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Hypertension is extremely common in patients with Chronic Kidney disease (CKD), with an estimated prevalence being around 60–95 %. Hypertension has a very complex relationship with CKD sharing similar pathophysiology and being almost inseparable. Hypertension is an important modifiable risk factor for CKD as well as is a consequence of CKD. Worldwide, approximately a billion adults (≈ 26.4 %) in 2000 had hypertension and this is projected to increase by nearly 60 % to 1.56 billion by the year 2025. Hence we expect a corresponding increase in the global burden of CKD.

The chief strategy in the management of patients with CKD is to primarily stabilize the renal function and target to prevent end-stage renal disease (ESRD). This requires aggressive control of predisposing modifiable risk factors like hypertension, diabetes, and other chronic diseases. CKD patients, chiefly those with a glomerular filtration rate (GFR) < 60 ml/min (CKD stages 3–5) have hypertension. Blood pressure (BP) is poorly controlled in most of the patients with CKD; with only 10 % achieving the target BP level of < 130/85 mmHg. Hypertension with CKD/ESRD amplifies the risk of Cardiovascular disease (CVD) which contributes for more than 50% of the mortality in CKD patients.

**BP MONITORING IN CKD**

Peripheral brachial BP measurement by sphygmomanometry has been the gold standard for ages. Though this peripheral BP measurement is convenient and cost effective, it however does not represent accurately the central aortic pressure which represents the true BP burden on the major vital organs. The systolic BP and the pulse pressure amplify from the aortic root to the peripheral brachial artery whereas the diastolic and mean BP remain unchanged. Emerging literature does suggest that central aortic BP, ambulatory and Home BP measurements are more powerful predictors of CV outcomes than our routine traditional office peripheral brachial BP in various patients including CKD.

**CENTRAL BP MONITORING AND ITS OUTCOMES IN PATIENTS WITH CKD**

In patients with CKD Isolated systolic hypertension is very common and it is due to the impact of aortic stiffness on central BP. Hence aortic stiffness is very strongly associated with increase in CV mortality. Invasive and non-invasive methods are both
used to measure central blood pressure with directly aortography being the gold standard. Non-invasive methods include client applanation tonometry and cuff oscillometric methods. Central aortic systolic, diastolic, pulse, and mean blood pressures can be obtained from central aortic pressure waveforms, which are estimated through a mathematical transformation of the radial or carotid arterial pressure waveforms captured by non-invasive applanation tonometry. Radial artery applanation tonometry is more comfortable for patients and easier to use in regular clinical practise as compared to the carotid one. These pressure wave forms are the summation of the forward transmissions generated by systolic, and the backward wave reflections generated by the peripheral vascular system.

Previous studies by Roman et al and Italian Dicamanio study have shown that central carotid pulse pressure was shown to be a strong independent predictor of all-cause and CV mortalities while peripheral brachial BP and pulse pressure failed to show any significant predictive value.

Pulse wave velocity (PWV) is presently the gold standard in calculating arterial stiffness. Increasing PWV is seen with aging, sustained systolic–diastolic hypertension in middle age, metabolic syndrome, isolated systolic hypertension, impaired glucose tolerance or diabetes mellitus, proteinuria, CKD and ESRD.

Aortic PWV is measured by capturing arterial waveforms typically from two sites namely carotid and femoral, and by measuring the distance w between the two site and the time required for the waves to travel central aortic stiffness assessed by PWV is a strong independent predictor of all-cause and CV mortalities in ESRD patients. They have also been shown to be good predictors of all-cause and CV mortalities.

In CKD, a higher PWV, proteinuria, and smoking are strong independent predictors for the progression to ESRD. The CRIC study found that central aortic stiffness by PWV significantly correlated with proteinuria in diabetics.

Study By Ignace et al concluded that stage 5 CKD patients showed improvement in arterial compliance by reduction in PWV and AIX, at 3 months after kidney transplant, suggesting a possible cause–effect relationship between impaired renal function and arterial stiffness.

Emerging data does suggest that measurements of central BP and arterial compliance are newer promising predictors of CV outcomes when compared with traditional peripheral brachial BP. Measuring central BP and arterial compliance will become an increasingly important part of routine clinical assessment of BP, CV risks, disease progression and treatment effects in high-risk populations such as patients with CKD.

**AMBULATORY BP MEASUREMENTS (ABPM) IN CKD AND IT’S ROLE IN PREDICTING CV OUTCOMES AND PROGRESSION OF CKD**

CKD is associated with an altered circadian BP rhythm, characterised by increase in non-dippers and even and some whose BP increases during the night (“reverse-dippers,” or “risers”). CKD have a peak BP nearly at mid-night and nocturnal increase in BP. The prevalence of non-dipper status increases progressively as the renal function deteriorates reaching more than 75% in patient with advanced (stage 5) CKD. Abnormal circadian BP by ABPM is common in CKD, and the efficacy of ABPM for predicting renal and CV outcomes are high. It demonstrates that nearly 50% of CKD patients have morning hypertension during the first 2 h after awakening. Majority of these patients with morning hypertension also have sustained elevation of nocturnal BP resulting in a high night time/daytime BP ratio, implicating that morning hypertension in CKD is of a sustained type, and not the surge type as reported in other populations.
ABPM accurately diagnoses White coat hypertension (WCH) and masked hypertension in CKD. About 18% of patients with CKD have WCH whereas 20% have masked hypertension.15,16 Night time SBP and DBP both are strong predictors for both renal and CV end points. Non-dippers and reverse dippers have a double risk of CV events. ABPM provides more readings, often more than 50 measurements, compared with a typical routine clinic BP of three readings, thereby providing a true and reliable estimate of BP burden on the circulation and the target organs and effects on CV outcomes.17 This information is useful in treating BP in CKD patients more efficiently and identifying their dipping status which aids in stratification of risk for CVD and CKD progression.

HOME BLOOD PRESSURE MONITORING (HBPM) IN CKD
HBPM is superior to office measurements for the diagnosis of hypertension. It is useful in both Diagnosis and management of hypertension in hemodialysis patients which is difficult due to massive volume shift. HBPM correlates with target organ damage, increased CV risk and progression of CKD, proteinuria and decline in eGFR. Morning HBPM has the strongest correlation with annual decline in eGFR. HBPM is very attractive since it provides greater patient empowerment in their own care with superior control and attention to lifestyle modifications.

CLINICAL UTILITY OF CENTRAL, ABPM AND HBPM IN CKD PATIENTS
Utility of central BP monitoring in CKD provides more accurate prediction of true BP burden of vital organs. As well as superior assessment of burden of BP-related cardiovascular risk, atherosclerosis and vascular injury. Whereas ABPM and HBPM are useful in identifying Masked BP, White coat hypertension, Predicting CVD risk and Progression of CKD and optimisation and adjustment of medications.

SUMMARY
Hypertension and CKD go hand in hand and use of traditional clinic BP measurements do not correlate well with CKD progression and CVD outcomes. ABPM will be the new gold standard for BP measurement. Central BP monitoring is newer promising modality since it allows arterial stiffness to be measured easily an noninvasively and it is well established that arterial stiffness is an independent predictor of CV events. HBPM is a cost effective, convenient tool in accurately predicting the true burden of hypertension.

REFERENCES


12. Pickering TG, Miller NH, Ogedegbe G, Krakoff. *Annual Conference of Hypertension Society of India - HSICON 2018* - which will be held from 31st August to 2nd September 2018 at Hotel Udaya Samudra, Kovalam, Thiruvananthapuram, Kerala. The Scientific committee has formulated a vibrant and interactive scientific program. We will have a fruitful discussion on current global and national scenario of management of hypertension along with entertainment for the mind and a feast for those who are oriented to gustatory amusement.

Association of Physicians of India, Kerala Chapter in collaboration with Physicians Club of Trivandrum invite you to participate and enjoy this exceptional educational treat and to have exchange of thoughts and knowledge.

Thank you,

**Invitation**

**Dear Colleague,**

It gives us great pleasure to invite you to the **27th National Annual Conference of Hypertension Society of India - HSICON 2018** - which will be held from **31st August to 2nd September 2018** at **Hotel Udaya Samudra, Kovalam, Thiruvananthapuram, Kerala.**

The Scientific committee has formulated a vibrant and interactive scientific program. We will have a fruitful discussion on current global and national scenario of management of hypertension along with entertainment for the mind and a feast for those who are oriented to gustatory amusement.

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Prevalence of Mutated Allele CYP2C93* Among Type 2 Diabetes Patient Undergoing Adverse Drug Reaction due to Sulfonylurea Treatment

Bornali Dutta

INTRODUCTION

Single nucleotide polymorphism (SNP) is the most common human genetic polymorphism playing a crucial role in pharmacogenetics. SNP describes the occurrence of at least two different allele for one gene differing at only one specific DNA position. It also includes deletion and insertion of a single nucleotide. It was only in the last century the clinicians documented the huge influence of genetics on the response of an individual to a specific drug treatment, these finding concerned three widely used drugs the antimalarial drug primaquine, the toxicity of primaquine was reported for a small percentage of Caucasians (1%) and African Americans (5-10%). There was an acute haemolysis after primaquine treatment; subsequent studies revealed a genetic background that is absence of enzyme glucose 6 phosphate dehydrogenase in erythrocytes of affected individuals accounted for primaquine toxicity. Also in 1950 a rare inherited deficiency of plasma cholinesterase was found to explain the prolonged muscle relaxation after treatment with muscle relaxant succinylcholine. Due to inherited differences in the acetylation of drug Isoniazid, peripheral neuropathy was observed in tuberculosis patient taking Isoniazid. Occurrence of SNPs differs between different races like Caucasians, colored and Asians.

However all early examples for pharmacogenetic polymorphism concern genetic variation of the pharmacokinetic pathway, more specifically the drug metabolising enzymes. The reason for this is the huge impact on drug treatment response, because of the importance of detoxification of drugs, first and foremost, the polymorphism of the most important human enzyme of oxidative drug metabolism the cytochrome P450 monooxygenase, first describe in the 1970s for CYP2D6 and CYP2C9 isoform and in 1980 CYP2C19 still seems to have extensive clinical consequences. The aim of pharmacogenetics is to allow for individualized medicine, that is, based on the patient genetic make-up (the genotype of the CYP isoform) the phenotype (i.e. the metabolizing activity of the administered drug) is predicted. Cytochrome P450 2C9 (CYP2C9) enzyme, the most abundant of the CYP2C enzyme family and comprises approximately one-third of the total hepatic P450 content. It is involved in the metabolism...
of more than 100 drugs, including coumarin, anticoagulants, sulfonylureas and some nonsteroidal anti-inflammatory drugs, but is largely responsible for the metabolism of oral hypoglycaemic agents such as tolbutamide, glibenclamide, glimepiride, glipizide and many CYP2C9 variants have been associated with reduced enzyme activity, with CYP2C9*2 and CYP2C9*3, having the most clinical relevance. However, the effect of functional CYP2C9 polymorphisms on the risk of ADRs with oral hypoglycaemic therapy in patients has not yet been widely studied.

Cytochrome P450 (CYP) is a complex gene superfamily consisting of heme containing enzymes that comprises of over 70 families. In humans, more than 50 distinct families of cytochrome P450 enzymes have been identified. The enzymes belonging to the families CYP1, CYP2 and CYP3 catalyze the oxidative biotransformation of exogenous compounds, including many drugs, procarcinogens and alcohols. The other CYP450 enzymes are involved in the metabolism of endogenous compounds such as fatty acids, prostaglandins and steroids. The genes encoding CYP2C9 exhibit genetic polymorphism, with 34 variant alleles for CYP2C9. Many of these variants, the most common being CYP2C91*, CYP2C92* and CYP2C93*, seems to be important, CYP2C92* is formed by a C430T substitution on exon 3 which leads to Arg (144) Cys conversion resulting in the formation of an enzyme with decreased activity. The CYP2C93* allele is due to an A1075T substitution on exon 7 of CYP2C9. This result in an altered protein with and Ileu (359) Leu substitution, which exhibits further reduced enzyme activity than the CYP2C92* variant. The frequency of polymorphic alleles shows marked inter-ethnic variation. The frequency of alleles CYP2C92* and CYP2C93* have been studied in different global populations. In Caucasians, the frequency of CYP2C9 mutant alleles is higher (2*: 12.%.3*: 8.3%).

Altered activity of CYP450 is one of the main causes of inter-individual variability in oxidative metabolism of drugs.

India contains an admixture of the Aryan, Dravidian, Kolarian and the Mongoloid races. Although the populations of Assam share a mixed ethnic origin having descended from the Aryans and Mongolians, it is difficult to distinctly trace back the origin of the Inhabitants of the Assam. An admixture of populations by inter-race marriage is prominent and leads to widespread genetic complexity. Patients of all areas of Assam and North eastern region visit Gauhati Medical College & Hospital. So it is difficult to trace their origin.

Type2 diabetes among adults in Guwahati, Assam, in north eastern India shows a high prevalence rate (8.2% age> 20 years). Several classes of drugs are available to treat T2DM, but its clinical response exhibits significant variation. Sulfonylureas have been a mainstay of Type 2 DM pharmacotherapy for over 50 years. It is well recognized that interindividual variability exists in sulfonylurea response (i.e., pharmacodynamics), disposition (i.e., pharmacokinetics) and adverse effects. The field of pharmacogenomics has been applied to sulfonylurea clinical studies in order to elucidate the genetic underpinnings of this response variability. Historically, most studies have sought to determine the influence of polymorphisms in drug-metabolizing enzyme genes on sulfonylurea pharmacokinetics in humans. More recently, polymorphisms in sulfonylurea drug target genes and diabetes risk genes have been implicated as important determinants of sulfonylurea pharmacodynamics in patients with Type 2DM. As such, the purpose of this study is to discuss sulfonylurea pharmacogenomics in the setting of Type 2 DM, specifically focusing on polymorphisms in drug metabolism enzyme genes (CYP2C9) and their relationship with interindividual variability in sulfonylurea response and adverse effects.

**MATERIAL METHODS**

The study was carried out in the department of Medicine and Endocrinology of GMCH including both in-patient and out-patient and Biotechnology Hub NIPER. The study was a
prospective observational type. The study was based only on type 2 diabetic patient taking oral hypoglycaemic agent’s sulfonylurea (glimepiride, glipizide, gliclazide and glibenclamide) experiencing adverse drug reaction and without adverse drug reaction

**Inclusion criteria:** All Type2 diabetes mellitus patients’ ≥18 years of age on oral hypoglycaemic agent’s sulfonylurea (glimepiride, glipizide, gliclazide, glibenclamide).

