REVIEW OF LITERATURE

The state of hypertension has a cause-effect relationship with oxidative stress which may translate to genetic damage and further compromise the health of the hypertensive patients. Hence, in the present study, assessment of dyslipidemia, oxidative stress and genetic damage as a function of some drug metabolizing gene variants in peripheral blood leukocytes of Atenolol treated hypertensive patients and of normotensive healthy controls was carried out. Here under the background information about the disease and the drug, Atenolol is first presented followed by the review of literature appropriate as per these aspects.

2.1 Background Information about Hypertension

2.1.1 Definitions

According to National Health and Nutrition Examination Surveys (NHANES III, 1993) blood pressure is “the force exerted by the blood on the wall of a blood vessel as the heart pumps (contracts) and relaxes”. While its regulation is through the physiological functions of cardiovascular, neural, renal and endocrine systems (Chopra et al., 2011). When the pressure of the blood in arteries increases beyond certain limits, it leads to hypertension. The Indian Hypertension Guidelines (IHG III, 2013) and Joint National Committee (JNC 7, 8) on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian et al., 2003; James et al., 2014) define hypertension as systolic blood pressure (SBP) of ≥140mmHg and/or diastolic blood pressure (DBP) of ≥90 mmHg though there are slight differences in respect of the blood pressure categories:

<table>
<thead>
<tr>
<th>Indian Hypertension Guidelines, (2013)</th>
<th>Seventh and Eighth reports of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (Chobanian et al., 2003; James et al., 2014)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Category</strong></td>
<td><strong>Systolic (mm Hg)</strong></td>
</tr>
<tr>
<td>Optimal</td>
<td>&lt;120</td>
</tr>
<tr>
<td>Normal</td>
<td>&lt;130</td>
</tr>
<tr>
<td>High-normal Hypertension</td>
<td>130-139</td>
</tr>
<tr>
<td>Stage 1</td>
<td>140-159</td>
</tr>
<tr>
<td>Stage 2</td>
<td>160-179</td>
</tr>
<tr>
<td>Stage 3</td>
<td>&gt;180</td>
</tr>
</tbody>
</table>


2.1.2 Diagnosis

Two or more blood pressure measurements at least on two different occasions, one to three weeks apart after the initial screening, should be recorded before diagnosis of hypertension can be made as large spontaneous variation are typical of blood pressure (IHG III, 2013). For each record, an average of three blood pressure readings is taken. If systolic blood pressure is equal to or greater than 140mmHg and/or diastolic blood pressure is more than or equal to 90mmHg, it indicates a state of hypertension. However, the history and physical examination of the patient can assist to discern if there is any secondary cause of hypertension (Simces et al., 2012).

2.1.3 Prevalence

2.1.3.1 Global Level- Hypertension along with its co-morbidities are a primary contributor to mortality worldwide. In 2000, more than one-quarter of the world’s population (~1 billion) had hypertension which has been predicted to increase to 1.56 billion by 2025 (IHG, 2013). In 2010, 9.4 million deaths and 7% disabilities in USA alone were probably from hypertension (Lim et al., 2013) and the World Health Statistics attribute 12.80% of the global deaths to high blood pressure (WHO, 2012). In the developing countries, there is a similar scenario with hypertension becoming one of the leading causes of death and disability.

2.1.3.2 National Level- As a primary non-communicable disease, hypertension accounted for 10% of all deaths in India in 2011 (Patel et al., 2011) while in a cross-sectional study from eight states of India, Joshi et al. (2014) had reported 46% prevalence of hypertension. Rather higher prevalence (28.10-31.50%) of hypertension predominates in urban compared to rural residents of Tamil Nadu, Jharkhand, Chandigarh and Maharashtra (Bhansali et al., 2015). In a meta-analysis of 12 studies from urban India and ten studies from rural India, prevalence of hypertension was 40.80% and 17.90% , respectively (Midha et al., 2013); earlier it had been predicted that by 2025 there would be 213 million hypertensives in India in rural and urban areas (Reddy et al., 2005).On gender differences in a large multi-centric study from all over India, Gupta et al. (2013) had reported higher prevalence (39.10%) in females compared to 33.40% in males.
2.1.3.3 **Regional Levels**- Gupta *et al.* (2004) had reported 51.15% prevalence of hypertension in the urban *Punjabi* population. A study in 2013 the Amritsar district had reported 17.50% hypertensives (Kaur *et al.*, 2013) while Singh *et al.* (2014), have recently reported 35.90% prevalence among the urban *Sikh* population of Amritsar district.

2.1.4 **Clinical Types**

Clinical hypertension may be primary or secondary. Primary (essential) hypertension accounts for 90-95% incidences related to high blood pressure (Dabhade *et al.*, 2014). Though idiopathic, however its association with obesity and family history has been widely acclaimed. On the other hand, secondary hypertension (5-10% of cases) is related to dysfunction of the central nervous system, kidneys, lungs and the vascular and endocrine systems (Chiong *et al.*, 2008).

2.1.5 **Co-morbidities**

In developed countries, hypertension affects more than 25% of the adult population and though most prevalent, yet is a controllable disease (Williams, 2009). However if not appropriately treated, the condition results in high morbidity and mortality due to the development of complications like heart failure, renal disease and cerebral hemorrhage. Other co-morbidities include target-organ damage in the form of left ventricular hypertrophy and renal dysfunction, atherosclerotic disease, diabetes mellitus and the metabolic syndrome (Calhoun *et al.*, 2008; Sowers *et al.*, 2009). Rather, hypertensive patients have two times higher risk for coronary artery disease, four times higher risk for congestive heart failure and seven times higher risk for cerebrovascular disease as compared to normotensive individuals (Stamler, 1991; Flack *et al.*, 1995). In patients with unspecified and malignant hypertension, diabetes was the most common co-morbid condition, while abnormal lipid metabolism was most common in benign hypertension (Davila and Hiaing, 2008). Osteoarthritis and asthma have also been detected in patients with hypertension (Wang *et al.*, 2005). In fact about 80% of hypertensive patients have co-morbidies such as obesity, glucose intolerance, hyperinsulinia, low HDL-cholesterol, high LDL-cholesterol and triglycerides.
Simultaneous occurrence of ≥2 co-morbidities have been documented in about 50% of hypertensive patients (Saha et al., 2006).

2.1.6 Etiology

Essential hypertension is a multifactorial polygenic disease associated with environmental and genetic confounders (Wang and Peng, 2014). Various physiological mechanisms maintain the pressure of the blood in the body and hence any derangement, imbalancing the cardiac output and peripheral resistance can cause hypertension to develop (Beevers et al., 2001).

Changes in the life-style patterns such as sedentary life-style, stress, obesity, low potassium and calcium intake, sodium sensitivity and excessive alcohol intake can lead to development of hypertension (INTERSALT, 1988; Sever and Poulter, 1989; Sagare et al., 2011). Also ageing, genetic predisposition and a family history of hypertension increase the risk for this condition (Tabassum and Ahmad, 2011). The medical conditions of renal artery stenosis, thyroid disorder, chronic kidney disease and sleep disorders can also lead to hypertension.

2.1.7 Risk Factors

Hypertension is a complex trait with genetic and environmental factors playing complex roles in the maintenance of optimal blood pressure levels. Based upon its etiology, hypertension may be primary/essential hypertension (with no visible cause) or secondary hypertension with a definite cause. Almost 90% of cases of hypertension have essential hypertension (Dabhade et al., 2014). Diverse risk factors are associated with development of hypertension; these may be modifiable or non-modifiable.

