Today’s India represents one of the few growing economies of the world, while most developed nations are toiling to stabilize their economies. This new found success which started around mid nineties has put its citizens at a cross road where they are getting the benefits of modern science through improved sanitation, vaccination, availability of antibiotics and increased medical attention in substantially minimizing the mortality due to infectious diseases. But there is increased prevalence of lifestyle diseases, like heart disease, diabetes and cancer that are now responsible for a substantial number of deaths especially among the younger population.

The Indian scenario is quite worse. It is said that, the possibility of an Indian suffering from a lifestyle disease is four per cent greater than people from other nationalities. Changing lifestyle is mainly responsible for an increase in the number of people suffering from hypertension, stress and other heart ailments (Yusuf et al., 2001). Change in our diet from high nutritional food to unhealthy food, aided with reduced physical activity and exercise has contributed to the establishment of lifestyle diseases. Substance abuse, especially tobacco smoking and alcohol drinking are known to be important factors that increase the risk of life style diseases later in the life. Doctors term these diseases as 'affluent society diseases'. The World Health Organization (WHO) has identified India as one of the nations that is going to have most of the lifestyle disorders in the near future (Sanderson et al., 2007). India appears to be heading towards gaining another dubious distinction of becoming the lifestyle-related disease capital of the world.

Hypertension is a leading member of the life style group of non-communicable disease (NCD) and leading contributory cause of death worldwide (Sapru, 2008). A larger number of population surveys from different parts of the globe have consistently demonstrated hypertension to be a ubiquitous disease. This disease affects both sexes and the age of onset for this disease is slowly decreasing hence more and more patients are turning up at younger ages. These conditions not only cause enormous human suffering, they also threat the economies of many countries as they impact on the older and experienced members of the workforce.
The results of this investigation are presented in three different parts. First one is the prevalence of essential hypertension in rural area; the second one is auditing the prescription of antihypertensive therapy in these patients and the last one is association studies of ACE (I/D), ADD1 Gly460Trp and β-1ADR Ser49Gly polymorphism and monitoring the response of the patients to anti-hypertensive therapy based on the genotypes of the studied candidate genes. For making things simple the discussion of results is also provided in three sections.

Prevalence and determinants of hypertension:

The prevalence of hypertension in the world’s most populous countries, namely India and China has been analyzed. The overall Indian and Chinese population prevalence rates for hypertension among males are 20.6% and 22.6%, respectively (Kearney et al., 2005). The prevalence of hypertension has been increasing in India, both in rural and urban regions. Tribal populations in India have been observed to have a significantly lower prevalence than in other nontribal rural centers. The prevalence of hypertension in rural areas of north India during 1977-1997 was about 5.5% and it has increased to 20-33% in last decade (Anand, 2000, Shanthirani et al., 2003). Studies conducted elsewhere in India among the rural population showed a lower prevalence rate (14%). Poor control of this highly prevalent disease leads to the development of ischemic heart disease, heart failure, stroke and chronic renal insufficiency (Mittal et al., 2010).

It is pertinent to mention that the low prevalence in the past and a rapid increase in the prevalence in the last decade or so is fairly interesting. One reason for this could be the change in the life style and some of the factors listed above, like lack of physical activity and unhealthy food habits. However, one has to consider change in the very definition of the term hypertension. Earlier, hypertension has been defined as systolic blood pressure of >160mmHg and diastolic blood pressure of >105mmHg (Chobanian et al., 2003). However, after JNC-6 guidelines these norms were changed and are still followed (>140/>90mmHg). This clearly suggests that in earlier studies the cut off
values were higher and certainly would have contributed in having fewer people diagnosed with hypertension and hence lower prevalence rate.

To ascertain the prevalence of hypertension in rural setting a hospital based approach was conceived and executed at the Department of Medicine, M M Institute of Medical Science and Research Institute, a established health centers in rural area (Mullana) of Haryana. One of the advantages of selecting this institute was the reliability of the patients with rural background and proper diagnosis of the disease. Commute to hospital is a big problem for villagers and often a cause for improper follow up of the patients. This was not a concern in this study as a free bus service was in place that used to bring patients to the medical institute from the villages and then taking them back. One positive aspect of this service was that the follow up of the general patient was very good.

Our hospital based retrospective study showed a high prevalence of hypertension among all the patients that visited OPD of the Dept of Medicine, MMIMS, Mullana (Figure 6). The overall prevalence of hypertension was 38.2% according to JNC-7 (>140/>90mmHg) guidelines which is slightly higher than most of the reported studies in rural Indian populations. The next most frequent disease was ischemic heart disease followed by diabetic mellitus in rural population of Haryana, North India.

In addition to the high prevalence, a steady increase was observed in the number of hypertensive patients every year from 2003 onwards which pushed the essential hypertensive population in our study (Figure 7). Gupta and his colleagues conducted three serial epidemiological studies (criteria: $\geq 140/90$ mm of Hg) from Jaipur during 1994, 2001 and 2003 and demonstrated a rising prevalence of hypertension (30%, 36% and 51% respectively among males and 34%, 38% and 51% among females). In 2002, Hazarika et al reported 61% prevalence of hypertension among men and women of age $> 30$ years in Assam. One concern for the rural studies is the definition of what is rural. There are studies which are conducted in villages located on the outskirts of metropolitan cities. Some of the subjects might be living in rural areas but virtually work in the cities and are subjected to those stress and strain and hence could increase
the prevalence of this disease in rural background. Necessary precautions were taken in the present study to avoid such bias by adopting strict inclusion and exclusion criteria (3.7 and 3.8).

Some of the reported prevalence studies in rural Indian population in different years: 0.52% in Bombay (1959), 1.99% in Delhi (1959), 3.57% in Haryana (1978), 5.41% in Delhi (1983), 5.59% in Rajasthan (1984), 2.63% in Punjab (1985), 4.02% in Maharashtra (1993), 3.41% in Maharashtra (1993), 7.08% in Rajasthan (1994), 3.58% (1998) and 4.5% (1999) in Haryana. In south Indian rural subjects, that are almost urbanized, the prevalence has been reported to be as high as 17.8% (1993) and 12.46% (Gupta et al., 1994). Overall there is significant increase in hypertension in rural areas although the rise is not as steep as in an urban population (Deedwania, 2002). Different studies regarding prevalence of hypertension among urban Indian population showed significantly increased prevalence of hypertension, as high as 47%, among urban people in Kerala (Mandal et al., 2010).

