DISCUSSION

In the present case-control study, genetic damage in essential hypertensive patients was investigated and compared with that in age-, sex- and socio-economic status-matched normotensive healthy controls belonging to the rural Jat Sikh population sub-group of Punjab. Given that genetic damage is often caused by oxidative stress, which has a cause-effect relationship with hypertension (Yildiz et al., 2008; Baradaran et al., 2014), and furthermore as the consequences of unrepai red genetic damage can promote carcinogenesis (Camacho, 2012; Bridge et al., 2014) and precocious age-related changes (Best 2009; Khansari et al., 2009), such studies have significance as the study-outcomes can find use as prognostic and diagnostic biomarkers for disease-intervention and management strategies thereby delaying disease-progression and mortality.

Profiling of some metabolic genotypes was also carried out to discern if there was any association with disease in this sub-group, and whether levels of genetic damage were influenced by these genotypes, because population-group specifying can also modulate genetic profile (Chen et al., 2011; Stronks et al., 2013). The present cross-sectional study is the first investigated study of its kind to the best of knowledge in the rural Jat Sikh Punjabi sub-group. Ethnic-specific studies have gainful importance as differences in ethnic backgrounds occur because of genetic and cultural differences (Anand, 1999). Holmes et al. (2013) has specified that for the hypertension condition, ethnic differences also include life-style, socioeconomic status, family income and education level. Rather the interplay between environmental and genetic factors result in the disease. There is in fact 56-57% heritability component (Van Rijn et al., 2007) of the heterozygous hypertensive condition arising from defects in gene regulation of various pathophysiological pathways involved in disease causation or manifesting as expression effects of predispositional genes. The presence of genetic variation in the human genome and the database of 991 genes and 255 candidate SNPs associated with hypertension (http://www. bws.iis.sinica.edu.tw/THOD/ accessed on July 27, 2015) underlies the importance of such ethno-specific studies.

As genders are differently affected by interactions between genetic and environmental factors (Levin et al., 1998) and can influence the levels of genetic damage (Garm et al.,
both males and females were included in the study group. Other factors modulating genetic damage include age (Best, 2009; Eshkoor et al., 2011), occupational stress (Rim, 2012), life-style factors like alcohol consumption (Brooks, 1997) and smoking (Lee and Pausova, 2013). Detailed information on such variables has been recorded for the study participants. As the state of hypertension requires life-long treatment and the treatment of hypertension can last for decades, therefore the effect of drug-therapy can also modulate genetic damage. The cases of the present study were on monodrug atenolol (a beta-blocker), therapy for more than two years.

The patients, comprising 50.50% females (average age 59.47±1.13y) and 49.50% males (average age 63.47±1.11y) matched each other for marital status, diet, socioeconomic status, rural area, mobile-phone usage and physical activity as well as with controls (average age females 59.84±1.28y; males 60.80±1.24y). The age of onset of hypertension was 59.31±0.81y. In literature also, patients with essential hypertension generally have an onset age >46y (Guddad et al., 2012) and recently, it has been documented as ≥50y (Machado et al., 2014). The duration of the disease (treatment-time is similar because on disease-diagnosis, the drug treatment was initiated) was 2.28±0.06y matching between genders, which was also the case for disease-onset. The average blood pressure measurements for systolic blood pressure (SBP) in female cases (145.89±1.92mmHg) matched with those of male cases (145.15±2.03mmHg) as did respective diastolic blood pressure (DBP) measurements (85.73±0.83mmHg and 88.00±1.03mmHg). The calculated respective values of pulse pressure (PP; 60.16±1.48mmHg, 57.15±1.43mmHg) and mean arterial pressure (MAP; 105.58±1.09mmHg, 106.86±1.27mmHg) were also similar, implying no differences on stratification for genders. In literature also, no gender differences in blood pressure measurements have been documented by Iliescu et al. (2006). The important facet is that the recorded random blood pressure measurements are levels in treated cases-- these comprise the hypertensive group under study despite drug-therapy. The persistent higher blood pressure levels imply long-term and continuous manifestations of mechanical stress (Virdis et al., 2011) which can translate into oxidative stress (Montezano and Touyz, 2012) and cause macromolecular oxidation (Rahal et al., 2014) including damage to genetic material (Moller et al., 2014) which could also be triggered.
by atenolol-treatment as some studies (Telez et al., 2000; 2010) have reported its genotoxic potential.

Hence in the study group, there were no gender differences for blood pressure measurements, levels of genetic damage, dyslipidemia, oxidative stress and frequency of molecular genotypes. The demography also matched except that alcohol-consumption was confined to males; there were also no male or female smokers.

5.1 Obesity-Status in the Study Group

Association of obesity with hypertension varies with different racial and ethnic groups (Suglia et al., 2013) and sex-differences reveal higher incidence and/or prevalence of hypertension in obese females than in obese males (Fuzita and Hate, 2014). Obesity indicators derived from anthropometric variables included body mass index (BMI), waist circumference (WC) cut-offs, waist-hip-ratio (WHR) and waist-to-height ratio (WHtR). These are validated markers of obesity-determination (Maffeis et al., 2001). Though BMI, WC and WHR are positively associated with hypertension (Gus et al., 2004), Chobanian et al. (2003) have suggested that WHtR is a better obesity index for hypertension-prediction.

