± 1.08	0.208
Systolic BP (mmHg)	121 ± 5.68	137.69 ± 5.59	0.000
Diastolic BP(mmHg)	76.6 ± 8.33	86.38 ± 3.1	0.000
Fasting glucose (mg/dl)	4.65 ± 0.37	4.51 ± 0.29	0.382

All values expressed as mean ± SD, BMI—body mass index, HDL—high density lipoprotein, LDL—low density lipoprotein.