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Traditional Indian sports – A case-control study on *Kho Kho* players investigating genomic instability and oxidative stress as a function of metabolic genotypes

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ABSTRACT

The beneficial effects of physical exercise regularly for overall well being, or for recreational or professional purposes are widely accepted in clinical practice and have from time immemorial been the reasons for performing traditional sports. On the contrary, there is also evidence implying increased oxidative stress and genetic damage from intensive exercising. Depending on the intensity, time, frequency and characteristics of exercises, there can be differential induction of oxidative stress and provocation of oxidation of cellular macromolecules (including DNA) and cellular dysfunction which can likely accumulate with age, physical attributes and increase the susceptibility to disease on one hand, while stimulating cell signalling pathways leading to cell adaptation and improved resistance to stress, on the other. In order to observe if continuous sports activities as in *Kho Kho* increase oxidation capacity, which can also provoke oxidation of cellular macromolecules, the effects on oxidative/antioxidant changes and DNA damage in professional *Kho Kho* players modulated by individual genetic differences were assessed. *Kho Kho*, a traditional Indian game of 'Tag', is an all-time favourite which requires endurance, agility and strength. Healthy *Kho Kho* players (20.27 ± 0.28 y; sports age 6.78 ± 0.52 y) and controls (20.90 ± 0.45 y) were matched for age, gender, BMI, VO_2 max (maximal oxygen uptake), frequency of *GSTT1* (present/null), *M1* (present/null), *SOD2* (C199T) polymorphisms but differed for variant allele frequencies of *GSTP1* (A313G) and *SOD2* (C47T). Players compared to controls had significantly increased levels of DNA damage (1.8x, 44.66 ± 1.68 vs. 23.85 ± 1.79 μm, p = 0.000), lipid (MDA) peroxidation (2x, 1.72 ± 0.06 vs. 0.83 ± 0.16 μmol/l, p = 0.000) and total antioxidant capacity (1.09x, 1.69 ± 0.06 vs. 1.11 ± 0.03 mmol Trolox equivalent/l, p = 0.000) but with no differences for SOD activity (94.99 ± 2.42 vs. 93.36 ± 2.54 U/ml, p = 0.935). These results suggest that the players have increased genetic damage and oxidative stress probably from the intense physical activity in the absence of other exposure(s) as other attributes were comparable in the study group. The players may therefore be at increased risk for susceptibility to cancer, various diseases and precocious age-related changes and should be sensitized to health risks related to regular intensive physical exercise.

1. Introduction

Among recreational and semi-professional games is the Indian game of tag, *Kho Kho*, which has its etiology in the state of Maharashtra (Marathi *khō-khō*); it is a team sport where contact from the opponents is to be avoided. Earlier known as *Rathera* on account of being played on Indian chariots (*raths*), the game has emerged as a version of tag, a modified form of 'run-and-chase' in which the purpose is to chase/pursue and touch the opponent. Even though a team sport, *Kho Kho* at individual level requires the variables of stamina, endurance, strength and agility, and the skills of dodging, feinting and bursts of speed because the game is vigorous and combative in nature [1]. Running, skipping and weight-lifting are the training measures for maintaining endurance and strength required for the sport; the game is complicated and tactical and those with the mesomorphic somatotype and good muscle development and with better anerobic and aerobic fitness compared to volley ball players, perform well. The game has become popular because it does not require expensive equipment (only wooden posts, string, measuring tape and stop watch) and yet builds strength, stamina and agility, which gets tested in the time-frame (20–35 min) of the game (www.olympic.ind.in/images/KhoKho.pdf; accessed on July 13, 2018). In fact compared to players of *Kabbadi* (another indigenous game), the physical fitness

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variables of agility, speed, power and endurance, and the only variable of rhythmic ability among the coordinative ability variables, have been reported as significantly better in *Kho Kho* players. The lean body mass (LBM) value was also significantly higher in *Kho Kho* players compared to non-sports persons [2], and female *Kho Kho* players had greater LBM and ectomorph component compared to controls. In fact LBM is considered a main pre-condition for good performance in playing the game as LBM (rather than body weight) is associated with the physiological parameters of oxygen consumption, cardiac output and vital capacity [3].

In *Kho Kho*, as in other sports, there is increased requirement for oxygen uptake for performing the intense physical activity and for training during aerobic and anaerobic exercises so as to maintain endurance and stamina. However the increased oxygen consumption can alter the intracellular pro-oxidant homeostasis on increased production of reactive oxygen/nitrogen species (ROS/RNS) and can cause an imbalance of the oxidant-antioxidant system and lead to oxidative stress. Documentations exist on oxidative stress in those engaged in professional sports and on oxidation of cellular biomolecules by ROS and affecting genomic stability [4, 5]. But as exercises of different types, intensity and duration may have differential damaging effects on the cellular components of DNA and other macromolecules, and as there are relatively few studies which have addressed these issues for traditional sports, therefore this remains an area of active investigation and which the present study has tried to partially address. Such studies are also important in view of the "physical activity health paradox [6]" which argues that different domains of physical activity have different effects on health; rather the occupational physical activity does not provide the cardiovascular health benefits that leisure time physical activity does.

Recently, Kawakami *et al.* [7] have also reported decreased levels of DNA damage in individuals performing high levels of leisure-time physical activity but with low physical activity at work. On the other hand, significantly increased oxidative DNA damage was observed in ballet dancers after the dancing season compared to the pre-season levels and the authors [8] have suggested a need for measures to protect ballet dancers from potential occupational health risks.

In fact it is well known that oxidative damage to nucleic acids can cause base substitutions, additions, deletions, and other mutations and result in the development of various diseases [9]. Elevated ROS levels (nutrition-, environment-, exposure-mediated) can also stimulate cancer initiation by triggering DNA mutations, damage, and pro-oncogenic signalling [10]. Since damage to DNA, as from oxidative stress, is an initial step in the pathogenesis of numerous diseases including cancers, the biomarkers of DNA damage can be used to study the relationship of oxidative damage and risk in the development of cancer and various diseases [9, 10].

Therefore for the present study, the purpose was to investigate players of an indigenous sport (*Kho Kho*) for levels of genetic instability and oxidative stress as a function of inherited individual differences because genetic variants, besides affecting sports performance [11], can also modulate biomarkers of genetic damage and oxidative stress [12]. As genomic instability and oxidative stress underlie the manifestations of precocious ageing, age-related diseases, neurological disorders and malignancy [13, 14, 15], studies evaluating these biomarkers in sports-persons can find relevance in initiating strategies for circumvention of early disease-onset and carcinogenesis.