The study participants comprised 60.50% cases and 77.50% controls with general obesity, having higher BMI. In the context of central obesity, with WC 89.00% cases and 91.50% controls, with WHtR 91.50% cases and 90.50% controls and with WHR 98.50% cases and 97.50% controls were obese. An appraisal implies that at least in the present group, WHR turned out to be a better predictor of central obesity. It is further informative to note that more healthy controls were obese and hence more predisposed to the accompanying co-morbidities especially with respect to increased risk for hypertension (Re, 2009; Landsberg et al., 2013) and cardiovascular diseases (Poirier et al., 2006; Pérez et al., 2007).

Recent literature documentations include both, a lack of association of general obesity with hypertension (Oni et al., 2014) and also linear relationship of BMI and WHR with hypertension (Reddy et al., 2010; Pantelic et al., 2013). All the patients included in the present study group were obese on the basis of WHR. Hence as most of the participants of the present study including controls are obese, there is an increased likelihood for
cardiovascular risks (Narkiewicz, 2006). The increased obesity among controls could be because of dietary habits, sedentary life-style and genetic background (Chan and Woo, 2010).

### 5.2 Dyslipidemia and Hypertension

Significantly (p<0.0001) higher levels of total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLCL-C) were observed in hypertensive patients. The artherogenic indices (ratios of TC/HDL and LDL/HDL) and coronary risk index (ratio of TG/HDL) were also significantly higher in hypertensive patients making them susceptible to cardiovascular diseases (Fakhrzadeh and Tabatabaei-Malazy, 2012). These findings are similar to those cited in literature; a recent report on hypertensive patients had significantly higher lipid profile and male hypertensive patients had significantly higher levels of triglycerides as compared to female patients from Madhya Pradesh (Goyal and Sarwate, 2014). TG levels were also higher in males compared to those in female patients of the present study, though the values did not reach significance. Hypertensive patients from Bangladesh (Choudhary et al., 2014) and from Mashhad, Iran (Ghooshai et al., 2014) also had increased TC, LDL-C, VLDL-C and TG levels. Rural Karnataka hypertensive patients had also significantly higher TC, TG and TC/HDL ratio (Parmiladevi et al., 2011). Hypertensive patients maintaining inconsistent treatment-schedule, had significant instable serum TC, TG, LDL-C and HDL-C levels (Ijeh et al., 2010; Bambara et al., 2013).

Despite instable vs. consistent dyslipidemia, the hypertension condition has often shown association with an abnormal blood-serum lipid profile and the aberrant artherogenic and coronary artery risk indices imply susceptibility of dyslipidemic hypertensive patients to cardiovascular diseases (Qahtani et al., 2015) as such dyslipidemic persons have more likelihood for the occurrence of atherosclerotic lesions (Hong, 2010). Therefore the present study participants also are prone to heart diseases.

Atenolol treatment for hypertension also tends to cause dyslipidemia as observed by the study of Tziomalos et al. (2011). levels of HDL-C were increased in those on atenolol drug-therapy for hypertension (McDonough et al., 2013). The patients included in the
present study had been on monodrug (atenolol) therapy for >2y and were also
dyslipidemic which could be from the effects of the drug. Moreover, the drug is known
to induce CVD (Carlberg et al., 2004) and stroke (Kuyper and Khan, 2014).

5.3 Oxidative Imbalance and Hypertension

There exists a cause-effect relationship of oxidative stress with hypertension
(Grossman, 2008). A number of mechanisms can induce oxidative stress and thereby
hypertension. These primarily include oxidative-stress induced inactivation of nitric
oxide (Saez and Redon, 2003), imbalanced ROS triggered imbalanced sodium
metabolism (Redon et al., 2003) and decreased antioxidant levels (Beg et al., 2010).

Inactivation of NO by increased ROS causes endothelial dysfunction which enhances
re-modeling of arterial walls as well as platelet aggregation, smooth muscle cell
proliferation and vasoconstriction thereby contributing to increased blood pressure
values (Saez and Redon, 2003). Interference of sodium metabolism by ROS can also
contribute to the development of hypertension (Ando and Fujita, 2012). Oxidative stress
can also increase if levels of antioxidant enzymes decrease as have been observed in
both, animal models and in hypertensive subjects (Redon et al., 2003). The hypertensive
patients of the present study also had oxidative imbalance as observed from increased
levels of total oxidative stress (TOS), oxidative stress index (OSI) and malondialdehyde
(MDA which is a marker of lipid peroxidation) which were significantly (p≤0.001)
higher and TAC significantly (p≤0.001) lower. In an earlier study from the author’s
laboratory (Thukral, 2014), Punjabi Arora hypertensive patients also had 1.67 times
higher TOS levels (vs. 5.78 in present study) with 2.68 times OSI (vs. 6.71 times in
present study), 1.90X MDA (vs. 7.04 in present study) and 1.14X lower TAC levels (vs.
4.83 in present study) compared to respective levels in normotensive controls.

Oxidative stress in hypertension has often been reported (Table 35); increased
biomarker levels of homocysteine, uric acid, MDA, plasma thiobarbituric acid reactive
substances, carbonic unhydrase activity and carbonyl levels have been documented
(Kashyup et al., 2005; Nwanjo et al., 2007; Khanna et al., 2008; Meera and Marcus,
2012). Decreased antioxidant status in hypertensive patients includes lower levels of
TAS (Table 36), superoxide dismutase and catalase (Subash et al., 2010; Deoghare et
al., 2014). Other documentations have simultaneously reported increased oxidative stress and decreased antioxidant capacity associated with the hypertensive condition (Beg et al., 2011, Baradaran et al., 2014). In a preliminary study on serum samples of Punjabi Jat Sikh hypertensive patients, Kaur and Gandhi (2014) have also reported significantly (p≤0.001) increased levels of TOS, OSI, MDA and decreased levels of TAC compared to levels in healthy normotensive controls. The results on the present study participants also substantiate recent works on the increased presence of oxidative stress and lowered antioxidants in hypertensive patients (Kachhawa et al., 2014; Pena-Sanchez et al., 2014; Skibska and Goraca, 2015).

