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PREDICTION OF AEROBIC AND ANAEROBIC CAPACITY FROM SELECTED OXIDATIVE STRESS PARAMETERS, MUSCLE DAMAGE INDICES AND INFLAMMATORY MARKERS IN SOCCER AND HOCKEY PLAYERS

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Abstract

Introduction. The aim of the present study was to find the relation of exercise-induced biomarkers (antioxidant, muscle damage, and inflammatory markers) with endurance capacity and anaerobic power. The study also aimed to develop predicting regression models for maximal oxygen uptake ($\dot{V}O_{2max}$) and relative anaerobic power (W_{peak}) to specify the essential performance limiting elements. ***Material and Methods.*** Eighty-six endurance male players (i.e., football (n = 39) and field hockey (n = 47)) were selected as test subjects for the present study. Muscle damage indices (creatine kinase (CK), lactate dehydrogenase (LDH), cortisol), inflammatory markers (interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α)), antioxidant variables (malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx)) and performance variables (indicated as $\dot{V}O_{2max}$ and W_{peak}) were assessed using standard protocols. ***Results.*** The most significant (sig $p < 0.001$) prediction of $\dot{V}O_{2max}$ = (0.763) MDA+ (5.644) SOD+ (0.039) GSH- (0.154) GPx+ (0.002) LDH- (0.011) CK+ (0.038) cortisol+ (1.232) IL+ (1.135) TNF+ 20.018. The strongest correlations were found between $\dot{V}O_{2max}$ vs MDA ($R^2 = 0.852$), $\dot{V}O_{2max}$ vs IL-6 ($R^2 = 0.589$), $\dot{V}O_{2max}$ vs TNF-α ($R^2 = 0.385$). ***Conclusions.*** Artificial neural network perceptron model depicted stronger prediction of $\dot{V}O_{2max}$ ($R^2 = 0.872$) in comparison to W_{peak} ($R^2 = 0.271$), with MDA and CK as the major predictors for $\dot{V}O_{2max}$ and W_{peak}, respectively. Among all biomarkers, MDA, IL-6, and TNF-α were identified as the most valuable indicators to predict endurance capacity significantly. While MDA, SOD, GPx, IL-6, and TNF-α were strongly correlated with $\dot{V}O_{2max}$ and LDH, cortisol was strongly correlated with W_{peak}. Contrarily, exercise-induced biomarkers failed to predict anaerobic power.

Key words: antioxidant variables, muscle damage indices, inflammatory markers, correlation matrix, multiple regression model

Introduction

Both football and hockey are endurance-energy system-based intermittent games where high-intensity bursts of exergy are required along with the capability of fast recovery [1, 2]. Hence, to excel in games like football and hockey, the player needs high $\dot{V}O_{2max}$ and W_{peak} with proper lactate and cardio-respiratory response to support and sustain short, intense bursts of high energy while recovering more quickly [1, 2, 3]. Thus, $\dot{V}O_{2max}$ and W_{peak} are the two variables that can predict the game performance as a whole and be influenced by several biochemical biomarkers produced during the play [3, 4].

Previous studies have already reported that high-intensity short-term and long-term exercise could induce redox imbalance and an increase in oxidative stress markers along with inducing muscle damage indices (CK and LDH) and inflammatory markers (IL-6, TNF-α) [4, 5, 6, 7, 8]. Previously, Sarkar et al. [8] reported a significant increase in MDA, GPx, and a decrease in SOD, GSH following high-intensity exercise, which might happen due to the intense exercise-induced ROS generation which leads to redox imbalance [4, 5]. For CK and LDH, approx. 100- to 200-fold increase was reported after moderate/acute (60-70% HR_{max} for 45 min) and high intensity/chronic (70-80% HR_{max} for 30 min) training, which gradually rises till 24h post-exercise and may even elevate in some cases for 48 h or so [6, 9]. Similarly, the temporary hypoxic condition induces an inflammatory response via sympathetic response and the blood level of IL-6 and TNF-α might even remain elevated 48h post-exercise [7].

Previous studies like Dokumaci et al. [10] helped to understand the relation between performance variables and exercise-induced biomarkers. However, studies on predicting the performance from these exercise-induced biomarkers are scanty and not significant when it comes to ascertaining the relationship between these performance predicting variables. On the other hand, it is quite known now that antioxidant status, inflammatory markers, and muscle damage indices are factors limiting performance. Therefore, the present study is aimed to identify the relationship of antioxidant capacity, muscle damage indices and inflammatory variables with endurance capacity and anaerobic power, and to predict the performance variables regression equations.

Material and Methods

Selection of subjects

Eighty-six young Indian male team game players (i.e., football (n = 39) and field hockey (n = 47)) were recruited in the present study. Football group (n = 39; mean age = 15.6 ± 1.53 yrs) and Hockey group (n = 47; mean age = 15.8 ± 1.81 yrs). All participants had a minimum of 4 years of professional training experience and were only recruited after the clinical examination.

