REVIEW OF LITERATURE

The present study was carried out to assess genetic damage and whether the genetic damage was modulated by metabolic genotypes and obesity in coronary artery disease (CAD) patients. Genetic damage was assessed in peripheral blood lymphocytes (PBL) using the alkaline single cell gel electrophoresis (SCGE) assay and genotyping of GSTs (GSTT1, GSTM1, GSTP1 A313G and GSTP1 C341T) and CYP2D6 (CYP2D6*2, CYP2D6*4 and CYP2D6*10) was also carried out. Oxidative stress was determined (malondialdehyde, total antioxidant capacity and total oxidative stress) as were lipid levels in blood sera samples.

The literature has been reviewed for these aspects preceded by background information about coronary artery disease.

2.1. Cardiovascular Diseases

Diseases of the heart and blood vessels are known as cardiovascular diseases (CVD) and among non-communicable disease, almost 50% causes of mortality and morbidity are from the heart diseases alone (Pranavchand and Reddy, 2013). CVD are multifactorial resulting from interaction of common genetic and environmental factors and therefore are hard to define. However Pranavchand and Reddy (2013) have attempted to classify them on the basis of which part of the circulatory system involved. Coronary artery disease (CAD) predominates among these as almost 90-95% all cases and deaths result from coronary artery disease.

Classification of Cardiovascular Diseases Conditions

<table>
<thead>
<tr>
<th>Problem associated with</th>
<th>Examples of cardiovascular disease conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart chamber</td>
<td>Congestive heart failure, corpulmonale (pulmonary heart disease)</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>Cardiomyopathy, myocarditis</td>
</tr>
<tr>
<td>Heart valves</td>
<td>Stenosis and regurgitation of mitral, aortic, tricuspid valves, rheumatic heart disease</td>
</tr>
<tr>
<td>Coronary artery/veins</td>
<td>Angina pectoris, myocardial infarction, coronary heart disease/coronary artery disease/ischemic heart disease</td>
</tr>
</tbody>
</table>
Electrical system | Tachycardia, brachycardia, ventricular/atrial fibrillation, long QT syndrome
Heart lining | Pericarditis, endocarditis
Congenital | Septal defects, transposition of great vessels
Others | Myxoma, hypertensive heart disease

Adapted from Pranavchand and Reddy (2013)

2.2. Coronary Artery Disease

Among cardiovascular diseases causing maximum death and disability throughout the world are coronary artery disease (WHO, 2011) and myocardial infarction (Roger et al., 2012). CAD (also known as coronary heart disease) results from chronic inflammation and atherosclerotic plaque build-up in the walls of coronary arteries (Ross, 1999). Myocardial infarction (MI) is a severe outcome of coronary artery disease with atherosclerotic plaque disturbances and coagulation in coronary arteries (Kim et al., 2008) while a common clinical indicator is angina. The characteristic features of CAD therefore include atherosclerosis and the amplification of fibro-fatty plaques (Libby, 2003). However there are differences in phenotypic characteristics of CAD and genetic etiology of the CAD sub-phenotypes also exists (Diemert and Schunkert, 2011).

Sub-phenotypes of CAD with Potentially Distinct Genetic Etiology (Adapted from Diemert and Schunkert, 2011)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Frequency</th>
<th>Heritability ($h^2$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td></td>
<td>0.75</td>
<td>Broeckel et al., 2002</td>
</tr>
<tr>
<td>Plaque rupture/plaque erosion</td>
<td>?</td>
<td>?</td>
<td>Burke et al., 1999</td>
</tr>
<tr>
<td>Left main disease/proximal manifestation</td>
<td>10%-30%</td>
<td>0.47</td>
<td>Fischer et al., 2005</td>
</tr>
<tr>
<td>Diffuse/focal CAD</td>
<td>40%</td>
<td>0.41</td>
<td>Fischer et al., 2005</td>
</tr>
<tr>
<td>CAD with ectatic lesions/coronary aneurysms</td>
<td>5%</td>
<td>0.54</td>
<td>Fischer et al., 2005</td>
</tr>
<tr>
<td>Coronary calcification</td>
<td>15%</td>
<td>0.51</td>
<td>Fischer et al., 2005</td>
</tr>
<tr>
<td>In-stent restenosis</td>
<td>?</td>
<td>?</td>
<td>Shah et al., 2008</td>
</tr>
</tbody>
</table>
2.3. Etiology of CAD

Coronary artery disease is a complex multifactorial, polygenic condition and contributors in coronary artery disease development are genetic and environmental factors. These may be considered as traditional and non-traditional risk factors (Pranavchand and Reddy, 2013). Age, gender, family history, diabetes, smoking, hypertension, dyslipidemia and obesity are traditional risk factors with family history independent of other risk factors and more among older men and post-menopausal women and those belonging to lower or middle social classes and living in urban areas. Behavioral risk factors of smoking, physical inactivity, unhealthy diet and excessive alcohol consumption account for ~80% of cases of CAD and cerebrovascular disease (WHO, 2009) and are related to the four major physio-metabolic changes of hypertension, obesity, hyperglycemia and dyslipidemia (WHO, 2011).

Non-traditional markers include increased levels of homocysteine, fibrinogen, C-reactive proteins and low levels of HDL-cholesterol, insufficient coenzyme Q10 (CoQ10), nitric oxide and vitamins D and K (Kullo and Cooper, 2010) which have independent genetic components ranging from 25-90% (Chilton, 2004; Lusis et al., 2004).

Genetic Determinants- The heritable component in CAD lies between 30-60% as observed in family clustering and from twin studies (Llyod-Jones et al., 2004; Ginsburg et al., 2007; Dandona and Roberts, 2009). Candidate gene-based and gene-centric studies simultaneously with genome wide association studies (GWAS) have revealed ~300 genetic loci associated with CAD (Lieb and Vasan, 2013; Pranavchand and Reddy, 2013). Among these, the 9p21.3 locus has been reported to be consistently the associated CAD locus (Schunkert et al., 2008) and three of its SNPs (single nucleotide polymorphisms) have been observed to be associated with CAD in North Indians also (Kumar et al., 2011). The genetic loci explain only ~10.6% of CAD heritability (Schunkert et al., 2011; Deloukas et al., 2013) indicating the complex genetics of the disease. Genetic predisposition and genetics of non-traditional disease markers related to CAD such as dyslipidemia, hypertension and diabetes also independently can contribute to disease risk (Kitsios et al., 2011).
2.4. Development of CAD

Inflammation plays an important role in the development of atherosclerosis and interleukin 18 (IL-18), a pro-inflammatory cytokine has been observed in unstable plaques (Mallat et al., 2001). Interaction between genetic background and environmental factors results in CAD (Kathiresan and Srivastava, 2012; Stylianou et al., 2012) and oxidative stress and inflammation are involved in its pathogenesis (Sugamura and Keaney, 2011; Tousoulis et al., 2011). Elevated oxidative stress results in oxidized low density lipoprotein (Ox-LDL) which can cause atherosclerotic abrasions by development of foam cells and streaks (Halliwell and Cross 1994). DNA oxidative stress has also a role in the development of atherosclerosis with augmented production of reactive oxygen species (Harrison et al., 2003). Isoprostanes (end-product of lipid peroxidation) also play a role in pathophysiology of the disease and increase in F2-isoprostane has shown association with cardiovascular risk factors and lead to increase in oxidative stress (Young, 2005). The development of atherosclerotic plaques in the coronary arteries occurs from endothelial dysfunction, which causes susceptibility to accumulation and subsequent deposition of lipids as per the injury hypothesis (Mano et al., 1996). In the first stage, the intima (inner layer) of the blood vessels is affected (hardened) which progresses to the media at the bifurcations of major arteries (Tegos et al., 2001). Inflammation and lipid deposition lead to the formation of a hard fibrous cap in the intima (Chilton, 2004) with the process initializing during teenage with onset of symptoms after an interval of about ten years (Pranavchand and Reddy, 2013). Cholesterol is transported in the arterial wall by LDL particles, which are overwhelmed by macrophages and lead to plaque-formation; the latter is associated with atherosclerosis. The plaque breaches over time and triggers blood clotting producing stenosis in blood vessels which if severe, lead to heart attack, stroke and peripheral vascular disease (AHA, 2009). The block in the coronary artery leads to clinical manifestations varying from mild conditions of stable or unstable angina to the severe form of acute myocardial infarction, ischemia with depleted supply of oxygen to the heart, or sudden cardiac arrest (Pranavchand and Reddy, 2013). DNA damage has also been documented in development and progression of atherosclerosis with the
involvement of DNA repair genes in causing susceptibility to CAD (Wang et al., 2010; Gokkusu et al., 2013; Yu et al., 2014).

2.5. Obesity

Obesity is defined as a state, with an excessive accumulation of body fat in the measure that health of the individual may be compromised. It is an epidemic of 21st century and is a major contributing factor for the development of metabolic disorders (Ramachandran and Snehalatha, 2010). According to WHO (2006) there about 2-3 billion adult people would be considered overweight and 700 million would be obese by 2015.

As per World Health Organization (WHO, 2004), overweight is defined as body mass index (BMI) of 23.0-24.9kg/m$^2$ and obesity as BMI $\geq$25 kg/m$^2$. BMI and WC measurements are used to determine general and abdominal obesity, respectively. Obesity is distinguished by having large number of adipose tissue and grow in the size of adipocytes (Jo et al., 2009) which results in over-production of pro-inflammatory cytokines which includes tumor necrosis factor-alpha, monocyte chemotactic protein-1, interleukin-6, leptin and osteopontin affecting chronic inflammation (Zeyda and Stulnig, 2009). A study from Kaplan (2003) showed that together obesity and inflammation have been associated with hypertension, type 2 diabetes mellitus, cardiovascular diseases, cancer, stroke and other psychological problems.

2.5.1. Prevalence

Ogden et al. (2006) showed that in United States the prevalence of obesity increased from 11.00% in men and 16.00% in women during 1960s to 32.00% in men and 34.00% in women by 2003-2004 in adults of the age range of 20-74 years. In the study by Puoane et al. (2002), the results from the first SADHS (South African Demographic and Health Survey) it was observed that more than 29% men were overweight and 56% of women were obese. The study by Mikyung et al. (2010) showed 27.70% (27.2% men and 28.4% women) obesity was prevalent among Koreans.

In Indian population, the total prevalence of overweight was 30.5% (35.0 vs 32.0%) and obesity was 6.8% (7.8 vs 6.2%) being higher in females on comparison with males.
(Kalra and Unnikrishnan, 2012). A cross-sectional survey of the Indian population in Delhi was conducted by Gupta and Kapoor (2010) and observed that the obesity was more prevalent among females (42.7%) compared to males (20.7%).

Obesity according to BMI criteria was 11.90%, but as per the criteria of waist-hip-ratio was 33.80% in Punjabi population in the study reported by Gupta et al. (2012).

Kalra et al. (2011) reported the prevalence of general obesity was (33.6%) and central obesity was (42.10%) in the population of Nepal. As per Gupta et al. (2012), the prevalence of overweight in the Northern, Western, Eastern, Southern and central regions of India was (41.10%) and (45.20%), obesity (8.30%) and (15.80%), waist circumference (35.70%) and (32.50%) and waist-hip-ratio (69.00%) and (83.80%) in men and women, respectively.

2.5.2. Obesity in Association with Coronary Artery Disease

Relevant literature on the same aspect from 2004 onwards is reviewed here.

Abdominal obesity was strongly related to myocardial infarction (Yusuf et al., 2004). A study by Nanchahal et al. (2005) in United States observed that higher BMI and WHR were significantly associated with higher risk of coronary heart disease. Murphy et al. (2006) observed that 15000 middle-aged men and women established the association of obesity with fatal and non-fatal cardiovascular events in the 20 year follow-up study. Khalili et al. (2007) reported that in an Iranian population, the overweight was related to increased acute myocardial infarction risk. As per Baker et al. 2007, a higher BMI during childhood was associated with coronary artery disease in adulthood. Coronary artery disease was significantly higher among abnormally (WHR) obese patients compared to non-obese patients in United States (Siavash et al., 2008). Artham et al. (2009) reported an association of obesity with heart failure, coronary artery disease, arrhythmias. Kaur et al. (2010) reported a significant association of abdominal obesity (BMI and WHR) with coronary artery disease.

In coronary artery disease patients, central obesity was found to be associated with higher mortality rate (Coutinho et al., 2011). A cross-sectional study carried out in Japanese population revealed an independent association of obesity with acute myocardial infarction.
Vanessa et al. (2012) documented that fat accumulation central obesity in the neck region are independent risk factors for coronary artery disease. As per Jin (2013), risk of CAD development was higher in people with central obesity. In individuals with higher body mass index, higher cardiovascular outcomes were reported by Ghoorah et al. (2014).