**Exclusion criteria**
1. Patient on insulin
2. Patient with chronic kidney disease
3. Patient with liver disease
4. Pregnant women
5. Patient receiving concomitant medication that induce CYP2C9 activity: Amiodarone, cimetidine, cotrimoxazole, disulfiram, fluvastatin, fluvoxamin, Fluconazole, isoniazid, ketoconazole, metronidazole, sulfipyrazole, ticlopidine, Zafirlukast
6. Patient receiving concomitant medication that inhibit CYP2C9 activity: barbiturates, carbamazipine, phenobarbital, phenytoin, primidone, rifampin) CYP2C9 activity were excluded from the study.9

Blood samples was collected from the sample collection centre GMCH with prior Ethical Committee clearance (reference number MC/190/2007/Pt11/22 Date: 30/03/11) of Gauhati Medical College Hospital.

**Methods followed for identification of ADR**

a. Patient interviews
b. Record linkage studies

Patient medical records are used to match drugs prescribed with adverse effects Blood was withdrawn from both Type2 DM patients with ADR and without ADR. With written informed consent and was analyzed for detection of allele CYP2C9*, CYP2C92* and CYP2C93*. Patient adverse event history, history of medication and its course, duration concomitant medication details were recorded in the ADR analysis format followed by Indian pharmacovigilance programme.3ml of blood from Identified type 2 diabetic patients under sulfonylurea (glimepiride, gliclazide, glipizide, glibenclamide) therapy was collected having no complain against the drug as well as from the patient undergoing adverse drug DNA was isolated from the blood samples by DNA isolation kit (Hipure blood genomic DNA mini preparation kit, Himedia). Extracted DNA was kept at 80°C, PCR-RFLP (restriction fragment length polymorphism) technique was used for detection of variant form, CYP2C92* (Arg144Cys) and CYP2C93* (Ile359Leu) allele and the wild type allele CYP2C91* from the variant form by digestion with restriction enzyme.10

**RESULTS**

Various adverse drug reactions occurred in Type 2 DM patient taking sulfonylurea (glimepiride, glipizide, gliclazide, glibenclamide) Table 1. The mutated allele CYP2C93*responsible for major reduction of enzyme activity resulting in reduced metabolism of sulfonylurea was present in the patient (n=11, 10.2%) experiencing adverse drug reaction due to sulfonylurea (Figure 1). The mutated allele CYP2C92* with minor effect in metabolism was not present in any patient experiencing ADR due to sulfonylurea nor in the patients without ADR (Figure 1). The wild type allele CYP2C1*responsible for normal enzyme activity was present in the entire group of patient (n=53,100%) having no complain for sulfonylurea (Table 2). The test for association carried by Pearson Chi Square test found highly significant(Chi Sq=9.96, df =1 P=.002) association between allele and ADRs.

**DISCUSSION**

Pharmacological treatment of T2DM has made significant progress over the decades, and presently there is a wide option from which medications for this disease can be selected. This is also associated with an increase in the potential adverse drug reactions from the different compounds that are used. However,
Table 1: Spectrum of adverse drug reactions (ADRs) associated with sulfonyl urea

<table>
<thead>
<tr>
<th>Type of ADRs</th>
<th>Glimepiride N = 38</th>
<th>Glipizide N = 20</th>
<th>Glibenclamide N = 6</th>
<th>Gliclazide N = 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoglycemia</td>
<td>8(20.1%)</td>
<td>4(20%)</td>
<td>2(33.33%)</td>
<td>4(21.05%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0(0.0)</td>
<td>1(5%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Urinary retention</td>
<td>3(7.8%)</td>
<td>2(10%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Abdominal discomfort</td>
<td>4(10.5%)</td>
<td>3(15%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Elevation of liver enzymes</td>
<td>1(2.6%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Cholestasis</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Jaundice</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Generalized weakness</td>
<td>0(0.0)</td>
<td>1(5%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Edema</td>
<td>3(7.8%)</td>
<td>5(25%)</td>
<td>0(0.0)</td>
<td>1(5.26%)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>0(0.0)</td>
<td>3(15%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Agranulocytosis</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1(2.6%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Paresthesia</td>
<td>9(23.6%)</td>
<td>0(0.0)</td>
<td>2(33.33%)</td>
<td>7(36.8%)</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>1(2.6%)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
<td>0(0.0)</td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>8(21%)</td>
<td>1(5%)</td>
<td>2(33.33%)</td>
<td>4(21.05%)</td>
</tr>
</tbody>
</table>

Fig. 1: Allele distribution in the patients with T2DM experiencing suspected adverse drug reaction due to glimepiride, glipizide, gliclazide and glibenclamide
without knowing the genetic makeup of the potential users, it is difficult to predict the various ADRs which might arise from the different anti-diabetic agents currently under use. Knowledge of the genetic makeup of the individual will make it possible to provide personalized medications so as to provide more effective therapy together with the avoidance of possible ADRs which might develop from the various medications. So far, anti-diabetic therapy in T2DM has not taken into account, diversity that may contribute to the heterogeneity in the treatment outcomes.11

The ability of single nucleotide polymorphism to influence drug response may rely on the capacity of the variant to induce changes in the expression of proteins which may influence either the pharmacokinetic or pharmacodynamic profile and hence the clinical efficacy of the drug. Sulfonylurea are the class of drugs used in type 2 diabetic patient, sulfonylurea are metabolized by P450(CYP) isoform of CYP2C9. Polymorphism of CYP2C9 gene significantly affect the pharmacological response of diabetic patient to sulfonylurea because of the reduced metabolism of drugs which may be followed by increase in bioavailability and resulting in adverse drug reaction.

In this study we identified the adverse drug reaction due to sulfonylurea (glimepiride, gliclazide, glipizide glibenclamide). A total of 83 patient, of age Group 49.52±9.97, weight 60±6, Fasting blood sugar 142±62 Post prandial blood sugar 200±71, undergoing suspected adverse drug reaction due to the sulfonylurea (glimepiride, gliclazide, glipizide, glibenclamide) therapy was identified and considered for the study.53patients of age group50.09±10.125, weight 59.26±7.353, Fasting blood sugar 151.25±63.303, post prandial blood sugar 223.73±82.825 was identified who has no any complain regarding the sulfonylurea therapy.

Adverse drug reactions in patients (n=38) taking the drug sulfonylurea glimepiride was hypoglycemia, abdominal discomfort, edema, urinary retention others like elevation of liver enzymes and anemia (Table-1). Allele frequency in these patients was studied by PCR-RFLP technique. Allele CYP2C93* was detected in 5(13.2%) male patient and 1(2.6%) female patient. No CYP2C92* allele was detected in any patient of this group. Wild type allele CYP2C91* was detected in 25(65.7%) male and 7(18%) female. Another similar study by Georgia et el, [8] showed that presence of CYP2C93* allele increases risk for ADR in Type 2 DM patients treated with sulfonylureas. Among the patient taking the drug glipizide (n-20), ADR most common in this group were hypoglycemia, visual disturbances, Paresthesia, edema, gastrointestinal disturbances, generalized weakness, dyspepsia and anemia (Table 1). Allele CYP2C93* was detected in two (10%) male patient. No CYP2C92* allele was detected in any patient of this group. 12(65%) male patient. 6 (30%) female patients was detected with wild type allele. A similar study by Bhatt et al12 showed the presence of CYP2C9*3 allele significantly affected plasma glucose drop per milligram of drug values in patients taking glipizide and glimepiride resulting in ADR, while effects of CYP2C92* allele were insignificant.

In patients taking gliclazide (n=19) adverse drug reactions like hypoglycemia, visual disturbances, diarrhea, itching, paresthesia, vertigo and edema (Table 1) were experienced. Allele CYP2C93* was detected in 2(10.5%) male patient. No CYP2C92* allele was detected in any patient of this group. Wild type allele CYP2C91* was present in
17 (89.4%) patients. Only 6 patients taking glibenclamide and undergoing suspected adverse drug reactions like hypoglycaemia, abdominal discomfort, paresthesia, edema, and with urinary retention were encountered. Allele CYP2C9*3 was detected in one (16%) patient. No CYP2C92* allele was detected in any patient of this group. Five (83.3%) patients were detected with wild type allele CYP2C91*.

It has been observed that adverse drug reactions were prevalent among the T2DM patient of GMCH taking sulfonylurea (glimepiride, glipizide, gliclazide and glibenclamide) category of drugs. Causality assessment was done using Noranjo scale where it is based on the Score. Majority of ADRs were probable. (Glimepiride=81.5%, Gliclazide=63%, Glipizide 65%, Glibenclamide=85%).

Detection of allele CYP2C91*, CYP2C92*, & CYP2C93* in the groups who were experiencing suspected adverse drug reaction was done by polymerase chain reaction –restricted fragment length polymorphism showed the presence of variant form CYP2C9*3 in patients of T2DM of GMCH taking sulfonylurea and undergoing adverse drug reaction whereas no allele CYP2C9*2 was present in any of the samples. The wild type allele CYP2C9*1 which is considered to have the normal enzyme activity predominates in the groups. The Allele CYP2C93* that was present in the patient who suffered from adverse drug reactions like hypoglycemia and visual disturbances. Presence of allele CYP2C9*3 in the subjects with suspected adverse drug reactions explains us about the reduced metabolism of sulfonylurea resulting in higher bioavailability and lower clearance of the drug in the subjects and resulting in adverse drug reactions. The test for significance (chi square test) for association of allele with adverse drug reaction was done and was found to be statistically significant.

This study supports that polymorphism may affect the pharmacological response of diabetic patients to sulfonylureas because of its reduced metabolism followed by increase in drug bioavailability and risk of adverse drug reaction. In allele CYP2C93* there is a nucleoside change from adenine to cytosine at gene position 42614 which results in the amino acid substitution of isoleucine by leucine in protein position 359 and thus results in a loss of 70% enzyme activity compared to wild type allele CYP2C91*[13] sulfonylurea. The 2nd generation sulfonylurea (glimepiride, glipizide, gliclazide and glibenclamide) are also metabolized by the polymorphic CYP2C9.[14-17] Finding that CYP2C9 polymorphism influence the sulfonylurea response and adverse effects are intriguing, the utility of CYP2C9 genotyping prior to initiating sulfonylurea therapy is unclear. A recent population based study Rotterdam study showed that polymorphism in CYP2C9 gene affected the sensitivity to sulfonylurea.[18,19]

This study supports that polymorphism may affect the pharmacological response of diabetic patients to sulfonylureas because of its reduced metabolism followed by increase in drug bioavailability and risk of adverse drug reaction. Mutated allele CYP2C9*3 was present among the T2DM patient of Gauhati Medical college taking sulfonylurea and undergoing adverse drug reactions.

**CONCLUSION**

The mutated allele CYP2C9*3 (with 70% less enzyme activity) was present among the patients of T2DM group with adverse drug reactions due to sulfonylurea treatment. No mutated allele was detected in the group without adverse drug reactions. The mutated allele CYP2C9*2 with minor effect in metabolism was not present in any of patient with ADR due to sulfonylurea nor in the patients without ADR. The test of association carried out by Pearson Chi Square test found highly significant (Chi Sq=9.96, df =1 P=.002) association between allele and ADRs. The study further emphasized the need to assess the enzyme activity and examine the underlying genotype to minimize the occurrence of ADR and treatment failures with drugs that are metabolized by polymorphic CYP2C9.
study further emphasized the need to assess the enzyme activity and examine the underlying genotype to minimize the occurrence of ADR and treatment failures with drugs that are metabolized by polymorphic CYP2C9.

REFERENCES


2. Young-Ran Yoon, Ji-Hong Shon, Moon-Kyung Kim, Young-Chai Lim, Hye-Rang Lee, Ji-Young Park Frequency of cytochrome P450 2C9 mutant alleles in a Korean population. Young-Ran Yoon, Department of Pharmacology, Inje University College of Medicine and Clinical Pharmacology Center, Pusan Paik Hospital, Pusan, Kwangju, Korea.

3. Aisha siddiqi, dilshad ahmad khan, faroq ahmed khanAnd abdul khaliq naveed” impact of cyp2c9 genetic polymorphism On warfarin dose requirements in Pakistani population” Department of Pathology, Department of Biochemistry, Army Medical College Rawalpindi, National University of Sciences and Technology, Islamabad, Pakistan Pak. J Pharm Sci 2010; 23:417-422.


11. Brunetti A. Individualizing Care in Type 2 Diabetes Mellitus. Journal of Diabetes, Metabolic Disorders & Control vol- 1 issue 4


18. Chauhan N. Inter-individual variability of cytochrome P450 and pharmacokinetics in Indian population. 2007.

Association and Interrelation of Angiotensinogen, ACE, ACE-2, and Aldosterone Synthase Gene Polymorphism in Essential Hypertension

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ABSTRACT

Renin-Angiotensin-Aldosterone system (RAAS) plays a central role in Essential Hypertension. The components of RAAS are controlled by different enzymes which are coded by different genes. The genetic polymorphisms of different genes are associated with essential hypertension in different population. Identification of these factors may help in better understanding and control of the disease.

Aim: To investigate the association of angiotensinogen (AGT), ACE I/D and ACE2 rs2106809, and aldosterone synthase (CYP11B2344C→T) polymorphisms and their interrelation with essential hypertension.

Subjects and methods: A total of 250 hypertensives (160 males and 90 females) and 270 normotensives (158 males and 112 females) were enrolled in the study. 5 ml of venous blood was collected for biochemical and genetic analysis. We have analysed M235T, T174M and G6A polymorphism of AGT gene, Insertion/deletion (I/D) of 190 bp polymorphism of ACE gene, rs2106809 polymorphism of ACE2 gene and CYP11B2 C344T polymorphism of aldosterone synthase enzyme.

Results and conclusion: The DD genotype of ACE and TT genotype of ACE2 were significantly high among female hypertensives, while T allele of ACE2 was linked to male hypertensives. AGT gene polymorphism and Aldosterone synthase gene polymorphism with CT and TT genotype are associated with essential hypertension in our study population.