Apart from Indian studies, the prevalence of hypertension in developing countries varied from a low of 3% in rural Thailand and 5% in rural China to a high of 22% in the Philippines and 23% in Indonesia (Nogueira et al., 1992). Low prevalence (15.5%) of hypertension among the Greek population was reported by (Pitsavos et al., 2006). The prevalence rates of arterial hypertension are greater in Europe (29%) than in the United States (28%), Canada (27%), or Asian countries including Korea (22.9%) or China (20%) (Dorobantu et al., 2010). The prevalence of arterial hypertension in Romania can be integrated between lower values in Europe like those in Greece (31.1%), Sweden (38%), Italy (38%) and higher values such as those from Spain (47%) or Germany (55%) (Efstratopoulos et al., 2006).
The risk factors for developing hypertension could be broadly divided into two components:

- Genetic factors:
  - Anthropogenetic variables, like height, weight, waist circumference, hip circumference; candidate genes: ACE, ADD1 etc.

- Environmental variables:
  - Smoking, alcohol consumption, stress, etc.

The demographic characteristics of all the subjects have been summarized in Table 7. This includes anthropometric parameters, education and occupation. The systolic and diastolic blood pressure was extremely significant (p<0.0001) compared to normotensive population. Weight, BMI and hip circumference was higher in hypertension patients compared to normotensive population and the differences were highly significant (p=0.0057; p<0.0001; p=0.0001). There were 18.33% hypertensive patients who consumed alcohol which was slightly higher than the normotensive ones (13.22%). Similarly, high numbers of patients were smokers (22.85%) and the numbers were almost halved among the normotensive controls which showed significant difference (p=0.055). The two groups shared almost similar education and occupation. However, higher number of patients had family history of hypertension compared to normotensive controls but the difference was not statistically significant (Table 7).

The distribution of hypertensive population by age and sex showed that the prevalence of hypertension was highest (26.76%) in the age group of 41-50 years among males and 42.85% of females were in the age group of 51-60 years (Table 8). In both males and females prevalence of hypertension increased with increasing age up to 51-60 years. Overall the prevalence of hypertension among males (59.16%) was higher in comparison to females (40.83%). Similar findings were noted by Joshi et al (1993) and Gupta et al (1994) in subjects aged >20 years and Malhotra et al (1999) reported higher prevalence of hypertension among males (24%) then females (17%) in a rural community. There were studies with contradictory findings, i.e where the prevalence
was higher among females than their male counterparts, but the difference between the two groups were very small (Gupta et al 1978, 1997). Many Arab and Muslim countries, including neighboring Pakistan, showed higher prevalence of hypertension in females than males (Hamdan et al 2010). For each gender, arterial hypertension was raised in successive age groups. Age specific prevalence showed significant increase with age in both males and females (Table 8).

Studies from rural areas of developing countries reported prevalence of 2-15% using the old guidelines (Nissinen et al., 1988). The prevalence of hypertension in rural areas of Ethiopia (males 6%, females 2%), Zulu (males 7%, females 14%), Tanzania (males 2%, females 2%), Zambia (males 8%, females 10%), Nepal (males 8%, females 4%), and Tibet (males 12%, females 15%) show a wide geographic variations. These findings are in consonance with other regions of Asia where it has been reported that, at any one time, about half of all individuals have a high blood pressure (Gaziano, 2005). The prevalence of hypertension varied around the world from 3.4% in men in rural India as the lowest to 72.5% in elderly women in Poland as the highest reported (Laws et al., 2008).

It is evident from the foregoing discussion that a substantial variation exists in the prevalence of hypertension in the world. Given that people inhabiting these countries adapt to their geographical niche and in the process acquire some common characteristics. Therefore, anthropometric parameters in different ethnic groups make an interesting study with respect to life style disease. Additionally, features like waist circumference and hip circumference might be better markers reflecting the obesity of an individual than assessing the same by taking in account quantity and quality of food intake, which might be more subjective than anthropometric measurements which might portray a much better picture (Wang et al., 1994).

Anthropometric measurements presented with some interesting findings (Table 9), Body weight (0.0755) and Body mass index (p=0.0267) showed slightly significant difference in hypertensive males than normotensives males. Unlike in men, hip circumference (p< 0.0001), and waist circumference (p<0.0001) showed highly significant difference in hypertensive females compared to normotensives females.
Obesity is an important risk factor associated with hypertension. Over eating and physical inactivity in combination with genetic factors are the major causes for the development of obesity in humans. Severe obesity is clearly associated with increased mortality and the incidence of cardiovascular diseases (Erdem bileg et al., 2003). The ethnic origin of the population influences the predictive power of various anthropometric indices. Asian Indians are unique ethnic group in terms of body morphology and cardiovascular disease risk. Asian Indian immigrants have a higher rate of cardiovascular diseases than do other ethnic groups in Canada (Anand et al., 2000). In terms of body morphology, Asian Indians have lower BMIs and have higher central obesity and abdominal fat than do Europeans (Raji et al., 2001). In India, studies have reported the risk threshold levels of various anthropometric indices, but none have measured all four commonly used anthropometric indices in the same population to assess the relative importance of these indices for predicting cardiovascular risk factors (Kaur et al., 2008).

International organizations and experts have published age and sex specific BMI reference values and proposed them for international use. These are based on population data either from a single country or limited number of countries. The BMI cut offs for obesity in Asian adults have found to be lower than the internationally recommended ones, due to the presence of higher levels of body fat and risk factors at lower BMI levels among different Asian populations (Razak et al., 2007). It would be prudent to use threshold cut off values for various anthropometric variables that are developed using local populations instead of the ones proposed by the International groups. Several epidemiologic studies in Asian populations have shown that Asians have higher amounts of body fat at lower BMIs and waist circumferences than do western populations, perhaps leading to the greater prevalence of cardiovascular disease risk factors at lower BMIs in Asian populations than in western populations (Wildman et al., 2004).

