Prediction of Cardiovascular Disease Event in Type 2 Diabetes Mellitusby Gene Variants and Abnormalities of Lipid Profile

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Abstract
Background
Cardiovascular diseases continue to be a leading cause of morbidity and mortality among adults worldwide.A total of 180 subjects, aged 15 years and older were selected for the case control study from of Telangana and Andhra Pradesh states in India. For the study90 diabetic cases and 90 controls were selected.The study aim was to validate the association of gene variants in APOC III(rs121918381), AGT(rs699,rs4762)and lipid abnormalities in prediction of cardiovascular disease event in the discrete study population. The results showed increased values of lipid profile characteristics in both the diabetic cases and some of the controls. The gene variants of APOC III gene are present in 43.9% of the population and AGT gene of rs699 is present in 48.7%. The SNP rs4762 in AGT gene is not reported in our study population. It can be safely concluded that increased lipid profile characteristics and presence of the genetic variants are the risk factors for future cardiovascular event.

Keywords: Cardiovascular diseases, Risk factors, Lipid abnormalities, Gene variants, APOC III-Apolipoprotein C III, Angiotensinogen, T2DM –Type 2 Diabetes Mellitus.

Introduction

Diseases of the heart and circulatory system are called cardiovascular diseases(CVD).It is estimated that about 23.6 million people will die from cardiovascular disease by 2030 [1,2]. There are more than 1.2 billion people suffering with heart disease in India. Due to intake of high fat diets and physically inactive lifestyle 51 per cent of men and 48 per cent of woman are having heart disease [3]. They account for 60 percent of the heart disease patients' worldwide. Theyare the main cause of death and with most of them occurring in low-and middle-income countries. It has emerged as the number one killer among Indians. They are responsible for about 25 per cent of deaths in the age group of 25- 69 years.

There multifactorial link between diabetes and CVD is complex. They arethe important risk factors for each other [4]. The abnormal lipid profile is the leading cause of death in type 2 diabetes [5]. Along with diabetes several modifiable and non-modifiablerisk factors define the increased risk for CVD [6,7]. They can be influenced by variants in genes or by lipid and lipoprotein abnormalities [7,8].

Lipid Profile

Lipids are transported in the blood mainly in the form of Cholesterol and Triglycerides. High Density lipoprotein (HDL), low-density lipoprotein (LDL), and Very low density lipoproteins (VLDL) are the different forms of cholesterol. The abnormalities in the plasma and blood levels of lipids are proved to be best cardiovascular risk biomarkers [10]. The hypercholesterolemia, hypertriglyceridemia independent of each also plays an important role in the prediction of cardiovascular event [11].

The other risk factorswhich influence lipid profile are smoking, metabolic factors such as overweight, obesity and smoking [10-13]. The dietary intake ofhigh fat and low fruit/vegetable while physical inactivity or sedentary life styles areresponsible for abnormal lipid profile.

In addition to these biochemical traits, people genetic abnormality in form of Single Nucleotide Polymorphisms in the Genes show increased risk for CVD [14,15].

Apolipoprotein C III (APOC III)

APOC III is the product of this gene that induces the development of hypertriglyceridemia (dyslipidemia)by delaying the catabolism oftriglyceride-rich particles [16]. Apolipoprotein C III is a major protein of very low density lipoprotein (VLDL). It is the product

of APOC III gene. The unusually high amounts of ApoC-III are present in patients with single nucleotide polymorphism rs121918381. The risk of atherosclerosis is increased in dyslipidemia lead to heart attack due to increase in VLDL.

Angiotensinogen (AGT)

The angiotensinogen (AGT) gene belongs to a family of SERPIN is associated with heart disease progression. The angiotensin protein produced by it is a part of the renin-angiotensin system that regulates blood pressure [16]. The first candidate gene linked to hypertension was human AGT gene.

In absence of genetic abnormality the CVD risk is decreased by improving glycemic control and treating lipid abnormalities and hypertension. Table 1 shown below is theBiochemical and genetics risk factors for the prediction of cardiovascular event.

Risk factors	Reference
High Blood Glucose Level	[22,23]
Lipid profile	
Triglycerides	[24]
Total Cholesterol	[25]
HDL	[26]
LDL	[27]
VLDL	[28]
Gene Variants	
APOC III	[29]
AGT	[30]

Table 1: Risk factors Predicting Cardiovascular event.

Materials and Methods

Leftover Fasting and Post prandial blood samples were collecting from people visiting diagnostic centers after taking their written consent. Among the 200 enrolled subjects the study included 180 subjects. 90 subjects (females 54 and males 36), with fasting blood glucose (FBG) value of more than 126 mg/dl or $\geq$ 7.0 mmol/L and PPBS value of more than 200 mg/dl or $\geq$ 11.1 mmol/Lwere enrolled as diabetic cases whereas another 90 (females 42 and males 48) with value less than the above value are designated as controls. They wereaged 15 years and older. This is in accordance to WHO criteria.

Fasting and Postprandial Blood glucose levels and Lipid Profile characteristics were selected for the study. APOC III genes were analyzed for presence of SNP rs121918381 and SNPs rs699, rs4762 in AGT gene.

