Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 1967-1973 | ISSN:2251-6727

The Association of ACE Insertion/Deletion Polymorphisms with Type 2 Diabetes Mellitus and Hypertension

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(Received: 16 September 2024 Revised: 11 October 2024 Accepted: 11 December 2024)

KEYWORDS

Diabetes Mellitus,Angiotensin-converting enzyme (ACE),Renin-angiotensin-aldosterone system,genetic polymorphism

ABSTRACT:

Background: Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia and various complications, including diabetic nephropathy, neuropathy, and retinopathy. Hypertension is a common comorbidity in DM patients, exacerbating cardiovascular risks. The Renin-Angiotensin-Aldosterone System (RAAS) plays a significant role in regulating blood pressure and has been linked to both hypertension and type 2 diabetes mellitus (T2DM). The angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism is a notable genetic variant within RAAS that may influence susceptibility to these conditions.

Objective: To investigate the association between the ACE I/D polymorphism and the prevalence of hypertension in patients with T2DM.

Methods: This study included 30 patients with diagnosed T2DM and hypertension from MGM Medical College, Navi Mumbai. Genotyping for the ACE I/D polymorphism was performed using polymerase chain reaction (PCR). Clinical parameters such as fasting blood sugar, postprandial blood sugar, glycated haemoglobin (HbA1c), and lipid profiles were assessed. The genotypes were categorized into I/I, I/D, and D/D, and their associations with clinical parameters were analysed.

Results: The distribution of ACE genotypes among the subjects was 77% ID and 23% DD. The ID genotype was significantly associated with higher levels of fasting blood sugar (170.4 ± 54.5 mg/dL vs. 109.5 ± 11.1 mg/dL, p=0.003), postprandial blood sugar (261 ± 74.9 mg/dL vs. 174.1 ± 33.5 mg/dL, p=0.003), and HbA1c (7.8 ± 1.0% vs. 6.7 ± 0.6%, p=0.004) compared to the DD genotype. Although no significant differences were observed in age, BMI, or blood pressure between the genotypes, ID genotype carriers exhibited more pronounced derangements in lipid profiles compared to DD carriers.

Conclusion: The ACE I/D polymorphism is strongly associated with hypertension in T2DM patients. Specifically, the ID genotype correlates with elevated fasting blood glucose, postprandial blood glucose, and HbA1c levels. These findings suggest that the ID genotype may increase susceptibility to hypertension and diabetes-related complications. Further research with larger sample sizes is needed to confirm these associations and explore their implications for personalized treatment strategies.

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Introduction:

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism. Recognized as one of the oldest known diseases, it was first documented in ancient Egyptian manuscripts over 3000 years ago. The distinction between type 1 and type 2 diabetes was made in 1936, with type 2 diabetes later identified as a key component of metabolic syndrome in 1988. Today, DM is a rapidly growing global health concern, affecting populations in both developed and developing countries.[1]

The condition is associated with numerous long-term complications, including macrovascular and microvascular disorders such as diabetic nephropathy, neuropathy, and retinopathy. Key risk factors for DM and its complications include chronic hyperglycemia, hypertension, dyslipidemia, smoking, obesity, aging, and insulin resistance. Type 2 diabetes (T2DM), the most prevalent form, is characterized by insulin resistance, relative insulin deficiency, and hyperglycemia.[2]It is also influenced by genetic and environmental factors, including hormonal changes, genetic abnormalities, other diseases, and certain medications.[3]

Hypertension, a common comorbidity of T2DM, is a significant public health issue linked to an increased risk of cardiovascular diseases such as coronary heart disease, stroke, and heart failure.[4]The interplay between hypertension and metabolic syndrome highlights the importance of genetic factors, particularly those in the Renin-Angiotensin-Aldosterone System (RAAS), which regulates blood pressure and electrolyte balance.[5]

The angiotensin-converting enzyme (ACE) plays a critical role in RAAS, converting angiotensin I to the potent vasoconstrictor angiotensin II. The ACE gene, located on chromosome 17q23, exhibits a well-studied insertion/deletion (I/D) polymorphism in intron 16, resulting in three genotypes (II, ID, and DD).[6,7]The D allele, particularly in homozygous DD individuals, has been linked to elevated ACE levels and increased angiotensin II activity, contributing to conditions like hypertension and T2DM.[8]However, the relationship between ACE I/D polymorphism and these diseases remains inconsistent across studies, potentially due to genetic and environmental interactions, including obesity status.[9]

Given the conflicting evidence and the limited data on the ACE I/D polymorphism's role in T2DM and hypertension, this study aims to investigate the association of the ACE I/I, I/D, and D/D genotypes in individuals with these conditions. Understanding these genetic influences could provide insights into disease mechanisms and inform targeted interventions.

