INTRODUCTION

Cardiovascular diseases, the diseases of the heart and blood vessels, are predominant causes of morbidity and mortality with the 2030 estimates that among non-communicable disease death>50% will be from cardiovascular disease (Roger et al., 2011). The Cardiovascular diseases (CVD) have inter-related metabolic pathways and include those of lipid metabolism, blood coagulation, blood pressure, inflammation and cell-cycle regulation (Pranavchand and Reddy, 2013). The types of CVD include heart beat irregularities, cardiac failure, coronary artery disease and sudden cardiac death (Park, 2000). In coronary artery disease, damage of the coronary arteries results in atherosclerosis/arteriosclerosis (Ye et al., 2008), and so the disease is known as atherosclerotic heart disease or coronary artery disease (CAD) or coronary heart disease (Fischer et al., 2005).

The accumulation of plaques in the arteries causes atherosclerosis which can lead to myocardial infarction (MI). In fact, the clinical phenotypes of CAD range from stable or unstable less severe angina, to severe types like acute myocardial infarction (AMI), including ischemia (low oxygen supply to heart) and also can cause sudden cardiac death (Kitsios et al., 2011). Hence there is phenotypic heterogeneity of CAD and in fact the sub-phenotypes have distinct genetic etiology (Fischer et al., 2005).

In India, the prevalence of CVD/CAD is 7-13% in urban and 2-7% in rural areas (Gupta et al., 2008) with an overall prevalence of 5.4% (Murthy et al., 2012). Mahajan et al. (2012) documented 1.5-fold significantly (p<0.05) higher CAD prevalence in urban areas as compared to rural areas. The prevalence of CAD in rural population of Punjab was 4.6% with equally affected males and females (Kahlon et al., 2014). The increased prevalence of CAD in Indians is attributed to family history, age, gender, diet, lack of physical activity, diabetes, smoking, hypertension, dyslipidemia and obesity (Pranavchand and Reddy, 2013).

In fact, CAD manifests as a paradigmatic complex disorder resulting from multitude physiologic, genetic and environmental factors (Kitsios et al., 2011). It includes both, modifiable (high blood pressure, cholesterol, overweight/obesity, tobacco use, lack of
physical activity and diabetes) and non-modifiable factors of age, gender, ethnicity and family history (Yusuf et al., 2004; WHO, 2007) with latter, independent of others (Zintzaras and Kitsios, 2006). Among the major risk factors contributing to CAD are smoking, hypertension, increased body mass index (BMI), increased abdominal obesity, dyslipidemia and diabetes (Nadeem et al., 2013). However even patients with normal body weight and central obesity have higher mortality rate from CAD (Coutinho et al., 2013). In fact, obesity is a key player in the pathogenesis and development of cardiovascular disease (Lavie et al., 2009). The life-time risk for CAD increases in middle age (Allen et al., 2012) implying the role of blood pressure in its etiology. Also persons with metabolic syndrome have increased risk for CAD (Malik et al., 2004). The elevated levels of triglycerides and decreased levels of HDL-C (atherogenic dyslipidemia) are an essential component of the metabolic syndrome and have an important role in the progression of the CAD (Marroquin et al., 2004; Iribarren et al., 2006; Rein et al., 2010). Besides abnormal lipid levels, endothelial dysfunction, oxidative stress and chronic inflammation also can influence advancement of atherosclerotic heart disease (Cai and Harrison, 2000). Hence a number of factors with their causal effects pose a risk for the manifestation of the disease.

Besides these factors, the non-traditional disease markers with their genetic make-up and variable behavior in different environments also contribute independently towards the disease-risk (Kitsios et al., 2011). The behavioral risk factors such as smoking-status, sedentary life-style, unhealthy diet and alcohol over-drinking are responsible for almost 80% cases of CAD and cerebrovascular disease (WHO, 2009; Ainsworth et al., 2011). It has also been observed that there is slightly higher CVD mortality associated in individuals with fewer CVD-protective health-habits (Ford and Caspersen, 2012; Matthews et al., 2012).

The American Heart Association in its 2020 impact goals has made commitment to improve cardiovascular health of Americans by 20% and to reduce deaths from CVD and stroke by 20% (Labarthe and Dunbar, 2012). The concept of cardiovascular health relates to seven health metrices which include the four health behaviours of not smoking, being physically active, having a healthy diet-pattern and energy balance by
normal body weight, and the three health factors of optimal levels of total cholesterol, blood pressure and fasting blood glucose without drug treatment.

