

Examining Acute Changes in Some Serum Biochemical Markers of Brain Tissue Damage After Free and Greco-Roman Style Wrestling

[Serbest ve Grekoromen Güreşi Takiben Beyin Doku Hasarını Gösteren Bazı Serum Biyokimyasal Markerlerindeki Akut Değişimin İncelenmesi]

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ABSTRACT

Purpose: This study aimed to examine the acute changes in some serum biochemical markers due to overloading in one match of Greco-Roman and free style wrestling competitions.

Method: In a repeated measures design, serum samples were collected before and 20 minutes after the first matches of the wrestlers during local wrestling competitions; subsequently analyzed for S-100B and heart-type fatty acid-binding protein. Study groups composed of Greco-Roman (n=15) and free style (n=16) wrestling groups of ≥19 year-old, healthy, male wrestlers. All matches were video-recorded for trauma analyses for each player.

Results: Study results showed increments of 109% (p=.007) and 145% (p=.001) in serum S-100B; 63% (p=.023) and 198% (p=.002) in heart-type fatty acid-binding protein levels from pre to post match in the free and Greco-Roman style groups, respectively. The comparison of the increases obtained in the measured parameters did not reveal significant differences between the groups.

Conclusion: Due to the significant increases in serum markers, there may be a possible additive effect of traumas in causing brain injuries/head traumas in free and Greco-Roman style wrestling. However, different style wrestling did not affect serum markers indicating brain tissue damage differently.

Key words: Free Style Wrestling, Greco-Roman Style Wrestling, S-100B, heart-type fatty acid-binding protein.

ÖZET

Amaç: Bu çalışma serbest ve grekoromen güreş müsabakalarında tek bir güreş karşılaşmasındaki aşırı yüklenmeye bağlı olarak bazı serum markerlerindeki akut değişimin incelenmesini amaçladı.

Yöntem: Bölgesel güreş müsabakaları esnasında, tekrarlı ölçüm yöntemiyle güreşçilerin ilk karşılaşmaları öncesi ve 20 dakika sonrasında kan örneği alındı; S-100B ve H tip yağ asidi bağlayıcı protein düzeyleri ölçüldü. Çalışma grupları grekoromen (n=15) ve serbest stil güreş gruplarına (n=16) ait ≥19 yaşında, sağlıklı, erkek güreşçilerden oluştu. Tüm müsabakalar her bir güreşçinin maruz kaldığı darbelerin analizi için kamera ile kaydedildi.

Bulgular: Sonuçta serum S-100B düzeylerinde sırasıyla serbest ve Grekoromen stilde %109 (p=.007) ve %145 (p=.001), H tip yağ asidi bağlayıcı protein düzeylerinde ise %63 (p=.023) ve %198'lik (p=.002) artışlar saptandı. Ölçülen parametrelerdeki artışlar karşılaştırıldığında, gruplar arası anlamlı farklılık belirlenmedi.

Sonuç: Serum markerlerindeki anlamlı artışlara bağlı olarak, serbest ve grekoromen stil güreşte darbelerin beyin yaralanması/kafa travması için olası bir etkisinin olabileceği düşünülebilir, fakat farklı stil güreş beyin doku hasarını gösteren serum markerlerini farklı olarak etkilemedi.

Anahtar sözcükler: Serbest stil güreş, Grekoromen stil güreş, S-100B, H tip yağ asidi bağlayıcı protein.

Introduction

Research has documented that athletes belonging to different sports branches have suffered from injuries during sports activities (1-7). Although many of these injuries are musculoskeletal in nature, both the peripheral nervous system and the central nervous system are at risk for injury.

Athletes are exposed to different injury risks during competitions and continue their activities even if they are sometimes injured, which may negatively affect their performances. The injury occurred during the sports activity may be healed. However, if it is not healed, and if the athlete starts his performance before complete recovery, his sports life, or his own life may end. For example, professional wrestling resulted in deaths of one male and two female wrestlers due to head trauma in Japan (8). The frequency of head trauma varies depending on the sport. As in football and boxing, head and neck zones may be injured during wrestling. Wrestling requires a competition between two rivals and during a wrestling match patterns of injury are consistent with the person-to-person, combative contact between wrestlers. Wrestling matches are performed as free and Greco-Roman styles in accordance with the FILA requirements (9,10). The Greco-Roman Style and Freestyle basically differ from each other in that "in Greco-Roman wrestling, it is strictly forbidden to grasp the opponent below the belt line or to trip him or to use the legs actively to perform any action; however, in Freestyle wrestling, it is permissible to grasp the legs of the opponent and to trip him and to use the legs actively to perform any action" (10).