2.6. Genetic Damage and Coronary Artery Disease

The presence of DNA and chromosomal damage in heart disease patients has been well-reported. The studies have investigated organ damage (in heart besides and muscle cells as well as in plaques) and lymphocytic damage in blood cells. The relevant literature is reviewed here from 2001 to-date.

Chromosomal damage in PBL of patients having coronary ischemic artery disease (group I) or valvular heart disease (group II) was significantly (p=0.02) higher micronuclei (MN) frequency than in group III (control) individuals and the increase in MN frequency was increased with more number of affected vessels being significantly higher (p=0.008) in patients with two-vessel disease compared to those with single-vessel disease (Botto et al., 2001). Positive correlation (p=0.032) of MN was observed with severity of CAD and systolic blood pressure (p=0.009). A relationship between higher MN frequency and CAD severity was also observed.

Patients after percutaneous transluminal coronary angioplasty (PTCA) had higher oxidative DNA damage in their PBL (group I) compared to that in patients who underwent elective coronary angiography (group II) for diagnostics (Andreassi et al., 2002) There were significantly (p=0.04) higher frequency of binucleated cells with MN in the cytokinesis block micronucleus (CBMN) assay in patient groups I and II compared to that in healthy controls (group III). Similarly DNA-single strand breaks (p=0.001), pyrimidine-(p=0.002) and purine (p<0.0001)-damage assessed by the SCGE assay were also higher than in the control group. Significant increase (p=0.004) in Fpg-sensitive sites was observed with increase in affected vessels and also after PTCA. The extent of CAD measured by Duke Score showed significant increase (p=0.0001) in binucleated cells with micronuclei (MNBN) frequency post-PTCA in group I patients and as well as for percent DNA in tail (p=0.0003), probably related to the ischemia-reperfusion injury.
Botto et al. (2002) investigated DNA damage in CAD patients (n=13; males=10 and females=3; mean age 61.80±2.90y) and in healthy controls (n=11; males=6 and females=5; mean age 63.40±2.00y) from Italy, using the comet assay. Results of the study showed significantly (p<0.001) elevated levels of DNA strand breaks (3.6-fold), oxidized purines (6.7-fold) and altered purines (4-fold) in patient group as compared to that in controls. Increase in affected vessel showed positive correlation with elevated oxidized purines (r=0.76, p=0.003). Patients with dyslipidemia showed significantly (p=0.03) elevated (4.2-fold) levels of oxidized purines as compared to values in patients with normal lipid levels.

Andreassi and Botto (2003) in their review article discussed the role of somatic mutations and genetic instability in the pathogenesis of atherosclerosis and suggested that chemo-protective therapeutic agents could be useful in the prevention of oxidative DNA damage in atherosclerosis disease.

Demirbag et al. (2005a) studied DNA damage in peripheral blood lymphocytes of 53 patients with acute coronary syndrome (ACS) in 48 patients with stable angina and in 35 healthy controls using the alkaline comet assay. Plasma levels of total antioxidant capacity (TAC) were also determined. Patients with stable angina had significantly higher damage in comparison to controls. DNA damage was also significantly higher in patients with acute myocardial infarction (AMI) in comparison to patients with stable angina. There were significantly (p<0.05) lower TAC levels in patients with ACS than in the other group (1.21±0.31 versus 1.46±0.29 mmol Trolox eq/l). TAC and D-dimer (marker of thrombotic syndromes) were independent predictors of DNA damage in patients and the authors concluded that increased DNA damage could be related with plaque instability.

In a study on peripheral blood leukocytes of CAD patients, significantly (1.4-fold) higher DNA damage was observed by using alkaline SCGE assay (Demirbag et al., 2005b). There was significantly elevated levels of DNA damage with increasing number of affected blood vessels. Levels of antioxidants (TAC) showed 1.2-fold significantly lower level in CAD patients as compared to levels in healthy controls. The authors concluded that there is negative relationship between TAC levels and DNA
damage, as the TAC lower, DNA damage increases in patients with affected number of blood vessels.

In peripheral blood lymphocytes of congenital heart disease (CHD) patients there was significantly elevated levels of chromosomal aberrations, chromatid breaks, acentric fragments, chromatid breaks and micronuclei frequency as compared to values in healthy controls (Andreassi et al., 2006).

In slow coronary artery flow patients (n=23) there was increase (though non-significant) in DNA damage and lower TAC levels compared to those in 23 controls (Demirbag et al., 2006). Positive correlation (p<0.001) of DNA damage with age and negative correlation with TAC (p<0.001) and HDL-C (p<0.029) levels were observed. The findings indicated no association of slow coronary artery flow with increase in DNA damage and lower TAC levels.

In a review article (Mahmoudi et al., 2006) the authors have stated that atherosclerosis is associated with DNA damage in the circulating cells as well as in the cells of the vessel walls with a likelihood of damage from reactive oxygen species.

Andreassi et al. (2007) observed that radioactive exposure during interventional cardiovascular procedure as in 74 patients caused a significant increase in MN frequency viz. in patients undergoing radiation exposure during invasive cardiovascular procedures. There was also significant increase in baseline levels of MN in females (p=0.004) and in diabetics (p=0.03). It was concluded that chromosomal changes could be induced by burden of low radiations.

Gur et al. (2007a) reported that there was significant increase (p<0.001) in lymphocytic DNA damage and of C-reactive protein (CRP) in patients with cardiac syndrome X (n=23) with significant decrease in TAS in comparison to those with non-cardiac syndrome X (n=21) and in controls (n=20). DNA damage showed correlation with TAS (p=0.017), high-sensitivity C-reactive protein (p=0.006) and diabetes (p=0.006). The authors hypothesized that increase in oxidative stress and chronic inflammation could be a cause of DNA damage in patients with cardiac syndrome X.
In another study, Gur et al. (2007b) investigated lymphocytic DNA damage as an independent predictor of aortic intima-media thickness (IMT) in 70 patients with non-atherosclerotic heart disease. On the basis of thickness of thoracic aorta, grade 1 was normal with grades 2-4 having increasing thickness. There was significant (p<0.001) increase in DNA damage with increasing IMT. IMT also had significant (p<0.001) correlation with DNA damage, high-sensitivity C-reactive protein (p<0.015), total antioxidant status (TAS, p<0.001), total cholesterol (TC; p<0.001) and LDL-C (p<0.001) on bivariate correlation analysis while there was negative correlation of TAS level with DNA damage (p<0.001).

Nair et al. (2007) investigated DNA damage by using a 32P-postlabeling-immune affinity method to estimate 1, N 6-ethenodeoxyadenine (εdA) and 3, N 4-ethenodeoxycytosine (εdC) in smooth muscle cells (SMC) of abdominal aorta of atherosclerotic patients (n=13) and in healthy controls (n=3). Atherosclerotic smokers had higher εda (p=0.06) and εdc (but not significant) levels than ex-smokers. There was significant increase in εdc levels in ex-smokers (p=0.03) and in smokers (p=0.07) in comparison to non smokers though levels of εda or εdc showed poor correlation with 8-hydroxy-2'-deoxyguanosine (8-OHdG) but positive correlation with aromatic DNA adducts.

The relation of increased chromosomal damage using the CBMN assay in patients with CAD was studied by Federici et al. (2008). There was significantly (p=0.04) increased MN frequency in patients with acute coronary syndrome than in those with angina. Significant association of MN was observed with diabetes (p=0.02) and with post-coronary artery bypass grafting (p=0.03). The upper tertile of MN showed significant (p=0.03) association with higher risk for cardiovascular events with two-fold increase hazard ratio. It was concluded that stratification of CAD could be done with stratification of chromosomal damage.

Satoh et al. (2008) in peripheral blood of metabolic syndrome (MS) patients with CAD (n=57; AMI=26, stable angina pectoris AP=31) and of healthy controls (n=21) analyzed circulating endothelial progenitor cells (EPCs) for oxidative DNA damage and telomere shortening. CAD patients showed significantly (p<0.01) higher level of 8-hydroxyl
deoxyguanosine; AMI case had more damage than AP cases as mean fluorescent intensity (MFI) of EPCs. CAD patients had significantly (p<0.01) lower telomerase activity in EPCs than in controls whereas within the patient group, AMI patients had significantly (p<0.05) lower values than AP patients. Patients with MS showed significantly (p<0.05) higher 8-hydroxyl deoxyguanosine levels and lower telomerase activity and telomere length than in patients without metabolic syndrome. It was also observed that in CAD patients, oxidative DNA damage and MS were significant independent predictors of telomere shortening.

On comparing levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and biopyrrins in patients with CAD (n=70) and without (n=61) ischemia heart failure, Nagayoshi et al. (2009) observed 8-OHdG and biopyrrins levels to be higher in CAD patients. It was also observed that 8-OHdG urinary levels regulated severity of atherosclerosis while there was no association of biopyrrin levels with severity of affected vessels. Dyslipidemia was however also higher in CAD patients.

Simon et al. (2010) investigated the relation of biochemical and epidemiological risk factors with DNA damage in patients with CAD and the cardio-metabolic syndrome (n=72) and in healthy subjects (n=40). There was high frequency of binucleated cells with micronuclei (BNMN) in patients and though diabetes, smoking, alcohol-intake, hypertension, dyslipidemia, abdominal obesity, sedentary life style, non-vegetarian diet, high socioeconomic status and residing in urban area influenced the MN frequency, it was not of statistical significance. Dyslipidemia was however significantly (p<0.05) higher as were total-C, TG and LDL-C levels in the patient group compared to values in control group whereas HDL-C levels were significantly (p<0.05) lower in the former.

Ramakrishnan et al. (2011) assessed DNA damage in patients with CAD (n=56) using the CBMN assay and reported significantly (p<0.001) 3.6-folds higher frequency of BN with MN (increasing significantly from those with no affected vessels) in comparison to controls (n=25). Hypertension and diabetes showed significant (p<0.01) correlation with MN frequency while male patients had increased MN frequency compared to that in female patients.
Lymphocytes of CAD patients (42-78y) did not show increased chromosomal damage and proliferation index as assessed with the CBMN assay (Erkol et al., 2012). On treatment with H$_2$O$_2$, the cells showed no changes in the distribution of MN frequency. It was concluded that levels of chromosomal instability and proliferation index did not discriminate CAD patients from controls.

Kliemann et al. (2012) have documented that genetic damage could be a useful predictor for CVD risk on the basis of a study carried out on 34 children and adults. Hypertension showed significant positive correlation (p=0.031) with increased CVD risk. Patients with moderate and high CVD risks showed significantly higher level of LDL-C and total-C whereas triglyceride (TG) level was significantly higher in patients with high CVD risk in comparison to those with low- and moderate- risks. There was two-fold increase in damage index (DI) and damage frequency (DF) in moderate from lower level risk group individuals; in the high-risk group there was two-fold increase in DI and DF than in the moderate risk group. There was no effect on increase in MN, nucleoplasmic bridges, and nuclear buds with increased CVD risk. BMI, body fat, total-C, LDL-C and TG showed positive correlation with DNA damage. There was a two-fold increase in DNA damage in hypertensive subjects with effect of DI (p=0.031) and DF (p=0.005) in comparison to normotensive subjects.

### 2.7. DNA Damage in Plaques, in Blood Vessels and in Heart Tissues

Smooth muscle cells (SMC) from tunica media were assessed for levels of DNA adducts in 76 cases with frequent atherosclerosis changes and in 57 controls (with fewer changes). There was significant increases in DNA adducts (p<0.001), total-C (p<0.001) and LDL-C (p<0.01) in case group in comparison to controls with smokers having elevated levels of DNA adducts as compared to non-smokers in the control group while TG, high density lipoprotein (HDL-C) and vitamin A levels showed no significant differences. Plasma levels of cotinine (p<0.01) and LDL-C (p<0.05) showed significant relation with DNA adducts. Vitamin A levels showed significant (p<0.05) negative correlation with DNA adducts while GSTM1 showed no effect on DNA adducts but NAT2 showed borderline significance (p=0.068) in cases. LDL-C (p<0.001), vitamin A (p<0.01) smoking behavior (p<0.05) and NAT2 (p<0.05) showed significant effect in
the development of DNA adducts. The authors concluded that formation of DNA adducts could lead to development of atherosclerosis (Binkova et al., 2002).

On investigating loss of heterozygosity (LOH) and microsatellite instability in 30 atherosclerotic lesions from myocardial infarction autopsies, Hatzistamou et al. (1996) had reported the presence of microsatellite instability in 33% LOH cases and in 23% of cases. The authors summarized that LOH and microsatellite instability could be involved in the development of atherosclerosis probably through microsatellite somatic mutations.