INTRODUCTION

Essential hypertension (EH) is a complex multi-factorial condition influenced by genetic factors. The Renin- Angiotensin-
Aldosterone system (RAAS) is an important regulatory system for maintaining normal blood pressure and electrolyte balance.\textsuperscript{1} Dysregulation of RAAS has a role in the pathogenesis of EH. Many antihypertensive drugs have developed targeting RAAS.\textsuperscript{2}

Angiotensinogen (AGT) is an $\alpha$-2- globulin of hepatic origin, a plasma protein produced constitutively and released into the circulation mainly by the liver. AGT gene is present in 1q42-43 locus.\textsuperscript{3} Single-nucleotide polymorphism (SNPs) have been observed to be associated with serum AGT level which may affect EH.\textsuperscript{4} Frequency distribution and disease association of M235T and T174M has been shown to vary between different ethnic groups. G-6A (rs5051) which is a point mutation in the promoter region which affect essential hypertension in certain ethnic groups.

ACE is encoded by a 21 Kb long gene that has been mapped to chromosome 17q23.\textsuperscript{5} Insertion/deletion (I/D) polymorphism of a 287 bp Alu repeat sequence in intron 16 of the ACE gene is associated with altered levels of ACE and its activity in plasma.\textsuperscript{6} The DD genotype has been shown to be associated with high serumACE production and activity while II and ID genotypes relate to low and intermediate levels and activities, respectively.

Angiotensin-converting enzyme 2 (ACE2), a recently described RAAS component has been found to play a protective role in regulation of BP homeostasis and cardiac function. ACE2 hydrolyses angiotensin II to angiotensin 1–7 which is a vasodilator and partially hydrolyses angiotensin I and the gene is found in X chromosome (Xp22).\textsuperscript{7} ACE2 rs2106809 mutation (C$\rightarrow$T) has been reported to be associated with clinical manifestation of hypertension.\textsuperscript{8}

Aldosterone mediates sodium balance and arterial pressure by influencing intravascular volume and arterial thickness. Aldosterone synthase involved in the terminal step of aldosterone synthesis. Mutation of Aldosterone Synthase Gene (ASG) CYP11B2-344(C$\rightarrow$T) in promoter region upregulates aldosterone production causing EH.\textsuperscript{9}

All the major components of RAAS are proteins and are controlled by genes. The role of individual genetic polymorphism in EH has been described in earlier studies.\textsuperscript{6,8,9} However, it is likely that the polymorphisms in different genes may have a joint effect on the risk of EH which has not been studied adequately.

Therefore, the research has been undertaken to investigate the association of different polymorphisms of AGT, ACE, ACE2, and aldosterone synthase gene and their interrelation with the clinical manifestations and risk of EH.

**MATERIALS AND METHODS**

The present study was conducted at VSS Institute of Medical Sciences and Research, Burla, Sambalpur, Odisha from August 2015 to July 2017 in which 250 adult patients (age $>$ 18 years) of EH and 270 normotensive patients were included after permission from Institutional Ethical Committee. The diagnosis of EH was made according to JNC-7 criteria.\textsuperscript{10} As it is a genetic analysis we included all grades of EH.

Patients with secondary hypertension (renovascular, renoparenchymal, pheochromocytoma etc.) were excluded from this study. Patients of hypertension with diabetes mellitus were also excluded from this study.

After enrolment, data on age, sex, family history, education, intake of additional salt during any sort of food intake, diet (vegetarian/non-vegetarian), Blood pressure was measured by sphygmomanometer in sitting position on the right arm. Data were recorded on a proforma. Height was measured in centimetres and weight in kilograms. Body Mass Index was calculated using the formula (weight in Kg)/(height in metres)$^2$ and individuals with BMI 23 kg/m$^2$ and $\geq$25 kg/m$^2$ were classified as overweight and obese, respectively.\textsuperscript{11}

Blood was collected from all patients for
complete blood count, serum sodium, serum potassium, fasting blood glucose, serum urea, serum creatinine, lipid profile. Urine analysis was done in all cases. Abdominal USG and ECG were done at the time of admission. Genetic analysis was done at Regional Medical Research Centre (RMRC), Bhubaneswar. For this purpose 10ml of EDTA blood was collected and sent to the RMRC laboratory in cold chain. We have analysed M235T, T174M and G6A polymorphism of AGT gene, Insertion/deletion (I/D) of 190 bp polymorphism of ACE gene, rs2106809 polymorphism of ACE2 gene and CYP11B2 C344T polymorphism of aldosterone synthase enzyme. The scheme of genetic analysis has been summarised in Figure 1.

The genomic DNA was extracted from the whole blood using the standard phenol chloroform method. The extracted DNA was resuspended in 100 micro-litre of DNase free water and kept at -20°C until use.

i. The M235T and T174M polymorphisms were analysed together in a single PCR reaction using a forward primer: 5’GAT GCG CAC AAG GTC CTG-3’ and a reverse primer 5’-CAG GGT GCT GTC CAC ACT GGC TCG C-3’.12

ii. To determine the I/D polymorphism of ACE gene, a flanking primer pair 5’-CTGGAGACCACTCCCATCCTTTCT-3’ and 5’GATGTTGGCCATCACATTGCA CGAT-3’ was used to amplify the segment of the ACE gene containing the mutation.13

iii. The CYP11B2 C-344T polymorphism was determined by PCR- RFLP. The primers used were 5’-CAG GGC TGA GAG GAG TAA AA-3(forward) and 5’-CAG GGG GTA CGT GGA CAT TT-3’ (reverse).14

iv. ACE2 rs2106809 polymorphism was detected using the forward primer 5’-GAAAGCCAGATGCTTTAACAAG-3’ and the reverse primer 5TTTTCCATATCTCTATCTGAT CG-3’.15

All the PCR amplifications were performed in a 20 ml reaction mixture containing 5 picomoles each of forward and reverse primer, 1.9nM
of each dNTP, 10mM Tris-HCL, 50mMKCl, 2.75mM MgCl2, 0.01% Gelatin, 1.5U Taq DNA polymerase (Bangalore Genei) and 3 ml of template DNA. The PCR cycling conditions were carried out with an initial denaturation for 10 minutes at 96°c, followed by 35 cycles of denaturation at 94°c for 1 minute, annealing at 66°c for 1 minute and extension at 72°c for 1 minute, followed by a final extension for 10 minutes at 72°c. The products were separated by electrophoresis on 2% agarose gels and visualized after staining with ethidium bromide (0.5 mg/ml). The reaction mixture composition of ACE-2 was the same as ACE, with the only exception that the MgCl2 concentration was dropped to 1.5 mM.

Unpaired t-test or chi-square test or Fisher’s exact test was used to compare the characteristics of the two groups and to compare the characteristics according to different genotypes in females one-way ANOVA was used. Genotypes and alleles were compared using chi-square test or Fisher’s exact test as applicable. Graph Pad version 5 was used for the above analysis. Logistic regression analysis was carried out to identify the independent risk factors using SPSS version 17. As ACE-2 gene is localised in X-chromosome, ACE-2 polymorphism was analysed separately in males and females.

RESULTS

Characteristics of subjects

A total of 250 hypertensives (160 males and 90 females) and 270 normotensives (158 males and 112 females) individuals were included in the study. The mean age of patients was 49.47 ± 10.38 years and that of controls was 48.82 ± 11.04 years. The mean age of male patients and controls were 49.20 ± 9.76 years and 47.17 ± 9.25 years and that of female patients and controls were 49.87 ± 11.47 years and 51.30 ± 13.15 years respectively. All the subjects were age and sex matched. Systolic blood pressure (SBP), diastolic blood pressure (DBP), frequency of family history and overweight were higher in patients in total as well as male and female groups. Body Mass Index (BMI), triglycerides and alcohol consumption rate were higher in patients of the total group whereas high density lipoprotein (HDL) levels were high in the control group. In males, the triglyceride levels and alcohol consumption rate were high and in females BMI, creatinine levels and frequency of hyperlipidemia were high in the patient groups compared to the controls (Table 1).

Genotyping results

1. AGT polymorphism (Table 2): The genotype distributions of M235T polymorphism were in Hardy-Weinberg equilibrium in both patients and controls in the total population as well as in males and females. No difference was observed in the genotype distributions or allele frequencies in any group. In univariate analysis, in females the T allele was observed to increase the chances of risk in additive (TT vs MM) and dominant
2. ACE and ACE-2 Polymorphisms (Tables 3 & 4): The banding pattern of ACE I/D revealed three genotypes such as the 490 bp band for the homozygous ancestral genotype (Insertion/Insertion, II), 190 bp band for the homozygous derived genotype (Deletion/Deletion, DD) and both 490 and 190 bp bands for the heterozygous genotype (Insertion/Deletion, ID). The distribution of the genotypes in the studied population showed no significant deviation from Hardy-Weinberg equilibrium. On analyzing the genotypes according to different genetic models, a significant association of the mutation with hypertension was found in additive (DD versus II) \( \left( p=0.006, \text{OR}=2.47, \text{CI}=1.24–4.74 \right) \) and recessive (II/ID versus DD) \( \left( p=0.003, \text{OR}=2.50, \text{CI}=1.35–4.64 \right) \) models. When gender-specific analysis was carried out associations were observed in females in co-dominant (DD versus ID, \( p=0.04, \text{OR}=3.46, \text{CI}=1.05–11.41 \)); ID versus II, \( p=0.019, \text{OR}=2.46, \text{CI}=1.47–6.37 \) as well as other genetic models. In males, no association could be observed. When frequencies of both the alleles were compared, D allele was significantly higher in patients than in controls in the total \( \left( p=0.04, \text{OR}=1.34, \text{CI}=1.01–1.77 \right) \) as well as female population \( \left( p=0.001, \text{OR}=2.36, \text{CI}=1.46–3.84 \right) \).

ACE-2 polymorphism showed deviation from Hardy-Weinberg equilibrium.
3. Aldosterone synthase (CYP11B2 C-344T polymorphism) (Table 5): On analysing the CYP11B2 C-344T polymorphism, the frequencies of TT, TC and CC genotypes were found to be 51.47, 39.71 and 8.82% among the hypertensives and 72.95, 19.26 and 1.93% among normotensives, respectively, while the frequencies of T and C alleles were 71.32 and 28.68% among the former and 85.51 and 14.49% among the later group of subjects. The frequencies of TT, TC and CC were hypertensive males: 48.89, 40.00 and 11.11%, normotensive males: 80.51, 16.95 and 2.54%, hypertensive females: 56.52, 39.13 and 4.35% and normotensive females: 62.92, 35.96 and 1.12%. The allele frequencies of T and C were: in male patients: 68.89 and 31.11%, male controls: 88.98 and 11.02%, female patients: 76.09 and 23.91% and female controls: 80.90 and 19.10%. All the genotype distributions were in Hardy–Weinberg equilibrium (HWE). The genotype patterns of the CYP11B2 C-344T polymorphism between patients and controls groups were found to be significantly different in the pooled and male populations (P < 0.0001 in both cases). From univariate analysis, the polymorphism was found to be associated with hypertension in the entire population.

(x^2=11.6) but no association was found in males.
in dominant \((P < 0.0001, \text{OR} = 2.542, \text{CI}: 1.68–3.84)\), recessive \((P = 0.0019, \text{OR} = 4.911, \text{CI}: 1.63–14.78)\) and additive \((P = 0.0002, \text{OR} = 6.471, \text{CI}: 2.13–19.67)\) models. In males, significant differences were found between the genotype patterns \((P < 0.0001)\) and allele frequency distributions. The associations were observed in dominant \((P < 0.0001, \text{OR} = 4.381, \text{CI}: 2.45–7.61)\), recessive \((P = 0.0123, \text{OR} = 4.792, \text{CI}: 1.35–16.99)\) and additive \((P = 0.0008, \text{OR} = 7.197, \text{CI}: 2.003–25.86)\) models. The \(C\) allele was also found to be higher in the entire group \((P < 0.0001, \text{OR} = 2.372, \text{CI}: 1.68–3.36)\) as well as in male patients \((P < 0.0001, \text{OR} = 3.648, \text{CI}: 2.25–5.91)\).

**Logistic regression analysis results**

From logistic regression analysis, it was found that in males, MM and MT genotypes of M235T polymorphism \((p<0.001)\) GG genotype of G-6A polymorphism \((p=0.001)\) and in females, the MT and TT genotypes of M235T polymorphism \((p=0.005)\) and the TM and TT genotypes of T174M polymorphism \((p=0.003)\) were associated with hypertension. No polymorphism could be identified as risk factor in the total group. Low HDL/LDL levels were high in the total group \((p=0.022)\) and in males \((p=0.001)\) which may be linked to higher levels of LDL in the respective control groups.

Three factors were observed to be independent risk factors for hypertension, viz., ACE I/D polymorphism, ACE2 rs2106809 polymorphism. In males, ACE2 rs2106809 polymorphism was linked to hypertension. However, low HDL/LDL ratio was associated with normotensives. In females, however, the polymorphisms, ACE I/D and ACE2 rs2106809 polymorphisms only, were identified as independent risk factors.

**Linkage disequilibrium and haplotype analysis results**

By linkage analysis it was observed that all the three polymorphisms are in linkage disequilibrium. The strongest linkage was between M235T and T174M (Total population: \(D' = 0.9924, p < 0.0001, \text{Exp(B)} = 0.4356; \text{Males: } D' = 0.8028, p < 0.0001, \text{Exp(B)} = 0.4204; \text{females: } D' = 0.3842, p = 0.0018, \text{Exp(B)} = 0.0694)\), followed by that between M235T and G-6A (Total: \(D' = 0.9920, p < 0.0001, \text{Exp(B)} = 0.5438; \text{Males: } D' = 0.8554, p < 0.0001, \text{Exp(B)} = 0.5832; \text{females: } D' = 0.4604, p = 0.0016, \text{Exp(B)} = 0.4848)\) and the least between T174M and G-6A (Total: \(D' = 0.9985, p < 0.0001, \text{Exp(B)} = 0.0011; \text{Males: } D' = 0.9324, p < 0.0001, \text{Exp(B)} = 0.0120; \text{females: } D' = 0.3628, p = 0.0088, \text{Exp(B)} = 0.0129)\). (Haplotype analysis revealed that after crude analysis none of the haplotypes were linked to hypertension except in males wherein the MTG haplotype was significantly high in hypertensives (Odds ratio: 1.66, 95% CI: 1.22–2.20, \(p = 0.001\) (\(p = 0.005\) considered significant: 0.05/number of haplotypes, i.e., 0.05/7). However, after adjustment for risk factors, the TMG haplotype was found to be associated with hypertension in the total (Odds ratio: 3.14, 95% CI: 1.48–6.70, \(p = 0.0034\)) and female populations (Odds ratio: 3.02, 95% CI: 1.43–6.38, \(p = 0.0039\)) and the MTG haplotype in the male population (Odds ratio: 1.60, 95% CI: 1.18–2.10, \(p = 0.0036\)).

