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Clinical Implication of ApoB (12669G/A) Gene Polymorphism and Risk of Cardio Vascular Disease in Indian Population

Original Article

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ABSTRACT

Background: *Cardiovascular disease is rising day by day due to having high fat diet and due to genetic alterations.* ***Materials & Methods:*** *Study included 70 CVD patients and their peripheral blood samples were collected for genotyping by venipuncture under aseptic condition in EDTA vials (2ml) as well as in serum vials (3ml) for biochemical parameters. Genomic DNA extraction was done by phenol chloroform method from blood samples collected in EDTA vials from cases as well as controls for genotype study.* ***Results:*** *The difference of genotype between cases and controls was found to be significant (p=0.0003). Study observed that high percentage of GA 29 (41.4%) and AA 8 (11.4%) genotype was found in patients compared to controls GA 10 (20%) and AA 0 (0%) while lower GG 33 (47.2%) genotype in patients compared to control GG 40 (80%) genotype. Compared to the GG genotype, the OR 3.51 (1.49-8.25) and 20.55 (1.14-369.6) for the heterozygous GA and homozygous AA genotypes were estimated, suggesting a possible dominant effect of Apo B polymorphism on CVD risk. In smokers, compared to the GG genotype, the OR 2.19 (0.69-6.88) and 1.71 (0.29-9.87) for the heterozygous GA and homozygous AA genotypes. In alcoholism, compared to the GG genotype, the OR 2.66 (0.93-7.57) and 8.4 (0.92-76.19) for the heterozygous GA and homozygous AA genotypes. Patients with mutant homozygous AA, heterozygous GA genotypes showed 123.3±14.34 (mg/dl) and 76.92±24.09 Apo B level in CVD patients compared to wild type GG homozygous genotypes were 70.82±17.12.* ***Conclusion:*** *It was observed that Apo B gene polymorphism and smoking behaviour found to be associated with increased risk of CVD in Indian population.*

Key words: *Cardio vascular disease, apoB polymorphism, Clinical importance.*

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INTRODUCTION

Cardiovascular disease patients are rising day by day due to over consumption of fats or due to genetic alterations. It is a major cause of morbidity and mortality in young age patients. It not only delayed clinical diagnosis but also increased risk manifold and resulted in unintended death of patients. Therefore, an early identification and treatment are much needed to accelerate disease prevention and morbidity expansion.[1] Numerous physiological biomarkers have been identified that are associated with increased cardiovascular risks. Some of them are simple traditional biomarkers based on serum lipid profile and risk factors. More often, levels of plasma, serum, and blood are proved to be best cardiovascular risk biomarkers.[2] Atherosclerotic cardiovascular disease is now considered the major cause of chronic illness and most common type of dyslipidemia in patients with high plasma triglyceride levels, lower HDL levels and a predominance of small dense LDL particles, very often together with normal LDL cholesterol levels.[3] Several studies have been reported that the total plasma ApoB level is even a better predictor of cardiovascular events than the LDL cholesterol level.[4,5] Studies performed Beekman M et al have shown that genetic component accounts for 50 – 60% of the variation in the plasma ApoB levels.[6] The ApoB gene

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is located on the short arm of chromosome 2, and has at least 24 polymorphic sites.[7] The ApoB polymorphism has been reported to be associated with atherosclerosis development more than 20 years ago.[8] Apo B is the sole component of low-density lipoprotein (LDL) particles and plays an important role in the homeostasis of LDL cholesterol in plasma. Genetic alterations in the apolipoprotein B gene (APOB) reduce the binding of apo B to LDL receptors and the clearance of plasma LDL, causing a disorder known as familial ligand-defective apo B.[9] The APOB gene variations influence circulation cholesterol concentration and affect susceptibility to coronary artery disease.[10]

METHODS

Study Subjects and selection criteria

Present study included 70 CVD patients and their peripheral blood samples were collected by venipuncture under aseptic condition in EDTA vials (2ml) as well as in serum vials (3ml). Blood collected in EDTA vials was used for DNA extraction and serum vial samples were used for biochemical analysis. Study was carried out on in the department of biochemistry in association with department of medicine, suffering from hyperlipidaemia and 50 normal healthy subjects, of V.F.H.T Medical College and associated Memorial hospital Haldwani. Patient (age 30-65 years) with total cholesterol more than 240mg/dl and triglyceride more than 250mg/dl were taken as case, They were free from diabetes mellitus, hypothyroidism and hypertension. They were not taking any drug that causes hyperglycemia or hypoglycemia or affect the lipid metabolism. Subjects with total cholesterol less than 200mg/dl and total triglyceride less than 200mg/dl were taken as control.

