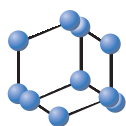


RESEARCH ARTICLE

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Alpha Adducin (ADD1) Gene Polymorphism and New Onset of Diabetes Under the Influence of Selective Antihypertensive Therapy in Essential Hypertension



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Abstract: Background: Different antihypertensive therapies (especially diuretics) are reported to induce the new onset of diabetes in some hypertensive patients. α -adducin-1 (ADD1) gene is salt sensitive gene which has its role in etiology of hypertension *via* salt sensitivity. Therefore, the G460T polymorphism of ADD1 gene may be associated with new onset of diabetes under the influence of diuretic and other antihypertensive therapies.

Aim: To assess the correlation between genetic polymorphism (ADD1 G460T polymorphism) and glycaemic disturbance under influence of diuretic and other antihypertensive drug therapies.

Materials and Methods: We recruited study subjects, 270 normotensive as control (150 male and 120 females), 270 hypertensive patients (95 male and 175 females) and 240 hypertensive with new onset of diabetes patients (80 male and 160 females). All study samples were genotyped for ADD1 polymorphic alleles and analyzed the relationship between different genotypes with respect to anthropometric and clinical parameters along with drug therapies.

Results: Clinical and anthropometric parameters (such as age, SBP, DBP, FBG, height, weight, WC, HP, W/H ratio, and BMI) of study population were found highly statistically significant ($p < 0.05$) at base value. Further, genotype wise comparison of all the above parameters revealed most of them as non-significant ($p > 0.05$). Whereas, comparison between genotype and different antihypertensive drug therapy of hypertensive patients, specifically, diuretic therapy as mono in male ($p = 0.0227$) and female ($p = 0.0292$) and in combination with BBs in both male ($p = 0.0023$) and female ($p = 0.0079$) revealed a higher FBG level in variant T allele. In case of hypertensive with new onset of diabetes patients, only female population showed a slightly statistically significant ($p = 0.0413$) difference in FBG level with diuretic mono therapy. Other antihypertensive drug therapies were safe and effective either as mono or in combination therapy.

Discussion: Anthropometric parameters may be the indicative factors for hypertension and diabetes. Variant T allele of ADD1 gene may be considered as the risk factor for the development of diabetes in hypertensive patients. Diuretics as mono therapy and in combination with BBs may be considered as the risk factor for new onset of diabetes in EH patients carrying variant T allele (either as TG or TT).

Keywords: Hypertension, antihypertensive therapy, diuretics, new onset of diabetes alpha adducing gene (ADD1), gene polymorphism, pharmacogenomics.

1. INTRODUCTION

In the present scenario, both hypertension and diabetes mellitus are the most common killer diseases among top ten

non-communicable diseases having high mortality rates in Asia, America and Europe [1, 2]. Hypertension alone is a risk factor for the induction of other complications such as angina pectoris, brain stroke, heart failure and end stage renal disease [3]. According to World Health Organization (WHO), the prevalence of hypertension is escalating year by year throughout the world and around 17 million peoples (31% of all deaths in the world) die per year due to cardio-

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vascular diseases, among which, hypertension alone contributing for approximately 9.4 million deaths per year [4]. As per the Joint National Committee VIII guidelines, recommended the drugs for the first line drug therapy as follows angiotensin converting enzyme inhibitors (ACE inhibitors), diuretics, beta blockers (BB), calcium channel blockers (CCB) and angiotensin receptor blockers (ARB) [5]. Many debates conducted on the duration of onset of diabetes mellitus after the chronic treatment of BBs, diuretics and CCBs [6-10]. Some of the clinical studies reported that an antihypertensive treatment either as a mono-therapy or as a combination therapy for more than 3 to 6 years showed to induce the onset of diabetes mellitus instead of anti hypertensive effect [11, 12]. All antihypertensive drugs have almost similar beneficial outcome for the treatment of high blood pressure but differ in their potential of causing adverse events such as disturbance in blood glucose homeostasis leading to induction of diabetes [13]. This disturbance in glucose homeostasis and elevation in blood glucose level during antihypertensive therapy is also associated with future cardiovascular events [14].

The interaction of rennin-angiotensin system and gene polymorphism may be responsible for regulation of onset of diabetes in hypertensive patients after treated with diuretics and beta blockers. The inter-individual difference in drug response of anti hypertensive therapy and new onset of diabetes in some patients can be explained on the basis of genetic polymorphism related to metabolic functions. Hence, Genetic polymorphism of genes (which influence the pathophysiology of hypertension and diabetes) may be responsible for new onset of diabetes under the influence of certain antihypertensive drugs due to alteration in physiological processes.

Gly460Trp polymorphism of alpha adducin-1 gene (ADD1) may be one of those genes which may affect the physiological processes related to hypertension and diabetes under the influence of antihypertensive therapy, especially with diuretics and BBs. ADD1 is the part of adducin family which is a heterodimeric cytoskeleton protein [15]. It is a salt-sensitive gene associated with increased renal sodium re-absorption *via* activation of $\text{Na}^+\text{-K}^+\text{ATPase}$ pump. The polymorphic alleles of ADD1 gene may show variations in the amount of sodium reabsorbed and potassium excreted counter to sodium ion and results in physiological change [16]. Studies have shown that 460W allele is associated with more sodium re-absorption than 460G allele; therefore, 460W allele is at more risk of developing salt-sensitive hypertension [17]. Therefore, 460W allele must be associated with excretion of more potassium *via* $\text{Na}^+\text{-K}^+\text{ATPase}$ pump as a counter ion to sodium. Potassium is considered to have a major role in glucose homeostasis *via* affecting the release of insulin as well as insulin-mediated glucose uptake into skeletal muscle. It has been well studied that hypokalemia may result in glucose disturbance [18, 19].

It is also found that, 460W allele is more prominent blood pressure reduction as compared to 460G allele after diuretic therapy [20]. More and quick reduction in blood pressure is associated with higher glomerular filtration rate (GFR) which is further associated with diabetes. So 460W allele may be associated with diabetes in hypertensive pa-

tients after diuretics treatment [21]. The T allele (460W) of salt sensitive ADD1 gene was found to be associated with higher risk of diabetes with diuretic therapy as compared to other alleles, although the significance level was very low, but it might be due to less population size [22].

The ADD1 gene polymorphism may be susceptible to the risk of diabetes by one or more of the above mentioned mechanisms. However, some studies deny the role of ADD1 gene polymorphism in risk of new onset of diabetes.