In total population, the \(CYP11B2\) CT/TT \((p = 0.006, \text{Exp(B)} = 1.098, 95\% \text{CI}: 1.028–1.172\) were independently associated with essential hypertension. In males \(CYP11B2\) CT/TT genotypes \((p=0.001, \text{Exp(B)} = 1.242, 95\% \text{CI}: 1.118–1.380)\) and low HDL levels \((p = 0.034, \text{Exp(B)} = 3.983, 95\% \text{CI}: 1.129–14.050)\) were associated with hypertension. High HDL/LDL ratio was however associated with hypertensives in the total group \((p = 0.009, \text{Exp(B)} = 0.264, 95\% \text{CI}: 0.097–0.720)\) as well as in males \((p = 0.001, \text{Exp(B)} = 0.042, 95\% \text{CI}: 0.003–0.264)\). Aldosterone levels were also found to be high in hypertensives in both the groups \((p = 0.040, \text{Exp(B)} = 1.078, 95\% \text{CI}: 1.008–1.158\) and \(p = 0.020, \text{Exp(B)} = 1.886, 95\% \text{CI}: 1.090–3.198, \) respectively).

Interrelation between different genes: It has been found that AGT has a gender specific effect on hypertension rather than affecting hypertension in whole population. From the spectrum of RAAS genetics the SNP of AGT such as M235T, G6A are associated more with male hypertensives while T174M is
higher in female subjects. The persons with DD genotype of ACE are with high risk of hypertension whereas II genotype is at least risk. ACE-2 rs 2106809 T allele in combination with ACE DD genotype has increased risk (2.3 fold increase) of hypertension in females. Aldosterone synthase CYP11B2 C344T genetic polymorphism was found in total hypertensive and male hypertensive patients with TT and CT genotype. No association with AGT and CYP11B2 C344T was detected.

**DISCUSSION**

The present study showed that multiple genetic polymorphisms influence the risk of development of EH.

RAAS plays the central role in EH. Human AGT is 453 aminoacid long and is acted upon by rennin to release first 10 aminoacids protein called angiotensin-I that persists in blood for only 30 minutes to 1 hour and converted to angiotensin-II by ACE. The later causes vasoconstriction, sodium and water retention causing hypertension. ACE-2 hydrolyses angiotensin-II to angiotensin 1-7 which is a vasodilator, hence plays a protective role in EH. The AGT level has been observed to be high in hypertensives than normotensives. The present study showed that three SNPs M235T (Met replaces Thr at amino acid residue 235), T174M (Thr replaces Met at amino acid residue 174), and G-6A (point mutation in the promoter region) are found to be associated with hypertension.

Both homozygous and heterozygous state of M allele (MM and MT genotypes) of M235T polymorphism was associated with high risk of developing disease and T allele in double dose to be protective in case of males. On the contrary, both in homozygous and heterozygous states of the T allele conferred risk (MT and TT genotypes) in females and M allele in double dose was protective. Similar to our observation, T allele have been reported to be associated with hypertension in the pooled study group of Tamil Nadu, India\(^{16}\) and in a Han population of China\(^{17}\) whereas association of M allele has been found with ischemic heart disease in the total group and male patients in Netherlands and in female hypertensives of Hyderabad, India\(^{18}\).

The TM and MM genotypes of T174M polymorphism have been identified as risk factors in females in the present study, i.e., the M allele have been found to confer risk in both homozygous and heterozygous states in females but not in males or in the total group. The GG genotype of the G-6A polymorphism was significantly high in male hypertensives in the present study, i.e., the GG homozygosity conferred risk of hypertension in the male gender, but this association was not observed in the total group or in females. In Tamil Nadu, India, too, no association of this polymorphism with hypertension was reported. All the three polymorphisms were strongly linked to each other, the strongest linkage was between M235T and T174M, followed by M235T and G-6G and the least was between T174M and G-6A. Because haplotypes allow a more accurate and sensitive analysis compared to individual polymorphisms alone, therefore we carried out haplotype analysis form which it was found that the TMG haplotype conferred about 3 times greater risk in the total population and females whereas in males the MTG haplotype conferred about 1.6 times greater risk.

The effect of M235T polymorphism on blood pressure is controversial. Although the Met→Thr substitution at position 235 alters the immunological recognition of the protein, no difference in glycosylation, secretion, or enzymatic properties, between the two recombinant angiotensinogens have been found in expression studies. The functional role of the substitution at residue 235 in AGT cannot be either established or ruled out on the basis of the available experimental evidence. Although no functional evidence is available, it has been speculated based on modeling analysis that the T174M polymorphism may lead to abnormal function of the human angiotensinogen protein. The exact mechanism is uncertain but it is unlikely that a blood pressure increase is mediated by
increased plasma angiotensinogen concentration, because previous studies have shown no association between T174M polymorphism and plasma angiotensinogen concentrations.19 Other functional polymorphisms, in linkage disequilibrium with the T174M polymorphism, may contribute to the development of salt-sensitive hypertension.

The promoter polymorphism AGT G (−6)A is located in region AGCE1 (hAG core promoter element 1 in position −25 to −1 bp) which binds the ubiquitously expressed nuclear factor AGCF1. Substitution mutation in this location affects the promoter activity and AGT gene promoter with −6A has increased promoter activity compared with −6G leading to increased transcription.

Interestingly, however, we observed a gender specific association of the polymorphisms with hypertension. It is established that hypertension is a gender dependent trait and is influenced directly and indirectly by gonadal hormones. The gender specific effects of the polymorphism are due to the differential regulation of angiotensinogen gene expression by sex hormones. It was also observed that a binding of transcription factors Upstream stimulatory Factors 1 and 2 effect AGT expression and consequently blood pressure differentially since expression in females is dependent on USF 1 and in males upon both USF1 and 2.

ACE and ACE2 both play an important role in blood pressure regulation and act in a counteracting fashion to maintain a normal blood pressure level in the human body. In the present study we have observed a significant association between DD genotype in essential hypertension. The persons with DD genotype were at greatest risk followed by ID and those with II genotype were at least risk of hypertension. In males, however, no association could be observed. Similar to our observation, gender-specific association have also been observed in other populations. ACE2 rs2106809 polymorphism with ACE DD have high risk of hypertension where as with II and ID there is less risk.

A strong association between C allele of CYP11B2 (−344C/T) gene and hypertension (adjusted \( P = 0.006 \)) was found in the present study which is in agreement with the earlier Japanese study20. Gender wise analysis of the data revealed that males harbouring C allele were at greater risk (adjusted \( P = 0.000 \)) for developing hypertension, while no association could be observed in females. The aldosterone levels in our study population were significantly high in patients in the pooled group and males compared to controls even after adjusting for other factors which was not observed in females. Further, the association of genotype-aldosterone levels observed in our study population is the highest in CC and lowest in TT. There have been other studies reporting the reverse trend with highest levels in TT and lowest in CC19 and still others reporting no association.21 The significance of the role of this polymorphism in the expression of aldosterone synthase is controversial.22 The −344C/T polymorphism at the SF-1 site is thought to alter the sensitivity of aldosterone synthase to angiotensin II. Although the −344C allele binds the SF-1 five times more than the T allele, but the polymorphism in itself may have no impact on the transcriptional regulation of aldosterone synthase. It is possible that the increased transcription factor (SF-1) availability at other functional sites (e.g. positions−71, −64) might result in altered expression of aldosterone synthase. Other mechanisms, including linkage of the polymorphism with a quantitative trait locus elsewhere in the regulatory region need to be explored or it might only become functional through epigenetic interaction with other genes.

The differences observed in genotypic associations with pathophysiological conditions among different populations may be due to race, age, gender, sampling methods, genetic epitasis, linkage with other polymorphism(s) and environmental factors. The gender-specific association may be due to linkage of the polymorphism to some other variant(s) in some autosomal or sex chromosome or due to the effect of gonadal hormones or some
environmental factors. Since essential hypertension is also influenced by environmental factors, some of these factors were also examined. In the total population, alcohol consumption was identified as risk factors for essential hypertension. In males low HDL levels was observed to contribute to the risk but in females no environmental factors could be identified. However, high HDL/LDL ratio which is generally a protective factor was associated with hypertensives which may be due to high levels of LDL in male controls.

There are certain limitations of our study. First is the relatively small sample size. A greater sample size could have given more accurate results. Secondly few other genetic analysis of RAAS including rennin, and estimation of cortisol, cortisone and AGT level may add to the knowledge.

CONCLUSIONS

In spite of the limitations, it can be concluded that multiple genetic factors affect EH. In males the M allele of M235T polymorphism, G allele of G-6A polymorphism, CYP11B2 C-344T polymorphism are associated with EH. In females the T allele of M235T polymorphism, M allele of T174M polymorphism, ACE DD and ACE-2rs2106809 polymorphism are associated with EH. In males low HDL levels were associated with essential hypertension in our study population.

REFERENCE


ABSTRACT
Renovascular hypertension is the most important cause of secondary hypertension and is a potentially curable cause of hypertension. In majority of the cases, the renovascular hypertension is due to renal artery stenosis secondary to either atherosclerotic arterial disease or fibromuscular dysplasia. It could be unilateral or bilateral. The evaluation should be restricted to high risk patients. Duplex Doppler ultrasound (US) is an attractive technique as a noninvasive screening test as it is a relatively inexpensive, that does not require contrast, and can be used in patients with any level of renal function, though it is operator dependent. The newer noninvasive imaging modalities like CT or MR angiogram have made the evaluation easy though expensive. Medical treatment remains the standard of care, which includes optimization of antihypertensive therapy along with smoking cessation and cardiovascular (CV) risk reduction including lipid lowering and antiplatelet therapy. Selected patients may need angioplasty, stenting or both. Open Surgical procedures are reserved for patients who have complex anatomical lesions of renal artery, for patients who require nephrectomy and those requiring surgery of aorta. As early recognition and treatment may result in reversal of the disease and control of blood pressure, a high index of suspicion is needed.

INTRODUCTION
Renovascular hypertension (RVH) is the most important cause of secondary hypertension. Renovascular hypertension is defined as the presence of systemic hypertension secondary to a stenotic or obstructive lesion within the renal artery. Cure of renovascular hypertension is defined as restoration of blood pressure to below 140/90 mm Hg while taking no antihypertensive medications.1 RVH is precipitated by a hemodynamically significant stenosis of a renal artery or arteries (that is, a stenosis greater than 75% of the vessel lumen or 50% with post-stenotic dilation). The frequency of renovascular hypertension is less than 1% in patients with mild to moderate elevation of blood pressure2 but in contrast the prevalence is much higher (10-25%) in patients with acute, severe or refractory hypertension. The prevalence of HTN in the general Indian population is reported to be 29.8%.3 In a clinically selected population, the prevalence of renovascular hypertension was similar in blacks and whites.1 Although most hypertension cases are “essential”—meaning that no primary cause can be identified—
approximately 3%-5% of patients with hypertension have renovascular etiologies. RVH is a potentially curable cause of hypertension and major advances in vascular imaging has lead to early and easy non invasive identification of vascular lesions. Effective and well tolerated antihypertensive drug therapy has lead to satisfactory medical management of RVH. Selected patients may need angioplasty, stenting or both.

**AETIOLOGY**

There are many intrinsic and extrinsic lesions listed as causes of renovascular hypertension. Among them, atherosclerotic renal artery stenosis and fibromuscular dysplasia are the major causes of renovascular hypertension.

**Atherosclerotic renal artery stenosis (ARAS)** is the most common cause of renal arterial compromise accounting for 70-90% of cases of renal artery stenosis. ARAS usually involves the ostium or proximal renal arteries. It is more common in older patients, patients with other CV risk factors. The lesion is bilateral in at least 1/3 of patients even at the time of initial diagnosis. The prevalence estimates for ARAS in a systematic literature review of 40 studies involving a total number of 15,879 patients was in suspected renovascular hypertension - 14.1%, Hypertension and diabetes - 20%, Coronary angiography - 10.5%, Coronary angiography in hypertensive patients - 18%, Coronary angiography and suspected renovascular disease - 17%, Heart failure - 54%, Peripheral vascular disease - 25%, Abdominal aortic aneurysm - 31% and End-stage renal disease - 41%. In the population with myocardial infarction, patients with hypertension, proteinuria, and renal insufficiency had 3.4-, 13.5-, and 4.8-fold increased risk of renal artery stenosis. Autopsy data suggest that the prevalence of ARAS increases with age, diabetes, peripheral arterial disease, coronary artery disease, systemic hypertension, and dyslipidemia, positive family history and smoking. ARAS is a progressive disease characterised by increasing stenosis, organ dysfunction and poor prognostic implications.

**Fibromuscular dysplasia (FMD)** on the other hand is not as common as ARAS and accounts for 10-30% cases. It is more common in young women and is associated with few cardiovascular risk factors. It affects the distal 2/3 of renal arteries and its branches. Unlike vasculitis, FMD is noninflammatory and the cause remains unknown. Genetic factors play a role. It is usually associated with good prognosis and progressive disease with total occlusion is rare. FMD involves not only the renal arteries but other medium-sized arteries, mainly the extracranial carotid, vertebral, mesenteric, and lower-extremity arteries. While aneurysms are most common with renal FMD, dissection is most common with carotid artery FMD occurring in 75% of cases.

Other nonatherosclerotic causes include renal artery aneurysms, congenital or traumatic arteriovenous fistulas, polyarteritis nodosa, takayasu arteritis, neurofibromatosis, trauma, acute arterial thrombosis or embolism, aortic/renal artery dissection, hypercoagulable states, congenital bands and radiation induced fibrosis. Transplant renal artery stenosis is the most common vascular complication following renal transplant and contributes to transplant failure.

