Research Article

Hemicentin-1 (Gln5345Arg) Gene Polymorphism in Patients with Essential Hypertension

Kamna Srivastava[1*], Rajiv Narang[2], Jagriti Bhatia[3], Daman Saluja[1]

[1]Dr. B R Ambedkar Centre for Biomedical Research, University of Delhi, Delhi, India

[2]Department of Cardiology, All India Institute of Medical Sciences, Delhi, India

[3]Department of Pharmacology, All India Institute of Medical Sciences, Delhi, India

***Corresponding author:** Dr. Kamna Srivastava, Dr. B R Ambedkar Centre for Biomedical Research, University of Delhi, Delhi-110007, India, Tel: 0091-11-27666272; Fax: 0091-11-27666248; E mail: kamna605@gmail.com

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Abstract

Background: Oxidative stress is one of the risk mechanisms for macular degeneration and essential hypertension. Hemicentin-1 gene was shown to be associated with Age related Macular degeneration, however its role in Essential hypertension has not been reported. We have made the objective to study Hemicentin-1 (Gln5345Arg) gene polymorphism in patients with essential hypertension and the levels of normal protective enzymes such as endothelial Nitric oxide (NO) and malondialdehyde (MDA) in patients with essential hypertension.

Methods: The polymorphism of the Hemicentin-1 **(**Gln5345Arg) gene polymorphism in 250 patient with essential hypertension and 250 controls is studied by PCR-RFLP technique. The plasma levels of MDA and NO were determined using standard procedures.

Results: Observed and expected genotypes showed Hemicentin-1 gene variants were conforming to the Hardy–Weinberg equilibrium law. The observed allele frequencies in patients were 0.824 and 0.176 for G and A alleles respectively, whereas 0.896 and 0.104 was observed in controls. Circulating levels of NO was lower in patients with AA genotypes as compared to AG and GG. Lipid peroxidation in terms of levels of circulating MDA was significantly higher in patients with AA genotypes as compared to patients with AG and GG genotypes.

Conclusion: There was statistically significant association found between Hemicentin-1 (Gln5345Arg) gene polymorphism and essential hypertension. Individuals with AA genotypes have lower levels of nitric oxide and higher lipid peroxidation as compared to the individuals with AG and GG genotypes indicating that the individuals with AA genotypes may be at substantially higher risk of developing essential hypertension.

Keywords: Essential hypertension; Hemicentin-1 (Gln5345Arg) gene; Malondialdehyde; Nitric oxide

1. Introduction

Human essential hypertension (EHT) is a multifactorial trait with a complex genetic basis. This complex disease is due to the consequence of an interaction between various environmental and genetic factors and it plays a major role in blood pressure (BP) variation [1]. Hemicentin was first identified in C. elegans as him-4 (High incidence of males and is now a recognized player in maintaining the architectural integrity of Myocardium [2]. This myocardial remodeling is imparted by an effect on cardiac fibroblast migration. HMCN1 (Hemicentin-1) is a Protein Coding gene, encoding a large extracellular member of the immunoglobulin superfamily and mapped to 1q25.30-1q31.1 [3-4]. Several groups have screened Hemicentin-1 gene for potential Age related Macular Degeneration (AMD) associated variants; however, no evidence for any significant allele associations has been reported so far [5-7]. AMD pathogenic mechanisms, such as oxidative damage, chronic inflammation, and angiogenesis regulation are also involved in the pathogenesis of essential hypertension [8-9]. Rodrigo et al., [10] has hypothesized that high blood pressure is a pathological state associated with oxidative stress. ROSs interact with lipid bilayer of cell membrane resulting in lipid peroxidation. Malondialdehyde (MDA) is a stable end product of lipid peroxidation. Thus, excess free radicals and the resultant oxidative stress along with elevated MDA explain the long-term vascular complications in essential hypertension, which is a major cause of mortality and morbidity [11-12]. Endothelium dysfunction is considered to be an intrinsic element in the pathogenesis of diabetes complications. The free radical nitric oxide (NO), accounting to the biological activity of endothelium-derived relaxing factor, is well recognized for its association with vascular disease [13-14]. Taking into consideration of the above facts, the present study was undertaken with an objective to study the polymorphism of the Hemicentin-1 **(**Gln5345Arg) gene polymorphism and assess the intergenotypic variations in the levels of endothelial Nitric oxide and malondialdehyde to determine the role of oxidative stress in patients with essential hypertension. These parameters in the disease process, were important to study as they might have utility to delay progression and in the treatment of essential hypertension which is major risk factor for CVD and strokes.

