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Original Article

# Hypothalamo-pituitary-adrenal axis activity and related factors in adolescent during a tennis tournament

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**Abstract**

**Aim:** Adolescence is an important period of development and during this period young people are more susceptible to mental illnesses. Therefore, a comprehensive knowledge of the factors that mediate stress response can help developing our understanding of these diseases. The aim of this study is to examine the activity of hypothalamo–pituitary–adrenal axis and determine factors affecting this activity in adolescents.

**Material and Methods:** The adolescents between the ages of 14 and 17, who participated in the official amateur tennis tournament, were included in the study. The data form, including questions for the assessment of the stress level and sociodemographic variables was filled. Then, salivary cortisol levels were measured before (-5 minutes) and after (+5 minutes) the first match of the official tennis tournament.

**Results:** The mean salivary cortisol levels were 40.8±53.8 ng/ml, before the game and 98.8±150.5 ng/ml, after the game (p=0.008). The salivary cortisol levels were statistically higher, after the game, in males, those who won, and those who felt anxious compared the before.

**Conclusion:** The results of this study showed that salivary cortisol level can be used in the assessment of stress response in adolescents and various factors such as gender, mood, and state of winning are effective on cortisol response.

**Keywords:** Adolescent; hypothalamo-pituitary-adrenal axis; salivary cortisol; stress; tennis; tournament

## INTRODUCTION

All of the stimuli that compromise the homeostasis of the organism are called 'stress'. In human body, two main neuroendocrine systems are activated get activated in order to obtain a new state of balance after being exposed to stress. These are the sympathetic nervous system and the hypothalamo-pituitary-adrenal axis (HPA) (1). In the HPA system, cortisol secretion is regulated by a hormone released from the pituitary gland (2). Although cortisol acts on a wide range from glucose metabolism to the immune system, it has an important role in the behavioral area of the response to stress (3). The cortisol response has also been shown to be included in stress-producing environments, such as preparedness to competition and confrontation (4,5) and therefore, sports competitions have been a research area to evaluate cortisol response to stress (5,6). In this respect, many competing sports branches such as wrestling, tennis, and rugby have been discussed in studies (4,7,8). Even, going even a step further, the studies emphasized that the features of HPA can be used to determine the level of preparation of athletes for training and competitions (9) and cortisol level can be used as performance factor (10).

Adolescence is an important developmental period that involves hormones, physical changes, and many mental and mental changes. A number of psychiatric diseases including depression, schizophrenia and anxiety disorder start in this period and continue in adulthood (11). In this period when young people are more vulnerable mentally (11), knowing the HPA system, which is one of the main factors involved in stress response, and the factors affecting the response created by this system, may provide various benefits in the psychiatric approach and preventive mental health. The level of cortisol is one of the parameters used in the evaluation of the HPA system activity and it involves different methods of measuring. Serum cortisol measurement is an invasive method and its application requires certain steps (12). In this context, determination the cortisol level in saliva allowed the amount of cortisol to be easily determined in a non-hospital setting by a non-invasive method (13).

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The level of circulating cortisol is not constant; it fluctuates during stress or exercise (14). In fact, cortisol level varies depending on the type of physical exercise (15), and cortisol release also shows individual differences (16). In parallel with this variability, cortisol response changes in a competitive environment. Given that cortisol increase is important for affecting mental and physical performances, and those with sufficient increase of cortisol are usually won the competition (17,18), determining the factors affecting cortisol response including age, gender, and mental state can guide the evaluations to increase performance and success.

In this study it is aimed to determine the hypothalamo-pituitary-adrenal axis activity and factors affecting this response in adolescents during a tennis tournament. In this way, it is aimed to contribute to the literature regarding the activity of HPA system in adolescents. While the amount of cortisol varies depending on psychological stress prior to a sports performance, it is regulated throughout the performance in relation to both physical and psychological stress (19,20). Tennis causes physical and psychological stress as a type of sport that usually is performed in the face of the opponent, without contact and involves periods of higher intensity activities interspersed between lower intensity activities (21). For this reason, the study was conducted on adolescents participating in the tennis tournament.

