Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[2] January 2020 : 106-110

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Effect of EDN1 Genotype in Essential Hypertension in A North-Eastern Indian Population

Gogoi Krishna[1]*, Deka Manab [2], Das Jharna [1], Goswami Amal Dev [3]
[1, 3] Research Scholar, Department of Bioengineering & Technology, Gauhati University, Guwahti-781014, India
[2] Professor and Director GUIST, Gauhati University, Guwahati-781014, India
[3] Physician, NEMCARE Hospital , Guwahati-781005, India
[1*]Corresponding Author's E-mail: krishna.rsguist@gmail.com
Running Title: EDN1 genotypes in Essential Hypertension

ABSTRACT
Essential hypertension is a complex cardiovascular disease with multifactorial etiology involving different genes and environmental factors. In this study we investigated the possible association of EDN1 gene polymorphisms rs5370 and rs1800541 and essential hypertension in an Assamese population from North-Eastern region of India. In this case-control study, a total number of 400 subjects- 200 hypertensive and 200 normotensive subjects were enrolled. The genotyping was performed by polymerase chain reaction followed by gene sequencing. Tetra-amplification refractory mutation resistant PCR was performed for rs5370. The results revealed higher risk of Essential Hypertension among the T-allele carriers of rs5370 (GT+TT) with OR 1.83 (1.23-2.73), p=0.002. The T-allele carriers were significantly overweight OR 3.34 (2.22- 5.04), p>0.001. The study did not find any association between rs1800541 and Essential Hypertension. Our study suggests that rs 5370 is an important genetic predictor in development of EH in North-Eastern Indian population.
KEYWORDS: *Essential hypertension, EDN1, rs5370, rs1800541, Overweight*

Received 13.12.2019 Revised 01.01.2020 Accepted 13.01.2020

INTRODUCTION

Essential hypertension (EH) is a Non-Communicable Disease of major interest as uncontrolled hypertension may lead to cardiac, renal and neurological complications [1]. It is a complex cardiovascular disorder involving genetic and environmental factors. It's a challenge to determine the exact interactive role of genetic and environmental factors in causation of hypertension. With advances in molecular techniques, several gene loci have been identified related to renin angiotensin system (RAS), sympathetic nervous system and endothelins in gene-association studies. Endothelial dysfunction is an important etiological factor in development of cardiovascular diseases and an enhanced expression of Endothelin-1(ET-1) has been reported in hypertension, chronic heart failure and other cardiovascular diseases like atherosclerosis [2,3,4]. ET-1, produced following cleavage of Big endothelin, is a very potent vasoconstrictor found in many organs in our body including brain, kidneys and vascular endothelium [5]. As ET-1 plays an important role in pathophysiology of vascular endothelial and cardiac disease, the EDN-1 gene encoding ET-1, located in chromosome 6p24.1 is an obvious choice in candidate gene-association study of cardiovascular diseases [6]. The interaction between EDN-1 gene polymorphisms and hypertension varied between studies from different countries worldwide [7,8,9,10,11,12].
To the best of our knowledge gene-association study targeting EDN1 polymorphisms in EH have not been undertaken in the North-Eastern part of India till date. We assessed the interaction of rs5370 and rs1800541 single nucleotide polymorphisms (SNP) of Endothelin (EDN-1) gene in essential hypertensive (EH) patients in an urban population from North-Eastern region of India.

MATERIAL AND METHODS

The present study was a case-control observational study carried out from June 2014 to May 2019. The subjects between 18 – 65 years of age, were enrolled following measurement of office blood pressure

(BP) at the physician's office located at permanent health care centres in the city. The control group comprised of normotensive volunteers with normal BP. Hypertension was categorized as per clinical guidelines of American Heart Association/American College of Cardiology [13]. Subjects with systolic blood pressure (SBP) ≥ 140 or diastolic blood pressure (DBP) ≥90 were categorized as hypertensive. The individuals with secondary hypertension, with other cardiac or systemic diseases like renal damage, pregnant ladies, children, hypertensive on lipid lowering drugs were excluded from the study. All the subjects underwent a general physical check-up, anthropometric measurements and elecrocardiography. Baseline characteristics - age, sex, body mass index (BMI), SBP and DBP, of all the participants are shown in Table-1. During this study period a total number of 400 participants – 200 with EH and 200 Normotensives, were enrolled. Venous blood was collected in EDTA vacutainers (Becton Dickinson, USA) from all the subjects for DNA extraction. The laboratory works were carried out at the Department of Bioengineering and Technology, Gauhati University .The study was approved by the Gauhati University Institutional Ethics Committee and all subjects were consented prior to enrollment in the study.