Generalized training program

The systematic training program was formulated by qualified coaches under the guidance of scientific experts. Three hours of training was done for each athlete on a daily basis for six days per week, with Sunday a compulsory day off. Daily training (3 hours) was divided into two sessions (morning and evening sessions of 90 min each). Each training session started with a warm-up and ended with a cool-down session (both warm--up and cool-down sessions involved 15 min of slow running). Physical training included different strength, speed, endurance and flexibility exercises. Game-specific (football, hockey) skill training (i.e., dribbling, passing, hitting/shooting, tackles, movement techniques, etc.) was done regularly during the training session.

Anthropometric variables

General physical characteristics, i.e., body height (cm) and body weight (kg) were measured using the Seca Alpha stadiometer (model – 213, Seca Deutschland, Germany) and Seca Alpha weighing scale (model – 770, Seca Deutschland, Germany), respectively. Body mass index (BMI) was calculated using the standard formula [11]. Body fat % (BF%) was calculated with the standard formula of Brozek et al. [12], after measuring the biceps, triceps, subscapular, and suprailiac skinfold thicknesses using a Harpenden skinfold caliper (with constant tension).

Biochemical analysis

Blood collection and plasma sample preparation: Blood samples were collected at 6:00-8:00 AM from the antecubital vein for serum preparation (without anticoagulant) in the pre-prandial state (after 8-10 h of fasting) to avoid possible differences due to diurnal variation. Samples were then centrifuged (REMI centrifuge, R-8C) at 3000 rpm for 15 min at 4°C to ensure complete separation of serum [13].

Skeletal muscle damage indices (CK, LDH): Creatine kinase (CK) and lactate dehydrogenase (LDH) activity was estimated from serum samples and analyzed via Advia 1650 analyzer (Siemens Healthcare Diagnostics, Deerfield, IL, USA) [14].

Serum cortisol: Cortisol was estimated using ELISA kit from The Diagnostic Automation, Inc. (AccuDiag), where a specific monoclonal anti-cortisol antibody was used to get the result [15].

Inflammatory markers (IL-6, TNF-α): Commercially available 96-well ELISA kits were used to measure the protein levels of IL-6 (Diaclone SAS., Besancon Cedex, France) and TNF-α (Diaclone SAS., Besancon Cedex, France) in the serum. The assay was performed according to the manufacturer's instructions. The absorbance of IL-6 and TNF-α was measured at 620 nm with an Anthos 2020 microplate reader (Biochrom Co., England). Each sample was assayed in duplicate and data were expressed as picograms per milliliter of serum [16].

Oxidative stress variables (MDA, SOD, GSH, GPx): Malondialdehyde (MDA) was measured by reacting with thiobarbituric acid (TBA) to form TBA-MDA under acidic conditions at an elevated temperature at 532 nm and expressed as μmoles of MDA/100 ml serum. Superoxide dismutase (SOD) was estimated by inhibiting the auto-oxidation of pyrogallol at 420 nm. The SOD activity was expressed as U/min/mg protein and 1U of the enzyme is defined as the enzyme activity that inhibits auto-oxidation of pyrogallol by 50%. Reduced glutathione (GSH) content was estimated from a yellow-coloured complex after reacting to DTNB with an absorbance maximum at 412 nm expressed as mg/100 ml serum. The glutathione peroxidase (GPx) enzyme degrades the H_2O_2 in the presence of GSH. The remaining GSH was measured via its reaction with DTNB. GPx activity was expressed as μmoles of GSH consumed/min/mg protein. All oxidative stress markers (MDA, SOD, GSH, GPx) were measured by following the standard procedure of Kuyumcu and Aycan [13]. Protein estimation was done by following the Lowry method where the Folin-Ciocalteau reagent was used to produce a blue-purple coloured complex, with maximum absorption at 660 nm [8].

Determination of physiological variables

Maximal oxygen consumption ($\dot{V}O_{2max}$) was measured using an incremental exercise protocol on a bicycle ergometer (Ergoline, VIA Sprint 150P, Germany). Athletes were asked to start pedaling without any load for 1 min, and initially 25-watt workload was applied for 2 min, and then it progressively increased by 25 watts every 2 min until complete exhaustion [3]. During the incremental test, breath by breath automated pre--calibrated metabolic gas analyzer (MetaMax 3B, CORTEX Biophysik GmbH, Leipzig, Germany) was used to determine $\dot{V}O_{2max}$ which was determined by maintaining the following criteria: i) a plateau in $\dot{V}O_2$ (2 ml/kg/min) despite the increasing workload, ii) respiratory exchange ratio (RER) ≥ 1.1, iii) > 90% of age--predicted HR_{max} ± 5%, iv) voluntary exhaustion [3, 17]. Relative anaerobic peak power (W_{peak}) was estimated by dividing the absolute anaerobic power with body weight. The anaerobic power output was assessed using the Running-based Anaerobic Sprint Test (RAST) [18]. Athletes were instructed to sprint 6 times over a 35-meter distance, with 10 sec intervals between each sprint. A polar heart rate monitor (Polar RS800CX, Polar Electro OY, Kempele, Finland) was used to measure the HR_{max} throughout the whole exercise protocol [3].