2. Methodology

2.1 Study population

The sample size of the study subjects (250 patients and 250 controls) is adequate for the present study determined by using standard statistical method in case-control groups, at the significance level of 0.05 at power 80% on the basis of prevalence of minor alleles referred form previous studies [1]. All the patients in the age range between 35 years to 65 years were screened randomly from the outpatient department (OPD) clinics of hypertension, Department of Cardiology, All India Institute of Medical Sciences (AIIMS), New Delhi, India. Healthy controls were recruited for the study from similar socioeconomic geographical background residing in North India from

past three generations. Blood pressure measurements were taken according to Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC-VII) the definition of essential hypertension and healthy controls. All the hyperten-sive patients were not suffering from any known disease, secondary hypertension, diabetes and kidney disease. This study complied with the Declaration of Helsinki. Approvals of ethics committee of AIIMS, New Delhi and ACBR, Delhi were obtained.

2.2 Sample collection and processing

Fasting (12 hours) venous blood samples (5 ml) were obtained from the study subjects in an ethylene diamine tetra acetic acid (EDTA) vial. Plasma were separated and genomic DNA was extracted from whole blood using the Flexigene DNA kit (QIAGEN®) according to the manufacturer's instructions.

2.3 Genotyping

Genotyping of the Hemicentin-1 **(**Gln5345Arg) gene polymorphism were identified by using PCR - RFLP techniques. The primers were designed by using GENERUNNER version 3.05 software (Hastings Software Inc. Hastings, NY, USA) based on gene bank sequence (NCBI Reference Sequence: NG_011855.1).

Forward Primer**:** 5′-CAA GTG TAT CTG TCC ACC AGG TC-3′

Reverse Primer: 5′ TGT CTG TAA TGC TGT TGA GGT TG-3′.

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood leukocytes using a Flexigene DNA kit (Qiagen). DNA fragment was amplified by PCR using primers designed using Generunner program. The PCR conditions were standardized to run at 2 minutes at 95°C, followed by 34 cycles of 30 seconds denaturation at 94°C, 45 seconds annealing at 58°C, and 55 seconds extension at 72°C. RFLP analysis was conducted on 159–base pair (bp) PCR amplified product following restriction fragment length polymorphism using the restriction endonuclease SalI. The digestion patterns are as follows: wild-type (GG), 159 bp; heterozygous variant (GA), 159 bp and 137 bp; and homozygous variant (AA), 137 bp. The reliability of our genotyping was confirmed by direct sequencing of amplified DNA from randomly selected samples (20%) in both the study subjects, with no difference observed in results between the two techniques.

2.4 Estimation of circulating nitric oxide levels

Plasma levels of NO were estimated in the study subjects by the method of Ding et al [12].

2.5 Estimation of circulating malondialdehyde levels

Plasma levels of MDA were estimated in the study subjects by the method of Satoh [11].

2.6 Statistical analysis

The sample size was found to be adequate in the present study which was calculated using PS-Power and sample size calculation version 3.0 software, with an α error of 5% and a power 80% at the significance level of 0.05. Statistical analyses were performed with commercial software (SPSS, ver.11.5; SPSS Inc., Chicago, IL). Chi-square goodness of fit was used to verify the agreement of observed genotype frequencies with those expected (Hardy–Weinberg equilibrium). The analysis of variance (ANOVA) was used to calculate the difference between genotype groups using Bonferroni's method for multiple comparisons between genotype classes. An odds ratio at [95% confidence intervals (CI)] was calculated as an index of the association of the genes with the disease. Data were presented as mean ± SD.