Hence in this case-control study, blood samples were assessed for genetic damage, oxidative stress and metabolic genotyping. Peripheral blood leukocytes (PBL) were investigated for DNA damage using the alkaline single cell gel electrophoresis (SCGE/comet) assay which assesses double- and single-strand DNA breaks and detects alkali-labile sites [16]; it has also found use in indirect measurement of oxidative stress [17]. Levels of products of oxidation assessed in blood serum included lipid peroxidation (Malondialdehyde, MDA), Total Antioxidant Capacity (TAC; it determines the antioxidative effects of bilirubin, uric acid, vitamin C, polyphenols and proteins) and the activity of superoxide dismutase (SOD2), an endogenous antioxidant enzyme. Genotyping of the metabolic genes (involved in xenobiotic metabolism and biotransformation reactions) of the Glutathione-S-transferases (*GSTT1, M1, P*) and of Manganese superoxide dismutase (*$MnSOD_2$, SOD2*) was performed to discern whether the levels of the circulating biomarkers are affected by the presence of some of the single nucleotide polymorphisms (SNPs). Comparisons with the similarly studied biomarkers in healthy persons (controls not engaged in physical activity and without exposures) formed the basis of the present case-control study design.

2. Materials and methods

The study was a part of a research proposal on 'Studies on Sports Excellence' which was approved by the Institutional Ethics Committee (IEC). The participants under voluntary, written informed consent were contacted from the sports ground of the university (players), and healthy controls (not physically active in sports/occupation), from the general population. Inclusion criteria comprised unrelated healthy adults with no accidental/incidental/occupational exposure(s), no recent past (~6 mo) illnesses, not on any prescribed/non-prescribed medications, representing both genders, belonging to the same geographic region (*Punjab*), and with the same socioeconomic status. Exclusion criteria were contrary to these.

The participants provided information on general demographics, lifestyle patterns, and exposure and medical histories and sports' related information (from players) on a specially-designed questionnaire. Anthropometric measurements of height, weight and waist and hip circumferences were taken using standard procedures [18]. Obesity status was determined on the basis of Body Mass Index (BMI, kg/m^2; [19]), and on the cut-offs [20] of waist circumference (WC, cm) and waist-hip-ratio (WHR). The Step Bench test [21] was performed by the participants and using the metronome, maximal velocity oxygen uptake (VO_2 max) was obtained, and the participants were categorized for aerobic capacity [22]. Venous blood (5 ml) collection was carried out before training sessions (and at similar times from controls). The samples were dispensed into vials with anticoagulants (heparin for comet assay; 0.5M ethylene diamine tetraacetic acid (EDTA) for molecular genetic work) and without anticoagulant (for serum separation), and were transferred to the laboratory on ice.

2.1. Genetic damage assessment

Genetic damage was assessed using the alkaline SCGE/comet assay [16] using locally available chemicals after samples had been assessed for cell viability (>90%) by the Trypan-Blue Dye–Exclusion test [23]. Whole blood (25 μl) was mixed with 100 μl of low melting point agarose (LMPA, 0.5%), transferred onto glass slides (2/sample) pre-coated with normal melting point agarose (1% NMPA) and covered with cover slips. After 10 min at 4 °C, the cover slips from the slides were removed and a layer of NMPA (100 μl, 0.5%) was poured on the glass slides and allowed to set. The cell preparations were lysed (2–3 h, 4 °C) in freshly prepared chilled lysing solution (2.5 M NaCl, 100 mM EDTA-2Na, 10 mM Tris HCl, pH 10.0–10.5 with 1% Triton X-100 and 10% DMSO added just before use). The slides were then placed in a horizontal gel electrophoresis tank (20 min, 4 °C) containing freshly prepared electrophoresis buffer (300 mM NaOH, 1 mM Na2EDTA, pH 13.0) to allow for DNA unwinding. Electrophoresis was carried out for 20 min at 25 V (300 mA, 0.75 V/cm). The slide preparations were then washed thrice in neutralization buffer (0.4 M Tris–HCl, pH 7.5) to remove excess alkali and detergents, air-dried, fixed (15% trichloroacetic acid, 5% zinc sulphate, 5% glycerol), washed in distilled water and again air-dried at room temperature. Staining was carried out with silver nitrate ([24]; 75 mg sodium bicarbonate, 0.1 mg silver nitrate, 0.1 mg ammonium nitrate, 0.5 mg silicotungstic acid) under continuous shaking till there was the development of greyish colour on the slides, followed by washings in stopping solution (1% acetic acid for 5 min) and distilled water and then air-drying at room temperature.

The slides were coded and scored blind under a transmission binocular microscope (Olympus LX-41) for nucleoids (supercoiled loops of DNA linked to the nuclear matrix, [25]). DNA loops with breaks lose their super coiling under electrophoresis and the DNA extends towards the anode appearing as a comet [26]. The comet assay provides quantification of DNA damage at single cell level. In the present study, as per the original alkaline SCGE assay [16], the manual measurement of DNA migration length was carried out though presently using image analysis systems and comet assay software programmes, the measurement of per cent DNA tail is considered more appropriate. Nonetheless, the visual scoring system continues to be considered as an alternative to the image analysis systems [27]. Therefore the extent of DNA migration (from the centre of the nucleoid to the end of the comet tail) was measured at 40× using a calibrated occulo-micrometer fitted on the eyepiece of the microscope. For each participant, 100 nucleoids (50 per slide) were scored for mean DNA migration length (μm) and per cent nucleoids with tails were documented as DF-Damage Frequency [28].

2.2. Serum preparation

Anticoagulant-free blood samples were centrifuged at 1500 g for 10 min to obtain the serum which was used for spectrophotometric (Thermo-Scientific Spectronic 20D^{+}) assessment of levels of Malondialdehyde (MDA), Total Antioxidant Capacity (TAC), and for Superoxide Dismutase (SOD) activity.

2.3. Assessment of malondialdehyde levels

The levels of MDA (as a marker of lipid peroxidation) were assessed as per the protocol of Devasagayam *et al.* [29]. For this to the serum sample (50 μl), were added Tris-HCl (0.1 ml), ascorbic acid (0.1 ml) and ferrous ammonium sulphate (0.1 ml), and a final volume (1.0 ml) was obtained by adding distilled water. After incubation (37 °C, 15 min), thiobarbituric acid (1.0 ml) and trichloroacetic acid (TCA) were added and the contents were kept at 100 °C for 15 min (for the development of

Table 1
Demographic characteristics of *Kho Kho* players and the controls.

