SUMMARY

With a rather high prevalence even in rural India, essential hypertension is a major public health concern because of the associated co-morbidities of cardio-, reno- and cerebro-vascular systems. The multifactorial etiological nature of hypertension underlies an interplay between genetic and environmental factors, both modifiable and non-modifiable. Among the modifiable factors, obesity has shown consistency with hypertension risk as two-thirds of the prevalence is directly attributable to it. The condition requires life-long treatment for the management of blood pressure levels, which thereby can help in delaying/preventing its co-morbidities. However, the drug-treatment itself may initiate secondary health consequences. Many of the drugs target the underlying physiological basis of hypertension of increased oxidative stress, endothelial dysfunction and vascular dysfunction, which are under genetic regulation. The metabolic genotypes of cytochrome P450 (CYP2D6) and Glutathione-S-transferase (GST), involved in drug-metabolism and antioxidant action, have also shown association with hypertension as oxidant-antioxidant imbalance with resultant oxidative stress have been reported in hypertension. This can cause oxidative damage to cellular macromolecules, with pre-cancerous lesions arising on oxidation of nucleic acids and resulting in DNA damage, adding malignancy to other co-morbidities associated with hypertension. However, early detection of DNA damage can facilitate interventions for age-related changes and malignancies. Against the backdrop of sparse studies relating CYP2D6 and GST gene polymorphisms with hypertension and inter-individual variation from these to genetic damage, the present study was undertaken. Using a case-control design, Jat Sikh hypertensive patients on treatment with the beta-blocker drug, Atenolol, were assessed for genetic damage, oxidative stress and were genotyped for some variants of GST (T1, M1 and P1) and CYP2D6 (*2, *4 and *10) genes under informed consent and after ethical approval from the Institutional Ethics Committee.

The ethnic-specific studies are significant in distinguishing biological, environmental, or social causes of disease (Gromann and Ginsberg, 2004). Ethno-specific related factors of family income, education and insurance status can influence the etiology and state of hypertension (Frist, 2005). In fact ethnic differences of health behaviors, access
to health care, and environmental exposures can influence hypertension (Cooper, 1998). As it is ethnic-specific study, so it rules out bias on the basis of diverse genetic backgrounds both for molecular genetic analysis for molecular genetic analysis and genetic predispositional confounders of disease and genetic damage (Sahebi et al., 2013).

Unrelated 200 Jat Sikh diagnosed hypertensive hospital-cases and 200 normotensive healthy controls from the general population, belonging to the rural areas of Amritsar district, comprised the study group. On a specially-designed questionnaire, record of the general demographic, anthropometric and clinical information of each participant was documented after informed consent. The mercury sphygmomanometer was used for blood pressure measurements; the anthropometric measurements of height, weight, hip circumference and waist circumference (WC) were made to assess general obesity from body mass index (BMI). For central/abdominal obesity, cut-offs of waist circumference, waist-to-height ratio (WHtR), and waist-to-hip ratio (WHR) were consulted. Intravenous peripheral blood samples from each participant were aliquoted and processed for genetic damage assessment (after cell viability test) and for genotyping; blood sera samples were used for biochemical analysis (oxidative stress assessment and lipid profiling). Genetic damage was ascertained using the single cell gel electrophoresis (SCGE)/comet assay. Lipid profile was determined using standard kits on a semi-automated blood analyzer and oxidative stress parameters of total antioxidant capacity (TAC), total oxidative stress (TOS) and lipid peroxidation malondialdehyde (MDA) levels were estimated spectrophotometrically using standard protocols. The genomic profile of CYP2D6*2 (A2850G, rs16947), CYP2D6*4 (G1846G, rs189209), CYP2D6*10 (C100T, rs106585) and GST P1 (A313G, rs1695) variants was assessed by PCR-RFLP method. Multiplex-PCR was performed for genotyping GST T1 and GST M1 variants. The observations of the study were subjected to statistical analysis (Chi-squared test, Students’ t-test, odds-ratio, correlation, regression, analysis of variance, principal component factor analysis (PCA), multifactor dimensionality reduction (MDR) analysis and haplotype analysis) performed on SPSS, MedCalc, MDR and Haploview softwares. An online Webasso-test was used to find the inheritance models for different gene variants.
Hypertensive patients (50.50% females, 49.50% males) aged 61.59±0.80y and normotensive controls (47.00% females, 53.00% males) aged 60.36±0.89y were matched for baseline characteristics of age, gender, socioeconomic status, dietary habits, alcohol consumption, residential area, mobile phone usage and occupational groups. BMI was significantly higher in controls (29.39±0.35kg/m²; 26.60±0.32 kg/m² patients) though central adiposity (WC, WHtR and WHR) had no differences. The patients compared to controls had significantly (p≤0.001) higher systolic blood pressure (SBP 145.52±1.39mmHg vs. 130.00±0.38mmHg) and diastolic blood pressure (DBP 86.86±0.66mmHg vs. 76.79±0.32mmHg). The age-of-onset of hypertension was 59.31±0.81y and the patients were on once-a-day oral dose of Atenolol (50mg) for an average of 2.28±0.06y. Despite treatment, 12.5% of patients had stage I hypertension, 19.00% stage II and 9% stage III. Dyslipidemia was apparent among patients with significantly (p≤0.001) higher levels of total cholesterol (TC 1.17X), low density lipoprotein cholesterol (LDL-C 1.32X), very low density lipoprotein cholesterol (VLDL-C 1.34X) and triglycerides (TG 1.34X) than controls. Total oxidant status (TOS 5.78X) and oxidative stress index (OSI 6.07X) were also significantly (p≤0.001) higher in patient group as were lipid peroxidation (MDA) levels (~7.04 fold higher) with total antioxidant capacity lower (~ 4.83 folds, p≤0.001) than in controls. More than half of the patients (53.50%) had metabolic syndrome (62.00% females, 44.00% males) while its presence was only 4% in controls.

