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Full Length Research Paper

The effect of competition on gamma-glutamyl transferase, creatinine and protein levels of taekwondo players

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Post-exercise proteinuria and increased urinary gamma-glutamyl transferase (GGT) levels can be predictive of exercise induced renal damage. In the literature, numerous studies exist on exercise induced proteinuria, but studies investigating the effects of exercise on urinary GGT levels are quite few in martial arts. The purpose of this study was to determine that changes in serum and urinary GGT activity and creatinine levels, and also urinary protein levels in order to assess any potential exercise induced tubular damage on taekwondo players. The study was performed on 18 female and 17 male taekwondo players who were participants in İstanbul Taekwondo Championships. Blood and urine samples collected pre- and 1 h post competition were analyzed for serum GGT and creatinine, urinary GGT, creatinine and protein levels. The post competition serum creatinine level (p = 0.002), urinary GGT (p = 0.010) and protein levels (p = 0.000) were higher than the pre- competition levels in female players. The post competition serum creatinine level (p = 0.006), urinary GGT (p = 0.005) and protein levels (p = 0.000) were higher than the pre-competition levels in male players. We suggest that high-intensity short duration exercise does not lead to increase in serum GGT levels, but lead to increase in serum creatinine levels, as well as induced excretion urinary GGT and protein levels.

Key words: Exercise, gamma-glutamyltransferase, serum, urine, enzyme, protein, creatinine, taekwondo.

INTRODUCTION

Taekwondo is a martial art and an international sport, and was accepted as a new Olympic sports in the 2000 Sydney Olimpic Games. Taekwondo competition consist of 3 rounds each being 2 min and 1 min rest period. The basis of Taekwondo competition consists of the body kicks which are made in high speed (on the safe-guard), quick and powerfull kicks and punches, sometimes kicking techniques on the face of the opponent is used. At the end of these punches and kicks, the physiological changes on the athletes are very important. Increased serum enzyme activity after the exercise was first reported in 1958; subsequent studies have established that many factors determine the degree to which the serum activities of a variety of enzymes increase during and after the exercise. The intensity, duration, type of exercise and individual variability are the most important determining factors (Noakes, 1987). The mechanism of exercise-induced serum enzyme rise has been attributed to either a metabolic effect on muscle fibers, producing increased membrane permeability or to a mechanical stress on muscle fibers, resulting in membrane damage and fiber necrosis (Karamızrak et al., 1994). The serum creatinine concentration depends not only on the

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glomerular filtration rate, but also on a number of confounding factors, particularly muscle mass, consumption of cooked meat, tubular secretion of creatinine and physical exercise load (Gailiünienė et al., 2007). Increases in the urinary excretion of renal enzymes is an important indicator of kidney damage. The degree of proteinuria depends on the type, intensity and duration of exercise (Poortmans, 1985). High-intensity, short-duration exercise has been shown to induce both glomerular and tubular orginated proteinuria, while moderate-intensity, long-duration exercise causes proteinuria (Castenfors, 1977; Steward and Posen, 1980; Poortmans, 1984; Waller et al., 1989). Gamma-glutamyltransferase (GGT) is an enzyme located on the outer membrane of numerous cell types. The primary role of the cellular GGT is to break down the extracellular reduced glutathione (GSH) in order to provide precursor amino acids especially cysteine, required for the intracellular GSH synthesis. GGT is present in most cell types and blood. Serum GGT activity was used as a marker for liver diseases and excessive alcohol consumption in clinical practice for a long time (Whitfield, 2001). In the kidney, the primary site of GGT activity is the outer surface of the microvillus membrane in the proximal tubule (Tate and Meister, 1985). Serum GGT may be also an early predictor for the development of chronic kidney disease (Ryu et al., 2007) or oxidative stress (Lee et al., 2004). In previous studies, we also observed that some types of exercise cause abnormal urinary GGT levels (Ayca et al., 2006, 2008). In this study, we aimed to assess the exercise-induced changes in serum, urinary GGT and creatinine, and also urinary protein levels in taekwondo players. In addition to these, we evaluated the relations between body mass index (BMI), training year, match number and biochemical parameters.

MATERIALS AND METHODS

Subjects

The study was performed on 18 female and 17 male players who were participants in Istanbul Taekwondo Championships. All subjects voluntered to participate in this study. The exclusion criteria were the presence of any history of serious/chronic disease, or any family history of renal disease and the presence of risk for a potential drug-induced nephrotoxicity. Taekwondo players complying with these criteria and gave signed consent after being informed about this study, were included in the study. The study was performed in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the Medical Faculty of Marmara University.