2.1.7.1 Modifiable Risk Factors- Intervention strategies can modify these risk factors which mainly include excess salt-intake, overweight and obesity, physical inactivity and excess alcohol consumption (Sagare et al., 2011; Singh et al., 2012).

2.1.7.2 Non-modifiable Risk Factors- These are irreversible and cannot be modified; they include age, gender, family history and ethnicity (Abed and Haddaf, 2013).

Among the main modifiable and non-modifiable risk factors which contribute to development of hypertension are life-style, age, gender, family history, ethnicity and obesity. Some salient details of these factors are given below:
Life-style: Life-style patterns contributing to the development of hypertension are alcohol consumption, smoking, physical inactivity and dietary habits. The risk of developing hypertension from alcohol consumption is via stimulation of the sympathetic nervous system; renin-angiotensin-aldosterone system is also altered including cortisol and intra-cellular calcium levels with nitric oxide inhibited and also because of the development of insulin resistance (Ceccanti et al., 2005). With excessive sodium-intake, fluid retention occurs raising blood pressure (Kaur and Khannab, 2012). Reduction in blood pressure through physical activity act by reducing visceral fat, plasma renin and catecholamine activity and sympathetic stimuli; sodium elimination gets improved as well and this increases the parasympathetic tone.

Age: With aging, structural changes of the heart and heart vessels occur which can lead to SBP (Latiffah et al., 2008). Diastolic blood pressure on the other hand, increases upto the age of 50y and then starts to decrease, simultaneously causing pulse pressure to rise with age. Large arterial stiffness and peripheral vascular resistance also are casual factors in causing age-related systolic blood pressure while increased DBP results only from peripheral vascular resistance (Pinto, 2007). Other pathophysiological age-related events in hypertension include baroreceptor sensitivity, increased responsiveness to sympathetic system stimuli, altered renal and sodium metabolism, and altered rennin-aldosterone relationship (Weber et al., 1990).

Gender: The genders are differently affected by gene-environmental interactions. Although expression of autosomal genes does not differ between sexes, yet some genes have a different impact and it is feasible that contribution of genes towards hypertension is different in males and females (Levine et al., 1982). Generally, the blood pressure measurements are higher in males as compared to females of same age. The onset of menopause in females tends to raise blood pressure which may even be more than in males.

Family history: Family history of hypertension is an important predispositional factor (Shirakawa et al., 2006; Hottenga et al., 2005) which may manifest from contribution of genes involved in variable physiological, biochemical and anatomical pathways. In fact the polygenic natures of the conditions influence inter-individual differences in blood
pressure. Due to the complex network of mechanisms that regulate blood pressure, it is possible that gene variants that help to increase blood pressure may have an opposite effect on blood pressure according to the genetic and environmental backgrounds (Barlassina et al., 2002).

**Ethnicity:** Ethnic backgrounds differ because of genetic and cultural differences (Anand, 1999). In hypertension, ethnic differences include life-style, socioeconomic status, family income and education (Holmes et al., 2013).

**Obesity:** General and central obesity are important causal factors of hypertension. Increased cardiovascular risk and hypertension often manifest in those with central obesity (Narkiewicz, 2006). Excessive weight gain related to increased visceral adiposity accounts for 78% and 65% risk of developing hypertension in men and women, respectively (Garrison et al., 1987). The increase in heart rate in obese individuals is from decreased parasympathetic tone, increased sympathetic nervous system activity and activation of renin-angiotensin-aldosterone system (Hall et al., 2007). Also the adipose tissue dysfunction in obesity eventually leads to hypertension (Dorresteijn et al., 2012).

### 2.1.8 Genetics of Hypertension

Familial aggregation studies have reported hypertension heritability to vary from 34-36% on single blood pressure measurements (Levy et al., 2000) to 56-57% depending on long-term blood pressure average (Pilia et al., 2006; VanRijn et al., 2007). In 29 genome-wide association studies (GWAS), meta analysis (including association of 2.5 million SNPs with SBP and DBP) identified only 29 SNPs significantly associated with hypertension out of which 16 were new SNPs (Ehret et al., 2011). The increases in gene mapping studies make it difficult to follow all the candidate genes for hypertension. There were as many as 837 gene variants associated with hypertension as per (Dia et al., 2013) while in the Text-mined Hypertension, Obesity and Diabetes candidate gene database (T-HOD) currently there are 991 genes and 255 candidate gene SNP sites (http://www. bws.iis.sinica.edu.tw/THOD/ accessed on July 27, 2015). The genes selected for the present study *CYP2D6* and *GST*, also are included in this database.
2.1.9 Treatment

Treatment for hypertension is aimed at reducing blood pressure levels to less than 140/90mmHg in hypertensive patients younger than 60y and to less than 150/90mmHg in ≥60y old patients (James et al., 2014). This can circumvent or delay the associated co-morbidities and so protect from organ-damage and consequently improve the quality of life by reducing the overall morbidity risk.

2.1.9.1 Pharmacological Treatment- According to Indian Guidelines on Hypertension (IHG III, 2013), the first line of treatment for management of hypertension recommends five main classes of drugs which include angiotensin converting enzyme inhibitors (ACE inhibitors), angiotensin II receptor blockers, calcium-channel blockers, diuretics and beta-blockers. Depending on a host of factors like age, presence of target organ damage, other co-existing diseases, etc, an appropriate drug is prescribed.

Angiotensin converting enzyme (ACE) inhibitors- These are the drugs that block the production of hormone angiotensin II from angiotensin I by blocking angiotensin converting enzyme. Angiotensin II circulates in the blood and constricts blood vessels, causing the heart to work harder to enable pumping of blood into the main arteries causing the pressure of blood to rise.

ACE inhibitors block the production of angiotensin II, preventing constriction of blood vessels and so lowering of the blood pressure occurs (Sweitzer, 2003). the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme, which stimulates major source of reactive oxygen species (ROS) production, is also limited by ACE inhibitors and hence these inhibitors also provide protection from oxidative stress (Münzel and Keaney, 2001).

Angiotensin II receptor blockers (ARBs)- The block angiotensin II receptors and so angiotensin II cannot bind to its receptor, its effects is inhibited (Terra, 2003) there by reducing blood pressure and oxidative stress. ARBs are prescribed for hypertension, cardiovascular and renal diseases.

Calcium-Channel Blockers (CCBs) or antagonists- These invoke vasodilation and so inhibit the entry of calcium ions via voltage-dependent Ca^{2+} channels causing calcium
levels to be reduced thereby decreasing contraction and results in lowering blood pressure (Dustan, 1989).

**Diuretics**- Diuretics decrease sodium reabsorption by facilitating the absorption of sodium from distal tubule back to the interstitium. This results in an increased fluid loss through urine causing extracellular fluid to decrease and renin release increases, cardiac-output is decreased and hence, blood pressure gets lowered (Conway and Lauwers, 1960).

**Beta (β) –blockers**- Beta-1-Adrenergic receptors are present in heart, blood vessels and lungs and these are stimulated by neurotransmitters (catecholamines-epinephrine and nor-epinephrine) released from nerve endings of sympathetic nervous system. The neurotransmitters help the body to bear stress and anxiety. Stimulation of beta-1 adrenergic receptors by catecholamines causes the G-protein to couple with adenyl cyclase and cAMP gets generated. The cAMP in turn activates protein kinase A, which increases the calcium ion entry into the cytosol by phosphorylation of membrane calcium channels. The increased calcium concentration results in positive inotrophic and chronotropic effects (Opie and Yusuf, 2008) that lead to increased heart rate, blood pressure and heart muscle contraction. The beta-blocker drugs block the beta-1 adrenergic receptors and present their binding to excess of catecholamines in sino-arterial node of heart and blood vessels and hence blood pressure reduction occurs (Frishman, 2003). However as per the British Hypertension Society Guidelines, beta-blockers have been demoted from first-line to fourth-line drugs (BHS, 2011) as these do not protect against stroke which other drugs provide. Some commonly used beta-blocker drugs include Acebutolol, Atenolol, Bisoprolol, Carvedilol, Celiprolol, Labetalol, Metoprolol, Nadolol, Nebivolol, Oxprenolol, Pindolol and Timolol.