Spandidos et al. (1996) had documented 20% microsatellite instability (of one out of seven microsatellite markers) in 30 specimens of atheromatous plaques and the authors suggested that decreased fidelity in DNA replication and repair could be associated with disease development.

Atherosclerotic plaques had increased DNA strand breaks as well as oxidized lipid deposits and 55 times higher level of 8-oxo-dg in the carotid artery when compared with the levels in mammary arteries (Martinet et al., 2002). There was further also observed the up-regulation of DNA repair enzymes. It was concluded that oxidative DNA damage and DNA repair increased significantly in atherosclerotic plaques.

2.8. Oxidative Stress and Coronary Artery Disease

Imbalance between oxidants and antioxidant leads to cellular damage which marks the characterization of oxidative stress (Stocker and Keaney, 2004). Antioxidants have protective role against free radicals, which results from reperfusion-induced damage and lipid peroxidation (Ghiselli et al., 2000). Reactive oxygen species (ROS) play an important role in the development of myocardial infarction (Loeper et al., 1991) and cause lipid peroxidation in the membrane (Salvemini and Cuzzocrea, 2003). Oxidative modification of LDL molecules causes atherosclerosis by increased production of oxygen free radicals that further leads to acute myocardial infarction (Boullier et al., 2001). Kumar et al. (2008a, b) observed increased oxidation of LDL, raised malondialdehyde (MDA) levels and of conjugated dienes (probably because of dyslipidemia) and an abnormal lipid profile. Elevated plasma MDA levels were also
associated with inflammatory markers in patients with coronary artery disease (Jung et al., 2004). Antioxidants protect against free radical defense and inhibit thrombosis, myocardial damage and arrhythmia during acute myocardial infarction. The antioxidant scavenger system balances tissue-oxidant and antioxidant activity by superoxide dismutases, catalases, glutathione peroxidases and vitamins A, C and E and other carotenoids (Srinivas et al., 2000).

**Studies on Oxidative Stress and Coronary Artery Disease from 2001-to-date are reviewed here:**

Cavalca et al. (2001) investigated the role of oxidative stress and homocysteine (Hcy) levels in coronary artery disease patients. Significantly (p<0.0002) increased level of homocysteine was observed in patients than its values in controls. The total-MDA and free-MDA levels were significantly higher in CAD patients (p<0.001). It was concluded that CAD had an association with mild/moderate hyper homocysteinemia and with increased MDA concentration as the free MDA levels were different in those with stable and unstable (being higher) angina; the authors suggested that this could be a new diagnostic tool.

Oxidative stress was reported in patients with coronary heart disease though they were clinically stable under medical treatment (Weinbrenner et al., 2003). The patient group (n=22) had significantly (p=0.001) higher (2-folds) levels of plasma oxidized LDL-C (OxLDL) and significantly (p=0.021) lower (2.2 folds) level of autoantibodies against OxLDL, significantly (p=0.032) higher (5 folds) activity of superoxide dismutase and glutathione peroxidase (GSH-Px; 1.9-folds, p<0.001) as compared to controls. It was reported that there was increased risk of having CHD in highest tertile of OxLDL as well as with increased GSH-Px levels.

Nabatchican et al. (2014) investigated if a correlation existed between oxidative stress and onset of CAD in early onset CAD patients (n=80, <55y) with mean age of 44.90±4.90y and late onset CAD patients (n=80, >65y; mean age 71.70±2.50y). The FRAP (total ferric reducing antioxidant power) assay evaluated the antioxidant status of plasma, and the activity of paraoxonase (PON1; metabolizes organophosphates and has antioxidant activity) was also determined. Levels of TG, total-C, LDL-C, PON1 activity
and FRAP values did not differ significantly between the early-onset and late-onset CAD patients whereas HDL-C level was lower and LDL-C/ HDL-C level higher in early-onset than in late-onset CAD patients. The FRAP levels in both patient groups were significantly lower than reference level in healthy subjects. The authors suggested that oxidative stress measurements in conjunction with lipid measurements should be monitored in young CAD patients as related to in older subjects.

On investigating association of serum antioxidants with risk of developing CAD, it was found that there was significantly (p<0.001) lower level of vitamin E in the patient group as compared to control values (Rajasekhar et al., 2004). The total-C/HDL-C and LDL-C/HDL-C ratios were significantly (p<0.001) higher and lower HDL-C (p<0.001) levels were observed in the patient group. There was significant (p<0.01) negative association of vitamin E with CHD and it was suggested that vitamin E supplementation could lower the risk of developing CHD.

On investigating the correlation of oxidative stress with CAD and with different risk factors, Vassalle et al. (2004) observed significant (p<0.001) increased levels of 8-epiPGF2α (marker of lipid peroxidation) in CAD patients with more number of affected vessels. The total oxidant power (PAO) was significantly (p<0.05) lower in CAD patients in comparison to that in controls. There was significant correlation of elevated 8-epiPGF2α and lowered PAO levels with the extent and severity of CAD and with the various risk determinants of atherosclerosis (hypertension, gender, hypercholesterolemia).

Hui et al. (2006) investigated whether oxidative stress and inflammation played a role in rupturing of plaque in patients with coronary heart disease. The lesions included plaques with smooth borders (group I patients), irregular lesions (group II), and long lesions (group III). Significantly higher levels of MDA-LDL and hs-CRP in group II patients were observed and there was also positive correlation with the group II plaques. The authors concluded that oxidative stress and inflammation could be causative in plaque rupture.

Risal et al. (2006) assessed association between oxidative stress and cardiovascular disease (CVDs). Results of the study showed elevated levels of thio barbituric acid
reactive substance (TBARS) in CVD patients as compared to controls, whereas total antioxidant activity (TAA), Vitamins C and E levels were lower in CVD patients as compared to controls. The authors concluded that in cardiovascular disease, oxidative stress is not an etiological factor instead it is a consequence.

A study was carried out in Serbia by Kotur-Stevuljevic et al. (2007) to investigate the relationship between oxidative stress (MDA, O2-, SOD) and high sensitivity C-reactive protein (hsCRP) in CAD patients. It was revealed that there were increased levels of MDA and superoxide anion (O2-) in combination, in CAD patients; there was also significant association of MDA and O2- with CAD development. It was concluded that oxidative stress is involved in atherosclerosis development that leads to CAD progression.

In a case-control study, Sarkar et al. (2007) reported that there were significantly (p<0.001) higher levels of LDL-C and MDA and lower levels of HDL-C in CAD patients with alcohol-drinking and non-alcoholic drinking habits when compared to controls. There was significantly (p<0.001) lower concentration of glutathione peroxidase (GPx) and Superoxide dismutase (SOD) in high alcoholic CAD patients than in non-alcoholic CAD patients and controls. Se, Zn and Cu were significantly lowered (p<0.001) in CAD patients with high alcohol consumption. There was negative correlation of MDA with Se and Zn levels but positive correlation with Cu levels in both, alcoholic and non-alcoholic CAD patients. The low antioxidant and trace elements levels in alcohol drinkers could be making them susceptible to the development of CAD.

The correlation between oxidative stress MDA, nitric oxide (NO), and CAD was investigated by Soydinc et al. (2007) and significant increase in serum levels of MDA (p<0.001) and of NO (p<0.05) in CAD patients (n=45) were observed compared to levels in healthy controls (n=45). However no significant relationship between MDA and NO levels was observed. Also there were no significant differences in levels of NO in CAD patients with one-, two- or three- vessels though hypertensive CAD patients had significantly (p<0.05) higher NO levels than normotensive CAD patients. The authors concluded that the high MDA levels in patients play a role in the relationship between oxidative stress and CAD.
A significant increase (p<0.01) in nitrite/nitrate levels in MI patients was observed compared to values in controls (Surekha et al., 2007) whereas the TAS levels were significantly (p<0.01) lowered in MI patients.

Georgiadou et al. (2008) investigated the relationship between plasma osteopontin and oxidative stress in patients having coronary artery disease (n=71; males=60 and females=11; mean age 61.70±10.00y) and grouped into two groups, group I patients with significant CAD (n=58) and group II with non-CAD (n=13). Osteopontin levels showed independent association with MDA and suggested that this interaction might play a role in the disease-progression.

Guzik et al. (2008) investigated the expression and activity of calcium-dependent nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in human atherosclerotic coronary arteries. The study group comprised of CAD subjects (n=12). CAD patients showed elevated NADPH oxidase 5 (NOX5) proteins as compared to non-CAD individuals and the NOX5 activity was increased 7-folds in CAD patients.

Normolipidemic AMI patients (n=165) were assessed for their oxidative stress and antioxidant status (Kumar et al., 2008a). Significant (p<0.001) increase in malondialdehyde and conjugated dienes levels was observed in AMI patients as compared to controls. Lipid profile parameters (TC, LDL-C and TG) and atherogenic indices (HDL-C/TC and LDL-C/HDL-C) were also significantly elevated in AMI patients.

In another report, Kumar et al. (2008b) reported that the patients with acute myocardial infarction (n=165) but normal lipid profile had significantly lower (p<0.001) endogenous antioxidants (albumin, bilirubin and uric acid) and significantly increased (p<0.001) lipid peroxidation (MDA and conjugated dienes) levels in comparison to values in control individuals.

Maharjan et al. (2008) compared the levels of oxidative stress and lipids in Ischemic heart disease (IHD) patients (n=29; 20 males and 8 females) and controls (n=30; 17 males and 13 females) from Nepal. There were significantly elevated TC (1.1-fold), TG (1.2-fold), VLDL (1.2-fold), LDL (1.2-fold), TBARS (1.4-fold) and significantly lower
HDL-C (1.1-fold) levels as compared to control values. Correlation analysis revealed negative correlation between TBARS and TAA, and positive correlation between TG and TC levels in controls.

Sarkar and Rautaray (2008) reported association of Ox-LDL and paraoxonase in patients with ischemic stroke. In the patient group, Ox-LDL was significantly (p<0.001) elevated and paraoxanase reduced as compared to control values. It was concluded that higher Ox-LDL and lower paraoxonase activity may contribute to the development of oxidative stress which may further lead to ischemic stroke.

Vassalle et al. (2008) assessed oxidative stress levels in elderly coronary artery disease patients (n=69; 73.91% males and 26.09% females) on the basis of gender. Higher levels of serum hydroxyl peroxides (HP) in women were present as compared to values in men. Dyslipidemia showed 5.8-fold increased risk of developing CAD in men.

Oxidative stress and cardiac biomarkers were assessed in patients with acute myocardial infarction (n=50) and in 50 healthy controls (Deepa et al., 2009). Significant (p<0.001) decrease in antioxidants i.e. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH), vitamin C, vitamin E, β-carotene and HDL-C was observed in AMI patients compared to values in healthy controls. Total-C, TG, LDL, VLDL, creatin kinase, creatine kinase isoenzyme MB, C-reactive protein, thiobarbituric acid reactive substances and ceruloplasmin levels were however increased. These observations implied that there was imbalance between oxidants and antioxidants with more values in patients with AMI as compared to values in controls.

Sarkar and Rautaray (2009) investigated the relationship between paraoxonase and lipid profile in patients (n=100) with ischemic stroke and controls (n=100). The patient group had significantly increased total-C, TG and LDL-C levels and lower paraoxonase and HDL-C levels in comparison to controls.

Paraoxonase (PON1) activity, MDA levels and protein oxidation were evaluated in patients with coronary artery disease (Mohan and Priya, 2010). Significant (p<0.001) increase in 8-isoprostane, protein carbonyl, MDA and significantly (p<0.001) lower paraoxonase activity in CAD patients was observed compared to control values. It was
suggested that lower paraoxonase activity lessens the cardio-protective effect of HDL-C and raises the risk of cardiovascular complications and makes these risk factors in patients with CAD.

Sezen et al. (2010) investigated the correlation of oxidative stress in coronary artery ectasia (CAE, a rare abnormality of coronary arteries, commonly seen with CAD occurrence at the same time) in 44 CAE patients. Significantly (p=0.018) increased levels of TAS were observed in CAE group than in individuals with normal coronary artery. TOS (p=0.037) and TAS (p=0.039) levels independently related to CAE while no differences in oxidative stress were observed within the CAE group.

Life-style modification (LM) of six months was studied on lipid profile, oxidative stress and serum-stimulated human coronary artery endothelial cells (HCAEC) viability in CAD (n=30; 15 with and 15 without interventions) patients (Srimahachota et al., 2010). Oxidized LDL, protein carbonyl cholesterol, TG and ROS levels showed decreases while the HCAEC viability increased in the group with life-style modification (low fat, high antioxidant, fiber diets, moderate exercise and stress management). The authors concluded that LM could improve the lipid profile, reduce oxidative stress, and hence had a lower risk for future cardiovascular events in patients with CAD.