Therefore, in the present study, we attempted to access the role of ADD1 gene polymorphism during antihypertensive treatment causes the onset of diabetes.

2. MATERIAL AND METHODS

2.1. Methods

2.1.1. Ethics

The study was approved (IEC/670) by the Institutional Ethics Committee of M. M. University and written consent was obtained from all the participants.

2.2. Design and Sample Size

The study was carried out on the basis of cross-sectional survey by M. M. Institute of Medical Science & Research, Mullana (Haryana), there are around 38,500 inhabitants residing in 17 villages of this rural area. The survey recorded a total of 2672 individuals afflicted with essential hypertension. These patients will be referred to as hypertensive (HT) with a disease prevalence of 6.94 %. Out of 2672 patients, we have selected 510 patients who visited regularly in hospital OPD. A sample size of 164 patients will be sufficient to represent the hypertensive population residing in the rural area under investigation with a power of 80% and a *P*-value of 0.05. Patients who agree to participate were explained the nature and the objectives of the study, and informed consent was obtained individually. The information about patient's identity was not included with other data and only the consulting physician had the access to this information.

2.3. Study Population

In the present investigation, the subjects under study (n=510) were divided into 2 groups with age and sex matched, 1) Essential Hypertensive (EH) 2) Essential hypertensive with the onset of diabetes (EHNOD). 1) The first group comprised of 270 hypertensive patients had 95 male and 175 females 2) Second group consisted of 240, hypertensive with diabetes patients, out of which 80 male and 160 females. In order to made comparison with normal individuals, we have selected normal individuals (n=270) with age and sex matched from the same place and denoted as the third group consists of 150 male and 120 females. Various parameters like age, sex, body mass index (BMI), blood pressure, education, and family history were recorded in a given questionnaire.

2.4. Inclusion Criteria

Patients residing in rural area of Haryana for more than two generations were recruited. They were the age of 18

years to 75 years with an average blood pressure limits >140mmHg systolic blood pressure (SBP) and > 90 mmHg diastolic blood pressure (DBP) on three separate occasions.

- 1) For essential hypertensive group [EH]: Only those patients who had essential hypertension and were treated with selective antihypertensive medication (Diuretics, BBs, CCBs) as mono or in combination for about 3 years or more.
- 2) For essential hypertensive with new onset of diabetes mellitus [EHNOD]: Only those patients who had EH (as per above criteria) with onset of diabetes mellitus (diagnosed according to WHO criteria as either a fasting plasma glucose > 7.0 mmol/L and/or random (non-fasting) blood glucose > 11.1 mmol/L) and were treated with selective antihypertensive medication (Diuretics, BBs, CCBs) as mono or in combination for about 3 years or more.
- 3) Normotensives [NT]: Those who were residing in the rural area of Haryana without any history of hypertension or diabetes between the ages of 18 years to 75 years.

2.5. Exclusion Criteria

All individuals with the age less than 18 years or above 75 years are spared. Essential hypertensive patients who were treated with other than diuretics, BBs and CCBs alone or in combination were excluded. Pregnant and lactating mothers were removed from the study.

2.6. Blood Pressure Measurements

The blood pressure recordings were taken by the physicians in OPD. We verified blood pressure with a mercury sphygmomanometer at least twice a week before enrollment. Patients were made to sit quietly for at least 15 minutes in a chair. Intakes of caffeine, exercise, smoking and drugs (which may affect blood pressure) were avoided for at least 30 minutes prior to the measurement. Three readings were taken at 5 minutes interval, and the average was recorded. If there was gross variation in them, a fourth reading was obtained and used for the diagnosis of hypertension. Blood Pressure was measured in both arms and in the event of any difference, the higher reading was taken. An appropriately sized cuff (cuff bladder encircling at least 80 percent of the arm) was used to ensure accuracy in palpitory obliteration of radial pulse pressure which was obtained before auscultating for BP measurement. By the auscultatory method, appearance and disappearance of Korotkoff sound were taken as an indicator of systolic and diastolic BP, respectively [23].

2.7. Collection of Blood Sample

The blood samples were collected in tubes containing EDTA as an anticoagulant. The samples were transported on ice to the laboratory and were processed on the same day [24].

2.8. Glucose Monitoring

The blood glucose levels were monitored after the patient complaint and had a symptom of diabetes mellitus that were treated with chronic therapy of diuretics, beta blockers and

calcium channel blockers alone or in combination in the treatment of essential hypertension.

2.9. Genotyping for ADD1

DNA samples were isolated from peripheral blood lymphocytes by the standard modified inorganic method as described by Miller *et al.* [25], and quantified following standard spectrophotometric analysis. The isolated DNA samples were stored at -20°C till further analysis. The substitution of nucleotides from G-to-T resulted in the genetic variant of amino acid residue at 460 and was located at nucleotide position 614 of exon 10 of the ADD1 (Gen bank accession number L29294). The Gly460Trp polymorphism of ADD1 (rs4961) was detected using an Amplification Refractory Mutation System polymerase chain reaction (PCR). The following primer sets were used:

F614G 5'-GGGGCGACGAAGCTTCCGAGGTAG-3';
(Forward-G allele specific primer)

F614T 5'-GCTGAACTCTGGCCCAGGCCGACGAAGCTTCCGAGGATT-3'; (Forward-T allele specific primer)

R614 5'-CCTCCGAAGCCCCAGCTACCCA-3', (Reverse primer)

The polymerase chain reaction was carried out in thermal cycler (Bio-Rad, Japan) in total volume of 25 µl in 0.2 ml PCR tubes containing 2 µl (40 ng) of template DNA, 5.5 µl master mix (containing Taq polymerase, loading dye, dNTPs, MgCl₂ and buffer 10X), 1 µl (F614G primer, F614T primer and common primer (R614)), and 16.5µl nuclease free water. Initial denaturation was carried out at 94 °C for 10 minutes followed by 35 amplification cycles at 94 °C for 20 seconds, primer annealing at 60° C for 30 seconds, initial extension at 72 °C for 30 seconds and final extension step at 72 °C for 3 minutes. The amplified DNA samples were separated using gel electrophoresis (4% agarose gel) and observed under UV documentation chamber to find type of genotype present *i.e.* GG, TG, or TT genotype [23, 26]. The size of PCR products were 220 bp and 234 bp for the 460Gly and 460Trp alleles, respectively (NuSieve, 3:1 agarose, FMC Bioproducts).