2.1.9.1.1 Atenolol- As the patients in the present study were on Atenolol (monodrug) therapy, details of this drug are given below:

**Atenolol (CAS 29122-68-7) (4-[2 – hydroxy – 3 –[(1- methyl ethyl) amino] propoxy])**- Atenolol is a cardio-selective beta-blocker molecule with molecular weight of 266.34 daltons and it is an organic compound (phenyl acetamide) a derivative of
phenyl acetic acid and having chemical formula of: \( \text{C}_{14}\text{H}_{22}\text{N}_{2}\text{O}_3 \) (http://www.Atenolol Drug Bank accessed on May 20, 2015) With the following chemical structure:

![Chemical structure of atenolol](image)

It is a polar hydrophilic compound which gets mainly excreted by the kidneys. A daily dose of 25-50mg is generally prescribed (Karaman et al., 2014) and only 50-60% is absorbed reaching the to systematic circulation while 6-16% of the absorbed dose is plasma present-bound being retained for consistent drug levels. The peak blood levels of atenolol reaches within 2-4h after drug-intake and its elimination half-life if of 6-7h (Wander et al., 2009).

**2.2 Literature**

In this section, literature in the light of study objectives is reviewed viz. on obesity, oxidative stress, genetic damage, atenolol genotoxicity and molecular genetic markers as related to hypertension.

**2.2.1 Obesity and Hypertension**

As obesity assessment is one of the objectives of the present study, literature for the past six years (2009 onwards) relating hypertension to obesity is reviewed here under:

Sanya *et al.* (2009) investigated the relationship between adiposity indices of body mass index (BMI) and waist hip ratio (WHR) with hypertension in 404 individuals (15-85y; 204 males, 200 females) staying in Ibadan, Nigeria. A significant linear relationship between BMI and WHR with blood pressure of participants was observed with hypertensive patients having significantly \((p<0.05)\) higher BMI and WHR values as compared to the values in normotensive participants.
Studies on relationship of overall obesity and abdominal adiposity (WHR) with blood pressure in non-treated 303 Indian males and 357 females (Reddy et al., 2010) revealed that average SPB and DBP increased linearly with increase in BMI and WHR. However, there were some gender differences as BMI and WHR were positively associated with blood pressure in males, but in females only BMI showed positive association, a consistent linear relationship between adiposity and blood pressure was also observed.

In a cross-sectional study, Khalesi and co-workers (2012) documented a significant positive correlation of SBP and DBP with BMI, WC (waist circumference) and WHR in 183 hypertensive primigravidae from Rasht (Iran). BMI, WC and WHR emerged as important indicators of hypertension on step-wise regression analysis while logistic regression analysis revealed WC as the most important anthropometric variable associated with risk of hypertension.

In 197,191 children (7-17y) whose data were included in a Chinese National Survey in 2010, Bin et al. (2013) observed prevalence of high blood pressure to be significantly more in overweight and obese children in each group. Hypertension and diabetes tendency showed increase with increase in BMI in another study on 25-70y old 163 subjects (Singh and Christina, 2013).

A study from Amritsar district (Punjab) by Kaur et al. (2013) showed that BMI was significantly associated with both, SBP and DBP in males and in females while WHR was significantly associated with only DBP in both, males and females.

Among 682 school-going children aged 12-18y from Surat, Shah et al. (2013) reported that gender, obesity and family history showed positive association with hypertension.

In 8,543 African-Americans, Hispanics or white non-hispanics, there was observed a higher risk for development of hypertension in all sexes and all racial/ethnic groups who became obese in adulthood (Sugalia et al., 2013). An overall increased risk of hypertension for those who gained weight in adulthood was also reported. However, this risk was not there for those who maintained normal weight.

Physical fitness, BMI and WHR were studied for relationship with high blood pressure in 231 hypertensive patients (138 males and 93 females) aged 60-80 years, living in
their own houses in central, eastern and southern Serbia (Pantelic et al., 2013). A statistically significant correlation was observed between BMI, WHR and hypertension (p<0.05). The authors concluded that overweight was an important factor that contributed to high blood pressure.

On relationship of obesity and hypertension in 130 Nigerian hypertensive patients, Oni et al. (2014) observed a negative correlation of BMI with SBP and with DBP; the authors concluded that obesity was not consistently associated with poor control of hypertension.

### 2.2.2 Oxidative Stress and DNA Damage

There exists a cause-effect relationship between hypertension and increased oxidative stress and further it is known that oxidative stress can and does manifest into genetic damage (Grossman, 2008). In the normal state also the physiological, metabolic and other biochemical reactions release reactive oxygen species (ROS). These have important physiological functions, yet because of their reactive nature there is a tendency for them to cause oxidative damage to the lipids of the cell membrane, to proteins and to DNA if these are in excess (Kehrer et al., 1993).

Despite homeostatic mechanisms maintaining a balance between endogenous oxidants and antioxidant defenses (Scandalios et al., 1997), imbalance occurs between the levels of oxidative stress and antioxidants during the hypertensive state (McIntyre et al., 1999). Interaction of ROS with nitrogenous bases of DNA strands can cause oxidative DNA damage via base or sugar modifications, covalent cross-links or single- and double-strand DNA breaks (Loft et al., 1996). This effect may further be compounded by the available treatment modalities for the disease which may potentiate/reduce oxidative stress or directly induce a genotoxic response.

Hereunder, literature of the last decade on oxidative imbalance and genetic damage in hypertensive patients is reviewed:

### 2.2.3 Oxidative Imbalance

The antioxidant status as observed from a study carried out in 50 hypertensive patients compared to levels in 50 normal subjects by Kashyup et al. (2005) revealed that levels
of antioxidants (SOD, glutathione peroxidase (GPx), reduced glutathione, total glutathione, oxidized glutathione, total thiols, non-protein thiols, reactive nitrogen intermediates, total antioxidant power, vitamin A, ascorbic acid and glutathione S-transferase) were significantly decreased in the hypertensive individuals. A significant decrease in nitric oxide levels and ferric-reducing activity of plasma (FRAP) were also observed in hypertensive patients. The differences revealed reduced levels of antioxidants in hypertensive patients as compared to normal control individuals. The levels of total cholesterol, LDL-C, malondialdehyde (MDA), VLDL-C, uric acid and plasma homocysteine were also observed to be significantly increased in hypertension.

Lee et al. (2005) had observed about three times higher levels of DNA damage as 8-hydroxy-2’-deoxyguanosine (8-OH-dG), caused by reactive oxygen species in hypertensive patients (n=38) compared to levels in normal controls. However on treatment for two months with carvedilol (a drug with known antioxidant effects), there was significantly reduced DNA damage in hypertensive patients.

Serum malondialdehyde (MDA) levels were significantly increased and of superoxide dismutase activity significantly decreased in essential hypertensive patients (n=50) compared to conditions in age-and sex-matched control individuals (n=20) (Tandon et al., 2005). The authors concluded that adequate control of blood pressure, levels of oxidative stress and antioxidant status could be improved with antioxidant therapy.