In another study, Jawalekar et al. (2010) investigated the involvement of oxidative stress parameters and of lipid profile in atherosclerosis. There were significantly (p<0.001) elevated TG, total-C, LDL-C and VLDL-C levels while lower HDL-C levels were observed in the patient group as compared to control values. The total-C/HDL-C, TG/HDL-C and LDL-C/HDL-C ratios were also elevated in patients. MDA levels and carbonyl content were significantly (p<0.001) higher in CAD patients as compared to controls. The authors concluded that there is the role of oxidative stress in cardiac- and vascular-abnormalities.

In a case-control study carried out by Rahsepar et al. (2011) for association of pro-oxidant-antioxidant balance with extent of coronary artery disease, significantly (p<0.001) lower value of pro-oxidant-antioxidant balance (PAB) in controls was observed as compared to that in the patients group implying higher levels of oxidative
stress in CAD patients which was however not related to extent of CAD (stenosed vessels).

Rao and Kiran (2011) investigated the association between elevated oxidative stress and lipid profile in patients with CAD and in healthy controls. Significantly (p=0.001) elevated MDA, LDL-C, TG and total-C levels were present in patients compared to values in controls whereas HDL-C was significantly (p=0.001) lower in CAD patients as compared to controls. MDA level showed insignificant but positive correlation with Total-C, LDL-C, HDL-C and negative correlation with TG. It was concluded that elevated oxidative stress and abnormal lipid profile may play an important role in the atherosclerosis.

In a case-control study, Kim et al. (2012) checked levels of urinary 8-iso-prostaglandin F2α (8-iso-PGF2α) and 8-hydroxy-deoxyguanosine (8-OHdG) and their use as oxidative stress markers in CAD patients. The study comprised CAD (n=35) and non-CAD cases with stable angina (n=69). CAD patients showed significantly (p=0.001) 1.5-fold elevated 8-iso-PGF2α levels as compared to non-CAD patients whereas 8-OHdG levels were not different between the two groups as well as in patients with single- and multi-vessel CAD. It was concluded that there was independent association of 8-iso-PGF2α with significant CAD.

In an interventional study, Lee et al. (2012) investigated the effect of coenzyme Q10 on oxidative stress and antioxidant enzyme activity in patients with CAD. Results of the study showed negative correlation of coenzyme Q10 concentration with MDA levels (r=-0.35, p=0.02) and positive correlation with CAT and SOD (r=0.43, p=0.01; r=0.039, p=0.01 respectively). Plasma coenzyme Q10 to total cholesterol ratio showed significant (p=0.02) correlation only with SOD activity whereas plasma coenzyme Q10 to LDL-C showed significant positive correlation with CAT and SOD (r=0.35, p=0.04; r=0.45, p=0.01 respectively). Glutathione peroxidase activity showed no relationship with coenzyme Q10 concentration. It was concluded that supplementation of coenzyme Q10 (150mg) can lower oxidative stress and increase antioxidant enzyme activity in patients having coronary artery disease.
Movahed et al. (2012) investigated correlation of serum bilirubin and levels of MDA in CAD patients. The patient group had significantly (p<0.0001) lower levels of HDL-C in comparison to controls, though TG was elevated in patients (but not significantly) whereas TAC, MDA and homocysteine levels showed significantly (p<0.001) high levels in patients as compared to the control group. There were significantly higher levels of total bilirubin (p<0.001) and direct bilirubin (p<0.02) in controls as compared to patients. MDA levels showed significant negative correlation with TAC (p=0.001), bilirubin (p=0.002) and HDL-C (p=0.01). There was positive correlation of bilirubin with TAC (p=0.01) and HDL-C (p=0.03) but negative with homocysteine (p=0.01).

Young patients (n=42) with acute myocardial infarction and smoking habits were investigated for oxidative stress (Aksoy et al., 2012). Among the oxidative stress markers of paraoxonase (PON), arylesterase (ARE), total antioxidant capacity (TAS), total oxidative stress (TOS), oxidative stress index (OSI) and lipid hydro-peroxidation, the patients had significantly higher levels of TOS and OSI as compared to values in controls. Median TOS levels in patients (6.0μ mol H$_2$O$_2$ eq/l) was very significantly (p<0.0001) higher than in controls (4.1 μ mol H$_2$O$_2$ eq/l) whereas TAS showed significantly (p=0.02) higher levels in controls (1.7±0.1 μ mol Trolox eq/l) as compared to levels in CAD patients (1.6±0.1 μ mol Trolox eq/l). A positive correlation of OSI with TOS and negative correlation with TAS was observed; with severity of CAD, there was a significant and positive correlation with OSI and TOS. In fact, OSI was the only predictor of CAD severity among the various indices investigated. The authors observed that oxidative stress plays an important role in CAD pathogenesis in young smokers.

Vassalle et al. (2012) investigated whether there was any association of reactive oxygen metabolites (ROM) with mortality rate and adverse cardiovascular events in CAD patients. There was significant (p<0.01) increase in ROM levels in multi-vessel disease in comparison to those with one-vessel disease and levels were also higher in females. The authors suggested that in CAD patients, ROM could serve as an additional prognostic tool for cardiovascular events in CAD patients.

In a study by Bhat et al. (2013) significantly (p<0.001) elevated levels of lipids (TC 1.3-fold, TG 1.3-fold, LDL-C 1.7-fold, VLDL-C 1.3-fold), MDA (2.1-fold), TOS (3.2-fold)
and lower TAS (1.4-fold) and HDL-C (2.4-fold) levels in CAD patients (n=31) as compared to levels in healthy corresponding controls (n=19). The authors suggested that an imbalance between oxidant and antioxidant levels in CAD patients in coronary artery disease can cause other pathological conditions.

In a case-control study, of CAD cases (n=30) and healthy controls (n=30) to assess oxidative stress and lipid profile, Kulkarni and Phalak (2013) reported that the patients had significantly (p=0.0001) elevated total-C, LDL-C and TG levels but lower HDL-C levels as compared to values in healthy controls. Significantly higher levels of MDA were also observed and there were significantly (p=0.001) lower reduced glutathione levels in patients as compared to control values. It was concluded that increased oxidatively-modified LDL with elevated TG, and lower HDL-C with oxidative stress, are engaged in the development of CAD.

2.9. Glutathione S-Transferases

The glutathione S-transferases GSTs (EC 2.5.1.18) are a multigene family of the phase II metabolic enzymes involved in the detoxification and activation of a large variety of chemicals (Olvera-Bello et al., 2010). GSTs bind with toxins, detoxify endogenous compounds and inactivate secondary metabolites like endogenous end-products formed during oxidative stress (Sireesha et al., 2012). Xenobiotic metabolizing enzymes with inherited genetic polymorphisms have important roles in an individual’s susceptibility to various diseases (cancers, asthma, coronary artery disease, and atherosclerosis) and on drug effects (Mo et al., 2009). GSTs provide targets for antiarrhythmic and antitumor drug therapies (Matsushita et al., 1998) and also metabolize cancer chemotherapeutic agents, insecticides, herbicides, carcinogens and oxidative stress by-products. Nucleophilic attack of the sulphydryl group of reduced glutathione (GSH) on electrophilic substrates (having electrophilic carbon, nitrogen or sulphur atom) lowers the activity against cellular macromolecules (Armstrong, 1997). There are two binding sites on active GST; G-site and H-site. G-site is for glutathione-binding and the later is for substrate-binding. In humans, GSTs are classified into eight types; Alpha (GSTA) on chromosome 6, mu (GSTM) on chromosome 1, Pi (GSTP) on chromosome 11, Theta (GSTT) on chromosome 22, Tau (GSTZ) on chromosome 14, Sigma (GSTS) on
chromosome 4, Omicron (GSTO) on chromosome 10 and Kappa (GSTK) on chromosome 7 (Katoh et al., 2008) with additional sub-classes in each class (Townsend and Tew, 2003). There are three families of GSTs viz; cytosolic mitochondrial GSTs and membrane-associated protein in eicosonoid and glutathione metabolism (MAPEG). The first two are soluble enzymes and play an important role in defense against chemicals and oxidative stress.

### 2.10. **GST Genetic Polymorphisms and Coronary Artery Disease**

The genetic polymorphism of GST genes have also shown association with heart disease/CAD and reports on these studies are described here.

In a case-cohort study by Li et al. (2000), association of GSTM1 and GSTT1 genotypes with smoking-related CHD was studied. There was higher CHD risk associated with smoking among the GSTM1 null compared to the GSTM1 present genotypes. The GSTT1 present genotype was however weakly protective in non-smoker CHD patients.

An increased rate of developing atherosclerosis was observed in smokers with the GSTM1 null genotype (de Waart et al., 2001). Progression of atherosclerosis was measured after Vitamin E/placebo-conducted trial over two years. Smokers with the null genotype had a higher progression measured as the common carotid intima media thickness. Vit E group revealed no effect of GSTM1 genotype on atherosclerosis progression and the authors concluded that smokers with the GSTM1 null genotype had rapid development of atherosclerosis.

Salama et al. (2002) investigated susceptibility of GSTM1, GSTT1, CYP2E1, mEH, PON1, and MPO with atherosclerosis. Study comprised smokers with atherosclerosis (n=51; male=30, female=21, mean age 48.50±10.80), controls with smoking habit (n=28; male=17, female=11, mean age=49.20±11.20), non-smoker controls (n=21; male=13, female=8; mean age=47.50±9.80) for chromosomal aberration study group, whereas atherosclerotic patients with smoking habit (n=120; male=66, female=54; mean age=58.30±10.80), controls with smoking habit (n=90; male=47, female=43;
mean age=58.00±11.30). Results of the study showed 15.4-fold risk of developing atherosclerosis in individuals with *GSTM1* null genotype in combination with *CYP2E1*5B, whereas *GSTM1* null genotype in combination with *mEH* YY only showed 3.48-fold, and *GSTT1* null in combination with *mEH* YY showed slightly lower (3.4-fold) risk of developing atherosclerosis. Smokers with *GSTM1* null showed higher frequency of chromosomal aberrations.

Higher frequency of *GSTM1* null genotype in controls (52.70%) was noticed compared to that in patients (41.20%) and the authors (Wilson et al., 2003) suggested that the null genotype provided significantly protection from CAD development. Smokers and non-smokers showed similar pattern of respective genotype distribution with *GSTM1* null higher in controls (51.20% and 60.00%) as compared to patients (37.00% and 46.20%). *GSTT1* presented no significant association with CAD and hence the authors proposed that *GSTM1* null played a role against CAD-development.

Girisha et al. (2004) studied the association of *GSTT1* and *GSTM1* polymorphisms with CAD in North Indian population from Lucknow, India. The study group comprised 200 patients (male=169, female=31, mean age 44.31±12.11y) and 200 healthy controls (male=163, female=37, mean age 44.58±13.36y). There were 2.4-fold more *GSTT1* null genotypes in controls whereas; *GSTM1* null was only 1.1-fold higher in patient group. *GSTM1* genotype showed no significant association with CAD while *GSTT1* null showed protection against CAD.

Tamer et al. (2004) reported significant association of null *GSTM1* (1.1-fold) and *GSTT1* (1.4-fold) null genotypes for higher risk of developing coronary heart disease. Patients with smoking habit and *GSTM1* null genotype had 1.6-fold increased risk of developing CHD, while higher risks were observed in those with the *GSTT1* null (2.7-fold) and *GSTM1/GSTT1* null genotypes; (3.2-fold). The authors concluded that smoking in association with *GSTM1* and *GSTT1* null genotypes could influence CVD as well as DNA damage, the latter by direct binding to form DNA adducts.
Park et al. (2004) documented higher frequencies of GSTM1 null (60.00%) and GSTT1 null (52.50%) in patients with rheumatoid arthritis and the GSTM1 null in combination with GSTT1 null were 35.00%. The GSTP1 variant types (Ile/Val and Val/Val) also had 35.00% frequency. It was observed that the GSTT1 null genotype showed significantly (p<0.04) higher intima-media thickness (IMT) as compared to that in the GSTT1 present genotypes. The GSTM1 null also showed higher IMT but results were only marginally significant. However, GSTM1 null in combination with GSTT1 null showed significantly (p<0.008) higher IMT as compared to it in the GSTM1 and GSTT1 present genotypes. Univariate regression analysis also showed a significant association of GSTT1 null genotype with IMT.

