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Reference Interval for Enzyme Lactate Dehydrogenase in Male and Female Athletes

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Abstract: ***The present study is aimed to find out the reference interval for Lactate dehydrogenase (LDH) in male and female athletes. A total of 320 male athletes and 252 female athletes were volunteered to participate in the present study. Athletes were undergone training in Sports Authority of India. The mean age of the male and female athletes was 23.1±3.8 year and 20.8±3.8 year. Serum separated from the whole blood was used to analyze Lactate dehydrogenase (LDH) (Spinreact, Spain) by enzymatic method. According to IFCC (International Federation of Clinical Chemistry) and CLSI (Clinical and Laboratory Standard Institute) C28-A3 guideline, the reference interval for male and female athlete population is 138.4-746.0 IU/L and 140.5-599.5 IU/L respectively by non-parametric method with the help of add-in "Reference Value Advisor", a set of freeware macros for Microsoft Excel® . The male and female subgroup showed significant difference and robust method is used to frame the reference interval of LDH level in the athletes.***

Keywords: Reference interval, Lactate dehydrogenase, Athletes, Enzyme, Training

1. Introduction

Lactate dehydrogenase (LDH) enzyme activities are found in every tissue with its highest activity in skeletal muscle, liver, heart, kidney, brain, lungs and erythrocytes [26]. LDH enzyme level in serum is a biochemical marker for muscular damage and it is an oxidoreductase that catalyses the interconversion of pyruvate and lactate with the interconversion of NADH and NAD+ [3, 4]. Muscle fibres of athletes undergoing training may damage with metabolic and mechanical factors, it causes membrane damage of muscle fibres and the leakage of intracellular muscle component LDH into the extracellular fluid blood [6]. The metabolic factor is that the exhausted muscle fibres exhibit a decrease in the membrane resistance that causes an increase in the internal free calcium ions, which promotes the activation of the potassium channel [10, 11, 20]. The other mechanism of increased LDH could be due to the local tissue damage with sarcomeric degeneration from Z-disk fragmentation [12]. Hence, the circulating level of LDH is increased because of atrophy/loss of muscle fibre with the skeletal muscle damage. Thus, researchers reported that the increase of LDH level along the physical exercise is caused by the membrane damage of muscle fibres following acute and chronic muscle injuries [5, 7].

Some blood biomarkers have been proposed to be associated with overtraining in humans and one possible biomarker that could be used is the LDH level in the blood [9]. In any case, athletes are among the physically fittest persons in any community but still to get the better performance in their sport, their training adaptations should be monitored [8]. Biochemical testing of athletes plays an essential role in monitoring the training status and it helps to track the athlete's adaptation to training. It is evidenced by several studies that the biochemical level of athletes varies with the non-athlete. Hence, the reference interval for any biochemical of athletes should be framed to get the actual cause and to prevent misinterpretation of the result [18]. The aim of this study is to determine the reference intervals of LDH in various sports of male and female Indian athletes according to IFCC (International Federation of Clinical Chemistry) and CLSI (Clinical and Laboratory Standard Institute) C28-A3 guideline[2] and also to find the difference in LDH level of various games.

2. Literature Survey

Enzymes are protein in nature and are the functional assessment of athlete's fitness [17]. Brancaccio et al reported that enzyme levels in athletes are altered with adaptations to physical training that includes the training volume and intensity and concludes that lactate dehydrogenase (LDH) give an indication of the degree of metabolic adaptation to physical training of skeletal muscles. There are studies informing that there are ethnic differences about the enzyme level in human beings [17]. The amount of enzyme released from skeletal muscle tissue is reported by many studies after physical activity in sedentary population and in athletes [4, 6, 10, 12]. Reference interval for only some biochemical parameters in athletes is available and there is no reference interval for LDH is available [18, 36]. Hence, Authors framed the reference interval of LDH for athletes in the present paper in different sports.