Nwanjo et al. (2007) had evaluated the levels of oxidative stress in the form of malondialdehyde and non-enzymatic antioxidant activity in the form of vitamin E (α-tocopherol), vitamin C (ascorbic acid) and reduced glutathione (GSH). They reported that MDA levels were significantly higher while non-enzymatic antioxidant markers were significantly reduced in 150 hypertensive patients compared to levels in 120 healthy normal controls implying that hypertension is associated with increased oxidative stress and decreased antioxidant capacity.

In a cross-sectional study including 31 hypertensive patients and 35 normal healthy individuals (Rodrigo et al., 2007), oxidative stress-related parameters of ferric-reducing ability of plasma (FRAP) and 8-Isoprostane were revealed as significantly (p<0.001) higher in patients, while erythrocyte antioxidant enzyme status in the form of reduced
glutathione (GSH), oxidized glutathione (GSSG), superoxide dismutase, catalase (CAT) and glutathione peroxidase (GSH-Px) were significantly (p<0.001) reduced. A strong association was observed between blood pressure levels and the oxidative stress parameters of plasma and urine 8-Isoprostane.

In a similar study, Khanna et al. (2008) observed that oxidative stress levels in the form of malondialdehyde were significantly increased and antioxidant activity of enzymes, superoxide dismutase, glutathione, and non-enzymatic antioxidant levels (vitamin E, vitamin C) were significantly reduced in hypertensive patients (n=50) compared to levels in normal controls (n=20). On treatment with antihypertensive medication (50mg atenolol with 12.5mg hydrochlorothiozide for 3 months), there was a reversal in their levels.

Plasma thiobarbituric acid reactive substances (PTBARS) and lipid parameters (total cholesterol, triglycerides, LDL-C, VLDL-C) levels were also significantly higher in the hypertensive patients group (n=32) as compared to those in a normal healthy control group (n=30) as observed by Maharjan et al. (2008). The authors suggested that as oxidative stress was significantly increased in the hypertensive state, maintaining optimal oxidative stress levels in hypertensive patients would be helpful in preventing the occurrence of diseases related to hypertension.

On investigating the levels of MDA, GSH, SOD, CAT and GPx in treated, untreated and border-line arterial hypertensive and normal control group individuals, Paduraru et al. (2008) reported that CAT and GPx activities were reduced in the untreated hypertensive patient group while SOD and CAT activities were lower in bordeline hypertensive patient group compared to levels in the control group. After treatment with atorvastatin (enzyme HMG-CoA reductase inhibitor) and captopril, the decreased antioxidant activity in both the groups was normalized.

Increased systemic inflammation as c-reactive protein concentration (hs-CRP) and decreased total antioxidant status (TAS) in essential arterial hypertension (n=72) patients compared to levels in a normal control group (n=72) were also documented by Kuklinska et al. (2009).
In a group (32-66y) comprising 70 hypertensive patients and 86 normotensive individuals, Biswas and Kumar (2010) reported that levels of malondialdehyde and carbonic anhydrase activity were significantly higher in the hypertensive group and so concluded that there is increased oxidative stress in hypertensive patients.

Redox imbalance was evaluated by Vasconcelos et al. (2011) by investigating levels of SOD, CAT, GPx, GSH, vitamin C, transferrin, ceruloplasmin, and carbonyl groups in 20 hypertensive individuals and 21 normal controls. The levels of GPx and ceruloplasmin were significantly higher and of CAT, GSH and MDA were significantly reduced in the patient group implying redox imbalance in the hypertensive patients.

There was decreased activity of superoxide dismutase, glutathione and glutathione peroxidase with significantly increased MDA levels in hypertensive patients compared to those in normal controls indicating increased oxidative stress and decreased antioxidant enzyme activity in the hypertensive group (Amanullah et al., 2012).

Biomarkers of oxidative stress (MDA, TAC, NO, ADMA, 8-OHdH, NT and CoQ10) in serum samples of 18 dipper-hypertensive, 20 non-dipper hypertensives and 22 normal healthy controls were assessed by Gonenc et al. (2012). In both, dipper and non-dipper hypertensive patients compared to normals, levels of MDA were significantly increased and of TAC significantly decreased. On the other hand, ADMA and NT levels were higher and of CoQ10 decreased in non-dipper hypertensives compared to those in the control group. The authors concluded that oxidative stress was significantly higher in hypertensive patients (dipper and non-dipper) compared to that in controls.

Levels of catalase activity, serum malondialdehyde, total bilirubin, uric acid and plasma vitamin C –levels in hypertensive patients (n=71) compared to those in normal healthy controls(n=40) were assessed by Mera and Marcus (2012).Three sub-groups of hypertensive patients included Group B (SBP/DBP 140-159/90-99mmHg), Group C (SBP/DBP 160-179/100-109mmHg), and Group D (SBP/DBP >180/110mmHg) while controls were placed in Group A. Catalase activity significantly decreased with increase in blood pressure through Groups A to D while MDA levels were significantly increased. Bilirubin levels increased and hemoglobin levels decreased through groups B to D. The vitamin C levels were significantly decreased in group C to B in comparison
to levels in group A. These findings indicated endothelial dysfunctioning in hypertensive patients which could lead to atherosclerosis and cardiovascular diseases.

In 96 hypertensive cases sub-categorized on the basis of severity of hypertension and 96 healthy normotensive controls, there was observed significantly higher serum oxidant load and significantly reduced serum antioxidants (FRAP assay) in all the patient categories as compared to controls (Padhy et al., 2012). A significant positive association was observed between SBP, DBP and serum oxidant load, while negative association was observed for SBP, DBP with serum antioxidant capacity demonstrating a strong possible role of oxidative stress in inducing the severe hypertensive state.

2.2.4 Genetic Damage

All the available studies in literature investing genetic damage in hypertension are reviewed here:

In a very earlier study, Pero et al. (1976) found a greater potential of hypertensive patients for accumulating DNA damage and their study-results revealed that N-acetoxy-2-acetylaminofluorene (NA-AAF)-induced chromosomal damage was linearly related to diastolic blood pressure.

Levels of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was significantly higher in untreated hypertensive patients aged 46-58y as compared to controls in a study from Tanzania (Hiroko et al., 2001).

DNA damage (presented as Arbitrary units) was significantly (p<0.001) higher in white-coat (1.54X) and sustained (3.41X) hypertensive patients compared to controls (Yilidz et al., 2008). The total antioxidant status (TAS) was however significantly decreased in both the patient groups compared to levels in the control group. However, sustained hypertensive patients had significantly highest DNA damage and minimum TAS levels.

Subash and co-workers (2010a) reported a significant increase in urinary 8-OHdG levels in both, newly diagnosed untreated hypertensive (n=30) and treated hypertensive (n=75) patients, as compared to normal control (n=75) South Indian subjects. There was significant reduction in 8-OHdG levels after one year antihypertensive treatment. while
the level of total antioxidant status (TAS) was significantly decreased in both the patient groups compared to the levels in normotensive controls.

In a another study, Subhas et al., 2010(b) investigated 30 newly diagnosed hypertensive patients, 50 patients on antihypertensive treatment and 50 normotensive control individuals for DNA damage (Damage Index (DI) by SCGE assay) and TAS levels. Their results revealed significantly (p≤0.05) higher DI in both the newly diagnosed and treated hypertensive patients as compared to controls; between the patient groups the level of DNA damage was significantly higher in the newly diagnosed patients. TAS levels were also significantly reduced in both the patient groups compared to controls. A significant association of DNA damage was only observed with TAS.