Study Characteristics				*Kho Kho* players (n = 18) (%)	Controls (n = 10) (%)	P values	
Gender		Males		11 (61.11)	08 (80.00)	0.546	
		Females		7 (38.88)	02 (20.00)		
Age (y)				20.27 ± 0.28	20.90 ± 0.45	0.239	
Warm-up time (min)				23.33 ± 1.51	–	–	
Daily training sessions during camps (h)				4.33 ± 0.38			
Time-since-exercising/Sport Age (y)				6.78 ± 0.52			
Professionally active sport age (range)				3–11 y			
[a]Body Mass Index-BMI (kg/m^2)	Underweight (<18.0)			–	–	0.516	
	Normal (18.00–22.90)			15 (83.33)	09 (90.00)		
	Overweight (23.00–24.90)			01 (5.55)	01 (10.00)		
	Obese (>25.00)			02 (11.11)	–		
	Average			21.49 ± 0.58	20.78 ± 0.57	0.433	
[b]Waist Circumference-WC (cm)	Non-obese	Females	<80	7 (38.88)	02 (20.00)	0.182(f)	
		Males	<85	11 (61.11)	08 (80.00)	0.646(m)	
	Obese	Females	≥80	–	–	0.546(t)	
		Males	≥85				
	Average			32.69 ± 0.57	80.77 ± 2.74	**0.000**	
[b]WHR (Waist-Hip-Ratio)	Non-obese	Females	<0.81	–	–	0.182(f),	
		Males	<0.88		01 (10.00)	0.479(m),	
	Obese	Females	≥0.81	7 (38.88)	02 (20.00)	0.665(t)	
		Males	≥0.88	11 (61.11)	07 (70.00)		
	Average			0.89 ± 0.01	0.93 ± 0.02	0.164	
[c]VO_2 max (Aerobic Capacity; ml kg^{-1} min^{-1})	Fair	Females	36–39	–	02 (20.00)	–	**0.045**
		Males	42–45	–	–		
	Good	Females	40–43	05 (27.77)	–	0.327(t)	
		Males	46–50	–	01 (10.00)		
	Excellent	Females	44–49	01 (5.55)	–	0.723(m);	
		Males	51–55	03 (16.66)	05 (50.00)	0.905(t)	
	Superior	Females	>49	01 (5.55)	–	0.11(m);	
		Males	>55	08 (44.44)	02 (20.00)	0.386(t)	
	Average			54.37 ± 2.21	50.37 ± 2.06	0.243	
GST	***GSTT1* (rs17856199)**	Present		11 (61.11)	02 (20.00)	0.119	
		Null		08 (44.44)	08 (80.00)		
	***GSTM1*(rs366631)**	Present		11 (61.11)	06 (60.00)	0.774	
		Null		08 (44.44)	04 (40.00)		
	***GSTP1* (A313G) rs1695**	AA		18 (100.00)	05 (50.00)	**0.005**	
		AG		–	05 (50.00)		
		GG		–	–		
		A		36 (100.00)	20 (75.00)	**0.020**	
		G		–	05 (25.00)		
SOD2	***SOD2* (C47T) rs4880**	CC		05 (27.77)	07 (70.00)	**0.019**	
		CT		04 (22.22)	03 (30.00)		
		TT		09 (50.00)	–		
		C		14 (38.88)	17 (85.00)	**0.002**	
		T		22 (61.11)	03 (15.00)		
	***SOD2* (C399T) rs1141718**	CC		18 (100.00)	10 (100.00)	0.185	
		C		36 (100.00)	36 (100.00)	0.906	

p values in bold are statistically significant ($p < 0.05$); VO_2 max - maximal oxygen uptake; m - males, f - females, t - total.
[a] [19].
[b] [20].
[c] [22].

colour) followed by centrifugation (290 g) for 10 min. The absorbance of the chromogen was recorded at 532 nm against the blank and the concentration (μmol/l) of MDA was calculated [C = E/K*L, where E = extinction absorbance, K = molar extinction coefficient (1.5×10^5), L = length of cuvette, C = concentration].

2.4. Total antioxidant capacity

For determining the levels of TAC in the serum, the standard method of Erel [30] and as modified by Gupta *et al.* [31] was used. To 50 μl of the serum sample, 200 μl of reagent comprising acetate buffer (0.4 mmol/l, pH 5.8) was added and kept for incubation (5 min) followed by the addition of 20 μl of $ABTS^+$ (2,2-azinobis 3-ethylbenzothiazoline-6-sulphate) in acetate buffer (30 mmol/l, pH 3.6). The absorbance of $ABTS^+$ was recorded on a microplate reader (BioRad 680XR) at 660nm and TAC concentration (mmol Trolox equivalent/l) was calculated Factor × [(Δ A Blank) − (Δ A Sample)] where ΔA = A2 (Final Absorbance) − A1 (Initial Absorbance) of blank, sample or of standard and the Factor = Δ A Standard/(Δ A Blank − Δ A Standard).

2.5. Superoxide dismutase (SOD) activity

SOD activity was determined [32] by mixing serum sample (50 μl) in EDTA solution (1.3 ml), nitroblue tetrazolium (0.5 ml), Triton-X100 (0.1 ml) and hydroxylamine hydrochloride (0.1 ml); the SOD activity (U/ml) was recorded at 560 nm using Tris-HCl buffer as blank.

2.6. Molecular genetic analysis

Genomic DNA was isolated from whole blood by the organic method [33] and was quantified under 0.8% agarose gel electrophoresis [34]. A total of five SNPs were studied viz. three variants of the *GST* genes (*GSTM1, GSTT1, GSTP1* (A313G)) and two of *SOD2* (C47T, C339T). For genotyping of *GSTM1* and *GSTT1*, multiplex-PCR was carried out using intron 3 of *HLADRB1* as an internal control [35, 36]. Genotyping of *GSTP1* [35] and *SOD2* [37] was performed using PCR-RFLP analysis. The details of the PCR reaction mixtures and the thermal cycler (Applied Biosystems, India) conditions are described below.

For *GSTT1* and *GSTM1*, multiplex PCR was set up using the F 5′-TTCCTTACTGGTCCTCACATCTC-3′, R 5′-TCACCGGATCATGGCCAGCA-3′ for *GSTT1*, F5′-GAACTCCCTGAAAAGCTAAAGC-3′, R 5′-GTTGGGCTCAAATATACGGTGG-3′ for *GSTM1* using F5′-TGCCAAGTGGAGCACCCAA-3′, R5′-GCATCTTGCTCTGTGCAGAT-3′ for internal control (Intron 3 of *HLADRB1*). The PCR mixture for the 15 μl reactions comprised 8.4 μl PCR water, 1.5 μl 10XTaq buffer, 0.3 μl dNTP mix, 0.4 μl each of *GSTM1, T1* and of internal control forward and reverse primers, 1.5 μl 10% DMSO, 0.3 μl *Taq* polymerase and 0.6 μl DNA sample (50 ng/μl). The conditions for amplification included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 35 s, 61 °C for 35 s, 72 °C for 35 s and a final extension at 72 °C for 10 min. The amplified *GSTM1* gene fragment comprised 220 bp, and 450 bp for *GSTT1* gene and with which the internal control fragment of 796 bp was also amplified, as observed on 1.20% agarose gel electrophoresis.