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 18.0 (SPSS Inc., Chicago, Il, USA) was used to analyze the data. Data were expressed as mean ± standard deviation (SD). The Shapiro-Wilk test and Levene tests were carried out to check the data normality and homogeneity, respectively. Two-way/mixed ANOVA was applied to check the main effect of group and moment among all the groups at a time. Pearson's product-moment correlation matrix and scatter graph plots were done to observe the correlation between performance variables with biomarkers. Multiple regression was done to carry out the prediction model of $\dot{V}O_{2max}$ and W_{peak}. Artificial Neural Network (ANN) Multilayer perceptron process was done for predicting the independent variable importance for two performance predicting variables ($\dot{V}O_{2max}$ and W_{peak}). A 95% confidence interval was considered as the level of significance.

Results

Table 1 depicts the comparison of morphological parameters, antioxidant variables, muscle damage indices, inflammatory markers, cortisol, endurance capacity, and anaerobic power

output of trained endurance team game athletes. Only muscle damage indices (both LDH and CK) and cortisol were found to be altered significantly ($p < 0.01$), along with a significant ($p < 0.05$) change in body weight and BMI, when means were compared between the groups.

Table 1. The results of the regression analysis for static stability factors in FR

Variables	Football (n = 39)	Hockey (n = 47)	Combined (n = 86)	F value	Level of Sig.
Height (cm)	167.01 ± 5.98	169.23 ± 5.41	168.22 ± 5.75	1.615	0.202
Weight (kg)	55.99 ± 7.00	60.18 ± 6.33	58.28 ± 6.93	4.052	0.019*
BMI (kg/m^2)	20.03 ± 1.85	20.97 ± 1.43	20.54 ± 1.69	3.371	0.037*
Body fat (%)	14.70 ± 4.98	14.73 ± 5.78	14.71 ± 5.40	0.000	1.000
MDA (µmoles/ 100ml serum)	30.30 ± 2.93	30.62 ± .3.20	30.48 ± 3.07	0.114	0.893
SOD (U/min/ mg protein)	0.08 ± 0.001	0.09 ± 0.01	0.09 ± 0.01	0.000	1.000
GSH (mg/ 100ml serum)	45.98 ± 1.38	46.74 ± 2.60	46.40 ± 2.16	1.339	0.265
GPx (µmol/ min/ mg protein)	11.59 ± 1.38	11.17 ± 1.29	11.36 ± 1.31	1.140	0.322
LDH (U/L)	243.77 ± 29.37	218.79 ± 29.60	230.12 ± 31.88	7.047	0.001**
CK (U/L)	32.87 ± 8.05	27.45 ± 4.26	29.91 ± 6.79	7.342	0.001**
Cortisol (µg/dl)	7.64 ± 1.73	8.94 ± 1.73	8.35 ± 1.84	5.645	0.004**
IL-6 (pg/ml)	1.99 ± 0.22	1.93 ± 0.36	1.96 ± 0.30	0.504	0.605
TNF-α (pg/ml)	3.03 ± 0.35	2.93 ± 0.37	2.97 ± 0.36	0.850	0.429
VO_{2max} (ml/kg/min)	49.74 ± 2.91	50.20 ± 3.25	49.99 ± 3.09	0.244	0.784
W_{peak} (watt/kg)	8.23 ± 1.40	7.94 ± 1.64	8.07 ± 1.53	0.395	0.674

Values are mean SD, * = p < 0.05, ** = p < 0.01, *** = p < 0.001, NS = not significant, MDA = malondialdehyde, SOD = superoxide dismutase, GSH = glutathione, GPx = glutathione peroxidase, LDH = lactate dehydrogenase, CK = creatine kinase, IL-6 = interleukin 6, TNF-α = tumour necrotic factor alpha, VO_{2max} = maximal oxygen consumption, W_{peak} = relative peak anaerobic power output.

Table 2 depicts the Pearson's correlation matrix of antioxidant markers, muscle damage indices, inflammatory variables, and cortisol with maximal oxygen consumption ($\dot{V}O_{2max}$) and relative peak anaerobic power output (W_{peak}). $\dot{V}O_{2max}$ was found to be positively and significantly correlated with three oxidative stress/antioxidant marker variables (MDA, SOD, GPx) and both inflammatory markers (IL-6 and TNF-α). W_{peak} was found to be positively and significantly correlated with LDH and negatively and significantly correlated with cortisol.

Table 2. Pearson's correlation matrix of oxidative stress/ antioxidant markers, muscle damage indices, inflammatory markers, and cortisol with maximal oxygen consumption and relative peak anaerobic power output

Variables	VO_{2max}	W_{peak}
MDA	0.923**	-0.088
SOD	0.434**	0.044
GSH	-0.006	-0.030
GPx	0.269**	0.059
LDH	0.094	0.178*
CK	-0.064	0.080
Cortisol	-0.141	-0.178*
IL6	0.767**	-0.033
TNF-α	0.620**	-0.023

** = correlation is significant at the level of 0.01 (2-tailed), * = correlation is significant at the level of 0.05 (2-tailed), MDA = malondialdehyde, SOD = superoxide dismutase, GSH = glutathione, GPx = glutathione peroxidase, LDH = lactate dehydrogenase, CK = creatine kinase, IL-6 = interleukin 6, TNF-α = tumour necrotic factor (alpha), VO_{2max} = maximal oxygen consumption, W_{peak} = relative peak anaerobic power output.

