Diurnal variation of salivary cortisol and amylase activity in fatigued state

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Abstract

Saliva has great potential as a bio-fluid for point-of-use measurement of the human stress response. The purpose of this study was to evaluate the diurnal variation of salivary cortisol (CORT) and salivary amylase activity (SAA) under the condition of a fatigued state. The subjects were 11 emergency medical doctors who worked for the Department of Emergency and Critical Care Medicine in 24 h-shifts, and their diurnal variations were measured three times in one month during the 24h-shift workday. As a control experiment, 6 subjects' diurnal variations were measured during a holiday period. The saliva samples were collected 4 times in total during an examination. The mean CORT of a workday showed its lowest value of 1.07 ± 0.55 ng/ml before sleep (24:00), and was highest at 4.76 ± 3.16 ng/ml at the start of a daytime duty (9:00) ($p < 0.01$). In contrast, the mean CORT of a holiday showed its lowest value of 1.48 ± 1.00 ng/ml in the afternoon and a highest value of 4.74 ± 2.76 ng/ml the next morning ($p < 0.05$). The mean SAA of a workday showed its lowest value of 59.3 ± 31.5 kU/l during a daytime duty (12:00 – 14:00) and was highest at 112.7 ± 90.1 kU/l at the start of night-time duty (18:00). These salivary biomarkers CORT and SAA on both workdays and holidays showed diurnal variation. In particular, the diurnal variation of CORT followed a well-defined 'bath-tub' shaped curve. A negative correlation of –0.36 was observed between the individual's CORT and SAA in workdays and of –0.62 in holidays, as determined by using the dynamic programming method. It was suggested that the fatigued state of humans is possibly reflected in the diurnal variations of CORT and SAA.

Keywords: diurnal variation, cortisol, amylase, fatigued state, dynamic programming method.

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1 Introduction

A variety of scale-questionnaires have been used as screening methods in the area of mental healthcare. However, some reports have mentioned that evaluations using scale-questionnaires can be subjective and there are not sufficient basic data on the reliability of scale-questionnaires. Thus an objective evaluation method is required for evaluation of various mental health factors for use in preventive medicine and intervention strategies [1, 2].

Corticotrophin-releasing hormone (CRH) is one of the most important mediators of the stress response, coordinating the adaptive behavioral and physiological changes that occur during stress [3]. A salivary biomarker has the advantage that it is non-invasive, which makes multiple sampling easy and does not introduce distress. Salivary cortisol (CORT) has advantages for analysis of stress in that (i) this hormone has a simple relationship with CRH; (ii) the concentrations of these hormones correlate significantly between plasma and saliva [4]. Levels of the enzyme salivary α-amylase activity (SAA) have been found to increase slightly with increased salivary flow rates and large increments in amylase concentration have been observed during sympathetic control by Speirs et al. [5]. Recipients of beta-blockers significant decrease their secretion of SAA; this provides direct evidence for the sensitivity of SAA to change in adrenergic activation [6]. Currently, it is considered that measurement of this SAA is a useful tool for evaluation of the sympathetic nervous- adrenalmedullary (SAM) system [7, 8]. A hand-held type SAA monitor was previously fabricated by the authors, which allows for noninvasive, easy and real-time analysis of this enzyme [9, 10].

Amanda [11] found that salivary the cortisol response to awakening in patients with Chronic Fatigue Syndrome was significantly less than that in healthy volunteers. Therefore the normal diurnal variation of salivary biomarkers will be influenced by an employee's chronic fatigue situation.

The objective of this research is to reveal the influence of chronic fatigue on the diurnal variation of salivary biomarkers, which reflect the human stress system. Emergency critical medical doctors were selected as subjects who usually work for many hours under conditions that induce a chronic fatigue state [12]. During a 24h-shift in an emergency and critical care ward, saliva samplings were periodically performed. Two kinds of salivary biomarkers were analysed in order to evaluate diurnal variations of CORT and SAA, and to examine the similarity between these two biomarkers.

2 Material and method

2.1 Subjects

A total of 11 emergency critical medical doctors (7 males and 4 females, 38.4 ± 8.2 years, mean ± SD) participated in this study (Table 1). The subjects worked for the Department of Emergency and Critical Care Medicine 65 hrs a week on average including a 24 h shift 4 times per month. None of the subjects had physical or mental diseases, or were taking corticosteroids or oral contraceptives.

The study protocol was approved by the Ethical Committee of Tokyo Metropolitan Hiroo General Hospital. The study protocol was fully explained to all of the subjects in both spoken and written forms. Signed informed consent was obtained from each subject who enrolled in the study.

Table 1: Characteristics of subjects who were enrolled in the study.

