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The Correlation of Racing Performance with Pre-Race Blood Values in Thoroughbred Horses

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Abstract : Recently, the horse racing industry in Korea has developed rapidly with good betting turnover. One of the most important factors in enhancing the performance of racehorses is the study of equine exercise physiology. This study was performed to analyze the results of pre-race blood test on race day and evaluate its correlation with the race performance in Thoroughbred racehorses. Twenty-one, healthy 3- to 6-year-old Thoroughbred horses were used to collect blood samples and hematological and biochemical measurements were performed. Results were analyzed to identify its correlation with racing performance. Analysis of hematological profile of pre-race blood of the racehorses revealed that red blood cell indices including RBC, Hb, and PCV levels were elevated. Additionally, levels of AP, AST, and GGT were also elevated in pre-race blood of horses on race day. RBC, Hb, and PCV levels of high performance racehorses were significantly lower than those of low class racehorses. Moreover, the analysis of correlation between blood test results and racing ability-related categories have shown negative correlations for rating, placing strike rate, prize money per race, RBC, Hb, and PCV and positive correlations for G3F and PCV. Additionally, AST value was positively correlated to prize money per race. Thus, the results of this study show that red blood cell indices and AST are good parameters for evaluating racing performance of horses before the race.

***Key words* :** Performance of Racehorses, Pre-Race Blood Value, RBC, Hb, AST.

Introduction

Evaluation of hematological parameters of racehorses is one of the basic methods to examine the current physiological state, the presence of disease, and the degree of training. In particular, the red blood cells (RBC) of the racehorses play an important role in transporting oxygen to the tissues and carbon dioxide to the lungs. On an average, 1 μL of horse blood contains 9×10^6 matured red blood cells and has a lifespan of about 150 days (23).

As erythrocytes play an important role of oxygen transport in racehorses, several studies were performed to evaluate the correlation between hematological parameters and the training state and athletic performance (13,22). It has been shown that the PCV (packed cell volume) increases with age, degree of training, and the level of racing performance in racehorses (13). Stewart and Steel reported that Thoroughbred racehorses with low RBC indices did not win races compared to the normal group in Australia (22). However, researchers have found that the RBC indices can be influenced by excitement, time since last exercise, nutrition status, and transport (21). Distribution of red blood cells in the cardiovascular system can also vary by degrees of spleenic storage (14). In human athletes, a positive correlation between VO_{2max} and blood Hb concentration was found (5,6). Additionally, a study by Calbet *et al.* on effect of exercise on hemoglobin concentration in horse, showed that increasing Hb concentration resulted in significantly higher endurance time and speed and lower blood lactate concentration (1). Another human study showed that levels of RBC, Hb and PCV were reduced in endurance athletes group compared with other groups (18).

Several studies on the correlation between serum biochemical parameters and the training and athletic ability of racehorses have been reported. The most common enzymes that are used to indicate muscle damage are aspartate amino transferase (AST), creatine kinase (CK), and lactate dehydrogenase (LDH). The activities of these muscle derived enzymes have been shown to increase following races in gallop (15). Furthermore, it has been reported that the activities of muscle derived CK and AST is specifically increased during muscle damage. The half-life of CK is very short, so that the blood test has to be performed within 6 hours after exercise. On the other hand the activity of AST remains elevated for several days after exercise. Therefore, AST is an attractive marker than CK for confirming muscle damage caused by training in racehorses (23). Besides, a long-term training study of racehorses by Tyler-McGowan *et al.* showed that the levels of serum GGT (gamma-glutamyl transferase) were increased and that of AP (alkaline phosphatase) were decreased with training in these horses (24).

However, there have not been many studies on the relationship between the hematological and serum biochemical

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parameters and the performance of racehorses in clinically normal conditions (2).

The purpose of this study was to examine the hematological and serum biochemical parameters of the Korean racehorses using pre-race blood sample after normal training. Additionally, we aimed to identify parameters that can be used to evaluate the training level and exercise capacity in these racehorses. This was carried out by analyzing the correlation between hematological (RBC, Hb, and PCV), serum biochemical (AP, AST, GGT, CK, and LDH) parameters and race performance of these horses.

