

Association of Angiotensinogen Gene Variants with Type 2 Diabetes Mellitus Related Hypertension

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Abstract

Hypertension associated with Type 2 Diabetes Mellitus is a global public health problem. Diabetic people with obesity are at high risk for this condition. This paper is a part of case control study for the genetic and non-genetic risk factors of diabetes and its related complications carried out with a total of 180 subjects. Out of these, 90 were diabetic cases and 90 were controls. The main rationale of the study is to evaluate the reported polymorphisms rs699(C/T) and rs4762(C/T) of Angiotensinogen and the biochemical characteristics as the risk factors for Hypertension in the discrete diabetic population of Telangana and Andhra Pradesh states in India.

Results showed that all the lipid profile characteristics were increased, whereas HDL showed decreased value in all cases. The SNP study revealed Angiotensinogen gene with SNP rs699 T→C and the amino acid changed from Arg→Trp is present in 48% of the diabetic population. The SNP rs4762 was not identified in our study population.

Our findings suggest that the polymorphic variants of AGT gene-M235T show and T174M does not show association with hypertension in our discrete diabetic population.

Keywords: Angiotensinogen (AGT); Type 2 Diabetes Mellitus (T2DM); Hypertension

Introduction

Hypertension and Type 2 Diabetes Mellitus are strong risk factors for each other [1-5].

Incidence of both of them increases with age. Microvascular, macrovascular morbidity and mortality caused by diabetes and hypertension is worse and affects the quality and span of life [6]. Approximately 70% of patients with hypertension are affected with diabetes and the frequency of Hypertension is about twice in diabetic individuals in comparison to those without diabetes [7-10]. Increased peripheral vascular resistance is seen. With increasing age the incidence of hypertension and T2DM increases [11, 12]. Every fifth person among diabetics is having hypertension and is responsible for diabetic macrovascular and microvascular [13,14]. It substantially contributes to Morbidity and Mortality of 4.4% diabetic people in India and population worldwide [15] but also they are the independent risk factors for each other. About one-third of the patients with hypertension develop T2DM later in life on the other hand hypertension is prevalent 1.5-2.0 times more in those with T2DM than in those without [16]. This study is an attempt to validate the association of angiotensin polymorphisms rs699 and rs4762 reported in population worldwide and other parts of India in our discrete diabetic population and also to assess the role of Biochemical parameters [17] and presence of SNPs as risk factors for Hypertension in diabetic and normal persons [18].

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Type 2 Diabetes Mellitus

Type 2 Diabetes Mellitus, a metabolic disorder is an emerging global health problem affecting many people and is responsible for about 5% of all the deaths occurs each year. It is resulting from a defect in insulin secretion resulting in insulin deficiency, insulin utilization due to resistance, or both. This condition can be depicted by elevated blood glucose levels with hindrance to carbohydrate, fat and protein metabolism [19-21]. About a total of 1.56 billion people are going to be diabetic by 2025 and by 2030 around 366 million will have diabetes [22]. For all the age-groups worldwide the prevalence was estimated to be 2.8% in 2000 and 4.4% in 2030.

Hypertension (HTN)

The Condition in which the arteries have persistently elevated blood pressure is called Hypertension. It is the most common non-communicable chronic diseases affecting developed and developing countries around the world. Type 2 Diabetes mellitus and hypertension [22,23] is responsible for 57% of all stroke deaths and 24% of all coronary heart disease (CHD) deaths [24]. The most important cause of premature death worldwide according to WHO rating is HTN [25]. The global burden of HTN in economically developed countries in economically developing countries will rise from 972 million to 1.56 billion in 2005 [26]. Studies from 1969-2011 have reported

AGT gene

The Angiotensinogen gene, AGT is an essential component of the renin-angiotensin system (RAS) that regulates blood pressure, body fluid and maintains electrolyte homeostasis. It is a protein Coding gene that codes angiotensinogen precursor or Pre-angiotensinogen. On cleavage it produces peptide hormone derivative angiotensin. Defects in this gene in the form of polymorphism are associated with the variation in plasma angiotensinogen levels. The human AGT gene was the first candidate gene linked to hypertension [34] that is expressed in many tissues, which includes liver, adipose tissue, heart, vessel wall, brain, and kidney. It is a member of the serpin gene super family that stretches over only 12 kb on the chromosome 1 and has 5 exons. The two important regulatory domains include the promoter and enhancer [35]. It is not only well conserved in vertebrates but also has homologs in invertebrates [36]. Studies on the polymorphism in the AGT in multiple populations for conferring risk to hypertension reported varying results [37,38].

