Sports activities have always been considered an essential component of an individual’s
general welfare adding to the physical, emotional, mental, social and psychological
well-being (Carling et al., 2005). Rather, education is also considered incomplete
without sports being an integrated part of the curriculum or as an extra-curricular
activity. Sports is defined as an activity which includes an element of competition and
should not be judged to pose an undue risk to health and safety of athletes or
participants, and in no way, be harmful to any living creature (SportAccord, accessed on
April 12, 2014). Interest and participation in sports activities often manifests as a
professional activity or remains recreational. Recreational and low-intensity exercise
programs are therapeutic as there is a tendency to minimize the risk of diseases such as
to cardiovascular diseases, neurodegenerative diseases and ageing, which can be
triggered by reactive oxygen species (Radak, 2008; Reichhold et al., 2009; Kürkčü et
al., 2010). Professional sports are competitive, requiring various attributes like high-
level endurance-training, muscle strength, speed, agility and co-ordination to harness
the capabilities for a good performance (Carling et al., 2005). In fact, exercise at
competitive levels may compromise health due to dehydration, substrate depletion,
muscle damage, inflammation and increased production of free radicals (da Costa et al.,
2011).

The free radicals are generally produced continuously during regular metabolic
processes in the cells as a consequence of both, enzymatic and non-enzymatic reactions.
Enzymatic reactions include those involved in the respiratory chain, phagocytosis,
prostaglandin synthesis, and in the cytochrome P-450 system. The non-enzymatic
reactions are the reactions of oxygen with organic compounds as well as those initiated
by ionizing reactions (Rahman, 2007). In the normal physiological state, the production
of free radicals maintains an equilibrium being balanced by the anti-oxidant defenses of
the body (Andersson et al., 2009). Increased metabolic activities like exercising,
vigorous physical activity and infections which increase macrophages can initiate an
increase in oxygen consumption, causing leakage of mitochondrial electron transport
chain and increased production of many reactive oxygen species such as superoxide,
hydrogen peroxide and hydroxyl radicals (Sjodin et al., 1990; Akkus, 2011). If the natural antioxidant defense fails to scavenge these free radicals then an imbalance results, causing a state of oxidative stress within the body (Leeuwenburgh and Heinecke, 2001; Vollard et al., 2005) which has a number of physiological implications.

Besides increased oxygen consumption other pathways associated with exercise-induced increased production of reactive oxygen species (ROS) involve the activation of xanthine oxidase (Koyama et al., 1999) and NADPH oxidase (Hessel et al., 2000), ischemia reperfusion (Ji, 1999), enhanced purine oxidation, damage to iron containing proteins, disruption of calcium ion (Ca\(^{2+}\)) homeostasis (Jackson, 2000) and catecholamine auto-oxidation (Zouhal et al., 1998; Reichhold et al., 2009).

In fact, intense exercising requires greater than 10folds oxygen consumption of the resting level (Tsai et al., 2001; Shi et al., 2007; Andersson et al., 2009) while in active muscles, this may increase to 100 times (Andersson et al., 2009). Fortunately, a strong line of defense for ameliorating the toxic effects of these reactive species is active in the two phases, phases I and II (Jancova et al., 2010). In phase I, the cytochrome P450 (CYP450) enzymes as well as oxidases and reductases bring about hydroxylation, oxidation and reduction of metabolites into active metabolic intermediates while the phase II includes N-acetyl transferases (NAT), glutathione S-transferases (GSTs), methyl transferases, sulphotransferases and glucuronyltransferase enzymes which transform xenobiotic and endogenous compounds by conjugating reactions into more hydrophilic compounds which are easily excretable (Jancova et al., 2010). However, there is variability in the regulation of the removal of toxic metabolites depending on the metabolic activities in each phase or in the co-ordination of two phases which is influenced by genetic variability (Balmukhanov et al., 2013). Genetic polymorphism of any of the genes coding for detoxifying and antioxidant enzymes can modulate the effect of chemical carcinogens (Norppa, 1997). Moreover oxidative stress can induce oxidative damage to cellular components of lipids, proteins and also with DNA by the direct interaction of ROS with DNA and with various lipids causing lipid peroxidation (Djordjevic, 2004; Blomhoff, 2005), genetic variability in these genes can influence even the level of genetic damage (Norppa, 2004).
Among the detoxifying enzymes, the glutathione S-transferases (GSTs) are important detoxifying enzymes (Dusinska et al., 2012). The GSTs are a multigene family of isoenzymes (Dusinska et al., 2012) comprising eight distinct gene families encoding the cytosolic soluble GSTs namely- alpha, mu, theta, pi, zeta, sigma, kappa and omega (Jancova et al., 2010) which protect from toxic products generated by reactive oxygen species as well by lipid hydroperoxides, aldehydes, etc. (Tujague et al., 2006). The \textit{GSTM1} (null), \textit{GSTT1} (null), \textit{GSTP1} (GG) gene polymorphisms have shown an association with increased risk for diseases especially those associated with the inflammatory response and increased oxidative stress (Agalliu et al., 2006; Andersson et al., 2009; Manfredi et al., 2009). The \textit{GSTM1} and \textit{GSTT1} null genotypes have no enzyme activity as these are deletion mutants (Pemble et al., 1994). There are two gene polymorphisms of \textit{GSTP1} (at codons 105 and 114) which cause a significant change in enzyme activity. The \textit{GSTP1} homozygous mutant (Val/Val) has lesser enzyme activity (da Silva et al., 2008) than the homozygous wild (Ile/Ile) genotype whereas the heterozygous (Ile/Val) with one Val has intermediate conjugating activity (Watson et al., 1998).