GSTT1 null genotype was a strong predictor of untimely cardiovascular morbidity and mortality in type -2 diabetes patients (Doney et al., 2005). GSTT1 null individuals with smoking habits showed significant (p=0.001) 2-fold increased rate of cardiovascular events as compared to individuals with GSTT1 present genotype with smoking habit while there was 2.7-fold increased risk with smoking habit as compared to GSTT1 null genotype individuals with smoking habits and also when compared to GSTT1 present individuals with smoking habit. The GSTT1 null genotype also showed significant association with diabetic retinopathy (p=0.005) and nephropathy (p=0.01).

An association of genetic polymorphism of GSTM1, GSTT1 and CYP1A1 with smoking-related CAD was observed by Manfredi et al. (2007). The study group comprised CAD patients (n=169) and non-CAD (n=53) controls. CYP1A1 genotypes were not significantly different between the two groups. GSTM1 (58.60%) and GSTT1 (43.80%) null showed higher frequency in CAD patients as compared to the non-CAD group (45.30% and 24.50%, respectively). The GSTM1 null in combination with GSTT1 null genotypes had 3.9-fold increased risk of CAD. Smokers with GSTM1 and GSTT1 null showed 1.4 times higher number of stenosed vessels as compared to smokers with GSTM1 and GSTT1 present genotypes. It was concluded that there is increased susceptibility to smoking-related CAD in smokers.
Murgia et al., (2007) carried out a case-control study comprising 39 CVD patients (age range 42-86y; mean age 67.90±8.80y) and 67 healthy controls (age range 52-78y; mean age 64.60±5.70y). There was significant (p=0.006) increase in MN frequency in the cases and significantly (p=0.036) lesser frequency of \( GSTT1 \) null genotype was observed in CVD patients. \( GSTT1 \) positive genotype showed 6.3-fold increased risk of developing CVD. There was significantly (p=0.001) higher mortality risk for CVD in individuals with elevated MN frequency.

In a similar type of study, Kim et al. (2008) investigated the effect of \( GSTT1 \) and \( GSTM1 \) on smoking-related CAD in 775 subjects from Korea who were divided into three groups with group ‘A’ having much higher risk of CAD than groups ‘B’ and ‘C’. \( GSTT1 \) and \( GSTM1 \) null presented no significant alteration among three groups. The \( GSTM1/GSTT1 \) null genotypes with smoking habit exhibited 2-fold increased risk of CAD than non-smokers with \( GSTM1 \) non-null genotype. On collective effect of \( GSTT1 \) null and \( GSTM1 \) null, the risk of having CAD was 2.8-folds higher. It was suggested that \( GSTT1/GSTM1 \) null genotypes added towards the pathogenesis of CAD in smokers.

In a case-only study carried out on 231 patients having type2 diabetes (males 63.60% and females 36.40%; mean age 66.10±9.70y), out of which 80.00% had CAD. (Manfredi et al., 2009), significant relation of coronary atherosclerosis development with male gender (p<0.001) and smoking (p=0.003) was observed. There was 3.1-fold increased risk of developing 3-vessels CAD in individuals with both \( GSTT1 \) and \( GSTM1 \) null genotypes while \( GSTM1 \) null-\( GSTT1 \) null genotypes and smoking showed significant (p=0.03) interaction with increased number of diseased coronary vessels. The authors concluded that \( GSTT1 \) and \( GSTM1 \) gene polymorphisms are risk factors in developing CAD in type 2 diabetic patients with smoking habits.

In a study on 172 patients (males 56.40% and females 43.60% with mean age of 66.60±14.80y) and 105 healthy controls (males 49.50% and female 50.50% with mean age of 64.50±12.80y) Turkanoglu et al. (2010) investigated \( GST \) polymorphism and GST activity and its association with ischemic stroke. The \( GSTT1 \) null genotype had
about 5 times likelihood of hypertensive stroke compared to 2.4-fold risk in \( \text{GSTM1} \) null genotype showed lowest serum activity of GST in comparison to subjects with \( \text{GSTT1}/\text{GSTM1} \) present genotype. The results revealed that the \( \text{GSTT1} \) and \( \text{GSTM1} \) null genotypes and the hypertensive state may have significance in ischemic stroke development.

Based on meta-analysis of 19 studies comprising 8020 cases and 11501 controls, Wang et al. (2010) reported a relative risk for CHD of 1.5-fold for \( \text{GSTM1} \) null and 1.3-fold for the \( \text{GSTT1} \) null genotypes. Computation analysis of eight studies revealed 2.38 relative risk of developing CHD in individuals with both \( \text{GSTM1} \) and \( \text{GSTT1} \) null genotypes. The meta-analysis suggested that the \( \text{GSTM1} \) null genotype posed a higher risk for CHD.

The combined \( \text{GSTT1} \) null/\( \text{GSTM1} \) null genotype frequency was significantly (\( p=0.014 \)) lower in CAD patients (3.80%) than (10.20%) in controls as studied by Nomani et al., (2011) in Iranian population. While the \( \text{GSTT1} \)-present genotype had 2.2-fold increased risk for CAD, the frequencies of \( \text{GSTM1} \) and \( \text{GSTP1} \) genotypes did not differ significantly from controls. Significantly higher level of HDL-C was observed in those with the \( \text{GSTM1} \) null genotype as compared to those with the \( \text{GSTM1} \) present genotype. It was concluded that lowered frequencies of \( \text{GSTM1} \) and \( \text{GSTT1} \) null genotypes might have a role in the pathogenesis of CAD in the Iranian population.

Ramprasath et al. (2011) assessed association of \( \text{GST} \) variants with CAD in type 2 diabetes mellitus (T2DM) patients. The group comprised T2DM patients without CAD (\( n=222 \)), T2DM with CAD (\( n=290 \)) and controls (\( n=270 \)). \( \text{GSTT1} \) null genotype showed 1.9-fold higher prevalence in T2DM CAD patients as compared to its prevalence in T2DM patients without CAD. \( \text{GSTT1} \) present showed significantly lower HDL and LDL-C levels as compared to levels in the \( \text{GSTT1} \) null genotypes. \( \text{GSTM1} \) null showed no correlation with lipid levels in both, T2DM CAD and T2DM without CAD patients. It was observed that the \( \text{GSTM1} \), \( \text{GSTT1} \) and \( \text{GSTP1} \) variants could contribute to development of T2DM and that the \( \text{GSTT1} \) variant alone was involved in the development of T2DM-associated CAD in the South Indian population.
Phulukdaree et al. (2012) assessed association of GST polymorphism in young South African Indians having CAD with cigarette smoking and non-smoking habit. GSTM1 null genotype individuals showed significant (p=0.0377) 2.6-fold increased risk of developing CAD. GSTM1 null individual with smoking habit showed significant (p=0.0221) 1.7-fold increased risk of developing CAD. It was concluded that GSTP1 and GSTM1 null genotypes have association with CAD in young South African Indians with smoking habit.

Cora et al. (2013) investigated whether there was any relation between GSTT1 and GSTM1 genotypes in patients with myocardial infarction in Turkish population. The results of the study showed GSTM1 null genotype had a protective role against myocardial infarction, there was significantly (p=0.002) higher frequency of GSTT1 null genotypes in patients having myocardial infarction as compared to control group.

Yeh et al. (2013) reported no association of GSTM1, GSTT1, GSTP1 and GSTA1 variants with CAD in a Chinese population. Though a high prevalence of GSTT1 null genotypes in CAD patients with 1.6-fold increased risk of developing CAD was observed, however after adjustment for age, sex, smoking, alcohol use, diabetes mellitus, total-C and HDL-C levels, no association of GST variants with CAD was observed.

2.11. Modulation of Genetic Damage in CAD Patients by GST Genetic polymorphism

The right atrial appendage of 41 patients who had undergone open heart surgery were analyzed for smoking-related DNA adducts and GSTM1, GSTT1 and vitamin D receptor (VDR) polymorphisms (Van Schooten et al., 1998). Heart tissue of smokers had significantly higher DNA adduct levels compared to those of ex- or non-smokers. Also patients with severe CAD had higher DNA adduct levels in comparison to those with mild CAD. There was no modulation of DNA adduct levels by GST genotypes though the VDR genotype associated with lower vitamin D levels. In contrast VDR genotype showed significant (p=0.025) relation with CAD severity. The study concluded that
there is no relationship between smoking-related, bulky hydrophobic DNA adducts with \textit{CYP1A1 MspI} and \textit{GSTM1} genetic polymorphisms.

Masetti \textit{et al.} (2003) investigated the association of chromosomal damage and \textit{GST} genotypes in CAD patients with/without the smoking habit. The group comprised CAD patients (n=308; 263 males, 45 females) with mean age 61.40±10.40y and control (n=122) non-CAD patients (60 males, 62 females) with mean age of 60.40±11.40y. There was no significant difference in distribution frequency of \textit{GST} among CAD and non-CAD patients. However smokers with \textit{GSTM1/GSTT1} null genotype had 2.2-fold/3.4-fold risk of developing CAD. The \textit{GSTM1} null in combination with \textit{GSTT1} showed 4-fold increased risk of developing CAD after adjustment for sex, dyslipidemia, hypertension, diabetes and family history of CAD. Smokers with \textit{GSTT1} or \textit{GSTT1} in combination with \textit{GSTM1} showed 2.7-fold and 3.1-fold increased risk of developing CAD, respectively. MN frequency showed significantly higher frequency (1.6-fold) in three-vessel diseased patients as compared to those with one-vessel disease. \textit{GSTM1} null showed 1.3-fold higher MN frequency as compared to \textit{GSTM1} and \textit{GSTT1} present genotypes. Smokers with \textit{GSTM1/GSTT1} null showed 2.4/1.4 times higher MN frequency as compared to \textit{GSTM1/GSTT1} present genotypes respectively. Smokers with \textit{GSTT1} and \textit{GSTM1} null genotype showed 3.5 times higher MN frequency as compared to \textit{GSTT1} and \textit{GSTM1} present genotype. Non-smokers patients showed no significant differences. Author’s suggested there is higher risk of CAD in smokers with \textit{GSTM1} null genotype.

Murgia \textit{et al.} (2007) reported that there was higher mortality risk (MR) for CVD in subjects with increase in MN frequency. Results of the study showed that there was 2.4-fold significant (p<0.006) increase in MN frequency in CAD patients as compared to frequency in controls. Subjects with MN frequency >1/1000 showed significantly (p=0.01) lesser survival than MN ≤1/1000 mortality risk. \textit{GSTT1} positive genotype was significantly (p=0.02) higher in patient group although no association of \textit{GSTM1} and \textit{GSTT1} genotypes with MN was observed. There was six fold increase in MN frequency in patients with \textit{GSTT1} positive genotype as compare to \textit{GSTT1} negative genotype. The results showed increased MN frequency in CVD and the authors concluded that MN
could be suitable biomarker for CVD death and that the *GSTT1* positive genotype increased MR for CVD.

### 2.12. GST Genetic Polymorphisms, Oxidative Stress and CAD

Martin *et al.* (2009) investigated 67 CHD cases (mean age 64.00±8.10y) and 63 healthy controls (mean age 56.50±12.80y) for markers of oxidative stress viz. homocysteine level (Hcy), C-reactive protein (CRP), oxidized low density lipoprotein (OxLDL) and total antioxidant capacity (TAOx) levels. There was significantly (p=0.003) higher levels of homocysteine (9.66±3.35) in cases, less in controls and this was also the case for female patients. In male patients, TAOx level showed significantly elevated level (p=0.001) while comparing TAOx level significantly (p=0.0001) in comparison to pooled control females. *GSTM1* null showed 3.8-fold increased risk of developing CHD while protective effect of *NQO1* in CHD patients was observed (OR=0.18). A 3.5-fold increased risk of developing CHD in smokers with *GSTM1* null genotype was there. The authors concluded that deficiency in enzyme activity of *NQO1* and *GSTM1* and biomarker levels were strongly associated with CAD.

### 2.13. Genetic Damage as a Function of GST Polymorphisms

This has also been reported in persons exposed at workplace and those with various lifestyle patterns to find if *GST* genotypes influenced induction of genetic damage. The relevant literature is reviewed here.

In a case-control study carried out by Ichiba *et al.* (1994) on chimney sweeps (n=69) and controls (n=35) to investigate DNA adducts, micronuclei and genetic polymorphisms of *CYP1A1* and *GST1*. In chimney sweeps, there was non-significant increase in DNA adduct levels as compared to controls and also there was no significant difference for DNA adduct levels in subjects with *GSTT1* (-) and *GSTT1* (+) genotypes. However sweeps with *GSTT1* (-) had 50% higher adduct levels as compared to controls and marginally significant (p=0.07) while those with *CYP1A1* m1/m1 and *GST* (-) genotypes had 60% higher DNA adducts (p=0.04) as compared to corresponding controls. *CYP1A1* m1/m2 (Ile/Val) in comparison to individuals with *CYP1A1* m1/m1 (Ile/Ile) genotype also did not had significant difference for frequency of micronuclei in
B or T-lymphocytes. The GST1 (-) sweeps showed no alteration in micronuclei frequency as compared to GST1 (+) sweeps. However, the DNA adduct levels could significantly (r=0.25; p=0.01) with frequency of micronuclei in T-lymphocytes.

