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The Effect of Psychological Stress on Salivary Testosterone in Puberty Children

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Abstract

BACKGROUND: Psychological stress is a condition that is experienced by many adolescents which affect the Hypothalamic-Pituitary-Gonadal axis. Testosterone is known as a sex steroid hormone that is susceptible to acute stress and can be measured through saliva. Disruption of the reproductive system can affect the sexual maturation process.

AIM: To understand the difference in salivary testosterone levels in puberty children before and after given a stressor.

METHODS: A quasi-experimental intervention study was conducted at Antonius Bangun Mulia junior high school, Medan, North Sumatera, in July-October 2017. Subjects were students aged 12-14 years with sexual maturity G2 for boys and M2 for girls. Psychological stress intervention was generated by the Wechsler intelligence scale for children fourth edition (WISC IV). Saliva was collected before and after the intervention. The analysis was done with Wilcoxon test and a P value < 0.05 was considered significant.

RESULTS: Forty-two subjects of 24 male students and 18 female students with sexual maturation Tanner II (54.8%) and Tanner III (45.2%). This study obtained that there was a statistically significant difference in salivary testosterone levels before and after the subject was given a stressor (P = 0.015, CI 95%). This difference also was seen within sexual maturation Tanner II (P = 0.045, CI 95%). No difference was observed in testosterone levels based on gender, male students (P = 0.065, CI 95%) and female students (P = 0.112, CI 95%).

CONCLUSION: Stress can affect salivary testosterone levels. There was a statistically significant difference in salivary testosterone levels before and after psychological stress in puberty children.

Introduction

Puberty is an important stage in the child's developmental process that describes a complex biological process of sexual development and the reactivation of the Hypothalamus-Pituitary-Gonadal (HPG) axis [1]. Studies have shown that variation in age and development of puberty are influenced by genetic factors and environmental factors, such as psychological stress [2]. Although the current study has not determined the incidence of stress in adolescents, US survey in 2013 found that teenagers experienced higher stress than adults during school years [3], and academic exams were considered to be the stressor that evoked anxiety [4].

The widely accepted approach to understand about stress is the process of interaction from resolution request from the environment (transactional model) [5]. Psychological stress itself is defined as a state of perceived threat to homeostasis [6]. Stress is almost considered as a symptom that occurs in adult individuals, but some studies in Brazil show a prevalence of stress in children of 30% to 60% with similar characteristics in adult individuals [7].

The response to stress involves the activity of the autonomic nervous system and the Hypothalamus-Pituitary-Androgen (HPA) axis. The HPG axis has some component structures and neural circuits against the HPA axis, and both of these axes work by maintaining a regulatory balance, in which the relationship is mutual [8] [9]. Acute or chronic stress,

either physically or psychologically, is known to cause HPA axis activation and HPG suppression [10] [11]. Several studies have found different results; it is thought that the mechanism involved is the role of glucocorticoid rather than suppressing but maintain luteinizing hormone secretion [11] [12].

Testosterone is a sex steroid hormone that can be measured in both men and women. From research, it is known that testosterone levels are influenced by obesity, drugs, ageing, physical stress, psychological stress, and actual stress (such as surgery or fasting) [4] [13] [14]. Testosterone levels during puberty depend on age and maturity stage [1] [9]. A study in Germany has found that testosterone levels are susceptible to acute stress and increased in both male and female during acute stress [12]. Measurement of testosterone levels in saliva reflects free testosterone serum levels, although at a condition in which there is a change in the amount of saliva in the mouth [1] [12]. The advantages of this method in pediatric patients are it is noninvasive, and it allows repeated examination with stable results. Examination of morning testosterone levels from 07.00 to 09.00 am recommended for stable results [15]. This study aims to determine whether there is any difference in salivary testosterone levels in puberty children after given stressor.

Methods

A quasi-experimental intervention study was conducted to see the difference in salivary testosterone levels among adolescents before and after given stressor at Antonius Bangun Mulia junior high school, Medan, North Sumatera, in July-October 2017. Samples were students aged 12-14 years with sexual maturation G2 in boys and M2 in girls. The exclusion criteria were students with the long-term use of steroids or glucocorticoids, history of chemotherapy or radiotherapy, orchitis, inflammation of oral cavity, and malnutrition. Samples were obtained by simple random sampling method. This study was approved by the Health Research Ethical Committee, Medical School, University of Sumatera Utara.

