“STUDY OF ALPHA ADDUCIN GENE POLYMORPHISM IN ESSENTIAL HYPERTENSIVE NORTH INDIANS”

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Hypertension is a major public health problem, with India heading towards becoming the “Hypertension Capital of the world” (Patnaik et al. 2007). Essential hypertension is a heterogeneous group of diseases with the common characteristics of elevated blood pressure (BP). Essential hypertension is a major risk factor for several cardiovascular diseases. It is a complex trait resulting from the interactions of multiple genetic & environmental factors. Genetic variations of these factors could play a role in the genesis of EH which represents a major risk factor for ischemic heart disease, stroke, peripheral vascular disease and progressive renal damage (Mesrati, 2007). EH rises with age, and it aggregates with other cardiovascular risk factors, such as dyslipidaemia, glucose intolerance, hyperinsulinaemia, abdominal obesity, and hyperuricaemia.

Abnormalities in serum lipid and lipoprotein levels (dyslipidemia) are recognized as major modifiable cardiovascular disease (CVD) risk factors (Kannel et al. 1971) and have been identified as independent risk factors for essential hypertension giving rise to the term dyslipidemic hypertension (Williams et al. 1988 & Halperins et al. 2006). Dyslipidemia is more common in untreated hypertensives than normotensives, and lipid levels increase as BP increases (Borghi et al. 2002 & Neaton et al. 1992). With the current trend of increasing incidence and prevalence of hypertension, CVD, and other non-communicable diseases coupled with the persistence of high rates of communicable diseases in most developing countries, these countries have been said to be experiencing a “double burden of disease.”

Other environmental factors influence this disease like high dietary intake of sodium, alcohol, and stress. One can speculate that hypertension develops as a consequence of “errors” in well-coordinated regulatory systems of blood pressure. Errors in the cascade of molecular, biochemical & genetic processes, which regulate blood
pressure, have finally enough potential to result in hypertension. Numerous environmental factors surrounding the organism during its development should influence the expression of genetic information. However, despite the considerable research effort, it is still difficult to identify all genes and other genetic determinants leading to essential hypertension and other cardiovascular diseases usually become a medical problem in adulthood, their roots might be traced back to earlier stages of ontogeny.

One of the major components of the blood pressure regulation physiology is the renin-angiotensin system (RAS). The RAS has several sub-components-angiotensin converting enzyme (ACE, which converts angiotensin I to Angiotensin II, α-adducin (ADD1, which plays a role in renal sodium reabsorption and the next one is the angiotensinogen (AGT) gene which plays important role in BP regulation in essential hypertension. The functional Gly460Trp (G460T) variant of the α-adducin gene, accompanied by an amino acid substitution of tryptophan in place of glycine at residue 460 (National Centre for biotechnology Information Single nucleotide polymorphism cluster ID rs4961) has been associated with renal sodium retention & salt-sensitive hypertension, through enhancement of the activity of the sodium pump. This leads to increased renal tubular sodium reabsorption and may eventually increase blood pressure. Several studies shows an increased prevalence of hypertension in the presence of the Gly460Trp variant of the α-adducin gene (Joshi et al. 2007 & Manunta et al. 2006) but no relation with blood pressure has been reported. (Joshi et al. 2007) One reason may be that several other mechanisms compensate changes in blood pressure leading to hypertension in some but not in all carriers of the mutation.

The aim of this study is to evaluate the level of lipid profile in serum and electrolytes in serum and urine in essential hypertensive and normotensive
subjects. And the second aim of the present study was to investigate the relationship between the adducin gene polymorphism and blood pressure in essential hypertensive & normotensive young subjects.

Case control study: Hypertensive patients (n= 110) were enrolled from the outdoor patient Medicine OPD of King George Medical University (KGMU), Lucknow India, under the supervision of expert clinician. Age/Sex-matched normal controls (n= 100) were screened from healthy staff members of University, Blood bank of KGMU and our Colleges. This study was approved by the Institutional Ethical Committee of KGMU and a written informed consent was taken from all subjects enrolled in the study. Controls showing a normal blood pressure were included in the study whereas those having a history of coronary artery disease or other metabolic disorders were excluded. Subjects with blood pressure > 120 systolic and >80 diastolic or no any type of secondary hypertensive cause, were categorized in the Hypertensive group. A self-administered questionnaire was used to record the clinical history of blood pressure, associated complications such as hypertension as well as family history.