The athletes who were the participants in Championships, were weighed in the evening the day before. The competitions were made by considering 2 min × 3 rounds × 1 min the resting, according to the rules of the World Taekwondo Federation.

On the heavinesses which were not ended in the day long, the blood and urine protocol was applied by consideration of the last competition of the athletes.

Blood and urine samples were collected pre- and 1 h post the competition. Blood samples were drawn into polypropylene tubes with EDTA and centrifugeted at 3000 rpm for 15 min. Plasma and urine samples were stored in aliquots at -80°C for serum and urine GGT, creatinine and urine protein analysis. BMI in players was calculated as weight (kg) / height $(m)^2$.

Determination of plasma GGT activity

Plasma GGT activity was assayed according to Szasz's method (Szasz, 1969), using γ-glutamyl p-nitroanilide as donor substrate and glycylglycine as glutamate acceptor for the transpeptidation reaction. Standard assay included final reagent concentrations 4 mM of γ-glutamyl p-nitroanilide, 40 mM glycyl-glycine in 185 mM Tris buffer, pH 8.2. The rate of production of p-nitroaniline was followed at 405 nm in spectrophotometer (Photometer 4010, Boehringer Mannheim). The results were calculated using a molar extinction coefficient of p-nitroaniline at 405 nm of 9.900 M^{-1} cm^{-1} and expressed as U/L.

Determination of plasma creatinine levels

Plasma creatinine was determined using the Jaffé reaction method (Bonnes and Taussky, 1945). Briefly, under alkaline conditions, creatinine forms a picric acid-creatinine complex. The color intensity of this complex was measured at 520 nm with a spectrophotometer (Photometer 4010, Boehringer Mannheim). Results was calculated using a standard creatinine solution (2 mg/dl).

GGT assay in urine

For determination of urinary GGT activity, urine samples were centrifugated (Fete, clinical centrifuge) at 250x g for 10 min. Supernatants were diluted distilled water (1:10). GGT activity was determined by the Szasz method (Szasz, 1969) using gamma-glutamyl p-nitroanilide as the substrate and glyclyglycine as acceptor as describe earlier. The results were expressed as U/L. At the same time, in order to minimize errors arising from the effects of changes in urine flow, GGT levels are given as ratios againsts urinary creatinine levels (Coratelli et al., 1984).

Creatinine assay in urine

Urine creatinine levels were measured using the Jaffé reaction (Bonnes and Taussky, 1945).

Protein assay in urine

Urine protein levels were determined according to the Bradford method (Bradford, 1976) using bovine serum albumin as the standard.

Statistical analysis

The results were peresented as mean ± standart deviation (SD) and range. Paired sampled t-test and Pearson correlation test were used (SPSS for Windows 14.0 pocket program) in statistical analysis. P values < 0.05 were considered significant.

RESULTS

Age, physical characteristics, training year and match

Table 1. Age, physical characteristics, training year and match number of the taekwondo players.

Variables	Female (n = 18)		Male (n = 17)	
	Mean ± SD	Range	Mean ± SD	Range
Age (year)	20.22 ± 2.34	17 - 25	23.47 ± 3.08	18 - 29
Weight (kg)	58.33 ± 9.88	45 - 83	65.94 ± 10.09	54 - 86
Height (cm)	166.44 ± 6.16	157 - 175	172.18 ± 6.76	162 - 189
BMI (kg/m²)	21.11 ± 2.45	17.58 - 28.72	22.17 ± 2.59	19.36 - 28.41
Training year	7.58 ± 4.53	2 - 15	11.47 ± 3.78	5 - 16
Match number in competition	2.11 ± 0.90	1 - 4	1.35 ± 0.49	1 - 2

Data presented are mean ± SD (range) of players.

Table 2. Serum and urinary findings of female players (N:18).