Many studies have proven that the relationship between BMI and percentage body fat differs among various ethnic groups (Bozkirli et al., 2007). Women had higher BMI and body fat including waist circumference and hip circumference than men in our study population (Tables 9 and 10). Similar trend was reported among the Turkish
population (Bozkirli et al., 2007), however, contradictory findings were reported among the Japanese population (Erdembileg et al., 2003). The present results indicate that body fat deposit can be an important determinant of gender differences in hypertension and other metabolic diseases. In the studied rural population of Haryana, weight of females was heavier than males and vice versa in case of height (Table 9 & 10). Additionally differences were also observed with respect to the hip circumference and WHR among the two sexes.

Most of the studies reported the relationship between BMI and the percentage of body fat depends on age and sex, and differs across ethnic groups. Wang and colleagues in 1994 showed that Chinese people originating from Shanghai region but living in New York have lower BMI but a higher percentage of body fat than white people of the same age and sex. Similarly Indonesian and Polynesian people have lower BMI than white people. There were negative associations also reported among Americans black and whites (Deurenberg et al., 1998).

The series of body composition analyses using a standard format confirmed that there are obvious differences in the relationship between BMI and the percentage of body fat across ethnic groups. The relative percentage of body fat shows large variation among Asian populations. From the analyses undertaken, Hongkong Chinese, Indonesians, Singaporeans, urban Thai and young Japanese had lower BMIs at given body fat compared with Europeans, whereas Beijing (Northern) Chinese and rural Thai had similar values to those of Europeans (Nishida et al., 2004). These variations depend upon the environmental and physiological variables, such as the amount of physical activity, as observed in the differences between rural and urban populations in India and Thailand.

A representative Pakistani national survey showed 25% of the population to be overweight according to the Asian-specific BMI cutoff values and 10.3% were obese and they observed that hypertension was strongly correlated with BMI (Humayun et al., 2009). Asians, however, comprise many ethnic subgroups that differ in body composition, genotype, age structure, lifestyle, culture, religion and socioeconomic status. This would lead to greater ethnic differences in the association between BMI and
disease risk across Asian populations (Nguyen et al., 2009). The multi regression model is the best model for identifying predictor of hypertension in such population studies. In our study population, the strong predictor of essential hypertension was Body Mass Index and smoking (Table 11). There were other established studies from India that showed similar findings with age, obesity, BMI and high socioeconomic status (Sharma et al., 2006). In other study conducted in eastern India, reported old age, high BMI and vegetarian diet as important predictors of hypertension but the one from Chennai, South India found only age and BMI as predictors of hypertension (Sharma et al., 2006). In a study from Assam, the significant determinants of hypertension were age, sex, extra salt intake, BMI and WHR. Gender specific analysis showed that heavy drinking increased the risk of hypertension in men (Hazarika et al., 2004).

Epidemiological studies have found a progressive increase in the prevalence of elevated blood pressure with increasing adipose tissue (Kaur et al., 2008). Different anthropometric measurements like BMI, WC and WHR were investigated for assessing obesity. However, the relationship between the best obesity measures, blood pressures and hypertension still remain unsolved (Ghosh et al., 2007). Prevalence of obesity has been found to be very high in females compared to males in India. In the present study, females had higher prevalence of abdominal obesity than males (Table 10), similar observations have also been reported in populations living in Rajasthan, Wardha, Bengal, Delhi, Dhaka, Chennai and Jaipur (Haldiya et al., 2010).

Apart from India, a study from Mexico reported excessive weight and lack of physical exercise as predictor of hypertension in male (Rosas et al., 2003). In another study from Yaounde city, Cameroon hypertension was significantly associated with obesity, alcohol intake and smoking but not with gender (Shey-wiysonge et al., 2004) Age, BMI and smoking were related with hypertension in the urban population of Istanbul which is similar to our study. Age and obesity were the key predictors of hypertension in Brazil and Mexico whereas in Hungary, over weight and low physical activity were significantly associated with hypertension (Zoltan et al., 2002). These studies suggest that predictors of hypertension may be different in various geographical regions. In light of knowledge generated regarding predictors of hypertension all over
the world, it is pertinent to examine the hypertension in specific population group (Sharma et al., 2006).

The high prevalence of hypertension in our study seems to be having a strong genetic component as blood pressure has been strongly associated with BMI and family history (Table 19). This might have been facilitated by unusually high use of saturated fat as the preferred medium of cooking. Other reason could include changes of social factors and lifestyle habits. Unfortunately many hypertensives, even among health care workers go undetected. Sedentary lifestyle and central obesity are two important risk factors for hypertension and cardiovascular disease. A positive relationship between the use of smoking and blood pressure in men has been reported previously. However, in this study, smokers among men seem to increase the risk of hypertension (Table 11). The increased prevalence could also be because of increased awareness about the disease through electronic media like TV and more people are seeking intervention at an early stage. One good thing about this disease is that if it is detected early and corrective measures are instituted rigorously then this disease could be efficiently managed. One example for such observation could be the regular practice of yoga, which is now conventionally accepted to control this disease and its complications (Yeligar et al., 2010; Moser et al., 2007).

Pharmacoepidemiology of anti-hypertensive therapy

Drug therapy is the most commonly used method of any disease treatment in general practice. General practice data bases have been used as an effective method for pharmacoepidemiological research. Many classes of antihypertensive drugs are available to physicians. Each class has its own mechanism of action and may act on a different target organ. Some drugs, such as nitrates were used extensively in the past; diuretics and beta blockers generally were considered appropriate monotherapy as first line treatment (WHO, 1999). Calcium channel blockers and angiotensin-converting enzyme inhibitors are being used increasingly in the management of cardiovascular disease and hypertension (ESCG, 2003). In particular, calcium channel blockers often are indicated in isolated systolic hypertension in the elderly, whereas ACE inhibitors are
used is in hypertension when coexisting conditions, such as heart failure and renal failure, are present (Whitworth, 2003)