Materials and Methods

Study Population

The study enrolled 30 patients diagnosed with T2DM and hypertension, as defined by the American Diabetes Association (ADA) and American College of Cardiology/American Heart Association (ACC/AHA) guidelines. Inclusion criteria included a fasting blood glucose (FBG) ≥ 126 mg/dL and blood pressure (BP) ≥ 130/80 mmHg. Written informed consent was obtained from all participants, and the study was approved by the Institutional Ethical Committee. Each participant completed a bilingual questionnaire (in English,Hindi,Marathi) to collect information on socio-demographic characteristics, history of diabetes, and other co-morbid conditions. Additionally, weight and height measurements were taken for all participants, and their Body Mass Index (BMI) was calculated using the formula: weight (kg) / height (m^2).

Clinical and Biochemical Parameters

A detailed history was taken for all patients, including demographic data, medical history, and the duration of T2DM and hypertension. Biochemical investigations included lipid profile, fasting blood sugar (FBS), postprandial blood sugar (PPBS), glycated hemoglobin (HbA1c), and ACE gene I/D polymorphism.

Sample Collection and Analysis

Seven millilitres of peripheral blood were collected from each participant. Of these, 2 mL was placed in EDTA Vacutainers for DNA isolation and HbA1c determination, 3 mL in plain tubes for lipid profile, and 2 mL in fluoride tubes for glucose estimation. Plasma samples were separated and stored at −20°C until analysis. Lipid profile was determined using an Autoanalyser (Beckman Coulter AU480), and HbA1c was measured by Ion exchange High Performance Liquid Chromatography (HPLC) using the Bio-Rad D-10 Analyser.

Genotyping of ACE I/D Polymorphism

Genomic DNA was extracted from peripheral blood using the Hipura Blood DNA Extraction Kit. The

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extracted DNA was used for PCR amplification of the ACE I/D polymorphism using the following primers:

- Forward: 5'-CTGGAGACCACTCCCATCCTTTCT-3'
- Reverse: 5'-GATGTGGCCATCACATTCGTCACGA-3'

PCR amplification was carried out using a 5 µL DNA template, 2.5 µL 10X PCR buffer, 0.75 µL 50mM MgCl2, 0.5 µL 0.5mM dNTP mix, 1 µL 10 pmol primer solution, and 0.2 µLTaq DNA polymerase. The PCR cycle consisted of an initial denaturation at 95°C for 5 minutes, followed by 30 cycles of 95°C for 1 minute (denaturation), 58°C for 1 minute (annealing), and 72°C for 2 minutes (extension). A final extension was performed at 72°C for 5 minutes.

The PCR products were separated by 2% agarose gel electrophoresis and visualized under UV light. The DD genotype was identified by a 190 bp band, the II genotype by a 490 bp band, and the ID genotype by both 190 and 490 bpbands.The descriptive data and laboratory results were reported as means ± standard deviation.

Results

Biochemical Parameters in Different ACE Genotypes

The biochemical parameters of patients with the DD and ID genotypes of the ACE gene are shown in Table 1. Significant differences were observed between the DD and ID genotypes in terms of fasting blood sugar (FBG), postprandial blood sugar (PPBS), and HbA1c levels ($p<0.05$). Patients with the ID genotype exhibited significantly higher FBG and PPBS levels compared to those with the DD genotype. Additionally, the HbA1c levels were also higher in the ID group.

While lipid profile parameters such as cholesterol, triglycerides, HDL, LDL, and VLDL were slightly elevated in the ID group compared to the DD group, the differences were not statistically significant ($p>0.05$). No significant differences were observed between the genotypes in terms of age, BMI, height, weight, or blood pressure (SBP and DBP).