## MATERIAL and METHODS

### Study procedure

The study was designed as a cross-sectional study, and approved by the Clinical Research Ethics Committee (No: 2020/19). The children and adolescents between the ages of 14 and 17, who participated in the amateur tennis tournament organized by the Provincial Directorate of Youth and Sports, were included in the study. The data form, including questions for sociodemographic variables, was filled by the subjects that agreed to participate in the study. And then, adolescents were asked to evaluate individually whether the pre-match feelings applied to the anxious/nervous or good/normal categories. In order to measure cortisol levels, saliva samples were collected from the participants 5 minute before and after the first game. Saliva samples collected in 18 games in three consecutive days in the tournament. The saliva samples were collected at 10-11-12-13-14-15-16 o'clock from 29 of 36 players; 5 players weren't able to give samples and 2 player results are excluded because they are very extreme (salivary cortisol concentrations >1000 ng/ml). Equal numbers of samples were collected every point of collection, in order to ensure equal distribution of cortisol levels in saliva.

### Measurement of cortisol in saliva

Cortisol measurements were carried out according to Ozgocer et al. (22). Briefly, cortisol-bovine serum albumin (BSA) stock solution was diluted with carbonate buffer and added at 200 µL/well to a 96-well microtiter plates. The plates were incubated overnight at +4 ° C and washed 5 times with wash buffer using eight-channel pipette. Sites not occupied by the coating antigen were blocked for 2 h at 37 ° C by blocking buffer (200 µL/well, 1% BSA in phosphate buffered saline, pH 7.2). Plates were washed 5 times and standard solutions or samples (40 µL/well) and diluted primary antibody were added in duplicate (40 µL/well) and incubated at 37 ° C for 45 min. Before adding the samples into microtiter plates, they were thawed, centrifuged at 4000 g for 10 min, and the supernatant, diluted 5x in assay buffer, was used for ELISA analyses. Plates were washed 5 times and incubated at 37 ° C for 30 min following addition of biotinylated anti-rabbit antibody (100 µL/well). Plates were washed 5 times and incubated at +4 ° C for 15 min following addition of streptavidin peroxidase solution (100 µL/well). Then, the plates were washed again for 5 times and incubated in dark for 10 min after adding the substrate solution (tetramethylbenzidine, 150 µL/well). Reaction was terminated by adding stop solution was added (50 µL/well). Absorbance was measured at 450 nm using a microplate reader (Synergy HT, Biotek, USA) and the results were quantified by the software provided (Gen5, Biotek, USA).

### Statistical analysis

In the analyses of the data SPSS 25.0 software was used. Quantitative data were expressed as mean and min-max, and qualitative data were expressed as numbers and percentages. The compliance of the data for normal distribution was tested by the Kolmogorov-Smirnov test. Since the data did not complied with the normal distribution, Mann Whitney U Test and Wilcoxon Signed-Rank Test were used for statistical analysis. Effect size calculated with effect size calculator. A value of $p < 0.05$ was considered statistically significant.

## RESULTS

A total of 29 child and adolescent tennis players were included in the study. Fifteen participants were male and 14 were female. The average age was 15.2 (min-max: 14-16) for females, 15.3 (min-max: 14-17) for males and 15.2 (min-max: 14-17) for the whole study group. Fourteen children were in the winning group and 15 were in the losing group according to the match score. Seven children in the winning group and 8 children in the losing group were male. Those with a history of psychiatric or medical illness and chronic drug use were excluded from the study, due to the risk of influencing the analysis. The sociodemographic characteristics of the participants included in the study are given in Table 1.

### Comparison of saliva cortisol levels between variable groups

When the change between genders is evaluated; although the average saliva cortisol level in males was higher than females both before and after the game; the difference was not statistically different ($p > 0.05$). In the comparison of saliva cortisol levels between the losing and winning groups, the mean cortisol level was higher in the winning group before and after the game than the losing group; however, the difference was not statistically significant ($p > 0.05$).