Athletes are very frequently exposed to head injuries that affect brain function and there are many different ways to assess the severity of head trauma. In recent years, quantitative biochemical markers have been employed to diagnose a variety of diseases. S-100B has been investigated as a possible biochemical marker of brain injury in recent years (11) and used as a diagnostic and prognostic tool (12). S-100B has high specificity for nervous tissue although it is recognized that nonnervous tissues such as fat and muscle also release protein S-100B (13). Increased levels of S-100B are associated with a poor neuropsychological outcome (2,12). Heart type fatty acid binding protein (H-FABP) is highly expressed in neurons of the mature brain and involved in arachidonic acid incorporation in the brain (14). H-FABP increases after physical exercise by healthy subjects (15). It is accepted as a much better predictor for brain injuries than S-100B.

Athletes are prone to brain tissue injuries/head traumas during wrestling matches conducted in both Greco-Roman and Free styles, thus, early detection of these injuries is of vital importance. In addition, it is a matter of debate whether or not different style wrestling causes injury to brain tissue. Therefore, in this study we wanted to examine some serum markers of brain tissue damage

(S-100B and H-FABP), which may detect the brain trauma due to overloading following a single match of free and Greco-Roman style wrestling.

Material And Method

Participants: Totally 31 male wrestlers (≥ 19 years old) meeting the inclusion and exclusion criteria voluntarily participated in the study during local Free and Greco-Roman Style Wrestling competitions in Manisa and İzmir in 2009. All the athletes were informed about the study and they all signed a consent form. Athletes who smoke, have chronic illnesses and musculoskeletal problems, take alcohol and any medication were excluded from the study. The study was approved by the ethical council of Celal Bayar University, Faculty of Medicine.

Study Design: Before the study started, all participants were examined for a medical screening. Their body composition measurements were completed by means of bioelectrical impedance method (Tanita 300 MA, Tanita C.O., Tokyo-Japan) before the first matches of the wrestlers. All the measurements and tests were completed under identical conditions before the matches. Their blood samples were taken before and 20 minutes after the matches. Competitive wrestlers wrestled for three two-minute periods in one match. When one wrestler defeated his opponent in the first two periods, without the third period, he was announced as a winner. When the points of the wrestlers were equal at the end of the first two periods, the winner of the third period was announced as the winner of the match. When one wrestler defeated his opponent by means of fall-down, he won the match. All first matches were video-recorded for analyses for each player.

Wrestling match analyses: All first matches of the wrestlers were recorded using a camcorder (Canon MV 900 Slim stylish 2.7 wide LCD25800, Japan) placed at a 3-meter-distance and 2-meter-height to the wrestling mat. After the completion of the matches, two experienced wrestling referees analyzed the matches. They determined the wrestling games conducted by the opponent of the wrestler being analyzed and the number of each type of wrestling game the wrestler being analyzed was exposed to throughout the match was scored; thus, according to the FILA requirements, wrestling games were classified as high level of difficulty (5 points-), moderate level of difficulty (2-3 points), and low level of difficulty (1 point).

Blood analyses: Venous blood samples (10 ml) of the wrestlers were collected following a 10-hour fasting just before and after 20 minutes of the match. Blood samples were analyzed in the Biochemistry Laboratory of Celal Bayar University Hospital. Serum was separated from the samples by centrifugation at 1000 g for 10 minutes at +4°C. The serums were stored at -20°C until the batch assessment.

Serum S-100B was analyzed using ELISA method (Bio

Vendor, Modrice, Czech Republic). The lowest detection limit of the system is 5pg/ml. Intra assay CV value for 410.9pg/ml is 4.5%; inter assay CV value for 474.5pg/ml is 3.1%. Serum human H-FABP was analyzed using ELISA method (Hycult Biotechnology b.v., Udens, The Netherlands). The lowest detection limit is 4ng/ml. Intra assay and inter assay CV values are <10%.

Statistical analyses: Data were analyzed using SPSS package program (version 15.0) with non-parametric tests because of low numbers of subjects in different groups and lack of homogeneity of variance. Pre-and post match values of the participants were compared using Wilcoxon Signed-Rank Test and inter-group comparison was conducted using Mann-Whitney U test. Statistical significance was accepted at $p < 0.05$ level.

Results

In the statistical analyses, data gathered from totally 31 athletes belonging to Greco-Roman and Free style wrestling groups were used. In the tables, pre and post match physical and physiological parameters of the wrestlers were given as median, minimum-maximum values, and percent changes. Initial physical characteristics of the athletes participated in the study were given as median (min-max) values in Table 1. Initial serum S-100B and H-FABP levels of Greco-Roman and Free Style wrestling group given as median (min-max) values were similar (Table 2; $p > 0.05$).