For *GSTP1* (exon 5, A313G, rs1695; Ile105Ala) genotyping, F 5′-ACCCCAGGGCTCTATGGGAA-3′, R5′-TGAGGGCACAAGAAGCCCCT-3′ primers were used. The PCR mixture (15 μl) constituted of 10.7 μl PCR water, 1.5 μl 10xTaq buffer, 0.5 μl dNTP mix, 0.5 μl each of forward and reverse primers, 0.3 μl *Taq* polymerase and 0.5 μl DNA sample. The PCR conditions comprised an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 1 min, 63 °C for 35 s, 72 °C for 35 s and a final extension at 72 °C for 10 min. Restriction digestion was done with *Alw 26I* at 55 °C for 16 h and the results on electrophoresis (2.20% agarose gel) were genotyped as AA - 176 bp, AG - 176+91+85 bp and GG - 91+85 bp.

For genotyping of *SOD2* (exon 2, C47T, rs4880; Val16Ala), F 5′-ACCAGCAGGCAGCTGGCGCCGG-3′, R 5′-GCGTTGATGTGAGGTTCCAG-3′ primers were used with PCR conditions of 9.3 μl PCR water, 1.5 μl 10XTaq buffer, 0.4 μl dNTP mix, forward and reverse primers (each 0.5 μl), 1.5 μl 5%DMSO, 0.5 μl *Taq* polymerase and 0.8 μl DNA sample. The thermal cycler was programmed for an initial denaturation at 95 °C for 5 min, followed by 30 cycles of 95 °C for 35 s, 71 °C for 35 s, 72 °C for 35 s and a final extension at 72 °C for 10 min. Restriction digestion with *NgoMIV* at 37 °C for 16 h revealed CC - 107 bp, CT - 107+89+18 bp and TT - 89+18 bp genotypes on 2.50% gel electrophoresis.

For the second *SOD2* variant (exon 3, C399T, rs1141718; Ile58Thr), the F 5′-AGCTGGTCCCATTATCTAATAG-3′, R 5′-TCAGTGCAGGCTGAAGAGAT-3′ primers were used with PCR components (15 μl reaction) of 10.3 μl PCR water, 1.5 μl 10xTaq buffer, 0.3 μl dNTP mix, 1.5 μl 5% DMSO, 0.3 μl each of forward and reverse primers, 0.3 μl *Taq* polymerase and 0.5 μl DNA sample. The amplification conditions included an initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 59 °C for 30 s, 72 °C for 35 s and a final extension at 72 °C for 10 min. The restriction digestion was done with *EcoRV* at 37 °C for 16 h. The genotyping was carried out as CC - 139 bp, CT - 139+117+22 bp and TT - 117+22 bp on 2.50% agarose gel electrophoresis.

2.7. Statistical analyses

Statistical analysis was carried out using SPSS (version 16.00) and MedCalc software for Windows. Categorical data are presented as numbers and percentages whereas the continuous variables are given as mean ± S.E.M. Chi-squared analysis was carried out for categorical and molecular genotypic data. Students' t-test was performed for comparison of means of continuous variables as data showed normal distribution on Kolmogorov-Smirnov testing. Analysis of variance (ANOVA) followed by *post hoc* Tukey was performed for multiple comparisons of means in different categories. Pearson correlation and univariate regression analyses were performed for association factors and predictor assessment for

Table 2
Gender stratification for the assessed parameters.

Assessed Parameters		*Kho Kho* Players			Controls			P value (Players vs Controls)
		Males (n = 11)	Females (n = 07)	Total	Males (n = 8)	Females (n = 2)	Total	
Trypan-Blue Dye Exclusion Test	Cell Viability (%)	90.38	85.92	88.15	87.90	92.14	90.02	–
SCGE Assay	Damage Frequency (AU)	85.8***1 ± 4.95	95.28 ± 2.35	89.50 ± 3.29	72.87 ± 2.96	73.50 ± 3.50	73.00 ± 2.39	**0.000**
	Mean DNA migration length (μm)	43.17*** ± 1.55	45.78 ± 0.99	44.66 ± 1.68	25.38 ± 1.21	22.14 ± 1.32	23.85 ± 1.79	**0.000**
Total Antioxidant Levels (mmol Trolox eq/l)		1.69*** ± 0.06	1.53*** ± 0.14	1.63*** ± 0.06	1.09 ± 0.04	1.19 ± 0.04	1.11 ± 0.03	**0.000**
Malondialdehyde (MDA) Levels (μmol/l)		1.72*** ± 0.06	1.63*** ± 0.08	1.69*** ± 0.05	0.66 ± 0.15[a]	1.48 ± 0.09[a]	0.83 ± 0.16	**0.000**
Superoxide Dismutase (SOD) Activity (U/ml)		94.99 ± 2.42	90.09 ± 3.82	93.08 ± 2.11	93.34 ± 1.71	91.11 ± 0.90	93.36 ± 2.54	0.935

p values in bold are statistically significant ($p < 0.05$).
***very highly significant ($p = 0.000$).
[a] $p = 0.03$.

Table 3
Genetic Damage, SOD Activity, TAC and MDA Levels Stratified for VO_2 max Categories and Molecular Genotypes of *GST* and *SOD2*.