Figure 1 illustrates the scatter-plot graphs showing a correlation matrix between significantly correlated exercise-induced biomarker variables with $\dot{V}O_{2max}$. A significant positive correlation between $\dot{V}O_{2max}$ vs MDA (R^2 Linear = 0.852), $\dot{V}O_{2max}$ vs SOD (R^2 Linear = 0.189), $\dot{V}O_{2max}$ vs GPx (R^2 Linear = 0.072), $\dot{V}O_{2max}$ vs IL-6 (R^2 Linear = 0.589), $\dot{V}O_{2max}$ vs TNF-α (R^2 Linear = 0.385), W_{peak} vs LDH (R^2 Linear = 0.032) and a significant negative correlation between W_{peak} vs cortisol (R^2 Linear = 0.032) were noted.

Table 3 depicts the regression equations predicting the maximal oxygen consumption ($\dot{V}O_{2max}$) and relative peak anaerobic power output (W_{peak}). Various regression prediction equations were formed by using oxidative stress/antioxidant marker variables, muscle damage indices, inflammatory markers, and cortisol separately and in a combined way. Regression equation for predicting $\dot{V}O_{2max}$ via oxidative stress variables ($F = 243.086$, $p = 0.000$; coefficients constant $t = 9.423$, $p = 0.000$); muscle damage indices ($F = 2.306$, $p = 0.079$; coefficients constant $t = 20.679$, $p = 0.000$); inflammatory markers ($F = 193.082$, $p = 0.000$; coefficients constant $t = 24.292$, $p = 0.000$), and all variables combined ($F = 118.129$, $p = 0.000$; coefficients constant $t = 7.585$, $p = 0.000$). When combined variables were directed to predict $\dot{V}O_{2max}$, MDA, IL-6 and TNF-α were found to be significant at 0.000, 0.014 and 0.002 levels, respectively. Regression equation for predicting W_{peak} via antioxidant variables ($F = 1.146$, $p = 0.337$; coefficients constant $t = 2.858$, $p = 0.005$); muscle damage indices ($F = 2.814$, $p = 0.041$; coefficients constant $t = 6.137$, $p = 0.000$); inflammatory markers ($F = 0.099$, $p = 0.906$; coefficients constant $t = 8.055$, $p = 0.000$); and all variables combined ($F = 1.520$, $p = 0.145$; coefficients constant $t = 2.545$, $p = 0.012$).

Table 4 depicts the independent variable importance to predict the two dependent performance variables ($\dot{V}O_{2max}$ and W_{peak}) through the Artificial Neural Network (ANN) Multilayer perceptron process. The ANN multilayer perceptron model shows that the prediction of $\dot{V}O_{2max}$ (testing relative error 0.086, $R^2 = 0.872$) was stronger in comparison to W_{peak} (testing relative error 0.904, $R^2 = 0.271$). Among all independent variables, MDA and CK were counted as the major indicators (with 100% normalized importance) for predicting $\dot{V}O_{2max}$ and W_{peak},