However, each drug class is associated with its own set of adverse events that may reduce treatment compliance. Various clinical trials and studies on antihypertensive treatment have been published over the past decade. Based on clinical evidence and cost effectiveness, guidelines developed by the Joint National Committee in the United States and the National Institute for Health and Clinical Excellence (NICE) in the United Kingdom recommended that diuretics (particularly-thiazides type diuretics) should be the drug of first choice for patients with no compelling indications (Fretheim et al., 2005). However, the results of various studies have shown that adherence to such guidelines and recommendations are not at all uniforms; indeed, they have been found to vary by time period and country, and by the characteristics of patients and physicians (Liu and Wang, 2008). A physician face the problem with a common and very well studied condition such as hypertension would thus be expected to turn to randomized trials for guidance regarding the choice of initial treatment. On the basis of evidence, where trials regularly find similar efficacy in lowering blood pressure and safely among available agents, the physician might be tempted to follow the recommendation of various guidelines to start with the least expensive drugs with proven ability to reduce rates of sickness and death from cardiovascular disease but the fact is opposite and differ substantially, from those of randomized clinical trials (Caro et al., 1999)

Unsatisfactory control of arterial hypertension has been reported in epidemiological studies from different countries (Maria et al., 2002). The response to different agents varies from person to person, however, to effectively control blood pressure; the clinician may increase the drug dose or change to another drug. There are non-empirical ways of predicting response such as using rennin measurement or the Cambridge ABCD rule. Plasma rennin can be measured, usually in a clinical research setting as a simple rule of thumb younger and older patients can be assumed to have high or low rennin levels, respectively (Cheung et al., 2003). It has long been suspected that inter-individual variations in drug efficacy and side effects may be influenced by
During different studied period (June 2003-September 2006 & January 2010-July 2011), the data shows that monotherapy prescription of antihypertensive therapy were more in numbers than combination therapy compared to other study conducted in Hong Kong who received more combination therapy rather than single one 49.2% (Lee et al., 1997). Among monotherapy pattern, the prescriptions of β-blockers were more in percentage compared to diuretics, ACE inhibitors and calcium channel blockers (2003-2006). Surprisingly (2010-2011) data revealed altered prescription pattern in monotherapy. The prescription of diuretics decreased from 21% to 12% and vice versa in case of ACE inhibitors which increased from 27% to 40%. Only 10% prescription of calcium channel blockers was found in rural population of Haryana compared to Nigerian study where this drug was the most frequently used one and the most efficacious antihypertensive drug class in black Africans (Adigun et al., 2003).

Among combination pattern, the usage of beta blockers with ACE inhibitors were prescribed more in numbers at both studied periods. The physicians prescribed the ACE inhibitors and β-blockers in top priority among our study patients (Figure 8). The JNC-7 report states that in the absence of compelling or specific indications for another drug, a diuretic should be chosen as initial therapy for hypertension. These recommendations are seconded by various health organization and societies (WHO & British Hypertension Society) (Guo et al., 2003). Despite these guidelines, diuretics were prescribed in only 12% of the prescriptions in this study (2010-2011) (Figure 8), similar to 13.2% in Government Medical College Hospital (GMCH), Chandigarh, 1.9% in Punjab University Health Center (Chandigarh), 24% in Hong Kong and 26.5% in a study from Bangalore (south India) (Xavier et al., 1999). However, the usage of β-blockers (42% of the prescriptions) was maximum (Figure 8), which is similar to those reported from GMCH (Chandigarh) (46.7%), in PUHC (Chandigarh) (46.2%), Hong Kong (51%) and much higher than that reported from Bangalore (19%) (Xavier et al., 1999). Lesser use of diuretics in the present study may be due to adverse effect of diuretics on glucose homeostasis and lipid profile. On the other hand β-blockers have
shown long-term protective effects against cardiovascular disease in hypertensive patients. The exact mechanism by which beta blockers exert their antihypertensive effect is uncertain. Possible actions include a reduction of cardiac output (negative inotropic and negative chronotropic effect), an effect on vascular resistance, as well as an inhibitory effect on the release of rennin (which is stimulated by the sympathetic nervous system) and central effects that may be influenced by the hydro or lipophilicity of the beta blockers (Elsik, 2007). They vary in their lipophilicity, receptor specificity, mode of elimination, half life, primary indications and cost.

Among three most prescribed drugs, the percentage reduction of systolic blood pressure falls maximum -15.41% with diuretic treatment followed by $\beta_1$ blocker -14.18% and lastly -9.58% with ACE inhibitor after 4 week follow up. A comparison between these drugs revealed statistically significant ($p<0.005$) differences between $\beta_1$ blocker and ACE inhibitor. Whereas in case of diastolic blood pressure, $\beta_1$ blocker (-13.96%) and ACE inhibitor (-12.43%) showed maximum reduction of blood pressure compared to diuretics (-9.12%). When compared with each other, the differences obtained in percentage reduction of blood pressure with various drugs were all statistically non significant (Figure 9 and 10; Table 15 and 16).

From the foregoing data, it is evident that physicians do not follow the JNC-7 guidelines. According to JNC recommendations first-line drug therapy for uncomplicated hypertension has evolved over time. Both JNC-5 and JNC-6 recommended diuretics as preferred first-line therapies. Subsequently, the JNC released its seventh report in 2003, which recommended thiazide diuretics to be prescribed alone or as part of combination therapy for most hypertensive patients (Jun et al., 2006). As a result of various clinical trials and studies, a range of clinical guidelines on anti-hypertensive treatment have been published over the past decade (Liu and Wang, 2008).

Observational studies have provided evidence on which drugs are used initially to treat hypertension but few have analyzed the patient characteristics that influence the choice of first-line therapy. Adherence to recommendations in the long term is unclear and studies from other countries show proper compliance with such guidelines. Rates of
Discussion

Change or discontinuation of antihypertensive therapy are high, and may suggest that poor control is due to poor adherence to prescribed medication (Cheung, 2003).

Unfortunately many misconceptions persist regarding antihypertensive drugs; some of these are based on exaggerated reports of negative side effects. Pressure from industry to make newer drugs seem better and may result in dissemination of the “dangerous” side effects of the older drugs. The public and doctors alike should not be pressured by pharmaceutical companies to change treatment practices.

Earlier studies suggested that an ideal combination must include antihypertensive drugs possessing complementary modes of action that provide a synergistic anti-hypertensive effect without any significant adverse effect, at low doses. Furthermore, the anti-hypertensive drug combination therapy should be able to minimize or counteract the reflex compensatory mechanism that often limit the fall in blood pressure.

Those patients who are unresponsive to first line monotherapy or who showed adverse effects at high dose need to change the pattern of drug or use alternative methods to control blood pressure like combination therapy in two and more drugs. One advantage of combination therapy is that low doses of two antihypertensive agents tend to be more effective and better tolerated than higher doses of either drug alone (Whitworth, 2003).

