INTRODUCTION

Essential hypertension is a heterogeneous disorder characterized by sustained systolic blood pressure of $\geq 140$ mmHg and/or diastolic blood pressure of $\geq 90$ mmHg (IHG III, 2013), accounting for more than 90% of all cases (Bolivar, 2013) and affecting one billion hypertensives worldwide (IHG III, 2013). There are public health ramifications because of this condition as essential hypertensive patients are susceptible to cardiovascular, cerebrovascular and renal problems often manifesting as heart failure, stroke and end stage renal disease (Carretero and Oparil, 2000). Almost 80% of the patients have obesity, glucose intolerance and dyslipidemia (Aronow et al., 2011). Hence, optimal life-long therapy is required to manage the condition and prevent/delay the progress to the co-morbidities.

The normal physiological regulation of blood pressure is a complex interplay of cardiac, vascular, renal, neural and endocrine factors (Chopra et al., 2011) which are modulated by combined action of several genetic and environmental, with biological imbalance in any regulatory mechanism resulting in hypertension. The effect can also be from demographic and environmental risk factors of age, obesity, alcohol and salt-intake, smoking and physical inactivity (Abdulsalam et al., 2014).

Though all the genetic components in essential hypertension are not apparent, as many as 837 gene variants have shown an association with predisposition to hypertension (Dai et al., 2013) from genome wide association studies (GWAS) and candidate gene approaches (Franceschini et al., 2013). The Hypertension Obesity Diabetes Database (http://www. bws.iis.sinica.edu.tw/THOD/ accessed on July 27, 2015) enlists as many as 991 genes and 255 candidate single nucleotide polymorphisms (SNPs) associated with hypertension. Nonetheless, the penetrance of the disease is affected by such factors as obesity, excess salt-intake and physical inactivity as these can modulate blood pressure regulation (Williams et al., 2003). The common hypertension predispositional genes include those of Angiotensinogen (AGT), Angiotensin I converting enzyme (ACE), Angiotensiogen II receptor subtype I (AGTRI), Alpha -I- Antichymotrypsin (ACT or SERPINA3) (Whitfield et al., 2009). Among other the metabolic genotypes of

Among the seven CYP 450 enzymes involved in the metabolism of more than 90% of drugs (Guengerich, 2006), the cytochrome CYP2D6 enzyme present in the liver, brain and heart, metabolizes environmental and endogenous substances and their activity is maintained by CYP2D6 genetic variants (Ingelman-Sundberg et al., 2007) Which number as many as 120 (Evans & Relling, 2004). The Glutathione-S-transferase genes comprise a multigene family of metabolic enzymes which reduce the activity of endogenous and exogenous electrophilic compounds, including potentially toxic carcinogens, by making them water-soluble and favouring their elimination (Hayes et al., 2005). There are eight GST gene classes viz. alpha (GSTA), mu (GSTM) and omega (GSTO) and their enzymes provide variability in metabolizing capacity. The GSTT1 (22q11.2), GSTM1 (1p13.3) and GST P1 (11q13) gene variants confer differential enzyme activity, ranging from reduced activity to complete loss-of-activity (http://www.ncbi.nlm.nih.gov/gene/2950 accessed on June 29, 2015).

However, besides the heritability component of 56-57% depending upon the average of multiple blood pressure measures in hypertension (VanRijn et al., 2007), the non-genetic factors are bigger contenders for inducing hypertension (Bhan et al., 2010). More often, environmental factors and an individual’s biological characteristics interplay in the causation of hypertension (Doris, 2002). The processes of inflammation and induction of oxidative stress, by increased production of free radicals and depletion of antioxidants, causing cellular damage underline a host of diseases. There is oxidative macromolecular damage in degenerative conditions like Amyotrophic lateral sclerosis, Alzheimer’s disease, Friedreich’s ataxia, cancer (Roberts et al., 2009), cardiovascular diseases (Elahi et al.,2009) diabetes (Halder et al., 2015), kidney disease (Tucker et al., 2015) and also hypertension (Rubattu et al., 2015).
The excessive built-up of free radicals from insufficient antioxidants creates an imbalance resulting in oxidative stress (Lobo et al., 2010). The tendency of reactive oxygen species to oxidize cellular macromolecules, primarily lipids, DNA and proteins, results in oxidative damage manifesting in the disease-state (Deavall et al., 2012).