**RISK FACTORS**

Obesity is an important component of metabolic syndrome contributing to hypertension, diabetes, and atherosclerosis, and to cardiovascular events, and cancers. Obesity contributes to atherosclerosis and RAS. The
Pathogenic factors implicated in obesity-induced kidney injury are incompletely understood, the most prominent effects on the stenotic kidney seem to involve exacerbation of micro vascular loss and inflammation.\textsuperscript{15}

Smoking is a known risk factor for atherosclerosis, and a smoking history is typical in patients with renal artery stenosis.\textsuperscript{16} Smoking is an independent risk factor for progression of diabetic nephropathy.\textsuperscript{17} Active smokers presented with renal artery stenosis at a much younger age and experienced cardio-renal events at a younger age than non-smoking participants \textsuperscript{18} and smoking increases risk for renal artery stenosis.\textsuperscript{19}

**Pathophysiology**

The chief mechanism underlying renovascular hypertension involves two factors: 1) Activation of Renin Angiotensin Aldosterone System 2) Presence or absence of contralateral kidney.

In unilateral renal artery disease, the ischemic kidney initiates hypersecretion of renin, which in turn accelerates conversion of angiotensin I to angiotensin II leading to increased secretion of aldosterone causing sodium and water retention. On the other hand, there is renin suppression and pressure diuresis by the non stenotic kidney, precluding volume retention from contributing to the angiotensin II mediated hypertension. By contrast, a solitary ischemic kidney or bilateral disease has little or no capacity to excrete sodium and water thus leading to volume retention playing an additive role in the hypertension. In renovascular hypertension, the transition from simply a hemodynamic reduction in blood flow triggering RVH to an inflammatory, pro-fibrotic state complicates the clinical decisions regarding optimal timing for renal revascularization.\textsuperscript{20}

When hypertension is caused by stenosis of the renal artery and allegedly related to activation of the renin-angiotensin-aldosterone system (RAAS), and when blood pressure falls to normal levels after successful revascularization, it is said to be true renovascular hypertension. It therefore remains a post hoc diagnosis. If blood pressure does not normalize after revascularization, it could be due to two reasons, 1) the patient suffered from renovascular hypertension but that structural vascular changes prevented blood pressure from falling 2) the patient had essential hypertension with atherosclerotic renal artery stenosis, with or without a renovascular component. Renovascular hypertension usually happens due to renal artery stenosis, whereas renal artery stenosis can be present without renovascular hypertension. The mechanism of hypertension and cardiac morbidity in patients with ARAS is complex and often is not strictly renin-dependent; the interplay between the direct vasculotoxic effects of renin, the proinflammatory and neurohormonal effects of circulating angiotensin II, and the endocrinologic effects of aldosterone leads to an increase in total blood volume.\textsuperscript{21} In a Long-term follow-up of patients with atherosclerotic renal artery disease, the authors concluded that atherosclerotic renal artery disease appears to be a marker for the severity of atherosclerosis rather than a causative factor for atherosclerosis progression.\textsuperscript{22,23} In patients with ARAS, there is a significant risk of renal atrophy among kidneys exposed to elevated systolic blood pressure and among those with high-grade ARAS and low renal cortical blood flow velocity as assessed by renal duplex scanning. The occurrence of renal atrophy is well-correlated with changes in the serum creatinine concentration.\textsuperscript{24}

Pickering et al. first reported in the Lancet in 1988, a series of 11 hypertensive patients with bilateral atheromatous renovascular disease who presented with a history of multiple episodes of pulmonary oedema. In patients with bilateral RAS the weighted prevalence of Flash Pulmonary Oedema (FPO) was 14.3\% compared with 3.5\% in those with unilateral RAS. Distinct pathophysiologic and clinical differences between unilateral and bilateral RAS have been identified. Three main pathophysiologic mechanisms contribute to the
development of FPO: defective pressure natriuresis with sodium and fluid retention, increased left ventricular end-diastolic pressure associated with left ventricular hypertrophy and stiffening, and failure of the pulmonary capillary blood–gas barrier. In this entity of bilateral RAS, renal revascularization is the treatment of choice.25

WHEN TO SUSPECT FOR RENOVASCULAR HYPERTENSION

The clinical clues which suggest the presence of renal arterial disease as the cause of hypertension and CKD include

• Age at onset of hypertension <30 years or >55 years
• Abrupt onset of hypertension
• Acceleration of previously well controlled hypertension
• Refractory hypertension
• Accelerated hypertensive retinopathy
• Malignant hypertension
• Systolic diastolic abdominal bruit
• Flash pulmonary edema
• Evidence of generalised atherosclerosis obliterans
• Asymmetry in kidney size on imaging studies
• Acute kidney failure on treatment with an ACE inhibitor or ARB.26

WHOM TO EVALUATE

Because of the potential risks of invasive procedures, only those patients who have a high likelihood of getting benefit from the procedure should be tested for RAS which include severe hypertension with progressive renal insufficiency, refractory hypertension, accelerated or malignant hypertension, unexplained recurrent flash pulmonary edema, hypertension with ACEI or ARB induced acute renal failure and severe hypertension with asymmetry of renal size. Testing should not be performed in patients who have a low likelihood of having clinically significant renovascular disease which includes those with mild to moderate hypertension in the absence of clinical clues or in those who responded well to medical therapy of hypertension.

INVESTIGATIONS

General laboratory assessment for hematologic and biochemical parameters are normal or consistent with the degree of renal impairment or level of CKD assessed by glomerular filtration rate. Urine analyses are typically bland, and the presence of significant albuminuria (or increase of urinary albumin/creatinine ratio) should raise concerns about other parenchymal renal disorders, including diabetic nephropathy.

If there is clinical suspicion, investigations should include tests which define the structural abnormalities like Duplex Doppler ultrasonography, computed tomographic angiography (CTA), magnetic resonance angiography (MRA) and conventional angiography and those that define functional status of the kidneys like captopril renography, renal vein renin levels and 99mTc-DTPA renography.

Renal arteriography which is an invasive procedure is the gold standard for the diagnosis of renal artery stenosis. Catheter angiography is now rarely used for diagnosis of ARAS as it carries a small but important risk of contrast induced nephropathy, cholesterol embolization, allergic contrast media reactions and arterial dissection.27 Commonly employed non invasive procedures include duplex doppler ultrasonography, computed tomographic angiography(CTA) and magnetic resonance angiography (MRA). Other procedures like renal scintigraphy, peripheral renin levels, renal vein renin sampling are not usually used as a screening test due to their low sensitivity and specificity.28-30

Duplex Doppler ultrasonography

It is safe, inexpensive and easily available and hence forms the first line of investigation in most patients with renal artery
stenosis. Direct visualization of the main renal arteries (B-mode imaging) is combined with measurement (via Doppler) of a variety of hemodynamic factors. Stenotic lesions can be detected by comparing the systolic flow velocity in the renal artery to that in the aorta, since the velocity of flow increases as an artery narrows; end-diastolic velocity also may be increased distal to a stenotic lesion. When the renal artery peak systolic velocity (PSV) is increased to 100-200 cm/s or the renal to aortic PSV ratio is greater than 3.5, proximal stenosis should be suspected. Likewise, visualization of renal artery without detectable doppler signal indicates renal artery occlusion. But the result is operator dependent with an accuracy of 60-90%. The stenotic lesion may be missed as the entire length of renal artery or an accessory renal artery may be overlooked. As per literature review the sensitivity and specificity of ultrasonography in detecting hemodynamically significant ARAS was 85% and 92% respectively. The advantages of duplex ultrasound for diagnosis of renal artery stenosis are it is inexpensive, non invasive, no contrast, no radiation, repeatable and can assess a stented artery. But its disadvantages are it is time consuming (>1 h), operator dependent, lack of standardisation in diagnosis of ARAS, limited data on ability to grade stenosis >60%, limited by abdominal adiposity or overlying gas, limited visualization of distal renal artery and accessory renal arteries and data cannot be acquired in up to 20% of patients.

**COMPUTED TOMOGRAPHIC ANGIOGRAPHY (CTA) AND MAGNETIC RESONANCE ANGIOGRAPHY (MRA) (FIGURES 1, 2, 3)**

CTA and MRA provide excellent imaging of the abdominal vasculature and associated anatomical structures and each method has its own advantages and drawbacks over the other as listed in the table no. 1 in assessing RVH.

**Nuclear Imaging (Figures 4, 5, 6)**

Renography may be done with radio-labeled agents that are excreted either by glomerular filtration -technetium-99 diethylenetriamine pentaacetic acid (99Tc-DTPA)- or partially by filtration but mainly by tubular secretion to measure renal blood flow -99Tc-mercaptoacetyltriglycine (99Tc-MAG3). When used alone, isotopic renograms provided about 75% sensitivity.
and specificity for the diagnosis of RVH [34]. In RVH, hypoperfusion of the kidney, leads to renin release from the juxtaglomerular cells which in turn leads to high levels of Ang II to maintain intraglomerular perfusion pressure by constricting mainly the efferent arteriole. Either a reduction of the uptake of dimercaptosuccinic acid (DMSA) or a slowing of the excretion of 99m DTPA or 99Tc-MAG3, can be used to identify the effect of the ACEI in removing the constructive actions of the high levels of Ang II on the autoregulation of glomerular filtration and on the maintenance of renal blood flow, respectively [34]. ACEI renography is highly accurate in patients with a moderate likelihood of RVH and normal renal function, wherein sensitivity and specificity are approximately 90% [35].

**Table 1: CT and MR Angiogram in identification of RAS**

<table>
<thead>
<tr>
<th></th>
<th>CTA</th>
<th>MRA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>94%</td>
<td>90%</td>
</tr>
<tr>
<td>Specificity</td>
<td>60-90% [32,33]</td>
<td>100% [32,33]</td>
</tr>
<tr>
<td>Can detect small accessory renal arteries</td>
<td>No iodinated contrast medium</td>
<td>No iodinated contrast medium</td>
</tr>
<tr>
<td>Can be used in implanted devices</td>
<td>Cannot be used in those with implanted devices</td>
<td>Gadolinium based contrast medium may lead to nephrogenic systemic fibrosis in those with moderate to end stage renal failure</td>
</tr>
<tr>
<td>Can cause contrast induced nephropathy in those with impaired renal function</td>
<td>Images difficult to interpret in heavily calcified arteries</td>
<td>Contraindicated in patients with contrast allergy</td>
</tr>
</tbody>
</table>

**Renal arteriography**

ARAS typically involves the proximal 1/3 of the renal artery at or near the renal artery ostium. Lesions may be either concentric or eccentric within the renal artery. Regarding FMD, medial hyperplasia is the commonest form and is characterised by ‘string of beads’ appearance in angiography. It involves the middle to distal portion of the artery in contrast to ARAS. Renal arteriography helps to determine the translesional pressure gradient across areas of stenosis thus estimating the hemodynamic significance before performing invasive therapeutic procedures like percutaneous transluminal renal angioplasty (PTRA) or stenting. It was found that those with a translesional gradient>20 mmHg were associated with a significant improvement in hypertension following therapeutic procedure [36].

**MANAGEMENT OF RENOVASCULAR HYPERTENSION**

Optimal blood pressure control with antihypertensive therapy and reducing CV risk with statin therapy and aspirin, smoking cessation is the initial step in the management of RVH. Blockade of renin-angiotensin system with ACEIs and ARBs is the most essential part in the treatment of RVH. If blood pressure control and renal function can be maintained with optimal medical therapy, little more is gained by elaborate diagnostic procedures. On the contrary, failure of medical therapy...
points to a potential benefit of improvement with interventional procedures.

INDICATIONS FOR INVASIVE TREATMENT

Renal artery stenosis is a potentially reversible cause of hypertension, and transcatheter techniques are essential to its treatment. Angioplasty remains a first-line treatment for stenosis secondary to fibromuscular dysplasia. Renal artery stenting is commonly used in atherosclerotic renal artery stenosis, although recent trials have cast doubts upon its efficacy. Angioplasty and Stenting for Renal Artery Lesions (ASTRAL) trial (2009) and the Stent Placement and Blood Pressure and Lipid-Lowering for the Prevention of Progression of Renal Dysfunction Caused by Atherosclerotic Ostial Stenosis of the Renal Artery (STAR) trial (2009), assessed the usefulness of renal-artery stenting with respect to kidney function and showed no significant difference in this key measure. The CORAL trial (2013) found no benefit of stenting with respect to the rate of the composite primary end point or any of its individual components, including death from cardiovascular or renal causes, stroke, myocardial infarction, congestive heart failure, progressive renal insufficiency, and the need for renal-replacement therapy. Hence current management of ARAS is primarily optimal medical management. Selection of patients for renal revascularization depends upon the likelihood of getting clinical benefit. Indications for invasive treatment of ARAS are controversial. Medical therapy with antihypertensives and statins and smoking cessation remains the cornerstone for the management of patients with atherosclerotic renal artery

Figs.: 7, 8, 9, 10, 11: Percutaneous transluminal renal angioplasty (PTRA) with stenting in ARAS
(Courtesy: Dr. G. Rajesh, Department of Cardiology, Government Medical College, Kozhikode)
stenosis and hypertension. However a select subgroup of patients at risk of progressive ischemic nephropathy and end-organ damage (pulmonary edema, recurrent heart failure, refractory/accelerated/malignant hypertension) may still benefit intervention. The Society For Interventional Radiology has recommended that intervention should be done based on patient’s clinical symptoms and for hemodynamically significant stenosis defined as 1) Greater than 50% diameter stenosis or greater than 75% reduction in cross sectional area and 2) Systolic pressure gradient greater than 10% of systolic pressure or a pressure gradient of 20 mmHg. It has been demonstrated that renal artery stenting (Figures 7-11) is superior to Percutaneous Renal artery Transluminal Angioplasty (PTRA) alone for the management of atherosclerotic RAS. Several randomised controlled trials have failed to prove that stenting is superior to medical management and the only indication with excellent results in multiple studies for revascularisation is in the context of recurrent flash pulmonary edema. The other indications are rapid deterioration of renal function in patients with previously stable renal disease and rapid increase in antihypertensive requirement in patients with previously well controlled hypertension.