The ability to identify patient characteristics associated with BP response to each drug class could increase control rates and improve on the current “trial and error” approach for selection of drug therapy for hypertension. Some of the predictive factors include age, higher baseline BP, excessive dietary salt ingestion, ethnicity, sex and measurements of the rennin-angiotensin-aldosterone system (Canzanello et al., 2008). According to baseline blood pressure and presence or absence of complications, it appears reasonable to initiate therapy and if blood pressure control is not achieved, the next step is to switch to low dose of a different agent or to increase the dose of the first agent or to move to combination therapy. If therapy has been initiated by low dose combination, a higher dose combination is added. This situation is laborious, frustrating
and time consuming for both the patient and the physician, and motivates the development of clinically applicable pharmacogenetics (Padmanabhan et al., 2010).

There are considerable variations in patient’s response to antihypertensive treatment (Teresa et al., 2003). Although significant reductions in blood pressure occur in most patients, many respond to treatment with a paradoxical increase in blood pressure and there is no way to identify such patient before treatment (Kurland et al., 2005).

However, the results of various studies have shown that adherence to such guidelines and recommendations are not at all uniform; indeed, they have been found to vary by time period and country, and by the characteristics of patients and physicians (Liu and Wang, 2008).

**Distribution of genotypes and their allelic frequency**

Each individual is characterized by a sequence of ~3 billion base pairs. One out of 300 base pairs varies from person to person, meaning that anyone of us can be distinguished from his/her neighbor by 10 million base pairs. It is estimated that only around 1% of the base pairs are non-synonymous and can account for modifications in proteins. In this way, only 10,000 of the 10 million base pairs are able to modify our proteins. Moreover, a large number of genetic polymorphism exists in various combinations and responsible for variability in protein levels and protein activity in blood and are considered as risk factors for multi-factorial disease (Schelleman et al., 2004).

Advances in molecular genetic technologies that have led to sequencing of the Human Genome now also offer the possibility of identifying the DNA sequence variants contributing to inter-individual differences in drug response including that influencing blood pressure response to antihypertensive therapy (Lander et al., 2001). Finding the genes and their variants that influence response to antihypertensive drugs has the potential to revolutionize approaches to the diagnosis, evaluation and treatment of hypertension (Turner et al., 2001).
A common problem in studies of complex disease is the difficulty in acquiring the relevant data for rational choice of candidate genes. This has led to the use of only some of many relevant genes and markers in genetic studies of association and linkage. Complex genetic disease arises out of a dynamic interaction between both environmental and genetic factors. Although some environmental effects contributing to human disease such as diabetes and hypertension are understood, our current knowledge of specific genes contributing to these same diseases is sorely lacking.

Why is prevalence of hypertension increasing in this modern era? The disease could be easily diagnosed and there are effective medicines to treat this disease. Despite these developments, the prevalence rates are increasing which influences the mortality rates in adult population worldwide (Chinese 76.8%, Malay 13.9%, Indian 7.9% and 1.4% in Singapore (Tan et al., 2005). One aspect that has attracted lot of attention in developed nations, but has not been studied much in Indian scenario, is the pharmacogenomics of antihypertensive therapy. The present investigation was undertaken as a modest attempt to study selected candidate gene polymorphisms in a rural (Mullana) population of Haryana.

Gene that encodes protein that either is targeted by anti-hypertensive drugs or corresponds to components in the counter-regulatory systems is an obvious candidate gene for pharmacogenetics investigations. Studies of polymorphisms in individual candidate genes indicate that the contribution of genetics to the inter-individual variations in the blood pressure response to antihypertensive therapy is 3-5%. However, in a study targeting 74 single nucleotide polymorphisms (SNPs) in 25 candidate genes showed that combinations of 4-5 SNPs could explain 44-56% of the variation in the blood pressure response to treatment (Kurland et al., 2005).

A number of genes whose biological function makes them logical candidates or markers for BP homeostasis have been studied in recent years. Among these are loci coding for rennin-angiotensin system (RAS; involving the REN, ACE, AGT and AT1 loci; this system plays a vital role in kidney function and renal homeostasis (BP regulation) and is also a target system for antihypertensive agents (Sakuma et al., 2004).
Linkage studies of hypertensive sibling pairs for the REN, ACE, AT1 and the SAR genes have all been negative (Gong et al., 2006).

Genome wide association studies (GWAS), have identified number of genetic markers that are functionally implicated in the etiology of hypertension (Gui-yan et al., 2006). However, the contributions of these candidate genes could not be validated in various populations residing in diverse geographical regions and belonging to different ethnic groups (Kobashi et al., 2006)). The major reason for this lies in the etiology of disease. Hypertension is a heterogeneous and complex genetic disease where multiple gene variants are involved in different ethnic groups. Each gene could be contributing only a fraction of the whole effect. Given that numbers of genes are involved in the etiology of hypertension and are involved in yielding a common phenotype. The combination of these factors makes the frequency of any polymorphism(s) contributing to disease phenotypes invariably higher in disease group compared to normal controls (Risch 2000). The best approach to reveal the effect of such small effects is by conducting case control study where sizeable numbers of disease cases are compared with normal control from the same population. Genotyping of rural hypertensive patients from (Mullana) Haryana, North India was used to test the role of ACE, ADD1 and β-1 ADR gene polymorphism(s) to which the risk has been attributed in number of investigations.

ACE I/D polymorphism were studied using a PCR based strategy (Figure 3). The insertion allele was identified by the amplification of a 490bp product while the deletion allele amplified a product of 190bp only. Presence of both the products in an individual identified the presence of ID genotype (Figure 11). No statistically significant differences were observed between two populations that is hypertensive patients and normotensives for ACE I/D polymorphism. The frequencies of different genotypes were found to be similar in patient and the control population (Table 18). The frequencies of both the alleles (I/D) are quite high in the control and cases, thus obviating the possibility that the frequency of the rare allele is a cause for concern in the studied sample. Lack of association between ACE I/D polymorphism and hypertension have been reported by investigators in Indian and other populations of the world (Table...
49) but the controversial results was observed in South Indian Tamilian population (Ramu et al., 2011). Ethnic background is known to influence the ACE I/D polymorphism globally. A significant association of the ACE high producing D allele with hypertension in African, Americans, Chinese and Japanese populations has already been reported (Bhavani et al., 2004). However, two studies from Australia and Pakistan recorded the association of I allele with hypertension. The association of I allele with hypertension in Pakistan population was attributed to limited number of individuals studied and to the presence of high levels of inbreeding (Gupta et al., 2009).