Gandhi and Jyoti (2010) using the alkaline comet assay, reported that DNA damage was statistically increased in treated hypertensive (n=35) patients compared to normotensive controls (n=15) in comparing the parameters of cells with tail and DNA migration length between the two groups. Multiple regression analysis and analysis of variance further revealed association of DNA damage with systolic and diastolic blood pressure levels.

Kiykim and co-workers (2010) evaluated the levels of oxidative DNA damage in the form of 8-OHdG in newly diagnosed untreated hypertensive patients (n=49) and age- and sex-matched normal control individuals (n=20). The results revealed that levels of 8-OHdG were significantly higher in hypertensive patients as compared to normal controls. Investigation of 8-OHdG after treatment with olmesarten (20-40mg/day) for 4 weeks revealed a significant reduction in 8-OHdG levels; however the levels were still higher than in the normal controls.

In a case-control study, Kaur et al. (2011) had documented significantly reduced levels of total antioxidant capacity (TAC) in treated hypertensive patients as compared to levels in normal controls. Oxidative stress index and DNA damage (using alkaline single cell gel electrophoresis assay) were also significantly higher in the patients and all the DNA damage parameters also showed correlation with systolic blood pressure.

In atenolol-- treated hypertensive patients (n=22) and healthy controls (n=10) from Punjab, Thukral and Gandhi (2012) had reported that percent DNA in tail and MDA
levels were significantly higher in the patients and suggested that the observed genomic instability could be a consequence of oxidative stress.

Thukral et al. (2012) in Baniya and Jat Sikh sub-population groups had also observed that DNA damage (DNA migration length, damage frequency and damage index) in hypertensive patients (n=44) was significantly higher compared to the respective levels in normal healthy individuals (n=28). Dyslipidemia was also significantly more in these patients.

In another Punjabi population group comprising 75 hypertensive patients on atenolol-therapy and 25 normal age- and sex- matched controls, Kaur and Gandhi (2013) have reported that leukocytic DNA damage observed using the alkaline single cell gel electrophoresis (SCGE) assay was significantly higher in the hypertensive group.

In a preliminary study, on assessment of oxidative DNA damage using the modified enzymatic comet assay as well as the oxidant status, lipid profile, total antioxidant capacity and malondialdehyde levels in essential hypertensive patients on atenolol-therapy and normotensive controls belonging to the Jat Sikh Punjabi population subgroup, Kaur and Gandhi (2014) reported a significant increase in oxidative DNA damage, oxidative stress and malondialdehyde levels in the hypertensive patients. The lipid profile of the patients was also altered as levels of total cholesterol, LDL-C, VLDL-C and triglycerides were significantly raised and of HDL-C and antioxidant capacity were significantly reduced.

2.2.5 Atenolol and Genotoxicity

The studies in literature on the genetic damaging effects of Atenolol are all reviewed:

On perusing the effect of five beta-adrenoreceptor blocking agents (propranolol, practolol, pronethanol, acebutolol, atenolol) on activity of various rat liver enzymes (microsomal biphenyl-2-hydroxylase, ethoxyresorufin de-ethylase and many mixed function oxidase enzymes), Ioannides et al. (1979) administered male rats with single daily doses (5 mg, 50 mg and 150 mg kg\(^{-1}\) day\(^{-1}\)) of these drugs for three days. In liver microsomes, the normal therapeutic dose (5mg/kg) of none of the beta-adrenergic blocking agents (including atenolol) showed any inductive effect.
Alkaline sucrose gradient analysis of four beta-blockers including DL-1-(2-nitro-3-methyl-phenoxy)-3-test-butylamino-propan-02-ol, DL-1-(2-nitro-5-methyl-phenoxy)-3-tert-butylamino-propan-2-ol, DL-propranolol and DL-atenolol by Presta et al. (1983) revealed that three Beta-blockers including DL-propranolol caused dose-dependent DNA fragmentation in vitro to nuclei isolated from livers of male and female Wistar rats while DL-atenolol did not. Similarly, in vivo administration of DL-propranolol caused DNA damage in liver cells of females Wistar rats but no damage was observed on DL-atenolol treatment.

Okine and co-workers (1983a) had observed that a 5-day treatment with atenolol, practolol, pronethanol and propranolol at a dose of 150 mg/kg/day induced marked increase in guanylate cyclase activity in the liver, gastric and intestinal mucosae of rats. An association with decrease in cyclic guanosine monophosphate (GMP) levels was also observed. However as the ratio of cAMP/cGMP did alter in any of the tissues, it was hypothesized that any tissues, it was hypothesized that any oncogenicity of beta-blocking agents was not due to their pharmacological action.

In an another study by Okini and co-workers (1983b), the mutagenic potential of nine beta-adrenergic blocking agents including atenolol was evaluated by the Ames and micronucleus tests. The authors concluded that there was lack of mutagenic or carcinogenic potential associated with beta-adrenergic blocking drug activity.

Bandyopadhyay et al. (1990) studied the cytotoxic interactions in the form of lactate dehydrogenase release of cardio-active cationic amphiphilic compounds such as propranolol, verapamil, sotalol, atenolol and procainamide in rat hepatocytes. It was observed that sotalol, atenolol and procainamide failed to induce ultra-structural changes in hepatocytes after 24 h of incubation even up to the concentration of 400 µmol/L.

The formation of N-nitroso derivatives of six beta-adrenergic–blocking agents (atenolol, metoprolol, nadolol, oxprenolol, propranolol and sotalol) and their genotoxic effects in rat and human hepatocytes were assessed by Rabbiano et al. (1991). The N-nitroso derivatives of atenolol and metoprolol were not toxic even at the highest soluble concentration of 1mM to both, rat and human hepatocytes, and in fact the same results were obtained with parent beta-blocker drugs of atenolol and metoprolol.
Dysmorphogenesis induced by median concentrations of propranolol, alprenolol, metoprolol, pindolol, acebutolol and atenolol in 50% of rat embryos (EC50) was observed by Klug et al. (1994). Propranolol and metoprolol showed higher interfering potency to normal development of the embryo as compared to atenolol.

The N-nitrosoderivatives formed by in vitro reaction of five beta-blocking drugs (propranolol, metoprolol, nadolol, atenolol and sotalol) with sodium nitrite were tested for their ability to cause clastogenic effect in vivo on partially hepatectomized rats given 100 mg/kg dose of the five nitroso-derivatives by gavage (Martelli et al., 1994). Statistically significant increased frequency of micronucleated hepatocytes was observed but not in the bone marrow or the spleen. The authors suggested that there probably occurred in vivo detoxification of the five n-nitroso derivatives.

Zavanella et al. (1994) studied the tumor-promoting activities of propranolol and atenolol in two-stage protocol of hepato-carcinogenesis in male and female 344 Fischer rats and documented that spontaneous pre-neoplastic or neoplastic lesions were not initiated by propranolol or atenolol. Liver tumor-promotion in DEN-initiated rats given atenolol also did not occur.

Genotoxicity of atenolol, both in vivo and in vitro in the form of ability to induce sister chromatid exchanges (SCE) and micronuclei (MN) in cultured peripheral blood lymphocytes (PBL) was investigated by Telez et al. (2000). Induction of MN was also studied by fluorescence in situ hybridization (FISH) with centromeric probe. In vivo treatment comprised patients on antihypertensive drug atenolol and the in vitro assessment was carried out on PBL of controls by adding atenolol in culture medium in a concentration similar to that present in plasma. The SCE frequency did not differ between groups but frequency of MN was significantly higher in patients as compared to controls, both in vivo and in vitro besides exhibiting inter-individual variability in drug-sensitivity. It was suggested that chronic exposure to atenolol resulted mainly in the loss of chromosomes and that atenolol probably acted as an aneugenic.