DNA extraction and Genotyping

Genomic DNA extraction was done by phenol chloroform method from blood samples collected in EDTA vials from cases as well as controls. Genomic DNA was analysed on 1% agarose gel and observed under UV transilluminator. Isolated DNA was then polymerized to determine the genotypes at EcoR1 (rs1042031) polymorphic site of apoB by polymerase chain reaction by using site specific primers and using PCR master mix. For EcoR1 Site primers are Forward primer (F) 5'- CTG AGA GAA GTG TCT TCG AAG -3' Reverse primer (R) 5'- CTC GAA AGG AAG TGT AAT CAC -3' were used. PCR was performed in 50 µl reaction volume containing 3 µl of 100 ng template DNA, 0.25 µl, 25 pmol each primer 2.5 µl, 10 mM dNTPs, 1.5 µl of 20mM $MgCl_2$, 0.3 µl of 5 U/ µl Taq polymerase with 2.5 µl of 10X Taq Buffer (Fermantas) and 14.7 µl of nuclease-free ddH2O. The PCR was performed with initial denaturation at 94^0C for 10 minutes, followed by 40 cycles of denaturation at 95^0C for 40 seconds, annealing at 58^0C for 40 seconds, extension at 72^0C for 40 seconds and the final extension was at 72^0C for 10 minutes. PCR product of 480 bp subjected to EcoR1 for digestion and after digestion by EcoR1, two fragments of 223 bp and 257 bp were observed under agarose gel electrophoresis under UV light.

Statistical Analysis

Genotype frequencies between the cases and controls were evaluated using the Chi square test and values below 5 were analyzed by Fisher exact test. The associations between apoB genotypes and risk of CVD were estimated by computing the odds ratios (ORs) with 95 % confidence intervals (CIs). Mann- Whitney U test and Kruskal Wallis test were used to analyse the quantitative data. p value less than 0.05 considered to be statistically significant.

RESULTS

Study population:

All demographic features of the subjects are depicted in Table 1. In brief, total of 70 CVD patients and 50 number of healthy control were analyzed. Cases include 51 (72.8%) males and 19 (27.2%) females of age ≤50 group 29 (41.4%) and >50 group 41 (58.6%) with mean ±SD in cases of 52.57±7.42. 50 (71.4%) and 42 (60%) cases were smoker as well as alcoholic.

Table 1: Distribution of selected characteristics among CVD patients and healthy controls.

Variables	CVD patients (%)	Healthy controls (%)
Total no.	70 (100%)	50 (100%)
	Gender	
Males	51 (72.8%)	42 (84%)
Females	19 (27.2%)	8 (16%)
	Age at diagnosis (Years)	
≤50	29 (41.4%)	25 (50%)
> 50	41 (58.6%)	25 (50%)
Mean ± SD age (years)	52.57±7.42	50.88±9.04
	Smoking status	
Yes	50 (71.4%)	25 (50%)
No	20 (28.6)	25 (25%)
	Alcoholism	
Yes	42 (60%)	27 (54%)
No	28 (40%)	23 (46%)

Genotype distribution among cases, controls and CVD risk:

The difference of genotype between cases and controls was found to be significant (p=0.0003) (table 2). Study observed that high percentage of GA 29 (41.4%) and AA 8 (11.4%) genotype was found in patients compared to controls GA 10 (20%) and AA 0 (0%) while lower GG 33 (47.2%) genotype in patients compared to control GG 40 (80%) genotype.

Odds ratio with 95 % confidence intervals was calculated for each group to estimate the degree of association between the apoB genotype and risk of CVD in Indian patients depicted in Table 3. Compared to the GG genotype, the OR 3.51 (1.49-8.25) and 20.55 (1.14-369.6) for the heterozygous GA and homozygous AA genotypes were estimated, suggesting a possible dominant effect of apoB polymorphism on CVD risk.

Risk associated with smoking and alcoholism was calculated in CVD patients and found to be associated with more risk of CVD in smokers and alcoholism (table 4). In smokers, compared to the GG genotype, the OR 2.19 (0.69-6.88) and 1.71 (0.29-9.87) for the heterozygous GA and homozygous AA genotypes. In alcoholism, compared to the GG genotype, the OR 2.66 (0.93-7.57) and 8.4 (0.92-76.19) for the heterozygous GA and homozygous AA genotypes.

Serum apoB level with respect to apoB (12669G/A) genotypes:

It was observed that the apoB (12669G/A) polymorphism were significantly found to be associated with serum apoB level in CVD patients (0.0002). Patients with mutant homozygous AA, heterozygous GA genotypes showed 123.3±14.34 (mg/dl) and 76.92±24.09 apoB level in CVD patients compared to wild type GG homozygous genotypes were 70.82±17.12 (table 5).

Biochemical parameters in CVD patients and healthy controls:

Other markers such as cholesterol LDL, HDL, TG, apoB were compared between CVD patients and healthy controls were shown in table 6. High cholesterol, LDL, TG and apoB were observed in CVD patients while low HDL were observed compared to healthy control group. 310.4±48.90, 207.5±67.09, 408.8±110.2, 79.34±25.57 cholesterol, LDL, TG, apoB level were observed in CVD patients while 145.8±47.97, 83.62±38.06, 130.4±70.70 and 64.53±21.46 level were observed in healthy controls. Low HDL level (29.60±7.07) were observed in CVD patients while higher HDL level (36±9.34) were observed in healthy controls and differences were found to be significant.

