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High Serum Cortisol Levels as a Potential Indicator for Changes in Well-Regulated Daily Life among Junior High School Students

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Problematic smartphone use among adolescents has become a social concern and is associated with poor sleep quality. The relationship between life habits, such as smartphone use and sleep duration, and levels of immunological and neuroendocrine biomarkers, including the stress hormone cortisol, in adolescents seems to be important to objectively comprehend their health and well-being in school life. However, such a relationship has not been well documented. We therefore studied rural junior high school students in Japan to elucidate the relationship between serum cortisol (SC) levels and their life habits. A total of 155 students in the seventh grade in 2016 were recruited as subjects. Of them, 140 students with eligible responses and blood samples (12-13 years; 80 boys, 60 girls) were finally included in the study (response rate 90.3%). A questionnaire survey concerning wake-up time, sleep duration, and the length of time using a smartphone per day was conducted. Blood samples were collected from peripheral veins of participants under fasting conditions between 8:30 and 11:00 a.m. The Spearman rank correlation coefficients were as follows: between SC and wake-up time, 0.199 ($p = 0.018$); between SC and sleep duration, 0.185 ($p = 0.029$); and between SC and time spent on smartphones, 0.172 ($p = 0.042$). The multiple regression analysis showed that high SC levels were significantly associated with late wake-up time and with short sleep duration. We therefore propose that measuring SC levels is useful for early detection of the change in the well-regulated daily life among junior high school students.

Keywords: adolescent health; cortisol; junior high school students; life habits; psychological medicine
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Introduction

Problematic smartphone use, that is associated with Internet addiction and mobile gaming addiction, among adolescents has become a social concern in Japan (Hashioka et al. 2019). Indeed, problematic smartphone use is associated with poor sleep quality (Xie et al. 2018). The relationship between life habits and levels of immunological and neuroendocrine biomarkers, including the stress hormone cortisol, in adolescents seems to be important to objectively comprehend their health and well-being in school life. Particularly, cortisol is believed to have a role in habit formation (Fournier et al. 2017). To our knowledge, there have been only a few studies (Chun et al. 2018; Hunter et al. 2018) that investigated the relationship between life habits, that are related to smartphone use and sleep, and cortisol levels in adolescents, even though the lifestyle and hormone levels are dramatically changed in growth and development during puberty (Benyi and Savendahl 2017). Because cortisol is a primary mediator between psychosocial stress and health (McEwen 2000), serum cortisol (SC) levels in junior high school students appear to reflect the extent of their health and well-being. As the members of the "Sukoyaka" school health committee, that is comprised of teachers, parents, community members, and the health professionals, within the Izumo civic Daiichi junior high school district in Izumo, Japan, we studied the students of the junior high school to elucidate the relationship between their life habits and serum levels of cortisol.

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Methods

Subjects

Subjects were seventh-grade students of the Izumo civic Daiichi junior high school in 2016. There was a total of 177 students in the seventh grade of the school in 2016. Fifteen students with long-absence (absent for more than 30 days per year) were excluded. Seven students disagreed to be enrolled in this study. Blood samples and answers to the lifestyle questionnaire from 140 students (summarized in Table 1) out of 155 students who agreed to participate in this study were available. We obtained informed consent from the students and their parents for participating in this study that was approved by the medical ethics committee of faculty of medicine, Shimane university on June 12th, 2012. The approved title was "Epidemiological studies for life habits and stress assessment of infants, primary school children and junior high school students in Izumo" with the approved number 1029.

The questionnaire used

We collected data on life habit using self-answered questionnaire. We conducted the survey in October, 2016 and gathered data on school year, sex, wake-up time, sleep duration, and the length of time using a smartphone per day. For the question concerning wake-up time "What time do you wake up?", the four options were 1) by 6:30, 2) 6:30-7:00, 3) 7:00-7:30, and 4) after 7:00. For the question concerning sleep duration "How much do you sleep?", the four options were 1) over 9 hours, 2) 8-9 hours, 3) 7-8 hours, and 4) less than 7 hours. For the question concerning the length of time using a smartphone per day "How long do you use a smartphone per day?", the seven options were 1) do not use, 2) less than 30 minutes, 3) 30 minutes-1 hour, 4) 1-2 hours, 5) 2-3 hours, 6) 3-4 hours, and 7) over 4 hours.

Measurement of serum cortisol

Blood samples were collected from peripheral veins of participants under fasting conditions between 8:30 and 11:00 a.m. at the school on September 16, 2016. Blood samples of the students who was absent on this day were collected at hospital A on October 14, 2016. The samples were centrifuged to obtain serum. The obtained serum samples were frozen at -20°C and were transported to SRL, Inc. (Tokyo, Japan) where cortisol levels of the thawed serum samples were measured by using radioimmunoassay. A normal range of serum cortisol in individuals aged 2 months to 13 years is 2.4-22.9 μg/dL (Jonetz-Mentzel and Wiedemann 1993).

Statistical analysis

The Spearman rank correlation coefficient analysis was performed to determine the relationships between the students' SC levels and their wake-up time, sleep duration, or the length of time using a smartphone per day. Furthermore, we conducted the multiple regression analysis via the forced entry procedure to examine these relationships. We also evaluated correlations among wake-up time, sleep duration, and the length of time using a smartphone per day using the Spearman rank correlation coefficient analysis. The analyses were performed by using the software SAS 9.4 (SAS Institute Japan, Tokyo, Japan). A p-value less than 0.05 was considered statistically significant.