**Table 1. Sociodemographic characteristics of the participants**

| Variable | mean±SD (min-max) |
|---|---|
| **Age** | 15.2±0.8 (14-17) |
| **Number of siblings** | 2.4±0.8 (1-4) |
| **Age of mother** | 43.9±4.2 (34-52) |
| **Age of father** | 46.2±3.9 (38-54) |
| **How many days in a week you do he training?** | 2.3±1.1 (1-7) |
| **How many years you have been doing sports?** | 4.6±3 (1-8) |
| **How many tournament did you participated before?** | 4.5±4.8 (1-14) |
| | **n (%)** |
| **Gender** | |
| Female | 14(48.3) |
| Male | 15(51.7) |
| **In which order are you among siblings?** | |
| 1. | 13 (44.8) |
| 2. | 12 (41.4) |
| 3. | 3 (10.3) |
| 4. | 1 (3.4) |
| **Education level** | |
| Mid school | 5 (17.2) |
| High school | 24 (82.8) |
| **Family type** | |
| Nuclear family | 25 (86.2) |
| Wide family | 4 (13.8) |
| **Level of income** | |
| Low | 4 (13.8) |
| Medium | 20 (69) |
| High | 5 (17.2) |
| **How many hours a day do you do training?** | |
| Less than 1 hour | 17 (65.5) |
| 1-2 hours | 7 (24.1) |
| 2-3 hours | 2 (6.9) |
| 3-4 hours | 1 (3.4) |
| **Mother's education level** | |
| Primary school | 3 (10.3) |
| High school | 3 (10.3) |
| University | 23 (79.4) |
| **Mother's occupation** | |
| Housewife | 13 (44.8) |
| Officer | 16 (55.2) |
| Worker | 0 |
| Other | 0 |
| **Father's education** | |
| Primary school | 1 (3.4) |
| High school | 5 (17.2) |
| University | 23 (79.4) |
| **Father's occupation** | |
| Non working | 0 |
| Officer | 20 (69) |
| Worker | 3 (10.3) |
| Other | 6 (20.7) |

When children were categorized according to how they felt before the game, 16 (55.1%) children stated that they felt good (normal) and 13 (44.9%) subjects stated that they felt anxious (nervous). The saliva cortisol levels were higher in the children who stated that they were normal before the match, than those who stated that they felt anxious; however the difference was not statistically significant (p> 0.05). On the other hand, after the match, cortisol level was higher in individuals who described themselves as nervous as the opposite, but the difference was not statistically significant (p> 0.05).Comparison of saliva cortisol levels before and after the game between the variables are given in Table 2.

**Comparison of salivary cortisol levels before and after the match according to the variables**

The mean salivary cortisol levels were 40.8±53.8 ng/ml, before the game and 98.8±150.5 ng/ml, after the game. In whole group; post-match salivary cortisol levels were significantly higher than pre-match status (p=0.008). Pre- and post-match salivary cortisol levels in adolescents during a tennis tournament is shown in Figure 1.

In 24 (82.7%) people, the level of post-game cortisol increased compared to the previous level, while in the opposite of 5 (17.3%), cortisol level decreased after the game. In the latter group, 3 were female, 3 of them participated in the tournament for the first time, 1 of them participated for the second time, and 1 of them participated in more than three tournaments. In addition, all 5 children were in the group stating that they felt normal before the match and 3 of them were in losing group. Among the other 24 children with higher levels of cortisol after the game, 13 (54%) participated in three or more tournaments and 11 (%46) stated that they felt normal before the match.

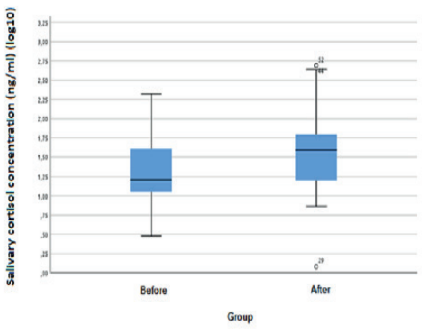


**Figure 1.** Pre- and post-match salivary cortisol levels in adolescents during a tennis tournament

When cortisol level change is evaluated within each child; the salivary cortisol levels were statistically higher, after the game, in males, those who won, and those who felt anxious (respectively, p=0.047, p=0.013, p=0.003) compared the before. The salivary cortisol levels were also higher after the game, in girls, those who lost, and those who stated that they felt normal before the game; however the differences were not statistically significant (p>0.05). While the effect size of male gender, anxious

**Table 2. Comparison of salivary cortisol levels before and after the game between the variables**

| Variable | n(%) | Salivary cortisol level before the game (ng/ml) Median(min-max) [Mean±SD] | Salivary cortisol level after the game (ng/ml) Median(min-max) [Mean±SD] |
|---|---|---|---|
| **Gender** | | | |
| Female | 14(48.3) | 14(5.8-170) [36.6±55.1] | 35.1(1.2-368.6) [61.1±99.4] |
| Male | 15(51.7) | 20.4(3-209.2) [44.1±54.4] | 48(10.1-492.1) [129±179.2] |
| **p value** | | 0.27 | 0.30 |
| **Game result** | | | |
| Winning | 14(48.3) | 18.8(7.9-170.6) [41.8±50.9] | 58.6(1.20-492.1) [158.6±192.2] |
| Losing | 15(51.7) | 15.4(3-209.2)[39.6±58.8] | 37.1(7.3-62.8)[34.4±20.3] |
| **p value** | | 0.48 | 0.08 |
| **Mood** | | | |
| Normal | 16(55.1) | 18.8(3-209.2) [48.5±62.2] | 38.8(1.2-492.1) [88.2±148.4] |
| Anxious | 13(44.9) | 13.7(5.8-135.1)[29.4±38.4] | 57.5(14.2-485.7)[114.3±159.4] |
| **p value** | | 0.45 | 0.51 |