Lipid peroxidation manifested as malondialdehyde levels (Shahzad et al., 2012), increased oxidant status (Deoghare et al., 2014), decreased antioxidant capacity (Hendre et al., 2013) as well as oxidation of proteins (Rahal et al., 2014) and even of nucleic acids (Pisoschi and Pop, 2015) have been reported in hypertensive patients. Though damage to cellular macromolecules has a direct effect, the oxidation of nucleic acids causing DNA damage and/or failure of DNA repair has far-reaching, detrimental effects in terms of inducing age-related changes, neurological disorders and malignancies (Rao, 2009) thereby adding to the co-morbidities associated with hypertension. An earlier diagnosis of lesions in the genetic material can assist in appropriate intervention and management strategies.

Only one study relating CYP2D6 polymorphism with hypertension in Egyptian cases has came to attention (Ali et al., 2013). However, clinical outcomes of the drug-therapy in relation to CYP2D6 polymorphism, have been extensively studied (Bijl et al., 2009; Blake et al., 2013; Ayyappadhas et al., 2015; Wu et al., 2015). In the general population, the CYP2D6 polymorphisms have also been well documented in different regions but data on its clinical significance are limited (Teh and Bertilsson, 2012).

On the other hand, a number of studies have reported association of GST polymorphisms with essential hypertension (Lee et al., 2012; Dhameja et al., 2013; Ge et al., 2015; Han et al., 2015) though on meta-analysis equivocal or inconsistent results have emerged on the association of genetic variants of GST, T1, M1 and P1 with hypertension.

Hence, the inter-ethnic differences in allele frequencies of CYP2D6 (Abraham and Adithan, 2001; Bernard et al., 2006; Yasuda et al., 2008) and of GST (Buch et al., 2001; Polimanti et al., 2013, Sharma et al., 2014) in population groups from different parts of the world and with even intra-ethnic differences are important for studying their allelic distributions and to better understand their biological significance (Miranda-Vilela et al., 2010).
Against this backdrop and in view of the prevalence of hypertension in the state of Punjab even in the rural areas, the present case-control study was planned to investigate genetic damage and oxidative stress in hypertensive patients and normotensive healthy controls genotyped for some variants of the CYP2D6 and GST genes.

DNA damage can be assessed in different tissues and diverse sensitive assays are available. However the assessment of DNA damage in peripheral blood leukocytes (PBL) is appropriate for investigations on hypertensive patients. Peripheral blood leukocytes as the cell population under study, are optimal because of their circulation throughout the body. Therefore other disease-pathophysiological mechanisms in all the body systems as well as the mechanical stress of the pressure of blood on vascular walls, with the potential to induce genetic damage may be manifested in PBL. Other advantages of this cell-population include easy availability of these cells in large numbers without requiring cell cultures; also their diploid nature, being in the same phase of cell-cycles (Collins et al., 2008); they also represent an accurate picture of the in vivo state of cells (Hensley et al., 2012).

A simple, sensitive and reliable technique for assessment of DNA damage at single cell level is Single Cell Gel Electrophoresis assay or the comet assay (Ozkan et al., 2009). Initially, the neutral comet assay was used to assess DNA damage which scores double-strand breaks only (Ostling and Johanson, 1984). The alkaline version of the technique (Singh et al., 1988) has however found favour because it also assesses single-strand breaks, double-strand breaks, alkali-labile sites, DNA-DNA cross links and incomplete excision sites (Moller et al., 2000; Liao et al., 2009). The assay has wide and varied applications and which are not limited to genotoxic testing (Singh et al., 1988; Tice, 1990), repair-kinetics (Olive et al., 1999), population biomonitoring and disease-states (Cortés-Gutiérrez et al., 2011).

