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Study of Association of 8002 G>A And -138 Del a Polymorphisms of Endothelin 1 (EDN 1) Gene with Essential Hypertension

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Abstract: Essential hypertension is a complex, multi factorial disorder involving both genetic and environmental factors. Genome wide scans have identified several genes (around 300) and loci, associated with intricate pathways involving highly sophisticated systems in the human body, mainly the sympathetic nervous system (SNS), the Renin-Angiotensin system (RAS) and the vascular endothelium. Of the genes that exert action on endothelium, Endothelin 1 (EDN-1) gene product is a strong vasoconstrictor resulting in vasoconstriction, there by playing an important role in blood pressure regulation. Two polymorphisms in END-1 gene viz. 8002 G>A polymorphism in intron 4 and -138 Del A polymorphism in exon 1were analyzedin the present study. A total of 226 hypertensive's (119 males; 107 females) and 226 (141 males; 85 females) normal controls where analyzed for polymorphisms inEDN-1 gene using polymerase chain reaction restriction fragment length polymorphism (PCR RFLP) analysis. The results revealed higher risk for hypertensive's with AA genotypes in relation to the other two genotypes put together (GG + GA) in general, (χ^2=3.685, p=0.055; OR=1.548; CI 0.991 to 2.417); for females (χ^2=3.08, p=0.079; OR=1.886; CI 0.930 to 3.817); for subjects with positive family history (χ^2=3.116, p=0.078; OR=1.909; CI 0.933 to 3.902) and for those with non-vegetarian dietary habit (χ^2=3.275, p=0.070; OR=1.580; CI 0.963 to 2.592). The risk for individuals with AA genotype was 1.54 times higher when compared to GG and GA genotypes put together and this risk increased to 1.88 times if they happen to be females; 1.9 times if they have a familial incidence of hypertension and 1.5 times if they have non vegetarian food habits. Considering -138 Del A polymorphism only 4A4A genotype was obtained for both patients and controls, which implies that the alleles areeither fixed in our population or the variants of the allelemay be very rare in occurrence.
Keywords: Essential Hypertension, Endothelin, Polymorphism and RFLP.

I. INTRODUCTION

Essential hypertension is a complex, multi factorial disorder, which enhances the risk for a variety of adverse sequelae including heart failure, stroke, and kidney failure. Due to these attributes hypertension is often described as "Silent Killer".With the development of molecular genetic tools and markers, various case control studies and genome wide scans have identified several genes (around 300) and loci, associated with intricate pathways involving highly sophisticated systems in the human body, mainly the sympathetic nervous system (SNS), the Renin-Angiotensin system (RAS) and the vascular endothelium. The Endothelin 1 (*EDN-1*) gene product is a strong vasoconstrictor called endothelin which exerts its physiological functions via its receptors on the vascular smooth muscle cells (VSMC), by activating cascade of intracellular molecules that increase the calcium ion concentration, resulting in vasoconstriction. Two polymorphisms namely 8002 G>A polymorphism in intron 4 and-138 Del A polymorphism in exon 1of Endothelin 1 (*END-1*) were considered for the present study.

II. METHODOLOGY

5- 10 ml of venous blood was collected in EDTA vaccutainers from 226hypertensives (119 males; 107 females) and 226 (141 males; 85 females) normal controls. The data and samples were collected with the co-operation and consent of the subjects. The patient group met the 1999 World Health Organization criteria (WHO/ISH) for hypertension defined as a systolic blood pressure (SBP) ≥ 140mm Hg and diastolic blood pressure (DBP) ≥ 90mm Hg. The patients were already on antihypertensive drugs at the time of investigations and were recruited from Gandhi Medical College and Hospital, Hyderabad, India. All the subjects were examined clinically and through biochemical and other investigations and cases associated with thyroid disorders, diabetes mellitus, stroke, cardiac diseases, renal problems were excluded from the study. For comparison with patient group, age and sex matched controls were recruited from different organizations and through personal contact.DNA was isolated from the peripheral blood

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samples collected using rapid non-enzymatic method (Lahiri&Nurnberger 1991). The samples were amplified by Polymerase Chain Reaction (PCR) and where used for the analyses of polymorphisms in*EDN-1* gene usingrestriction digestion with Taq I followed by electrophoresis onagarose gels for 8002 G>A polymorphism in intron 4 (Kozak et al, 2002) and restriction digestion with Bsl I and genotyping using PAGE for -138 Del A polymorphism in exon 1(Vasku et al, 2002) of EDN-1 gene.

III. RESULTS AND DISCUSSION

Endothelial dysfunction owing to increased vascular tone and neutrophil adhesion is a major cause of hypertension, atherosclerosis and coronary artery disease (CAD). One of the most important mediators of vasoconstriction and neutrophil trafficking is endothelin-1. Positive correlations of circulating endothelin-1 levels and blood pressure in patient with essential hypertension have been reported by Brugada et al (1997). The mutant alleles in endothelin-1 have been associated with hypertension (Asai et al, 2001; Jin et al, 2003; Tiret et al, 1999) and cardiovascular disorders (Buhler et al, 2007; Colombo et al, 2006, Lee et al 2008) where as more recently Fang et al, 2017 did not find any significant association with essential hypertension among Chinese population. Since there are no reports from Indiaon the association of polymorphisms of *EDN-1* gene with essential hypertension, the present study was undertaken to evaluate the contribution of the polymorphisms of this gene to hypertension in Indian patients.