Higher peroxidation of lipid levels observable as dyslipidemia and increased MDA levels concurrently with elevated of TOS and OSI and decrease of TAC observed in the patients of the present study, imply the state of oxidative stress in these patients with proneness for oxidation of cellular macromolecules, including DNA and consequently increased genetic damage (Rahal et al., 2014).

5.4 Genetic Damage and Hypertension

Damage to cellular macromolecules (proteins, lipids and nucleic acids) by oxidation from oxidative stress is well known (Sesti et al., 2012). Oxidation of DNA can lead to single-strand breaks (SSB), double-strand breaks (DBP) and alkali-labile sites (Von Sonntag, 1987). The alkaline single cell gel electrophoresis (SCGE/comet) assay performed on peripheral blood leukocytes of hypertensive patients and controls in the present study showcased significantly higher genetic damage in patients. The nature of genetic damage assessed by the assay, besides including SSB, DSB, alkali-labile site (ALS), also include DNA-DNA cross-links, DNA-protein cross-links and apoptotic-sites (Cortes-Gutierrez et al., 2012; Udupa et al., 2014). The observations of the study imply oxidative damage to DNA which has shown 3.43X significant increase in per cent tail DNA being representative of amount of damaged DNA/breaks (Collins et al., 2008). Tail moment (TM) was 3.07X increased from controls and of Olive tail moment (OTM) was 5.09X-these are indicators of tail intensity and length (Olive et al., 1990). The per cent tail DNA reflects the widest range of DNA damage (DSB, SSB, ALS) which is linearly related to break-frequency (Collins et al., 2008). As TM is the product
of tail length and per cent DNA in tail, it expresses both, tail length and tail intensity (Olive et al., 1990). The product of tail length and fraction of DNA in tail is known as Olive tail moment (Cotelle and Ferrard, 1999). Increase in quantitative measures of damage viz. damage index (DI=8.17X) and damage frequency (DF=5.07X) were also observed significantly higher (p≤0.001) in the hypertensive patients. DF is the frequency distribution of number of nucleoids with tail (Franke et al., 2005). DI (which categorizes the nucleoids into a class without tail (class 0) to nucleoids from classes one-to-four with varying tail-lengths) depends on the amount of DNA electrophoresed into the tail (Collins et al., 2008). The stage III hypertensive patients of the present study had the highest level of DNA damage among the patients, implying that higher blood pressure measurements are associated with increased DNA damage (Subash et al., 2010a).

The significantly increased genetic damage observed in the *Jat Sikh* hypertensive patients of the present study find reflections in observations on DNA damage in hypertensive patients belonging to the *Punjabi Arora* group (Thukral, 2014). A comparison between sub-groups revealed significantly (p≤0.001) higher levels of per cent tail DNA, TM and OTM in *Arora* patients though quantitative measures of DNA damage viz. nucleoids with tails (DF) and comet score (DI) were significantly (p<0.001) higher in the *Jat Sikh* patient group (Table 33). Differences for controls were vice-versa as percent tail DNA was significantly higher in *Arora* male and female controls. The reasons for the significant differences in DNA damage between groups with higher damaging effects among *Arora* compared to *Jat Sikh* patients on one hand and healthy *Jat Sikh* compared to *Arora* normal controls on the other, may be due to their dietary preferences, alcohol-consumption and physical inactivity. The effect of these factors on genetic damage have also been cited in literature (Abed and Haddaf, 2013).

The significantly increased genetic damage in hypertensive patients of present study also finds consistency with results of other studies (Table 34). Yilidz and co-workers (2008) had reported statistically significantly (p<0.001) higher genetic damage as assessed by the SCGE assay in Turkish patients with white-coat hypertensive (1.54X) and sustained hypertension (3.41X) as compared to normal controls. Subash et al.
had observed significantly higher \( p<0.001 \) genetic damage in south Indian hypertensive patients as compared to control individuals; the 8-hydroxydeoxyguanosine (8-OHdG) were also significantly higher \( \text{Subash et al., 2010b} \). Hiroko et al. (2001) had also observed that levels of 8-OHdG were significantly higher in hypertensive patients compared to those in normotensive controls. Pero et al. (1976) had revealed greater potential of hypertensive patients for DNA damage. Earlier similar studies from the same laboratory as the present work, had also revealed significantly higher levels of genetic damage as assessed by SCGE assay in hypertensive patients as compared to levels in normotensive controls \( \text{Gandhi and Jyoti, 2010; Thukral and Gandhi 2012; Thukral et al., 2012; Kaur and Gandhi, 2013; Kaur and Gandhi, 2014} \).

The observed increased oxidative stress and decreased TAS levels, the continuous mechanical stress from increased blood pressure and duration of disease as well as its treatment could be main causal factors of observed genetic damage.