Santella et al. (1995) studied the relationship between GSTM1 genotype with polycyclic aromatic hydrocarbon-DNA and protein adducts in coal-tar treated psoriasis patients (n=57; males 56% and females 44%). Patient group showed higher frequency (56%) of GSTM1 present as compared to controls (53%). GSTM1 present controls showed lower protein and DNA adducts but not significantly lowered. In patients with GSTM1 null genotype, there were lower levels of protein and DNA adducts as compared to those in GSTM1 present genotypes. Multiple regression analysis revealed no significant relation of DNA adducts with exposure (p=0.09), smoking (p=0.80) and the GSTM1 genotype (p=0.08).

In a study by Schroder et al. (1995), 1.2-folds higher sister chromatid exchange rate in GSTT1 null genotype subjects was observed compared to GSTT1 positive subjects (p<0.05). Smokers with GSTT1 null genotype also had higher sister chromatid exchange rate.

Hou et al. (1995) investigated aromatic DNA adducts in relation to GSTM1 and NAT2 genotypes in non-smoker bus-maintenance workers exposed to diesel exhaust (n=47; 27-65y) and in controls (n=22; 23-61y). There was no significant difference in DNA adduct levels between GSTM1 null and GSTM1 present genotypes. However, among slow acetylators, there were significantly (p=0.03) higher levels of DNA adducts in GSTM1 null genotyped individuals as compared to the GSTM1 present individuals among slow acetylator.

The association between GSTM1 null genotype with DNA adducts was examined by (Rothman et al., 1995). There were lower levels of DNA adducts in individuals with GSTM1 null genotype (n=28) as compared to those with the GSTM1 present genotype (n=19) though the difference did not reach significant level. The GSTM1 null genotype however was not associated with DNA poly aromatic hydrocarbon- DNA adducts (r=0.03, p=0.84).
Bernardini et al. (1996) reported no significant differences in the mean frequencies of sister-chromatid-exchange per cell in individuals with GSTT1 null (n=10) as compared to individuals with GSTT1 positive genotype (n=14) and also for GSTM1 null (n=10) as compared to individuals with GSTM1 positive genotype (n=15). The sister-chromatid-exchange rate was not associated with GSTT1 and GSTM1 genotypes separately or in combination.

Male lung cancer patients (n=138) and healthy controls (n=297) were studied for levels of hydrophobic DNA adducts (Ryberg et al., 1997). Patients with GSTP1 homozygous mutant (GG) had significantly (p=0.006) 2-fold higher levels of DNA adducts than patients with the homozygous wild type (AA) genotype. GSTM1 null showed association with cancer risk and the GSTM1 null in combination with GSTP1 (AG or GG) showed significant (p=0.011) higher levels of DNA adducts than in those with other groups of combinations.

Elevated base-line chromosomal aberration frequency was detected in individuals with homozygous deletion of GSTM1 gene (n=36) as compared to GSTM1 positive (n=26) individuals. GSTM1 null in combination with GSTT1 null exhibited significant (p=0.012) higher chromosomal aberration as compared to GSTM1 positive- and GSTT1 null- genotyped individuals (Scarpato et al., 1997).

Butkiewicz et al. (1998) investigated the effect of GSTM1, CYIA1 (exon 7) and CYP2D6 polymorphism on DNA adduct levels in healthy males controls (n=170; mean age 39.16±11.98y). Mononuclear white blood cells and granulocytes were collected and DNA adduct levels were measured by 32P-post labeling. In individuals with the GSTM1 null genotype, significantly elevated DNA adducts in WBCs (p=0.045) and in granulocytes (p=0.031) were present as compared to GSTM1 positive individuals. Individuals with CYP2D6 homozygous wild type (p=0.037) as well as heterozygous genotype showed significantly (p=0.014) elevated DNA adducts as compared to levels in the homozygous mutant group. In smokers, the GSTM1 null genotype showed association with DNA adducts.

Ollikainen et al. (1998) analyzed sister chromatid exchanges (SCEs) induced by styrene-7, 8-oxide (SO) in cultured human lymphocytes (n=10; males=6 and females=4,
age range 34-57y) of healthy non-smokers and the influence of \textit{GSTT1} genotype. Results of the study showed 1.7X higher SCEs (p=0.022) in SO-treated cells from individuals with \textit{GSTT1} null genotype as compared to those with the \textit{GSTT1} present genotype. It was suggested that there is possible role of \textit{GSTT1} in detoxification of SO in humans.

In a study carried out on Italian pesticide-exposed green-house workers (n=34) and matched unexposed controls (n=33), the \textit{GSTM1} positive and \textit{NAT2} fast acetylator genotype individuals had significantly (p<0.05) higher micronucleated cells (MNCs) (Falck \textit{et al.}, 1999).

A study by Lucero \textit{et al.} (2000) on green-house workers exposed to pesticides (n=64) and healthy unexposed controls (n=50) to assess frequency of micronuclei in peripheral blood lymphocytes and in buccal epithelium cells and its relation with \textit{GSTT1} and \textit{GSTM1} genotypes revealed lowered cytokinesis block proliferation index in exposed individuals with the \textit{GSTT1} present genotype.

Butkiewicz \textit{et al.} (2000) investigated the polymorphism of \textit{GSTP1} (Ile/Val) and \textit{GSTM1} genes and levels of polycyclic aromatic hydrocarbons (PAH) DNA-adducts in mononuclear white blood cells (WBCs) from healthy volunteers (n=170). \textit{GSTP1} (Ile/Val) alone showed no significant difference for DNA adducts while the \textit{GSTP1} (AA or AG) in combination with \textit{GSTM1} null genotypes had significant (p=0.015; 1.6-folds higher) DNA adduct levels in smokers as compared to levels in the \textit{GSTM1} null/\textit{GSTP1} (AA) genotype individuals. These results indicated that genetic polymorphisms of \textit{GSTM1} and \textit{GSTP1} genes could modulate PAH DNA-adduct levels.

Kocabas \textit{et al.} (2000) determined the influence of \textit{GSTM1} genotype on DNA damage and chromosomal aberrations after induction of bleomycin in cultured human peripheral blood lymphocytes. The \textit{GSTM1} null genotype showed no significant difference for damaged cells as well as for chromosomal aberrations, without bleomycin treatment as compared to levels in the \textit{GSTM1} present genotypes.

In a case-control study by Rundle \textit{et al.} (2000) investigated the relationship between PAH DNA adducts and \textit{GSTM1} genotype in breast tissue of women (n=227) grouped
into tumor (n=83), non-tumor (n=77) and benign controls (n=84). Odds ratio revealed no association of \textit{GSTM1} with breast cancer. \textit{GSTM1} null genotype predicted significant PAH-DNA adduct levels in malignant (p=0.003) and non-malignant breast tissue (p=0.05) in cases. However control group showed no such association (p=0.341). Hence the \textit{GSTM1} showed interaction with case-control status on PAH-DNA adduct levels (p=0.002) in breast tissue.

The relationship of DNA adducts of 1, 3-butadiene with \textit{GST} genotype (Zhao \textit{et al.}, 2001) was examined in 15 male workers (mean age 43.50±11/5y) exposed to butadiene monomer unit and in unexposed controls (n=11, mean age 43.20±11.30y) working in a heat-production unit. The \textit{GSTM1} null workers had significantly (0.026) higher DNA adducts as compared to \textit{GSTM1} present genotype individuals. The \textit{GSTM1} null in combination with \textit{GSTT1} present genotype had significantly (p=0.049) elevated levels of DNA adducts as compared to the levels in the \textit{GSTM1} and \textit{GSTT1} present genotype. In linear regression analysis, \textit{GSTM1} showed significant relation to DNA adduct levels.

Piipari \textit{et al.} (2003) studied the effect of \textit{GSTs} and cytochrome P450, separately and in combination, in active smokers (n=31). There was no association of \textit{GSTM1} or \textit{GSTP1} genotype with aromatic DNA adducts was observed.

In a case-control study carried out on coke-oven workers (n=18), DNA adduct levels were detected and checked for association with \textit{CYP1A1 Msp1}, \textit{GSTP1} (Ile/Val), \textit{GSTM1} and \textit{GSTT1} genotype and compared in those with healthy subjects (n=21). \textit{GSTT1}, \textit{GSTM1} and \textit{GSTP1} (Ile/Val) polymorphism showed no significant difference for DNA adduct levels (Teixeira \textit{et al.}, 2002).

Styrene-exposed individuals (n=48) and unexposed healthy individuals (n=14) with \textit{GSTM1} positive genotype had significantly elevated DNA damage (by SCGE assay) as compared to levels in the \textit{GSTM1} null genotype; whereas for \textit{GSTT1}, the results were reverse and the \textit{GSTP1} wild type showed significant protection from damage as compared to subjects having \textit{GSTP1} variant allele (Buschini \textit{et al.}, 2003).

Tuimala \textit{et al.} (2004) investigated the effect of genetic polymorphism of DNA repair protein and xenobiotic-metabolizing enzymes (XMEs) on levels of chromosomal
aberrations (n=145) and sister-chromatid-exchanges (n=60) in healthy caucasians (61 Finnish and 81 Hungarian). Regression models after adjustment for age, sex, smoking and genotypes revealed that GSTT1 null individuals had slightly elevated sister-chromatid-exchange levels which however did not reach significance level.

The GSTM1 and GSTT1 polymorphisms were studied for the effect on MN frequency in human lymphocytes (Kirsch-Volders et al., 2006). In a pooled analysis of data from seven laboratories, there was significantly (p=0.016) lower MN frequency in individuals with the GSTT1 null genotype as compared to levels in the GSTT1 positive genotype while this protection was reversed with age in 60y-old subjects. Overall, the MN frequency increased with age and gender, being higher in females.

Association of metabolic genotypes with DNA damage in fruit growers exposed to pesticides was investigated by Liu et al. (2006). Results of the multiple regression analysis showed significant association of age, high-pesticide exposure, low-pesticide exposure, CYP3A5 and GSTP1 with increased tail moment. Gene-environmental interaction analysis revealed that GSTP1 influenced DNA damage. It was suggested that individuals with GSTP1 exposed to pesticides express elevated DNA damage.

Lin et al. (2009) examined the association of GSTM1 with oxidative DNA damage in hemodialysis patients (n=488) and healthy controls (n=372). The GSTM1 had higher damage (63.10%) in patients as compared to controls (60.20%). The GSTM1 null patients had significant association with elevated levels of 8-OHdG as compared to the GSTM1 non-null genotypes. Mortality risk was also doubled in patients with GSTM1 null genotype. It was concluded that individuals with GSTM1 null genotype are vulnerable to oxidative stress as compared to those with the GSTM1 non-null genotype.

In individuals occupationally-exposed to cytostatics (expt1, n=72) and anesthetics (expt 2, n=76), and in healthy unexposed (expt 3, n=76) a comparison was made on the basis of GST gene polymorphism for chromosomal aberrations (Musak et al., 2009). Results revealed higher frequency of chromosomal aberrations (CAs) and chromatid type aberrations (CTAs) and chromosome-type aberration (CSAs) in individuals with the GSTT1 and GSTM1 null genotype. Individuals in expt 2 with the GSTM1 and GSTT1 null genotype also had higher frequency of CAs, CTAs and CSAs as compared to that
in individuals with the positive genotypes. The GSTP1 present or variant form however showed no variation for any aberration type.

Abhishek et al. (2010) investigated genotoxic effects of pesticides and its association with GST polymorphism in pesticides-exposed workers (n=40) and healthy (n=27) controls. GSTT1 null individuals had significantly increased DI (1.3 fold) and 1.5 fold per cent DNA in tail (p<0.05) whereas in GSTM1 null genotyped individuals, these were lower and did not reach significance. DF showed significantly (p<0.05) lower level in GSTM1 null genotype as compared to that in GSTM1 positive genotyped individuals. GSTT1 null in combination with GSTM1 null genotype showed higher value for DI (1.3-fold), DF (1.1-fold), per cent DNA in tail (1.4-fold, p<0.05). Hence only the GSTM1 null genotyped showed association with DNA damage in exposed group.