Parameters	Pre-competition		Post-competition		P value
	Mean ± SD	Range	Mean ± SD	Range	
Serum					
GGT (U/L)	6.94 ± 2.13	3.50 - 11.00	7.25 ± 2.26	4.00 - 11.50	0.355
Creatinine (mg/dl)	0.73 ± 0.10	0.49 - 0.90	1.01 ± 0.32	0.72 - 2.00	0.002*
Urine					
GGT (U/L)	9.78 ± 6.67	2.50 - 28.20	18.96 ± 14.51	2.50 - 47.00	0.010*
Creatinine (mg/dl)	534.89 ± 204.57	191.00 - 961.00	569.24 ± 181.40	251.00 - 956.00	0.701
Protein (µg/ml)	216.89 ± 83.99	59.00 - 389.00	471.12 ± 196.92	48.00 - 739.00	0.000*
GGT (U/g creatinine)	2.01 ± 0.39	0.84 - 7.46	3.42 ± 0.67	1.00 - 10.96	0.048*

*Statistical significance is expressed as $p<0.05$.

Table 3. Serum and urinary findings of male players (N:17).

Parameters	Pre-competition		Post-competition		P value
	Mean ± SD	Range	Mean ± SD	Range	
Serum					
GGT (U/L)	8.12 ± 2.30	4.50 - 12.00	7.94 ± 2.62	4.00 - 13.50	0.355
Creatinine (mg/dl)	0.73 ± 0.19	0.44 - 1.30	1.06 ± 0.36	0.70 - 1.87	0.006*
Urine					
GGT (U/L)	6.24 ± 2.39	2.90 - 13.00	13.00 ± 8.07	2.00 - 31.20	0.005*
Creatinine (mg/dl)	415.01 ± 247.29	71.00 - 960.00	546.38 ± 213.57	229.00 - 985.00	0.103
Protein (µg/ml)	148.35 ± 72.07	17.00 - 300.00	411.50 ± 161.03	119.00 - 609.00	0.000*
GGT(U/g creatinine)	2.18 ± 2.11	0.69 - 9.52	2.59 ± 1.63	0.22 - 7.00	0.544

*, Statistical significance is expressed as $p < 0.05$.

number of the taekwondo players are shown in Table 1.

The age range was 17 to 25 years for the female and 18 to 29 years for the male players. The range of training year was 2 to 15 years for the female and 5 to 16 years for the male players. The mean of match number in competetion was 2.1 ± 0.90 for the female and 1.35 ± 0.49 for the male players.

The serum and urinary findings of female and male players are shown in Tables 2 and 3, respectively. The post competition serum creatinine level and urinary GGT (U/L) and protein levels were higher than the pre-competition levels in male players ($p < 0.05$). The post competition serum GGT, urinary creatinine levels were higher than the pre-competition levels in female players

Table 4. Correlation findings between biochemical parameters and match number of taekwondo players.

Parameters	Female (n = 18)		Male (n = 17)	
	r value	P value	r value	P value
Serum GGT (U/L)	0.333	0.177	0.017	0.948
Serum Creatinine (mg/dl)	-0.128	0.624	-0.103	0.704
Urine GGT (U/L)	-0.142	0.583	-0.190	0.481
Urine creatinine (mg/dl)	0.292	0.255	0.087	0.750
Urine protein (µg/ml)	0.459	0.064	0.146	0.590
Urine GGT (U/g Creatinine)	-0.159	0.543	-0.223	0.407

Table 5. Correlation findings between biochemical parameters and training year of taekwondo players.

Parameters	Female (n = 18)		Male (n = 17)	
	r value	P value	r value	P value
Serum GGT (U/L)	-0.103	0.685	0.353	0.164
Serum creatinine (mg/dl)	0.207	0.426	0.199	0.459
Urine GGT (U/L)	-0.435	0.081	-0.154	0.568
Urine creatinine (mg/dl)	0.291	0.257	0.126	0.641
Urine protein (µg/ml)	-0.153	0.557	-0.008	0.978
Urine GGT (U/g Creatinine)	-0.496	0.043*	-0.121	0.656

*Statistical significance is expressed as p<0.05.

Table 6. Correlation findings among pre- and post- competition serum creatinine and GGT levels and BMI of taekwondo players**.**

Parameters	Pre-competition		Post-competition	
	r value	P value	r value	P value
Female				
Serum creatinine (mg/dl)	-0.295	0.234	0.098	0.709
Serum GGT (U/L)	-0.060	0.812	0.027	0.914
Male				
Serum creatinine (mg/dl)	0.206	0.427	-0.165	0.541
Serum GGT (U/L)	0.394	0.117	0.612	0.009*

*Statistical significance is expressed as p<0.05.

but these increases are not statistically significant (p > 0.05). The post competition serum creatinine level and and urinary GGT (U/L) and protein levels were higher than the pre-competition levels in male players (p < 0.05). The post competition serum GGT level, urinary creatinine and GGT (U/g creatinine) levels were higher than pre-competition levels in male players, but these increases are not statistically significant (p > 0.05).

