Distribution and effect of APO E genotype on plasma lipid in non-obese and obese in north Indian population

Authors

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Abstract

Introduction: Apolipo protein E (apo E) plays key role in lipid metabolism, obesity and accordingly in development of diabetes and coronary heart disease (CHD).
Methods: Apo E polymorphism was performed using polymerase chain reaction- restriction fragment length polymorphism in 80 non-obese and 80 obese patients was collected from northern India. Levels of lipids and glucose were determined in these subjects.
Results: The genotype and allele frequencies of APO E polymorphism in non-obese showed no significant statistical difference compared to obese patients. In obese group, the subjects with E4E4 genotype had significantly lower levels of HDL and higher levels of triglycerides, LDL, HDL/LDL, TC/HDL and TG/HDL compared to E2E3/E3E3 genotype. E4 allelic carrier had significantly higher levels of LDL, Total cholesterol and LDL/HDL compared to non-carrier.
Conclusions: The APO E gene polymorphism is associated with altered plasma levels of HDL, LDL, VLDL and triglycerides in north Indian population.
Keywords: Obesity, Dyslipidemia, Genetic polymorphism, APO E gene polymorphism, lipid profile.

Introduction

According to WHO, Obesity is one of the most common and most neglected, public health problems in the world¹. Obesity is the undesirable positive energy balance and weight gain. However, obese subjects differ not only in the amount of excess fat that they store, but also in the area of distribution of that fat within the body. The distribution of fat induced by weight gain affects the risks related to obesity, and the kinds of disease that result. The prevalence of overweight and obesity has been increasing in countries of the South Asian region, with adverse effects on health²,³. Obesity can be defined as the presence of an excess of body fat. A surrogate marker for body fat content is the body mass index (BMI), which is determined by weight (kilograms) divided by height squared (square meters). The value of BMI ≥ 23 kg/m² used to define overweight and ≥ 25 kg/m² to define obese,
Obesity is a high-risk factor for cardiovascular disease (CVD). Cardiovascular morbidity and mortality of obesity are related to classic risk factors, namely dyslipidemia, hypertension, and impaired glucose metabolism. These risk factors, known as the predictors of future cardiovascular disease, make up what is known as the metabolic syndrome. Obesity is determined by many genetic and environmental factors. The Dyslipidemia commonly seen in obese patients may be due to altered cholesterol metabolism. Obese patients observed with elevated cholesterol synthesis compared with normal-weight individuals.

The apolipoprotein E gene (APO E) is a 35 KD glycosylated protein produced mainly by liver and is located on chromosome 19q 13.2 and consists of four exons and three introns. APO E gene is polymorphic having three common alleles, designated as ε2, ε3 and ε4 which code for E2, E3 and E4 proteins, respectively. The three homozygous (E2/E2, E3/E3, E4/E4) and three heterozygous (E3/E2, E4/E2 and E4/E3) phenotypes are found in the general population. The three alleles differ in such properties as its binding affinity to apo E and low-density lipoprotein receptors, and its affinity for lipoprotein particles. The main role of apo E in lipid metabolism is as a ligand for receptor-mediated clearance of chylomicron and VLDL remnants and also in reverse cholesterol transport. Lipoproteins play an important role in the development of atherosclerotic cardiovascular disease in humans and the levels of lipoproteins in blood are determined by apolipoproteins present on their surface. The three common isoforms of apo E (E2, E3 and E4), differ at their amino acid residues 112 and 158 on it. E2 has cysteine residues at both sites 112 and 158 (Cys 112, Cys 158) and E4 has arginine residues at both sites (Arg 112, Arg 158), however, E3 has a cysteine at position 112 and an arginine at position 158. The amino-terminal region of apo E is responsible for binding of apo E to the LDL receptor whereas the carboxy-terminal mediates the binding of apo E to the surface lipoproteins. The apo E2 and apo E4 are metabolically different from apoE3. The apo E4 has arginine at position 112 and binds selectively to TG-rich lipoproteins like VLDL but apo E2 and E3 bind only to HDL. The VLDL-apo E4 particles are removed faster from plasma than VLDL-apo E3 particles resulting in a down regulation of the LDL receptor.

To the date, there are no information available for APO E polymorphism in non-obese and obese patients and its role in lipid metabolism in north Indian population. In the present study, we investigated the frequency distribution of APO E gene polymorphism in non-obese and obese patients and its association with lipid parameters in north Indian population.

**Material and Methods**

**Study Population and Design**

This study was conducted in subjects residing in north Indian population. A total of 80 non-obese healthy and 80 obese subjects were recruited from out-patients Department of Medicine, Sharda Hospital, Greater Noida, UP, India. The study was conducted in the Department of Biochemistry, School of Medical Sciences & Research, Sharda University, knowledge park-III, Greater Noida and the department of Biochemistry, Santosh Medical College & Hospital, Ghaziabad and IHBAS, New Delhi. Control group comprised of age and sex matched healthy volunteers. The study was approved by Institutional Ethics Committee and written informed consent was obtained from all study subjects.