2.10. Statistical Analysis

Data analysis was done with the help of an SPSS version 14.5. Continuous variables are expressed as means ± SD. Intergroup comparisons are made using ANOVA with multiple comparison with post-hoc test. Allele frequencies were calculated from genotype frequencies and were compared using chi-squared (χ^2) statistics. *P* value ≤ 0.05 was considered statistically significant.

3. RESULTS

3.1. Study Populations and Baseline Characteristics

A study population comprised of 510 (EH and EHNOD) patients and 270 normotensive individuals. Among patients, 270 were EH and 240 were EHNOD after treated with chronic anti hypertensive therapy. Out of 270 in hypertensive group, 175 were females and 95 were males same in the case of EHNOD, they were 160 females and 80 males. As per Table 1, all baseline parameters showed sig-

nificant changes ($p < 0.0001$) in comparison of different groups. Height could not show any significant change in multiple comparisons between normotensive versus essential hypertensive and essential hypertensive with new onset of diabetes. In blood pressure levels (SBP and DBP), there was statistically higher significance among all three groups. In fasting blood glucose levels (FBG), the average FBG levels were 207.29 ± 81.02 mg/dl in EHNOD than 107.20 ± 7.85 mg/dl in EH whereas average normotensive showed 97.1 ± 6.6 mg/dl. It was statistical higher significant. Among anthropometric parameters, BMI, waist circumference (WC) and waist-hip ratio (W/H) were showed higher statistical significant ($p < 0.0001$) when compared between all three groups in each parameter. The average duration of hypertension in both the groups [EH and EHNOD] were 4.70 ± 4.11 and 7.91 ± 4.52 years respectively and total duration of diabetes after treatment with anti hypertensive therapy was 5.72 ± 4.32 years respectively in EHNOD.

The gel picture (Fig. 1) represented the different genotypes of ADD1 gene. Table 2 represented genotype and the allelic distribution of EH versus normotensive group. GG homozygote was higher in percentage than TG and TT genotypes in both the groups [EH and EHNOD]. Non- statistical significance was found in patients and control according to Hardy-Weinberg equilibrium. Frequency of G allele was 0.813 than T allele (0.187) of EH group same in the case of EHNOD, it was 0.813 of G allele and 0.707 had T allele

whereas in normotensive group, the T allele was 0.167 and G allele was 0.833.

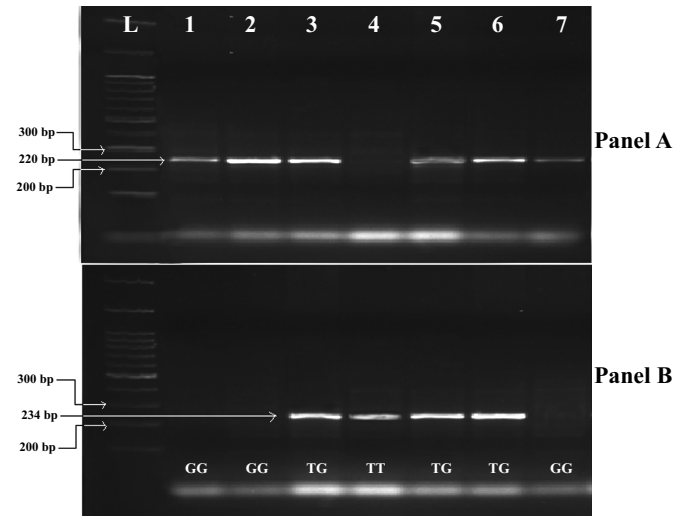


Fig (1). Representative image of agarose gel electrophoresis of showing the Gly460Trp polymorphism of ADD1 gene. Panel A represents the G allele specific primer amplification (F614G) and Panel B, T allele specific (F614T). Lane L represents the 100 bp DNA ladder. Appearance of band in both panel shows TG genotype (lane 3, 5, 6) while lane 1, 2, 7 represents GG genotype and lane 4 represents TT genotype.

Table 1. Base line parameters of total population and gender based specific.

Genotypes	Total population				Male				Female			
	Nor-motensive (n=270)	Essential Hypertensive (n=270)	Essential Hypertensive with new onset of diabetes (n=240)	p value	Nor-motensive	Essential Hypertensive	Essential Hypertensive with new onset of diabetes	P value	Nor-motensive	Essential Hypertensive	Essential Hypertensive with new onset of diabetes	p value
Age (yrs)	51.8±11.9	53.4±10.8	54.9±9.2	0.005	52.2±11.7	54.7±13.5	53.9±9.1	0.0368	51.3±12.2	52.6±9.0	55.4±9.3	0.0001
SBP (mmHg)	123.3±4.9	152.5±10.2	155.4±8.1	0.0001	123.5±5.6	153.7±6.3	156.19±5.7	0.0001	123.1±4.0	151.9±11.8	155±9.1	0.0001
DBP (mmHg)	81.5±3.7	91.8±2.6	92.3±2.4	0.0001	81.3±4.1	91.2±2.15	92.5±2.2	0.0001	81.7±3.2	92.1±2.7	92.2±2.5	0.0001
FBG (mg/dl)	97.1±6.6	107.2±7.8	207.2±81.0	0.0001	96.8±6.0	106.7±7.1	186.25±65.2	0.0001	97.6±7.2	107.4±8.2	217.8±86.1	0.0001
Weight (kg)	63.4±8.8	71.9±7.5	75.9±8.6	0.0001	67.8±8.5	74.8±8.34	82.2±6.9	0.0001	58.1±5.7	70.4±6.59	72.7±7.6	0.0001
Height (cm)	158.5±9.3	156.8±8.7	158.0±8.3	0.088	164.6±7.0	165.0±6.9	166.84.2	0.0002	150.8±5.2	152.4±6.0	153.6±6.1	0.0005
BMI (kg/m ²)	25.1±1.8	28.5±2.3	30.3±1.7	0.0001	24.9±1.7	27.3±1.6	29.5±1.5	0.0001	25.5±1.9	29.2±2.3	30.7±1.7	0.0001
WC (cm)	76.3±7.5	94.1±7.0	103.1±11.3	0.0001	77.3±6.8	88.6±6.7	110.5±12.5	0.0001	75.1±8.1	97.0±5.3	99.4±8.6	0.0001
HC (cm)	90.5±7.1	105.5±10.2	104.9±12.6	0.0001	87.3±6.64	94.2±4.96	114.2±12.7	0.0001	94.5±5.5	111.7±6.2	100.2±9.7	0.0001
W/H Ratio	0.85±0.0	0.90±0.0	0.99±0.0	0.0001	0.89±0.0	0.94±0.0	0.97±0.0	0.0001	0.79±0.0	0.87±0.0	0.99±0.0	0.0001
Duration of hypertension (yrs)	-	4.70±4.1	7.91±4.5	-	-	5.89±5.2	7±4.3	-	-	4.06±3.1	8.36±4.57	-
Duration of diabetes (yrs)	-	-	5.72±4.3	-	-	-	-	-	-	-	5.97±4.5	-

Table 2. Distribution of ADD-1 genotypes and alleles in cases and normal population.