Genetic variants of metabolic genes such as GST, CYP2D6, ERCC, XRCC1, TDG, XPA and PON1 have the potential to modulate genetic damage (Dhillon et al., 2011). Disease-susceptibility can also be influenced by metabolic genotypes (Ma et al., 2011). The lipoprotein lipase gene involved in adipose tissue metabolism in dyslipidemia (Liu et al., 2005), leptin gene resistance is related to pathophysiological mechanism in
obesity (http://www.genecards.org/cgi-bin/carddisp.pl?gene=LEP accessed on July 11, 2015). Furthermore, as essential hypertension is a condition requiring life-long drug treatment, inter-individual variation to drug response may also be influenced by CYP2D6 and GST gene polymorphisms. Therefore the contribution of the present study in molecular genotyping of CYP2D6 and GST genes can add knowledge to the genetics of hypertension and also provide a database for susceptible-genotypes of essential hypertension.

The prevalence of hypertension is 51.15% in the state of Punjab (Gupta et al., 2004) and therefore nearly half the population is vulnerable to end-stage renal disease, stroke and other co-morbidities, if uncontrolled. In the Amritsar district its prevalence has been reported as 17.50% (Kaur et al., 2013) while Singh et al. (2014) have reported 35.90% prevalence in urban Sikh population of Amritsar.

The Sikhs comprise more than 60% of the sub-population groups of the state with Jat Sikhs among the majority of (http://www.indiaonlinepages.com/population/punjab-population.html accessed on July 11, 2015). In view of their occupation in agricultural practices, most live in rural areas. Therefore, the study group comprised Jat Sikhs belonging to rural areas of Punjab. It needs further to be emphasized that as Punjab is an affluent state which has rapidly undergone economic transition (Pal and Palacios, 2008) there has been accompanying predominance of sociodemographic factors of age, gender, occupation and educational status, which are crucial in the causation of hypertension (Abdulsalam et al., 2014). This state of affairs has also pervaded the rural areas. Hence it was thought appropriate to undertake the present study in those staying in the rural areas of Punjab.

The present case-control study hence, is an attempt to evaluate genetic damage in peripheral blood leukocytes of unrelated Jat Sikh treated hypertensive patients (n=200) and normotensive (n=200) controls from rural areas of Amritsar district as a function of obesity and dyslipidemia and find any correlation of genetic damage with their genetic profiles of CYP2D6 (*2, *4 and *10) and GST (T1, M1 and P1) variants. Such studies integrating investigations on genetic damage and oxidative stress as a function of some metabolic genotypes are rare. In hypertensive patients and normotensive healthy
persons, such studies documented in literature have only investigated some of these aspects. Rather no such studies have been carried out on the Jat Sikh rural Punjabis.

The significance of ethnic-specific studies lies in distinguishing biological, environmental, or social causes of disease (Gromann and Ginsberg, 2004) rather than the ethno-specific related factors of family income, education and insurance status can influence the etiology and state of hypertension (Frist, 2005). Moreover, ethnic differences of health behaviours, access to health care, and environmental exposures have earlier been also reported to influence hypertension (Cooper, 1998). Also to rule out any bias on the basis of diverse genetic backgrounds for molecular genetic analysis and/or for genetic predispositional confounders of genetic damage, ethno-specific case-control studies have advantage (Sahebi et al., 2013).

In the present study, as a first of its kind, complex gene-gene and gene-environment interactions prevalent in essential hypertensive patients (n=200) belonging to the Punjabi Jat Sikh population sub-group on monodrug Atenolol (beta-Blocker) therapy, have been investigated for genetic damage and oxidative stress and comparison of the study-outcomes made with a matched normotensive control group (n=200)

The case-control study was planned with the following objectives:

(1) to assess from anthropometric variables, the obesity-status of patients and controls,

(2) to investigate DNA damage in patients and controls using the alkaline single cell gel electrophoresis (SCGE)/comet assay,

(3) to study the genomic profile of CYP2D6 (*2, *4 and *10) and GST (T1, M1 and P1) variants in hypertensive patients and normotensive controls,

(4) to examine association, if any, of the polymorphisms of GST and CYP2D6 genes with DNA damage.