Renal artery stenting should be offered only to patients with truly resistant hypertension (SBP > 150 mm Hg measured by strict guidelines, patient receiving more than three blood pressure medications including a diuretic if tolerated) and hemodynamically significant RAS based on angiography (>80% stenosis) or hemodynamic assessment (>24 mm Hg systolic gradient) and Renal Artery Stenting should be offered only in experienced centers with low mortality and morbidity.

RENAL ARTERY STENOSIS SECONDARY TO FIBROMUSCULAR DYSPLASIA

The first line of treatment for RAS secondary to FMD is PTRA. PTRA is deferred only in those selected patients who show a good response to medical treatment and in those whom the risk of procedure outweigh the benefits. But stenting is is usually not done in FMD unless angioplasty yields suboptimal results or a complication such as renal artery dissection issues.

TRANSPANT RENAL ARTERY STENOSIS (TRAS)

TRAS is the most common vascular complication following renal transplant and contributes to transplant failure. It is mostly due to vascular rejection. Arterial kinking and clamp injury may also play a role. Treatment is usually with interventional procedures. Technical success in transplant renal artery interventions requires knowledge of the anastomotic site.

MANAGEMENT OF RENO VASCULAR HYPERTENSION - SUMMARY

1. Antihypertensive Drug Therapy
   - Blockade of the Renin-Angiotensin System using ACEIs, ARBs
   - Direct Renin Inhibitors (Aliskiren)
   - Calcium Channel Blocking Agents
   - Diuretics
   - Mineralocorticoid Receptor Blockade
   - Additional Classes: Beta-Blockade, alpha-receptor blockade, sympatholytic agents, vasodilators

2. Cardiovascular Risk Reduction
   - Removal of tobacco use
   - Treatment of dyslipidemia
   - Treatment of obesity: obstructive sleep apnea
   - Management of glucose intolerance / diabetes

3. Renal Revascularization: Selected Cases
   - Endovascular revascularization
     - PTRA: primarily fibromuscular dysplasia
- PTRA with stenting: Atherosclerotic disease

**Surgical**: Renal artery bypass / endarterectomy (now usually reserved for complex aorto-renal disease, aneurysmal disease, failed endovascular stent procedures)

**CONCLUSIONS**
Renovascular hypertension is the most important cause of secondary hypertension. It is generally due to occlusive lesions of the renal artery or its branches. Atherosclerotic arterial disease or fibro muscular dysplasia accounts for the vast majority of cases. It could be unilateral or bilateral. The evaluation should be restricted to high risk patients. Duplex Doppler ultrasound (US) still remains as a useful non-invasive screening test. The newer non-invasive imaging modalities like CT or MR angiogram have made the evaluation easy though more expensive. Medical treatment remains the standard of care, which includes optimization of antihypertensive therapy along with CV risk reduction including lipid lowering and antiplatelet therapy and smoking cessation. Selected patients may need angioplasty, stenting or both. Surgical procedures are reserved for patients who have complex anatomical lesions of renal artery, for patients who require nephrectomy and those requiring surgery of aorta. As it is a potentially curable cause of hypertension, high index of clinical suspicion remains the key for early recognition and treatment.

**KEY WORDS**
Hypertension, Renovascular hypertension (RVH), Atherosclerotic renal artery stenosis (ARAS), Fibromuscular dysplasia (FMD), ACE inhibitors, Angiotensin Receptor Blockers (ARBs), Angioplasty, Stenting.

**REFERENCES**


Abstract Submission

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2. The subject of the mail must contain HSICON 2018 – abstract submission, to recognize your mail easily.
3. There is no limit to the number of abstracts an author may submit.
4. The abstract must be typed in single space in English, Arial, 12 font size and must be not more than 350 words.
5. The abstract should contain Title, authors (presenting author name underlined), affiliation, contact address with phone number and email ID.
6. The content of the abstract should briefly state: Background, Objectives, Methods, Results, Conclusion. Author(s) with potential conflicts of interest if any, must be disclosed.
7. The submission of the abstracts constitutes the authors’ consent to publication, if the abstract is accepted. Abstracts are published as submitted. Please proof-read your work carefully to avoid errors.
8. The decision of Scientific Committee will be final as to where to accommodate the paper.
ABSTRACT
Resistant hypertension is a common clinical problem faced by primary care clinicians and specialists. It is not rare, involving perhaps 20% to 30% of study participants. The prognosis of resistant hypertension is unknown, but cardiovascular risk is undoubtedly increased as patients often have a history of long-standing, severe hypertension complicated by multiple other CV risk factors such as obesity, sleep apnoea, diabetes, and CKD. The diagnosis of resistant hypertension requires use of good blood pressure technique to confirm persistently elevated blood pressure levels. Pseudo resistance, including lack of blood pressure control secondary to poor medication adherence or white coat hypertension, must be excluded. Resistant hypertension is almost always multifactorial in aetiology. Successful treatment requires identification and reversal of lifestyle factors contributing to treatment resistance; diagnosis and appropriate treatment of secondary causes of hypertension; and use of effective multidrug regimens. Observational assessments have allowed for identification of demographic and lifestyle characteristics associated with resistant hypertension, and the role of secondary causes of hypertension in promoting treatment resistance is well documented; however, identification of broader mechanisms of treatment resistance is lacking. In particular, attempts to elucidate potential genetic causes of resistant hypertension have been limited. Recommendations for the pharmacological treatment of resistant hypertension remain largely empiric due to the lack of systematic assessments of 3 or 4 drug combinations. Studies of resistant hypertension are limited by the high CV risk of patients within this subgroup, which generally precludes safe withdrawal of medications; the presence of multiple disease processes (e.g., sleep apnoea, diabetes, CKD, atherosclerotic disease) and their associated medical therapies, which confound interpretation of study results; and the difficulty in enrolling large numbers of study participants. Expanding our understanding of the causes of resistant hypertension and thereby potentially allowing for more effective prevention and/or treatment will be essential to improve the long-term clinical management of this disorder.

INTRODUCTION
Resistant hypertension is defined as blood pressure that remains above goal in spite of the concurrent use of 3 antihypertensive agents of different classes at optimal dose amounts, out of which one should be a diuretic. Arbitrary, resistant hypertension is defined in order to identify patients who are at high risk of having reversible causes of hypertension and/or patients who, because of persistently high blood pressure levels, may benefit from special diagnostic and therapeutic considerations. Patients whose blood pressure is controlled but require 4 or more
medications to do so should be considered resistant to treatment.

**PREVALENCE**
The prevalence of resistant hypertension is unknown; however, it is not uncommon.

Uncontrolled hypertension is not synonymous with resistant hypertension. The former includes patients who lack blood pressure control secondary to poor adherence and/or an inadequate treatment regimen, as well as those with true treatment resistance.

African-American participants had more treatment resistance & black women had the lowest control rate (59%) and non-black men the highest (70%).

**PROGNOSIS**
The prognosis of patients with resistant hypertension is impaired as such patients typically present with a long-standing history of poorly controlled hypertension and commonly have associated CV risk factors such as diabetes, obstructive sleep apnea, left ventricular hypertrophy (LVH), and/or CKD.

**PATIENT CHARACTERISTICS**
Strong predictor of lack of blood pressure control was older age, with participants >75 years being less than one fourth as likely to have systolic blood pressure controlled compared with participants ≤60 years of age. The next strongest predictors of lack of systolic blood pressure control were the presence of LVH and obesity (body mass index [BMI] >30 kg/m2). In terms of diastolic blood pressure control, the strongest negative predictor was obesity, with blood pressure being controlled less often compared with lean participants (BMI <25 kg/m2).

Older age, higher baseline systolic blood pressure, LVH, and obesity all predicted treatment resistance, needing 2 or more antihypertensive medications. Overall, the strongest predictor of treatment resistance was having CKD as defined by a serum creatinine of ≥1.5 mg/dL. Other predictors of the need for multiple medications included having diabetes mellitus.

**GENETICS/PHARMACOKINETICS**
Compared with normotensive controls, 2 β ENaC and γ ENaC gene variants were significantly more prevalent in the patients with resistant hypertension. It was associated with increased urinary potassium excretion relative to plasma renin levels but was not related to baseline plasma aldosterone or plasma rennin activity.

The CYP3A5 enzyme (11β-hydroxysteroid dehydrogenase type 2) plays an important role in the metabolism of cortisol and corticosterone, particularly in the kidney.

**PSEUDO RESISTANCE**

**Poor Blood Pressure Technique**
Inaccurate measurement of blood pressure can result in the appearance of treatment resistance. Two of the most common mistakes—measuring the blood pressure before letting the patient sit quietly and use of too small a cuff.

**Poor Adherence**
Poor adherence to antihypertensive therapy is a major cause of lack of blood pressure control. During 5 to 10 years of follow-up, less than 40% of patients may persist with their prescribed antihypertensive treatment.

**White-Coat Effect**
White-coat effect is as common in patients with resistant hypertension as in the more general hypertensive population (prevalence = 20% to 30%). Also, as with more general hypertensive patients, patients with resistant hypertension on the basis of a “white coat” phenomenon manifest less severe target organ damage and appear to be at less cardiovascular risk compared with those patients with persistent hypertension during ambulatory monitoring.
**Lifestyle Factors**

**Obesity**

Obesity is a common feature of patients with resistant hypertension. Mechanisms of obesity-induced hypertension are complex and not fully elucidated but include -

- impaired sodium excretion,
- increased sympathetic nervous system activity, and
- Activation of the renin-angiotensin-aldosterone system.

**Dietary Salt**

Excessive dietary sodium intake contributes to the development of resistant hypertension both through directly increasing blood pressure and by blunting the blood pressure–lowering effect of most classes of antihypertensive agents.

**Alcohol**

Heavy alcohol intake is associated with both an increased risk of hypertension, as well as treatment-resistant hypertension. Cessation of heavy alcohol ingestion reduces 24-hour ambulatory systolic and diastolic blood pressure, while dropping the prevalence of hypertension from 42% to 12%.

**Drug-Related Causes**

Several classes of pharmacological agents can increase blood pressure and contribute to treatment resistance. The effects of these agents, however, can be highly individualized, with most persons manifesting little or no effect, while other individuals may experience severe elevations in blood pressure.

Given their widespread use, nonnarcotic analgesics, including nonsteroidal anti-inflammatory agents (NSAIDs), aspirin, and acetaminophen, are the most common offending agents in terms of worsening blood pressure control. NSAIDs & selective cyclooxygenase-2 (COX-2) inhibitors can blunt the blood pressure–lowering effect of several antihypertensive medication classes, including diuretics, ACE inhibitors, angiotensin receptor blockers (ARBs), and β-blockers.

Although NSAIDs have an overall modest effect on blood pressure levels, in susceptible individuals significant fluid retention, increases in blood pressure, and/or acute kidney disease may occur. These effects occur secondary to inhibition of renal prostaglandin production, especially prostaglandin E2 and prostaglandin I2, with subsequent sodium and fluid retention. Elderly patients, diabetics, and patients with CKD are at increased risk of manifesting these adverse effects.

Other medications are sympathomimetic compounds such as decongestants and certain diet pills, amphetamine-like stimulants, modafinil, and oral contraceptives. Glucocorticoids, such as prednisone, induce sodium and fluid retention and can result in significant increases in blood pressure. Corticosteroids with the greatest mineralocorticoid effect (eg, cortisone, hydrocortisone) produce the greatest amount of fluid retention, but even agents without mineralocorticoid activity (eg, dexamethasone, triamcinolone, betamethasone) produce some fluid retention. Herbal preparations containing ephedra (or ma huang) have been associated with worsening blood pressure. Licorice, a common ingredient in oral tobacco products, can raise blood pressure by suppressing the metabolism of cortisol, resulting in increased stimulation of the mineralocorticoid receptor. In anemic patients with CKD, erythropoietic agents may increase blood pressure in both normotensive and hypertensive patients.

**Secondary Causes**

Secondary causes of hypertension in patients with resistant hypertension, are older patients with greater prevalence of sleep apnea, renal parenchymal disease, renal artery stenosis, and possibly primary aldosteronism. Others, include pheochromocytoma, Cushing’s syndrome, hyperparathyroidism, aortic coarctation, and intracranial tumors.
Obstructive Sleep Apnea

There was a significant gender difference, with sleep apnea being more common and more severe in the men compared with women patients.

A well-described effect is that the intermittent hypoxemia, and/or increased upper airway resistance associated with sleep apnea, induces a sustained increase in sympathetic nervous system (SNS) activity. Increases in SNS output would be expected to raise blood pressure through increases in cardiac output and peripheral resistance as well as by increased fluid retention.

Primary Aldosteronism

Primary aldosteronism is common in patients with resistant hypertension with a prevalence of approximately 20%, based on suppressed renin activity and a high 24-hour urinary aldosterone excretion in the course of a high dietary sodium intake.

Generalized activation of the renin-angiotensin-aldosterone system has been described with obesity, while other studies suggest that adipocytes may release secretagogues that stimulate aldosterone release independent of angiotensin-II.

Pheochromocytoma

Pheochromocytoma represents a small but important fraction of secondary causes of resistant hypertension. The prevalence of pheochromocytoma is 0.1% to 0.6% of hypertensives in a general ambulatory population. The occurrence of a sustained increase and the degree of blood pressure variability are related to the level of norepinephrine secretion by the tumor.

The diagnosis of pheochromocytoma should be entertained in a hypertensive patient with a combination of headaches, palpitations, and sweating, typically occurring in an episodic fashion.

The best screening test for pheochromocytoma is plasma free metanephrines (normetanephrine and metanephrine), which carries a 99% sensitivity and an 89% specificity.

Cushing’s syndrome

Hypertension is present in 70% to 90% of patients with Cushing’s syndrome. Although the main mechanism of hypertension in Cushing’s syndrome is overstimulation of the nonselective mineralocorticoid receptor by cortisol, other factors such as sleep apnea and the insulin resistance syndrome are major contributors to hypertension in this disease.

Although the exact prevalence of resistant hypertension in patients with Cushing’s syndrome is unknown, the overall CV risk in Cushing’s syndrome is substantial because the disorder is associated with other major risk factors such as diabetes mellitus, the metabolic syndrome, sleep apnea, obesity, and dyslipidemia, in addition to hypertension.

Surgical excision of an adrenocorticotrophic hormone (ACTH) or cortisol-producing tumor effectively lowers blood pressure. The most effective antihypertensive pharmacological agent in Cushing’s syndrome is a mineralocorticoid receptor antagonist (spironolactone or eplerenone).