Table- 1describes the percentage distribution of *EDN-1*intron 4 (8002G>A) polymorphism with respect to epidemiological factors in hypertensive patients and control groups. The overall genotypic frequencies of GG, GA and AA were 23.9 %, 50.0 % and 26.1 % in hypertensives, while they were 28.3 %, 53.1 % and 18.6 % in controls respectively. The distribution of these genotypic frequencies did not vary significantly between hypertensives and control groups and also in the various cohorts within hypertensive group. The frequencies of alleles G and A were 0.48 and 0.52 in hypertensives while they were 0.54 and 0.46 in controls. The genotypic frequencies of *EDN-1*intron 4 (8002G>A) polymorphism were in consistent with Hardy- Weinberg equilibrium in both hypertensive and control groups.

Table-2 describes the risk estimations of *EDN-1*8002G>A genotypes in various cohorts for developing hypertension. It was observed that higher risk for hypertension was conferred by the AA genotype in relation to the other two genotypes put together (GG + GA) in general, (χ^2=3.685, p=0.055; OR=1.548; CI 0.991 to 2.417); for females (χ^2=3.08, p=0.079; OR=1.886; CI 0.930 to 3.817); for subjects with positive family history (χ^2=3.116, p=0.078; OR=1.909; CI 0.933 to 3.902) and for those with non-vegetarian dietary habit (χ^2=3.275, p=0.070; OR=1.580; CI 0.963 to 2.592). These observations reveal that individuals with AA genotype had 1.54 times higher risk when compared to GG and GA genotypes put together and this risk increases to 1.88 times if they happen to be females; 1.9 times if they have a familial incidence of hypertension and 1.5 times if they have non vegetarian food habits. Other combinations did not show any significant results.

A. EDN-1-138 Del A polymorphism in exon 1

Vasku et al (2002) reported that the double heterozygote i.e. AG3A4A was at significantly less risk in patients with chronic heart failure in a study involving 8002G>A and -138 Del A polymorphisms of *EDN-1* gene. Significant differences in plasma endothelin levels were reported between 3A3A and 4A4A genotypes by Tanaka et al (2004). Lee et al (2008) reported that genotypic frequencies were significantly different in patients with variant angina as 4A4A- 1%, 4A3A- 36.1%, 3A3A- 62.9% as compared to controls (4A4A- 1.8%, 4A3A- 14.4%, 3A3A- 83.8% respectively). The allele frequency of 4A and 3A reported as 0.191 and 0.809 in patients were significantly deviating from that of control group (0.09 and 0.91).The frequency of mutant allele (3A) was lower in the angina group in the controls. The odds ratio value reported by Lee et al (2008) for the development of variant angina with 3A allele was 0.42 (95% CI 0.236-0.748) concluding that increase in protection against the disease is associated with mutant allele. A more recent study by Fang et al (2017) showed a lack of association between -138 Del A polymorphism with essential hypertension among the Chinese. In the present study only 4A4A wild type genotype was obtained for both patients and controls, implying that the alleles areeither fixed in our population or the variants of the allelemay be very rare in occurrence. This also implies that it is not an informative polymorphism in our study. Therefore replication studies involving large sample size may be valuable in this direction.

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Table 1: Percentage distribution of *EDN-1* (8002 G>A) genotypes with respect to epidemiological factors studied in Hypertensives and Controls

	Hypertensives							Controls						
	GG		GA		AA			GG		GA		AA		
	n	%	n	%	n	%	Total	n	%	n	%	n	%	Total
Total	54	23.9	113	50.0	59	26.1	226	64	28.3	120	53.1	42	18.6	226
Males	27	22.7	62	52.1	30	25.2	119	41	29.1	72	51.1	28	19.9	141
Females	27	25.2	51	47.7	29	27.1	107	23	27.1	48	56.5	14	16.5	85
Early onset	16	19.0	43	51.2	25	29.8	84	-	-	-	-	-	-	-
Late onset	38	26.8	70	49.3	34	23.9	142	-	-	-	-	-	-	-
Familial	30	25.9	58	50.0	28	24.1	116	31	34.1	47	51.6	13	14.3	91
Non-Familial	24	21.8	55	50.0	31	28.2	110	33	24.4	73	54.1	29	21.5	135
Obese	6	19.4	20	64.5	5	16.1	31	0	0	5	71.4	2	28.6	7
Non-Obese	48	24.6	93	47.7	54	27.7	195	64	29.2	115	52.5	40	18.3	219
Smokers	12	25.0	24	50.0	12	25.0	48	18	32.7	26	47.3	11	20.0	55
Non-Smokers	42	23.6	89	50.0	47	26.4	178	46	26.9	94	55.0	31	18.1	171
Alcoholics	18	21.2	48	56.5	19	22.4	85	21	29.2	33	45.8	18	25.0	72
Non-Alcoholics	36	25.5	65	46.1	40	28.4	141	43	27.9	87	56.5	24	15.6	154
Vegetarians	3	10.3	19	65.5	7	24.1	29	14	25.4	29	54.7	10	18.9	53
Non-Vegetarians	51	25.9	94	47.7	52	26.4	197	50	28.9	91	52.6	32	18.5	173