3. Methods

A total of 320 male athletes and 252 female athletes were volunteered to participate in the present study. Athletes were undergone training in Sports Authority of India. The mean age of the male and female athletes was 23.1±3.8 year and 20.8±3.8 year. The study was approved by the ethical committee of Sports Authority of India, India. The written

consent was obtained from all participants. The partitioning criteria of the reference population are based on sex and different sports [30].

The pre-analytical factors such as an overnight fast, venous blood were collected at 8.30 am with off training session for one day before the sample collection. The subjects in the present study are apparently healthy, without any disease and without major injury or hospitalization within the past 3-4 months. These requirements are fulfilled with the provided questionnaire.

Sample collection: Athletes were in a sitting position and the tourniquet was set around the upper arm then the needle was inserted in the vein of antecubital fossa region immediate after the blood was filled in syringe, the tourniquet was removed [29]. Blood was collected in the serum separator tube, inverted the tube for 5-8 times and allowed the blood to clot for upright position for at least 30 minutes. Then the blood was centrifuged for 15 minutes at 2200-2500 rpm for serum separation and the serum was used to analyze Lactate dehydrogenase (LDH) (Spinreact, Spain) by enzymatic method. The instrument used for the LDH estimations Erba Manheimm Biochemistry Analyser (Chem-7).

All results are expressed as mean (Standard Deviation). The analysis was performed by the statistical software Statistical Package for Social Sciences (SPSS) MS Windows 9.0. ANOVA (One way analysis of variance) was used to determine the difference exists among the means and LSD post-hoc test was used to determine which means differ. Clinical and Laboratory Standard Institute (CLSI) approved guideline C28-A3 was followed to frame the reference interval. Statistical significance was set at the 0.05 level.

"Reference Value Advisor", a set of freeware macros for Microsoft Excel® is used to calculate reference intervals in the present study [1, 27].

4. Results

Table 1 shows the mean value of LDH in male and female athletes of different sports discipline. Two sample t-test LDH value of male and female test shows significant difference. ANOVA test was done for LDH value of different sports discipline of male and female athletes, the results show a significant difference (Table 3 & 4). Table 5 and 6 displays the Reference Interval (2.5^{th} & 97.5^{th} Percentile) of LDH value in different sports of male and female athletes.

Table1: LDH level in Male and female players

Game	Number of athletes		Mean±SD	Mean±SD
	M	F	M	F
Total	320	252	393.2±157.2	342.3±122.6
Cycling	22	12	466.6±107.4	392.3±100.3
Long Distance Running	11	20	545.3±93.6	389.8±90.9
Middle Long	20	23	392.5±161.3	396.6±90.7
Distance Running				
Rowing	45	20	386.6±93.9	359.5±60.0
Sprint	41	21	386.1±84.7	356.9±82.7
Swimming	20	20	360.6±168.6	267.3±101.6
Walker	20	3	525.0±132.4	531.0±176.0
Waterpolo	10	11	528.5±127.3	485.7±123.6
Hockey	111	112	368.4±185.6	316.8±130.1
Wushu	20	10	230.4±47.2	219.4±26.05

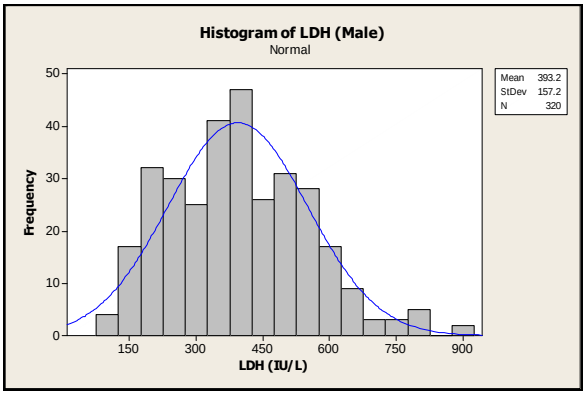
Table 2: Two sampleT-test between male and female subjects