3. Results

3.1 Baseline characteristics of the study subjects

The baseline characteristics of patients with essential hypertension (N = 250) and healthy controls (N = 250) are listed in Table 1. Clinical characteristics were comparable between patients and controls. There was non-significant difference between number of male and female in the study subjects. Systolic blood pressures (SBP) in patients were significantly higher (151.0 ± 12.5 mm Hg) than that of controls (151.0 ± 12.5 mm Hg). Similarly, Diastolic blood pressures (DBP) in patients were higher (94.8 ± 8.8 mm Hg) than controls (80.4 ± 2.9 mm Hg). High Density Lipoprotein cholesterol (HDL) and Low Density Lipoprotein cholesterol (LDL) were comparable in patients and controls.

3.2 Distribution of genotype and allele frequencies in the study subjects

Biallelic Single Nucleotide Polymorphisms and their genomic locations and related mapping data were obtained from the National Center for biotechnology Information (NCBI). The genotypic frequencies, allelic frequencies, odds ratio and relative risk estimations for the three polymorphic sites are given in Table 2. Testing genetic equilibrium between the observed and expected genotypes showed Hemicentin-1 gene variants were confirming to the Hardy–Weinberg equilibrium law. The individuals with 'AA' genotype of Hemicentin-1 was found to be significantly higher in patients with essential hypertension as compared to healthy controls. There was a significant difference between the hypertensive group and the controls as regard to A/A genotype (p = 0.0138). The A allele was found in a higher frequency in hypertensive patients than in the controls. (p = 0.0031). The observed allele frequencies in patients were 0.824 and 0.176 for G and A alleles respectively, whereas 0.896 and 0.104 was observed in control groups, which resulted in significant differences in the allele distribution among the case and control groups.

3.3 Intergenotypic Hemicentin-1 (Gln5345Arg) variations in systolic and diastolic blood pressure in patients with essential hypertension

A statistically significant intergenotypic variation in the systolic blood pressure (SBP) in patients with GG, AG and AA genotypes was found to be 139.5 ± 26.5 mmHg, 145.9 ± 28.5 mmHg and 160.6 ± 32.5mmHg respectively. There was statistically significant results found between the genotypes and SBP when compared with GG Vs AG (p=0.001), GG Vs AA (p=0.0001) and AG Vs AA (p=0.004), The intergenotypic variation in the diastolic blood pressure (DBP) in patients with GG, AG and AA genotypes was found to be 92.22 ± 11.2mmHg, 95.31 ± 18.2mmHg and 99.26 ± 05.4 mmHg respectively and was statistically significant (p=0.001) (Table 3).

3.4 Intergenotypic Hemicentin-1 (Gln5345Arg) variations in circulating Nitric oxide and MDA levels in patients with essential hypertension

Circulating levels of nitric oxide was lower in patients with AA genotypes as compared to AG and GG Table 4. However, this trend was not observed in the control group (data not shown). Lipid peroxidation in terms of levels of circulating MDA was significantly higher in patients with AA genotypes as compared to patients with AG and GG genotypes. Pearson correlation test shows the observed statistically significant negative correlation between MDA and NO ($r = -0.8$, $P < 0.001$) in our study. However, no significant correlation was observed in control groups between MDA and NO.

Table 1: Baseline characteristics of the study subjects.

Parameters	**Patients (N = 250)**	**Controls (N = 250)**	**P**
Sex (M/F)	154/96	150/100	0.45
Age (years)	50.2 ± 12.0	52.1 ± 7.0	0.26
BMI, (Kg/m^2)	18.4 ± 2.9	18.03 ± 3.4	0.88
Smoking, Heavy (n)	120	25	0.01*
Alcohol consumption (n)	96	45	0.01*
Heart Rate (Beats/min)	74.6 ± 8.6	72.3 ± 4.5	0.38
Blood glucose (mg/dl)	90.7 ± 15.5	91.4 ± 15.7	0.89
Blood Urea (mg/dl)	20.6 ± 3.9	19.3 ± 3.2	0.15
Serum Creatinine	0.97 ± 0.2	0.95 ± 0.2	0.22
LDL cholesterol (mg/dl)	88.5 ± 20.9	91.8 ± 26.8	0.26
HDL cholesterol (mg/dl)	41.5 ± 6.5	38.8 ± 6.5	0.13
Total cholesterol (mg/dl)	169.8 ± 30.8	170.4 ± 23.8	0.56
Triglyceride (mg/dl)	160 ± 37.0	159 ± 42.0	0.35
Systolic blood pressure (SBP) mm Hg	151.0 ± 12.5	120 ± 3.8	0.0001*
Diastolic blood pressure (DBP) mm Hg	94.8 ± 8.8	80.4 ± 2.9	0.0001*
Nitric Oxide (uM)	6.7 ± 3.59	15.2 ± 5.6	0.0001*
MDA (uM)	6.0 ± 3.5	14.7 ± 5.9	0.0001*

BMI, Body mass index; HDL, High density lipoprotein; LDL, Low density lipoprotein. Patients group were compared with controls with t-test of significance or by chi-square test; *$p < 0.05$ is considered to be significant.