5.4.1. Genetic Damage in Treated and Untreated Patients

Genetic damage in patients was very significantly increased viz. 3.43X percent tail DNA, 3.08X TM, 5.09X OTM, 8.17X DI and 5.07X DF compared to levels in matched controls. On comparison of patient groups (treated vs. untreated), the untreated patients \( \text{Table 2d} \) had 1.41X percent tail DNA, 1.11X TM, 1.40X OTM and 1.02X DF compared to treated hypertensives. Similarly, Subash et al. (2010b) had also observed significant increase in damage index in newly diagnosed untreated patients (2.41X) and in treated hypertensive patients (1.60X) compared to normal controls. The drug-treatment in treated hypertensive patients could be protecting against DNA damage. As documented by Saiz et al. (2004), the antihypertensive treatment helps to improve antioxidant status and treatment helps to reduce 8-OHdG levels in hypertensive patients. The antioxidant nature of atenolol has also been documented in literature on one hand (Khanna et al., 2008; Sankar et al., 2010) while on the other, the genotoxicity of Atenolol has also been observed as increased MN and CA frequencies and fragile site expression (Telez et al., 2000, 2010).

Studies in literature have amply demonstrated the strong link of mechanical stress with oxidative stress in hypertension \( \text{Hirata and satonaka, 2001; Virdis et al., 2011} \) and
induction of genetic damage from oxidative stress (Deavall et al., 2012) such observations have been observed in the patients of the present study.

5.4.2 Best Genetic Damage Indices

Principle component factor analysis (PCA) for the best predictor of genetic damage among the various indices scored in the present study revealed that per cent DNA in tail and tail moment were better predictors followed by Olive tail moment. Studies in literature have also reported that per cent tail DNA is a better indicator of genetic damage (Lee et al., 2004; Kumaravel and Jha, 2006). The index per cent tail DNA reflects break-frequency and hence compared to the other parameters of TM and OTM, it is the most insightful of genetic damage parameters (Collins et al., 2008). The underlying basis is that as DNA in tail can comprise single-strand breaks, double-strand breaks, alkali-labile sites, hence it is the most appropriate for assessing gross DNA damage. However, when analyzed for best genetic damage predictor in total study group (patients and controls), only one factor was generated (with > 0.4 factor loading) including all the genetic damage parameters revealing the importance of all the parameters for evaluating genetic damage, atleast in the present group

5.5 Hypertension and Metabolic Genotypes

Genetic polymorphisms of CYP2D6 (*2, *4 and *10) and GST (T1, M1 and P1) were analyzed in patients and controls and their association with disease was also investigated.

5.5.1 Hypertension and CYP2D6 Genetic Variants

The cytochrome P450 enzyme, debrisoquine 4-hydroxylase also known as CYP2D6, is involved in phase I metabolism of most drugs, primarily antidepressants, and in the selective serotonin re-uptake. The gene’s locus is on chromosome 22q13 (Heim and Myer, 1990) which is highly polymorphic with more than 120 alleles (Evans & Relling, 2004). Among these are the fully functional (*1 and *2) alleles having extensive metabolizing activity (extensive metabolizers; EM), *10, *17 and *36 alleles have reduced activity (intermediate metabolizers; IM), the *4, *5, *6, *7, *8, *11, *12, *13 and *14 alleles are non-functional (poor metabolizers) while ultra-rapid metabolizer
phenotypes have duplication of alleles (Tharanga et al., 2013). By virtue of the allelic combinations, there is a wide range of enzymatic activity of the gene, varying from none to those with ultra-rapid metabolism.

In the present study, the *2, *4 and *10 alleles were studied. The minor allele frequency of CYP2D6 *2 (0.37), *4 (0.38) and *10 (0.46) in patients varied from that in controls (0.42, 0.26 and 0.42, respectively) and though matching for CYP2D6 *2 and *10 alleles, yet differed significantly (p<0.0009) for CYP2D6*4 from respective values in control group. This implies a significant association of this allele with hypertension. Pair-wise linkage disequilibrium analysis however showed no association among the allelic variants. Rather, the genotypic frequencies of CYP2D6 *2 and *10 were also not in Hardy-Weinberg equilibrium. The deviation from the Hardy-Weinberg equilibrium can be explained by the actions of natural selection, mutations, migration, finite population size and non-random mating. In the present study one or more factors from these can explain the shift in genotypic frequency from equilibrium (Rodriguez et al., 2009; Andrews, 2010). It also needs to be recalled that patients of the present study were on treatment at local hospitals and hence comprised hospital-based sampling. The unrelated controls were either healthy relatives accompanying other patients at the same hospitals or were from the general population. This bias in sampling could be a reason for the alleles to be not in Hardy-Weinberg equilibrium. The other possibility is that the ethic-specific group has this inherent genetic make-up. Dodgen et al. (2013) had also observed that the frequency of CYP2D6*10 allele deviated from Hardy-Weinberg equilibrium in South African healthy citizens residing in Pretoria city.

In other studies from India, the allelic frequencies of CYP2D6*2, *4 and *10 (Table 40) were compared with those in the present study. Significant (p<0.0001) variations were observed with higher minor allele frequencies of CYP2D6 *2, *4 and *10 alleles in Jat Sikhs while the allele frequency of CYP2D6*2 did not differ from that in the South Indian population (Theophilus et al., 2006). In the hypertensive Arora group (Thukral, 2014), the allele frequencies significantly (p<0.0001) differed from that in the present study group. The allele frequencies of CYP2D6 *2, *4 and *10 also differed significantly from Punjabi Ramgarhia Sikhs (Mahajan, 2014) and Jat Sikhs (Bhatt,
The differences from the Punjabi Jat Sikh could be because of their residential areas because in present study, Jat Sikhs only from rural areas were investigated and the observed differences in allele frequencies can be due to non-random mating behavior in rural areas. Incidentally, the minor allele frequencies among the frequency in other population groups are highest in the present study group.