Parameter	DD	ID	Pvalue
Age(years)	**48.2 ± 12.4**	**53.69 ±11.08**	**0.1**
BMIkg/m^2	**25.3±3**	**25.1± 3.5**	**0.4**
FFBS	**109.5± 11.1**	**170.4± 54.5**	**0.003***
PPBS	**174.1 ± 33.5**	**261 ± 74.9**	**0.003***
HBA1c	**6.7± 0.6**	**7.8± 1**	**0.004***
Cholesterol	**163.6± 43.21**	**179.5± 52.2**	**0.2**
Triglyceride	**152.5± 54.9**	**163.8± 77.2**	**0.3**
HDL	**38.7± 7.8**	**32.6±11.4**	**0.09**
LDL	**105.5± 42.1**	**94.2± 36.4**	**0.2**
VLDL	**31.6± 9.1**	**32.6± 16.7**	**0.4**
Height	**155.2 ±7.3**	**156± 6.77**	**0.4**
Weight	**61.4±10.6**	**61.1± 10.2**	**0.4**
SBP	**126.4 ±6.2**	**128.8 ±6.3**	**0.1**
DBP	**87.1± 4.8**	**86.6 ±5.3**	**0.4**

*p<0.05isstatisticallysignificant

Table 1: Showing the Comparison of biochemical parameters of subjectwith ID andDDGenotype

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Genotyping of ACE I/D Polymorphism

The genotyping results revealed that the majority of participants were heterozygous (ID genotype), while a smaller proportion carried the homozygous DD genotype. Representative gel electrophoresis images (Figures 1 and 2) show the distinct band patterns for each genotype, confirming the presence of the I/D polymorphism.

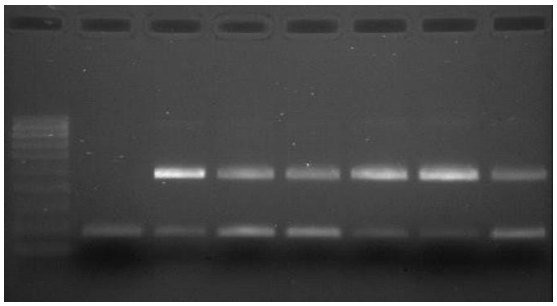

Fig.1DetectionofI/DpolymorphismofACE genein2

%agarosegelelectrophoresisshowheterozygousIDat190and490bpgenotypeof I/D polymorphism(100bpladder)

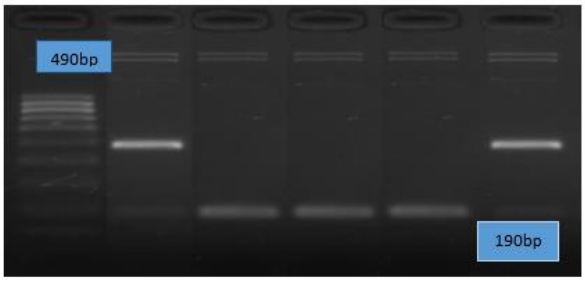

Fig 2: Detection of I/D polymorphism of ACE gene in 2 % agarose gel electrophoresisLane 2,3,4 show homozygous at 190bp DD genotype and Lane 1 and 5 , show at 190 and490bpIDgenotype(100bpladder)

Discussion

Diabetes mellitus (DM) is an increasingly spreading public health problem affecting people in both developing and developed countries. Diabetes is a chronic disease with long-term effects, such as macrovascular and microvascular complications. Microvascular problems include diabetic nephropathy (DN), neuropathy, and retinopathy. Chronic hyperglycemia, hypertension, dyslipidemia, smoking, obesity, ageing, and insulin resistance are only a few of the risk factors for DM and its complications. [2]

Hypertension (or HTN) is a condition in which the arterial blood pressure is abnormally high. It is also known as high blood pressure. Normal blood pressure is described by the Joint National Committee 7 (JNC7) as a systolic blood pressure of 120mmHg and a diastolic blood pressure of 80mmHg. A systolic blood pressure of 140mmHg or a diastolic blood pressure of 90mmHg is considered hypertension. Blood pressure readings between 120 and 139 mmHg systolic and 80 to 89 mmHg diastolic are considered prehypertension. [10]

Type 2 diabetes mellitus (T2DM), hypertension, obesity, and dyslipidemia are all symptoms of metabolic syndrome. It's very popular among people in developing countries. When associated with diabetes, hypertension is a normal occurrence and a significant risk factor for cardiovascular disease (CVD) and coronary heart disease (CHD). [11, 12]

In T2DM patients, the RAAS has been related to the development of cardiovascular and renal problems. [13]The renin-angiotensin-aldosterone system (RAAS) plays a critical role in blood pressure regulation and electrolyte balance. The genetic susceptibility to hypertension has been researched extensively using RAAS gene polymorphisms. RAAS blockade decreases insulin resistance, which is critical in the pathogenesis of type 2 diabetes. The I/D polymorphism of the ACE gene determines an increased plasma and serum ACE level.