Tabacova et al. (2003) observed that intrauterine growth retardation in humans from prenatal toxicity of atenolol at lower doses was more as compared to that in rats and rabbits.
In 2009, Amin studied the effect of atenolol-treatment on 30 hypertensive patients and reported that atenolol-treatment was associated with increased levels of serum creatine-kinase MB, being directly proportional to duration of the drug usage (Amin, 2009). As creatine-kinase MB is one of cardiac markers that is released from the cardiac muscles when they are damaged due to infarction, the author proposed that a direct correlation of atenolol with disease development (myocardial infarction) could occur.

Telez et al. in 2010 in continuity to their previous work; Telez et al., (2000) studied the genotoxicity of atenolol in human peripheral blood lymphocytes by observing chromosomal aberrations and expression of fragile sites (FS) in 11 hypertensive patients and nine normal controls. Results revealed that atenolol induced structural chromosomal aberrations but no cytotoxic effects on the basis of mitotic index.

The effect of atenolol on Allium cepa was studied (Jangala et al., 2012) by giving four treatments of 10 µg/ml atenolol (two spurt treatments of 2h, 3h and 17h to the root meristems of A. cepa). All the four treatments induced aberrations such as metaphase-anaphase disturbances, induced chromosomal breaks, bridges, stickiness, micronuclei, polyploidy, chromosomal lagging and condensation.

Rocco and co-workers (2012) studied the in vitro effects of nine pharmacological compounds (atorvastatin, sildenafil citrate, gemfibrozil, ibuprofen, atenolol, ofloxacin, carbamazepine, bezafibrate and diclofenac) present in waste-water of Italian-treatment plants on 40 semen samples using the comet assay, diffusion assay, TUNEL test and RAPD-PCR techniques. Statistically significant reduction in sperm DNA integrity and increased fragmentation values were induced by all the nine compounds.

The genotoxic effect of amlodipine, atenolol and captopril on alkaline phosphatase and on genomic DNA of kidney, liver and spleen cells of 150 Swiss albino mice was investigated by Claude et al. (2013) using the RAPD-PCR technique. Alkaline phosphatase activity was increased in the liver, spleen and kidney cells by all the three drugs while DNA damage was induced by amlodipine alone in liver, spleen and kidney cells Atenolol and captopril however did not induce DNA damage.

The effect of atenolol on membrane fatty acid saturation and oxidative stress in heart and skeletal muscle mitochondria of 134 mice was studied by Gomez et al. (2014).
Atenolol-treatment reduced visceral adiposity, decreased mitochondria oxidation, glyoxidation and lipoxidation in both, cardiac and skeletal muscles. Oxidative DNA damage in cardiac muscles was also reduced as atenolol treatment increased the amount of the extracellular-signal-regulated kinase signaling protein and decreased either membrane fatty acid saturation or oxidative stress in cardiac and skeletal muscles.

2.2.6 Selection of Genetic Polymorphism

Cytochrome P450 (CYP450) and glutathione-S-transferase (GST) are two important genes encoding enzymes for drug- and xenobiotic-metabolism. Variations in these genes have been known to modify an individual’s susceptibility to disease and/or any genotoxicity from drug therapy.

In the present study, the polymorphisms of CYP2D6 *2, *4, *10 and GST T1, P1, M1 genes were chosen on the basis of their functional relevance, either with blood pressure regulation antihypertensive drug response/oxidative stress-control metabolism. The CYP2D6 *2, *4, *10 polymorphisms is also associated with differential blood pressure response to different antihypertensive drugs, especially beta-blockers (Yuan et al., 2008; Ayyappadhas et al., 2015) as the patients in the present study were on monodrug beta-blocker (atenolol) therapy.

Among phase-II enzymes, GST are among the most important detoxifying enzymes and GST T1, P1 and M1 were selected for the present study as null genotypes of GST T1 and M1 had shown association with hypertension (Onike et al., 2008; Eslami and Sahebkar, 2014) and as polymorphisms in GST genes play an important role in xenobiotic metabolism (http://www.ncbi.nlm.nih.gov/gene/2950 accessed on July 11, 2014).

Therefore the literature available on investigations of CYP2D6 and GST gene polymorphisms in hypertensive patients from 2006 onwards is reviewed here under:

2.2.7 Hypertension and CYP2D6 Gene Polymorphisms

The clinical response to nebivolol treatment (Lefebvre et al., 2006) in 218 hypertensive patients increased plasma concentrations of D-nebivolol (ten folds) and of L-nebivolol (15 folds) in poor metabolizers (PM) compared to levels in extensive metabolizers
However, the blood pressure response to nebivolol was comparable in EM and PM hypertensive patients.

Zateyshchikov et al. (2007) investigated the association between CYP2D6 and ADRB1 gene polymorphisms with clinical response to betaxolol (a beta-1 adrenergic blocker) in 81 essential hypertensive patients and reported significant association between Pro34Ser variant of CYP2D6 and response to betaxolol therapy. A significant decline in SBP was observed in Pro34 allele carrier patients and the authors suggested that the effect of the drug on heart rate and blood pressure significantly depended upon variability in genes involved in drug metabolism.

In 125 mild-to-moderate Chinese hypertensive patients, Liu and coworkers (2007) observed that CYP2D6*10 significantly altered the pharmacokinetics and ADRB-1 (Ser49Gly and Gly389Arg) changed the pharmacodynamics of metoprolol antihypertensive therapy. In gene-dose effect manner, levels of metoprolol were associated with CYP2D6*10 variant and ADRB-1 carriers of Gly49 and Arg389 had stronger decrease in blood pressure levels.

In another study from China on 300 essential hypertensive patients, the same dose of metoprolol caused different therapeutic effects in patients with different CYP2D6 (*2, *5, *10) and ADRB-1 gene polymorphisms. Reduction in systolic blood pressure was significantly different between intermediate and extensive metabolizers and between poor and extensive metabolizers (Yuan et al., 2008), being maximum in intermediate metabolizers followed by that in poor metabolizers while extensive metabolizers had least reduction in SBP.

The association between CYP2C19*2 (681G>A) and *3 (636G>A) genetic polymorphisms with development of essential hypertension in 527 hypertensive subjects and 663 unrelated healthy controls was investigated by Shin et al. (2012). The CYP2C19*3 (636 A) allele showed association while CYP219*3 (636G) provided protection against hypertension.

The CYP2D6 (G1934A) and CYP2C9 (A1075C, C430T) allelic variants were in increased frequency in hypertensive patients in the study by Borodulin et al. (2012).
On genotyping $CYP2D6$ (*1, *4) in 123 hypertensive and 429 healthy individuals from Egypt, Ali et al. (2013) reported higher frequency of wild 1/1 genotype and lower frequency of 4/4 mutant genotype in hypertensive cases.

Meta-analysis by Blake et al. (2013) on relationship between pharmacokinetics of metoprolol and $CYP2D6$ metabolizer phenotypes revealed genotype-dependent metabolism of metoprolol. There were significant differences for plasma metoprolol concentration, area under the concentration time curve and elimination half-period for extensive, poor and ultra-rapid metabolizers.

The $CYP2D6$ phenotypes and genotypes of 49 hypertensive patients on metoprolol-therapy were studied by Duricova et al. (2013). Patients with lower enzymatic activity had normal serum metaprolol concentration and the resting heart rate was also lower before metoprolol- intake. It was concluded that both, genotype and phenotype, are important to optimize metoprolol therapy.