All students who fulfilled the inclusion criteria were enrolled in this study after given consent. We interviewed both parent and student to obtain demographic data. Student's stature was measured using microtoise. Sexual maturation was evaluated by trained personnel according to Tanner sexual stage. The testicular volume of male students determined by using orchidometer, and breast budding on female students through inspection and palpation. Saliva was collected at 08.00 am, previously students had been instructed not to eat, drink, or brush their teeth at least one hour. Students were asked to rinse their mouth with water, and then passive drooling method was used to collect approximately 1.5 ml of saliva.

Furthermore, students were asked to complete the questions from WISC IV which consist of mathematical and verbal questions within 15 minutes. The use of mathematical and verbal tests from WISC IV in children can lead to mild to moderate stress [10] [12]. After finished, saliva was collected in the same way as before. All saliva samples were stored in an ice box with temperature 2-8^{o}C until processed in the laboratory. Salivary testosterone examination was performed using enzyme-linked immunoassay kit DRG® Salivary testosterone (SLV-3013).

Data analysis was done with statistical software. Wilcoxon test was used to determine the difference in salivary testosterone levels before and after given stressor. Statistical calculation was done at 95% confidence interval and P-value <0.05 was considered significant.

Results

Of all 63 students, 50 students fulfilled the inclusion criteria. Five students were unwilling to take part in the study, one student was absent, and two students could not be sampled due to the oral lesion. Data collection and sampling were conducted on 42 students who were willing to participate and got approval from parents. Of 42 students aged 12-14 years consist of 24 male (57.1%) and 18 female (42.9%), we found that students median age were 12.8 (min-max 12-13.9) years. Table 1 shows the distribution and baseline characteristics of students, including weight, height, and stage of puberty.

Table 1: Baseline characteristics of students

Characteristics	Samples n=42
Gender	
Male, n(%)	24 (57.1%)
Female, n(%)	18 (42.9%)
Age (years), median (min-max)	12.8 (12-13.9)
Weight (kg),median (min-max)	39.4 (27.6-52.3)
Height (cm),median (min-max)	147.9 (130-156)
Stage of puberty	
Tanner II, n(%)	23(54.8%)
Tanner III, n(%)	19 (45.2%)

Figure 1 can be seen the boxplot of pre- and post-stress testosterone levels distribution. Median values for pre-stress testosterone levels were 5.79 pg/mL, whereas post-stress 11.40 pg/mL. Boxplots for both testosterone levels show that the data of testosterone value was not symmetrical. The value of the first quartile (Q1) for pre-stress testosterone was 2.90 pg/mL, and the third quartile (Q3) was 14.58 pg/mL with minimum value 1.9 pg/mL and maximum value 36.80 pg/mL. Some of the outlier values of pre-stress testosterone levels cause abnormal data distribution.

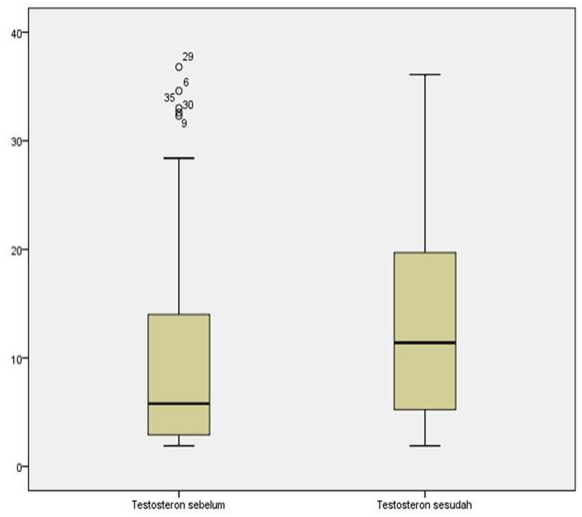

Figure 1: Boxplot of testosterone levels before and after stress

The Q1 value for post-stress testosterone was 5.22 pg/mL, and the Q3 was 19.83 pg/mL, with minimum value of 1.90 pg/mL and maximum value 36.10 pg/mL. The post-stress testosterone levels more evenly distributed, and there was no outlier value.