**Table 3. The comparison of salivary cortisol levels (ng/ml) before and after the game according to the groups**

| Variable | n | Before the game Median(min-max) [Mean±SD] | After the game Median(min-max) [Mean±SD] | p value | Cohen's d |
|---|---|---|---|---|---|
| **Whole group** | 29 | 16.3(3-209.2) [40.8±53.8] | 39.1(1.2-492.1)[98.8±150.5] | **0.008*** | **0.008*** |
| **Female** | 14 | 14(5.8-170) [36.6±55.1] | 35.1(1.2-368.6) [61.1±99.4] | 0,06 | 0,06 |
| **Male** | 15 | 20.4(3-209.2) [44.1±54.4] | 48(10.1-492.1) [129±179.2] | **0,047*** | **0,047*** |
| **Winning** | 14 | 18.8(7.9-170.6) [41.8±50.9] | 58.6(1.20-492.1) [158.6±192.2] | **0,013*** | **0,013*** |
| **Losing** | 15 | 15.4(3-209.2)[39.6±58.8] | 37.1(7.3-62.8)[34.4±20.3] | 0,27 | 0,27 |
| **Normal** | 16 | 18.8(3-209.2) [48.5±62.2] | 38.8(1.2-492.1) [88.2±148.4] | 0,27 | 0,27 |
| **Anxious** | 13 | 13.7(5.8-135.1)[29.4±38.4] | 57.5(14.2-485.7)[114.3±159.4] | **0,003*** | **0,003*** |

* p<0.05

mood and post-match winning status on cortisol release was moderate, the effect size of other groups was low. The comparison of saliva cortisol levels (ng/ml) before and after the game according to the groups were given in Table 3.

## DISCUSSION

In this study, factors affecting HPA activity and response in adolescents, in a competitive environment, were evaluated. In the literature, it is seen that cortisol and hormonal responses given to the competitive environment during childhood/adolescence and adulthood are variable. In a study evaluating cortisol levels in a golf tournament it was found that in adults, cortisol levels increased during competition and there was a relationship between somatic anxiety scores and cortisol response, and it was emphasized that somatic anxiety was confirmed by physiological anxiety measurements (6). Contrary to the expected increase in the cortisol level in adults (23), the level of cortisol decreased in most prepubertal children during a dual table tennis tournament, and this was thought to be related to a more relaxed psychological state among competitors during minimally physically taxing table tennis competitions against peers (24). In another study conducted by Mchale et al. (2018) it was found that cortisol levels decreased after the match in the non-physical teamwork in girls and boys in the prepubertal period, and a significant positive correlation was observed between competitive cortisol and androstenedione change (25). However, the different results were reported in some other studies. In another study conducted on girls in the prepubertal age playing basketball, in 2016, it was found that cortisol levels increased after the match significantly (26). There are a very few number of studies conducted on adolescents and gender-oriented studies are in the foreground. In a study conducted on 9 male tennis players including adolescent athletes consisting

of 4 seniors and 5 juniors (18.7 ± 1.8 years), it was found that the level of saliva cortisol increased significantly after the match in comparison to 10 minutes before the match (27). In our study, when all subjects are evaluated together (29 children); mean salivary cortisol levels of children were significantly higher after the game. This result may be due to the psychological and physical stress they were exposed during the game, or it may indicate that the metabolic effects due to pre-match stress continue for a certain period of time.

Another important result obtained from our study is that, when salivary cortisol concentration is handled individually, the increase in the amount of cortisol was not found in all children participating in the tournament. In 24 children, cortisol response increased after the match, while in 5 children, the opposite cortisol concentration decreased. We noticed that children with lower cortisol levels after the game had participated in fewer tournaments than other children. This may have caused the stress response to be higher before the match. The level of importance, awareness, and outcome expectation regarding the tournament in children who have already participated in the tournament is naturally different. Because as the effort is increased, the desire for reward increases. This is associated with increased stress. This emphasizes the need for feedback that includes children's feedback from previous competitions, experiences gained and the meaning attached to this process. In addition, all of these 5 children were in the group that normally defined the mood before the game. It shows that competition is not the only variable in a competitive environment, and various factors such as emotional response of adolescent to the environment may also affect stress and therefore cortisol response. In another study conducted on children at the age of 8–11 years in a football game, 41 of a total of 83 samples were examined and it was found that cortisol level was increased in 41 samples, decreased in 25 samples and did not change in 16 samples (28). In addition, in this study, a decrease in cortisol level was detected during intrasquad soccer scrimmage, in contrast to the football match; this result was attributed to the fact that this event caused little perception of stress (29).