The differences between pre-post match values in the measured biochemical parameters of the two groups were determined by using Wilcoxon Signed Ranks test. The analysis revealed significant increases in S-100B levels of both Greco-Roman group wrestlers (pre vs post median values: 35.0 pg/ml vs. 86.5 pg/ml; $\% \Delta = 145$; $p = 0.001$) and Free style wrestlers (pre vs. post median values: 28.8 pg/ml vs. 65.5 pg/ml; $\% \Delta = 109$; $p = 0.007$). We also detected significant changes in H-FABP levels of the Greco-Roman group wrestlers (pre vs. post median values: 1421.3 pg/ml vs. 4468.7 pg/ml; $\% \Delta = 198$; $p = 0.002$) and free style wrestlers (pre vs. post median values: 1298.7pg/ml vs. 5379.4 pg/ml; $\% \Delta = 63$; $p = 0.023$), (Table 3).

The changes (post-match – pre-match values) obtained at the end of the wrestling match in the serum S-100B and H-FABP levels of the two groups were compared by means of Mann-Whitney U test, but no significant differences were determined (Table 4).

Table 5 presents the number of types of games Greco-Roman and Free Style Wrestlers exposed to during the match. Considering this, we can express that Greco-Roman style wrestlers were exposed to totally 81 games in differing difficulty levels (54 low, 24 moderate, 3 high); whereas Free style wrestlers were exposed to totally 157 games in differing difficulty levels (104 low, 114 moderate, 10 high).

Discussion

Our study shows that wrestling in either Greco-Roman or Free Style results in significant increases in the serum markers of brain tissue damage (S-100B and H-FABP); however, these two wrestling styles are not different from each other in increasing these markers.

Sports and recreational activities are associated with either musculoskeletal or peripheral and central nervous system injuries. The musculoskeletal system and head are the most vulnerable areas during sports activities and competitions. Acutely injured brain tissue may release substances into the blood where they may be traced. Several different markers for brain injury have been investigated in recent years. Among these biochemical markers, S-100B is probably the most frequently investigated. Ingebrigtsen and Romner (17) have concluded that the S-100B protein is currently the most promising marker for evaluation of traumatic brain injury in patients with minor head injury. S-100B is a protein present in high concentrations in glial cells. However, more recent studies have shown that S-100B in serum may be raised not only in patients with overt cerebral injuries (traumatic or ischemic), but also in patients with extra cerebral injuries (eg, fractures, multitrauma) (17) or during surgery (18) and in healthy subjects after different types of exercise (eg, running and boxing) (19). However, the number of studies investigating the change in S-100B levels in sport-related mild head injuries/concussions

Table 1: Initial physical characteristics of subjects (median-min-max)

Parameters	Greco-Roman Style Group (n= 15)		Free Style Group (n= 16)	
	Median	Min-max	Median	Min-max
Age (yr)	19.0	19-30	20.0	19-26
Height (cm)	175.0	164-186	174.0	160-186
Body weight (kg)	83.8	59-111.5	73.4	59.6-111.3
BMI (kg/m ²)	28.6	21.5-35.2	23.8	20.3-33.6
Body fat (kg)	13.6	4.0-24.6	5.65	2.00-26.8
Percent body fat (%)	15.6	6.80-22.2	8.25	3.20-24.1
Fat free body weight (kg)	69.3	55.0-86.9	66.4	54.6-84.5

Table 2: The comparison of initial S-100B and H-FABP levels of subjects (median-min-max)

Parameters	Greco-Roman Style Group (n= 15)		Free Style Group (n= 16)		p
	Median	Min-max	Median	Min-max	
S-100B (pg/ml)	35.0	5.0-125.5	28.8	5.0-142.7	0.211
H-FABP (pg/ml)	1421.3	125.3-6114.9	1298.7	265.5-19390.1	0.906

Table 3: Changes in serum S-100B and H-FABP levels of the groups following a wrestling match

Parameters	Greco-Roman Style Group (n=15)				Free Style Group (n=16)			
	Pre Median (Min-Max)	Post Median (Min-Max)	%Δ	p	Pre Median (Min-Max)	Post Median (Min-Max)	%Δ	p
S-100B (pg/ml)	35.0 (5.0-125.5)	86.5 (48.0-166.9)	145	0.001**	28.8 (5.0-142.7)	65.5 (26.1-173.5)	109	0.007**
H-FABP (pg/ml)	1421.3 (125.3-6114.9)	4468.7 (1351.3-12629.9)	198	0.002**	1298.7 (265.5-19390.1)	5379.4 (2121.9-12945.2)	63	0.023*

*p< 0.05; **p< 0.01

Table 4: The comparison of the changes obtained in the groups following a wrestling match

Test/unit	Greco-Roman Style Group (n=15)	Free Style Group (n=16)	p
	Median (Min-Max)	Median (Min-Max)	
S-100B (pg/ml)	48.5 (14.7-145.5)	29.2 (-92.9-138.4)	0.114
H-FABP (pg/ml)	2662.0 (-1506.1-11628.9)	2819.6 (-7530.8-11593.9)	1.000