Table 2: Genotype and Allele Frequencies of Hemicentin-1 (Gln5345Arg) gene in patients with essential hypertension.

Subjects	**Genotypes, n(%)**			**Chi-square Test YATE'S correction; P value**	**Adjusted odds ratio and Relative risk at 95% Confidence Intervals for genotypes:**
	GG	**GA**	**AA**		
Controls (n= 250)	200 (80)	48 (19.2)	2 (0.8)	GG Vs GA = 7.25; p = 0.007 GG Vs AA = 4.54; p = 0.003 GG Vs GA+AA = 9.4; p = 0.002	Odds ratio GG Vs GA = 1.76 [1.16-2.68] GG Vs AA = 4.7[0.98-22.46] GG Vs GA+AA = 2.08[1.43-3.02]
Patients (n= 250)	170 (68)	72 (28.8)	8 (3.2)		Relative risk GG Vs GA = 1.15 [1.04-1.27] GG Vs AA =1.03 [1.00-1.07] GG Vs GA+AA = 1.17 [1.06-1.3]
Subjects	**Allele frequency**			A Vs C = 3.25; p = 0.0012	**Adjusted odds ratio at 95% Confidence Intervals for alleles; P value**
	G	A			
Controls	0.896	0.104			Odds ratio G Vs A=1.84 [1.27-2.66]; p=0.0001
Patients	0.824	0.176			Relative risk G Vs A= 1.4 [1.12-1.76]; p=0.0001

Patients groups were compared with controls with chi-square (χ^2) test at one degree of freedom with Odds ratio adjusted for age and sex in both genotypes and alleles. $p < 0.05$ is considered to be significant. Hardy Weinberg equilibrium value in controls, $X^2 = 0.228$; in patients, $X^2 = 0.0124$, at one degree of freedom, $P>0.05$.

Table 3: Intergenotypic Hemicentin-1 (Gln5345Arg) variations in systolic and diastolic blood pressure in patients with essential hypertension.

Blood pressure (mmHg)	**Average Blood pressure values in genotypes**			**p-value**
	GG	**GA**	**AA**	
SBP	139.5 ± 26.5	145.9 ± 28.5	160.6 ± 32.5	0.001
DBP	92.22 ± 11.2	95.31 ± 18.2	99.26 ± 05.4	0.001
Comparison of Genotypes*	**p-value**			
	SBP		DBP	
GG Vs GA	0.001		0.01	
GG Vs AA	0.0001		0.0001	
GG Vs GA+ AA	0.004		0.01	

SBP, Systolic blood pressure; DBP, Diastolic blood pressure were compared with respect to genotypes with t-test of significance test at one degree of freedom adjusted for age and sex. $p < 0.05$ is considered to be significant. *analysis of variance (ANOVA) using Bonferroni's method for multiple comparisons between genotype classes.

Table 4: Intergenotypic Hemicentin-1 (Gln5345Arg) variations of in the levels of Nitric oxide and Lipid Peroxidation in patients with essential hypertension

Parameters	**Average Nitric oxide and Lipid Peroxidation values in genotypes:**			**P values**
	GG	**GA**	**AA**	
NO (µM)	10.10 ± 4.83	6.54 ± 2.76	4.15 ± 0.9	0.0001
MDA (µM)	4.842 ± 1.65	5.7 ± 3.35	7.49 4 ± 3.08	0.0001
Comparison of Genotypes*	NO , **P values**		MDA**, P values**	
GG Vs GA	0.0001		0.001	
GG Vs AA	0.0001		0.001	
GG Vs GA+AA	0.0001		0.001	

+SBP, Systolic blood pressure; DBP, Diastolic blood pressure. SBP and DBP were compared with respect to genotypes with t-test of significance test at one degree of freedom adjusted for age and sex. $p < 0.05$ is considered to be significant. *analysis of variance (ANOVA) using Bonferroni's method for multiple comparisons between genotype classes.