As shown in Table 4, low correlation has been found between the match number and post competition serum GGT levels in female players, but this correlation is not statistically significant (r = 0.333, p > 0.05). There were no correlation between the match number and post competition serum GGT levels in male players (r = 0.017, p > 0.05).

There were no significantly correlations between match number and serum and urinary creatinine levels, urinary GGT and protein levels (p > 0.05). There were no significantly correlations between the training year, and serum and urinary biochemical parameters in female and male players (p > 0.05, Table 5).

As shown in Table 6, there were no correlation

between the body mass index (BMI) and pre- and post competition serum creatinine level in female players ($r = -0.295$, $p > 0.05$; $r = 0.098$, $p > 0.05$, respectively). And also there were no correlation between the BMI and pre- and post-competition serum creatinine level in male players ($r = 0.206$, $p > 0.05$; $r = -0.165$, $p > 0.05$, respectively). There were no correlation between the BMI and pre- and post-competition serum GGT level in female players ($r = -0.060$, $p > 0.05$; $r = 0.027$, $p > 0.05$, respectively). And also there were no correlation between the BMI and pre-competition serum GGT level in male players ($r = 0.394$, $p > 0.05$) but there were correlation between BMI and post competition serum GGT level in male players ($r = 0.612$, $p < 0.05$).

DISCUSSION

Taekwondo competition requires short, quick and all outbursts of maximal power during the match. These intense exercise-induced changes in biochemical parameters start up problems after the match whether athletes are not shown. Taekwondo competition has been 2-min combat, 1 min rest and 3 periods; during the time allowed, there are many interruptions during combat. The typical time structure is 10 s of activity with a 5 s interval (Topal et al., 2011). The results of this study show that high-intensity short duration exercise in taekwondo sportmen do not increase serum GGT levels, but increases serum creatinine levels. It also induced excretion of urinary GGT and protein.

Numerous studies have been published on the effects on biochemical, immunological, hematological and cardiac parameters of exercise (Bellighieri et al., 2008; Miao et al., 2010; Künstlinger et al., 1987; Biondi et al., 2003; Wu et al., 2004; Haili et al., 2011). Exercise-induced proteinuria is generally benign. It occurs most often among athletes participating in such sports as running, swimming, rowing, football and boxing. The normal range of protein excretion in healthy individuals is 150 to 200 mg of protein per day, of which albumin constitutes 10 to 20 mg. Various physiologic settings including exercise can induce a transient increase in urinary protein excretion (Saeed et al., 2012) Albumin secretion was noted to increase 20 to 25 times in short-duration exercises, while it increased only 2 to 6 times during long-duration exercises. While all kinds of exercise can cause an increase in protein secretion, it can be concluded that the significance of increase depends on the duration and intensity of the exercise (Robertshaw et al., 1993).

In our study, urinary protein excretion were found to increase significantly after competition for both of genders. In general, proteinuria is due to increased filtration, reduced protein reabsorption, or both. In our study, we did not identify the mechanisms leading to postexercise proteinuria. But the renin-angiotensin system (RAS) and prostaglandins are thought to play a major role in post-exercise proteinuria (Saeed et al., 2012).

The incidence of renal dysfunctions during exercise, as reported in many scientific studies, ranges from 11 to 100% depending on the type and amount of exercise and the athlete's state of hydration. These alterations include proteinuria, hematuria and reduction in renal blood flow, and glomerular fitration rate (Bellighieri et al., 2008). In another study, a group of 51 healthy athletes took part in a 100 km race over 14.25 h. All of them had to reach the finish together, urine and blood samples were collected before and immediately after running as well as 6 h after the race. The serum concentrations of potassium, protein and albumin decreased significantly (before the race versus 6 h after the race) but remained within physiological ranges (Gerth et al., 2002).