It was investigated by Chen et al. (2010) whether genetic polymorphisms of GSTT1 and GSTM1 influence MN frequency in peripheral blood lymphocytes (PBL) of individuals residing near a site for recycling e-waste (n=58; 35.00±9.40y; 75.90% males and 34.10% females) and in healthy controls (n=80; 43.7±10.60y; 51.30% males and 48.70% females). On stratification of micro nucleated and binucleated cell frequencies on the basis of GSTT1 and GSTM1, no significant difference was found between the two groups. GSTT1 null in exposed group had significantly (p=0.013) higher (6.6-fold) micronucleated binucleated cells. No association of GSTM1 and GSTT1 genotype was however observed with micronucleated binucleated cells.

Elevated mean frequencies of CBMN (1.1-fold), sister-chromatid-exchanges (1.1-fold) and per cent HFC (1.2-fold) in GSTM1 null road construction workers (n=105) were observed to levels compared to levels in non-exposed (n=105) controls (Kumar et al., 2011). It was concluded that there was association of GSTT1 and GSTM1 null genotypes with frequencies of sister-chromatid-exchanges, HFC and CBMN.

Association of GSTM1, GSTT1 and GSTP1 (Ile/Val) with DNA damage in workers exposed to organophosphate pesticides (n=115) and in healthy controls (n=115). Singh et al. (2011) revealed significantly (p=0.03; 1.1-fold) increased TM in exposed individuals with GSTM1 null genotype as compared to levels in those with the GSTM1
positive genotype. *GSTP1* (Ile/Ile) also showed significantly (p=0.02) higher TM as compared to individuals with heterozygous (Ile/Val) or mutant (Val/Val) genotypes. It was concluded that *GSTM1* null and *GSTP1* (Ile/Ile) were related to DNA damage in occupationally-exposed workers to organophosphate pesticides.

Skjelbred *et al.* (2011) investigated the link between *GSTM1, GSTT1, GSTP1, NAT1, NAT2* and *EPHX1* and chromosomal aberrations in peripheral blood lymphocytes of healthy Norwegian males (n=651, mean age 41y). Chromatid-type aberrations (CTAs) were significantly (p<0.05) elevated in the *GSTT1* null genotyped individuals.

In 127 individuals (49.60% males and 50.40% females, mean age 32.04±8.02y) with the *GSTT1* null genotype, significantly higher frequencies of CA (p=0.026) and MN (p=0.003) were observed by Kadioglu *et al.* (2012). On the other hand no difference was observed in those with *GSTM1* null or in those with the *GSTP1* mutant genotype. It was suggested that cytogenetic frequency in healthy individuals can be influenced by *GSTT1* gene polymorphism.

Singh *et al.* (2012) reported influence of *CYP2C9, GSTM1, GSTT1* and *NAT2* genetic polymorphism on DNA damage in individuals occupationally exposed to organophosphate pesticides (n=134) and healthy non-exposed controls (n=134). DNA damage was assessed using comet assay. Occupationally exposed workers showed (2.3-folds) significantly (p<0.001) elevated tail moment (TM) as compared to controls. *GSTM1* null exposed showed significantly (p=0.02; 1.1-fold) increased TM as compared to *GSTM1* positive exposed individuals. *GSTM1* null in combination with *GSTT1* null showed (1.1-fold) significant (p=0.02) increase in TM as compared to level in the positive genotype of the exposed group.

Cheng *et al.* (2013) in PBL of 1, 3 butadiene (BD)-exposed workers (n=44) and (n=39) healthy controls assessed chromosomal damage using SCE and CBMN assays. There was about 1.5 times increased MN in BD-exposed individuals than in controls but no difference for SCE in exposed and controls. In aged individuals there was 1.45 times increased MN frequency than in the younger group; in the group working for many years, 1.40 times increased MN frequency was observed compared to that in those who had just started work. There was about 1.5-fold increased MN frequency in *GSTM1* null
genotypes and 1.4-fold increase in those with GSTT1 null genotypes in comparison to levels in respective GSTM1 or GSTT1 present genotypes. The risk was increased up to 2.1-fold when GSTT1 null and GSTM1 null were present in combination compared to that in the GSTT1, GSTM1 present genotypes. It was concluded that the genotoxic effects of BD exposure could be modulated by polymorphism in the GSTT1 and GSTM1 genes.

2.14. Genetic Modulation and Genetic Damage in CAD

A single study of this type in CAD patients has come to attention. The MN frequency showed association with CAD and also with XRCCI Arg399Gln and XPD Lys751Gln polymorphisms (Guven et al., 2007). Significantly (p=0.018) increased MN frequency was observed in CAD patients as compared to control values. The XPD 751Gln (Gln/Gln genotype) showed significantly (p=0.02) higher MN frequency as compared to patients with XPD751Lys (Lys/Lys genotype). The XRCCI 399 Gln (Arg/Gln or Gln/Gln genotype) patients also had significantly (p<0.05) higher MN frequency than in those with XRCCI 399Arg (Arg/Arg genotype). There was also significant increase in MN in patients having dyslipidemia (p=0.023) and diabetes (p=0.011) as compared to values in controls.

2.15. Genetic Modulation and Oxidative Stress

Oxidative stress levels have also been modulated by genetic polymorphism of GSTs; only very sparse literature has come to attention which is reviewed here.

Otto-Knapp et al. (2003) investigated the role of GSTM1 deficiency with antioxidative enzymes of catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), super oxide dismutase (SOD) and glutathione S-transferase (GST) after ozone-exposure. In those with GSTM1 null genotype there as significant up-regulation of SOD activity (p=0.011) as compared to those with GSTM1 positive genotype. The authors suggested that there is impact of lack of GSTM1 on antioxidants enzymes after ozone-exposure.

Aydemir et al. (2007) while working on seminal plasma and spermatozoa from infertile patients, compared levels of ROS, MDA, protein carbonyls and glutathione (GSH) and
GST activity. Men with \textit{GSTM1} null genotype had significantly elevated levels of oxidative stress and damage markers in contrast to those with the \textit{GSTM1} positive genotype through the genotypic distribution of \textit{GSTM1} showed no significant difference between patients and controls.

Ueno \textit{et al.} (2009) examined if there was any influence of GST genetic polymorphism on MDA, LDL-C, soluble CD40, ligand E-selection and soluble ICAM1 levels in patients (n=143) with angiographically proven coronary artery disease. Patients with \textit{GSTT1} present genotype showed significantly elevated levels of MDA and LDL-C as compared to those with the \textit{GSTT1} null genotypes.

The relationship of \textit{GSTP1} Ile105Val polymorphism with oxidative stress in cancer patients under treatment with Docetaxel (Mir \textit{et al.}, 2009) revealed significant correlation between \textit{GSTP1} Ile/Ile. It was suggested that oxidative stress played an important role in the pathogenesis of Docetaxel-induced peripheral neuropathy.

In a case-control study (Lagadu \textit{et al.}, 2010) 8-oxo-7, 8-dihydro-2'-deoxyguanosine levels and its association with antioxidant vitamins and genetic susceptibility were studied in oesophageal cancer patients (n=17) and healthy controls (n=43). There was significant (p<0.0001) positive correlation of \textit{GSTP1} (Val/Val) with levels of 8-oxodG.

Maurya and Rizvi (2010) observed significant increase in plasma GST activity as a function of age and GST activity and plasma antioxidants showed a significant positive correlation. It was concluded that elevated activity of GST was a manifestation of increased production of ROS and lower antioxidant capacity during aging.

Lankin \textit{et al.} (2011) checked oxidized low-density lipoprotein (oxLDL) as a new diagnostic biomarker of oxidative stress in individuals from Estonia (n=782) and Russia (n=1433). The oxLDL and LDL-C levels showed significant (p<0.05) positive correlation in both the study groups. The Russians were at high-risk on the basis of elevated levels of oxLDL/HDL-C ratio.

Patients with combined \textit{GSTT1} and \textit{GSTM1} null genotypes had significantly higher levels of MDA and lower GST activity as compared to levels in \textit{GSTT1} and \textit{GSTM1}
positive genotypes in 60 (Datta et al., 2010). GSH levels were also lowered in combined null group as compared to levels in the present group.

Positive correlation of ferritin, nitric oxide and apolipoprotein B with increase in stenosis was observed in 476 CAD patients (Tayal et al., 2012). Results of the study showed nitric oxide as a predictor of CAD.

Li et al. (2013) reported that reduced expression of GSTP1 contributed to oxidative stress and in the development of hepatocellular carcinoma in chronic hepatitis B condition.

In a cohort study investigated by Parsons et al. (2013) the effect of GSTM1 polymorphism on oxidative stress and disease progression in HIV-infected patients (n=139, males 56.00% and females 44.00%; mean age 48.32±6.31y). Oxidative stress (8-oxo-dG, MDA, oxidized glutathione and complexes I and IV), apoptosis and HIV disease markers were studied. Patients with GSTM1 present genotype had lower complex I activity as well as lower 8-oxo-dG and MDA levels as compared to levels in individuals with the GSTM1 null genotype.

2.16. Cytochrome P450

Individual susceptibility to environmental, chemical, and drug toxicity is influenced by polymorphism in drug-metabolizing enzymes, especially the cytochrome P450s (Johansson and Ingelman-Sundberg, 2011). There are two classes of CYP enzymes depending on the penetrance for interindividual susceptibility for xenobiotics (Rodriguez-Antona and Ingelman-Sundberg, 2006). Class I includes CYP1A1, CYP1A2, CYP2E1, and CYP3A4 which do not have functional polymorphism but metabolize pro-carcinogens and drugs. The Class II is highly polymorphic and only metabolize drugs and include CYP2A6, CYP2B6, CYP2C9, CYP2C19, and CYP2D6.

Of these, CytochromeP4502D6 (CYP2D6) is a highly polymorphic gene located on chromosome 22q13.1 with its enzymes localized in the inner membrane of cellular organelles of the mitochondria and endoplasmic reticulum. These enzymes are monooxygenases which are responsible for catalyzing several endogenous substrates, xenobiotics as well as metabolism of about 25% of the most commonly prescribed
drugs including antidepressants, antipsychotics, β-adrenergic blocking agents, antihypertensives, antiarrhythmics and opioids. The polymorphism of CYP2D6 significantly affects the pharmacokinetics of about 50% of the clinically useful drugs, which are CYP2D6 substrates. More than 130 Single Nucleotide Polymorphisms (SNPs) have been identified with in the CYP2D6 gene, including non synonymous, silent, promoter and intronic changes. Presence of these SNPs can alter enzymatic activity of CYP2D6 which plays a major role in inter-individual variability in drug response. These genetic variants have been correlated with four major phenotypes: poor metabolizers (PMs), carrying two defective alleles completely lacking enzyme activity; intermediate metabolizers (IM) are heterozygous for a defective allele or have two alleles with decreased enzymatic activity; extensive metabolizers (EM), have two functional alleles, while ultrarapid metabolizers (UM), have >2 active gene copies (Johansson and Ingelman-Sundberg, 2011).

2.17. Population Distribution of CYP2D6 Genetic Polymorphism

Adithan et al. (2003) assessed the frequencies of CYP2D6*3, *4, *5 and *10 alleles in the Tamilian population (n=106). The frequency of *10 allele was maximum (10.2%), followed by *5(0.9%), and *4 (6.6%) in the study group. Of these six allelic, *1/*10 was predominant (21.70%) with other genotypes being *1/*4 and *10/*10 each with 6.60%, *4/*10 (5.70%) and 0.90% each of *1/*5 and *4/*5. Poor metabolizers condition of *4/*4 or *5/*5 were lacking.

Theophilus et al. (2006) determined genetic polymorphism of CYP2D6 (*2, *3, *4, *5, *10, *14 and *17) in 447 South Indians belonging to four states. Results of the study showed higher frequency for CYP2D6*2 (34.8%) followed by, *10 (10.2%) *4 (7.3%), *5 (1.9%) whereas *3, *14 and *17 were absent. The allelic combination *1/*2 (32.70%) showed higher frequency followed by, *1/*1 (19.40%) and *2/*2 (11.80%) in South Indian population. The poor metabolizer phenotype (*4/*4 and *4/*5) had 0.6% frequency and the authors concluded that in South Indians, the CYP2D6 genetic polymorphism were distinct as compared to other world populations.
2.18. CYP2D6 and Coronary Artery Disease

Only two reports on CYP2D6 gene polymorphism with heart disease/CAD have come to attention.

Teh et al. (2004) in a cohort study of CVD patients (n=128; mean age 58±10.17y) genotyped them for CYP2D6 (*1, *4, *5, *9 and *10). The CYP2D6*3 was absent and 11 allelic combinations were identified with high frequency CYP2D6*1/*10 (41.41%), followed by those of CYP2D6*1/*1 (28.13%) and the homozygous CYP2D6*10/*10 (14.84%). In patients having primary hypertension with ischemic heart disease, the CYP2D6*1/*10 allelic combination was most common. Also only the CYP2D6*1/*10 was out of Hardy-Weinberg equilibrium. The authors suggested that in cardiovascular disease there is possible contribution of CYP2D6.