Genotyping of SNPs: DNA was extracted from whole blood using QIAamp DNA mini kit (Qiagen, Hilden, Germany) as per manufacturer's protocol. For genotyping rs5370 tetra-amplification refractory mutation resistant Polymerase Chain Reaction (T-ARMS PCR) was performed as in earlier studies [14]. Standard PCR with Self-designed primer sequences forward: GCATGTGTGTTGTGCCAGTC and reverse: AGCAAAAGGTGCATGGAACAC (annealing temperature 58.4°C) were used to amplify rs1800541 polymorphism, which produced a band of 271bp. The products were run on 2% agarose gel containing 0.5 ug/ml of Ethidium bromide at 100V for 30 minutes. For rs5370 polymorphism the band sizes were a control band of 385 bp + 191 bp for T-allele and 385 bp + 242 bp for G-allele (Fig1). The rs5370genotypes were confirmed by sequencing the outer 385 bp product. The rs1800541 polymorphism was identified by sequencing the 271bp PCR product (Eurofins, Bangalore).

Statistical analysis: The continuous variables are presented as mean ± standard deviation and were compared by student's t-test. For comparing the categorical variables and genotype frequency of SNPs between cases and controls a Chi square/Fisher's exact test was performed and odds-ratio (OR) and 95% confidence interval (CI) were calculated. Hardy-Weinberg testing was performed using online calculator available at www.wpcalc.com (https://wpcalc.com/en/equilibrium-hardy-weinberg/). Online statistical calculator (www.snpstats.com) was used to analyze the genotype results. The statistical tests were considered significant at p value <0.05, based on two-tailed probability.

RESULTS

The baseline characteristics of the study groups are presented in Table-1. The mean age of the participants was 43.67± 7.53. The difference between mean age of cases (46.09±6.46 years) and controls (41.24±7.74 years) was significant (p <0.05). The gender male carried an increased risk of hypertension with OR 1.59(1.064, 2.402) with p=0.01.

The distribution of genotypes is presented in Table-2. Obtained genotype frequency for both the SNPs was in Hardy-Weinberg equilibrium ($_{HWE}$P >0.05). For rs5370 polymorphism the minor allele (T-allele) frequency (MAF) was 0.3 and for rs1800549 polymorphism the MAF (G-allele) was 0.18.

There was a significant difference in allele distribution for rs5370 between cases and controls, p = 0.007. We assessed the association under different genetic models -dominant, recessive, codominant and log-additive (Table 3). The SNP rs5370 was associated with increased risk of hypertension under co-dominant, dominant and log-additive model. As per the results of analysis of dominant model the carriers of rs5370 (GT + TT) had an increased risk for hypertension with OR 1.83(1.23-2.73), p=0.002. A significant association was found between rs5370 T-allele carriers and overweight individuals (BMI ≥25) with an OR 3.34 (2.22- 5.04), p>0.001(not shown in table). Polymorphism rs1800541 did not have any significant association with hypertension (p =0.86). We performed linkage disequilibrium (LD) analysis for the two genotypes and found the two genotypes to be in very weak LD with D=0.003, r=0.02, p =0.53.

DISCUSSION

We evaluated the effect of two polymorphisms in EDN-1 gene, rs 5370(G>T) and rs 1800541(T>G) in development of essential hypertension (EH) in a North-Eastern Indian population. We found that mean BMI, age and blood pressure were significantly higher in the EH group similar to earlier reports [15]. Global data shows that individuals of male gender and obesity are at higher risk of developing hypertension and metabolic disorders [1]. The same was reflected in findings of the present study.

To the best of our knowledge this is the first study on effect of ET-1 gene polymorphism rs5370 and rs1800541 in EH from North-Eastern region of India. The significant findings of the present study were T-allele carriers were at increased risk of developing EH and its significant association with overweight.

Both GT and TT genotypes showed significantly increased risk of EH compared to GG genotype of rs5370. In this study the MAF (T-allele) for rs5370 was 0.3 which is concordant with the Alfa project report of MAF 0.38 among South Asian population [6].

The rs5370 or K198N polymorphism leads to replacement of guanine by thymine at 6: 12296022 (GRCh38) location in exon 5 of ET-1 gene [6]. As a result Lysine in 198th position of the pre-proendothelin peptide is replaced by Asparagine. Earlier researchers have reported a significantly high content of plasma ET-1 in T- allele carriers [2]. African Americans who were T-allele carriers showed fewer improvements in maintaining ambulatory SBP compared to the non-carriers following behavioral intervention on exposure to psychosocial-stress factors [8]. The risk of hypertension in the low – aerobically fit T-allele carriers was two-times higher than the aerobically fit carriers [9]. Significant association between rs5370 and hypertension has been reported by other researchers [7]. The finding of significant association between T-allele carriers with obesity and EH in the present study is similar to earlier reports [10 and 11].

