SUMMARY

Sports as a career has lucrative benefits and in institutes of higher learning, they are increasingly in demand. The therapeutic benefits are well known, and recreationally sports assist in the all-round development. However, regular exercising and rigorous training sessions are physically strenuous activities requiring excessive oxygen uptake to meet the transient energy-requirements. In professional sports, this excessive oxygen uptake is a regular feature and it can be arduous in terms of free radical production and a causal factor in oxidative stress if antioxidants cannot mitigate the free radicals. Lesser known is that regular and excessive oxygen uptake results into oxidative stress which can cause genetic damage. Besides variants of genes associated with DNA damage and repair which can affect the capacity to repair genetic damage, metabolic and antioxidant genotypes may modulate genetic insult. Against this background, genotyping of some glutathione S-transferase (GST) and superoxide dismutase (SOD2) genes and genetic damage levels were investigated in sportspersons and in healthy controls. The study was conducted after approval from the Institutional Ethics Committee and voluntary written consent of the participants.

The present case-control study (n=400), the first of its kind from this region, has been carried out to assess primary genetic damage in peripheral blood leukocytes of players of different sports (n=200) and in age- and sex-matched healthy controls doing no strenuous activity (n=200) using the single cell gel electrophoresis (SCGE) assay. Genetic polymorphisms of glutathione S-transferases (GSTT1, GSTM1, GSTP1A313G (rs1695)) and superoxide dismutase [SOD2 C47T (rs4880), SOD2 C399T (rs1141718)] were also examined for any modulation of genetic damage in players and controls. The GSTM1 and GSTT1 genotyping was accomplished using a multiplex PCR method and the GSTP1 and SOD2 gene variants were genotyped using the PCR-RLFP method. Oxidative DNA damage assessment was carried out by the enzymatically modified comet assay and the determination of lipid and lipoprotein levels by using standard kits. In order to find support for genetic damage (if any), oxidative stress biomarkers were spectrophotometrically assessed in blood sera samples as oxidative stress can cause
genetic damage if not neutralized by the inherent antioxidant defense system. The data obtained were statistically analyzed using SPSS.

Study participants comprised university- and college-level students (aged 18-25y) contacted from Guru Nanak Dev University (Amitsar), Khalsa College (Amritsar), Dayanand Anglo-Vedic College (Jalandhar) and Janta College (Jalandhar). General information and demographic details (age, gender, population sub-group, socioeconomic status, dietary and lifestyle patterns) were recorded on a pre-designed questionnaire. Participants had no history of medical illness or exposures for at least the past six months and had no habits of smoking and alcohol-intake. Sports’ specific information included queries about involvement at university-and state-level competitions and details about sports age, training schedules, exercise types and warm-up sessions. Venous blood sample (~5ml) from each participant was collected and aliquoted into three different vials with heparin, ethylene diamine tetraacetic acid and without any coagulant.

Anthropometric measurements (height, weight, hip circumference and waist circumference) were taken for each participant using standard methods to assess obesity. The step-bench test (using a metronome) was performed by each participant to calculate the maximal oxygen uptake (VO$_{2max}$) which assesses the aerobic capacity.

The study group was matched for age, gender, body mass index, fat-free mass and obesity on the basis of cut-offs of waist-height-ratio (WHtR) whereas differed for waist circumference (WC) with more obese in sports group (31.50%) in comparison to obese controls (19.00%), and for waist-hip-ratio, by which more controls were obese (82.50%) compared to obese in sports group (64.50%). The dietary pattern also differed with more preference for non-vegetarian diet (45.00%) in players, and higher intake of juice by controls (36.50%) in comparison to that by players (26.00%).