VO_2 max Categories and Molecular Genotypes		*Kho Kho* Players						Controls					
		Number	Damage Frequency (AU)	Mean DNA migration length (μm)	Superoxide Dismutase (SOD) activity (U/ml)	Total Antioxidant Capacity (TAC) (mmol Trolox eq/l)	Malondialdehyde (MDA) Levels (μmol/l)	Number	Damage Frequency (AU)	Mean DNA migration length (μm)	Superoxide Dismutase (SOD) activity (U/ml)	Total Antioxidant Capacity (TAC) (mmol Trolox eq/l)	Malondialdehyde (MDA) Levels (μmol/l)
Fair		–						02	73.50 ± 3.50	22.89 ± 2.67	91.11 ± 0.90	1.19 ± 0.04	1.48 ± 0.09
Good		05	96.60*** ± 2.92	40.25 ± 0.36[a]	92.36 ± 4.86	1.78 ± 0.09	1.65 ± 0.11	01	66.00 ± 0.00	21.33 ± 0.00	99.32*** ± 0.00	1.15 ± 0.00	0.60 ± 0.00
Excellent		04	86.50*** ± 4.36	44.67 ± 1.70	95.11* ± 4.13	1.62 ± 0.12	1.73 ± 0.07	05	75.40 ± 4.41	24.08 ± 1.11	93.74 ± 1.19	1.12 ± 0.05	0.55 ± 0.22
Superior		09	86.88*** ± 5.97	47.09 ± 1.26[a]	92.43*** ± 3.03	1.54 ± 0.11	1.68 ± 0.07	02	70.00 ± 2.00	23.76 ± 0.71	89.37 ± 5.83	0.98 ± 0.01	0.97 ± 0.07
GSTT1	Present	11	87.72** ± 4.96	45.60*** ± 1.35	92.94 ± 2.94	1.67* ± 0.10	1.63* ± 0.07	02	81.00 ± 3.00	24.16 ± 1.62	91.78 ± 1.44	1.18 ± 0.00	0.68 ± 0.65
	Null	07	92.28*** ± 3.52	43.15*** ± 1.33	93.30 ± 3.10	1.55** ± 0.04	1.77*** ± 0.05	08	71.00 ± 2.46	23.34 ± 0.85	93.18 ± 1.72	1.09 ± 0.04	0.86 ± 0.15
GSTM1	Present	11	87.72*** ± 4.96	45.60 ± 1.35	92.94 ± 2.94	1.67 ± 0.10	1.63 ± 0.07	06	73.66 ± 3.01	23.53 ± 1.06	94.20** ± 1.48	1.14 ± 0.03	0.84 ± 0.21
	Null	07	92.28*** ± 3.52	43.15 ± 1.33	93.30* ± 3.10	1.55 ± 0.04	1.77 ± 0.05	04	72.00 ± 4.45	23.45 ± 1.06	90.93 ± 2.61	1.06 ± 0.07	0.79 ± 0.29
GSTT1(P)/ *GSTM1*(P)		11	87.72** ± 4.96	45.60 ± 1.35	92.94 ± 2.94	1.67* ± 0.10	1.63 ± 0.07	02	81.00 ± 3.00	24.16 ± 1.62	91.78 ± 1.44	1.18 ± 0.00	0.68 ± 0.65
GSTT1(P)/ *GSTM1*(N)		–						–					
GSTT1(N)/ *GSTM1*(P)		–						04	70.00 ± 2.79	23.22 ± 1.51	95.42 ± 1.93	1.12 ± 0.04	0.93 ± 0.17
GSTT1(N)/ *GSTM1*(N)		07	92.28*** ± 3.52	43.15 ± 1.33	93.30 ± 3.10	1.55 ± 0.04	1.77 ± 0.05	04	72.00 ± 4.45	23.45 ± 1.06	90.93 ± 2.61	1.06 ± 0.0	0.79 ± 0.29
SOD2 (C47T) rs4880	CC	05	86.00*** ± 8.19	45.20 ± 2.78	83.94 ± 2.66[b]	1.59 ± 0.22	1.66 ± 0.13	07	71.14 ± 2.84	23.02 ± 0.92	93.34*** ± 1.98	1.09 ± 0.04	0.76 ± 0.14
	CT	09	95.44*** ± 1.97	44.11 ± 1.22	100.37*** ± 0.52[b/c]	1.62 ± 0.06	1.69 ± 0.03	03	77.33 ± 4.05	24.62 ± 1.04	91.85 ± 0.83	1.16 ± 0.01	0.98 ± 0.48
	TT	04	80.50 ± 9.07	45.19 ± 1.71	88.11 ± 4.15[c]	1.68 ± 0.09	1.71 ± 0.16	–					

***very highly significant ($p = 0.000$).
*significant ($p < 0.05$).
ANOVA followed by post-hoc Tukey, P - present, N - null.
a = 0.002.
b = 0.000.
c = 0.001.

Table 4
Pearson correlation and univariate regression analyses of study characteristics with genetic damage, SOD activity and molecular genotypes.

Study Characteristics	Pearson Correlation/ Regression Coefficient	Damage Frequency	Mean DNA Migration Length	Total Antioxidant Capacity (TAC)	Malondialdehyde (MDA) Levels	Superoxide Dismutase (SOD) Activity
Age	r	0.101	−0.240	−0.057	−0.310	0.088
	β	0.101	−0.240	−0.057	−0.310	0.088
	p	0.611	0.219	0.772	0.109	0.656
Gender	r	0.333	0.027	0.027	0.282	−0.251
	β	0.333	0.027	0.027	0.282	−0.251
	p	0.083	0.893	0.893	0.146	0.198
Time-since-Excercising	r	0.107	−0.267	−0.401	−0.271	0.294
	β	0.107	−0.267	−0.401	−0.271	0.294
	p	0.674	0.284	0.099	0.277	0.237
Warm-up Time	r	0.447	−0.495	−0.207	−0.100	−0.276
	β	0.447	−0.495	−0.207	−0.100	−0.276
	p	0.063	**0.037**	0.411	0.693	0.267
Diet	r	−0.058	0.209	0.149	0.060	0.143
	β	−0.058	0.209	0.149	0.060	0.143
	p	0.771	0.287	0.449	0.760	0.468
Body Mass Index	r	−0.027	0.275	0.178	−0.073	−0.026
	β	−0.027	0.275	0.178	−0.073	−0.026
	p	0.892	0.157	0.365	0.711	0.894
Waist Circumference	r	−0.558	−0.910	−0.703	−0.807	−0.037
	β	−0.558	−0.910	−0.703	−0.807	−0.037
	p	**0.002**	**0.000**	**0.000**	**0.000**	0.850
VO_2 max	r	−0.053	0.381	0.225	0.104	0.081
	β	−0.053	0.381	0.225	0.104	0.081
	p	0.787	0.045	0.250	0.597	0.681
Mobile Phone Usage	r	0.258	0.241	0.080	0.161	0.151
	β	0.258	0.241	0.080	0.161	0.151
	p	0.184	0.216	0.686	0.412	0.443
GSTT1	r	−0.203	−0.452	−0.245	−0.359	0.032
	β	−0.203	−0.452	−0.245	−0.359	0.032
	p	0.299	**0.016**	0.209	0.060	0.872
GSTM1	r	0.074	−0.083	0.022	−0.119	−0.062
	β	0.074	−0.083	0.022	−0.119	−0.062
	p	0.708	0.674	0.910	0.548	0.753
SOD	r	−0.203	−0.185	−0.086	−0.120	0.130
	β	−0.203	−0.185	−0.086	−0.120	0.130
	p	0.301	0.347	0.665	0.543	0.511

p values in bold are statistically significant ($p < 0.05$).