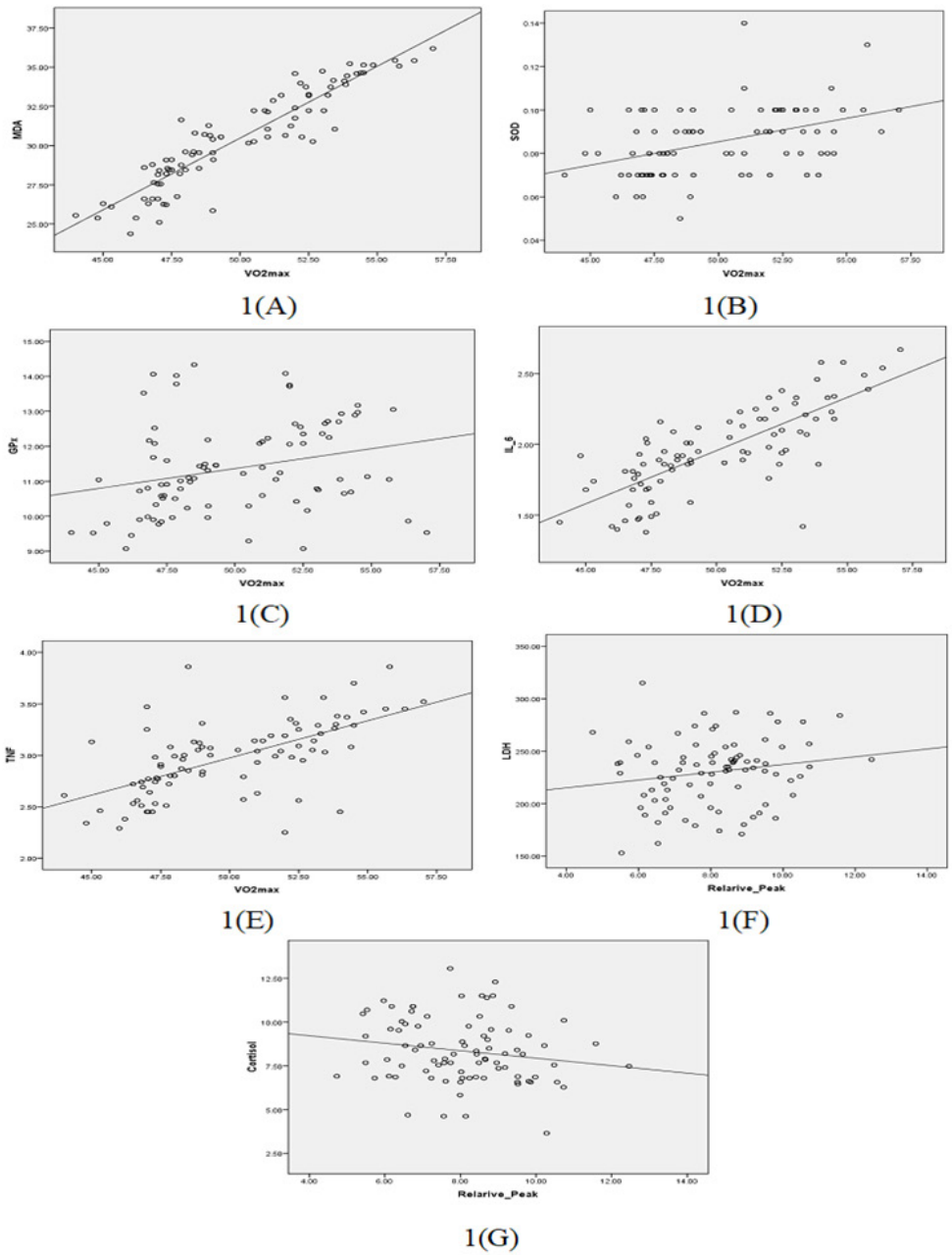


Figure 1. Scatter plots of (A) $\dot{V}O_{2max}$ vs MDA, (B) $\dot{V}O_{2max}$ vs SOD, (C) $\dot{V}O_{2max}$ vs GPx, (D) $\dot{V}O_{2max}$ vs IL-6, (E) $\dot{V}O_{2max}$ vs TNF-α, (F) W_{peak} vs LDH, and (G) W_{peak} vs Cortisol

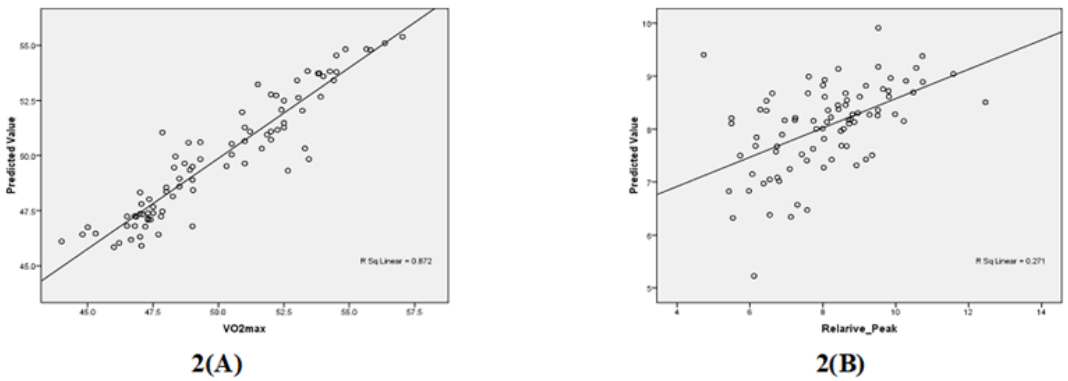


Figure 2. The regression prediction for (A) $\dot{V}O_{2max}$ and (B) W_{peak} against the predicted value

Table 3. Regression equations predicting endurance capacity and anaerobic power output

Categories	Multiple Regression Equation	R	R^2	SEE
Antioxidant	VO_{2max} = (0.932) MDA + (4.800) SOD + (0.004) GSH - (0.091) GPx + 22.009	0.924	0.853	1.194
	W_{peak} = - (0.087) MDA + (11.153) SOD - (0.004) GSH + (0.126) GPx + 8.534	0.163	0.027	1.527
Muscle damage	VO_{2max} = (0.010) LDH - (0.066) CK - (0.242) cortisol + 51.619	0.199	0.040	3.048
	W_{peak} = (0.007) LDH - (0.003) CK - (0.113) cortisol + 7.566	0.219	0.048	1.505
Inflammatory	VO_{2max} = (6.280) IL + (3.096) TNF + 28.503	0.834	0.696	1.711
	W_{peak} = - (0.140) IL - (0.050) TNF + 8.493	0.034	0.001	1537
Combined	VO_{2max} = (0.763) MDA + (5.644) SOD + (0.039) GSH - (0.154) GPx + (0.002) LDH - (0.011) CK + (0.038) cortisol + (1.232) IL + (1.135) TNF +20.018	0.932	0.868	1.152
	W_{peak} = - (0.112) MDA + (8.354) SOD + (0.000) GSH + (0.141) GPx + (0.007) LDH - (0.007) CK - (0.122) cortisol + (0.314) IL - (0.174) TNF + 8.798	0.279	0.078	1.509