Among the antioxidant enzymes, the superoxide dismutases (SOD, EC 1.15.1.1), act as the first line of defense which protect cells from oxidative stress by catalyzing dismutation of superoxide anions to hydrogen peroxides (Johnson and Giulivi, 2005). There are three superoxide dismutases: Cu-Zn SOD (SOD1), homotetrameric Mn-SOD (SOD2) and glycosylated SOD (SOD3). Earlier known as manganese superoxide dismutase, named as superoxide dismutase 2, mitochondrial (HGNC, 2014), the enzyme detoxifies superoxide anions, reducing the free radicals and thereby reduces the hazard from oxidative stress (Clair, 2004). The superoxide dismutase 2, mitochondrial (SOD2) gene is localized on chromosome 6q25.3 and has two variants, A16V and I58T. The 16A and 58I homozygotes induce better mitigation of oxidative stress compared to that by the 16Val and 58Thr homozygotes and heterozygotes (Zhang et al., 1999; Wang et al., 2001; Clair, 2004; Cai et al., 2005).

Increased or decreased oxidative stress levels can increase or decrease propensity for genetic damage and hence genetic polymorphism studies of genes regulating oxidative
stress (such as GSTs and SODs) can be meaningful in evaluating whether the genetically-predisposed genotype(s) has increased genetic damage. This has significance in studying genetic damage levels associated with sports activities since increased oxygen consumption during exercising/training is known to induce oxidative stress which can cause cellular macromolecular damage to lipids, proteins and DNA (Mastaloudis et al., 2004; Tsai et al., 2004).

The implications of unrepaired and persistent genetic damage are deleterious in terms of being mutagenic and/or carcinogenic (Valko et al., 2006) and as genetic damage can lead to malignancy, neurological disorders and age-related diseases (Halliwell, 2000; Marnett, 2000; Khansari et al., 2009) thereby predisposing the sports persons to these morbid outcomes through oxidative stress because of their professionally-related excessive oxygen consumption during exercising. Genetic damage and oxidative damage in different tissues as well as in physiological and biochemical changes resulting from intensive and strenuous activity have been reported in swimmers (Inoue et al., 1993), runners (Tsai et al., 2001, Mastaloudis et al., 2001, 2004) and in rowers (Sardas et al., 2012) besides being associated with intense physical activity.

However it needs to be emphasized that genetic polymorphism studies have more often been observed for associations between different sports performance measures. The angiotensin-converting enzyme gene (Folland et al., 2000), the α-2-adrenergic receptor (Wolfarth et al., 2000), the beta-1-adrennergic receptor (Defoor et al., 2006), the beta-2-adrenergic receptor (Macho-Azcarate et al., 2002; Wolfarth et al., 2007) and the bradykinin receptor β2 (Williams et al., 2004) have been reported for better endurance sports. Other genetic polymorphisms of α-actinin-3, creatine kinase, nitric oxide synthase 3, were observed to be associated with athletics performance (Rankinen et al., 2006).

An interesting study on the varying frequencies of GST, SOD2, ACE, MTHFR and CYP1A1 genetic polymorphic variants in sub-groups of athletes by Vedyakov and Tonevitskii (2006) that polymorphisms in the metabolizing genes could lead to retention of aggressive metabolites from phase I detoxification affecting the performance of athletes and thereby could influence longevity in sports. It was
suggested that those with an unfavorable genetic profile, an approach could be applied for regulating alternative dietary patterns and cooking processes for each individual athlete tailored according to their performance-associated metabolic genotypes.

Therefore sports persons in the course of their training regimen and during competitions are routinely exposed to exogenous oxidative agents (dust, air pollutants, solar radiation, etc.) on one hand and to the endogenous oxidative agents (free radicals like hydrogen peroxide, super oxide, etc.) triggered by increased oxygen consumption during exercising on the other, they are at a risk for genetic damage.

As such studies on sportspersons engaged in legally-popular sports like handball, baseball, softball, hockey and athletics have not come to attention, therefore the present study was planned. Using a case-control design, aimed to evaluate whether there is any DNA and/or any oxidative DNA damage in sportspersons (n=200) compared to that in healthy individuals (n=200). For investigating whether genetic predisposition in respect of metabolic and antioxidant genotypes can modulate the genetic damage response from strenuous activity, the players were also genotyped for GST (GSTM1, GSTT1, and GSTP1) and SOD2 (C47T and C339T) variants.

**Objectives:**

1. To assess primary DNA damage using the alkaline single cell gel electrophoresis/comet assay in peripheral blood leucocytes of sportspersons.

2. To assess oxidative DNA damage using the enzymatically modified comet assay.

3. To study the genetic polymorphism of Glutathione S-transferase (GSTT1, GSTM1, GSTP1) and Manganese Superoxide dismutase 2, mitochondrial (SOD2 C47T and C339T) genes in sportspersons.

4. To study the association of polymorphisms of these genes with levels of DNA and oxidative DNA damage.

5. To compare the results obtained for the above objectives with those from a healthy, age- and sex-matched control group.