TABLE 1: BASELINE CHARACTERISTICS OF THE SUBJECTS

Parameter	Cases(n=200)	Controls (n=200)	P value
Age (in years)	46.09±6.46	41.24±7.74	<0.05
Sex (Male/female)	135/65	113/87	0.01
BMI(kg/m^2)	25.99±4.0	23.46±3.38	<0.05
SBP(mmHg)	153.0±12.8	115.14±7.41	<0.05
DBP(mmHg)	89.96±9.42	75.11±5.48	<0.05

Data are mean ± standard deviation for continuous variables,
Student's t-test performed for continuous variables
Categorical variable Gender- Odds ratio was calculated

TABLE 2: GENOTYPE DISTRIBUTION IN CONTROLS AND CASES

SNP	Genotype or allele	Control n(%)	Cases n(%)	p
rs5370	GG	120 (60)	90 (45)	0.007
	GT	65(32)	84 (42)	
	TT	15 (8)	26 (13)	
	G-allele	305 (76)	264(66)	
	T-allele	95 (24)	136(34)	
rs1800541	TT	133 (66.5)	138 (69)	0.86
	GT	57 (28.5)	53 (26.5)	
	GG	10 (5)	9 (4.5)	
	T-allele	323 (81)	329 (82)	
	G-allele	77 (19)	71 (18)	

Chi-square test

TABLE 3: ASSOCIATION BETWEEN SNPS AND DISEASE STATUS

SNP	Model	Genotype	OR (95% CI)	P value
rs5370	Co-dominant	T/T	1	0.007
		G/T	1.72(1.13-2.63)	
		G/G	2.31 (1.16-4.62)	
	Dominant	G/G	1	0.002
		G/T-T/T	1.83 (1.23-2.73)	
	Recessive	G/G-T/T	1	0.06
		T/T	1.84 (0.94-3.60)	
	Additive	-	1.59 (1.18-2.15)	0.002
rs1800541	Co-dominant	T/T	1	0.48
		G/T	0.75 (0.47-1.2)	
		G/G	0.98 (0.35-2.76)	
	Dominant	T/T	1	0.27
		T/T-G/G	0.78(0.49-1.22)	
	Recessive	T/T-G/T	1	0.9
		G/G	1.06(0.28-2.97)	
	Additive	-	0.85 (0.58-1.23)	0.39

SNP: single nucleotide polymorphism;
OR: odds ratio; CI: confidence interval;
p values were calculated after adjusting for age and sex.

The present study did not find any significant association of variant rs1800541 in the promoter region of

the gene and EH. The reported MAF (G-allele) of rs1800541 is 0.18 in the present study. As per the Alfa project report, the G-allele frequency varies from 0.0 to 0.37 among different sets of population, with a global average of 0.28 [6]. An earlier study compared blood pressure and left ventricular mass (LVM) in two groups of youths who were ethnically different(European American and Black) and found that rs1800541 in low SES group had significantly higher LVM, while rs5370 had no effect at all[12]. That the polymorphism was in very weak LD with rs5370 in the present study suggests that rs1800541 is probably in a strong LD with some other variations.
The limitation in the present study was its small sample size.

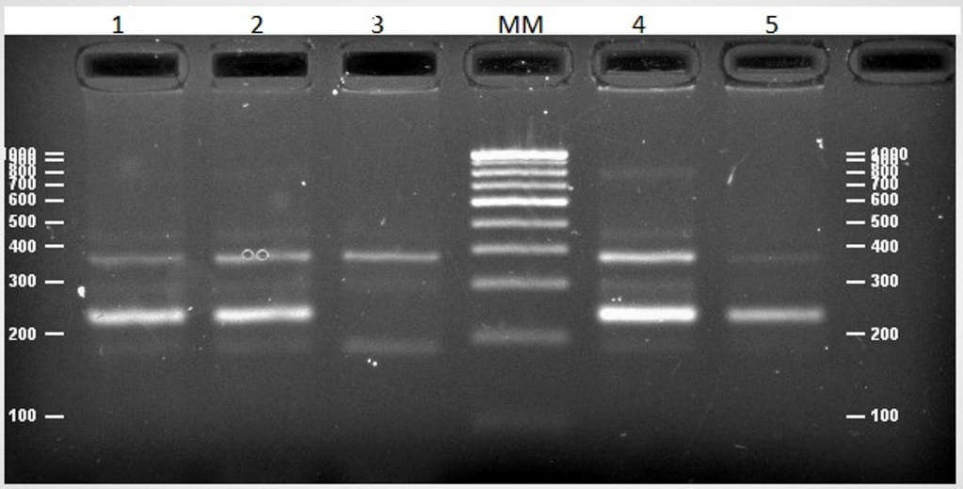

FIG1: Gel Image of T-ARMS PCR of rs5370
MM: 100 bp Molecular marker; Lane1,2,4: GT genotype (385bp+242bp+191bp;
Lane 3: TT genotype (385bp+191bp); Lane 5: GG genotype(385bp+242bp)