Renal Parenchymal Disease

CKD is both a common cause and complication of poorly controlled hypertension. Most of this population was receiving antihypertensive drug therapy, but achievement of current goal levels (<130/85 mm Hg) was uncommon. Treatment resistance in patients with CKD is related in large part to increased sodium and fluid retention and consequential intravascular volume expansion.

Renal Artery Stenosis

Renovascular disease is a common finding in hypertensive patients undergoing cardiac catheterization, with more than 20% of patients having unilateral or bilateral stenoses (with a degree of obstruction ≥70%). Studies of treatment-resistant hypertension commonly reveal a high prevalence of previously unrecognized renovascular disease, particularly in older patient groups.
More than 90% of renal artery stenoses are atherosclerotic in origin. The likelihood of atherosclerotic renal artery stenosis is increased in older patients; in smokers; in patients with known atherosclerotic disease, especially peripheral arterial disease; and in patients with unexplained renal insufficiency. Bilateral renal artery stenoses should be suspected in patients with a history of “flash” or episodic pulmonary edema, especially when echocardiography indicates preserved systolic heart function.

**Diabetes**

Diabetes and hypertension are commonly associated, particularly in patients with difficult-to-control hypertension. Pathophysiologic effects attributed to insulin resistance that may contribute to worsening hypertension include increased sympathetic nervous activity, vascular smooth muscle cell proliferation, and increased sodium retention.

**Evaluation**

The evaluation of patients with resistant hypertension should be directed toward confirming true treatment resistance; identification of causes contributing to treatment resistance, including secondary causes of hypertension; and documentation of target-organ damage. Accurate assessment of treatment adherence and use of good blood pressure measurement technique is required to exclude pseudoresistance. In most cases, treatment resistance is multifactorial in etiology with obesity, excessive dietary sodium intake, obstructive sleep apnea, and CKD being particularly common factors. Target-organ damage such as retinopathy, CKD, and LVH supports a diagnosis of poorly controlled hypertension and in the case of CKD will influence treatment in terms of classes of agents selected as well as establishing a blood pressure goal of <130/80 mm Hg.

**Medical History**

The medical history should document duration, severity, and progression of the hypertension; treatment adherence; response to prior medications, including adverse events; current medication use, including herbal and over-the-counter medications; and symptoms of possible secondary causes of hypertension. Daytime sleepiness, loud snoring, and witnessed apnea are suspicious for sleep apnea. A history of peripheral or coronary atherosclerotic disease increases the likelihood of renal artery stenosis. Labile hypertension, in association with palpitations and/or diaphoresis, suggests the possibility of pheochromocytoma.

**Assessment of Adherence**

Ultimately, adherence can only be known by patient self-report. Patients should be specifically asked, in a nonjudgmental fashion, how successful they are in taking all of their prescribed doses, including discussion of adverse effects, out-of-pocket costs, and dosing inconvenience, all of which can limit adherence. Family members will often provide more objective assessments of a patient’s adherence, but such input should generally be solicited in the presence of the patient.

**Blood Pressure Measurement**

Use of good blood pressure measurement technique is essential to the accurate diagnosis of resistant hypertension, including having the patient sit quietly in a chair with his or her back supported for 5 minutes before taking the measurement; use of the correct cuff size with the air bladder encircling at least 80% of the arm (the adult large cuff for the majority of patients); and supporting the arm at heart level during the cuff measurement. A minimum of 2 readings should be taken at intervals of at least 1 minute and the average of those readings should be taken to represent the patient’s blood pressure. The blood pressure should be measured carefully in both arms and the arm with the higher pressures generally should be used to make future measurements. Supine and upright blood pressures should be measured during follow-up to detect orthostatic complications with treatment.
Physical Examination

A fundoscopic examination should document the presence and severity of retinopathy. The presence of carotid, abdominal, or femoral bruits increases the possibility that renal artery stenosis exists. Diminished femoral pulses and/or a discrepancy between arm and thigh blood pressures suggest aortic coarctation or significant aortoiliac disease. Cushing’s disease is suggested by abdominal striae, particularly if pigmented; moon facies; or prominent interscapular fat deposition.

Ambulatory Blood Pressure Monitoring

Documentation of a significant white-coat effect requires reliable assessment of out-of-office blood pressure values. This is accomplished most objectively with the use of 24-hour ambulatory blood pressure monitoring.

A significant white-coat effect should be suspected in patients with resistant hypertension in whom clinic blood pressure measurements are consistently higher than out-of-office measurements; in patients who repetitively show signs of overtreatment, particularly orthostatic symptoms; and in patients with chronically high office blood pressure values but an absence of target organ damage (LVH, retinopathy, CKD). In such cases, 24-hour ABPM is recommended. A mean ambulatory daytime blood pressure of >135/85 mm Hg is considered elevated. If a significant white-coat effect is confirmed, out-of-office measurements should be relied on to adjust treatment.

Biochemical Evaluation

Biochemical evaluation of the treatment-resistant hypertensive should include a routine metabolic profile (sodium, potassium, chloride, bicarbonate, glucose, blood urea nitrogen, and creatinine); urinalysis; and a paired, morning plasma aldosterone and plasma renin or plasma renin activity to screen for primary aldosteronism. Even in the setting of ongoing antihypertensive treatment (excluding potassium-sparing diuretics, particularly aldosterone antagonists), the aldosterone/renin ratio is an effective screening test for primary aldosteronism, having a high negative predictive value.

A 24-hour urine collected during ingestion of the patient’s normal diet can be helpful in estimating dietary sodium and potassium intake, calculating creatinine clearance, and measuring aldosterone excretion. Measurement of 24-hour urinary metanephrines or plasma metanephrines is an effective screen when pheochromocytoma is suspected.

Noninvasive Imaging

Imaging for renal artery stenosis should be reserved for patients in whom there is an increased level of suspicion. This would include young patients, particularly women, whose presentation suggests the presence of fibromuscular dysplasia and older patients at increased risk of atherosclerotic disease. Due to poor specificity, abdominal CT imaging is not recommended to screen for adrenal adenomas in the absence of biochemical confirmation of hormonally active tumors (hyperaldosteronism, pheochromocytoma, Cushing’s syndrome).

Treatment Recommendations

Resistant hypertension is almost always multifactorial in etiology. Treatment is predicated on identification and reversal of lifestyle factors contributing to treatment resistance; accurate diagnosis and appropriate treatment of secondary causes of hypertension; and use of effective multi-drug regimens. Lifestyle changes, including weight loss; regular exercise; ingestion of a high-fiber, low-fat, low-salt diet; and moderation of alcohol intake.

Potentially interfering substances should be withdrawn or down-titrated as clinically allowable. Obstructive sleep apnea should be treated if present.

Maximize Adherence

Prescribed regimens should be simplified as much as possible, including the use of
a long-acting combination of products to reduce the number of prescribed pills and to allow for once-daily dosing. Adherence is also enhanced by more frequent clinic visits and by having patients record home blood pressure measurements. Involving the patient by having him or her maintain a diary of home blood pressure values should improve follow-up and enhance medication adherence, while involvement of family members will likely enhance persistence with recommended lifestyle changes.

**Nonpharmacological Recommendations**

**Weight Loss**

Weight loss, has a clear benefit in terms of reducing blood pressure and often allows for reduction in the number of prescribed medications. While difficult to achieve and even more difficult to maintain, weight loss should be encouraged in any patient with resistant hypertension who is either overweight or obese.

**Dietary Salt Restriction**

The benefit of dietary salt reduction is well documented in general hypertensive patients with observed reductions in systolic and diastolic blood pressure. However, in an evaluation of patients whose blood pressure was uncontrolled on a combination of an ACE inhibitor and hydrochlorothiazide, a reduced-salt diet lowered systolic and diastolic blood pressure. A dietary salt restriction, ideally to less than 100 mEq of sodium/24-hour, should be recommended for all patients with resistant hypertension.

**Moderation of Alcohol Intake**

Cessation of heavy alcohol ingestion can significantly improve hypertension control. Daily intake of alcohol should be limited to no more than 2 drinks (1 ounce of ethanol) per day (eg, 24 ounces of beer, 10 ounces of wine, or 3 ounces of 80 proof liquor) for most men and 1 drink per day for women or lighter-weight persons.

**Increased Physical Activity**

Aerobic exercise regimen (stationary cycling 3 times a week) lowers both diastolic & systolic BP. Reductions in diastolic blood pressure are maintained after 32 weeks of exercise, even with withdrawal of some antihypertensive medications. Based on these observed benefits, patients should be encouraged to exercise for a minimum of 30 minutes on most days of the week.

**Ingestion of a High-Fiber, Low-Fat Diet**

Ingestion of a diet rich in fruits and vegetables; high in low-fat dairy products, potassium, magnesium, and calcium; and low in total saturated fats (i.e., the Dietary Approaches to Stop Hypertension or DASH diet) reduced systolic and diastolic blood pressure.

**Treatment of Secondary Causes of Hypertension:**

When primary aldosteronism, pheochromocytoma, or Cushing’s disease is suspected or confirmed, treatment will be specific for that particular disorder. Effective management of these diseases may require referral to an appropriate specialist.

**Treatment of Obstructive Sleep Apnea**

Treatment of sleep apnea with continuous positive airway pressure (CPAP) likely improves blood pressure control.

**Treatment of Renal Artery Stenosis**

Angioplasty of fibromuscular lesions almost always benefits, and is often curative, of the associated hypertension and therefore is the recommended treatment of choice. Restenosis, however, may occur in excess of 20% of patients after 1 year. Endovascular angioplasty, with or without stenting, should be considered when drug therapy alone is unsuccessful. However, if the blood pressure remains poorly controlled in spite of optimal medical therapy, revascularization is recommended.
Pharmacological Treatment

Withdrawal of Interfering Medications

Medications that may interfere with blood pressure control, particularly NSAIDs, should be avoided or withdrawn in patients with resistant hypertension or lowest effective dose should be used with subsequent down titration whenever possible. When initiating treatment with these agents, blood pressure should be monitored closely while recognizing that adjustments to the antihypertensive regimen may become necessary. Therefore, if analgesics are necessary, acetaminophen may be preferable to NSAIDs in subjects with resistant hypertension, recognizing, however, that acetaminophen will provide little if any antiinflammatory benefit.

Diuretic Therapy

Evaluations of patients with resistant hypertension have been consistent that treatment resistance was a lack of, or underuse of, diuretic therapy. Blood pressure control was improved primarily through the use of increased doses of diuretics. Lack of blood pressure control was attributed most often to the use of a suboptimal medical regimen, which was modified most frequently by adding a diuretic, increasing the dose of the diuretic, or changing the class of prescribed diuretic based on the underlying renal function.

In most patients, use of a long-acting thiazide diuretic will be most effective. Chlorthalidone 25 mg daily provided greater 24-hour ambulatory blood pressure reduction. Given the outcome benefit demonstrated with chlorthalidone and its superior efficacy compared with hydrochlorothiazide, chlorthalidone should be preferentially used in patients with resistant hypertension. In patients with underlying CKD (creatinine clearance <30 mL/min), loop diuretics may be necessary for effective volume and blood pressure control. Furosemide is relatively short acting and requires twice-daily dosing. Alternatively, loop diuretics with a longer duration of action, such as torsemide, can be used.

Combination Therapy

This is particularly true of thiazide diuretics, which significantly improve blood pressure control when used in combination with most classes of agents. The combinations that included a thiazide diuretic were consistently more effective than combinations that did not include the diuretic.

It is appropriate to combine agents of different mechanisms of action. In that regard, a triple drug regimen of an ACE inhibitor or ARB, calcium channel blocker, and a thiazide diuretic is effective and generally well tolerated.

Combined α-β-antagonists, because of their dual combination of action, may be more effective antihypertensives, although head-to-head comparisons of maximal doses are lacking. An add-on antihypertensive benefit is achieved with aldosterone antagonists in patients uncontrolled on multidrug regimens. Centrally acting agents are effective antihypertensive agents but have a higher incidence of adverse effects. Lastly, potent vasodilators such as hydralazine or minoxidil can be very effective, at higher doses, but adverse effects are common. With minoxidil, reflexive increases in heart rate and fluid retention occur such that concomitant use of a β-blocker and a loop diuretic is generally necessary.

Ultimately, combinations of 3 or more drugs must be tailored on an individual basis taking into consideration prior benefit, history of adverse events, contributing factors, including concomitant disease processes such as CKD or diabetes.

A combined use of an ACE inhibitor and ARB or a dihydropyridine and non-dihydropyridine calcium channel blocker provides significant additional antihypertensive benefit compared with monotherapy with the different agents.

Mineralocorticoid Receptor Antagonists

Consistent with reports of a high prevalence
of primary aldosteronism in patients with resistant hypertension have been studies demonstrating that mineralocorticoid receptor antagonists provide significant antihypertensive benefit when added to existing multidrug regimens. Spironolactone (12.5 to 50 mg daily), lowers blood pressure significantly. Spironolactone lowers systolic and diastolic blood pressure, when added to the regimen of patients whose blood pressure was uncontrolled with at least 2 medications. Amiloride antagonizes the epithelial sodium channel in the distal collecting duct of the kidney, thereby functioning as an indirect aldosterone antagonist. Amiloride 10 mg daily, spironolactone 25 mg daily, or a combination of both were used as add-on therapy in patients whose blood pressure was uncontrolled on a 2-drug regimen. Amiloride was associated with significant increases in plasma renin activity while spironolactone was not.

Spironolactone and amiloride, both agents are generally safe and well tolerated. The most common adverse effect of spironolactone is breast tenderness with or without breast enlargement, particularly in men. Hyperkalemia is uncommon with either agent, but it can occur. Risk of hyperkalemia is increased in older patients, patients with diabetes and/or CKD, or when added to ongoing treatment with ACE inhibitors, ARBs, and/or NSAIDs. The mechanism of mineralocorticoid receptor blockade in the treatment of resistant hypertension likely involves more effective diuresis than is provided with thiazide diuretics alone.

Dosing

The patients taking at least one of their hypertensive agents at bedtime had better 24-hour mean blood pressure control and, in particular, lower nighttime systolic and diastolic blood pressure values. Nighttime blood pressure levels better predict cardiovascular risk than do daytime values. It may be that twice-daily dosing of nondiuretic blood pressure medica-

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Hypertension Specialist

Patients with resistant hypertension do benefit from referral to a hypertension specialist, if the blood pressure remains elevated in spite of 6 months of treatment. If a specific secondary cause of hypertension is suspected in a patient with resistant hypertension, referral to the appropriate specialist is recommended as needed.