The frequency of D allele of ACE I/D polymorphism in different hypertensive populations of India varied within 0.590 to 0.477 (Table 49). The highest frequency was reported in a Sikh group from Punjab which also showed an association between the D allele and the hypertension. Similar observations have also been made on populations from southern India (Ramu et al., 2011). The frequency of D allele in the studied patient and control populations were well within the reported range for the North Indian populations, but contrary to the earlier findings, no association between D allele and hypertension was observed in the rural population of Haryana. We believe the number of patients studied in other Indian populations showing positive associations with D allele were very small to allow any meaningful conclusion (Gupta et al., 2009).

Table 50: The Genotypic distribution and allele frequencies of the ACE I/D polymorphism in essential hypertension in different population of the world.

<table>
<thead>
<tr>
<th>Population studied (n)</th>
<th>Genotype distribution</th>
<th>Frequencies</th>
<th>Reference</th>
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<tr>
<td></td>
<td>DD DD DD ID ID ID ID II II II</td>
<td>D allele I allele</td>
<td></td>
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<tr>
<td>North Indian (106)</td>
<td>30 49 27</td>
<td>0.514 0.496</td>
<td>Present study</td>
</tr>
<tr>
<td>European:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Italian (86)</td>
<td>31 41 14</td>
<td>0.60 0.40</td>
<td>Teresa et al., 2003</td>
</tr>
<tr>
<td>Kyrgyz (180)</td>
<td>20 91 69</td>
<td>0.36 0.64</td>
<td>Polupanov et al., 2007</td>
</tr>
<tr>
<td>Dutch (257)</td>
<td>80 138 39</td>
<td>0.58 0.42</td>
<td>Schut et al., 2004</td>
</tr>
<tr>
<td>Turkish (109)</td>
<td>49 59 01</td>
<td>0.73 0.27</td>
<td>Agachan et al., 2003</td>
</tr>
<tr>
<td>Solvenian (413)</td>
<td>132 199 82</td>
<td>0.57 0.43</td>
<td>Galvnik et al., 2007</td>
</tr>
<tr>
<td>German (621)</td>
<td>167 309 145</td>
<td>0.517 0.483</td>
<td>Mondry et al., 2005</td>
</tr>
</tbody>
</table>

Asians:
To check the relationship between the genotype of ACE I/D and anthropometric variables on the basis of gender and compared with normotensives and hypertensive population, our data failed to find any association between these but in the case of blood pressure with ACE genotypes, the results showed positive associations when compared with both population (Tables 21, 22, 23, 24 and 25). However, in the case of ACE genotype with anthropometric variables and blood pressure in hypertensive population, the result was found to be non significant (Table 26).

**ADD1** is a heterodimeric cytoskeleton protein present in most tissues with α, β and γ subunits involved in cell to cell contact, cell membrane ion transport and signal transduction. **ADD1** is one of the many proteins that regulate Na\(^+\)-K\(^+\) ATPase activity. Abnormalities in adducin gene by mutation have been shown to influence Na\(^+\)-K\(^+\) ATPase activity leading to faster renal tubular Na reabsorption. Clinical and experimental studies have demonstrated the involvement of **ADD1** in the pathogenesis of essential hypertension both in human and animals (Manunta and Bianchi, 2006). The gene encoding human **ADD1** is mapped onto the chromosome location 4p16.3. The common molecular variant of the **ADD1** gene causing the substitution of tryptophan instead of glycine (Gly460Trp) at amino acid position 460 was found to be associated with increased risk of hypertension (Li et al., 2005) and therefore this SNP, Gly460Trp, was studied among hypertensive and normotensives population in this study.
The present study is the first report investigating the role of this important polymorphism in a rural population of Haryana, North India. The Gly460Trp polymorphism was detected using an ARMS PCR assay using allele specific primers (Figure 11). The G allele specific primer yielded a product of 220bp and T allele specific primer amplified a 234bp product (Figure 12). A sample yielding product of 220 and 234bps was identified as TG heterozygote (Figure 12). Our data failed to find any association between hypertensive and normotensives group and similar observations were recorded in an elaborative study conducted in a south Indian population (Ramu et al., 2010). The frequency of G allele in both the groups was higher than normotensives group. In our study, the frequency of 460 Trp allele was 0.33 in the normotensives and 0.26 in the hypertensive. Frequency of 460 Trp allele of ADD1 gene in our population are considerably lower than those of Japanese (52-66%), Korean (67%) and Chinese (42-56%) populations (Norihiro et al., 1998). However, frequency of 460Trp alleles is similar to that of populations from black South Africans and lower than populations from Europe (18% Italy, 20% in France, and 27% in Scotland) (Ranade et al., 2000). It might be safe to assume that the observed differences in the allele frequencies are reflections of the varied ethnicity of the studied populations.

There is lack of association between ADD1 genotypes and BMI, waist circumference and waist hip ratio (Table 31, 32 and 33). However, hypertensive and normotensives populations revealed statistically significant results between the two genotypes (TG & TT) of the ADD1gene and systolic blood pressure (Table 28), but when the data was split based on the gender of the subjects, both sexes revealed significant differences between all the genotypes of Û-adducin (ADD1) polymorphism with the systolic blood pressure (Table 28). The association was more fragmentary with diastolic blood pressure and the Û-adducin (ADD1) genotypes. In total population only, TT genotype showed negative association with diastolic blood pressure. When the data was grouped according to the gender, males revealed association with all three genotypes of Û-adducin gene and in females TT and TG genotype showed statistically significant differences (Table 29). It is rather difficult to assign any cogent reason for these observations as they are very diverse and they represent a rather small sub group of subjects (Table 30). However in the case of ADD1 genotype with anthropometric
variables and blood pressure in hypertensive population, the result was found to be non significant (Table 34).