R = correlation coefficient, SEE = standard error of the estimate, VO_{2max} = maximal oxygen consumption, W_{peak} = relative peak anaerobic power output, MDA = malondialdehyde, SOD = superoxide dismutase, GSH = glutathione, GPx = glutathione peroxidase, LDH = lactate dehydrogenase, CK = creatine kinase, IL-6 = interleukin 6, TNF-α = tumour necrotic factor alpha.

Table 4. Artificial Neural Network (ANN) Multilayer perceptron process for predicting the independent variable importance for two performance predicting variables ($\dot{V}O_{2max}$ and W_{peak})

VO_{2max}			W_{peak}		
Training	SSE	8.999	Training	SSE	39.568
	RE	0.151		RE	0.643
Testing	SSE	1.781	Testing	SSE	28.780
	RE	0.086		RE	0.904
R^2 Linear		0.872	R^2 Linear		0.271
Variables	Importance	Normalized importance	Variables	Importance	Normalized importance
MDA	0.443	100.0%	CK	0.217	100.0%
IL-6	0.218	49.2%	GSH	0.165	75.9%
TNF-α	0.100	22.6%	LDH	0.143	65.6%
Cortisol	0.070	15.8%	GPx	0.092	42.3%
SOD	0.049	11.0%	MDA	0.086	39.4%
CK	0.046	10.4%	SOD	0.082	37.8%
GSH	0.034	7.7%	TNF-α	0.081	37.5%
GPx	0.024	5.5%	Cortisol	0.073	33.5%
LDH	0.017	3.8%	IL-6	0.062	28.3%

SSE = sum of square error, RE = relative error, VO_{2max} = maximal oxygen consumption, W_{peak} = relative peak anaerobic power output, MDA = malondialdehyde, SOD = superoxide dismutase, GSH = glutathione, GPx = glutathione peroxidase, LDH = lactate dehydrogenase, CK = creatine kinase, IL-6 = interleukin 6, TNF-α = tumour necrotic factor alpha.

respectively. Top three major indicators for predicting $\dot{V}O_{2max}$ and W_{peak} were MDA, IL-6, TNF-α and CK, GSH, LDH, respectively.

Figure 3 shows the artificial neural network (ANN) multilayer perceptron processing for predicting the performance variables ($\dot{V}O_{2max}$ and W_{peak}). One hidden layer is shown in between the inputs and output where the significant connections were greater in numbers while predicting $\dot{V}O_{2max}$ in comparison to W_{peak}.

Discussion

The purpose of the present study is to report the relation between exercise-induced biomarkers (antioxidant variables, muscle damage indices, inflammatory markers) and endurance capacity and to develop the regression equation model to predict the maximal oxygen consumption ($\dot{V}O_{2max}$). The present study has reported that various exercise-induced biomarkers were able to successfully predict $\dot{V}O_{2max}$ but failed to predict W_{peak}. Among all exercise-induced variables, MDA, IL-6, and TNF-α were identified as the most important predictor markers to predict performance significantly, especially endurance capacity.

To excel in an endurance-based intermittent game like football and hockey, there is high demand for endurance capacity and anaerobic power output to maintain intense bursts of high energy for a short duration as well as to have faster recovery [1, 2, 3]. Thus, $\dot{V}O_{2max}$ and W_{peak} are the two variables that can predict the game performance as a whole [3]. The present study reported $\dot{V}O_{2max}$ values of 49.74 ± 2.91 ml/kg/min, 50.20 ± 3.25 ml/kg/min, and 49.99 ± 3.09 ml/kg/min respectively for football, hockey, and combined endurance athletes' groups where no significant inter-group changes were reported. On the other hand, the present study revealed W_{peak} values of 8.23 ± 1.40 watt/kg,