The sports group included 89% state level and 11% national players engaged in professional sports for >3-12y with an average sports age of 6.02±0.22y while maintaining a regular training regimen of six days per week which included 15-30 min warm-up time followed by 2-6h/day of regular training. Exercise types performed were body rotations, body stretching, jogging and sports’ specific exercises. The sports group
comprised those involved in playing handball \( (n=50, \text{sports age } 6.14\pm0.18 \text{ y}) \), hockey \( (n=50, \text{sports age } 6.72\pm0.26 \text{ y}) \), baseball/softball \( (n=50, \text{sports age } 5.88\pm0.22 \text{ y}) \) and athletics \( (n=50, \text{sports age } 5.34\pm0.22 \text{ y}) \). Handball, hockey and athletics are categorized as high-dynamic, low-static sports whereas baseball and softball are moderate-dynamic, moderate-static sports in respect of their exercise requirements.

Players had higher aerobic-capacity (\( \text{VO}_{2\max} \)) probably because of their regular training schedules with 94.17% males and 1.25% female in the superior aerobic-capacity category, 3.33% males and 35.00% females in excellent category, 2.50% males and 60.00% females in the good category and 3.75% females in the fair aerobic-capacity category. Control males had superior (37.90%), excellent (29.84%), good (29.03%) and fair (3.23%) aerobic-capacities. The control females were in good category (44.74%) with 1.32% in excellent, 51.32% in fair and 2.63% in poor categories. Interestingly among the control males, 23.50% had superior aerobic-capacity and hence can perform well in sports activities.

The alkaline SCGE assay on peripheral blood leukocytes was used to assess primary genetic. As there were 60% male and 40% female sportspersons (with similar representation in controls), gender stratification for genetic damage levels was done. Using the alkaline SCGE assay, primary genetic damage in players revealed significantly increased DNA damage viz. 3.5fold higher per cent tail DNA, 11.51-fold tail moment (TM), 6.55-fold Olive tail moment (OTM), 1.66-fold Damage Index (DI) and 1.42-fold Damage Frequency (DF) compared to the values in controls. There was a marked gender effect as in male players, tail moment (1.39-fold) and Olive tail moment (1.25-fold) were significantly higher from those in female players while no differences were observed for per cent tail DNA, Damage Index and Damage Frequency. Some gender effects were also apparent among controls. Hence the data on males and females were not pooled and were subjected separately for further analysis.

For oxidative DNA damage assessment in a sub-set of the study group, foramidopyrimidine glycosylase (FPG, for oxidized purines) and endonuclease III (Endo III, for oxidized pyrimidines) were incorporated in the SCGE assay. There was increase in total oxidized purines and oxidized pyrimidines for per cent tail DNA, TM
and OTM, all being significantly higher (p<0.000) in players from values in controls. Within the male and female players, respective per cent tail DNA of oxidized pyrimidines (5.63±0.81 vs. 3.52±0.44; p=0.027), of oxidized purines (6.99±0.83 vs. 3.92±0.58; p=0.004) as well as total oxidative DNA damage (12.67±1.46 vs. 7.44±0.69; p=0.003) were significantly higher in male players when compared to values in female players. The gender differences are probably on account of the excessive uptake of oxygen in males leading to higher oxidative stress and concurrently higher genetic damage.

Oxidative stress biomarkers revealed oxidant-antioxidant imbalance in players, where malondialdehyde (MDA) as a biomarker of lipid peroxidation (1.33-fold), total oxidant status (TOS, 1.25-fold), oxidative stress index (OSI, 1.13-fold) and increased total-antioxidant capacity (TAC, 1.09-fold) showed significant increase in players when compared to respective control values. The levels of superoxide dismutase (SOD) were however similar in both groups. In sports and control group, none of the oxidative stress biomarker levels differed between genders.

The lipidemic profile when assessed in study participants showed significantly (p=0.000) increased high density lipoprotein (HDL-C, 1.17-fold) levels and decreased low density lipoprotein (LDL, 1.17-fold), very low density lipoprotein (VLDL, 1.17-fold) as well as lipid ratios (TC/HDL-C, LDL/HDL-C, TG/HDL-C) in players in comparison to values in controls. When stratified by gender within players and controls, male players had significantly higher levels of total cholesterol (TC, 1.09-fold), LDL (1.17-fold) and of TC/HDL-C (1.07-fold), LDL/HDL-C (1.15-fold) than females but control males only differed with respect to higher levels of triglycerides (TG, 1.17 fold) and TC (1.08-fold) from females.