Results

The mean value of SC levels in all the 140 students was 6.68 ± 2.62 μg/dL (means ± standard deviation) as shown in Table 1. Our findings are summarized in Table 2.

Seventy students (50%) woke up before 6:29 and showed SC of 6.36 ± 2.67 μg/dL, fifty-six students (40%) woke up at 6:30-6:59 and showed 6.63 ± 2.35 μg/dL, and fourteen students (10%) woke up after 7:00 with 8.52 ± 2.83 μg/dL. Six students (4%) slept for more than 9 hours with 4.46 ± 2.92 μg/dL, forty-three students (31%) slept for 8-9 hours with 6.41 ± 2.30 μg/dL, and ninety-one students (65%) slept for less than 8 hours with 6.96 ± 2.68 μg/dL. Seventy-six students (54%) did not use a smartphone and showed SC of 6.30 ± 2.27 μg/dL, fifty-six students (40%) used a smartphone for less than 1 hour with 7.03 ± 2.94 μg/dL, and eight students (6%) used a smartphone for more than 1 hour per day with 7.82 ± 3.04 μg/dL.

The Spearman rank correlation coefficients were as follows: between SC and wake-up time, 0.199 ($p = 0.018$); between SC and sleep duration, 0.185 ($p = 0.029$); and between SC and the length of time using a smartphone, 0.172 ($p = 0.042$). These results indicate that high SC levels are significantly correlated with relatively late wake-up time, short sleep duration, and long time using a smartphone. The standardized partial regression coefficients were as follows: between SC and wake-up time, 0.756 ($p = 0.021$); between SC and sleep duration, 0.907 ($p = 0.017$); and between SC and the length of time using a smartphone, 0.657 ($p = 0.066$). The adjusted R-squared was 0.081. These results indicate that SC levels are significantly associated with wake-up time and with sleep duration. There

Table 1. Characteristics of the seventh-grade students studied.

	n (%)	Age	Serum cortisol (μg/dL)			
			Mean	SD	Minimum	Maxim
Boys	80 (57.1)	12-13	6.63	2.53	2.6	12.9
Girls	60 (42.9)	12-13	6.75	2.76	2.21	17.5
All	140 (100)	12-13	6.68	2.62	2.21	17.5

SD, standard deviation.

Table 2. Relationship between serum cortisol levels and life habits.

Life habit	n (%)	Serum cortisol (means ± SD, μg/dL)	Spearman rank correlation coefficient	p value*	Standardized partial regression coefficient	p value#
wake-up time			0.199	0.018	0.756	0.021
< 6:30	70 (50)	6.36 ± 2.67				
6:30-7:00	56 (40)	6.63 ± 2.35				
> 7:00	14 (10)	8.52 ± 2.83				
sleep duration			0.185	0.029	0.907	0.017
> 9 hours	6 (4)	4.46 ± 2.92				
8-9 hours	43 (31)	6.41 ± 2.30				
< 8 hours	91 (65)	6.96 ± 2.68				
time using a smartphone			0.172	0.042	0.657	0.066
no usage	76 (54)	6.30 ± 2.27				
< 1 hour	56 (40)	7.03 ± 2.94				
> 1 hour	8 (6)	7.82 ± 3.04				

SD, standard deviation.
*p value for the Spearman rank correlation coefficient analysis.
#p value for the multiple regression analysis.

were no significant correlations between wake-up time and sleep duration (p = 0.345), between wake-up time and the length of time using a smartphone (p = 0.212), or between sleep duration and the length of time using a smartphone (p = 0.948).

Discussion

Our finding that sleep duration was negatively associated with cortisol levels is consistent with the results of the HELENA multicenter cross-sectional study, in which European adolescents from nine countries (12.5-17.5 years old; n = 933; 53.9% girls) participated (Perez de Heredia et al. 2014). The negative correlation between sleep duration and cortisol levels was also demonstrated in other cross-sectional studies of different-aged subjects, namely children aged 5-12 years (n = 327) (Fernandez-Mendoza et al. 2014) and adults in their middle 40s (n = 30) (D'Aurea et al. 2015). Therefore, such a negative correlation may not be age-specific.

It has been implied that prolonged stress is associated with increased cortisol levels (Izawa et al. 2012) and that comorbidity of depression and anxiety affects cortisol levels (Liao et al. 2006). Accordingly, increased SC levels may be a detective indicator for the disruption of well-regulated daily life, that is associated with stress-induced depression/anxiety, in middle school students. However, it should be noted as limitations that we could not strictly control wake-up time, a period from wake-up time to blood collection time, or sleep duration. In the case where we perform the multiple regression analysis, these three factors do not seem to be independent. In addition, blood sampling itself could be stress to students.

Our results suggest that high SC levels indicate the disruption of well-regulated daily life, that can be characterized by comparatively late wake-up time, short sleep duration, and long-time use of smartphone, among junior high school students. It is also suggested that students with short sleep duration and long-time use of smartphone could show the increased SC levels. Although all the SC values measured in this study can be considered as within the broad normal range of age-matched individuals, SC levels may be a sensitive state marker for deteriorative changes in daily life of middle school students. In other words, observable life habits correlated with SC levels may be an objective indicator for the stress of school life. Further studies are clearly warranted to establish this theory.

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Conflicts of Interest

The authors declare no conflict of interest.

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