Subject	*Mean*	*SD*	*T-value*	*Level of significance*
Male (N=320)	393	157	4.35	0.000
Female (N=252)	342	123		

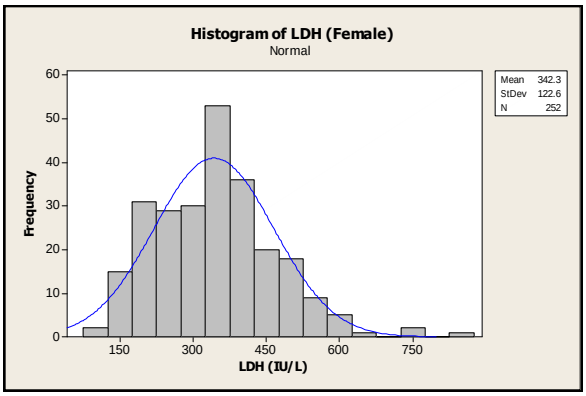
* P<0.05, **P<0.01, ***P<0.001

Table 3: ANOVA test of LDH level in different sports of male and female athletes

Sex		*Sum of squares*	*df*	*Mean square*	*F ratio*	*Sig.*
Male	Between groups	1505001	9	167222	8.290	0.000
	Within groups	6112074	303	20172		
	Total	7617075	312			
Female	Between groups	821917	9	91324	7.465	0.000
	Within groups	2948163	241	12233		
	Total	3770081	250			



Graph 1: Histogram of Male athletes



Graph 2: Histogram of female athletes

Table 4: Reference Interval of LDH in male athletes

Sport	Reference Interval	90% Confidence Interval		Outlier (Dixon's method)	Method
		Lower limit	Upper limit		
Total Athlete	138.4-746.0	85.5-164.8	673.4-791.3	0	Non-parametric
Cycling	250.3-708.8	205.7-308.2	629.5-778.8	0	Robust method with Box-Cox transformation
Long Distance Running	--	--	--	--	--
Middle Long Distance Running	Upto 705.1	-999999-189.22	624.6-746.5	0	Robust method with a Box-Cox transformation
Rowing	248.5-642.7	231.5-265.6	541.5-755.9	0	Non-parametric
Sprint	243.9-590.5	226.5-266.4	531.4-648.3	0	Robust method with a Box-Cox transformation
Swimming	41.2-778.1	0.96-135.2	661.8-931	0	Robust method with a Box-Cox transformation
Walker	256-823.3	198.5-341.8	733.4-915.5	0	Robust method with a Box-Cox transformation
Waterpolo	--	--	--	--	--
Hockey	136.7-809.6	125.2-154.2	742.8-924.5	0	Non-parametric Bootstrap method
Wushu	168.7-387.4	159.7-182.2	297.5-585.5	0	Robust method with a Box-Cox transformation

Table 5: Reference Interval of LDH in female athletes

Sport	Reference Interval	90% Confidence Interval		Outlier (Dixon's method)	Method
		Lower limit	Upper limit		
Total Athlete	140.5-599.5	131.8-158.2	570.1-729.9	0	Non-parametric
Cycling	--	--	--	--	--
Long Distance Running	226.3-613.6	198.5-269.8	535.8-697.9	0	Robust method with a Box-Cox transformation
Middle Long Distance Running	229.2-582.8	205.5-261.9	505.3-672.1	0	Robust method with a Box-Cox transformation
Rowing	206.6-473.3	151.4-263.3	442.7-498.6	0	Robust method with a Box-Cox transformation
Sprint	222.5-582.8	197.7-252.4	472.3-700.9	0	Robust method with a Box-Cox transformation
Swimming	106.5-617.2	81.1-135.4	392.9-880.5	0	Robust method with a Box-Cox transformation
Walker	--	--	--	--	--
Waterpolo	--	--	--	--	--
Hockey	129.5-608.6	92.2-143.8	568.4-830.1	0	Non-parametric Bootstrap method
Wushu	--	--	--	--	--