Estimations of Serum Sodium, Potassium by auto analyzer of Electrolytes and lipid profile (TC, HDL-C and TGL) were done by Gonson & Gonson Company Vitros 250 Dry Biochemistry Fully Auto analyzer (available in our biochemistry lab of KGMU). Low Density Lipoproteins (LDL) and Very Low Density Lipoproteins (VLDL) were also calculated by this instrument. Height and weight were measured to calculate body mass index (BMI). Systolic and diastolic blood pressures (BPS and BPD) were measured in the sitting position with an appropriately sized cuff after a 5 min rest. Clinical details of patients and controls were recorded.
Blood samples were collected using 0.5M Ethylenediaminetetraacetic acids (EDTA) as anticoagulant. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the standard phenol chloroform method and checked on 1% Agarose gel for quality of DNA. The quantity of DNA was estimated using double beam UV-Vis spectrophotometer. The DNA samples were stored at -80°C until further use. PCR Amplification of following fragments were performed in a 25µl reaction mixture containing 100 ng of template DNA, buffer, 200µM dNTP, 10pmol of each primer and 1.5 units Taq DNA polymerase.

The PCR cycling condition for ADD1 was as follows, an initial denaturation step at 95°C for 10 min, 35 cycles at 95°C for 30 Sec, 60°C for 30 Sec, and 72°C for 30 Sec, followed by one elongation step at 72°C for 5 min. The PCR cycling condition for AGT was as follows, an initial denaturation step at 90°C for 3 min, 10 cycles at 94°C for 1 min, 68°C for 1 min, and 72oC for 1 min, and 30 cycles at 90°C for 30 seconds and 68°C for 30 seconds and 72 for 30 seconds and final extension at 72°C for 10 min.

Amplified products were digested with Sau 96I enzyme for ADD1 at 37°C and Tth 111 enzyme for AGT at 65°C. The time of incubation (digestion) was 16 hour. After the digestion these products were electrophoresed on 12% polyacrylamide gels.

Urinary Sodium and Potassium were measured using commercially available kits by electrolyte auto-analyzer.

Allele frequencies and carriage rates of alleles in all the groups were compared using 2x2contingency table while genotype frequencies were compared using 2x3contingency table by Fisher’s exact test. The Hardy-Weinberg equilibrium at individual loci was assessed by chi-square (χ2) statistics using Prism (v 5.0). The
relationships of the \textit{ADD1} genotypes with the clinicopathologic parameters of patients were tested by t-test.

Total 215 samples were taken to our study but results of only 210 samples are presented in this thesis. Rest of the samples shows different problems (haemolization, degradation and smearing etc). For \textit{ADD1} gene, G allele frequency in North Indian (76.36%) patients was approximately similar to Caucasians (77.4% Staessen \textit{et al.} 2001), Turkish population of Emin (Alioglu \textit{et al.} 2010) \& North Indian Stroke patients of J. Kalita (76.7% Kalita \textit{et al.}, 2011), but higher than Han Chinese patients in two different studies of 2014 \& 2007. (54.22 in Liu \textit{et al.}, 64.8% in Huang \textit{et al.}) Tryptophan allele frequency (23.64%) is intermediate between Scandinavian 17% (Melander \textit{et al.} 2000) \& Han Chinese 35% (Huang \textit{et al.} 2007). T allele frequency is similar to these study populations Caucasians 23.2% (Staessen \textit{et al.} 2001), Turkish 23.5% (Alioglu \textit{et al.}. 2010) \& North Indian 23.3% (Kalita \textit{et al.}. 2011) but it was lower from the Koreans 65.15% (Shin \textit{et al.}), Caucasian 53% (Kato \textit{et al.}. 1998), Chinese in different studies 48% (He \textit{et al.}, 2001) \& 58%(Wang \textit{et al.}. 2014).

For \textit{AGT}, we screened 50 hypertensive (mean age 31.05 ± 7.39) \& 35 age matched controls (mean age 37.64 ± 7.75). We could not identify significant association between \textit{AGT} M235T gene variant and primary hypertension in case-controls but we found significant association between male populations. So we think/consider that our sample size is very small and it was not suitable for confirm the association between EH \& NH. The frequency of the T allele in our patient group (34%) of North Indian population was similar or nearest to the study of Shrivastava \textit{et al.} 2012 (31.2%) \& Caucasian population of Wierzbicki \textit{et al.} 2000 (30.21%). Several studies show similarity that there was no significant association between EH \& \textit{AGT} MT \& TT genotype. This were in England, Caucasians
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(Caufield et al. 1994, Hingorani et al. 1996), Glavnic et al. in Mongolian population, (Bautista et al. 2008, Ying et al. 2010 & Saab et al. 2011) Lebanese population & in India (Mohona et al. 2012). It has been suggested that the population heterogeneity in association of AGT (m235t) polymorphism with essential hypertension may be due to significant variations of population backgrounds (Ortiz et al. 2012).