Materials and Methods

Experimental Animals

The pre-race blood tests were performed on 21 Thoroughbred racehorses (aged 3-6 years old; comprising 11 colts, 3 females, 7 geldings) at Seoul Racecourse of Korea Racing Authority. All the horses used for the experiments were maintained and trained by one trainer in order to maintain constant feeding and environmental conditions. Also, all horses were prepared to participate in the race on race days. They were examined by the veterinary officer of Korea Racing Authority to verify their health condition before the race.

Blood Sample Collection and Analysis

Jugular vein blood samples were collected through the mandatory doping test, 3 hours before the race. Each blood sample was divided into two tubes, one sample was collected in EDTA-tube and processed for hematological tests, and other sample was collected in SST-tube and processed for serum biochemical tests. Hematological parameters were measured on XN-V (SYSMEX, Japan) whereas biochemical parameters were measured on DRI-CHEM3500i (FUJIFILM, Japan).

Hematological and Biochemical Values

Hematological parameters, RBC count, Hb, PCV and biochemical values, AP, AST, GGT, CK, and LDH were measured to examine their correlation with the racing performance.

Experimental Groups and Analysis of Racing Performance

To correlate the hematological and serum biochemical parameters with racing performance, the experimental animals were divided in two groups: the good result group (race time record was shortened) and the bad result group (race time record was extended). Furthermore, the experimental animals were divided into the high class group (1 to 4 classes) and the low class group (5 to 6 classes) to analyze the racing performance with blood values. Parameters for evaluating racing performance included ratings of each racehorses (the official racing performance values of racehorses from Korea Racing Authority), placing strike rate (percentage of coming in 3^{rd} place), prize money per race, sectional time (S1F: time record of 200 m from the start, G3F: time record of final 600 m to the winning post, G1F: time record of final 200 m to the winning post). Horses' classes, ratings, race results, and time records were collected from the official homepage of Korea Racing Authority (http://race.kra.co.kr).

Statistical Analyses

In order to compare the difference of means in measurements between two groups, data were analyzed by the Wilcoxon's rank sum test, one of the nonparametric tests assuming two independent groups have normal distributions. The significance of associations between blood values and rating, placing strike rate, prize money per race, sectional time was determined by calculation of Pearson correlation coefficient. P values under 0.05 or less were considered statistically significant.

Results

The result of the hematological and biochemical tests of the Thoroughbred racehorses on race days before the race were, WBC was 8.1 ± 1.1 $10^3/\mu L$, RBC was 11.0 ± 1.0 $10^6/\mu L$, Hb was 17.1 ± 1.5 g/dL, PCV was 47.8 ± 4.0%, glucose was 100.0 ± 21.5 mg/dL, creatinine was 1.2 ± 0.1 mg/dL, uric-acid was 0.4 ± 0.1 mg/dL, T-protein was 5.8 ± 0.3 g/dL, albumin was 2.9 ± 0.1 g/dL, A/G ratio was 1.0 ± 0.1, T-bilirubin was 2.2 ± 0.5 mg/dL, AP was 432.0 ± 55.6 IU/L, AST was 423.7 ± 258.1 IU/L, GGT was 34.0 ± 16.8 IU/L, CK was 117.5 ± 32.7 IU/L, BUN was 15.9 ± 2.2 mg/dL, and LDH was 282.6 ± 53.6 IU/L. Although most blood values were within normal range, the RBC count and PCV values were elevated. Additionally, Hb, AP, AST, and GGT levels were also elevated above the normal range (Table 1).