Genotypes	Groups		Groups	
	Normotensive	Essential Hypertensive	Normotensive	Essential Hypertensive with new Onset of Diabetes
Gly460Trp Genotypes				
Gly/Gly	190 (70.4 %)	182 (67.4%)	190 (70.4%)	165 (68.8)
Gly/Trp	70 (25.9%)	75 (27.8%)	70 (25.9%)	60 (25%)
Trp/Trp	10 (3.7%)	13 (4.8%)	10 (3.7%)	15 (6.3%)
P value	0.692		0.412	
Gly	0.833	0.813	0.833	0.813
Try	0.167	0.187	0.167	0.707
P value	0.383		0.380	

Table 3. Distribution of genotypes with respect to various parameters in gender based and total population of essential hypertension patients.

Genotypes	Total population				Male				Female			
	GG	TG	TT	p value	GG (59)	TG (34)	TT (2)	P value	GG (123)	TG (41)	TT (11)	p value
Age (yrs)	52.5±9.7	55.8±12.5	51.6±14.0	0.005	51.6±11.3	60.6±15.5	47.5±3.5	0.0055	52.91±8.9	51.95±7.4	54.09±13.1	0.733
SBP (mmHg)	152.4±10.7	153.0±8.7	151.6±11.6	0.008	152.3±6.7	156.3±4.5	150±5.6	0.0087	152.44±12.2	150.39±10.3	152.64±12.5	0.621
DBP (mmHg)	91.8±2.8	91.8±1.9	91.5±2.9	0.000	90.4±2.1	92.5±1.4	90±2.8	0.0001	92.41±2.9	91.22±2.1	92.09±2.8	0.061
FBG (mg/dl)	107.3±7.4	107.0±8.4	105.5±10.3	0.029	105.4±5.5	109.2±8.9	101.5±3.5	0.0296	108.26±8.0	105.24±7.5	107±10.6	0.121
Weight (kg)	71.7±7.5	72.4±6.7	71.4±11.4	0.158	75±8.4	73.9±8.0	85.5±4.9	0.1584	70.31±6.7	71.24±5.2	68.27±9.2	0.401
Height (cm)	156.2±8.8	158.0±7.5	159±11.5	0.009	165.1±7.3	163.9±5.2	179±1.4	0.0095	152±6	153.22±5.4	155.64±7.1	0.107
BMI (kg/m²)	28.6±2.1	28.5±2.5	28.1±2.7	0.841	27.3±1.	27.4±2.0	26.68±1.1	0.8412	29.23±2.1	29.47±2.6	28.3±2.9	0.341
WC (cm)	94.9±6.6	92.2±7.9	93.2±4.8	0.276	88.7±6.5	88.1±7.0	96±5.6	0.2763	97.91±4.2	95.59±7.1	93.73±5.0	0.004
HC (cm)	106.4±10.3	103.0±10.0	108.5±6.9	0.420	93.9±4.8	94.4±5.2	98.5±0.7	0.4207	112.41±5.9	110.12±7.1	110.18±5.9	0.0896
W/H Ratio	0.9±0.0	0.9±0.0	0.86±0.0	0.249	0.95±0.0	0.93±0.0	0.98±0.0	0.2493	0.87±0.0	0.87±0.0	0.85±0.05	0.3327
Duration of hypertension (yrs)	4.12±3.3	5.9±5.1	5.4±5.1	0.005	4.6±3.9	8.15±6.5	3±2.8	0.0057	3.81±3.0	4.2±2.7	6.55±5.0	0.0208

Table 3 represented the distribution of genotypes with respect to various clinical and anthropometric parameters of EH patients. In anthropometric parameters, only height showed statistical significant difference ($p = 0.009$) among different genotypes of total population and in male population only. In female specific, only waist circumference showed a slightly significant change ($p = 0.004$) in comparison with different genotypes. Blood pressure levels and fasting blood glucose both showed statistical good significant. The duration of hypertension was also showed statistically significant ($p = 0.005$) difference in male specific group and total population in comparison with different genotypes.

While, in case of essential hypertension with new onset of diabetes (Table 4), the age of total population showed statistical lesser significant ($p = 0.02$) when compared between different genotypes. According to gender wise, only

BMI of male population showed slightly significant ($p = 0.033$) between different genotypes as compared to other parameters. Duration of hypertension was also showed non-significant.

Table 5 represented the genotype distribution with respect to drug therapy in male and female population of hypertensive patients. In male patients, both the blood pressure [SBP ($p = 0.0087$), DBP ($p < 0.0001$)] showed the statistical extremely significant difference in comparison between different genotypes. A similar result was obtained in FBG level of male patients ($p = 0.0296$). In female patients, only DBP showed slightly significant change ($p = 0.0610$) with respect to different genotypes. After one year treatment of mono drug therapy (BBs/diuretics/CCBs), only fasting blood glucose levels of patients treated with diuretics showed statistical slightly significant difference in both the genders

Table 4. Distribution of genotypes with respect to various parameters in gender based and total population of essential hypertension with new onset of diabetes.