5.5.2 Metabolizer Phenotypes

The combinational genotypes of CYP2D6*2, *4 and *10 variants revealed higher frequency of IM in patients (94.50%) as well as in controls (90.00%). The rest were extensive metabolizes (5.50% in patients and 10.00% in controls) while poor metabolizes were lacking. Only a comparison could be made with the Arora, Ramgariha Sikh and Jat Sikh groups studied in the same laboratory as for the present study. Among Arora patients, there were also no PM while IM (79.00%) were lesser and EM (21.00%) more (Thukral, 2014). In Punjabi Ramgharia Sikhs (Mahajan, 2014) and Jat Sikhs (Bhatt, 2014) also, no PMs were observed and frequency of EM (33.75% and 21.50%) were higher and frequency of IM were lower (66.25% and 78.50%) from that in the present studied population. The information on metabolic phenotypes can assist in presenting an optimal drug-dose because inter-individual variability in drug-response can cause under- or over-dosing at same dose-level/dosage. These study results therefore have implications for therapeutics and for applications in clinical practice. Stratification of genetic damage according to metabolic phenotypes of CYP2D6 *2, *4 and *10, revealed significant increase in the CYP2D6 *4 metabolic phenotype, as DI was significantly (p<0.05) higher IM patients as compared to the EM patients. Reverse trends were observed in controls where percent tail DNA was significantly higher in EM compared to PM controls. This may be because enzyme activity can also be influenced by disease status (Ingelman-Sundberg et al., 1999). The patient and control groups were matched for phenotypes (IM and EM). As the concentration of reactive oxygen species and carcinogens that react with DNA, depends upon the rate of enzyme activity required for detoxification of carcinogens (Au turup, 2000) and the enzyme activity in IM is lesser as compared to that in EM (Tharanga et al., 2013); this could be one of the reasons for increased genetic damage in IM due to reduced detoxification as compared to that in EM patients.
5.5.3 Hypertension and GST Polymorphisms

Glutathione S-transfereases (GST) are a multigene family of phase II metabolic enzymes, which catalyze a variety of endogamous and exogamous substances. There is different metabolizing ability of enzymes (for carcinogens and anticancer agents) encoded by different GST alleles (http://www.ncbi.nlm.nih.gov/gene/2950 accessed on June 29, 2015). They also offer protection to DNA from oxidative damage (Syed et al., 2010). In fact, there is immense GST variation as GSTs show ethnic-dependent polymorphism (Sharma et al., 2014).

The GSTT1 (22q11.2), GSTM1 (1q13.3) and GSTP1 (11q13) polymorphisms have been extensively studied worldwide. Homozygous deletions in GSTT1 and GSTM1 cause complete loss of enzyme activity. The A→G transition at nucleotide 313 results in the isoleucine valine substitution at codon 105 in exon 5 of the GSTP1 gene, being responsible for lowering of enzyme activity.

The patients and controls of the present study matched for genotypic frequencies of GSTT1 and GSTM1 alleles. The allelic and genotypic frequencies of GSTP1 were however significantly (p≤0.0001) different, being higher for the frequency of heterozygous (AG) and homozygous mutant genotypes (GG) in patients. The genotypic and allelic frequencies were also significantly (p<0.0001) different from other Punjabi and Indian population groups, with highest minor allele frequency in the presently studied population though the allele frequency was matched with that in Punjabi Arora control group (Thukral, 2014).

Comparison of results of present study in context of association of GST with hypertension in other populations (Table 37, 38 and 39), showed that as in the present study group, the GSTT1 and GSTM1 were not associated with hypertension (Oniki et al., 2008; Miranda-Vilela et al., 2010; Hussain et al., 2012). The frequency of GSTT1 and GSTM1 null genotypes were however significantly (p<0.000) higher in the present Punjabi Jat Sikhs compared to that in other Indian population sub-groups.

5.5.4 Predictors of Hypertension Phenotype

The principal component factor analysis (PCA) and multifactor dimensionality reduction (MDR) analysis were performed to identify the best predictors for
hypertension. PCA analysis reduced the original 40 confounding variables into 16 factors that explained ~77% (76.74%) of the cumulative variance. The predicted factors included loadings of blood pressure parameters (SBP, DBP, PP, MAP), lipid profile parameters (Total cholesterol, triglycerides), atherogenic and coronary risk indices (Total Cholesterol/HDL ratio, LDL/HDL and TG/HDL-C ratio), obesity parameters (WC, WHtR and BMI) and molecular genetic variables (CYP2D6*2, CYP2D6*4, CYP2D6*10, GSTP1). In the literature also, studies have reported salt-intake, obesity, physical inactivity and excess alcohol-consumption as modifiable risk factors for hypertension (Sagare et al., 2011; Singh et al., 2010). On MDR analysis also, the combinations of lipid peroxidation (MDA) with oxidative stress index (OSI) and of genetic damage (Damage frequency), lipid peroxidation (MDA) and total antioxidant capacity (TAC) were predictors of hypertension. Gene-gene combinations generated by MDR analysis were CYPD6*4 and GSTP1, and the combination of CYP2D6*4, CYP2D6*10 and GSTP1. Individuals with the heterozygous genotypes of CYP2D6*4 (GA), CYP2D6*10 (CT) and GSTP1 (AG) were at increased risk of developing the disease.