The etiopathogenesis of atherosclerosis is influenced by environmental and genetic factors, and the latter may have a direct effect on these may act via cardiovascular risk factors causing the development and progression of CAD. Generally, cardiovascular events appear after the fifth decade of life in men and the sixth in women, while the process begins earlier in life during fetal development (Kazim et al., 2009).

Given the multi-staged process of CAD with varied pathoanatomic and clinical presentations (Hansson, 2005), there are many pathways which can contribute to disease-susceptibility and the atherosclerotic disease-spectrum (Westaway et al., 2011). The pathogenesis of atherosclerosis is inter-related to that of other cardiovascular diseases (Clearfield, 2010; Sitia et al., 2010; Frostegard, 2013) beginning with endothelial injury and leading to accumulation and deposition of lipids (Pranavchand and Reddy, 2013). The process of pathogenesis (Nair et al., 2007) therefore includes endothelial injury, increased arterial permeability to plasma lipoproteins and proliferation of smooth muscle cells (SMC) and platelet-aggregation. There occurs accumulation of lipids, fibrous elements and inflammatory molecules on the walls of the large arteries (Sanz et al., 2013; Tabas and Glass, 2013; Sakakura et al., 2013) beginning with the efflux of low-density lipoprotein cholesterol (LDL-C) in the sub-endothelial space where oxidized LDL-C gets fixed by macrophages turning into foam cells (Glass and Witztum, 2001) with the release of cytokines (interleukins and tumor necrosis factor). Fatty streaks develops, containing foam cells in the sub-endothelial space along with accumulation of lymphocytes and mast cells (Libby et al., 2011). SMCs migrate to intima and also form fatty streak (Glass and Witztum, 2001) producing extracellular matrix molecules which create a fibrous cap-cover over the fatty streak to form fibrous plaque (stable or unstable). These projects into the arterial lumen causing flow-limiting stenosis, tissue-ischemia and stable angina. Unstable plaques have macrophages and pro-inflammatory and prothrombotic molecules which expose the core of the plaque to circulating coagulation proteins, causing thrombosis and
sudden obstruction of the lumen of the arteries (Libby et al., 2011; Sakakura et al., 2013) resulting in the acute coronary syndrome.

Family-clustering and twin studies have demonstrated heritability estimates of 30-60% in the development of CAD (Llyod-Jones et al., 2004; Sivapalaratnam et al., 2010; Bachmann et al., 2012). Molecular genetic studies have revealed several Mendelian forms of CAD and candidate gene-based and gene-centric approaches have identified ~300 genes while genome wide association studies (GWAS) have shown 50 genetic loci associated with CAD (Kwon and Goate, 2000; Keavney, 2002; Lieb and Vasan, 2013). The chromosome 9p21.3 locus is the most consistently associated CAD locus across the globe (Roberts and Stewart, 2012) observed in North Indians as well (Kumar et al., 2011) and it also has association with various vascular phenotypes (Schunkert et al., 2008).

In fact, the genetic architecture of CAD is complex because the risk-variants are not related to traditional risk factors alone and the genetic loci explain only ~10% of the heritability of CAD (Schunkert et al., 2011; Deloukas et al., 2013). Ethnic variation is yet another predispositional aspect of the disease (Pranavchand and Reddy, 2013). Therefore the pathophysiology of CAD needs to be addressed by looking for new molecular mechanisms that predispose to it.

The development and progression of atherosclerosis is also mediated via DNA damage induced by oxidative stress (Kaya et al., 2012; Madamanchi et al., 2010) and there is also a contributory effect of DNA repair genes in CAD development (Yu et al., 2014) and of the 8-oxoguanosine glycosylase (hOGG1) variant in the development and severity of CAD (Wang et al., 2010).