Genotypes	Total population				Male				Female			
	GG	TG	TT	p value	GG (62)	TG (13)	TT (5)	P value	GG (103)	TG (47)	TT (10)	p value
Age (yrs)	55.8±8.9	52.0±9.8	56.4±8.8	0.0229	54.1±9.1	50.7±6.7	60.2±11.8	0.1401	56.8±8.7	52.4±10.5	54.5±6.9	0.0263
SBP (mmHg)	155.2±7.1	155.4±10.0	156.8±10.9	0.7776	156.1±5.7	154.3±5.3	160.8±4.7	0.1024	154.6±7.8	155.7±10.9	154.8±12.7	0.8048
DBP (mmHg)	92.2±2.3	92.1±2.7	93.33±3.0	0.2338	92.5±2.2	92.1±2.5	92.4±1.82	0.8253	92.1±2.3	92.1±2.7	93.8±3.55	0.1330
FBG (mg/dl)	207.4±79.7	213.7±87.4	179.93±65.6	0.3523	187.2±64.0	197.3±68.7	144.8±68.4	0.3038	219.5±85.8	218.3±92.1	197.5±59.8	0.7433
Weight (kg)	76.8±8.5	73.3±8.3	76.27±9.35	0.0261	82.3±6.4	80.9±9.8	84.6±5.9	0.5991	73.5±7.9	71.2±6.6	72.1±7.9	0.2254
Height (cm)	158.7±8.3	156.2±8.3	157.13±7.8	0.1206	166.6±3.5	167.8±7.1	166.4±2.1	0.6271	154.0±6.6	153±5.1	152.5±4.6	0.5502
BMI (kg/m ²)	30.4±1.7	30.0±1.8	30.78±1.6	0.1717	29.6±1.5	28.6±1.1	30.54±1.7	0.0333	30.9±1.6	30.3±1.7	30.9±1.5	0.1966
WC (cm)	104.0±11.4	101.5±10.9	99.2±10.7	0.1342	110.2±11.9	115.6±12.2	101±16.0	0.0769	100.3±9.4	97.6±6.5	98.3±7.8	0.2075
HC (cm)	105.9±13.2	103.2±10.9	100.53±11.1	0.1444	114.6±12.7	116.1±10.5	105±16.1	0.2289	100.7±10.6	99.6±8.0	98.3±7.8	0.6794
W/H Ratio	0.99±0.05	0.99±0.05	0.99±0.05	0.9999	0.96±0.04	0.99±0.05	0.96±0.0	0.0708	1±0.06	0.98±0.0	1±0.0	0.1302
Duration of hypertension (yrs)	7.8±4.5	8.2±4.51	7.0±4.0	0.6619	7.1±4.2	6.54±4.9	6.8±4.7	0.9075	8.32±4.75	8.6±4.3	7.2±3.9	0.6415

($p=0.022$: male and $p=0.029$:female patients) when compared between different genotypes. Parallel results were obtained in case of combination drug therapy (BBs with diuretic) showed moderate significant in fasting blood glucose levels. Other parameters failed to show any statistical significant difference.

The data (Table 6) represented the genotype distribution with respect to drug therapy in male and female of EHNOD patients. Baseline of SBP, DBP and FBG of both male and female genotypes were found non-significant when compared between different genotypes (GG/TG/TT). After one year treated with different drugs, no significant changes were observed in any of drug treatment of male patients between different genotypes in SBP, DBP and FBG levels. In case of female patients, a slight significant difference ($p=0.0413$) was found in FBG level with diuretic therapy only.

The genotype wise average fall in SBP in EH patients with different drug therapies is given in Fig. 2. The best results were obtained in the combination of BB with CCB in TT genotype followed by CCB mono therapy. In other genotypes, diuretic with CCB and BB with diuretic were also effective. BB mono therapy was least effective in all genotypes. Almost same trend was followed for average fall in DBP (Fig. 2). Here also BB+CCB were most effective combination followed by CCB mono therapy. In both blood pressures (SBP and DBP), the combination drug therapies were most effective than mono drug therapies. Overall, all drug therapies were effective in controlling blood pressure. Fig. 3 represents the average difference in the FBG level in EH patients after treatment with different antihypertensive drugs. A positive value represent the fall in FBG while negative value represents hike in FBG level. Most hike in FBG level was observed with diuretic treatment in TT genotype fol-

lowed by TG genotype and further GG genotypes. A combination of diuretic with BB also elevates the FBG level in TG genotype followed by TT genotype whereas GG genotype showed protective effect with fall in FBG level. Other drug therapies were safe and no rise in FBG was observed.

Similarly, in EHNOD patients, most falls in SBP (Fig. 2) was observed in CCB mono therapy followed by diuretic alone and in combination with BB in TT genotype. All other drug therapies were equally effective in all genotypes. While fall in DBP (Fig. 2) was best achieved in combination of diuretic with CCB in GG genotype followed by diuretic + BB combination and CCB monotherapy in TT genotype. All other combinations were equally effective in all genotypes. Fig. (4) represents the average fall in FBG level in different genotypes after different antihypertensive drug treatment. Effective fall in FBG level was achieved with all drug therapies except diuretic in which FBG level was observed to rise in TT genotype.

4. DISCUSSION

In the present study, we evaluated the possible association of different polymorphic alleles of ADD1 gene with antihypertensive therapy (especially diuretics as mono therapy or as combination therapy) and new onset of diabetes in EH patients in rural population of Haryana. The prevalence of essential hypertension in this population was found around 40% as reported in previous studies [27, 28], therefore, it was suitable to conduct the study on the given population to find the correlation of antihypertensive therapy and new onset of diabetes in EH patients using pharmacogenomics tool. Our results indicated variant T allele (either TG heterozygous or TT homozygous) of ADD1 gene as the independent risk factor for new onset of diabetes under the

Table 5. Distribution of genotypes with respect to various parameters in male and female of essential hypertensive patients before and after drug treatment.