Table 1. Hematological and serum biochemical parameters before the race in Thoroughbred horses

	Unit	Mean ± SD	*Reference
WBC	$10^3/\mu L$	8.1 ± 1.1	7.0~11.0
RBC	$10^6/\mu L$	11.0 ± 1.0	6.0~11.0
Hb	g/dL	17.1 ± 1.5	11.0~17.0
PCV	%	47.8 ± 4.0	32.0~48.0
Glucose	mg/dL	100.0 ± 21.5	75~115
Creatinine	mg/dL	1.2 ± 0.1	1.2~1.9
Uric-acid	mg/dL	0.4 ± 0.1	0~1.0
T-protein	g/dL	5.8 ± 0.3	5.2~7.9
Albumin	g/dL	2.9 ± 0.1	2.6~3.7
A/G ratio	-	1.0 ± 0.1	0.9~1.3
T-Bilirubin	mg/dL	2.2 ± 0.5	1.7~3.1
AP	IU/L	432.0 ± 55.6	143~395
AST	IU/L	423.7 ± 258.1	226~366
GGT	IU/L	34.0 ± 16.8	15~31
CK	IU/L	117.5 ± 32.7	28~134
BUN	mg/dL	15.9 ± 2.2	10~21
LDH	IU/L	282.6 ± 53.6	162~412

*Data from reference intervals established at the Equine Hospital of Korea Racing Authority

Table 2. The difference of means in hematological and biochemical results between good and bad result groups. Data are expressed as mean ± SD

	Unit	Good result group (n = 10)	Bad result group (n = 11)	P value
RBC	$10^6/\mu L$	10.7 ± 1.3	11.4 ± 0.6	0.1486
Hb	g/dL	16.5 ± 1.5	17.6 ± 1.3	0.1294
PCV	%	46.3 ± 4.3	49.2 ± 3.3	0.1485
AP	IU/L	422 ± 67	440 ± 44	0.4173
AST	IU/L	495 ± 355	410 ± 201	0.9159
GGT	IU/L	36.0 ± 15.2	32.3 ± 18.6	0.4375
CK	IU/L	118.9 ± 34.0	116.2 ± 33.1	0.9438
LDH	IU/L	273.9 ± 62.6	290.5 ± 45.6	0.4597

Table 3. The difference of means in hematological and biochemical results between the high and low class groups. Data are expressed as mean ± SD

	Unit	High Class Group (n = 8)	Low class Group (n = 13)	P value
RBC	$10^6/\mu L$	10.2 ± 1.1	11.5 ± 0.6**	0.0073
Hb	g/dL	15.8 ± 1.4	17.8 ± 0.9**	0.0037
PCV	%	44.3 ± 3.7	50.0 ± 2.3**	0.0030
AP	IU/L	414 ± 57	442 ± 54	0.2458
AST	IU/L	536 ± 389	398 ± 188	0.7999
GGT	IU/L	29.9 ± 11.9	36.6 ± 19.2	0.4460
CK	IU/L	114.5 ± 38.8	119.3 ± 29.9	0.2613
LDH	IU/L	276.5 ± 66.3	286.3 ± 46.8	0.5870

**indicates significance at $P < 0.01$

There was no significant difference observed between the good result group and the bad result group (Table 2). However, a significant difference between the high class group and the low class group was observed. The RBC, Hb, and PCV values in the high class group were 10.2 ± 1.1 $10^6/\mu L$, 15.8 ± 1.4 g/dL, 44.3 ± 3.7% respectively, whereas the low class group showed the RBC, Hb, and PCV values as 11.5 ± 0.6 $10^6/\mu L$, 17.8 ± 0.9 g/dL, 50.0 ± 2.3% respectively ($P < 0.01$, Table 3).

Analyzing the correlation between hematology and racing performance showed that RBC, Hb, PCV were negatively correlated with rating, placing strike rate, prize money per race ($P < 0.01$), whereas only PCV was positively correlated with G3F ($P < 0.05$, Table 4). The study of correlation between serum biochemical parameters and racing performance revealed that only AST was positively correlated with prize money per race ($P < 0.05$), whereas other biochemical values did not show statistical correlation (Table 5).