Table 5
Multivariate Regression Analyses of Study Characteristics and Molecular Genotypes of *GST* and *SOD2* with VO_2 max, Genetic Damage, SOD Activity, TAC and MDA Levels.

Study Characteristics	Damage Frequency			Mean DNA Migration Length			SOD Activity			TAC Levels			Malondialdehyde Levels		
	β	t	p	β	t	p	β	t	p	β	t	p	β	t	p
Age	0.564	1.566	0.178	0.130	0.263	0.803	0.225	0.529	0.619	0.270	0.653	0.543	0.186	0.359	0.734
Gender	0.306	0.191	0.856	0.171	0.078	0.941	0.051	0.027	0.979	−2.213	−1.205	0.282	0.895	0.389	0.713
Time-since -excercising	−0.283	−0.647	0.546	0.331	0.552	0.605	−0.097	−0.188	0.858	−0.416	−0.828	0.445	−0.790	−1.257	0.264
Warm-up time	0.220	0.222	0.833	−0.129	−0.095	0.928	−0.032	−0.027	0.979	0.017	0.015	0.989	−0.728	−0.509	0.632
VO_2 max	−0.660	−0.583	0.585	0.130	0.083	0.937	0.096	0.072	0.945	−1.971	−1.516	0.190	0.739	0.454	0.669
Diet	−0.488	−1.333	0.240	−0.201	−0.400	0.706	0.140	0.325	0.759	−0.226	−0.539	0.613	−0.259	−0.491	0.644
Body Mass Index	0.669	1.459	0.204	0.471	0.748	0.488	−0.430	−0.796	0.462	0.019	0.036	0.973	−1.185	−1.795	0.133
Waist Circumference	−0.529	−0.849	0.435	0.362	0.422	0.690	−0.192	−0.262	0.804	0.252	0.351	0.740	0.243	0.271	0.797
Waist-Hip-Ratio	−0.029	−0.065	0.951	0.040	0.065	0.951	−0.916	−1.730	0.144	0.646	1.251	0.266	−0.505	−0.781	0.470
Mobile Phone Usage	0.729	2.073	0.093	0.485	1.004	0.361	0.069	0.166	0.875	−0.165	−0.408	0.700	0.151	0.298	0.778
SOD2	−0.027	−0.053	0.960	0.420	0.595	0.578	−0.155	−0.256	0.808	0.683	1.157	0.300	−0.573	−0.775	0.474
GSTT1	0.107	0.296	0.779	−0.287	−0.578	0.589	−0.422	−0.988	0.369	0.812	1.950	0.109	−0.565	−1.084	0.328

genetic damage in players. Principal Component Analysis (PCA) of the data was done to extract main components from a large number of independent variables contributing to genetic damage and oxidative stress. Receiver Operating Characteristic (ROC) curve analysis was performed for discriminating players and controls on the basis of indices of genetic damage and oxidative stress. Significance was set at $p < 0.05$.

3. Results

The study participants were all unmarried college/university students and comprised *Kho Kho* players (n = 18) and age- and sex-matched controls (n = 10) in the age range of 18–25 y (Table 1). The study group included nearly 50% non-vegetarians, and all were non-smokers. Except for one player who took alcohol, none drank alcohol nor took any nutritional supplements. Nearly 55% of players and 30% controls

Table 6
Comparison of factor loadings by principal component analysis with Varimax rotation and communalities of the study characteristics including DNA damage and molecular genotypes.

Study Characteristics	Communalities	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Age	0.715	−0.030	−0.014	0.016	**0.826**	−0.004	0.176
Time-since-excercising	0.736	**−0.556**	0.025	**−0.450**	0.044	0.258	0.393
Warm-up time	0.877	**−0.891**	−0.188	−0.101	−0.009	0.177	−0.075
Diet	0.856	**−0.764**	0.102	0.386	0.105	0.232	−0.219
Body Mass Index	0.636	0.257	0.162	−0.077	0.147	−0.110	**0.710**
Hip circumference	0.839	**0.853**	−0.090	0.139	−0.264	0.042	0.111
Waist Circumference	0.862	**0.524**	−0.319	**0.632**	−0.024	−0.125	0.263
Waist-Hip-Ratio	0.908	−0.340	−0.297	**0.792**	0.268	−0.002	0.069
Gender	0.944	**−0.819**	0.088	0.279	−0.304	0.248	−0.184
VO_2 max	0.884	**0.836**	0.110	−0.127	0.346	−0.185	−0.050
Mobile phone usage	0.750	0.456	0.145	−0.125	**0.632**	0.176	0.274
Damage Frequency	0.845	−0.250	0.228	0.051	0.390	**0.684**	−0.327
Mean DNA migration length	0.873	**0.779**	−0.246	0.003	0.101	0.440	−0.050
Total Antioxidant Capacity	0.678	0.137	0.199	0.230	0.099	**−0.728**	−0.164
Malondialdehyde Levels	0.684	0.044	**−0.549**	0.028	0.436	−0.410	−0.146
SOD Activity	0.831	0.043	−0.054	−0.876	0.207	0.129	0.012
SOD	0.772	0.045	0.212	−0.384	−0.243	−0.213	−0.688
GSTT1	0.948	−0.026	**0.964**	−0.092	0.059	−0.074	−0.009
GSTM1	0.948	−0.026	**0.964**	−0.092	0.059	−0.074	−0.009
Eigen Values		5.48	3.2	2.23	1.97	1.41	1.27
% of Variance		28.86	16.88	11.74	10.37	7.46	6.69
Cumulative %		28.86	45.74	57.49	67.86	75.33	82.02

Values in bold have factor loading ≥0.4.

Table 7
Receiver operating characteristic (ROC) curve for genetic damage and oxidative stress indices.

Studied Indices	AUC	P value	Sensitivity	Specificity
Damage Frequency	0.864	**0.0001**	100.00	77.78
Mean DNA Migration Length	1.000	**0.000**	100.00	100.00
Total Antioxidant Capacity	0.944	**0.0001**	100.00	94.44
Malondialdehyde Levels	0.953	**0.0001**	90.00	88.89
Superoxide Dismutase Activity	0.578	0.113	100.00	50.00

p values in bold are statistically significant ($p < 0.05$), AUC - Area under curve.

used mobile phones. The players were professionally active with a sports age of 6.78 ± 0.52 y (3–11 y) and during the training period, daily sessions were of 4.33 ± 0.38 h duration. The warm-up (running, whole body rotations, stretching and jumping) time of the players ranged from 15–30 min (23.33 ± 1.51 min). Controls were healthy individuals with no recent illnesses or exposures and were not physically active in any sports or doing any strenuous manual work. The players and the controls were matched for age (20.27 ± 0.28 y vs 20.90 ± 0.45 y) and for general and central/abdominal obesity. All players were obese on the basis of WHR but only 11% were obese on BMI basis. As expected, players had better aerobic capacity (VO_2 max) and differed for categories from controls.