The results of PCA and MDR are important in defining the prevalent risk factors in hypertension in the present case-control study. Therefore for the Jat Sikh sub-group, there is likelihood of dyslipidemia, physical inactivity, obesity, increased blood pressure and genetic damage. The genetic polymorphisms of GST P1 and CYP2D6*2, CYP2D6*10 were observed to be important predictors of hypertension. Among the various modifiable risk factors for hypertension in literature, physical inactivity, obesity and smoking have been documented (Abed and Haddaf, 2013). Age, family history of disease, gender and genetic factors are the non-modifiable risk factors (Slama et al., 2002).

5.5.5. Metabolic Genotypes as Predictors of Disease-Status

Principal component factor analysis loaded CYP2D6*4 and GSTP1 in factor one explaining 19.79% variance for the presence of disease. MDR analysis had also revealed that among the two-gene combinations, the CYP2D6*4 and GSTP1 combination and in the three-gene combinations, the CYP2D6*4, CYP2D6*10 and
GSTP1 combination were best predictors for disease. Individuals with the heterozygous genotypes were however at increased risk for disease. In literature only one study has come to attention investigating association of CYP2D6 (*1 and *10) and hypertension (Ali et al., 2013) and has also shown a significant association between CYP2D6 genotype and hypertension. CYP2D6 is involved in the metabolism of some endogenous and exogenous substances and neurotransmitters like adrenalin (involved in blood pressure regulation) that predispose individuals to cardiovascular diseases. Inconsistent results for association of CYP2D6*10 and hypertension treatment response have been reported in the literature (Ran et al., 2002; Wang et al., 2009). Levinsson and Co-workers (2014) have also recently reported significant association of GSTP1 gene with hypertension. The heterozygous genotype for GST P1 is associated with reduced enzyme activity (Zimniak et al., 1994) and thus increased oxidative stress that can predispose individuals to hypertension (Oparil et al., 2003).

5.6 Predictors of Genetic Damage

5.6.1 Patients

On correlation analysis it was observed that genetic damage indices were significantly associated with disease-related variables (SBP, DBP, PP, MAP), obesity (BMI, WHtR), physical inactivity, lipid levels (TC, TG, HDL-C, LDL-C, VLDL-C), coronary risk index (TC/HDL) and the atherogenic index (LDL/HDL). However on performing univariate followed by multivariate and combined multivariate regression analyses, the retained factors of physical inactivity, BMI, SBP, PP, TC, HDL, TC/HDL, LDL/HDL and TAC were found to be predictors for genetic damage. Analysis of variance (ANOVA) has also demonstrated the association of these factors with genetic damage.

5.6.2 Controls

Genetic damage in controls also showed association with lipid profile, total antioxidant capacity, BMI, WHtR and dietary habits on correlation analysis. On performing univariate followed by multivariate and combined multivariate analyses, the genetic damage predictors were BMI, HDL-C, LDL-C, VLDL-C and TG/HDL ratio.
5.6.3 Total Study Group

In total study group on correlation analysis, association of all the genetic damage parameters was with oxidative stress parameters (TOS, OSI), obesity measure of BMI, blood pressure levels (SBP, DBP, PP, MAP), family history of the disease, lipid and lipoprotein levels. From ANOVA and regression analysis, genetic damage parameters were significantly associated with dietary pattern, BMI, SBP, DBP, TC, TG and oxidative stress parameters.

In accordance with the other studies, besides dietary habits (Kazimirova et al., 2004), gender and alcohol consumption (Fenech and Bonassi, 2011) have shown significant association with genetic damage. DNA damage parameters assessed by SCGE assay in hypertensive patients showed association with BMI and blood pressure (Khanna et al., 2008) Other studies have also documented that obesity (BMI) is associated with increased genetic damage and apoptosis (Donmez-Altuntas et al., 2014). The excess food intake leading to impaired respiratory capacity may further increase additional stress in the cell (Pintus et al., 2012) leading to DNA damage. Cholesterol is an important structural lipid that plays an important role in the absorption of fat-soluble vitamins including vitamin-E, which is a major membrane-bound antioxidant (Burton and Ingold, 1989) and in the control of their flow in and out of the cell (Stahl et al., 2002). So decreased cholesterol levels lead to decreased antioxidant capacity leading to increased oxidative stress and reactive oxygen species that can react with DNA (Li et al., 1994). Some studies have however reported negative association of lipid profile parameters with DNA damage (Martinet et al., 2001; Kikuchi et al., 2013). Blood pressure is related to oxidative imbalance which with decreased antioxidant capacity (Subash et al., 2010a) can also lead to genetic damage.