5. Discussion

Athletes undergo training that leads to physiological and biochemical changes to get his/her best performance during competitions. Training may result in functional overreaching (increment in sports performance) or non-functional overreaching (decrement in sports performance. Hence, monitoring of training load is very essential to get the functional overreaching. Physical activity alters the enzyme's activity in athletes and the Lactate dehydrogenase is one among the enzyme that present in blood and it is used to monitor the training status of athletes [15, 16, 21, 25]. LDH enzyme level is increased during muscular damage/non-functional overreaching state which can lead to a reduced physical performance [14, 19]. The molecular level changes that increase LDH level is the myofibrillar damage in particular sarcomeric Z-disk disruption [13]. Hence, monitoring of this enzyme periodically helps to prevent the stage of non-functional over reaching in athletes. To interpret the result of LDH value, the reference interval of that particular population is required.

In the present study, male and female athletes of ten different sports were participated. As reference interval of athlete population is varied from non-athlete population, Reference interval of total male and female athletes is given in table 5 and table 6. As the ANOVA test shows significant difference among different sports, it is essential to calculate the reference interval for each game.

The mean value of LDH is high in male athletes when compared with the female athletes of all sports [22]. Estrogen regulates and has a negative correlation with LDH activity and so the value of female athletes is less than the male athletes [22,23,24]. Estrogen possess a hydroxyl group on its "A" steroid ring, its structure is similar to that of the antioxidant vitamin E and it donates its hydrogen ion from this hydroxyl group and scavenges the free radical and thus acts as antioxidant [33]. Tiidus suggest that estrogen has a protective effect on exercise induced muscle damage in females as antioxidant, membrane stabiliser and influences neutrophil infiltration [32]. The other cause may be the muscle fibre's cross sectional area that is high in male than females which reflects the high LDH level in male than females [31]. Hence, this study agrees that the high estrogen level and the less muscle fibre cross sectional area in female may be the cause of low LDH activity in female athletes.

In the present study, to identify the 95% reference interval, the central 95% of the reference distribution was estimated using reference limits at 0.025 fractile (2.5^{th} percentile) for the lower reference limit and 0.975 fractile (97.5^{th} percentile) as the upper reference limit, the guidelines recommended by IFCC and CCLS guidelines. Data were transformed to Gaussian distribution using Box-Cox transformation and then Outliers were detected and removed using Dixon's method [28]. Reference limits of overall male and female athletes and hockey athletes were calculated according to the non-parametric percentile method (CLSI standard C28-A3). For the male and female subgroup with less number of sample sizes the "robust method" was used to construct the reference interval and where the sample size is less than 20, the reference intervals were not calculated according to IFCC/CLSI guideline. Reference interval for

male and female athlete population is 138.4-746.0 IU/L and 140.5-599.5 IU/L respectively and for the subgroups is presented in table 4 & 5. As of our knowledge, this study is the first study to frame the reference interval for LDH level in male and female athletes of various sports.

The reference interval of lactate dehydrogenase in sedentary population is 105 – 333 IU/L and the LDH level in athlete population is 2-3 fold higher than sedentary population [34]. Hence, the reference interval used for sedentary population cannot be used and may not be valid for athlete population. The framing of reference interval in athlete population is essential [35].

6. Future Scope

The present study can be useful for all coaches, athletes and sport population for interpretation of their test result. Future study can be done with phase wise reference interval of athletes that is during preparatory, pre-competitive and competitive phase of training.

7. Conclusions

Reference interval for male and female athlete population is 138.4-746.0 IU/L and 140.5-599.5 IU/L respectively. This study helps the coaches and athletes to prevent the misinterpretation of LDH values in athletes and also this research is an initiative to frame the reference interval of all the other biochemical parameters used to monitor athlete's training and nutritional status.

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