**Anthropometric Profile**

Body weight and height were measured with the subject barefoot and wearing light clothing. BMI was calculated as weight in kilograms over height in meters squared. Waist circumference was...
measured at the midpoint between the lower limit of the rib cage and upper border of the iliac crest. Blood pressure was recorded in a sitting position of the right arm to the nearest 2 mmHg using mercury sphygmomanometer. Two readings were taken 5 min apart and the mean was taken as the blood pressure. Body mass index (BMI) was calculated from height and weight measurements using the formula: BMI = body weight/(height)$^2$ in kg/m$^2$. According to the World Health Organization guidelines for Asians, individuals with BMI $\geq 25$ kg/m$^2$ are classified as obese.

Biochemical Estimations
Venous blood was drawn from each individual after an overnight fast of $\geq$12 h for estimation of metabolic variables. Serum lipids i.e. cholesterol, triglycerides, high density lipoprotein (HDL) was estimated by commercially available kit (Accurex Biomedical, India) using a Star 21 Plus Semi automatic Biochemistry Analyzer (Rapid Diagnostics, India).

APOE Genotyping
Genomic DNA was isolated using peripheral blood by gDNA isolation kit (Chromous Biotech, Bangalore, India). Genotyping of the fourth exon of APOE was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) using specific primers 5'-ACAGAATTCGCCCGCCTGGTAC-AC-3' and 5'-TAAGCTTGGCAGCCTGTCCAAGGA-3' as described by Pandey et al. (2007). PCR assay was performed on Bio-Rad cycler, USA, in a 20μl volume of 1x a high-fidelity master mix (Bio-Rad, USA) containing high-fidelity buffer, 0.04U/μl DNA polymerase, 1.5mM MgCl2, 200μM dNTPs. 7.5% dimethyl sulfoxide (DMSO), 10 ng of genomic DNA, 0.5 μM of each primer was used in final concentration. The cycling condition of PCR compromised of a 3 minute of denaturation at 95°C followed by 40 cycles of 95°C (30 sec), 63.3°C (30 sec.) and 72°C (30 sec) and final extension 72°C for 3 minutes. The PCR product (244 bp) was digested with 10 units of Hhal overnight at 37°C and run on 15 % polyacrylamide gel followed by the ethidium bromide staining. Their sizes were determined by comparison with Fast Ruler Ultralow Range DNA Ladder (Thermo Fisher Scientific, USA).

Statistical Analysis
The frequency distribution of genotypic and allele was compared between non-obese and obese using chi-square test. Odds ratios (OR) and its 95% confidence intervals (CI) were calculated using 2 X 2 contingency tables. An ANOVA was carried out to evaluate the association of APO E polymorphism with biochemical parameter and lipid profile. In all cases, a p-value of $<0.05$ was considered statistically significant. All analyses were carried out using GraphPad Prism 6 software (http://www.graphpad.com/quickcalcs/ttest1/) and vassarstats.net. (http://www.vassarstats.net).

Results
In this study, we recruited the total of 160 subjects, in which study group comprised of 80 obese subjects aged 17-55 years (mean age 28.81±12.1 years) and 80 non-obese healthy controls (mean age 21.69±7.9 years). Five different genotypes of APO E were observed in this study. The frequency distribution of E3E3 genotype was most frequent and comparable in non-obese (63.75%) and obese (53.75%). The frequency distribution of E3E4, E4E4, E2E4 genotype of APO E gene and E4 allele carrier were high in obese as compared with non-obese (30% vs. 23.75%, 3.75% vs.1.25%, 2.5% vs. 0% and 36.25% vs. 25%, respectively), increasing the susceptibility to obesity by three folds when E4E4 genotype was present (OR=3.08, 95% CI: 0.448-40.42), Similarly when E3E4 genotype was present (OR =1.15, 95% Cl: 0.5598-2.268) and when E4 allele carrier was present (OR=1.7, 95% CI:0.8809-3.416) (Table 1). To assess the possible impact of the APO E polymorphism on lipid metabolism, we determined the level of lipid profile in serum in different APO E genotypes in obese group (Table
2). In obese group, the subjects with E4E4 genotype had significantly lower levels of HDL and higher levels of triglycerides, LDL, VLDL, HDL/LDL, TC/HDL and TG/HDL compared to E2E3/E3E3 genotype. E4 allelic carrier had significantly higher levels of LDL, Total cholesterol and LDL/HDL compared to non-carrier (Table: 3). No significant genotypic effect on total cholesterol levels for the APO E genotype was observed in our study.