Table 2: Testosterone levels based on gender

	Testosterone levels (pg/mL) Median (min-max)		P*
	Pre-	Post-	
Gender			
Male	7.69 (1.90–36.80)	17.80 (1.90-36.10)	0.065
Female	5.77 (1.93–33.00)	8.55 (1.98-20.50)	0.112

*Wilcoxon test.

Normality test was done using Shapiro-Wilk test toward pre- and post-stress testosterone levels, and the result showed that data were not normally distributed. Bivariate analysis for pre- and post-stress testosterone was done with Wilcoxon test. From Table 2, testosterone levels were assessed based on gender. Pre-stress levels in male was 7.69 pg/mL (min-max 1.90-36.80), higher when compared with female 5.77 pg/mL (min-max 1.93-33.00). Post-stress levels in male (17.80 pg/mL, min-max 1.90-36.10) were also higher than female (8.55 pg/mL, min-max 1.98-20.50).

The higher the level of puberty, the higher the testosterone levels is. Table 3 shows pre-stress levels Tanner II of 5.40 pg/mL (min-max 1.90-34.60), lower than Tanner III of 6.80 pg/mL (min-max 1.95-36.80). Post-stress levels on Tanner II (10.90 pg/mL, min-max 1.90-36.10) were also lower than Tanner III (13.70 pg/mL, min-max 1.98-35.30).

Table 3: Testosterone levels based on puberty stage

	Testosterone levels (pg/mL) Median (min-max)		P*
	Pre-	Post-	
Stage of puberty			
Tanner II	5.40 (1.90 – 34.60)	10.90 (1.90 – 36.10)	0.045
Tanner III	6.80 (1.95 – 36.80)	13.70 (1.98 – 35.30)	0.198

*Wilcoxon test.

With the Wilcoxon test, we found that there was no statistically significant difference in pre- and post-stress levels in male (P = 0.065), as well as on female (P = 0.112). Maybe this was because the number of samples was too small for each gender and also was not normally distributed. The statistically significant difference in pre- and post-stress testosterone levels were found on subjects with sexual maturation Tanner II (P = 0.045), but not with puberty level Tanner III (P = 0.198).

Table 4: Testosterone levels difference pre- and post stress

n = 42	Median (min-max), pg/mL	P*
Testosterone levels pre-stress	5.78 (1.90-36.80)	0.015
Testosterone levels post-stress	11.40 (1.90-36.10)	

*Wilcoxon test

Discussion

Puberty can be viewed as a transitional period and is a very sensitive phase because of major changes in the neuroendocrine system. Development of puberty is also a time when the responses to the stressor will increase hormonal stimulation and reactivity, thus affecting emotions, behaviour and the process of sexual maturation [2] [3] [16] [17]. Research in America in 2013 found that hormones of the HPG axis are sensitive to stress stimuli in adolescence [16].

The onset of puberty is known to be affected by stress, where early life stress causes early puberty and prepubertal stress will result in late puberty [2] [18] [19]. In general, stress and reproduction have a negative relationship; under stressful conditions will inhibit reproductive function. However, existing studies have shown inconsistent results. Some studies in children and adults have found that in acute stress there will be an increase in testosterone levels that can be measured through saliva [4] [12] [16] [20]. Stress influence during puberty depends on whether the stress is acute or chronic, the degree of stress, and individual cognitive conditions [17].

Testosterone is a lipophilic molecule that can cross the blood barrier and salivary glands [21]. Level of morning testosterone is the highest level [15] [22], so it is often used as a basal testosterone index to get the picture of the maturation process [22]. A sampling of saliva by passive drool method gave better results than using salivettes [23] [24]. Examination of salivary testosterone with enzyme-linked immunosorbent assay (ELISA) method proved to have high sensitivity and specificity with stable results [15], but saliva samples were very sensitive to blood contamination [24]. Testosterone may increase or decrease depending on the subjective factors associated with the assessment of the situation so that the stress response is determined by prior experience and how the experience is interpreted [22].