Genetic profile of CYP2D6 (*2, *10) and GST (T1, M1) revealed no differences for allelic and genotypic frequencies between patients and controls. However for minor alleles of CYP2D6*4 (1.42X) and GST P1 (2.11X), significantly higher frequencies were there in hypertensive patients. The GST P1 genotypes also varied significantly (p≤0.001) with 2.54 times (35.50%) more patients having the homozygous variant (GG) genotype and 1.62 times (43.00%) more patients with heterozygous (AG) genotypes compared to those in controls. The distribution of CYP2D6*2 genotypes in patients and controls, and of CYP2D6*10 and GST P1 genotypes in controls were not in Hardy-Weinberg equilibrium (p≤0.001) probably because of hospital-based selective sampling of the ethnic-specific patient group, and the matched controls included from general population/healthy relatives of other patients accompanying them to the hospital.
Genetic damage parameters assessed viz. per cent tail DNA (3.43 folds; 14.32±0.61 vs. 4.17±0.21), tail moment (3.08 folds; 26.92±2.65 vs. 8.75±1.32AU), Olive tail moment (5.09 folds; 30.25±1.61 vs. 5.94±0.34AU), damage index (8.17 folds; 185.49±1.95 vs. 22.71±1.06AU) and damage frequency (5.07 folds; 91.92±0.47 vs. 18.12±0.76) were significantly (p≤0.001) higher in patients than in controls, respectively. PCA revealed that per cent tail DNA, tail moment and Olive tail moment were equal predictors of genetic damage in patients with a total variance of (47.20%) while damage index and damage frequency were equal predictors in controls. On the total study group, only one factor got generated comprising all the genetic damage parameters with factor loading more than 0.4 explaining a total variance of 68.32%. These observations imply that in the present study all the genetic damage parameters are valuable for defining genetic damage.

Treated (compared to an additional) untreated patient group had significantly lower genetic damage (per cent tail DNA and DI), though oxidative stress index showed significant (p≤0.05) increase in treated hypertensive females (5.85X) and males (9.38X) and in the total group (6.83X) compared to the respective values in untreated group. Total antioxidant capacity was also significantly higher in untreated male-(3.68X) and total (2.79X) patients compared to the values in the treated male patient and the total treated patient groups. Irrespective of the higher antioxidant capacity in untreated vs. treated patients, genetic damage was significantly higher, probably because of their different life-styles and dietary habits, alcohol consumption and physical inactivity.

On stratification of patient and control data for the genetic damage, oxidative stress and lipid profile parameters by gender, no significant differences were observed. Stratification by age, (40-60y vs. >60-90y) revealed only DI as significantly (p≤0.01) increased in >60-90y old patients. On stratification by duration of treatment (≥1-2y vs. 2-4y), TAC was significantly (p≤0.05) reduced with the increased duration of treatment. In patients with late age-of-onset of disease (61-85y) compared to those with earlier onset (35-60y), DI was significantly higher (p≤0.05) in those with later onset. In females stratified for menopausal status, there were no significant differences among post-menopausal (67.33%), peri-menopausal (14.85%) and pre-menopausal (17.82%) patients for all these parameters. In controls also, no differences were significant as a
function of menopausal status. Genetic damage increased across blood pressure categories (though non-significantly) with maximum damage in patients in the hypertensive stage III patients though TOS levels were significantly higher (p≤0.05) in stage II compared to those in stage III patients. On stratification by general- and central obesity-status, there was only significant increase in per cent tail in DNA in non-obese vs. obese patients while tail moment was higher in non-obese controls based upon general obesity while no significant differences were observed for central obesity. Stratification of the data by genotype revealed only DI as significantly higher (p≤0.05) in patients with the CYP2D6*4 heterozygous vs. homozygous wild type. In controls, per cent tail DNA and OTM were significantly (p≤0.05) higher in homozygous wild type and in heterozygous genotypes. The CYP2D6*2 heterozygous compared to homozygous wild type genotypes also had significantly higher DF.

There were 5.50% extensive and 94.50% intermediate metabolizers among patients based upon combinational genotypes of CYP2D6 (*2, *4 and *10), with 10.00% extensive and 90.00% intermediate metabolizers among controls. There were non-significant genetic damage differences within phenotypes in each group. Intermediate metabolizers compared to extensive metabolizers among patients as per CYP2D6*4 genotype alone, however had significantly (p≤0.05) higher DI; in the control group, the per cent tail DNA and OTM were significantly (p≤0.05) higher in extensive compared to poor metabolizers. On genotypic stratification also, the heterozygous genotype had significantly higher DI.