Discussion

Previous correlation studies used multiple factors such prize money per race and rating to evaluate the racing performance of the participating horses (7-9). In this study, we collected blood from racehorses after the training for the race, and immediately before the race, in order to perform blood and serum biochemical tests and assess the correlation between the measured values and racing performance. To ensure homogeneity in the experimental conditions for all racehorses, all participating horses were trained and managed by a single trainer from the identical racecourse. Therefore, several conditions and training methods for all the racehorses were identical. Furthermore, although different racehorses had different gender, age, and racing date, physical ability of each racehorses was objectified using factors (i.e., rating and prize money per race) as the racing outcomes of each racehorse were obtained under the most identical condition possible.

Rose and Allen reported that Hb concentration in racehorses increased proportionally with greater training intensity (16). Moreover, Snow *et al.* demonstrated that secretion of catecholamine and contraction of the spleen during the horse's movement promotes PCV level increase from 20% to 25% (21). As ≥ 50% of RBC in the horses is stored in spleen,

Table 4. The Pearson correlation coefficients between hematological results and race performance parameters

	Rating	Placing Rate	Prize Money per a race	S1F	G3F	G1F
RBC	–0.78**	–0.42**	–0.75**	0.09	0.35	0.22
Hb	–0.73**	–0.56**	–0.75**	0.18	0.38	0.27
PCV	–0.78**	–0.58**	–0.79**	0.13	0.44*	0.32

*indicates significance at $P < 0.05$, **indicates significance at $P < 0.01$

Table 5. The Pearson correlation coefficients between biochemical results and race performance parameters

	Rating	Placing Rate	Prize Money per a race	S1F	G3F	G1F
AP	–0.32	–0.12	–0.27	0.24	–0.03	–0.11
AST	0.40	0.05	0.46*	–0.34	–0.07	0.25
GGT	–0.21	–0.27	–0.28	0.20	0.07	–0.01
CPK	0.00	0.02	0.05	–0.20	–0.07	–0.12
LDH	–0.20	–0.02	0.00	–0.07	0.30	0.32

*indicates significance at $P < 0.05$

RBC-related values can be largely altered. Therefore, this is known to be a limiting factor for physical activity assessment in racehorses (12). Although the Hb concentration can confirm increased physical ability, contraction of the spleen and dehydration from accumulation of fatigue after training can affect these values and therefore the interpretation of these values should be carried out with caution. In this study, pre-race blood test results showed RBC and PCV values of 11.0 ± 1.0 $10^6/\mu L$ and $47.8 \pm 4.0\%$ respectively, which were higher than the normal value range. Similarly, the Hb concentration, with a value of 17.1 ± 1.5 g/dL, was higher than the normal range. The observed elevated levels of RBC, PCV, and Hb in this study could be due to the fatigue after training and dehydration (causing increased RBC level in general) and contraction of spleen from excitement of racehorses during blood collection.

AST and CK are muscle-derived enzymes exhibiting increased activity due to muscle damage after the training (21). Pre-race biochemical test results showed that the average AST activity was 427.3 ± 258.1 IU/L and was higher than the normal value range. Previous studies have shown that AST activity was increased by 35% after 1500 m canter training in horses (3), and by up to 50% after intense training (i.e. full pace gallop) (11). Furthermore, Tyler-McGowan *et al*. reported that excessively trained racehorses exhibited significantly increased AST activity (24). The racehorses in this study had 2-week training for the race, and therefore the biochemical test results for these horses exhibited increased activity of muscle-related AST enzyme.

AP and GGT are the enzymes from liver and biliary ducts, with an increased activity of AP indicating hepatic damage (4). Moreover, GGT activity is known to steadily increase in racehorses after the training (20). Another study also reported that excessively trained racehorses exhibited elevated GGT activity (24). In a previous study that assessed the correlation of GGT activity with physical ability of racehorses, showed that increased GGT activity in racehorses was associated with inferior physical ability. More specifically, a few racehorses with GGT activity of ≥ 100 IU/L exhibited remarkably reduced racing ability (19). Pre-race biochemical test results of the racehorses in this study showed that the average AP and GGT activities were 432.0 ± 55.6 IU/L and 43.0 ± 16.8 IU/L respectively, and they were higher than the normal range values. This is likely because of the training for the actual race that would have caused overall elevation of AP and GGT activities above the normal range. None of the racehorses in this study exhibited GGT activity of ≥ 100 IU/L (Maximum GGT: 84 IU/L, Race outcome: 5^{th} place /9 racehorses), and the correlation analysis outcome indicated no correlation between physical ability of the racehorses and AP or GGT activity.