CYP2D6 polymorphisms in CVD patients (n=91) on metoprolol-therapy for the past six months were studied by Ismail and Teh (2006). Four allelic combinations were observed viz. CYP2D6*1/*1, *1/*10, *10/*10 and *10/*9. The study includes higher number of extensive metabolizers (63.73%), followed by intermediate metabolizers (31.87%), ultra metabolizers (3.30%) and poor metabolizers (1.10%).

2.19. Dyslipidemia in CAD Patients

Triglycerides play an important role in the pathogenesis of atherogenesis (Manocha and Srivastava, 2002). There was significant risk of developing CHD with elevated triglyceride level and furthermore, with hyper-triglycridemia, levels of HDL-C lowered and there was increased risk for CHD.

The Asia-Pacific cohort studies collaboration (Zhang et al., 2003) conducted a meta-analysis of 29 cohorts comprising 3,52,033 individuals (42% females) with a mean age of 47 years. The mean cholesterol values after adjustment for age and sex for Australian and New Zealand (ANZ) participants was 5.52m mol/l and 4.87m mol/l, respectively. Asian men and women had similar mean cholesterol values of 4.80m mol/l and 4.82m mol/l respectively. In ANZ and Asian populations, the total cholesterol was strongly associated with risk of CHD and ischemic stroke.
Tarchalski et al. (2003) evaluated the lipid profile of 141 coronary atherosclerosis patients. On the basis of angiography, the Gensini Score (GS) was used as reference for the extent of atherosclerosis. Positive correlation of GS with levels of total-C ($p<0.001$), LDL-C ($p<0.001$) and TG ($p<0.005$), and negative with HDL-C ($p<0.001$) was observed. Patients with angina pectoris had positive correlation with total-C, LDL-C and TG but negative correlation with HDL-C.

Sarkar et al. (2006) studied paraoxonase activity and lipid levels of patient with premature coronary artery disease ($n=120$, 99 males and 21 females) and in normal subjects ($n=50$, 41 male and 9 female). A significant increase was observed in the concentrations of total cholesterol, triglycerides and LDL-cholesterol in premature coronary artery disease patients in comparison to values in healthy controls. Total cholesterol, triglycerides, LDL-C and HDL-C were significantly associated with CAD ($p<0.001$) in patients.

In a case-control study conducted by Phababpha et al. (2009) on CAD patients ($n=23$) to assess association of recorded dyslipidemia with coronary artery stenosis, significant ($p<0.05$) increase in TG and reduction in HDL-C levels in CAD patients was observed compared to values in the non-CAD group. There was significant correlation of number of stenosis vessels with levels of TG ($p<0.035$) and HDL-C ($p=0.036$). Plasma level MDA was also higher ($p=0.057$) in CAD patients probably causing increased oxidative stress. The authors observed that dyslipidemia was a good predictor for the severity and extent of coronary artery disease in CAD patients and that it could also lead to oxidative stress.

Risk factors in the development of CAD in South Asian Immigrants were studied by Dodani (2009). Previous data reported that 31.8% females and 28.8% males from South Asia had metabolic syndrome while South-African Caribbeans had lesser prevalence (23.4% females, 15.5% males) as also Europeans (14.4% females and 18.4% males). It was observed that though the role of HDL-C is in protection against CAD, yet its main effectiveness as pro-oxidant (rather more as a antioxidant) related with CAD has also been observed and is called as dysfunctional HDL.
2.20. Metabolic Syndrome

According to WHO criteria (WHO, 1999) metabolic syndrome (MS) is defined as ins

ulin resistance or reduced glucose regulation in combination of any of two or more constituents of blood pressure, lipid levels, obesity and urinary albumin/creatinine viz.

TG ≥ 1.7 mmol/l, HDL-C < 0.9 mmol/l in males and <1.0 mmol/l in females, blood pressure ≥ 140/90 mmHg, BMI >30 kg/m² or WHR >0.90 for males and >0.85 for females or ratio of urinary albumin/creatinine ≥ 30 mg/g. However in a report by National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) in 2001 metabolic syndromes was defined as the occurrence of any three or additional of the variables of blood glucose level (fasting) >6.1 mmol/l, TG ≥ 1.69 mmol/l, HDL-C ≤ 1.04 mmol/l in males and ≤1.29 mmol/l in females, blood pressure measurements ≥ 130/85 mmHg or WC ≥ 102 cm in males and ≥ 88 cm in females. According to International Diabetic Federation (IDF, 2005), the criteria for MS is same but makes it compulsory to include WC as abdominal obesity measure. The CAD patients and controls of the present study were also examined for the presence of MS based on IDF (2005) criteria but taking the cut-off WC ≥80 cm in men and ≥85 cm in women as per Snehalatha et al. (2003).

2.21. Genotoxicity of Prescribed Drugs

Medical substances by their intrinsic nature are biologically active with varying modes of action and by virtue of this, their mutagenic, genotoxic and/or carcinogenic potential cannot be ruled-out. Since chronic conditions require life-long treatment on diagnosis, the continuous administration of (prescribed) medications may themselves induce pre-cancerous (mutagenic/genotoxic) lesions/effects. In the present study, the patients were on a total of six prescribed drugs which included furosemide (diuretic), metaprolol (β-blocker), atenolol, plagerine, ecosprin. Their genotoxicity mutagenicity is reviewed here:

2.21.1. Furosemide (CAS No. 54-31-9; C₁₂H₁₁ClN₂O₅S)

Furosemide (4-chloro-2-((furan-2-ylmethyl) amino)-5-sulfamoylbenzoic acid) is a diuretic and is used in the treatment of edema related to congestive heart failure,
cirrhosis of the liver, and renal disease and hypertension (www.drugbank.ca/drugs accessed on March 22, 2014). The drug is an anthranilic-acid derivatives and it inhibits the sodium-potassium-chloride co-transporter (Mondal et al., 2012). About 90% of drug excreted in its purest form (Isidori et al., 2006). On bio-activation by cytochrome P450, it forms various reactive intermediates and among these, furosemide epoxide and furosemide-enedial can bind to cellular macromolecular causing side effects like hepatotoxicity and ototoxicity as well as being carcinogenic (Mondal et al., 2012). The drug did not cause lipid peroxidation in mice (Randle et al., 2008; Mondal et al., 2012) and drug showed antioxidant properties in vivo and in vitro (Lebedev and Petrenko, 1996; Lahet et al., 2003) while in mitochondria, it induced an oxidant effect (Rogers et al., 2000).

2.21.1.1 Genotoxicity- Earliest documentation of genetic toxicology potential of furosemide is by Subramanyam and Jameela (1977) who reported translocations in meiotic cells of male mice. Clastogenicity and chromatid anomalies induced by furosemide in vitro in cultured human lymphocytes as well as in bone marrow cells of mice and in root tip cells of Allium cepa have also been reported (Jameela et al., 1979). The frequency of sister-chromatid exchanges on treatment with Furosemide in human fibroblasts in vitro however did not show an increase while conflicting results were reported for chromosomal aberrations in vivo fibroblast and lymphocyte cells (Subramanyam and Jameela, 1977; Jameela et al., 1979) while their increase in Chinese Hamster Ovary and lung fibroblasts in vitro as well as in vivo in mouse germ cells (Reddy and Subramanyam, 1978; Jameela et al., 1979; Venkata et al., 1981). Furosemide was positive in the gene mutations in mouse lympho cells in the presence of S9 while in Salmonella typhimurium strains, furosemide showed no mutagenic effect irrespective of metabolic activation (Isidori et al., 2006). Kirpnick et al. (2005) reported inconclusive genotoxic effects of furosemide in the micronucleus in vitro assay, Salmonella typhimurium and chromosome aberration assay. In fact, Bucher et al. (1990) and Brambilla et al. (2009a, b) have documented inconclusive genotoxicity of furosemide.
Isidori et al. (2006) studied Furosemide and its photoproduct (a dimer) for mutagenesis and genotoxicity using the SOS chromotest and the Salmonella (Ames) mutagenicity assay. Although Furosemide was not mutagenic, the dimer was a frameshift mutagen showing a significant mutagenic activity in the Ames test.

A study by Mondal et al. (2012) assessing in vivo genotoxicity of Furosemide observed DNA damage, TUNEL positive cells and increased DNA fragmentation in mice hepatocytes in vivo. While structural chromosomal aberrations were not increased in bone marrow cells in which however mild DNA strand breaks were observed in the comet assay. It was concluded that Furosemide is weakly genotoxic in bone marrow cells compared to its potential in mice hepatocytes.

2.21.2. Metoprolol (CAS No. 37350-58-6; C_{15}H_{25}NO_{3})

Metoprolol  \{2-hydroxy-3-[4-(2-methoxyethyl)phenoxy]propyl\}(propan-2-yl)amine, a cardio selective beta-adrenergic antagonist, is used in the management of hypertension and edema by competing with catecholamines, the drug blocks beta1-andrenergic receptors which causes heart rate to decrease so there is decrease in cardiac output and blood pressure (Gorre and Vandekerckhove, 2010)

2.21.2.1. Genotoxicity-  Ames test and comet assay carried out in the algal and bacterial strains (Pseudokirchneriella subcapitata and Vibrio fischeri, respectively) revealed positive genotoxicity of Thiamethoxam and Metoprolol (Šojić et al., 2012).

2.21.3. Atenolol (CAS 29122-68-7; C_{14}H_{22}N_{2}O_{3})

Atenolol (4-{2^1-hydroxy-3^1-[(1-methyl ethyl) amino]prpoxy}) is a Beta adrenergic receptor used in cardiovascular therapeutics (Kerkar and Phadke, 2009). The drug is hydrophilic in nature, does not cross the blood brain barrier and has a longer elimination half life (6-7 hours).

2.21.3.1. Genotoxicity- Martelli et al. (1994) reported a lower in vivo clastogenic potency of N-nitroso derivatives of five beta-blocking drugs (propranolol, metoprolol, nadolol, atenolol and sotalol) compared to in vitro DNA-damaging potency. Okine et al. 
(1983) while studying the effect of atenolol, reported increased activity of guanylate cyclase and decreased activity of adenylate cyclase in liver, gastric and intestinal mucosae of rats. However, no oncogenic effects for beta-adrenoceptor drugs were observed. Ames test carried out in the *Salmonella typhimurium* strains and the micronucleus test in adult CF1 outbred mice revealed no significant dose-dependent increase in number of micronuclei (Okine *et al.*, 1983).

Telez *et al.* (2000) observed a non-significant increase in *in vitro* and *in vivo* sister chromatid exchanges (SCE) in hypertensive patients on Atenolol--drug therapy (beta-blocker) compared to control individuals. The frequency of micronuclei (MN) was also observed to be significantly increased in patients compared to controls. This indicated that long-term atenolol treatment resulted in chromosome loss.

A wide range of aberrations including chromosome breaks, bridges, chromosome lagging, disturbed meta-anaphases, micronucleus and polyploidy in were observed by Jangala *et al.* (2012) in *Allium cepa* root meristems treated with 10µg/ml atenolol at varying time intervals.

In a group of 30 hypertensive patients divided into two groups depending upon duration of treatment, Amin (2009) reported a significant positive correlation between serum creatine kinase-MB (a marker of cardiac injury) and duration of disease concluding that long term use of atenolol could cause cardiac injury.

### 2.21.4. Ecosprin (CAS No. 50-78-2; C₉H₈O₄)

Ecosprin (2-acetoxybenzoic acid) inhibits cyclooxygenase activity, an enzyme responsible for the formation of prostaglandins which plays a key role in pathogenesis of inflammation, pain and fever (Vane and Botting, 2003).

### 2.21.5. Plagerine (CAS No 113665-84-2; C₁₆H₁₆ClNO₃S•H₂SO₄)

Plagerine (methyl (+) - (S)-α- (2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5 (4H)-acetate sulfate) exerts antithrombotic action by interfering with platelet aggregation. About 50% of drug is absorbed orally.
The information in this review of literature elicits that oxidative stress, genetic damage and metabolic genotypes have important roles in the initiation/progression of CAD. The presence of obesity and dyslipidemia include other factors responsible for development of CAD. A number of studies in various population sub-groups have shown the effects of these factors in CAD patients. However studies on the Punjabi Ramgarhia Sikh sub-group have not come to attention. The present study was hence planned to study DNA damage and oxidative stress in Ramgarhia Sikh CAD patients from this region and to determine if metabolic genotypes modulated their levels.