Drug Treatment	Genotypes							
	Male			P value	Female			P value
	GG	TG	TT		GG	TG	TT	
Base line readings								
Number	59	34	2		123	41	11	
SBP	152.37±6.75	156.32±4.56	150±5.66	0.0087	152.44±12.26	150.39±10.36	152.64±12.56	0.6210
DBP	90.46±2.11	92.59±1.44	90±2.83	<0.0001	92.41±2.95	91.22±2.1	92.09±2.81	0.0610
FBP	105.49±5.59	109.21±8.93	101.5±3.54	0.0296	108.26±8.09	105.24±7.58	107±10.64	0.1216
Readings after one year								
Mono drug therapy								
BB	6	4	1		17	2	2	
SBP	133.33±5.68	135.25±3.95	132	0.5758	131±7.38	131.5±10.61	134.5±4.95	0.8238
DBP	86.17±2.93	87.75±3.86	86	0.4807	89.29±5.63	88.5±7.78	90.5±2.12	0.0935
FBP	106.33±5.57	110.75±7.46	110	0.3120	109.41±10.1	111.5±9.19	113.5±9.19	0.8413
Diuretic	7	3	1		15	4	2	
SBP	129.86±6.36	125.33±5.86	121	0.3233	128.27±6.69	127.5±9.75	125.5±4.95	0.8628
DBP	85.71±5.22	85.33±4.16	82	0.9146	87.13±6.52	88.25±6.24	88.5±6.36	0.9431
FBP	109.29±9.6	128.33±10.41	141	0.0227	110.07±11.15	117.75±12.5	135.5±17.68	0.0292
CCB	9	6			24	5	1	
SBP	127.33±6.12	131.17±6.37	-	0.2623	132.75±6.22	128.6±9.26	134	0.2223
DBP	87.11±4.81	86.17±4.45	-	0.7090	89.46±6.37	87.4±5.77	88	0.5106
FBP	103.44±5.59	101.83±6.79	-	0.9945	106.83±8.08	105.8±7.82	104	0.7964
Combination drug therapy								
Diuretic + BB	14	10			27	15	2	
SBP	121.79±4.39	124.7±5.06	-	0.1470	123.74±4.93	124.47±7.76	126.5±7.78	0.7973
DBP	82.43±3.52	84.1±3.7	-	0.2739	81.93±2.70	82.33±4.29	82.5±3.54	0.9193
FBP	105.29±5.88	115.5±8.7	-	0.0023	106.48±10.6	117.53±14.86	126.5±16.26	0.0079
BB + CCB	9	5			22	10	2	
SBP	123.67±4.42	121.6±5.5	-	0.4550	124.82±6.46	121.8±4.89	125±5.66	0.4178
DBP	83.22±4.24	83.2±4.32	-	0.9934	83.68±3.93	82.6±3.53	83.5±4.95	0.7643
FBP	99.67±6.32	101.6±6.43	-	0.5962	103.59±10.26	106.6±8.11	102.5±9.19	0.6896
Diuretic + CCB	14	6			18	5	2	
SBP	119.86±5.19	121.67±4.03	-	0.4584	120.83±4.67	120.6±7.83	126.5±7.78	0.3903
DBP	80.43±3.23	82.17±3.43	-	0.2923	81.17±2.53	83.4±5.18	83.5±6.36	0.3496
FBP	100.86±4.93	98.17±4.02	-	0.2556	100.89±10.44	99.8±9.65	100.5±6.36	0.9775

Table 6. Distribution of genotypes with respect to various parameters in male and female of essential hypertensive new onset of diabetes patients before and after drug treatment.

Drug Treatment	Genotypes							
	Male			P value	Female			P value
	GG	TG	TT		GG	TG	TT	
Base line readings								
Number	62	13	5		103	47	10	
SBP	156.19±5.74	154.38±5.33	160.8±4.76	0.1024	154.68±7.81	155.74±10.99	154.8±12.77	0.8048
DBP	92.58±2.28	92.15±2.54	92.4±1.82	0.8253	92.12±2.32	92.11±2.79	93.8±3.55	0.1330
FBP	187.27±64.09	197.31±68.71	144.8±68.43	0.3038	219.55±85.89	218.32±92.12	197.5±59.89	0.7433
Readings after one year								
Mono drug therapy								
BB	6	2	-		8	3	-	
SBP	134.33±4.41	131.5±9.19	-	0.5520	131.25±4.98	133.33±5.77	-	0.5667
DBP	84.83±3.49	82.5±3.54	-	0.4458	83.75±3.49	83.67±2.08	-	0.9716
FBP	100.83±26.55	101.5±37.48	-	0.9781	116.38±51.3	110.67±28.22	-	0.8620
Diuretic	5	2	2		10	4	3	
SBP	132.2±8.23	129.5±7.78	131.5±9.19	0.9284	129.5±8.4	127.75±6.65	132.67±6.66	0.7151
DBP	85.8±4.27	86.5±4.95	86±7.07	0.9859	86.5±4.86	83.25±3.40	85.33±4.16	0.4891
FBP	110.2±24.94	127.5±74.25	166.5±77.07	0.4299	117.5±40.52	142.5±43.27	193.33±38.19	0.0413
CCB	9	2	1		17	4		
SBP	130.78±6.36	128.5±6.36	131	0.6574	128.65±6.58	132.25±7.14	-	0.3437
DBP	83.67±3.12	84.5±3.54	85	0.7453	84.94±4.35	83.75±2.5	-	0.6087
FBP	104.33±16.43	103.5±17.68	105	0.9503	106.06±25.62	103.25±20.55	-	0.8412
Combination drug therapy								
Diuretic + BB	12	2			16	6	2	
SBP	128.25±7.2	132±5.66	-	0.5015	132.19±7.54	128.83±7.05	129.5±9.19	0.6211
DBP	85.33±4.83	85±4.24	-	0.9295	87.25±4.3	85.17±3.31	84.5±4.95	0.4570
FBP	115.58±36.48	137.5±36.06	-	0.4463	122.63±49.19	149.5±50.48	190.5±68.59	0.1537
BB + CCB	20	2	1		31	16	2	
SBP	126.15±5.47	125±7.07	130	0.7832	125.71±5.09	128.69±5.41	126.5±6.36	0.1909
DBP	84.3±4.11	82±2.83	85	0.4534	83.87±3.88	84.63±3.18	83.5±3.54	0.7753
FBP	106.4±18.88	108.5±17.68	170	0.8819	108.03±26.3	109.38±38.72	102.5±38.89	0.9558
Diuretic + CCB	10	3	1		21	14	3	
SBP	128.7±6.95	123.67±4.04	128	0.2659	127.33±4.76	130.43±7.27	128.67±7.23	0.3329
DBP	83.5±3.81	81.67±2.08	84	0.4512	81.86±3.14	84.43±3.90	83.67±3.79	0.1091
FBP	102.6±14.05	108.33±19.73	108	0.5794	103.1±16.34	107.43±49.31	110.33±39.63	0.9004