Table 5: Number of types of games Greco-Roman and Free Style Wrestlers exposed to during the match

Type of Game	Greco-Roman Style Group (n=15)	Free Style Group (n=16)
	Number	Number
Low level of difficulty	54	104
Moderate level of difficulty	24	51
High level of difficulty	3	2
Total number of games	81	157
Total wrestling time (min)	51.0	37.53

is limited. In a study, Otto et al (19), analyzed serum S-100B levels in athletes before and after different sport practices (running, boxing, soft heading of a soccer ball, and cycling on ergometer); they found that running and

boxing caused a rise of S-100B serum concentrations. The serum levels of S-100B increase rapidly after a traumatic brain injury. Some studies have reported a 10- to 15-fold increase above baseline levels followed by a sig-

nificant decrease during the next 4 to 6 hours (20-23). Highly increased values have been reported after multi-trauma and burns without head injury (17), and smaller increases have been detected after swimming (24), running, and boxing (19,24). In sports like boxing, wrestling, and football, trauma to the head and neck areas may lead to injuries and micro traumas in these zones (25-28). In a study with professional footballers, Straume-Naesheim et al (27) determined that at the end of the training season, the group of footballers who performed more intensive training programs had more increased S-100B levels in terms of the severity of the trauma to their heads than the other group of footballers. On the other hand, Stalnacke and Sojka (29) did not determine any changes in the S-100B levels of 18 professional footballers following a football match in which each footballer hit the ball falling from 18 m with their heads with a mean of 5 times. It has been reported that neuropsychological deficits occur at levels above 500ng/L of S-100B protein in serum after minimal head trauma (30). In our study which compared the serum S-100B levels before and after Greco-Roman and Free Style wrestling matches, serum S-100B levels were found to be increased in both groups, which may be accepted as the indication that wrestlers are prone to trauma during wrestling matches. However, such levels (500ng/L) were not reached by any of the wrestlers examined in our study. Considering the S-100B levels reached in our study, neuropsychological deficits are not expected as a result of either Greco-Roman or Free Style wrestling following a single match. In all wrestlers, we determined the serum S-100B levels within the reference value for the neuropsychological deficits to occur. Even so, due to the significant increases in serum S-100B levels in both groups, both style wrestlers appear to be prone to brain tissue damages/head traumas. However, it is unclear whether S-100B increase results from brain tissue damage since – as mentioned above – S-100B may increase due to various reasons. With all caution regarding the origin of the rise of S-100B is actually derived from brain tissue, vibration of brain at each wrestling game is likely to be a thinkable mechanism for the rise in S-100B during wrestling.

There are various fatty acid binding proteins (FABPs) and among these heart type fatty acid binding protein (H-FABP) is found in neurons of the mature brain and involved in arachidonic acid incorporation in the brain (14). Patient studies indicate that H-FABP is a more sensitive marker for minor brain injury than the currently used markers S-100B and neuron-specific enolase (31). However, we could not find any studies investigating the role of H-FABP as a brain tissue damage marker in athletes. Therefore, it is difficult to evaluate the increased levels of H-FABP in our study due to both Greco-Roman and Free style wrestling as a determiner for the brain injury.

Conclusion

To our knowledge, the present study is the first attempt to analyze some of the serum markers indicating brain tissue damage/head traumas (S-100B and H-FABP) in association with two different styles of wrestling. When two styles of wrestling were compared, the insignificant inter-group differences in the changes obtained in the measured parameters enable us to suggest that wrestling in either style may not cause different effects on the biochemical markers of brain injury/head traumas. The statistically significant intra-group increases determined in the biochemical markers make it possible to state that wrestling of a single match in either style may lead to increases in the serum markers of the brain cell damage/head traumas. However, one cannot express for sure that wrestling can lead to brain tissue damage. As indicated before, the increase in S-100B may be extra cerebral in nature. Moreover, one can also hypothesize that the exercise associated with wrestling may influence the permeability of the blood-brain barrier and that wrestling-related impact on the brain may then result in the demonstrable release of S-100B in the blood.

Although we did not determine brain injuries by means of computerized tomography/magnetic resonance, depending on the literature which reports increases in S-100B and H-FABP levels of the people exposed to brain traumas, we can indirectly draw such a conclusion. Due to the significant pre-post match increases, there may be a possible additive effect of traumas in causing brain injuries/head traumas in both style wrestling. However, comparing the results obtained from biochemical markers and computerized tomography would be of great importance for more precise evaluation. In addition, if repetitive elevation of serum S-100B and H-FABP levels are related to neuropsychological deficits – despite not having been proven – not only wrestlers but also athletes such as joggers, runners, and marathon runners should be examined carefully in future studies since the rise in these parameters may have resulted from the axial vibration of the brain at each step.

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