**Table 1: APO E gene polymorphism genotype and allelic frequency in total subjects.**

| Genotype | Total Subjects | Obese (n=80) | Non-obese (n=80) | p-value | OR (95% CI) |
|----------|----------------|--------------|------------------|---------|-------------|
|          |                | n | % | n | % |           |             |
| E2E3     |                | 8 | 10 | 9 | 11.25 | >0.9999 | 0.8765 (0.3169-2.28) |
| E2E4     |                | 2 | 2.5 | 0 | 0 | 0.4969 | *             |
| E3E3     |                | 43 | 53.75 | 51 | 63.75 | 0.0183 | 0.433 (0.2206-0.8782) |
| E3E4     |                | 24 | 30 | 19 | 23.75 | 0.7212 | 1.15 (0.5598-2.268) |
| E4E4     |                | 3 | 3.75 | 1 | 1.25 | 0.6202 | 3.078 (0.448-40.42) |
| E4Carrier|                | 29 | 36.25 | 20 | 25 | 0.1697 | 1.706 (0.8809-3.416) |

* = not significant value

**Table 2: Association of APOE genotypes with anthropometric and biochemical parameters.**

| Parameters (mg/dl) | E2E3 (n=17) | E3E3 (n=94) | E3E4 (n=43) | E4E4 (n=4) | p value |
|-------------------|-------------|-------------|-------------|-------------|---------|
| **Anthropometric characteristics** | | | | | |
| Height (m)        | 1.59±0.1   | 1.63±0.1   | 1.6±0.08    | 1.6±0.1    | **Ns** |
| Weight (Kg)       | 74.7±14.7  | 79.32±12.05| 78.16±10.9 | 79.33±21.9 | **Ns** |
| BMI (kg/m²)       | 29.17±1.94 | 29.90±4.4  | 29.12±2.7  | 30.44±4.3  | **Ns** |
| Hip circumference (cm) | 102±5.6 | 108.16±9.6 | 106.58±6.6 | 108±7.2 | **Ns** |
| Waist circumference (cm) | 98.25±10.2 | 100.41±9.0 | 99.04±10.8 | 99.33±12.8 | **Ns** |
| Waist hip ratio   | 0.96±0.1  | 0.93±0.08  | 0.93±0.08  | 0.91±0.06  | **Ns** |
| Waist height ratio| 0.61±0.04 | 0.61±0.07  | 0.60±0.06  | 0.62±0.03  | **Ns** |
| Systolic P         | 125±13.0   | 123.55±8.4 | 121.79±6.8 | 120±0      | **Ns** |
| Diastolic P        | 80±2.6     | 81.93±5.2  | 82.70±5.1  | 80±0       | **Ns** |
| **Biochemical parameters** | | | | | |
| Fasting blood sugar | 91.24±25.3 | 87.24±15.8 | 88.14±15.3 | 81.75±9.1 | **Ns** |
| Lipid profile      | | | | | |
| HDL                | 47.4±10.9  | 43.5±10.2  | 42.19±11.4  | 33.75±2.0  | * p=0.017 |
| LDL                | 29.56±7.1  | 27.45±2.8  | 30.31±4.6  | 18.51±9.2  | * p=0.015, * p=0.014 |
| Total Cholesterol  | 176.1±34.4 | 177.5±26.7 | 186.7±29.7 | 202.5±8.8  | **Ns** |
| Triglycerides      | 122.8±49.5 | 127.5±55.0 | 134.0±40.3 | 187.0±21.4 | * p=0.021, b p=0.034, c p=0.013 |
| VLDL               | 23.4±7.6   | 25.09±9.7  | 26.72±8.1  | 41.75±5.9  | * p=0.0003, b p=0.001, c p=0.0008 |
| HDL/LDL            | 2.39±1.1   | 2.68±1.0   | 3.06±1.2   | 3.75±0.3   | * p=0.033 |
| TC/HDL             | 4.0±1.3    | 4.31±1.3   | 4.74±1.4   | 5.52±0.7   | * p=0.039 |
| TG/HDL             | 2.86±1.1   | 3.16±2.0   | 3.39±1.3   | 4.65±1.4   | * p=0.013 |

Parameters represented in mean ± SD and ANOVA test applied.
n = number of subjects, ns – not significant
a – E2E3 vs. E4E4, b – E3E3 vs. E4E4, c – E3E4 vs. E4E4
Table 3: Association of E4 non-carriers and carriers with anthropometric and biochemical parameters.