In a study from South India (Kumar et al., 2013) on 279 hypertensive patients and 321 controls, no association of $CYP2C8^*2$, $CYP2C8^*3$, $CYP2C9^*2$ and $CYP2J2^*7$ polymorphisms with hypertension was observed. The metoprolol response to hypertension was also not affected by $CYP2D6^*4$ gene variants in an investigation by Ayyappadhas et al. (2014).

Recently, Wu et al. (2015) also did not observe any association of $CYP2D6^*10$ (100C>T) and of ADRB1 (1165G>C) with response to metoprololin 93 hypertensive patients. However, the CC variant of ADRB1 had better treatment outcome with metoprolol for hypertension.

### 2.2.8 Hypertension and GST Gene Polymorphism

In the last decade from 2007 onwards, 16 studies have documented investigations on association of GST gene variants and the state of hypertension. These studies are briefly reviewed here:

On genotyping hypertensive patients with congestive heart failure (n=94) and 204 healthy unrelated Portuguese for $GSTT1/M1$ and $MTHFR$, Marhino et al. (2007) documented that $GSTT1$ null genotype may have an important role in the protection
against hypertension. The *GSTT1* non-null genotype results in the formation of glutathione conjugates leading to GSH-depletion and so hydrogen sulphide levels are decreased that worsen hypertension pathology.

The association of *GSTA1*, *GSTM1* and *GSTT1* polymorphisms with incidence of hypertension was investigated by Oniki *et al.* (2008) in a health-screening programme. Hypertensive patients had significantly higher frequency of *GSTA1B* allele carriers with risk of hypertension significantly increased in those with *GSTA1B* and *GSTM1* null and *GSTT1* null genotypes.

Unrelated Han adult males (n=197) were investigated by Lin *et al.* (2009) for *GSTP1* gene polymorphism and its association with heart rate and blood pressure. The study failed to find any significant association.

Relationship between *GSTM1* genotype and resistant hypertension was studied by Gonzalez *et al.* (2009) in 49 patients of resistant hypertension, 232 patients with controlled hypertension and 110 healthy participants. Resistant hypertensive patients had 2-fold higher frequency of *GSTM1* null genotypes than in those with controlled hypertension.

On studying 91 hypertensive patients and 110 normal controls, Miranda-Vilela *et al.* (2010) in Brazil studied whether haptoglobin, *ACE*, *GSTM1* and *GSTT1*, *MnSOD* (Val9Ala), *CAT* (-21A/T) and *GPx* (Pro198Leu) genetic variants showed association with hypertension. The investigation revealed that only *Hp1*-1 and *MnSOD* Val/Ala genotypes showed significant association with hypertension.

In a Korean study comprising 227 newly diagnosed, untreated, subclinical hypertensive patients and 130 normal controls, Han *et al.* (2011) reported higher frequency of *GSTM1* null genotype and also of both, *GSTM1* and *GSTT1* null genotypes. It was proposed that the *GSTM1* null genotype could be a potential genetic factor for prediction of sub-clinical hypertension.

In an Italian group comprising 193 essential hypertensive patients and 210 healthy controls (Polimanti *et al.*, 2011), possible association of *GST* gene polymorphisms (*GSTA1, GSTM1, GSTO1, GSTO2, GSTP1* and *GSTT1*) and essential hypertension was
investigated. A significant association of only \textit{GSTT1} null genotype with increased risk for hypertension was revealed and the risk was significantly higher in female patients.

In 755 lead-exposed male workers with lead-induced hypertension and their relationship with blood lead levels, Lee \textit{et al.} (2012) documented significant association of disease with \textit{GSTT1} positive allele.

\textit{GSTM1} and \textit{GSTT1} gene polymorphisms however showed no association with disease in 30 hypertensive patients and 33 healthy controls in a study in UAE carried out by Hussain \textit{et al.} (2012).

In the Chinese Han population, 315 patients with ischemic stroke and 210 healthy controls were studied for \textit{GSTM1 and GSTT1} gene polymorphisms (Wang \textit{et al.}, 2012). Both, \textit{GSTT1} and \textit{GSTM1} null genotypes showed association with increased risk for stroke.

In hypertensive patients with \textit{GSTT1} and \textit{GSTM1} null genotypes (\textit{GSTM1+/GSTT1-}, \textit{GSTM1+}, \textit{GSTT1-}) as compared to non-null genotypes (\textit{GSTM1+/GSTT1+}), MDA levels were significantly higher (Dhameja \textit{et al.}, 2013). Also though \textit{GST} and FRAP levels decreased in those with null genotypes, the levels were statistically non-significant.

Eslami and Sahebkar (2014) performed a meta analysis of 12 published studies relating \textit{GST} polymorphism and hypertension and concluded that there were significant association for risk of hypertension with \textit{GSTM1} and \textit{GSTT1} null genotypes.

In 72 healthy individuals, Rafee \textit{et al.} (2014) observed significant effect of \textit{GSTT1} and \textit{GSTM1} genotypes on mean arterial pressure (MAP). The authors concluded that \textit{GST T1} and \textit{GST M1} are candidate genes that modify blood pressure.

Abbas \textit{et al.} (2014) investigated the association of \textit{ACE}, \textit{FABP2} and \textit{GST} gene polymorphisms with EH-risk in 138 essential hypertension and 116 sex-, age and ethnicity-matched controls from North India. Significant differences between patients and controls were observed for the frequencies of \textit{ACE}, \textit{GSTT1} null, \textit{GSTM1} non-null and \textit{FABP2} (Ala54/Ala54) genotypes implying their association with risk of hypertension.
Ge et al. (2015) on performing meta-analysis comprising 13 case-control studies on GSTT1 and 14 on GSTM1 gene polymorphisms for increased risk for hypertension, observed a statistically non-significant association between risk for hypertension and the GSTT1, GSTM1 null genotypes.

Han et al. (2015) investigated the effect of GSTM1 and GSTT1 polymorphisms on blood pressure, blood sugar and lipid profile following supplementation with Brassica oleracea acedphala (Kale) in 84 hypertensive patients from South Korea. Significant association of GSTT1 polymorphism on reduction of blood pressure after Kale juice supplement was observed. The levels of SBP were significantly lesser by 4.4% and DBP by 3.6% in the GSTT1 null genotype as compared to respective decrease of 6.0% and 4.2% in those having the GSTT1 present genotype.

2.2.9 Genetic Polymorphism and Susceptibility to Genetic Damage

Studies on the genetic polymorphism of GST and CYP2D6 genes in persons with exposure at workplace and/or smoking habits or with disease assessed for genetic damage are presented here:

DNA damage (Olive tail moment) in response to AhR, CYP1A1, GSTM1 and GSTT1 genetic polymorphisms in 240 coke-oven was observed to be significantly higher than in 123 non coke-oven workers (Chen et al., 2001). Among coke-oven workers, those with AhR Arg554/Arg554 genotype had higher DNA damage while in the control group higher DNA damage was observed in those with the CYP1A1 Mst1 TT genotype compared to those with CYP1A1 Mst1 CC/CT genotype.