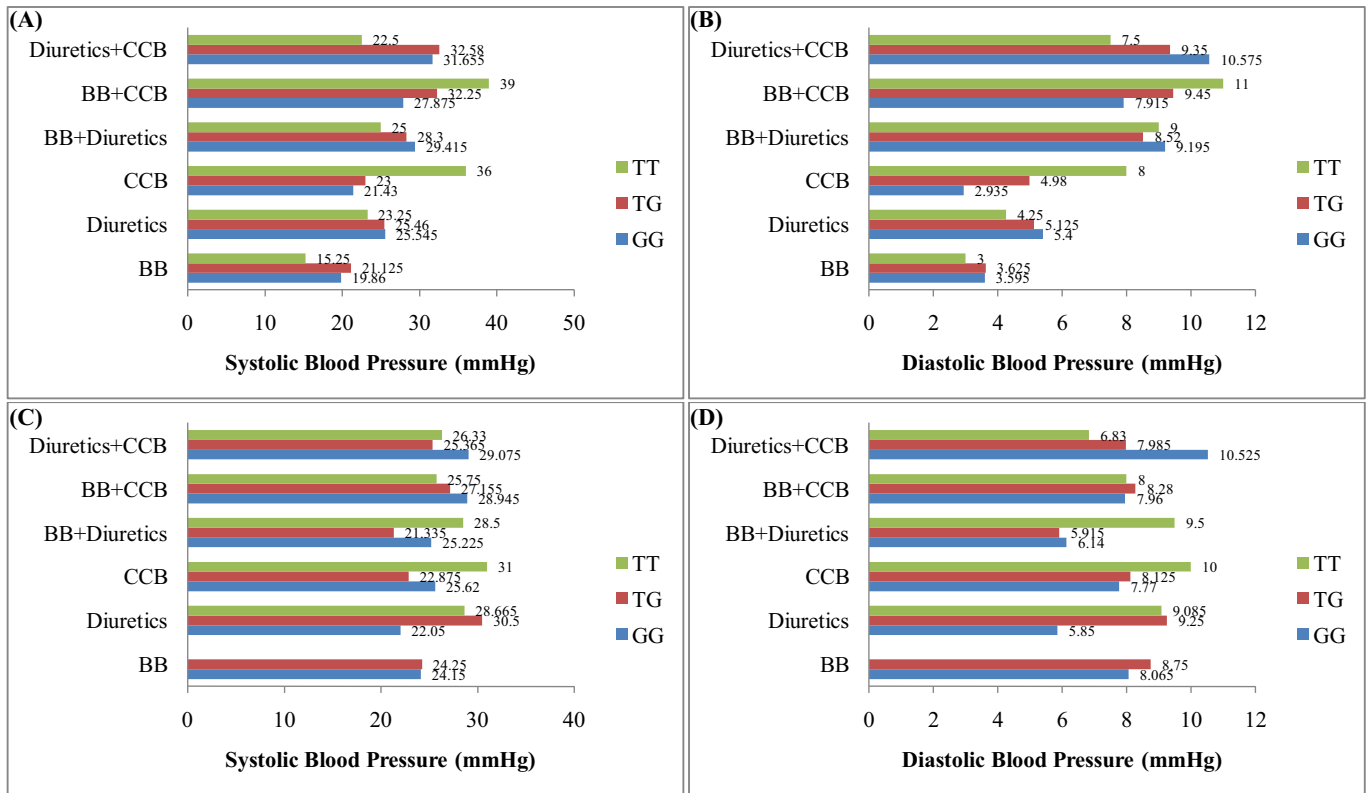


Fig (2). (A) Average fall in systolic blood pressure in EH patients among different genotypes with different antihypertensive therapies. (B) Average fall in diastolic blood pressure in EH patients among different genotypes with different antihypertensive therapies after one year. (C) Average fall in systolic blood pressure in EHNOD patients among different genotypes with different antihypertensive therapies. (D) Average fall in diastolic blood pressure in EHNOD patients among different genotypes with different antihypertensive therapies after one year.

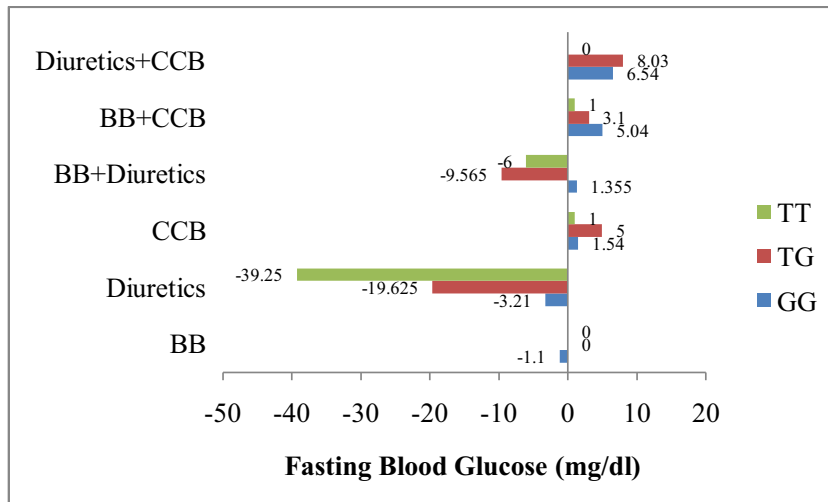


Fig (3). Average change in fasting blood glucose level in EH patients among different genotypes after different antihypertensive therapies treatment after one year.

influence of diuretic therapy. The study population was grouped as normotensives, EH and EHNOD groups.

The base line parameters (clinical and anthropometric) of these groups were compared with overall population, male and female population respectively (Table 1). Data showed a higher statistical significance difference ($p < 0.0001$) at p value for different anthropometric parameters (Weight, BMI,

WC, HC, W/H ratio) in all populations. The average duration of hypertension in EH and EHNOD groups were statistically non-significant. All clinical parameters (SBP, DBP and FBG level) were highly significant for EH and EHNOD groups when compared with normotensives. So, the study of base-line parameters suggested that different anthropometric parameters were indicative of risk factors for EH in both EH

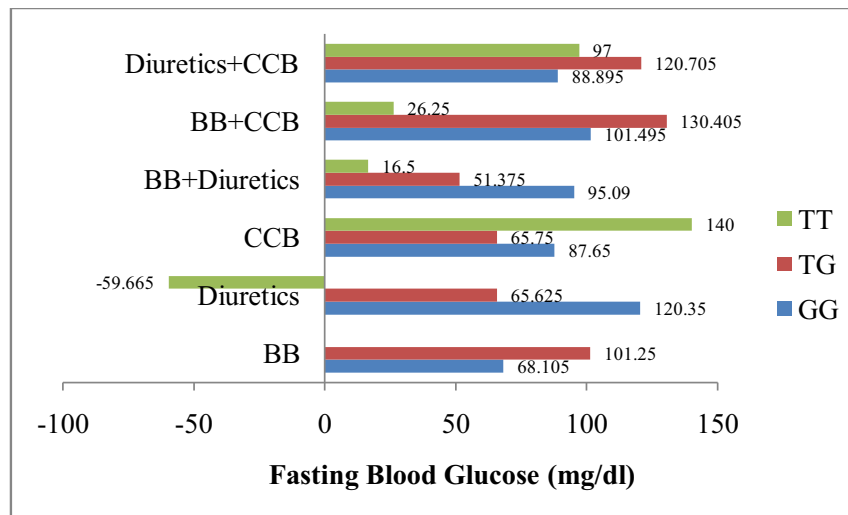


Fig (4). Average change in fasting blood glucose level in EHNOD patients among different genotypes after different antihypertensive therapies treatment after one year.

and EHNOD groups when compared with normal population. However, age and height were not significant for all comparisons and could not be taken as the indicator of risk factor for EH.