5.7 Genetic Damage as Function of Various Variables

Genetic damage data were stratified for the variables of age, obesity, disease related and molecular genotypes to study whether there were differential effects of these variables on genetic damage parameters. Stratification of genetic damage by age revealed significant (p≤0.05) increase in DI in patients with old age. In literature also, association of age with DNA damage (Harman, 2003; Schumacher et al., 2009; Soares et al., 2014)
has been reported. With age beroreceptor sensitivity, increased responsiveness to sympathetic stimulus and the altered renal and sodium metabolism can lead to increased blood pressure/hypertension (Weber et al., 1989). It has been reported that the hypertension condition is related to increased oxidative stress (Gonenc et al., 2012; Sinha and Dabla, 2015) which can lead to genetic damage (Klaunig et al., 2010). In the results of the present study, genetic damage parameters were highest in hypertension stage III category of patients. DI in the studied group showed significant (p≤0.05) elevated levels in patients with late-onset of disease probably also including age-related genetic damage. However on studying the menopausal categories for differential genetic damage, there were no significant differences for these parameters implying that despite hormonal differences in different menopausal categories, these differences did not modulate genetic damage at least in this group.

On the basis of obesity variables, per cent tail DNA was significantly higher in non-obese patients compared to obese patients and tail moment was significantly higher in non-obese controls. Increased body mass index (obesity) is associated with increased cholesterol levels (Ugwuja et al., 2013), increased cholesterol levels help in the absorption of vitamins including the antioxidant vitamin-E (Burton and Ingold, 1989) thereby reducing oxidative stress and hence causing lesser damage to DNA contrarily on the other hand, the increased number of adipocytes in the obese individuals incite an inflammatory response (Makki et al., 2013) inducing production of ROS, increasing oxidative stress (Fernández-Sánchez et al., 2011) and so have the potential to cause genetic damage. Obese individuals in various studies have been reported to have more genetic damage than non-obese individuals (Kocael et al., 2014). The converse in the present study may be due to the presence of other confounding factors.

Even though central adiposity (WC) has shown association with increased genetic damage (Bukhari et al., 2010; Wiegand et al., 2010), dyslipidemia is increased TGs and decreased HDL-C is associated with adiposity (Klop et al., 2013) and by extension to genetic damage and the increased blood pressure levels have shown association with increased genetic damage, the present study participants did not exhibit differential genetic damage response in those with the metabolic syndrome (based on WC, HDL-C,
TG and blood pressure levels). The presence of various confounding factors active in those with or without metabolic syndrome could be responsible for this.

Stratification by disease-specific variables of duration and treatment of disease and age-of-onset of disease revealed that TAC was significantly ($p \leq 0.05$) reduced in patients with increased duration of treatment. In this context though the treatment with atenolol, which has some antioxidant properties (Gomes et al., 2006), for over two years could have prevented the decrease in TAC, however as the patients were still hypertensive with blood pressure levels not controlled despite the treatment, the mechanical stress probably induced oxidative stress (Virdis et al., 2011) with decreased antioxidants causing homeostatic imbalance. The role of confounding factors cannot be overlooked.

Genotypic stratification and statistical differences for genetic damage revealed that DI was significantly higher in patients with $CYP2D6^*4$ heterozygous (GA) genotype compared to the values in homozygous wild type (GG) genotype. Conversely in controls per cent tail DNA and OTM were significantly ($p \leq 0.05$) higher in homozygous wild type as compared to genetic damage in heterozygous and homozygous mutant genotypes. Also in controls, the $CYP2D6^*2$ heterozygous compared to homozygous wild type genotypes, had significantly higher DF. The cytochrome P450 enzymes are involved in the detoxification of endogenous and exogenous substances, reactive intermediates formed in the detoxification reactions can react with DNA (Nebert and Dalton, 2006) causing damage to it, there observed differences for genetic damage as a function of genotype may be due to differential activity of the enzyme. The lower activity in variants and heterozygotes may not be enough to balance the oxidative stress and hence could be responsible for increased genetic damage. The $CYP2D6^*4$ heterozygous has intermediate enzyme activity vis-à-vis the homozygous wild with extensive activity while the homozygous variant has poor enzyme activity. However, as along with genetic polymorphism, activity of cytochrome P450 enzyme can also be influenced by disease-status, environmental and exogenous factors like diet and toxins (Ingelman-Sundberg et al., 1999), the differential genetic damage in the different genotypes of $CYP2D6$ could be also from these causes.
5.8 Metabolic Genotypes as Predictors of Genetic Damage

Gene-Gene interactions as genotypic predictors for genetic damage parameters by MDR analysis revealed two-gene combinations of \( CYP2D6^*10 \) and \( GSTP1 \) as best predictors for percent tail DNA, TM and OTM while \( CYP2D6^*4 \) and \( GSTP1 \) combination showed best prediction for DI and DF. In three-allelic combinations, the \( CYP2D6^*4 \), \( CYP2D6^*10 \) and \( GSTP1 \) combination emerged as the best predictor for all the genetic damage parameters of percent tail DNA, TM, OTM, DI and DF. In both two and three factor combinations it has been observed that individuals with the heterozygous genotypes are at increased risk for developing genetic damage. The \( GSTP1 \) is involved in ROS-detoxification and of endogenous and exogenous substances; it also promotes direct protein-protein interactions causing the function of C-Jun N-terminal kinase to be inhibited, which affects stress-responses and apoptosis (Karin and Gallagher, 2005). Hence the genetic variants of this gene modify its enzyme functions, by its reduced activity (Dusinska et al., 2012). On reductions of enzyme activity there is ROS which can lead to increased genetic damage. As observed in the present study, in other studies also GST P1 heterozygous genotype has shown associated with increased genetic damage (Dusinska et al., 2012).

The significantly increased genetic damage, oxidative stress and dyslipidemia in the present study, is in accordance with other studies reported in literature. The minor allele frequencies of \( CYP2D6^*4 \) and \( GSTP1 \) was also significantly higher in the patient group compared to control group.