Another result obtained from our study is; the cortisol levels after the match were significantly higher in men, in those who won the match, and who state that they were anxious at the beginning of the match. In addition, these variables had a moderate effect on cortisol release. Although there was an increase in the level of cortisol after the match in the girls, those who lost the match, and those who stated that their mood was normal, the difference was not statistically significant. These variables had a low effect on cortisol release. Our results show that male gender, anxious mood and winning status affected cortisol response more significantly. Anxiety and fear are the starting and maintaining factors of the war or run response. Therefore, it is an expected result that the state of feeling anxious before the match would have a more pronounced effect on the cortisol response.

When the related studies in the literature are examined; it is seen that the stress response varies according to variable conditions such as the nature of the stressful situation (competition, immigration etc.), gender, mood etc. In a study conducted on Judo sporters, it was found that the cortisol levels before the competition and after the competition increased significantly compared to the resting status (30). In addition, in this study, anxiety scores were found to be increased simultaneously with cortisol response, on the day of competition. This result was considered to be an indicator of the relationship between negative affection and cortisol level in a competitive environment (30). Another finding of this study showing a positive correlation between self-confidence (possibility of winning) supports our findings suggesting that winning status had a greater effect on cortisol increase. Indeed, winning a game is closely related to the endurance of the individual during competition. This endurance is affected by conditions such as mental health, self-confidence, and ambition of winning (31).

In a study examining the end-game cortisol levels of the individuals who participated in the wrestling competition, the cortisol levels of the winners were higher than the losers (7). In a study in which the psychophysiological stress was evaluated in the first tennis match in tennis players, 5 saliva samples were collected at rest and 10th and 60th minutes before and after the match. The cortisol levels measured on the day of the match were found to be higher in both winners and losers according to resting state. In addition, saliva concentration was determined at the highest level 10 minutes after the end of the match, even though there was a significant decrease 60 minutes after the end of the match, this value was still higher compared to the resting state and it was found to remain at high levels for a long time (32). In addition, in this study, contrary to our study, higher cortisol levels were found in women compared to men. In a study conducted on immigrants, no difference was found between daily cortisol levels between male and female gender, but it was emphasized that the relationship between perceived stress and adjustment variables and cortisol response was affected by gender (33). In a study evaluating the response of elite female and male volleyball players to cortisol, it was found that women showed higher fasting cortisol response than men (28). In studies conducted on adolescents, it has been shown that cortisol response varies due to different factors in stress environment, similar to adults. In a study conducted in a mathematics contest, it was shown that there was no relationship between cortisol response and gender, and the decrease in cortisol levels was almost twice larger in losing competitors than the winners (25). In our study, both pre-and post-match cortisol levels were higher in males compared to females, but the difference was not statistically significant. In addition, the pre/post-match change of cortisol levels were statistically significant in males, whereas the changes weren't statistically significant in females. These varying

results reported by different studies show that gender alone is not an effective variable on HPA activity and it would be more appropriate to evaluate all factors together while evaluating HPA activity.

## LIMITATIONS

Although the study has much strength, it also has some limitations. A limitation of this study is that the level of cortisol at resting status is unknown; therefore, a comparison of cortisol levels by this respect was limited. Since tennis is a sport that requires active performance and physical exertion directly induce cortisol release, it is difficult to determine the level psychological effects of competition. Another limitation is that sample was collected 2 times before and after the match and the change of cortisol level during the day could not be evaluated. In addition, a single type of sports was chosen as a source of stress in the study. These results cannot be generalized for cortisol response in other types of sports and different competitive environments.

## CONCLUSION

One of the important strengths of the study is that it involves adolescent age group, in which fast physical and hormonal growth occurs. Because of, the changes in the body cause the reactions against the events to change. Additionally, the salivary cortisol levels were statistically higher, after the game, in males, those who won, and those who felt anxious compared the before. Therefore, it is a powerful study in terms of evaluating the factors affecting stress response and the course of these factors in this age group. Our findings may helpful in identifying risky groups and determining the measures to develop adolescents' coping strategies.

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*Ethical approval: The study was approved by the Inonu University Clinical Research Ethics Committee (No:2020/19).*

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