The analysis of blood test results and the mean values of different groups based on the racing ability showed that none of the categories exhibited statistically significant differences. However, for the groups based on the class, the high-class group (1 to 4 classes) exhibited significantly lower levels of RBC-related values (RBC, Hb, and PCV) compared to the low-class group (5 to 6 classes) ($P < 0.01$). Moreover, the analysis of correlation between blood test results and racing ability-related categories have shown negative correlations for rating, placing strike rate, prize money per race, RBC, Hb, and PCV ($P < 0.01$) and positive correlations for G3F and PCV ($P < 0.05$). In other words, the group with greater racing ability showed low levels of RBC-related values. Additionally, higher rating, placing strike rate, and prize money per race and faster G3F record was associated with lower RBC count. Rose and Allen reported that increasing the training intensity in racehorses resulted in a proportional increase of Hb concentration (16), and Sahka showed that PCV and RBC levels are significantly increased after physical training (17). Therefore, although RBC-related values can increase with physical training in an individual racehorse, the analysis of correlation with factors that can compare racing ability between racehorses or represent racing ability showed that racehorses with greater racing ability exhibited significantly lower RBC-related values (RBC, Hb, and PCV) in the blood test performed 3 hours before the racing on the race day ($P < 0.01$). In humans, subjects who perform regular endurance training showed significantly lower average RBC-related values compared to the normal control ($P < 0.05$) (18). Mairburl demonstrated that "sport anemia" - the phenomenon of lower RBC count in athletes compared to a normal person - is due to an increased serum capacity after exercise. As RBCs can mechanistically rupture as they move through capillaries due to muscle contraction, older RBCs can easily undergo hemolysis inside the blood vessels (10). Therefore, our finding that higher-ranked racehorses exhibit significantly lower level of RBC compared to lower-ranked racehorses is in agreement with the findings in human studies. In addition, accumulation of fatigue from continuous training until the day before the race and dehydration in the horses with high RBC-related values may have exerted a negative effect on the racing ability. With higher than normal levels of RBC-related values (RBC, Hb, and PCV) and liver and muscle-related enzymes (AP, AST, and GGT), we could confirm high fatigue level of racehorses before the race. Therefore, accumulation of fatigue from training may have affected the racing ability of racehorses. Nonetheless, horses have a large number of RBC stored in their spleen, and therefore additional studies should be performed to assess the relationship between physical ability of racehorses and RBC-related values.

Muscle-related enzymes (CK and AST) in racehorses can exhibit increased activity specifically against muscle damage. However, because of the short half-life of CK, blood test for CK level should be performed within 6 hours from the physical exercise. On the other hand, blood AST level remains elevated for several days after muscle damage, making it an attractive marker of muscle damage due to the training (23). In this study, increased AST activity was associated with greater prize money per race. Unlike other enzymes that immediately reflect the fatigue level from training, AST level can be maintained for a certain period of time from accumulated training even if the horses recover from fatigue. Therefore, the correlation between increased AST activity of racehorses earned greater prize money per race is associated with sufficient training and appropriate rest or sufficient cooling-down after training.

Conclusion

This study shows that levels of RBC indices and enzymes derived from muscle and liver were elevated in pre-race blood of racehorses on race day. Also, the results suggested that levels of RBC, Hb, PCV, and AST were significantly correlated with racing performance. Therefore, blood test using parameters suggested on race day before the race is a good method for evaluating racehorses' fatigue and racing performance.

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