All the SNP genotypes (except for *SOD2* (C399T) with only wild type genotypes) showed Hardy-Weinberg equilibrium (HWE). Players and controls were matched for the genotypic distribution of *GSTT1, GSTM1* and *SOD2* (C399T). However the minor allele frequency (MAF) of *SOD2* (C47T) was higher in players and of *GSTP1* (A313G), in controls.

Genetic damage was significantly elevated in players ($p = 0.000$) as Damage Frequency (1.87 folds) and mean DNA migration length (1.79 folds) in PBL of players were increased as were the levels of TAC (1.09 folds, $p = 0.000$) and malondialdehyde (2.03 folds, $p = 0.000$) were also significantly elevated in players whereas SOD activity did not vary (Table 2). The biomarker levels did not differ by gender. On stratification of biomarker levels (Table 3) by VO_2 max categories (as increased aerobic capacity from increased oxygen uptake has liability for increased oxidative stress and susceptibility for genetic damage), players with superior compared to those with good aerobic capacity had significantly ($p = 0.002$) increased mean DNA migration length. Stratification by molecular genotypes (on account of differential metabolic capacities) revealed significant decrease in SOD activity in players having either of the homozygous genotypes (CC/TT) for *SOD2* (C47T) variant compared to those with the heterozygous (CT) genotype.

The mean DNA migration length showed association with the warm-up time, *GSST1* and waist circumference; the latter also influenced the total antioxidant capacity and malondialdehyde levels. These also emerged as significant predictors of genetic damage and oxidative stress on univariate linear regression analysis (Table 4). However following on with multivariate linear regression and also on combined multivariate analyses, no significant associations were revealed (Table 5).

The results of PCA (Table 6) revealed that out of 19 variables, the loading of six factors showed a total variance of 82.02%. Time-since-exercising, warm-up time, diet, hip circumference, gender, VO_2 max and mean DNA migration length contributed towards variance of 28.86% in factor 1 (Eigen value 5.48). The factor 2 comprised malondialdehyde, *GSTT1* and *GSTM1* which contributed a total variance of 16.88% (Eigen value 3.2). A variance of 11.74% was contributed by waist circumference and waist-hip-ratio (Eigen value 2.23) in factor 3. Mobile phone usage loaded in factor 4 (variance of 10.37%; Eigen value 1.97), damage frequency and total antioxidant capacity in factor 5 (variance of 7.46%; Eigen value 1.41) and body mass index in factor 6 (variance of 6.69%; Eigen value 1.27).

The receiver operating characteristic (ROC) curves were generated for identifying whether the indices of genetic damage and oxidative stress can discriminate between players and controls with high specificity and high sensitivity (Table 7, Fig. 1). Damage Frequency (AUC = 0.864), mean DNA migration length (AUC = 1.00), malondialdehyde (AUC = 0.953) and TAC (AUC = 0.944) levels emerged as significant indices discriminating players from controls with increased sensitivity and specificity.

4. Discussion

In the present study, significant ($p = 0.000$) increase with no gender differences was observed for genetic damage (~1.8×) and oxidative stress (~2.0×) in *Kho Kho* players in comparison to the controls. Warm-up time and sports age (duration of exercising) emerged as contributors of genetic damage. Also players with superior aerobic capacity had significantly increased mean DNA migration length compared to those with good aerobic capacity implying that increased oxygen uptake may

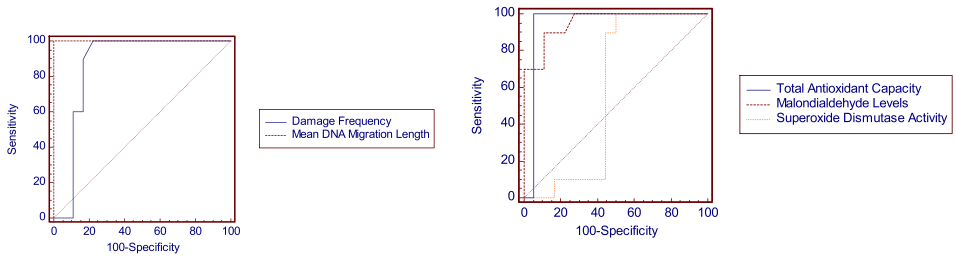

Fig. 1. Receiver operating characteristic (ROC) curve for genetic damage and oxidative stress indices.

partly be inducing an oxidative stress response and causing genetic damage. Similarly in literature, various sports activities have shown association with increased genetic damage [38, 39] and oxidative stress [40, 41] and with no differences reported between males and females engaged in acute exercising [42].

Fitness in competitive sports is characterized by aerobic (maximum amount of oxygen the body can use during intense exercise) and anaerobic (ability to mobilize energy during activities of intense nature) capacities, and sporting events require both. Aerobic capacity is dependent on cardio-respiratory performance and the ability for maximal use of oxygen from blood circulation.

Kho Kho as a game of chase, attack and defense, requiring speed, endurance, agility and strength also depends on both, anaerobic capacity for managing intensive work as bursting speed, and aerobic capacity for intensive exercises.

The increased oxygen uptake during exertion (as when performing arduous physical tasks and exercising and training for recreational and/or professional purposes) has on one hand, the potential for harmful health effects via generation of free radicals (reactive oxygen species, ROS). Interestingly on the other hand, the up-regulation of antioxidant defences can also occur as observed during training for sporting events [43]. Antioxidants (endogeneous or exogeneous) can mitigate effects of oxidative stress either by direct scavenging of ROS [44] or as secondary effects. It needs to be mentioned that optimal levels of free radicals during exercise also serve to increase the adaptive response; but if overwhelming, free radicals have the potential to induce extensive and oxidative damage, decrease performance, and cause ill health/disease [4].

In fact the mode, intensity and duration of exercise as well as population group and training status can impact extent of oxidation [4] as different sports require different types of exercises, and differ in energy requirements, levels of oxygen consumption, and the imposed mechanical stress on body tissues [45]. Therefore even though normal physiological processes and redox signalling depend on ROS, yet excess ROS from reduced antioxidants can cause oxidative stress and harm cell membranes and biomolecules, targeting proteins, lipids and nucleic acids.