*p<0.05

1) Allele frequencies: for Hypertensive's G allele = 0.48; A allele = 0.52; χ^2 for HWE = 0.143 for Controls G allele = 0.54; A allele = 0.46; $\chi^2$for HWE = 0.735

Table 2: Risk estimations for EDN-1 (8002 G>A) AA Vs (GG+GA) genotypes for developing hypertension

	AA Vs (GG+GA)	
Total χ^2 p	3.685*	0.055
OR CI	1.548	0.991-2.417
Female:	3.081*	0.079
	1.886	0.930-3.817
Family History:	3.116*	0.078
	1.909	0.933-3.902
Non-Vegetarian Diet:	3.275*	0.070
	1.580	0.963-2.592

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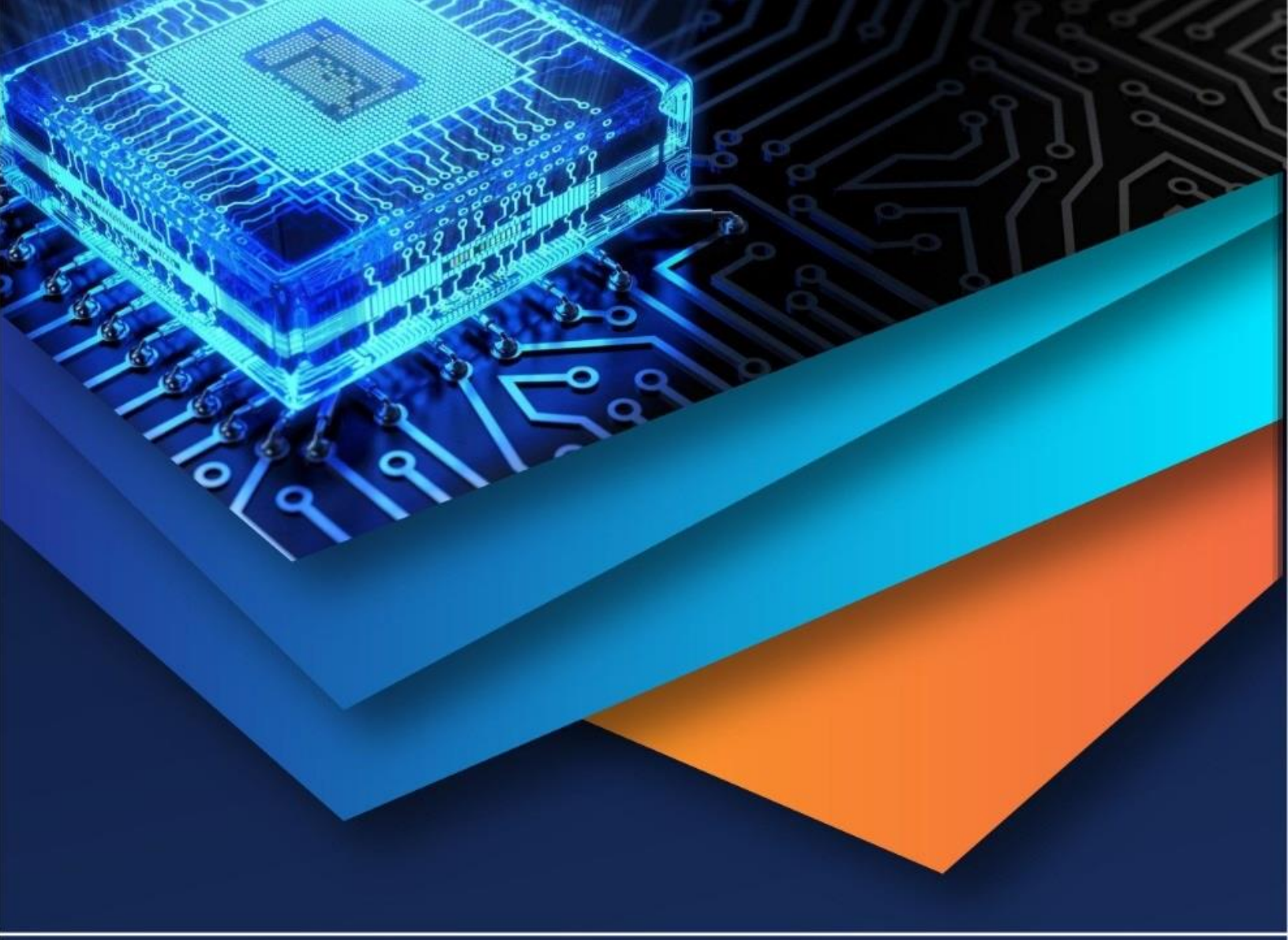

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