The comparison of the genotypic and allelic frequency distribution (G allele and T allele) among EH and normotensive group (Table 2) showed a higher percentage of GG genotype as compared to TG and TT genotypes and hence higher G allele frequency than T allele in EH but it was non-significant when compared with that of normotensive group. While the genotypic and allelic frequency distribution among EHNOD and normotensive group (Table 2) showed a significant difference ($p=0.0008$) in T allele frequency of EHNOD group when compared with normotensives. Therefore, the data showed a positive association between EHNOD and TT genotypic polymorphism which may indicate that T allele as the probable risk factor for new onset of diabetes in hypertensive population.

The genotype wise comparison of all anthropometric and clinical parameters in overall EH population (Table 3) revealed that few of the anthropometric parameters were non-significant like weight, BMI, WC, HC and W/H ratio. However, clinical parameters and anthropometric parameters of essential hypertensive were showing extremely statistical significant. In male population (Table 3), the statistical data suggested that p value were highly significant for age, SBP, DBP, FBG levels and duration of hypertension which signified the clear difference in wild type and variant type allele in terms of clinical parameters. But other anthropometric parameters such as weight, body mass index, WC, HC and W/H ratio were found to be statistical non-significant at p value. In female population (Table 3), it showed statistical significant difference in DBP, FBG levels, WC and duration of hypertension. All other anthropometric parameters were non-significant.

Similarly, the genotype wise comparison of different anthropometric parameters in overall population of EHNOD (Table 4) revealed significant difference in age, weight and

height. While in male patients (Table 4), it showed significant difference only in BMI and in female patients, it showed statistically significant difference only in age. All other parameters showed non-significant results.

Therefore, the above results revealed that there was a slight difference in wild type and variant type genotypes of ADD1 gene with respect to different clinical and anthropometric parameters. Although this difference was minor and from the data, it was not observed properly but it suggested that variant T allele as the risk factor for metabolic disease *via* salt sensitivity. However, contradictory results were given by Ranade *et al.*, 2000 [29] and Ramu *et al.*, 2010 on Indian population [16].

The effect of first line antihypertensive drug therapy on different genotypes of male and female population of hypertensive patients (as mono therapy or as combination therapy) was evaluated by comparing the genotype wise clinical parameters (SBP, DBP and FBG) at baseline and after one year of drug therapy with different drugs such as BB, diuretics, and CCB (Table 5). One year period for observation of patient's clinical parameters was followed to assess the long term effect of different antihypertensive drug therapies on these clinical parameters with respect to genotype of patients. Among mono drug therapy of BB, no significant difference was observed in SBP, DBP and FBG level in between GG, TG and TT genotype both in males and females. Monotherapy of CCB showed somewhat protective effect on blood glucose level with better management of hypertension. But after one year of diuretic treatment patients, FBG level was significantly different among different genotypes in both males ($p=0.0227$) as well as females ($p=0.0292$) of EH group. TG and TT genotypes showed elevated blood glucose level as compared to GG genotype in both male and female EH patients. The combination therapy of diuretic with BB also elevated the blood glucose level in T allele carrying male ($p=0.0023$) and female patients ($p=0.0079$). However, combination of diuretic with CCB and other drug combinations were safe and effectively controlled the blood pressure.

In EHNOD patients, the antihypertensive drug therapy effectively controlled the blood pressure in all genotypes. No significant difference was observed in FBG level of male population after one year treatment but a female patients showed a slight significant difference ($p=0.0413$) with diuretic therapy (Table 6). In both monotherapy (diuretics) and in combination therapy (diuretic with BB) of male and female patients, it showed fluctuations (given as standard deviations) in FBG levels as it was very high and the results were non-significant despite anti diabetic treatment in variant T allele (GT and TT genotypes) as compared to G allele. However, no such type of fluctuation in FBG level was observed with other mono drug therapy or combination drug therapies and these were found quite effective.

Therefore, from data (Fig. 2), we can suggest that all antihypertensive drug therapies either as monotherapy or in combination effectively controlled both the blood pressures (SBP and DBP) in EH patients and EHNOD patients. However, diuretic either as mono drug therapy or in combination with BB in both EH and EHNOD patients were reported to elevate FBG level in T allele (either as TG or TT genotype or both) of ADD1 gene. In EHNOD patients, diuretic mono therapy was associated with elevated FBG level in TT genotype but the combination of diuretic with BB was safe in this group.

From our findings, we may suggest that those patients who were treated with chronic diuretic therapy whether it was monotherapy or in combination therapy, the FBG levels may chance to elevate due to disturbance in glucose homeostasis. The probable mechanism may lie behind the fact that variant T allele of ADD1 was suggested to be associated with the enhanced renal Na^+ re-absorption through Na^+-K^+ ATPase pump [30, 31] which in turn eliminate more K^+ ions to balance the ionic potential across the renal membrane [16, 17]. But a higher K^+ elimination is associated with low insulin secretion and insulin insensitivity and hence leads to glycaemic dysregulation [31, 32] as described in Fig. (4).

Therefore, in our results, we can easily show the evidence that, those individuals who are having T allele may prone to high risk of essential hypertension as well as the new onset of diabetes mellitus after chronic diuretic therapy in essential hypertensive patients.

CONCLUSION

Our study suggested that variant T allele of the ADD1 gene may be considered as the risk factor for the development of new onset of diabetes in EH patients of Haryanvi population.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved (IEC/670) by the Institutional Ethics Committee of M. M. University, Haryana, India.

HUMAN AND ANIMAL RIGHTS

No animal were used in this research. All humans research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki

<<http://www.wma.net/policiespost/wmadeclaration-of-helsinki-ethicalprinciples->> for-medicalresearch-involving-human-subjects/>principles of 1975, as revised in 2008 (<http://www.wma.net/en/20activities/10ethics/10helsinki/>).

CONSENT FOR PUBLICATION

Written consent was obtained from all the participants.

AVAILABILITY OF DATA AND MATERIALS

The data is not revealed by the authors. Still the project is going further on large patients size. Moreover, patients profile is a confidential part, we can not disclose as per IEC. If any person have any query regarding this. They can contact me at given email address as corresponding author.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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