As different sports have different training regimens and intensity requirements, the levels of genetic damage, oxidative DNA damage and oxidative stress may be different as a function of aerobic capacity (superior, excellent, good, fair), sports age (5.06±0.13y vs. 9.24±0.14y) and training duration (3.67±0.59y vs. 5.95±0.62y). No differential differences however were observed for these parameters implying that increase in
aerobic capacities, sports age and training duration did not cause increase in genetic damage, oxidative stress and oxidative DNA damage.

On stratification for sports types, primary genetic damage (per cent tail DNA, TM, OTM, DI and DF) was significantly elevated in hockey players followed by that in runners and baseball- and- softball players with least in the handball players. Oxidative DNA damage levels however did not differ by sport types. There were also no gender differences for both primary and oxidative DNA damage as a function of sports. For oxidative stress biomarkers, hockey players had statistically significantly elevated MDA levels when compared to handball players. TAC levels were significantly higher in handball players compared to levels in runners. Levels in baseball and softball players were significantly higher in comparison to values in runners. OSI was significantly decreased in baseball and softball players when compared to its values in runners and hockey players. Lipid profile, when stratified for sport types, revealed statistically lower values for triglycerides in handball players compared to levels in baseball-softball players, in hockey players and in runners. Therefore as a function of sport types, hockey players had significantly elevated levels of genetic damage, MDA and TOS. Runners had significantly higher OSI and handball players had significantly lower TG levels compared to respective values in other players.

When genetic damage and oxidative stress were assessed as a function of obesity (based on WHtR) no significant differences between obese and non-obese players and controls were observed. A gender effect was observed however as non-obese male players had significant increase of per cent tail DNA, TM and OTM in comparison to values in non-obese female players while the trend was reverse in non-obese controls. Differences were however observed as a function of obesity for oxidative DNA damage. Non-obese male players had significantly higher levels of oxidized purine per cent tail DNA and oxidized purine TM when compared to values in obese male players. When stratified for gender, non-obese male players had significantly elevated oxidized purine per cent tail DNA and oxidized pyrimidine per cent tail DNA when compared to levels in non-obese female players. Furthermore the non-obese male players had significantly elevated levels when compared to values in non-obese female players for levels of TC,
LDL-C, TC/HDL-C and LDL-C/HDL-C. These results exhibit that genetic damage, oxidative stress and lipemic profile did not differ as a function of obesity while oxidative DNA damage was significantly higher in non-obese compared to obese male players.

Molecular genetic analysis revealed that the genetic variants were in Hardy Weinberg Equilibrium. The distribution of GSTT1, GSTM1, GSTP1 (A313G, rs1695) and SOD2 (C47T- rs4880) and C399T- rs1141718) genotypes was not statistically different between the player and control groups. Similarly no significant differences for genotypes were observed when compared on basis of gender and sport types.

In order to find out whether genetic polymorphisms modulated genetic damage in players and controls, influence of single and combinations of the different genotypes were assessed in players, controls and in the total participants. There was observed elevated genetic damage in GSTP1 (A313G) homozygous (GG) variant in comparison to that in the homozygous wild (AA) and the heterozygous genotypes (AG) in players and in the total participants indicating the probable role of GSTP1 variant (GG) in causing increased genetic damage. To study the combined effect of studied genotypes on genetic damage and oxidative stress parameters, a total of 34 combinations were obtained from which 14 combinations for players and 15 combinations in controls with ≥5 individuals were included for pair-wise comparisons within players and controls. It was observed that different combinations act differently for different parameters of genetic damage and oxidative stress biomarkers in players and controls.

As the study group comprised different population sub-groups viz. 12.50% Brahmins, 65.50% Jat Sikh, 12.50% Scheduled castes and 12.50% Backward classes, on stratification by these groups, neither genetic damage or oxidative stress nor genotyping data showed statistical significance within players and within controls. Therefore the data were not stratified by population sub-groups.