Testosterone is thought to be a stress-sensitive hormone and is susceptible to acute stress [12] [16] [20] [21]. Testosterone also plays an important role in secondary sexual characteristics and reflects the maturation process [21]. Chronic stress will decrease testosterone levels because glucocorticoids can increase apoptosis of Leydig cells in male individuals thus suppressing steroidogenesis in the testes [20] [25]. But different mechanisms occur in acute stress conditions, where glucocorticoids precisely maintain LH and FSH secretion, resulting in increased testosterone levels [12] [19] [25]. Endogenous release of testosterone can be triggered by stressful challenges and increases in 15 minutes to 60 minutes [10] [12] [21]. The WISC IV test is an IQ test given to children aged 6-16 years. From other research, it is known that series of mathematics and verbal questions from the WISC IV test for 15 minutes has been able to stimulate the occurrence of mild to moderate stress [12].

This study found a significant difference between saliva testosterone levels before and after being given psychological stressor in puberty children; salivary testosterone was an increase in both male and female students. This finding consistent with the theory of the relation of hormonal adaptation response to stress conditions, that is testosterone secreted under LH regulation not only has a key role in sexual differentiation and function but also relates to aggression, competitive behavior, proactive responses, and triggers social status search or motivation of social dominance [20] [25] [26] [27]. Increased testosterone levels in adolescent individuals are associated with stress and competition that occurs strongly in both sex [21]. Research on the adolescent subjects in Iran in 2016 found increased saliva testosterone levels in psychological stress conditions. In the study, it was said that the increase in testosterone was not due to psychological stress alone, but also influenced by sex factors, personality traits, and emotional control [4]. Similar results were also found in Germany study in 2014 against 62 students aged 14-15 years, which showed an increase in salivary testosterone after given stressor, and followed, by increased fine motor skills [12].

In Table 2 it can be seen that there is no statistically significant difference based on gender, but it appears that testosterone levels before and after given stressor is higher in the male. This finding is similar to studies in Germany and Iran. In both studies, although the increase was not statistically significant, there was a higher increase in the male subject [4] [12]. A reasonable explanation is because testosterone is the main hormone in men and the association of testosterone with puberty are found more closely in boys [21].

Sex differences were seen to affect behavioural patterns associated with testosterone reactivity, which were consistent with previous studies of sex factor differences on androgen levels, HPA axis responses, and neurotransmitters [28][29]. Other studies have suggested that testosterone levels in men are associated with sensitivity to social status motivation [30].

Social relationships are thought to have contributed to changes in testosterone levels [25]. A study in Japan in 2015 found adult individuals with increased levels of testosterone in the early stages of stress depending on stable social relationships, but the difference was found mainly in women [20]. Although no significant direct association was found between stress factors and saliva testosterone levels, the study showed that in women with low testosterone levels there is social support from surrounding environment that can make the physiological response to stress decreased. Whereas in women with fewer social support will increase the stress response resulting in increased testosterone levels. Limitation of that study is the least number of male subjects [20].

Age is another factor that affects testosterone, and its relationship is inversely, but there is an exception during puberty. Increased testosterone levels found in higher maturation stage. Puberty and testosterone have a very close relationship, where increased testosterone will accelerate the maturation process [21]. Sexual maturation assessment with Tanner method, performed by a physician, can be used to evaluate pubic hair growth, breast, and genital development [9]. This study also found that testosterone levels were higher in the Tanner III maturation group (Table 3), by the literature that testosterone was higher as the maturation stage increased [17]. However, a statistically significant increase was found in the Tanner II maturation stage, which is estimated because in this study the number of subjects in the Tanner II maturation stage was more than subjects with Tanner III.

Higher levels of chronic stress during adolescence are associated with cognitive impairment and the development of future psychiatric disorders [17] [21] [31]. Increased testosterone levels are found to correlate with the emergence of family problems and negative emotions in male, but not in female children [16].

Some limitations of this study were the small number of subjects. The limited number of samples conducted in one school alone has made the results of this study couldn't be generalised, so more extensive research is needed involving more schools and larger sample quantities. Second, IQ assessment or subject achievement index was not performed, wherein these factors influence the individual stress response that may have an impact on changes in testosterone levels. This was a cross-sectional study design, so it only describes the stress response without assessing the previous stress and disturbance factors.

In conclusion, stress can affect testosterone levels as measured through saliva. This study can consider that in children with puberty disorders, we need to evaluate the stress factor as one of the causal factors.

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