Pesticide-exposed 91 fruit growers and 106 unexposed control subjects were genotyped for CYP3A5, PON1, GSTM1, GSTT1 and GSTP1 by Li et al. (2006). DNA damage (tail moment) was significantly higher in subjects with high or low exposure to pesticides. The multiple regression analysis revealed a significant association of age, extent of exposure to pesticides and of CYP3A5 and GSTP1 null genotypes with DNA tail moment and it was concluded that those with susceptible metabolic (GSTP1) genotypes might experience an increased risk of DNA damage.
The results of the alkaline comet assay (Moretti et al., 2007) on 109 workers exposed to polycyclic aromatic hydrocarbon (PAH) and 82 controls genotyped for CYP1A1, EPHX and GSTM1 genes (which are involved in activation of PAH and in its detoxification) revealed significantly higher DNA damage in individuals exposed to PAH in graphite electrode manufacturing plant with non-significant differences for the analyzed genotypes.

da Silva and co-workers (2008) evaluated DNA damage in 108 agriculture workers exposed to pesticides and 65 normal controls with no pesticide exposure. Only the PON genotype among others (GSTT1, GSTM1, GSTP1, CYP1A1, CYP2E1) showed association with genetic damage (MN frequency was ~1.7 times higher); DI (~4.6 times) and DF (~5.6 times) higher in the pesticide-exposed group.

Assessment of primary and oxidative DNA damage, sister chromatid exchanges (SCE) and micronuclei (MN) in 39 under-ground road tunnel construction workers occupationally exposed to dust, gases and diesel exhaust and in 34 unexposed subjects (Villarini et al., 2008) genotyped for CYP1A1 and GSTM1 variants, showed significant differences in primary and oxidative DNA damage and SCE, while MN frequency was significantly higher in the exposed group though there were no effects of CYP1A1 and GSTM1 variants on genetic damage.

Lin et al. (2009) observed significantly higher levels of 8-OHdG in 488 maintenance haemodialysis patients as compared to levels in 372 gender-matched healthy controls. The GSTM1 null genotype was significantly associated with higher levels of 8-OHdG as compared to the levels in those with GSTM1 present genotype.

The influence of haptoglobin, manganese superoxide dismutase (MnSOD Val9Ala), catalase (CAT-21A/T), glutathione peroxidase 1 (GPx-1 Pro198Len), ACE (I/D) and glutathione S-transferase GSTM1 and T1 gene polymorphisms on DNA damage and oxidative stress induced by hydrogen peroxide (H$_2$O$_2$) in peripheral blood leukocytes from 135 healthy humans, was studied by Miranda-Vilela et al. (2010). The authors observed that DNA damage was significantly influenced by GPx-polymorphism and the Pro/Len genotype had higher genetic damage. Damage was significantly higher in those
with the Hp1F-1F/GSTM1+T+ and Hp1F-1s/M1+T1- genotypes with decreased levels in those having Hp1F-1s/GSTM1-T1- and Hp1F-2/GSTM1+T1+ genotypes.

Oxidative DNA damage (comet assay) and its relationship with GSTM1 and T1 polymorphisms was assessed in 49 healthy Korean male smokers (Lee et al., 2010). Those with GSTT1 null genotype had higher levels of DNA damage and of conjugated dienes with decreased plasma HDL-C and atherogenic index as compared to those with GSTT1 present genotypes.

Modulation of genetic (DNA) damage as a function of CYP1A1, CYP3A5, CYP2C9, CYP2D6 and PON1 gene polymorphisms was investigated by Singh et al. (2011) in 150 workers occupationally exposed to organophosphate pesticides and 134 healthy controls. Significantly higher (~13 times) DNA damage (comet assay) was present in the workers with CYP2D6*3 PM and PON1 (QQ and MM) genotypes.

In a similar study, Singh and co-workers (2012) evaluating DNA damage in CYP2C9, GSTM1, GSTT1 and NAT2 genotyped occupationally-exposed organophosphate pesticide workers (n=134) and equal number of controls (without any exposure), reported that DNA tail moment was significantly higher in the exposed group with significant increase in those having NAT2 slow acetylation and CYP2C9*3/*3 and GSTM1 null genotypes.

DNA damage was significantly higher (oxidized bases) and base-excision repair significantly lower in 388 individuals with at least five years of exposure to asbestos, stone wool or glass fibre compared to levels in 149 non-exposed individuals (Dusinska et al., 2012). Those with GSTT1 null genotype had more significant effects and the authors concluded that GST genetic polymorphism and activity were significantly associated with DNA stability and repair of oxidized bases.

The effects of PAH-exposure were modulated by the CYP1A1 M1 and M2 heterozygous and homozygous variant genotypes as individuals with these genotypes had increased tail moment and buccal micronuclei (BMN) frequencies (Giri et al., 2012) in a study on coal-tar workers (n=115) and healthy controls (n=105).
Kadioglu et al. (2012) assessed genetic damage in peripheral blood lymphocytes of a healthy Turkish group (n=127) in relation to GST gene polymorphisms. Those with GSTT1 null genotype had higher levels of chromosomal aberrations and MN frequency as well as of single-strand DNA breaks and the EndoIII and FPG sites.

Investigating the genetic polymorphism of GSTT1, GSTM1 and P1 in 65 infertile men with varicoceles and 30 healthy males from North-west China (Tang et al., 2012), sperm DNA damage (as assessed by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labelling; TUNEL assay, 8-OH-dG by HPLC) as well as levels of MDA and nitric oxide were significantly higher in those having GSTM1, GSTT1 and GSTT1/GSTM1 null genotypes. Surprisingly, levels of TAC were also significantly reduced in these individuals compared to those with the present genotypes.

Wlodarczyk and Novicka (2012) investigated DNA damage in 220 healthy non-smokers in response to CYPIA1, GSTM1, GSTT1, GSTP1 and XPD genetic polymorphisms. Genetic damage observed as per cent DNA tail was highest (6.7%) in those having the AA variant of XPD gene and the GSTM1 null genotype. The lowest levels (3.7%) were observed in GSTP1-AA/GSTM1(+) genotypes.

Urinary 8-OHdG levels in 148 non-smoker traffic policemen were significantly higher than in 135 normal controls (Prasad et al., 2013) and the authors also reported that the levels were increased significantly in those with the CYPIA1 M1 variant genotype and the null GSTM1 genotype.

Gomez-Martin et al. (2014) determined the levels of N-7 methyl deoxyguanosine as a biomarker of chemical methylating agent in 39 plastic green-house workers (exposed to low and high levels of pesticides) who were also genotyped for the paraoxonase-1(PON1) and GSTT1 and GSTM1 genes. Those with higher exposure to pesticides and with susceptible metabolic genotypes (individuals with GSTM1 null and PON1 192R) had higher DNA alkylation levels and higher risk of DNA damage.

Cho et al. (2015) assessed DNA damage and antioxidant status in 95 smokers (before and after grape juice-supplementation) depending upon glutathione-S-transferase genetic polymorphism. In those with the GSTM1 null genotype, significantly decreased diastolic blood pressure, lymphocyte DNA damage and plasma conjugated dines were
observed. After a period of 8-week of grape juice-supplementation, all the DNA damage parameters (tail per cent DNA, tail length, tail moment) were significantly decreased in those having the GSTM1 null genotype. The tail per cent DNA and tail moment were both significantly decreased in the GSTM1 present genotype. In both, null and present GSTT1 genotypes, genetic damage decreased and antioxidant effect of grape juice supplement was higher in GSTT1 present genotypes compared to the levels in GSTT1 null genotypes.

The perusal of literature therefore has revealed that imbalance between oxidative stress and antioxidant defenses in hypertension state results in increased oxidative stress that could lead to genetic damage. The genetic polymorphism of metabolic genes (CYP2D6 and GST) can also modulate treatment response and genetic damage. As no studies have come to attention on the potential functional consequences of CYP2D6 and GST gene polymorphisms in the Punjabi Jat Sikh hypertensive patients, the present case-control study was planned to investigate genetic damage in Punjabi Jat Sikh hypertensive patients and normal controls and to study the genetic profile of CYP2D6 (*2, *4, *10) and GST (T1, M1 and P1) gene variants and their association with genetic damage and with disease.