4. Discussion

Etiology of the essential hypertension remains far from clear. More than 150 candidate genes have been implicated in the regulation of blood pressure that is linked to several pathways [15]. An A16, 263G change in the HEMICENTIN-1 gene (NM_031935) was reported to be associated with the disease in the family originally found to demonstrate linkage to 1q25-3. This variation produces a non-conservative substitution of arginine for glutamine at codon 5345 (Gln5345Arg), an amino acid highly conserved at this position in eight other species analysed. This gene encodes a large extracellular member of the immunoglobulin superfamily and mapped to 1q25.30-1q31.1. This glutamine to arginine variation changes both the size and charge of the amino acid side chain within a very highly conserved calcium-binding EGF-like domain, likely to lead to a reduction or disruption of the domain function. [4]. HMCN1 (Hemicentin-1) is a Protein Coding gene, may play a role during myocardial remodeling by imparting an effect on cardiac fibroblast migration [16]. Essential hypertension is a possible risk factor for age related macular degeneration. Oxidative stress, and Essential hypertension is one of the strong risk factors for cardiovascular disorders [9]. AMD pathogenic mechanisms, such as oxidative damage, chronic inflammation, and angiogenesis regulation are also involved in the pathogenesis of essential hypertension. These two mechanisms represent a common pathway in the development of AMD and essential hypertension. Inter-individual variability in human blood pressure (BP) is genetically determined [1] with a heritability factor of approximately 30–60%. In our study, we found that GG genotype was most frequent as compared to AG and AA genotypes in the controls group, while amongst patients AG genotype was most frequent compared to AA and GG genotypes. On

further analysis we found that in our study the frequency of A and G alleles in patients were 0.824 and 0.176 respectively. The Odds ratio observed in our study is 2.0, indicating that the individuals with AA genotypes may be at substantially higher risk of developing essential hypertension than those with the other two genotypes at the locus in question. Importantly, we also observed intergenotypic variations in the mean systolic blood pressure in patients which once again indicates that the subjects having GG genotypes are more prone to develop essential hypertension. Levels of malondialdehyde was significantly higher in patients compared to controls. The increase in oxidative stress might be contributed by decline in the levels of Nitric oxide and increase in Plasma MDA levels in patients. This further strengthens the fact that increased Oxidative stress is associated with essential hypertension. MDA showed a significant increase ($P < 0.05$) in both individuals with AG and AA genotypes as compared to the GG in patient group, explaining the generation of free radicals with increased lipid peroxidation during the course of disease process. This potentiates the reduced bioavailability NO due to decrease NO synthesis and increased NO inactivation, contributing to endothelial dysfunction in essential hypertension [17]. Plasma nitrite, the end product of NO, was found to be lower in individuals with AG and AA genotypes as compared to the GG in patient group. It is suggested that a complex interaction exists between NO and ROS within the microenvironment of vessel wall, thereby contributing to the reduced bioavailability of NO leading to the pathogenesis of essential hypertension [17, 18]. We have observed statistically significant negative correlation between MDA and NO ($r = -0.8$, $P < 0.001$) in our study reveals the quenching phenomena of ROS on NO to form peroxynitrite with its decreased bioavailability. Thus, reduced NO production and raised MDA due to oxidative stress may exacerbate endothelial dysfunction and accelerate vascular complications in patient with essential hypertension atherosclerosis. Nevertheless, we hope the mentioned findings will provide a better insight into pathogenesis of essential hypertension and help in detecting complications at an early stage. To the best of our knowledge there is no such study available reporting the associations of essential hypertension with *Hemicentin-1* 5345R gene polymorphism.

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Author Contributions

Conceptualization and conceived by K.S.; methodology, K.S.; Screening and recruitment of study subjects, Clinical sample analysis, R.N.; resources, K.S., D.S., R.N. and J.B.; formal analysis, K.S.,D.S.,; writing, original draft preparation, K.S, D.S.; funding acquisition, K.S. and D.S. All authors have read and agreed to the publishing of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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