ABSTRACT

Coronary artery disease results from a multitude of physiological, genetic, and environmental factors with heritability estimates of 30-60% and other non-modifiable risk-factors of age, gender and ethnicity. It may also result from oxidative stress and genetic damage which can be modulated by metabolic genotypes. As study of genetic damage, oxidative stress and genetic polymorphisms of metabolic genotypes in CAD patients can provide novel insights, the present study was carried out. Unrelated CAD patients (n=200) from hospitals and healthy controls (n=200) from general population, belonging to Ramgarhia Sikh sub-group were contacted. Primary and oxidative DNA damage was assessed in peripheral blood leukocytes using the single cell gel electrophoresis (SCGE) assay and oxidative DNA damage by the modified comet assay. Serum samples were analysed for dyslipidemia and oxidative stress. Genotyping of GST (T1, MI, GSTP1 (A313G) and GSTP1 (C341T)) and CYP2D6 (*2, *4 and *10) was carried out by the multiplex PCR and PCR-RFLP methods. The cases and controls were matched with respect to marital status, dietary habit, mobile phone usage, alcohol-drinking, general obesity (BMI) as well as central obesity (waist circumference, waist-hip-ratio and waist-height-ratio).

There were 72.50% patients with myocardial infarction (MI), 15.00% with stable and 12.50% with unstable angina; disease-onset varied from 35-70 y (mean 51.76±0.59y) and 28.00% cases had family history. Treatment duration varied from 1-16y (mean 5.91±0.26y) and medications included furosemide (62.50%), atenolol (16.00%), metoprolol (45.00%), plagerine A (36.00%) and ecosprin (77.50%). Primary genetic damage revealed significantly (p<0.001) elevated levels for per cent DNA in tail (% DNA in tail, 2.7x), Tail Moment (TM, 5.3x), Olive Tail Moment (OTM, 3.9x), mean DNA migration length (3.2x), Damage Frequency (DF, 2.4x) and Damage Index (DI, 6.5x) in CAD patients as compared to controls. Oxidative DNA damage was also significantly (p<0.001) elevated in patients. TOS, OSI, CYP2D6*2 and obesity were revealed as significant predictors of genetic damage. Lipid peroxidation, oxidative stress and lipid levels were significantly (p<0.001) higher whereas total antioxidant status (TAS) and high density lipoprotein (HDL-C) were lower in patients as compared to controls. Malondialdehyde (MDA) showed positive correlation with GSTP1 (C341T), TAS with GSTT1 and total oxidant status (TOS) with CYP2D6*4 genotype.
There were no significant differences for genetic damage and oxidative stress levels within patients and controls on stratification by gender, age, obesity, hypertension categories, treatment time/duration of disease, and menopausal status (females). Patients on drug combinations of furosemide, ecosprin and plagerine A had significantly elevated levels of DNA damage as compared to patients on furosemide, ecosprin and metoprolol. Also MI patients had significantly (p<0.001) elevated levels of DNA damage as compared to damage in patients with stable and unstable angina.

In patients and controls, the genotypic frequencies of *CYP2D6*<sup>*2*</sup> and *CYP2D6*<sup>*4*</sup>, *CYP2D6*<sup>*10*</sup>, *GSTT1*, *GSTM1*, *GSTP1* (A313G) and *GSTP1* (C341T) were in Hardy-Weinberg equilibrium. For the *CYP2D6*<sup>*10*</sup> and *GSTP1* (C341T) genetic polymorphisms, the homozygous mutant genotypes were lacking. Genetic damage was significantly (p<0.05) elevated in those with *CYP2D6*<sup>*2*</sup> heterozygous (AG) and homozygous mutant (GG) compared to levels in the homozygous wild type (AA). For *CYP2D6*<sup>*4*</sup>, homozygous mutant (AA) group had significantly elevated levels of DI compared to levels in homozygous wild type (GG) and heterozygous (GA) genotypes. For *CYP2D6*<sup>*10*</sup>, *GSTT1*, *GSTM1* and *GSTP1* (C341T) genetic polymorphisms, no significant differences for DNA damage were observed while the homozygous wild type (AA) genotype of *GSTP1* (A313G) had significantly elevated levels of oxidative stress and DNA damage as compared to levels in the heterozygous (AG) group. The *GSTT1* null genotype when present in combination with other wild type genes, showed elevated risk for genetic damage.

Heterozygous *CYP2D6*<sup>*4*</sup> (GA/AA) and the *GSTM1* null genotypes present in single/double/triple gene-gene combinations revealed higher risk for disease.

The study results therefore exhibit that patients had significantly elevated levels of primary and oxidative DNA damage and oxidative stress as compared to the control group and there were no gender differences. Damage was slightly higher in patients with myocardial infarction as compared to patients with stable angina/unstable angina. Patients with *CYP2D6*<sup>*2*</sup> (AG/GG) and *GSTP1* (A313G) variant (AG/GG) genotype had elevated levels of DNA damage as compared to levels in the wild type group. *GSTM1* null showed higher risk for CAD in combination with other genes.