Metabolic syndrome (MS) was present significantly higher among patients (53.30%) compared to controls (4.00%). Stratification by MS (present vs. absent) in each group for genetic damage parameters, revealed no significant differences within groups. Probably other confounding factors could be interplaying in causing genetic damage.

On correlation analysis, it was observed that genetic damage indices were significantly associated with disease-related variables (SBP, DBP, PP, MAP), obesity-states (BMI, WHtR), physical inactivity, dyslipidemia (TC, TG, HDL-C, LDL-C, VLDL-C), and the coronary risk (TC/HDL) and artherogenic (LDL/HDL) indices in patient group. However, on performing univariate followed by multivariate and combined multivariate
regression analyses and ANOVA the factors included were physical inactivity, general obesity (BMI), blood pressure (SBP, PP), dyslipidemia (TC, HDL, TC/HDL, LDL/HDL) and antioxidant status as predictors of genetic damage.

In controls, genetic damage parameters showed association with lipid profile, total antioxidant capacity, BMI, WHtR and dietary habits on correlation analysis. On performing univariate followed by multivariate analyses and combined multivariate regression analysis, genetic damage predictors were BMI and dyslipidemia (HDL-C, LDL-C, VLDL-C, TG/HDL). For the total study group, correlation analysis revealed association of all the genetic damage and oxidative stress parameters with BMI, blood pressure (SBP, DBP PP, MAP), family history of the disease and all lipid levels and ratios. Regression analyses also showed significant association but with dietary pattern, BMI, SBP, DBP, TC, TG and oxidative stress parameters.

Therefore predictors of genetic damage among patients of present study are increased obesity, blood pressure, oxidative stress and dyslipidemia.

On MDR analysis the CYP2D6*4, *10 and GSTP1 was revealed as the best predictor for disease. Considering gene-gene interactions, MDR analysis revealed the two-factor combinations of CYP2D6*10 and GST P1 as the best predictor for percent tail DNA, TM, OTM and of CYP2D6*4 and GST P1, for DI and DF. In the three-factor combination, that of CYP2D6*4, CYP2D6*10 and GST P1 was the best predictor for all the genetic damage parameters. In fact the heterozygous genotypes of these genes were at maximum risk for genetic damage. As both CYP2D6 and GST enzymes are involved in detoxification of exo- and endogenous contaminants and in oxidative stress homeostasis, the heterozygous genotypes have decreased enzyme activity and hence, inappropriate detoxification capacity thereby increasing genetic damage.

Genetic profiling for disease-status revealed frequency of CYP2D6*4 and GST P1 except all genetic polymorphisms was significantly differed between patients and controls (as explained above). Among the inheritance models recessive model each of CYP2D6*4 and additive models of CYP2D6*2, CYP2D6*10 and GST P1 were observed as best-fit models for disease-prediction.
There was increased likelihood of developing hypertension (odds-ratio analysis) in those with CYP2D6*4 heterozygous (2.00X) or homozygous variant (2.33X) genotypes. The heterozygous CYP2D6*10 genotype also showed 2.40 times higher risk and for GSTP1 the risk was 4.49X for heterozygous and 7.02X for the homozygous variant genotypes even after Bonferroni correction. However, on adjustment for age, gender, socioeconomic status, BMI, family history of the disease, TAC and TOS, this significance was lost.

No haplotypes were generated for CYP2D6 (*2, *4 and *10) and GST (T1, M1 and P1) gene variants, implying that there was not any tendency of these variants to be inherited together in the studied population sub-group.

For elucidating prevalent risk factors among patients contributing to disease, PCA reduced the 40 risk factors to 16 comprising levels of lipids, blood pressure and genetic damage, obesity-related variables and molecular genotypes of CYP2D6*2, *4, and *10, and GST P1. On excluding genetic damage parameters (as genetic damage is inconsistently considered as a risk factor for hypertension), the analysis retained all these risk factors.

On studying gene-environment interactions for disease-status, MDR did not reveal any gene combination but MDA, OSI as two-factor and DF, MDA, TAC as three-factor combinations emerged as predictors of disease-status. These observations imply that individuals with higher levels of MDA, OSI, DF, and lower levels of TAC, were at increased risk for developing hypertension.

Gene-gene combinations for hypertension risk by MDR analysis, revealed that there is 6.68 times higher likelihood for the disease for CYP2D6 *4, CYP2D6*10 and GST P1 interaction and individuals with heterozygous genotypes for these variants are at maximum disease-risk.

These study results reveal significantly increased genetic damage, oxidative stress and dyslipidemia in the patient group. The minor allele frequencies of CYP2D6 *4 and GSTP1 showed increase in patients. The homozygous variant and heterozygous GSTP1 genotypes had significantly higher frequency. The contribution of CYP2D6 and GST gene polymorphisms add knowledge about genetics of hypertension and provides a database for susceptibility genotypes of essential hypertension.