Materials and Methods

Materials

The case control study was performed with the leftover peripheral blood sample of 180 subjects visiting diagnostic centres. The whole blood samples were collected from 90 diabetic patients and 90 normal subjects aged between 15-85 years after the overnight Fasting followed by postprandial sample (2hrs after intake of food) from Andhra Pradesh and Telangana States in India. The samples were collected in both EDTA and non-EDTA vacutainers in accordance to the protocol of some of the investigations. Each time 3ml Blood samples were collected using 5ml syringe in sitting position by tying a band to make the vein more prominent. Life style and family was assessed by simple questionnaire. On the basis of pre-prandial and post-prandial glucose levels 90 diabetic cases and 90 controls were selected. The biochemical test reports of diabetic cases showed Fasting Blood Sugar value of more than 126 mg/dl and post-prandial Blood Sugar value of more than 200 mg/dl (WHO recommended criteria for diabetes diagnosis)

Methods

The biochemical parameters were examined and recorded in both diabetic cases and control groups. The Pre-Prandial and Post-Prandial Blood Sugar value was estimated by glucose oxidase and peroxidase (GOD-POD) method. The method in which Glucose in sample is oxidized to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide further reacts with phenol and 4-aminopyrine by catalytic action of peroxide to form red quinonimine dye complex. The intensity of colour formed is directly proportional to amount of glucose present in sample. The colour formed is measured spectrophotometrically at 505nm. The HDL, LDL, triglycerides and total cholesterol were analyzed using Star 21plus auto-analyzer.

We have used 41 diabetic cases and 10 controls (51 total samples) for SNP study. The polymorphisms rs699(C/T) and rs4762(C/T) of Angiotensinogen [39-42] was selected for the SNP study among the different SNPs reported for hypertension at the time of initiation

of our study. The WBC was separated from other components of the blood samples and they were processed for genomic DNA extraction and purification using modified Sambrook, *et al.* protocol [43,44]. The other blood components were separated using Tris HCL pH = 7.5 (wash buffer) after lysing them using proteinase and lysis buffer while giving brief heat treatment at 65°C. Sodium acetate was used to precipitate the lysed proteins. The DNA was then purified. The AGT gene from both the diabetic cases and controls was amplified in the single nucleotide polymorphisms specific portion using Polymerase chain reaction (PCR) [45,46]. The conditions were optimized for the gene. The gene amplification was done with primers designed using Primer 3 Blast tool. The Qualitative Analysis of the amplified PCR products was done by agarose gel electrophoresis [47,48]. The gel was visualized and photographed in transilluminator after staining with ethidium bromide and subjected to sequencing by Sanger sequencing method [49].

Results

Our study showed that the lipid profile values HDL, LDL, Total cholesterol and Triglycerides was found to be high in both the diabetic cases and controls. The HDL levels showed the decreased value.

SNP study for validation of rs699 M235T and rs4762 T174M variants of AGT gene in the discrete diabetic population identified that only rs699 M235T and not rs4762 T174M as a risk factor for hypertension. The distribution of Laboratory values of subjects with and without rs699 M235T is shown in Table 1 and Table 2.

Parameter	T2DM cases Mean ± SD	Controls Mean ± SD	Normal Range	p value
FBS	152 ± 92.8	92 ± 11.7	70-110	0.8
PPBS	229.3 ± 67.6	131.2 ± 18.8	170-200	0.7

Table 1: Fasting and post-prandial blood Sugar in Diabetic 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 ± 28.6	10-40	0.09
VLDL	41.8 ± 20.7	28.6 ± 13.6	Upto140	0.09

Table 2: Laboratory values of Lipid Profile.

Discussion

Hypertension is a multifactor disease resulting from both genetic and non-genetic risk factors.

Studies from different ethnic populations show varied association between hypertension and the M235T, T174M variants of AGT gene. Highest frequency of 235T polymorphism has been observed in Africans (84-94%) followed by Asians (70-73%) and Caucasians (20-45%) [50] pre-prandial-postprandial glucose were higher in the patients with type 2 diabetes mellitus, LDL, triglycerides and total cholesterol were high not only in the diabetic cases but also in some controls. The HDL values were recorded low in those who had high triglyceride levels.

Conclusion

From the results of our study we conclude that the polymorphic variants of AGT gene-M235T show and T174M does not show association with hypertension in our discrete diabetic population The increased biochemical parameters in the controls make them susceptible to type 2 diabetes and to hypertension.

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Future Directions

The conclusive association of new SNPs reported in diabetic population of Andhra Pradesh and Telangana given by further extension of analyses work on a larger sample size.

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