This study attempted to know the serum lipid profile levels in essential hypertensive North Indians were higher as compare to healthy controls. In present study the results revealed that the mean value of serum total cholesterol, triglycerides, serum LDL-cholesterol, HDL-C and serum VLDL-C was significantly higher in hypertensive cases than those of controls. It has been shown in other studies that evidence of a relationship between serum lipids & blood pressure can be found even after adjusting for confounding variables such as age & body mass index. A review of seven studies including over 41,000 subjects showed that significantly correlations existed between blood pressure and TC, TG, LDL & VLDL-C. This study is in line with our study. The means of total cholesterol, LDL-C, VLDL-C and Triglycerides were higher and statistically significant in hypertensive subjects than normotensive subjects (p<0.05). The mean HDL-C in hypertensive subjects is lower than normotensive subjects and statistically significant (p< 0.05). Disorders in the metabolism of HDL & TG play a key role in EH progression. Another risk factor is sodium sensitivity which is an environmental factor that has received the greatest attention. Approximately one third of the essential hypertensive population is responsive to sodium intake. (Katori et al. 2006) It has been shown that mutations in ADD1 gene (Cusi et al. 1997) can lead to higher activity of the sodium pump (Zhang et al. 1998), resulting in increased renal sodium reabsorption that is followed by volume expansion and hypertension. In Caucasians a combination of a polymorphism...
in ADD1 gene (G460T) and the deletion of polymorphism of ACE is associated with a higher incidence of hypertension (Torielli et al. 2008). Furthermore co-occurrence of these two polymorphisms is associated with renal dysfunction (Matsuoka et al. 1996). Moreover the antihypertensive drug PST2238 allowed for lowering of the pump rate and a reduction of blood pressure (Manunta et al. 1998), supporting the suggestion of a role of α-adducin in essential hypertension.

Some study (Momtaz et al. 2000 & Lyalomhe et al. 2008) indicates higher serum sodium & low serum potassium in hypertensive as compare to controls but our results were not showed the difference in serum sodium & potassium between these two groups. Decreased sodium excretion was related to CVD, death & chronic heart failure (O’Donnell et al. 2011). A surprise finding in this study was that the excretion of Na⁺ was lower in essential hypertensives even though they had normal serum Na⁺ levels. This observation may also indicate that overall renal handling of Na⁺, Cl⁻ and K⁺ in this set of hypertensive patients was abnormal (Coleman et al., 1981). Potassium excretion was not different between these two groups.

ADD1& AGT polymorphism was not associated with essential hypertension but male population of essential hypertensives was significantly affected by this polymorphism. A larger sample may be needed to look for the association of ADD1 and AGT with essential hypertension, because hypertension is more prevalent in the North Indian population. Our study indicates that dyslipidemia in a form as manifested by abnormal cholesterol fractions are not uncommon in hypertensives. This study also demonstrates the mutations occurring in the adducin molecule may increase sodium retention, with the risk of developing essential hypertension and its associated cardiovascular and renal complications. We must encourage a change in
the life style of hypertensives; a healthy diet and more physical activity will result in a healthier community.

Genetic tests are employed to confirm diagnosis of a genetic condition and to help in the management of the disease. Uncertainty often leads to stress and the genetic test results can provide a much needed sense of relief. Genetic testing and counseling help people to make decisions about managing their health care. Testing negative for an abnormality helps to avoid unnecessary checkups. Testing positive for a mutation enables a person to look for preventive or treatment options.

By this we will be able to establish the relationship between phenotype and genotype in the north Indian population and develop certain prognostic markers for clinical purpose. The present study will provide a lead to the contribution of alpha adducin & angiotensinogen gene heterogeneity to the susceptibility and development of Essential hypertension. The purpose of our study is to bring a piece of light on gene environmental interactions potentially implicated in the pathogenesis of essential hypertension.

References


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