Creatinine is a substance formed by decomposition of creatinine in muscles, filtered through kidneys easily before entering urine. In sports medicine, creatinine as a renal filtration marker is used for evaluating general health status of sportmen. However, the use of serum creatinine as an indirect marker of glomerular filtration rate has been criticised because it is also affected by age, sex, race, diet and body mass. A correlation between creatinine and BMI, and a positive association between creatinine and physical exercise have been reported in the general population (Banfi and Del Fabbro, 2006). In our study, post-competition serum creatinine levels in female and male players were higher than pre-competition levels, but these changes were not statistically significant. Very few papers have been published on creatinine concentrations in athletes, before and/or during competitions. Banfi and Del Fabbro (2006) measured serum creatinine level in 151 elite male athletes from five different sports (rugby, soccer, alpine skiing, sailing and cycling) before the competitive season. In the whole group of athletes, a positive correlation between serum creatinine and BMI were found. In this study, we studied the effect of competition on creatinine level in competitive season. In our study, there were no significantly correlation among the BMI and pre- and post-competition serum creatinine levels in female and male taekwondo players. The reason for the lack of a correlation between creatinine levels and BMI in taekwondo sportmen may be peculiar status of professional athletes such as the contents of total body water or may be type of sport (Banfi, 2010).

In our study, the post-competition serum GGT levels of male players were lower than the pre-competition levels; but these decreases were not statistically significant. And the post-competition serum GGT levels of female players were higher than the pre-competition levels; but these increases were not statistically significant.

In renal damage, the urinary excretion of some renal enzyme such as N-acetyl-beta-D-glucosaminidase, leucine aminopeptidase, ALT increases. GGT is also

considered to be indicator of proximal tubule damage (Vanderlinde, 1981), because the primary site of GGT activity is on the outer surface of the microvillus membrane in the proximal tubules (Tate and Meister, 1985).

In our previous study (Ayca et al., 2006) done on female and male volleyball players, we did not find a significant increase in urine GGT levels of the players between pre and post- exercise. On the other hand, a significant exercise induced increase was observed in urinary protein excretion in male players, and creatinine excretion in female players. When the results were compared separately for setters and spikers; even though female players had no difference, the male spikers had a significant increase in GGT levels. In 2008 study with female gymnasts, GGT levels were slightly increased after training but more significantly after 24 h. Creatinine levels were slightly increased after training and in 24 h period as well. Proteinuria levels were increased slightly after training but significantly after 24 h (Ayca et al., 2008). It has been reported that urinary abnormalities were reported to resolve in 24 to 36 h after the exercise (Gilli et al., 2008). In this present study, urinary GGT (U/L) and protein excretion were found to increase significantly after competition for both of genders. The postcompetition increase in the urinary GGT levels (as U/g creatinine) was significant in female players. Increasing the enzymes excretion to urine can be due to renal damage mediated-ischemia or Rhabdomyolysis. In the first mechanism, complete or partial ischemia of kidneys may prevent oxygenation to tubular epithelium and trigger biochemical events (Witt et al., 1992). Rhabdomyolysis that myoglobin enters the renal tubules, causes renal cells damage and release of GGT (Senert et al., 1994).

Gür et al. (1994) indicated that the intensive running conditions of a half-marathon race may cause urinary abnormalities and these may occur independent of age, duration of running, training and athletic background of athletes. In this present study, there were no significantly correlation among the training year and serum and urinary biochemical parameters in female and male players. There were no significant correlation among match number and serum and urinary creatinine levels, and urinary GGT and protein levels in female and male players.

Salvaggio et al. (1991) performed a study for the association between body mass index and serum liver enzyme activity (GGT, ALT, AST) in 3167 subjects, 2373 men and 794 women. Their observations demonstrate an important relation between body weight (expressed as body mass index) and serum activity rates of liver enzymes. This relation is still evident after age, physical activity, and cigarette and alcohol consumption are adjusted for. BMI was related to serum activities of GGT, ALT and AST in men, but only to GGT and ALT in women.

In our study, there were no significant correlations among the body mass index and pre- and post-competition serum GGT level in female players. But there were significant correlations among body mass index and post-competition serum GGT levels in male players.

Conclusion

In conclusion, the present study suggests that high-intesity short duration exercise do not increase serum GGT levels, but increases serum creatinine levels. This type of exercise also, induces excretion of urinary GGT and protein levels.

Our study had a limitation, only 1 h post-competition serum and urine samples were taken from each players. If samples were collected at periodic intervals such as post-competition 6, 12 and 24 h, any significant increase/ or decrease could have been observed or the duration of any potential increse as well as the time of resolution could have been determined.

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