The overall results have shown that the \( CYP2D6^*4 \), \( CYP2D6^*10 \) and \( GSTP1 \) genotypes (alone or in combination) modulated the disease status as well as levels of genetic damage.

5.9 Salient Features of the Study

- This study is the first of its kind to explore complex gene-gene and gene-environment interactions prevalent in essential hypertensive patients belonging to Punjabi Jat Sikh population sub-group.
Genetic damage parameters *viz.* per cent tail DNA (~4x), tail moment (3x), Olive tail moment (6x), Damage Index (8x) and damage frequency (5x) were significantly (*p*≤0.001) higher in hypertensive patients compared to normotensive controls. Genetic damage was significantly higher in untreated patients compared to treated patients.

Oxidative stress parameters *viz.* total oxidant status (10x), oxidative stress index (17x) and malondialdehyde (10x) levels were significantly (*p*≤0.001) increased while total antioxidant capacity was significantly decreased in hypertensive patients compared to controls.

Allele frequencies of *CYP2D6*<sup>4</sup> and *GSTP1* were significantly different between patients and controls, as minor allele frequencies for *CYP2D6*<sup>4</sup> (0.38 vs. 0.27) and *GSTP1* (0.57 vs. 0.27) were higher in patients compared to controls.

Genotypic frequencies of *CYP2D6*<sup>4</sup> and *GSTP1* also varied significantly as homozygous variant genotypes and heterozygous genotypes were higher in patients compared to controls.

Patient and control group individuals were matched for metabolic phenotypes based on *CYP2D6*<sup>2</sup>, *4* and *10* combinational allelic variants.

Genotypes of CYP2D6<sup>2</sup> were not in Hardy-Weinberg equilibrium in both patients and controls and the genotypic frequencies of CYP2D6<sup>10</sup> and GSTP1 were also not in Hardy-Weinberg equilibrium in the control group.

Metabolic syndrome was more prevalent among patients (53.50%) compared to controls (4.00%).

Stratification of genetic damage and oxidative stress parameters for age, obesity measures, age at onset of disease, duration/treatment of disease, menopausal status in females and metabolic syndrome did not reveal any significant pattern of increase.

Genetic damage was highest in patients in the hypertension stage III category.
On the basis of genotypes, DI was significantly higher only in CYP2D6*4 heterozygous genotype (GA) compared to values in the homozygous wild (AA) genotype.

The combination of CYP2D6*4, CYP2D6*10 and GSTP1 as revealed by MDR analysis was best predictor allelic combination for genetic damage.

Correlation, regression and analysis of variance revealed lipid and lipoprotein levels, obesity variables and physical inactivity were predictors of genetic damage in patients, in controls and in the total study group.

Lipoprotein levels, blood pressure measures, genetic damage parameters were predictors of disease-status by PCA. After excluding genetic damage parameters, similar variables were loaded but also included CYP2D6*10 genotype.

Combination of DF, MDA and TAC was predicted as best combination for disease-status by MDR analysis, while when analysis was performed without genetic damage, the combination of MDA, TAC and TOS emerged as the best predictor.

Disease-risk on the basis of gene-gene interactions by MDR analysis revealed that CYP2D6*4, CYP2D6*10 and GSTP1 allelic combination as the best predicted combination for disease-risk.

For genetic damage and disease-status, MDR analysis similarly revealed combination of CYP2D6*4, CYP2D6*10 and GSTP1 as the best allelic combination. Individuals with heterozygous genotypes of these variants were at maximum risk for disease and for increased genetic damage.

5.10 Importance of the Study

As a first ethno-specific cross-sectional, case-control study of its kind, data on hypertensive and normotensive rural Jat Sikhs have been generated for genetic damage, oxidative stress, dyslipidemia and genotypes of CYP2D6 (*2, *4 and *10) and GST (T1, M1 and P1) gene variants. The study has contributed in identifying gene-gene and gene-environmental interactions in hypertensive Jat Sikhs, which has prognostic and
diagnostic applications. The increased obesity, genetic damage and oxidative stress in hypertensive patients substantiate similar earlier findings in literature while the association of metabolic genotypes is a new prospective of its kind adding information on genetic susceptibility to genetic damage and predisposition to hypertension. Furthermore the prevalence of metabolic syndrome in hypertensive patients in some healthy normotensive controls has implications because of their risk for cardiovascular disease. So these participants have been counseled for management and intervention strategies. Additional information on metabolic phenotypes can assist in appropriate drug-dosage prescriptions.

5.11 Clinical Significance of the Study

The main findings of the study of significantly elevated levels of genetic damage and oxidative stress in hypertensive patients imply about their future risk for occurrence of malignancy and/or precocious age-related changes and in the light of this, appropriate management and intervention/treatment strategies. Hence it may be prudent to routinely screen hypertensive patients for genetic damage. The association of increased genetic damage with CYP2D6 *4 and GST P1 genotypes may be useful for identifying those with liability to genetic damage. The presence of these genotypes in the hypertensive group adds to database on the gene variants pre-disposing towards hypertension among Jat Sikhs and can be useful in disease-diagnostics. The prevalent gene/environment factors in the patient group can also find use in predictive risk factors for disease in a similar population sub-group. The metabolic phenotypes based upon CYP2D6 (*2, *4 and *10) gene variants also has clinical significance for appropriate adjustment of drug-dose in the field of personalized medicine.