The present study studied Ser49Gly β-1 ADR gene polymorphism using the PCR-RFLP method. The CF/RF common primer pair amplified a PCR product of 564 bp which was digested with ECO 01091 enzymes. The presence of glycine residue in the DNA provided a site for the restriction enzyme to digest the fragment resulting in two sub fragments of 235 and 345 bp while the presence of serine residue removed the restriction site and hence the fragment remained intact of 564 bp. The heterozygote yielded three fragments of 564, 345 and 219bp (Figure 13).

Table 35 shows the allele and genotype frequencies of the A145G (Ser49Gly) polymorphism in the rural population of Haryana. It showed positive association between allele frequency and subject population, the data was statistically significant (p<0.023) and compared well with the Mexican (Mestizos) population (Fragaso et al., 2005) which is similar to this study having highest frequencies of Ser allele and the lowest frequencies of the Gly allele. However, contrary results have also been reported where Gly allele were higher than Ser allele in Caucasians, African-American, Asians and latino-hispanics populations (Leineweber et al., 2009). Interestingly, a positive association was also observed in a hypertensive South Indian population having Gly49Gly homozygous genotype higher than Ser49Ser homozygous genotype (Ramu et al., 2009).

Again there is lack of association between β-1 ADR genotypes and BMI, waist circumference and waist hip ratio (Table 39, 40 and 41). However, hypertensive and normotensives populations revealed statistically significant results between the two genotypes (SS & GG) of the β-1 ADR gene and systolic blood pressure (Table 36), but when the data was split based on the gender of the subjects, both sexes revealed significant differences between all the genotypes of β-1 ADR polymorphism with the systolic blood pressure (Table 36). The association was more fragmentary with diastolic blood pressure and the β-1 ADR genotypes. In total population only SS genotype showed negative association with diastolic blood pressure (Table 37). When the data was grouped according to the gender, males revealed association with all three
genotypes of \(\beta-1\) ADR gene and in females only SS and GG genotype showed statistically significant differences (Table 37). However in the case of \(\beta-1\) ADR genotype with anthropometric variables and blood pressure in hypertensive population, the results were found to be non significant (Table 42).

Essential hypertension is a complex genetic disease which assumes that multiple genes are involved in the etiology of this very common disease. Therefore, it should not be surprising to note that lot of variation has been reported in the role of any given polymorphism(s) or its genotypes in the etiology of the disease. This could also be a reason why single gene studies are not replicated in different populations as the confounding factors which could be genetic or environmental in nature may vary in those studies. One possible way to address this lacuna could be employing gene-gene or gene-environmental studies. Such analysis should yield much more meaningful data than comparison of genotypic or allelic distribution data in the diseased and control populations. The gene-gene interaction between three genes (ACE, ADD 1 and \(\beta-1\) ADR) revealed negative association in both populations based on (Table 43 and 44).

Similar observations have also been made in a South Indian Tamilian population (Ramu et al., 2011). It is important to mention that the sample size required for conducting gene-gene interaction studies between three different genes will be much higher than what has been investigated in this study. Therefore, the data presented should be considered as preliminary comparison and needs to be validated with a much larger study.

The present study demonstrated the blood pressure-lowering effect and the smoothness of blood pressure control of ramipril (5mg) after 4th week in 106 patients. About 30 hypertensive patients were having DD genotypes. Out of which 21 showed responding and 09 showed non responding effects at standard dose similar in case of ID they showed only 09 non responding effects out of 49 but in II genotype only 04 showed non responding effect out of 27 hypertensive patients and they are statistically non significant (Table 45).

The probability of showing more response in ID & II genotype in responder group may be due to the low the expression of ACE which leads to decrease serum
ACE activity and similar studies were also reported (Rigat et al., 1990; Tiret et al., 1992) while opposite results were seen in Stassen et al., 1997. These results can be justified with some of the studies have suggesting ACE polymorphism interferes in ACE serum concentration. Further DD genotype individuals would have the highest ACE serum concentrations, where those with genotype II would have the lowest. It is estimated that allele D would contribute with approximately half the variation of ACE plasma levels (Sandro et al., 2007). But non-response in II & ID in our study cannot be justified with above studies. These are some more studies in which too show II/ID alleles non responders to ACE inhibitors (Schelleman et al., 2004; Redon et al., 2005; Jiang et al., 2007). But it may respond to combinational drug therapy either with diuretic or beta blockers. There are however inconsistencies in trial findings and as a result the extent of effect modification of this polymorphism remain unclear (Scharplatz et al., 2004). So to achieve anti hypertensive response they may require high dose of ramipril or some other drug for effective management of hypertension.

Another forty eight patients received the combination therapy, about 13.34% showed non-responding effect in the combination of Ramipril (5mg) with metoprolol (25mg) and 27.78% showed non-responding effect in Ramipril (5mg) with hydrochlorothiazide (12.5mg) (Table 46).

Different individuals have different polymorphism exists in the human genome. From predictor model, our study suggested that ACE inhibitor individually couldnât control the blood pressure even at high dose so the physicians should prescribed combination therapy at small doses because as we know multiple polymorphisms involved in development of hypertension. Combination therapy may give the better results in the treatment of hypertension.

There were thirty two patients who were treated with diuretic mono therapy for the control of blood pressure (Table 47). It is evident from this table that patients with different Gly460Trp genotypes showed variable to this therapy. Maximum number of patients having GG or TG genotype responded favorably to this treatment but those who were having TT genotype showed maximum failed to respond to the diuretic monotherapy. However study on French and Italian populations revealed that the
Trp460 allele was associated with response to diuretic therapy (Cusi et al., 1997). Despite these unambiguous results and persuasive physiologic link, several studies failed to find an association between the alleles of \( \alpha \)-adducin gene and hypertension in Australians (Wang et al., 1999) and in Japanese patients (Tamaki et al., 1998).

Some patients respond better than others to \( \beta \)-blockers is well accepted by clinicians. In this study we found that the Ser49Gly & Ser49Ser genotype showed maximum number of responding effect when compared to Gly49Gly homozygote genotype with the blood pressure response to \( \beta \)-blocker (Table 48). This finding was consistent across every type of analysis conducted. It is already recognized that African-Americans subjects tend to have poorer responses to \( \beta \)-blockers than white subjects and because the allele frequencies of the \( \beta -1ADR \) polymorphism differ within ethnic groups. This could be one of the reasons but our study shows that the clinical difference in response to \( \beta \)-blockers is largely dictated by the \( \beta -1 ADR \) genetic polymorphisms. The primacy of genotype over ethnicity as a predictor of drug response has also been demonstrated with thiazide diuretics, an antihypertensive drug class historically thought to be more effective in African American subjects than the white subjects (Johnson et al., 2003).