| Parameters                          | E4 Non-Carrier (n=111) | E4 Carrier (n=49) | p value |
|-------------------------------------|------------------------|-------------------|---------|
| **Age**                             | 26.23±11.0             | 26.95±11.1        | Ns      |
| **Anthropometric characteristics**  |                        |                   |         |
| Height (m)                          | 1.65±0.1               | 1.65±0.09         |         |
| Weight (Kg)                         | 70.71±12.8             | 72.20±12.36       |         |
| BMI (kg/m²)                         | 25.96±4.6              | 26.53±3.95        |         |
| Hip circumference (cm)              | 101.62±9.6             | 101.63±8.7        |         |
| Waist circumference (cm)            | 91.75±11.2             | 92.75±12.1        |         |
| Waist hip ratio                     | 0.90±0.08              | 0.91±0.07         |         |
| Waist height ratio                  | 0.55±0.08              | 0.56±0.07         |         |
| Systolic P                          | 121.22±8.1             | 119.65±7.3        |         |
| Diastolic P                         | 80.25±5.5              | 80.40±5.2         |         |
| **Biochemical parameters**          |                        |                   |         |
| Fasting blood sugar (mg/dl)         | 87.85±17.5             | 87.61±14.8        | Ns      |
| **Lipid profile**                   |                        |                   |         |
| HDL (mg/dl)                         | 44.04±10.4             | 42.16±11.3        | Ns      |
| LDL (mg/dl)                         | 107.81±28.3            | 117.69±29.5       | p=0.0465|
| Total Cholesterol (mg/dl)           | 177.31±27.8            | 187.36±28.4       | p=0.0382|
| Triglycerides (mg/dl)               | 127.35±54.0            | 137.73±42.0       | Ns      |
| VLDL (mg/dl)                        | 25.09±9.7              | 27.48±8.4         | Ns      |
| LDL/HDL                             | 2.64±1.1               | 3.05±1.2          | p=0.0378|
| TC/HDL                              | 4.27±1.3               | 4.75±1.4          | p=0.0387|
| TG/HDL                              | 3.11±1.9               | 3.47±1.3          | Ns      |

Parameters represented in mean ± SD and ANOVA test applied, n = number of subjects, ns – not significant.

**Discussion**

Obesity is the main cause of dyslipidemia because obesity leads to impaired peripheral trapping and increased fluxes of free fatty acids from adipocytes to the liver and other tissues as well as hepatic overproduction of very low density lipoprotein, decreased circulating TG lipolysis and the formation of small dense LDL. The effect of the APOE polymorphism on the TC/HDL-C and apo A-I/apo B ratios is modulated by BMI z-score and adiponectin levels. Therefore, it is crucial to investigate the association of APO E gene polymorphism with dyslipidemia, considering the contribution of obese status.

A number of studies from different parts of India have been conducted to find out if there is any association of Apo E gene polymorphism and dyslipidemia. It was found that allele frequencies ranged from 0.031 to 0.094 for e2; 0.803-0.968 for e3 and 0.000-0.133 for e4. A comparable frequency of e4 allele was found in Eastern part of India. Moreover, the individuals with e3/4 combination as well as those homozygous for e4 allele (e4/4) had considerably higher prevalence of dyslipidemia as compared to other genotypes. A study conducted in Northern part of India also showed a high frequency of ApoE e3 allele (0.913). A significant association of Apo E e4 allele and e3/e4 genotypes with high-density lipoprotein cholesterol and low-density lipoprotein cholesterol suggests a relationship between ApoE polymorphism and development of CHD. Our results in the north Indian population living in Delhi showed that the polymorphism of the APO E gene was associated with HDL, LDL, TG and VLDL concentrations in the population. These results provide support for the notion that APOE E are involved in the regulation of TG and LDL metabolism and thus might play an important role in lipid and cholesterol transport. Several studies have reported positive associations of the APO E4 allele with increased LDL-C
concentrations or atherosclerosis. This is consistent with our present results. A mechanism may account for the association of the E4 allele with hypercholesterolemia (e.g., increased LDL-C). Because of the enhanced catabolism of lipoproteins that contain APO E4, more cholesterol is delivered to liver cells by apo E-mediated uptake in subjects with an E4 allele. Numerous studies revealed the influence of APO E genotypes on TG levels. The results of a meta-analysis indicated a consistent relationship between plasma TG levels and APO E allele in different populations where as we found TG concentrations were higher in E3E3, E2E3, E3E4, and E4E4 than in E2E3 in the present study. Several studies suggested the association between APO E genotype and HDL-C levels. We have found a similar association of APO E genotype with HDL levels in obese subjects.

Conclusion
In summary, we have shown that polymorphism of the APO E gene is associated with altered plasma LDL, HDL, TG and VLDL concentrations suggesting a role of APO E gene as a key peripheral contributor to the development of obesity and related metabolic dysfunctions. Further prospective investigations in large populations among various races are required to confirm these findings.

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Conflicts of Interests
The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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