CONCLUSION

In conclusion, we studied association between polymorphisms in EDN-1 gene and found that T-allele carriers of rs5370 polymorphism were associated with a genetic predisposition to EH and obesity in people from North Eastern region of India. That T-allele carriers were significantly overweight than the other genotypes, further suggests that changes in their life style at an early age might play a crucial role in preventing development of high BP. There was no significant association between rs1800541 and EH.

CONFLICT OF INTEREST: We do not have any Conflict of interests to declare.

REFERENCES

1. World Health Organization(2018).Non communicable diseases Factsheets. available at http://www.who.int/mediacentre/factsheets
2. Aggarwal PK, Jain V, Srinivasan R et al.(2009). Maternal EDN1 G5665T polymorphism influences circulating endothelin-1 levels and plays a role in determination of preeclampsia phenotype. *J of Hypertension, 27(10): 2044–2050*
3. Vasku A, Spinarova L, Goldbergova M et al. (2002).The Double Heterozygote of Two Endothelin-1 Gene Polymorphisms (G8002A and -3A/-4A) Is Related to Big Endothelin Levels in Chronic Heart Failure. *Exp Mol Pathology **73:** 230–233*
4. Bohm F, Pernow J (2007).The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. *Cardiovascular Res*, 76(1):8–18
5. Arinami T, Ishikawa M, Inoue A et al (1991). Chromosomal assignments of the human endothelin family genes: the endothelin-1 gene (EDN1) to 6p23-p24, the endothelin-2 gene (EDN2) to 1p34, and the endothelin-3 gene (EDN3) to 20q13.2-q13.3. *Am j hu genetics*, *48(5), 990–996.*
6. National Center for Biotechnology Information (NCBI)[Internet]. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information Available from: https://www.ncbi.nlm.nih.
7. Dzholdasbekova AU, Gaipov AE.(2010) The Association Between Polymorphism of Lys198Asn of Endothelin-1 Gene and Arterial Hypertension Risk in Kazakh People .*Eur J Gen Med .7(2):187-191*
8. Gregoski MJ , Barnes VA ,Tingen MS et al.(2012) Differential Impact of Stress Reduction Programs upon Ambulatory Blood Pressure among African American Adolescents: Influences of Endothelin-1 Gene and Chronic Stress Exposure. *Int J Hypertension : Article ID 510291*

9. T. Rankinen, T. Church, T. Rice et al. (2007) Effect of Endothelin 1 Genotype on Blood Pressure Is Dependent on Physical Activity or Fitness Levels. *Hypertension* .50(6):1120–1125
10. Tiret L, Poirier O, Hallet V et al.(1999).The Lys198Asn Polymorphism in the Endothelin-1 Gene Is Associated With Blood Pressure in Overweight People.*Hypertension.33:1169–1174*
11. Jin JJ, Nakura J, Wu Z et al (2003) Association of Endothelin-1 Gene Variant With Hypertension. *Hypertension. 41:163-167*
12. Dong Y, Wang X, Zhu H et al.(2004) Longitudinal Findings in Youth Endothelin-1 Gene and Progression of Blood Pressure and Left Ventricular Mass: longitudinal findings in youth *Hypertension. 44:884-890*
13. Whelton PK,Carey RM et al.(2018) 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults. *Hypertension. 71:e13-e115*
14. Hashemi M, Sadeghi-Bojd S, Aryanezhad S et al. (2017). Association of Endothelin-1 rs5370 G>T gene polymorphism with the risk of nephrotic syndrome in children. *J Nephropathol.;6(3):138-143*
15. Bhaskar LVKS ,Thangaraj K, Non KL, Kumar PK, Pardhasaradhi G, Singh L,Rao VR. (2010). Neuropeptide Y gene functional polymorphism influences susceptibility to hypertension in *Ind J Hu Hypertension, 24, 617–622*

CITATION OF THIS ARTICLE

G Krishna, D Manab, D Jharna, G A Dev. Effect of EDN1 Genotype in Essential Hypertension in A North-Eastern Indian Population. Bull. Env. Pharmacol. Life Sci., Vol 9[2] January 2020 : 106-110