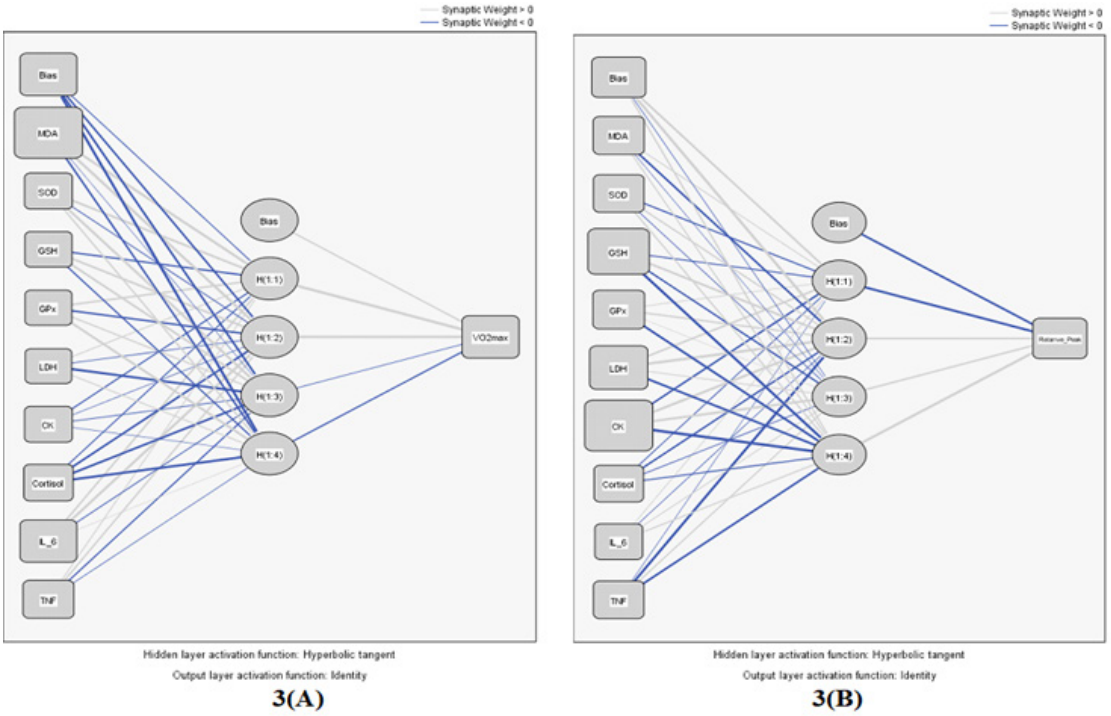


Figure 3. Artificial Neural Network (ANN) multilayer perceptron processing for regression prediction of (A) $\dot{V}O_{2max}$ and (B) W_{peak}

7.94 ± 1.64 watt/kg, and 8.07 ± 1.53 watt/kg respectively for football, hockey, and combined endurance athletes' groups where no significant inter-group alterations were observed. Present results show that in terms of energy demand, both the games of football and hockey were similar in nature and reported no such statistical difference in the measure of endurance capacity and anaerobic power. However, hockey players were observed to have slightly higher (0.9%) $\dot{V}O_{2max}$, while footballers had slightly higher (3.6%) W_{peak} in comparison to their counterparts but in a statistically insignificant manner. The present result is in agreement with the previous study by Sarkar et al. [3], where footballers still have the upper hand when it comes to relative anaerobic power measures.

The regressing model significantly predicted $\dot{V}O_{2max}$ against antioxidant variables ($F = 243.086$, $p < 0.001$; prediction coefficient $t = 9.423$, $p < 0.001$), muscle damage indices ($F = 2.306$, $p = 0.079$; prediction coefficient $t = 20.679$, $p < 0.001$) and inflammatory markers ($F = 193.082$, $p < 0.001$; prediction coefficient $t = 24.292$, $p < 0.001$). Separately, MDA ($t = 26.175$, $p < 0.001$), IL-6 ($t = 13.139$, $p < 0.001$) and TNF-α ($t = 7.699$, $p < 0.001$) were observed to predict $\dot{V}O_{2max}$ significantly in an independent manner. The combined regressing model significantly predicted $\dot{V}O_{2max}$ ($F = 118.129$, $p < 0.001$; prediction coefficient $t = 7.585$, $p < 0.001$) against combined exercise-induced biomarkers where MDA ($t = 13.363$, $p < 0.001$) and inflammatory marker variables (IL-6 ($t = 2.494$, $p = 0.014$) and TNF-α ($t = 3.123$, $p = 0.02$)) can predict $\dot{V}O_{2max}$ significantly. The regressing model significantly predicted W_{peak} against antioxidant variables ($F = 1.146$, $p = 0.337$; prediction coefficient $t = 2.858$, $p = 0.005$), muscle damage indices ($F = 2.814$, $p = 0.041$; prediction coefficient $t = 6.137$, $p < 0.001$) and inflammatory markers ($F = 0.099$, $p = 0.906$; prediction coefficient $t = 8.055$, $p < 0.001$). The combined regressing model was not able to predict W_{peak} significantly ($F = 1.520$, $p = 0.145$; prediction coefficient $t = 2.545$, $p = 0.012$) against combined exercise-induced biomarkers and also failed to identify any biomarker which can significantly predict anaerobic power alone. The present study was in agreement with the report of Sarkar et al. [8], where the researchers reported similar exercise-induced biomarkers for predicting $\dot{V}O_{2max}$ but failed to identify significant markers to predict endurance capacity. Therefore, the studied prediction observation is quite strong, where MDA, IL-6, and TNF-α were the identified predictor variables that can significantly predict $\dot{V}O_{2max}$ with the maximal weightage to MDA [10, 19, 20].