Change in the SNP leads to the change in amino acid in the adrenergic receptors has previously been shown to be associated with cardiovascular and drug response phenotypes. The \( \beta \)-blockers having the dual mechanism on different genotypes, Arg389 form of the receptor has been associated with increasing coupling of the \( \beta -1 \) adrenergic receptor to G protein, leading to greater adenylcyclase activation. The Ser49 form of the receptor has most consistently been associated with resistance to receptor down regulation. Therefore the Ser49-Arg389 haplotype would be expected to be most responsive to activation by catecholamine, and consequently a greater response to \( \beta \)-blocker with this haplotype would also be expected (Pacanowski et al 2008). Several studies have been reported on the Ser49 Gly or Arg 389 Gly \( \beta -1 ADR \) polymorphisms on blood pressure and heart rate responses to \( \beta \)-blocker treatment in hypertensive patients but divergent results have been obtained. Two studies found that the antihypertensive effect of the \( \beta ADR \) blocker treatment was genotype-dependent.
different in patients with the Arg389 or Gly389 β-1 ADR, three studies failed any Arg389 Gly β-1 ADR genotype-dependent differences in antihypertensive responses. (Johnson et al., 2003) reported in 40 hypertensive female and male patients the impact of the Ser49Gly and Arg389 Gly β-1 ADR on the antihypertensive effect of metoprolol with 200 mg twice daily. They found that patients homozygous for Arg389 β-1 ADR had significant greater reduction in 24 hr and day time diastolic blood pressure than patients with the Gly389 allele. In contrast with other three studies, Karlsson et al., (2004) treating 101 female and male hypertensive patients (from Sweden) for 12 weeks with 50mg/day, Filigheddu et al., (2004) treating 270 female and male patients with essential hypertension (North Sardinia) for 8 weeks with 50mg/day and O'Shaughnessy et al., (2000) treating 92 females and male hypertensive patients (England) for 4 weeks with 50 mg/day did not find any Ser49Gly or Arg389Gly β-1 ADR genotype-dependent difference in the β blocker treatment-evoked decreases in blood pressure (Brodde, 2008).

Relative to the number of available antihypertensive medications and the recognized heterogeneity in responses, studies addressing the genetics of response have been quite limited with respect to both the number of drug responses and the number of candidate genes evaluated. Given the extensive understanding of blood pressure regulatory pathways and the known and specific targets of antihypertensive drugs, this incongruency is rather surprising. The explanation probably includes the wide therapeutic index for most antihypertensive drugs, lack of correlation between drug levels and responses, reluctance by drug companies to risk fragmenting their markets, and previous lack of methods to directly measure and analyze DNA sequence variations. So far, all candidates gene studies have been designed as ‘association’ studies involving unrelated individuals (vs. linkage in family members) and were conducted in relatively small groups.

Identifying association between a gene and a complex genetic disease is difficult. One possible reason for this is the involvement of a large number of genes in the etiology of essential hypertension. Furthermore, these genes may interact with each other in different combinations to give rise to a similar disease phenotype. The
magnitude of this problem makes the frequency of any polymorphism contributing to a
disease phenotype marginally higher in disease group compared with unaffected
controls. Linkage analysis has limited power to detect such small effects and case
control studies with matched controls from the same population had greater probability
of detecting such minute effects. The inability to find association between ACE I/D
polymorphism with hypertension in the present study strongly point out that ACE gene
is not playing a predominant role in the pathophysiology of this disease in our
population and is not a good predictor of susceptibility to hypertension. Similar
observations have also been made in a Meta analysis studying the role of genetic
polymorphisms in hypertension. Since hypertension is a complex genetic disorder, it is
assumed that there could be other genetic and environmental factors that interact and
influence the development of this disease.

Despite numerous drug classes acting on a variety of blood pressure control
systems, less than 50% of treated hypertensive individuals have their blood pressures
adequately controlled. For agents from each class, blood pressure response is
continuously distributed, and standard deviations of response are as large as the mean
responses. It has long been suspected that inter-individual differences in response to
antihypertensive drugs and their side effects may be influenced by genetic or
environmental causes.

Hence this study concludes that hypertension is more prevalent in rural
population at age of above 40 yrs in both sexes. Obesity is an important risk factor for
the hypertension in both sexes. Therefore, there is need to focus attention towards
primary prevention of hypertension and more studies are required to assess the
prevalence, determinants preventive interventions of hypertension in rural areas. There
is a need for strengthening health education programs promoting hypertension
awareness, and emphasizing preventive measures. This study will focus attention on
this "silent killer" arousing awareness of health care services for surveillance and
opportunistic intervention and will provide a baseline for future studies. Perhaps
changes in traditional dietary habits and lifestyle patterns in combination with other
factors like smoking have made them prone to hypertension and possibly contributing in increasing the prevalence of hypertension in rural area.

The sample size used in this study represents adequately the hypertensive population under investigation but is limiting in addressing larger issues of gene-gene interaction or gene-environment interaction. In this context, the present study should be regarded as hypothesis generating and needs to be replicated with large sample size, longer duration and possibly encompassing other ethnic groups residing in this region.

The clinical implications for genetics of complex disorders are still in its cradle. No doubt in the developing countries the personalized drug therapy is a far reaching goal because of the increasing patient population and the high cost involved in tackling the increased burden of disease. Indian Government has initiated few steps towards this goal and it is felt that the small pharmacogenomic studies should be encouraged and data pooled to develop a clinically useful picture in future. Larger and coordinated studies will not be possible without the establishment of an infrastructure for national or international multicentric studies that fulfils all aspects of ‘good clinical practice' guidelines. This would clearly require funding by appropriate national/ international agencies.

Finally, it is concluded that drugs that are more specific for functional characteristics associated with individual patients polymorphism may contribute to a better response and reduced toxicity of pharmacotherapy. These emerging concepts might markedly change the management of essential hypertension in the next future.