Controlled Resistant Hypertension:

With the current definition of resistant hypertension, patients whose blood pressure is controlled but who use 4 or more medications should still be considered resistant to treatment. Whether or not to adjust the treatment regimen in this situation should be decided on an individual basis with the primary objective being to maintain blood pressure control but use fewer medications and/or use a regimen that minimizes adverse effects.

Research Challenges and Needs

Experimental assessment of patients with resistant hypertension is complicated by the associated high CV risk, which limits the safe withdrawal of medications and which restricts the types and duration of experimental interventions that can be used to explore proposed etiologies. Studies are further limited by concomitant disease processes such as diabetes, CKD, sleep apnea, and atherosclerotic disease. These concurrent diseases and their treatments are difficult to systematically control for and confound interpretation of study results. Overcoming such a challenge will likely require a consortium of hypertension centres allowing for multicenter participation.

Lastly, even among patients with resistant hypertension, subgroups of patients with different aetiologies undoubtedly exist. As an extreme example, the young patient with
combined systolic and diastolic resistant hypertension is undoubtedly different in terms of aetiology, prognosis, and likely effective treatment than the elderly patient with severe, isolated, resistant systolic hypertension. Meaningful differentiation of these subgroups will likely speed identification of respective causes of treatment resistance and development of specific treatment strategies. Much additional knowledge is needed to better identify and treat patients with resistant hypertension. Cross-sectional and outcome studies have identified patient characteristics associated with resistant hypertension, but underlying mechanisms of treatment resistance, particularly potential genetic mechanisms, have not been widely investigated. Efficacy assessments of specific multidrug regimens are needed to better guide therapy.

HIGHLIGHTS

- Failure to achieve goal BP (<140/90 mmHg) using 3 different drugs with pharmacologically complementary mechanisms, one of which is an appropriately dosed diuretic.
- All three drugs given in maximally tolerated doses. Failure to control blood pressure (BP) inevitably heralds renal deterioration as well as accompanying increases in cardiovascular morbidity and mortality.
- CKD itself is a predictor of cardiovascular events as a result of failure to achieve adequate BP control.
- BP control should be a role in management of CKD & diabetes mellitus.
- A resistant hypertension in CKD & DM, results poor prognosis, high mortality, more prone to terminal cardiovascular events.
- All failure hypertension should not be taken as refractory hypertension, as pseudorefractory & secondary hypertension may also simulate in one way or other. Even white coat hypertension should be clearly separated before putting a level of resistant hypertension.
- Compared with patients with white-coat hypertension, true resistant hypertension is associated with male gender, longer duration of hypertension, smoking, diabetes, target-organ damage (as measured by presence of LVH, impaired renal function, microalbuminuria, documented CVD.
- ABPM is desirable for correct diagnosis and management.
- For true resistant hypertension along with available drugs (excluding secondary hypertension, and ensuring normal renal functions), renal denervation should be considered when both kidneys are normal in terms of anatomy, vasculature without stenosis/stenting of both renal arteries.

REFERENCES

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31st August - 2nd September 2018
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Dr. Ramanujam was looking tense and not at ease while sitting in the small departmental seminar room of St. Peter’s Medical School.

He was waiting for the faculty and fellow colleagues to arrive for the first case presentation by him as per academic scheduled.

He was considered the brightest student with sound clinical expertise and skills with good clinical knowledge among the students of St. Peter’s Medical School.

The reason for nervousness was not about the case presentation but to speak in front of Dr. J. Shankar the legendary, clinician and researcher. Dr. Shankar was known to be excellent physician, and a teacher with a heart of gold, however with an additional quality of hard task master.

Dr. Ramanujam composed himself as he watched faculty and students arriving and occupying the seats in the auditorium. It is reassuring to see Dr. Shankar waived his hand for him and smiled before sitting on a chair just in front of him.

He projected his first slide and initiated the presentation as follows:

A 42 years old Parsi Lady. Ms. Perin non diabetic presented to the medicine OPD with complaints of weakness, lassitude and decrease in appetite for last 3 to 4 weeks. She was detected to be hypertensive for last 6 months and was on two drugs (Ramipril and HCTZ) with good control of blood pressure.

She visited several general practitioners for the complaints of weakness in the last 2 months. However effective cure was not found. She was referred to the medicine OPD by a general practitioner as he noticed a behavioural change and raised serum creatinine on one of the recent reports.

The lady was evaluated by the medicine team along with Dr. Ramanujam who is the chief resident on that day.

With this brief history Dr. Ramanujam also highlighted certain other prominent general examination findings which included a postural drop in blood pressure with corresponding tachycardia. She had equal blood pressure reading in both the arms with no brachio-femoral delay. Ms.Perin didn’t have any pallor/Icterus/cyanosis/oedema or rashes. However noted slight loss in skin turgor.

Systematic examination didn’t reveal any abnormalities in the chest, cardiovascular system. She didn’t show any signs of target organ damage. She was conscious, welloriented, however found to have little bit aberrent in comprehension. concentration with decrease attention span. There were no focal neurological deficits or any neck stiffness elicitable. Kernig’s sign was absent. Plantars were down going with reduced deep
tendon jerks. Abdominal examination did not show any organomegaly or renal bruits.

**LAB INVESTIGATION REVEALED**

CBC: Hb- 12.5gm%, WBC- 6500/Cml, Platlets- 4.50 lakhs/dl

Differentials count – Normal.

USG – KUB:- Normal size kidneys with no urinary obstructions, maintained CMD.

Renal Function Test: BUN- 80mg%, S Creatinine-1.5mg%, S Na+ 126, SK+ 2.4meq/L, S Cl- 72meq/L, S HCO3- 36 mmol/L.

Urine Routine: Normal

Lipid Profile: Normal

FBS & PPBS: 98mg% & 128mg% respectively.

Lately patient was put on Syp. Kesol 20meq twice a day as advised by Physician in view of hypokalemia. Dr. Ramanujam reasoned after analysing above clinical and biochemical parameters as of Intravascular volume depletion with hyponatremia, hypokalemia and hypochloremic metabolic alkalosis due to hydrochlorothiazide as antihypertensive.

**Dr. Shankar:**

Dr. Ramanujam can you elaborate a little bit about why you feel it is due to diuretics and how to clinch the diagnosis?

**Dr. Ramanujam:**

Ms. Perin was intiated on Ramipril and HCTZ to control blood pressure. HCTZ caused intravascular volume depletion due to its diuretic effect and resulted in hyponatremia and hypokalemia. Also noted was increased serum HCO3- level indicates contraction alkalosis with hypochloremia.

**Dr. Shankar:**

In a sense you are right. Let me explain in detail about consequenses of starting a thiazide diuretics as 1st choice in individual’s according to JNC 6 & 7 recommendation.

Occasionally you can have hyponatremia due to loss of Nacl due to blocking of Thiazide sensitive sodium chloride channel. However hyponatremia is triggered rather due to rather intact medullary tonicity due to intact NKCC2 channel. Also collecting duct are not affected where ADH mediated free water absorption takes place. Diuretic use results in intravascular vouleme depletion which in return triggers the release of ADH. The volume gets replenished due to effective free water absorptions by aquaporin-2 channels at the collecting duct.

The above effect will lead to hypochloraeic hyponatremia with hypokalemia. The decline in GFR is due to multiple factors like intravascular volume depletion due to diuretic use and heightened RAS activity. This leads to decline in blood flow to the afferent arteriole. Also ACE Inhibitors will suppress Ang-II activity which lead to decline in arterial tone of efferent arteriole in a glomerulus. The resultant effect is decline in the GFR( Glomerular filtration rate). This has both beneficial and adverse event. The beneficial effect being it provides nephron-protective effect which is desirable by not transmitting the systemic arterial pressure to kidney and reduce proteinuria. However the guidelines say if the GFR declines more than 25% from baseline and continue to decline further wth or without hyperkalemia then it is imperative to stop the ACEI. Also you have to be more cautious in using ACEI or ARB’s in presence of Chronic Kidney Disease . ACEI and ARB’s are contraindicated in bilateral significant renal artery stenosis due failure of auto regulation in kidneys.

Dr Sadique who was sitting in the audience was perplexed and raised his hand and asked “I am wondering why patient had these complaints after so many months of initiation of therapy. How to confirm the diagnosis?

**Dr Shankar**

It is a pertinent question Dr Sadique. Dr Ramanujam do you have any logical expla- nation for this.

Dr Ramanujam hesitated for a moment and revealed some startling facts.
Dr Ramanujam:

The question raised was quite relevant and was wondering before presenting the topic. On repeated enquiry Ms Perin disclosed a startling fact. She said she is habituated about taking laxatives quite often. She also used other medications to facilitate water loss so that she can maintain her weight in the range of 40 to 41kg. She felt she became overweight and resorted to the above tactics to keep her weight under control. On repeated digging she mentioned about putting her fingers in the mouth and induce vomiting following food intake. Her BMI was about 15.5.

Dr Shankar:

Dr Ramanujam what you described is a rare psychiatry disorder known as Anorexia Nervosa. This condition is reflection of abnormal perception of body habitus and they tend to think they are overweight and tend to loose weight to abnormal degree which lead to metabolic abnormalities. In presence of hypertension use of diuretics it might lead to abnormal life threatening electrolyte and metabolic disorders. Severe hypokalemia could be a risk factor for development of cardiac arrhythmias. Besides hypokalemia, malnutrition it self can trigger a devastating ailment known as Osmotic demyelination Syndrome(ODS) if there is an attempt to correct sodium too quickly. Rather in this case hyponatremia correction should take backstag as patient is adapted to the environment with no or minimal CNS manifestations. It will be wise to correct the potassium first and give good nutrition and correct hyponatremia slowly @ 4 to 6meq/L/ Day.

Dr Ramanujam after listening about so many bad consequences of Thiazide diuretics can you explain why Thiazide are considered first line antihypertensive not other groups like loop diuretics etc Dr Shankar remarked.

Dr Ramanujam chuckled and scratched his head for few seconds and showed his inability to answer this question.

When the question went to the audience there was a complete silence for a moment and before Dr Shankar can proceed further a soft feminine voice was heard from the back of the seat “Let me try Sir”. Recognising the voice of Dr Lucy Dr Shankar allowed her to speak. He was enthusiastic and wanted to hear from this brilliant lady doctor who joined the curriculum recently.

Dr Lucy exclaimed “before I proceed I need a marker and a white board to draw a figure”. It was supplied immediately and she drew few lines over the white board and started explaining:

On initiation of thiazide diuretics due to its class effect certain hemodynamic changes do occur. The effect starts within days and continues for few weeks. You can see from the above figure the changes happening like lowering the Mean arterial pressure( MAP), initial rise in total peripheral resistance (TPR), mild to moderate decline in cardiac output(CO), decline in plasma volume. After few weeks to months you can see all effects return to the baseline level except MAP and TPR which are responsible for lowering the BP.
**Dr Shankar:**

Excellent Dr Lucy. I hope this gives a logical explanation about why Thiazides are considered 1st line agents in JNC 6 and 7 guidelines. Besides it is cheap, easily available and has long time tested benefits with good safety profile. However in certain subset of patients you have to be extra careful in initiating this drug due to the above discussed adverse effects. Now going back to Dr Sadique’s question Dr Ramanujam how can you establish the diagnosis of surreptitious diuretic abuse in this case scenario?

**Dr Ramanujam explained:**

Many a times these patients will conceal the information about diuretic abuse while giving the history. However you have to be very clever to extract that from history if a suspicion arises. The main reason for suspicion would be to see evidence of intravascular volume depletion, low BMI, with biochemical parameters suggestive of hyponatremia, hypochloremic hypokalemia with metabolic alkalosis.

You have to differentiate them from other diseases like Incomplete Bartter’s Syndrome and Gittleman Syndrome.

To differentiate I asked for urinary electrolytes like Na+, K+ and Cl- in spot samples at different time period of the day without telling the relevance to the patient. Also simultaneously a blood and urine sample can be sent for estimation of diuretics. Obviously these studies are done after stopping the thiazide diuretics which was used for control of BP few weeks in advance. In this patient urine sample of midday showed high sodium and chloride excretion with detection of Torsemide in the blood and urine during that time.

**Dr Shankar:**

Why she is not a case of Conn’s disease (Primary Hyperaldosteronism)?

**Dr Ramanujam:**

Hypokalemia with hypertension with normal serum sodium level is a feature of Primary Hyperaldosteronism. Also here we will get supressed plasma renin activity with high plasma aldosterone level. Where as in diuretic abuse all the components of RAS will be elevated.

**Dr Shankar:**

How will you manage this patient?

**Dr Ramanujam:**

As you have pointed out sir first I shall stop all the diuretics and consel her. I shall start correcting her hypokalemia first and ensure about slow correction of hyponatremia later.

Also start counselling about the balanced diet she has to take in future. I shall take the help of a Psychiatrist and diet counsellor and form a multi disciplinary team to treat the patient.

Satisfied with the answers Dr Shankar got up from chair and thanked everybody for their participation and applauded the efforts of Dr Ramanujam.

Dr Ramanujam was beaming with joy to see his mentor appreciated his presentation. Everybody dispersed from room with much satisfaction.

**DISCLAIMER**

This article is a story of fiction. All charachters are hypothetical and doesn’t relate to any living or dead. The format of case presentation is loosely based and inspired from the articles published in quarterly journal of medicine by mentors Dr Halparin and Dr Kamel Kamel.

I must thank Mr Nagin & Ms Sheetal for their assistance in preparing the manuscript.

**REFERENCES**

2. M L Halperin, Kamel KS, Marc Goldstein; Fluid, Electrolytes and Acid Base Physiology A problem based Approach. 2017
Rosuvastatin range
Specially designed for Indians

Roseday®- 5/10/20/40
Rosuvastatin 5/10/20/40 mg

Roseday®-F5/F10/F20
Rosuvastatin 5/10/20 mg + Misoprostol 200 mg

Roseday®-A10/A10Forte
Rosuvastatin 10 mg + Aspirin 75/150 mg

Ref.: # Data on file
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