Among all exercise-induced biomarkers, only muscle damage indices i.e., CK (19.7%) and LDH (11.4%) were reported to be significantly ($p < 0.01$) higher among footballers, and cortisol (17.0%) was significantly ($p < 0.01$) higher among hockey players in comparison to their counterparts. Except for this profile of muscle damage indices (CK, LDH, cortisol), all other profiles i.e., antioxidant and inflammatory markers do not have any game-wise intragroup significant difference between football and hockey and have identified important predictors (MDA, IL-6, and TNF-α) for performance.

The present study shows that $\dot{V}O_{2max}$ has a significant ($p < 0.01$) positive correlation only with MDA, SOD, GPx, IL-6, and TNF-α (correlation coefficient = 0.923, 0.434, 0.269, 0.767, and 0.620, respectively) among all exercise-induced biomarkers. W_{peak} has a significant ($p < 0.05$) positive correlation with LDH (correlation coefficient = 0.178) and negative correlation with cortisol (correlation coefficient = -0.178). Scatter plots and regression analysis showed that exercise-induced biomarkers revealed $\dot{V}O2max$ vs MDA ($y = 0.930x + 21.641$, $R^2 = 0.852$, SEE = 1.191, $F = 975.064$, $p < 0.001$), $\dot{V}O_{2max}$ vs SOD ($y = 87.248x + 42.545$, $R^2 = 0.189$, SEE = 2.785, $F = 39.507$, $p < 0.001$), $\dot{V}O_{2max}$ vs GPx ($y = 0.635x + 42.783$, $R^2 = 0.072$, SEE = 2.979, $F = 13.211$, $p < 0.001$), $\dot{V}O_{2max}$ vs IL-6 ($y = 7.833x + 34.670$, $R^2 = 0.589$, SEE = 1.983, $F = 243.437$, $p < 0.001$), $\dot{V}O_{2max}$ vs TNF-α ($y = 5.327x + 34.154$, $R^2 = 0.385$, SEE = 2.426, $F = 106.259$, $p < 0.001$), W_{peak} vs LDH ($y = 0.009x + 6.101$, $R^2 = 0.032$, SEE = 1.509, $F = 5.556$, $p = 0.020$), and W_{peak} vs cortisol ($y = -0.148x + 9.310$, $R^2 = 0.032$, SEE = 1.509, $F = 5.572$, $p = 0.019$). The artificial Neutral Network

(ANN) multilayer perceptron model revealed the prediction for performance variables ($\dot{V}O_{2max}$ and W_{peak}). The top three major indicator variables for predicting $\dot{V}O_{2max}$ were MDA, IL-6, and TNF-α with 100%, 49.2%, and 22.6% normalized importance, respectively. On the other hand, CK, GSH, and LDH were identified as the top three major indicators for predicting W_{peak} with 100%, 75.9%, and 65.6% normalized importance. However, the ANN multilayer perceptron and regression analysis showed that the prediction of $\dot{V}O_{2max}$ (testing relative error 0.086, $R^2 = 0.872$) was stronger in comparison to W_{peak} (testing relative error 0.904, $R^2 = 0.271$). All significantly correlated variables show that the exercise-induced biomarkers increased with the improvement in performance ($\dot{V}O_{2max}$ and W_{peak}). Similar observations were made by Sarkar et al. [8], Baygutalp et al. [19], Dokumaci et al. [10], and Rosa et al. [20], where performance had a strong correlation with antioxidant status and inflammatory markers.

The present study has some limitations. The sample size was small (n = 86). A greater number of subjects might predict the variables even more precisely and in a statistically strong manner. Only oxidative stress, inflammatory markers, muscle damage markers and cortisol were measured and considered for predicting performance variables in the present study. Many other parameters were not considered due to the unavailability of data.

Conclusion

Biomarkers i.e., oxidative stress/antioxidant variables, muscle damage indices, inflammatory markers and cortisol were found to be the most valuable indicators for significantly predicting maximal oxygen consumption ($\dot{V}O_{2max}$). However, they failed to predict anaerobic power (W_{peak}) significantly. In particular, antioxidant variable (MDA) and inflammatory markers (IL-6, TNF-α) were identified as the best predictors among all exercise-induced biomarkers for significantly predicting endurance performance ($\dot{V}O_{2max}$). Artificial neural network perceptron model depicted stronger prediction of $\dot{V}O_{2max}$ ($R^2 = 0.872$) in comparison to W_{peak} ($R^2 = 0.271$), with MDA and CK as the major predictors (with 100% normalized importance) for predicting $\dot{V}O_{2max}$ and W_{peak}, respectively. Alternatively, the present study postulates that the athletes who revealed an increased level of MDA, SOD, GPx, IL-6, and TNF-α were counted to have more $\dot{V}O_{2max}$, and with a decreased level of cortisol and increased LDH, they were found to have more W_{peak}. The $\dot{V}O_{2max}$ and W_{peak} predicting regression equations can be used to specify essential performance limiting elements in football and hockey because of the dominating character of endurance capacity and explosive anaerobic power as demanding requisites. The present study will also help to track these variables with the help of other parameters through the presented prediction equations and to follow and maintain performance. All the exercise-induced factors, particularly MDA, IL-6 and TNF-α, also act as the limiting factors in terms of generating fatigue and limiting performance.

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