To study the association of confounding factors with genetic damage and oxidative DNA damage, regression analysis of data on male players revealed that genetic damage parameters showed significant association with aerobic capacity, sports age, fat-free mass, TAC, mobile phone usage and diet. For oxidative DNA damage indices,
warming-up, diet, socioeconomic status and waist circumference were significantly associated. In female players, for primary genetic damage parameters (per cent tail DNA and TM), significant association was observed for aerobic capacity and BMI and with WHtR for oxidative DNA damage indices. Combined multivariate analysis on data of male players revealed SOD2 (C47T) as predictor for per cent tail DNA. Correlation analysis revealed similar results. Therefore the predictors of genetic damage parameters were sports age, warm-up, aerobic capacity, fat-free mass, BMI, WHtR, mobile phone usage diet, socioeconomic status, TAC, GSTT1 and SOD2 (C47T).

The genotypic data on players and controls were also subjected to analysis to find if any of the inheritance models showed significant association. Only the co-dominant model of SOD2 (C47T) showed 1.36x likelihood of the TT genotype to be present in players in comparison to controls. The distribution of genotypes in combinations also showed significant differences in players with respect to control groups. On considering the combinations of the three GST genes, significant distributions of the GSTM1 present, GSTT1 present and GSTP1 (GC) genotype and the GSTM1 present, GSTT1 null and GSTP1 (AG) genotype was observed being higher in players. In SOD2 and GSTM1 combination, for double mutant of SOD2 (TT) and GSTM1 null genotypic combination were significantly predictive in players. On OR analysis of SOD2 and GSTP1 genotypic combinations, the SOD2(CC) and GSTP1 (AG) genotypic combination as well as the SOD2 (CT), GSTP1 (AG) and the SOD2 (TT) and GSTP1 (GG) genotypic combinations were present in higher frequency in the player group.

Considering the four genotypes together, significant associations were observed for the SOD2 (CT), GSTP1 (AG), GSTT1 present and GSTM1 present; SOD2 (CT), GSTP1 (GG), GSTT1 present and GSTM1 null; SOD2 (CT), GSTP1 (GC), GSTT1 present and GSTM1 null; SOD2 (CT), GSTP1 (GC), GSTT1 null and GSTM1 present and SOD2 (TT), GSTP1 (AA), GSTT1 null and GSTM1 present genotypes. Thus, with increasing genotypic combinations, higher frequencies were present in players compared to controls.

In players, principal component analysis was performed on 24 variables, 12 factors got extracted with cumulative variance of 76.24%. In factor 1, 12.94% variance was
contributed by gender, height, weight, and aerobic capacity. Factor 2 was loaded with variables of weight, waist circumference, waist-height-ratio with 9.26% variance. In factor 3, \textit{GSTM1} null and in factor 4, \textit{GSTTI} null contributed to 13.19% of the variance. Factors 5-10 included TOS, OSI, \textit{SOD2} (CT), age, sports age, time duration, WHR, \textit{GSTP1} (GG), diet, SOD levels, and warm-up with cumulative variance of 41.15%. Therefore in the player group of present study, these factors correlated with each other contributing towards the variance in the data.

In conclusion, significantly increased genetic damage, oxidative DNA damage and oxidative stress were observed in sports persons in comparison to the controls probably associated with the strenuous physical activity in players. Male compared to female players had increased genetic damage. Though genetic damage as a function of sports age, training duration and aerobic capacities did not differ within the respective categories, as a function of sport types, hockey players had maximum genetic damage followed by runners, baseball-softball players and least in handball players. Among combinational genotypes, the presence of \textit{GSTTI} and \textit{GSTP1} (AG) genetic variants were associated with genetic damage. The frequency of the \textit{SOD2} (C47T) gene differed between players and controls with 1.36x likelihood of genotype to be present in players. From genotyping results, the \textit{GSTP1} (A313G) showed significant genetic damage differences for variant homozygous (GG) with heterozygous (AG) and wild homozygous (GG) in players. Hence the study results reveal that presence of genetic polymorphism of \textit{GSTP1} can be determining factors for DNA damage provoked by strenuous physical activity in the players group of present study.