Reactive oxygen species (ROS), as an important marker of oxidative stress, have been reported to cause vascular dysfunction via endothelial-dependent vasodilatation, cell growth, inflammation and extracellular matrix formation (Machlin and Bendich, 1987; Pulido et al., 2005). Increased ROS formation was also reported in diseased arterial walls and in plaques (Bagi et al., 2003) and are the most likely agents inducing DNA damage in atherosclerosis (Mahmoudi et al., 2006) as oxidative DNA damage in
atherosclerotic lesions and tissues (Izzotti et al., 2001) has been documented. Botto et al. (2002) reported that in atherosclerosis and coronary artery disease, DNA damage plays an important role. Andreassi and Botto (2003) had observed that oxidative DNA damage could be contributing to development and progression of atherosclerosis. Rather, DNA damage and somatic genetic changes induced by oxidative-stress have been implicated in the development and progression of CAD (Wang et al., 2010; Zhou et al., 2012) which if unrepaired can cause mutations, and lead to disease (Simon et al., 2013). The single cell gel electrophoresis assay is a sensitive method for assaying DNA damage (single strand DNA breaks, double strand DNA breaks, alkali-labile sites, DNA-DNA cross links) at cell level (Collins et al., 2008) and has applications in DNA damage and repair assessment, genotoxicity testing, human biomonitoring, diagnostics and ecological monitoring (Collins, 2004). The modified assay using bacterial enzymes, has the sensitivity to detect oxidative DNA damage (Smith et al., 2006; Collins, 2004).

The presence of chromosomal deletions or duplications in part or whole, loss-of-heterozygosity, microsatellite instability, DNA strand breaks, DNA modifications, and DNA adducts emphasize the role of DNA damage in causing atherosclerotic plaques (Federici et al., 2008). The circulating lymphocytes also have such induced genetic lesions. Lymphocytic DNA damage in CAD patients has been observed by Satoh et al. (2008), Ramakrishnan et al. (2011) and Erkol et al. (2012).

The presence of metabolic genotypes of phase I and phase II genes can however modulate DNA damage (Murgia et al., 2007; Dusinska et al., 2012) and therefore promote/induce/inhibit progression to disease. Hayes et al. (2005) had documented that GST enzymes (glutathione-S-transferases; phase II) are inactivate numerous toxic composites (unsaturated aldehydes, quinines, epoxides and hydroperoxides) which are formed as secondary metabolites during oxidative stress. The GST genetic polymorphisms (GSTM1, GSTP1, and GSTT1) have variations in enzymatic activity by which oxidative stress and downstream genetic damage can be modulated (Hayes and Strange, 2000). Cytochrome P450 (CYP450; phase I) enzymes metabolize xenobiotics and endogenous substances whose metabolites maintain cardiovascular health (Elbekai and El-kadi, 2006; Zordoky and El-kadi, 2010a,b). Genetic polymorphisms of CYP
hence may also be modulating genetic damage. In literature, *GST* and *CYP2D6* genetic polymorphisms, have also shown in the modulation of DNA damage (Izzotti et al., 2001; Salama et al., 2002; Masetti et al., 2003) as well as an association with CAD and CVD (Girisha et al., 2004; Teh et al., 2004; Nomani et al., 2011). Ethnic dissimilarities among diverse populations can furthermore affect the development and progression of CAD (Makin et al., 2002; Chaturvedi, 2003).

Studies from India on genetic damage, genetic polymorphisms and CAD development in conjunction have not come to attention and in fact are limited to studies on genetic polymorphism in CAD (Ramprasath et al., 2011). In the Ramgarhia Sikh sub-group of Punjab, such studies are lacking and the present study was therefore planned in this direction given the affluent lifestyle and culinary practices of the people from this region on one hand, and the endogamous nature of mate selection on the other, which has been sustained at least among those participating in the present study.

Ethno-specific case-control studies have advantages typically required for population stratification, ruling out any bias on the basis of diverse genetic backgrounds for interpretation of molecular genetic analysis and/or of any genetic predispositions as confounders in genetic damage studies (Sahebi et al., 2013). Hence, within this framework and in the absence of studies on genetic damage and genetic polymorphism not coming to attention on CAD patients belonging to Ramgarhia Sikh sub-group from Amritsar district, the present case-control study as one of its kind, was designed to assess DNA damage using the single cell gel electrophoresis assay in CAD patients and healthy controls and to observe any modulatory effects of genetic polymorphisms on DNA damage and oxidative stress.

**Objectives**

The main objectives of the study were:

1. to investigate for DNA and oxidative damage using the standard alkaline and enzymatically-modified Single Cell Gel Electrophoresis assays in coronary artery disease patients (*n*=200) and in healthy controls (*n*=200).
2. to study the genetic polymorphisms of glutathione S-transferase (GSTT1, GSTM1, GSTP1 (A313G) and GSTP1(C341T) and of cytochrome P450 (CYP2D6*2, CYP2D6*4 and CYP2D6*10) in patients and controls.

3. to assess obesity in the study group from anthropometric measurements.

4. to find an association, if any, of genetic polymorphisms with DNA and oxidative damage in the coronary artery disease patients and controls.