Table 2: Genotype distribution and allele frequencies of apoB (12669G/A) among CVD patients and controls.

Variables	GG (%)	GA (%)	AA (%)	p value
Patients(n=70)	33 (47.2%)	29 (41.4%)	8 (11.4%)	0.0003
Controls(n=50)	40 (80%)	10 (20%)	0 (0%)	

Table 3: Risk of CVD associated with apoB (12669G/A) genotype.

Genotype	Healthy controls (n=100)	CVD patients (n=100)	OR (95% CI)
GG (ref)	40	33	-
GA	10	29	3.51 (1.49-8.25)
AA	0	8	20.55 (1.14-369.6)

Table 4: Risk of apo B genotype in CVD patients associated with smoking and alcoholism.

Genotype	Non-smokers (n=20)	Smokers (n=50)	OR* (95% CI)
GG (ref)	12	21	-
GA	6	23	2.19 (0.69-6.88)
AA	2	6	1.71 (0.29-9.87)
Genotype	**Non-alcoholic (n=28)**	**Alcoholic (n=42)**	**OR* (95% CI)**
GG (ref)	18	15	-
GA	9	20	2.66 (0.93-7.57)
AA	1	7	8.4 (0.92-76.19)

Table 5: Serum apoB level of CVD patients with different genotypes.

Genotype	CVD patients (n=70)	apoB level (mg/dl) (Mean ± SD)	p value
GG	33	70.82±17.12	
GA	29	76.92±24.09	0.0002
AA	8	123.3±14.34	

Table 6: Serum markers level of CVD patients and healthy controls.

Serum markers	Healthy controls (Mean ± SD)	CVD patients (Mean ± SD)	p value
Cholesterol	145.8±47.97	310.4±48.90	<0.0001
LDL	83.62±38.06	207.5±67.09	<0.0001
HDL	36±9.34	29.60±7.07	0.0006
TG	130.4±70.70	408.8±110.2	<0.0001
Apo-B	64.53±21.46	79.34±25.57	0.0005

DISCUSSION

In the present study we observed significant difference in distribution of apoB genotypes in CVD patients, an independent association of mutant AA genotype and GA heterozygous genotype apoB polymorphism were associated with increased risk of CVD. It was observed that the GA and AA genotype in patients showed more than 3 and 20 fold increase risk of CVD compared to healthy controls. Patients with smoking had GG and AA genotypes showed more than 2 and 1 fold higher risk of CVD while alcoholic patients with GA and AA genotypes showed more than 2 and 8 fold higher risk of CVD compared to non-smokers and non-alcoholism. It was observed that apoB polymorphism was found to be associated with increased level of serum apoB level. Patients with heterozygous GA and AA genotype showed higher serum apoB level compared to wild type homozygous GG genotype.

Low-density lipoprotein (LDL) is 75% lipid (cholesterol and cholesteryl esters) and 25% protein. A high level of LDL is a risk factor for cardiovascular disease.[11] Apolipoprotein B (ApoB) plays a central role in lipid metabolism as the main protein component of VLDL and LDL. It also serves as the ligand for removal of LDL from the circulation by LDL-receptor-mediated endocytosis.[12] ApoB is the protein primarily responsible for transporting cholesterol in LDL to tissues. Although it is uncertain what functional role ApoB plays in LDL, it is absolutely required for its formation. ApoB on the LDL particle acts as a ligand for LDL receptors in various cells throughout the body. High plasma ApoB levels are a factor contributing to plaques that cause atherosclerosis.[13] Various studies have shown an association between the Apo B gene polymorphisms with lipoprotein subfractions (TC, LDL-and TG).[14] The frequency of apoB mutated allele has been shown to be more dominant in CAD patients as in comparison with normal subjects in some hence, indicating that subjects having the mutated allele are more likely to be amenable to CAD development.[15] A number of Apo B polymorphisms have been found to be linked to serum lipoprotein levels in many children and adult populations.[16] In fact, genetic polymorphisms at the Apo B have been associated with elevated plasma concentrations of LDL cholesterol, and hence, it has been conjectured to be also linked to atherosclerosis and increased risk for CAD.[17] Nucleotide changes in the apolipoprotein B gene (APOB) reduce the binding of apo B to LDL receptors and the clearance of plasma LDL, causing a disorder known as familial ligand-defective apo B.[18]

CONCLUSION

Study conclude that apo B gene polymorphism was found to be associated with Cardio Vascular Disease and risk was linked with heterozygosity and mutant homozygosity. Smoking and alcoholism increases the risk of occurrence of CVD.

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