The DD genotype is linked to higher ACE expression and operation, which can predispose people to hypertension and/or type 2 diabetes, as well as their complications. [14]

The ACE protein coding gene encodes the main enzyme in the renin-angiotensin system, which can catalyse the conversion of angiotensin I to angiotensin II. The ACE gene is located on chromosome 17q23 and has a total length of 21 kb, with 26 exons and 25 introns. There is an insertion/deletion (I/D) polymorphism based on the presence or absence of a 287 bp fragment in intron 16. [15]

In the present study the serum ACE level was genetically determined by a 287 bp fragment of I/D

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polymorphism in the 16 th intron at chromosome 17. The insertion/ deletion (ID) of this sequence is represented as heterzygosity while DD represent the homozygosity of a 287 bp fragment sequence. In this study we determined that ID genotype and D allele of the ACE gene were strongly associated with hypertension and type 2DM as compared to DD genotype

Vasudevan et.al also found that the D allele of the ACE gene was in statistically significant association with HTN and type 2 diabetes in Malaysian population.[16] These findings are in agreement with some previous studies.[17,18]

AbdolrahimNikzamir et.al also indicated that ACE I/D polymorphism is associated with hypertension in patients with type 2 DM.[19]

Bengtsson Ket.al also suggested that D allele might increase the susceptibility to hypertension, particularly in hypertensive type 2 diabetic patients.[20] We also found that the mean of fasting blood sugar and post prandial blood sugar was elevated in ID genotype population as compare to DD genotype. Vasudevan et.al have also suggested that blood glucose levels gave significant differences in type 2 DM with hypertension.[16]

Yun-Fei Zhou et.al found that TG and LDL-C levels in patients with ID and DD genotype were significantly higher than those in patients with II genotype, the difference of blood glucose levels were not statistically significant in different genotypes. [15]

The present study also found that the mean of total cholesterol and triglyceride were raised in ID genotype as compared to DD genotype.

Emilia et.al was also found that the mean value of LDL were higher in DD genotype population.

The association between this polymorphism and lipid profiles was still at the matter of controversy. Carriers for D alleles have been associated with altered lipid values in Japanese (Suzuki et al., 1996), Israeli (Oren et al., 1999), Korean (Kim, 2009) and Mexican (Alvarez-Aguilar et al., 2007) populations. However, results from some other reports were contradictory (Nagi et al., 1998; Uemura et al., 2000; Um et al., 2003).

The mechanism by which ACE gene variants may influence lipid levels could be attributable to the specific localization of the renin-angiotensin system in adipose tissue (Schling et al., 1999), which suggests that it may also involved in lipid metabolism. Differences in ethnic background (Mao and Huang, 2013) and dietary patterns (Yang et al., 2013) may also become significant contributors.[21]

Vasudevan et.al has also determined that LDL-C levels, TG and TC have not shown any differences in subject with diabetes mellitus having hypertension as one of the complication.[16]

In the present study there were no significant difference found in age, BMI height, weight ,VLDL,between the genotype of ID and DD polymorphism.

Vasudevan et.al has suggested that was no significant differences found in age, and BMI between the genotypes of I/D polymorphism.[16]

Emilia et.al have also determined that there was , no significant differences between the ACE genotypes in all parameters (Age,BMI,Height ,Weight) for anthropometric characterization indicates that this polymorphism was not involved in the regulation of body mass and adipose tissue.[21]

In our study we carried out genetic analysis to study ID and DD polymorphism in type 2 DM with Hypertension. We also found that ID polymorphism is more predominant as compare to DD polymorphism. We observed DD polymorphism also but in very few subject . The finding of the subject is clearly indicates that ID is the main polymorphism present in subject with type 2 DM with hypertension.So the subject carrying ID genetic predisposition risk to develop diabetes mellitus in early stage when the patient is having hypertension

Similar findings were also reported by Rakesh K et.al. that ID genotype were significantly associated with the risk for T2DM and HTN, so that it is reasonable to expect that ID genotype of I/D polymorphism of ACE gene may be associated with T2DM and HTN.[22]

Conclusion

This study provides evidence that the ACE I/D polymorphism, particularly the D allele, is associated with an increased risk of developing T2DM with hypertension. The ID genotype appears to be the most common variant in this cohort, and it is associated with higher blood glucose levels and altered lipid profiles. These findings suggest that genetic screening for ACE polymorphisms may be useful in identifying individuals at high risk for developing these conditions. Further

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JCHR (2024) 14(6), 1967-1973 | ISSN:2251-6727

studies with larger sample sizes are needed to confirm these results and to explore the underlying mechanisms by which the ACE I/D polymorphism contributes to the pathogenesis of T2DM and hypertension.

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