In the present study, damage at DNA level in PBL scored in the comet assay was markedly increased in *Kho Kho* players as DNA migration length increased almost two-folds between players and controls. The comet tail results from single/double-strand breaks and alkali-labile sites with a longer tail implying more damage [25]. Damage Frequency also showed an increase of two times signifying more nucleoids with tails [26]. Support for the observations on genetic damage in the players has been documented in literature for other sports as this is the first study of its kind in *Kho Kho* players. Physical fitness/training induced significantly increased DNA damage [46], and both, chromosomal and DNA damage were increased in body builders [47], wrestlers [48] and in hockey, base/soft ball and judo players [49, 50]. Genetic instability has also been reported in athletes [51], in trained and untrained treadmill runners, football players, swimmers [52] and in those taking part in marathons [41].

DNA damage has also been shown [39] from strenuous and intense exercises. Oxidation of nitrogen bases (8-OHdG) has however shown increase in some events [53] and not in football players.

The players of the present study also had significantly increased MDA and TAC serum levels. The oxidative damage to poly unsaturated fatty acids (PUFA) in the cell membranes can cause lipid peroxidation and induce the formation of alkanes and MDA (a three-carbon chain aldehyde formed on degradation of lipids). It has emerged as a sensitive marker of lipid peroxidation whose presence has also been observed in numerous diseases. Significant increase in lipid peroxidation has been documented in athletes, swimmers, cyclists and handball players. However climbers had increased levels of MDA as well as TAC and other antioxidants but with reduction in antioxidants after short exercises which increased during recovery [54]. The levels of antioxidants may decrease after exercising to balance the increased free radicals generated during exercising [4].

Although higher oxygen uptake can stimulate endogenous antioxidants and cytosol enzymes (catalase, SOD), in the present study SOD activity was slightly decreased though not significantly. Literature perusal has also revealed no decrease in SOD activity but has revealed increased oxidized glutathione (GSH/GSSG) ratio, thiobarbituric acid reactive substances and creatine kinase and decreased total antioxidant status in triathletes compared to the sedentary subjects.

In literature, the discrepancies for genetic damage and oxidative stress levels in different sports players may be because of the differences in the biomarkers analyzed, the mode, duration and intensity of exercise, tissue sampling time, training status, dietary intake, demographics, lifestyle and individual differences.

Despite the fact that oxidative stress and genetic damage are normal events in the process of ageing, modulatory effects can be mediated by the genetic and environmental factors [55]. Genetic variants modulating xenobiotic mechanisms can also elevate oxidative stress [56] and also have the potential to affect sports performance [12]. The latter can also be influenced by physique as well as the biomechanical, physiological, metabolic, behavioural, psychological and social characteristics.

It is also known that genetic differences exist among different sportspersons [57]. However only sparse documentations have reported associations between molecular genotypes and oxidative stress and genetic damage indices as investigated in the present study. Akimoto *et al.* [18] in runners had reported that *SOD2* (C47T) significantly influenced creatinine kinase (CK) level and Haptaglobin (*Hp1F-1S* and *Hp1S-2*) genotypes influenced TBARS values and genetic damage. The present study *Kho Kho* players had increased SOD activity in those with the CT genotype of *SOD2* (C47T) in comparison to those homozygous (CC and TT). The superoxide dismutase 2 enzyme is a key component of the antioxidant defense system and the valine-to-alanine substitution (Val 16 Ala; rs4880) forms a beta-sheet secondary structure (not the alpha-helix) of the enzyme affecting its transport into the mitochondria and decreasing the antioxidant capacity, which in turn increases oxidative stress. However in the present study, the SOD activity between the players and controls did not differ. In literature also, different results for SOD activity among the sports persons have been documented. SOD activity was increased during exercise tests performed on cycle ergometer on treadmill while no significant differences were observed between levels in cyclists and controls.

There is evidence that free radicals are involved in disease-

pathogenesis (diabetes, CVD, pulmonary disease, cancer) and in various physiological processes (muscle contractile activity, aging); however, at the cellular level, these can cause increase in oxidation of proteins, lipids and nucleic acids. Damage to DNA from free radicals can cause strand breakage and base deletions [58]. The consequences of DNA damage can include transcription-arrest, replication errors and genomic instability which in turn can manifest in carcinogenesis [59]. Reactive oxygen species can also affect the DNA repair process, activate signal-transduction molecules and influence proteins required for the cellular activities of apoptosis and DNA repair, and in this manner participate in carcinogenesis [55]. During exercising (as in training and sports events) there occurs 10–15 fold increase in oxygen consumption and the inflammatory reactions can possibly induce tissue injuries and phagocyte activation, which can also initiate the production of free radicals [38]. This could well be occurring in the players of this traditional sport.

Data reduction analysis also revealed that DNA damage and MDA levels loaded onto first two factors with other variables and contributed to variance in players. Association and regression analysis revealed lipid peroxidation, TAC levels, *GSTT1* and WC as predictors of genetic damage.

In conclusion, the results of the study reveal that high-intensity exercises required for endurance, agility and strength in order to play *Kho Kho* can damage DNA, as observed in peripheral leukocytes using the comet assay. Though the domain of exercise-induced DNA damage is inherently complex and requires more research focussed especially for consequences of DNA damage [60], nonetheless oxidative stress observed as increased lipid peroxidation in players implies its role in the cellular DNA damage.

The product of the three interactive components of exercise viz. duration, frequency, and intensity represents the nature of physical activity [61] and these components are known to cause elevated oxidative stress with increased duration [62], intensity [63] and frequency [64], and more so during anaerobic than aerobic exercises [65].

Intensive physical activity is also known to increase the basal metabolic rate [66] and provoke oxidative stress and damage DNA [4] while it is beneficial and protective when it is moderate [67]. There appears to be a biphasic response (hormetic relationship) to physical activity whereby moderate activity induces beneficial effects which become harmful with intense activity [61]. The important point being ability to discern the threshold in different activities in order to marginalize the harm.

Therefore based on the present study findings and from those reported in literature, the *Kho Kho* players are probably at increased risk for future health complications because oxidative stress can induce molecular damage; this may accumulate with age, and the progressive physiological attrition can increase the susceptibility to disease and mortality [68] as direct or indirect oxidative damage on nucleic acids can cause dysfunction and detrimental effects to cells, organs and the entire body [9]. The ramifications of genetic damage include cell cycle arrest and/or apoptosis and can lead to cancer progression. The present study findings in respect of increased DNA and lipid peroxidation discriminating players from controls and as sensitive indices of future health (consequences) in the light of reported literature, highlight the need for sensitization of players of modern and traditional sports (and those engaged in physical activities) about health issues for implementing circumventional measures such as by altering physical training and increasing the antioxidant intake [69, 70].

Declarations

Author contribution statement

Gursatej Gandhi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

R. Sharma: Performed the experiments.

Gurpreet Kaur: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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