Experimental Procedure

Biochemical Analysis

The Blood glucose levels were measured by glucose oxidase peroxidase method [25]. Total cholesterol (TC) and high density lipoprotein (HDL) cholesterol concentrations were assayed using CHOD-POD/ Phosphotungtate method, triglycerides (TG) estimation was done by GPO/PAP method using Triglycerides-EGD kit low density lipoprotein (LDL) and very low density lipoproteins(VLDL) were estimated by using reagent kit [26-28].

Variant Analysis

DNA sample was prepared by isolation and purificationfrom the leucocytes component of blood samples using modified Sambrook et al. Protocol [29,30]. The DNA is the subjected to amplification in the variant region. The DNA was increased in volume by Polymerase chain reaction (PCR). It was carried out using primers specific to the gene region. The conditions were optimized for the maximum yield [31]. The products were checked for quality and quantity by Agarose Gel Electrophoresis (AGE) [31,32]. The gel was observed in ultraviolet light transilluminator and photographed. Thereafter sequencing the sequence of the DNA sample from diabetic case was compared with the control DNA sample.

Results and Discussion

The risk of cardiovascular morbidity and mortality substantially increases in Type 2 Diabetes patients with obesity hypertension and dyslipidemia. It has have become a growing health problem worldwidedue to over utilization of fats.

On comparison of our data with the guidelines of risk factors for cardiovascular disease given by the American Heart Association (AHA) showed that abnormalities are present in lipid profiles. In this present study, we evaluated the distribution of Triglycerides, Total cholesterol, HDL and LDL in the diabetic cases and control population of Telangana and Andhra Pradesh states in India.

The mean of the lipid profile characteristics werefound to be total cholesterol $=176.8 \pm 50.3$ mg/dl; triglycerides $=198.1 \pm 87.7$ mg/dl; HDL $=42.5 \pm 4.7$ mg /dl and LDL $=86.4 \pm 25.8$ mg/dl, VLDL $=41.8 \pm 20.7$. The lipid ratios like total cholesterol/HDL $=4.16 \pm 10.7$ cholesterol and the LDL cholesterol/HDL cholesterol ratio 2.0 $\pm$ 5.48. Table 2 shown below gives the list of the lipid profile characteristics of T2DM cases and Controls.

Parameter	T2DM Cases Mean ± SD	Controls Mean ± SD	Normal Range	*p*=value
Total Cholesterol	176.8 ± 50.3	163 ± 51.7	130-250	0.09
Triglycerides	198.1 ± 87.7	141 ± 56.9	50-150	0.1
HDL	42.5 ± 4.7	43.2 ± 5.9	35-70	0.05
LDL	86.4 ± 25.8	44.0 ± 28.6	Upto 140	0.09
VLDL	41.8 ± 20.7	28.6 ± 13.6	10-40	0.09

Table 2: Lipid Profile Characteristics.

Single Nucleotide Polymorphism was found in position 116832864 (rs121918381) of APOC III and in 230710048 (rs699) of AGT gene. The AGT gene SNP at position 230710231 (rs4672) is not found in our study population (Table 3).

Gene	Ref Sequence	% age of Positive population
APOC III	rs121918381	43.9
AGT	rs699	48.7

Table 3: Distribution of AGT and APOC III gene SNPs

APO C III SNP is positive in 18 T2DM cases. The amino acid change from Thr→Ala is due change in nucleotide sequence from A→G. Increased APO C III would lead to increased levels of VLDL, triglycerides, impaired FBS, commonly known as pre-diabetes. It regulates plasma lipid and lipoprotein metabolism. AGT gene with SNP rs699 is present in 20 T2DM case. The nucleotide change is from T→C and the amino acid change is from Arg→Trp. The AGT contains Angiotensinogen hormone. It causes increased sodium retention in the blood and increased sodium reabsorption in the kidney. The AGT variant is also associated with high risk for non-dipper, a condition characterized by blood pressure remain elevated during sleep. Non-dippers are at higher risk for developing cardiovascular because of high blood pressure during sleep.
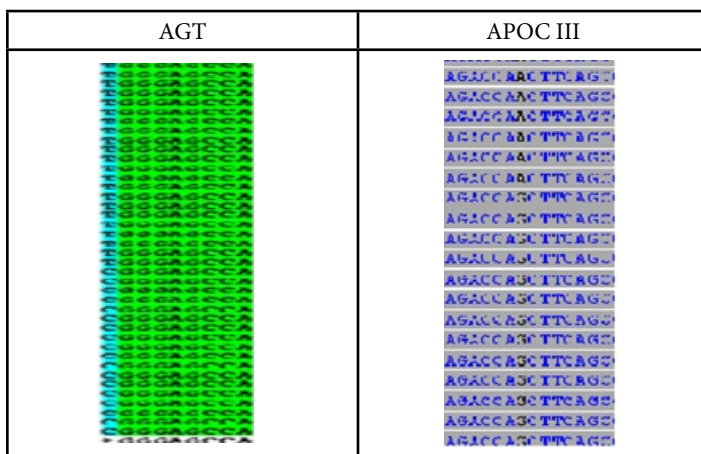
AGT	APOC III

Table 4: Box Shade of APOC III and AGT gene

Conclusion
From our study we can safely conclude that numerous genetic and non-genetic risk factors independently predict cardiovascular events but all of them together improve the overall predictive ability.

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