Condition	Workday (n = 11)	Holiday (n = 6)
Age (years, mean ± SD)	38.4 ± 8.2	40.0 ± 7.4
Male : Female	7 : 4	4 : 2
Carrier (years, mean ± SD)	13.1 ± 8.3	14.5 ± 8.0

2.2 Protocol

Diurnal variations during the workday (n = 11) were measured three times in a month for each subject. During the experiment, the subjects were not allowed to take any food or drinks except for water between meals. Moreover, the subjects took a sitting position for 30 min before the sample collecting. The saliva sample was collected 4 times in total: starting a daytime duty (9:00); starting a night-time duty (18:00); before sleeping (24:00); ending the night-time duty the next morning (8:45) (Fig.1). Extra saliva samples were collected during a daytime duty (12:00 – 14:00) and after the examination of critical patients during a night-time duty (0:00 – 8:45).

Diurnal variations during one day of holiday (n = 6) were measured as the control. The saliva sample was collected 4 times in total: morning (8:00); 2 h after lunch (14:00); before sleeping (24:00); the next morning (8:00) (Fig. 1).

All subjects recorded their activity by themselves (activity record) about actions, meals, rest and sleeping during the experiment.

2.3 Collection and analysis of salivary biomarker

In order to collect a saliva sample, a cotton roll was placed under the tongue for 3 min and then placed in a sampling cup. Then the saliva samples were centrifuged and the supernatant was stored at –20 °C.

CORT was analyzed using enzyme-linked immunosorbent assay kits (1-3002, Salimetrics LLC, PA) and a plate reader (450 nm measurement wavelength, ARVO MX, Perkin Elmer Inc., MA). The concentration was expressed as ng/ml.

SAA was analyzed using an enzymatic reagent (Espa AMY liquid II, Nipro Co., Japan) and a clinical automatic analyzer (Miracle Ace 919, Nipro Co., Japan). The saliva sample was diluted 1/100 with distilled water before addition to the substrate for the assay. The definition of one unit activity (U) per mass of enzyme is that this activity produces 1 μmol of the reducing sugar, maltose, in 1 minute.

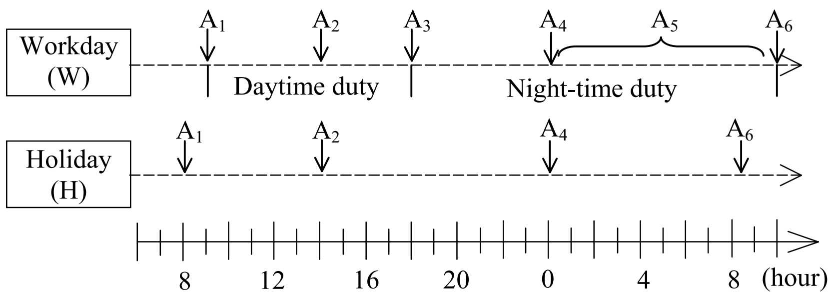

Figure 1: Evaluation protocol to determine the diurnal variation of salivary biomarkers of emergency critical medical doctors. A_1: starting daytime duty (W) or morning (H), A_2: during daytime duty (W) or 2h after lunch (H), A_3: starting night-time duty (W), A_4: before sleeping (W & H), A_5: during night-time duty (W), A_6: next morning (W & H).

2.4 Statistical analysis

Data were presented as mean values and standard deviation (mean ± SD). The paired *t*-test was used to assess the salivary parameters between workday and holiday, and to estimate differences in the salivary parameters with time. The dynamic programming method was used to estimate similarity with diurnal variations. Statistical significance was accepted at $p < 0.05$. Proprietary statistical software (Excel 2007, Microsoft Co., Japan) was used for the calculations.

3 Results

3.1 Diurnal variation of CORT

The mean diurnal variation of CORT is shown in Fig. 2. The mean CORT during a workday ($CORT_w$) showed a lowest value of 1.07 ± 0.55 ng/ml before sleeping and a highest value of 4.76 ± 3.16 ng/ml at the start of daytime duty. The mean $CORT_w$ of before sleeping was statistically lower than at the start of daytime duty, before ending night-time duty ($p < 0.01$), at the start a night-time duty and after the examination of critical patients during a night-time duty ($p < 0.05$). Also, the mean $CORT_w$ at the start of daytime duty was statistically higher than the time-point during daytime duty and ending the night-time duty ($p < 0.05$).

The mean CORT of holiday ($CORT_h$) showed a lowest value of 1.48 ± 1.00 ng/ml in the afternoon and a highest value of 4.74 ± 2.76 ng/ml at the next morning. The mean $CORT_h$ of before sleeping was statistically lower than next morning ($p < 0.05$).

Most of the diurnal variations of CORT followed a bath-tub shaped curve except in 2 cases. The similarity between the mean diurnal variation of $CORT_w$ and that of $CORT_h$ was 0.57 (Table 2). The similarity of an individual subject's diurnal variation of $CORT_w$ was 0.79 ± 0.38 and $CORT_h$ was 0.59 ± 0.51.

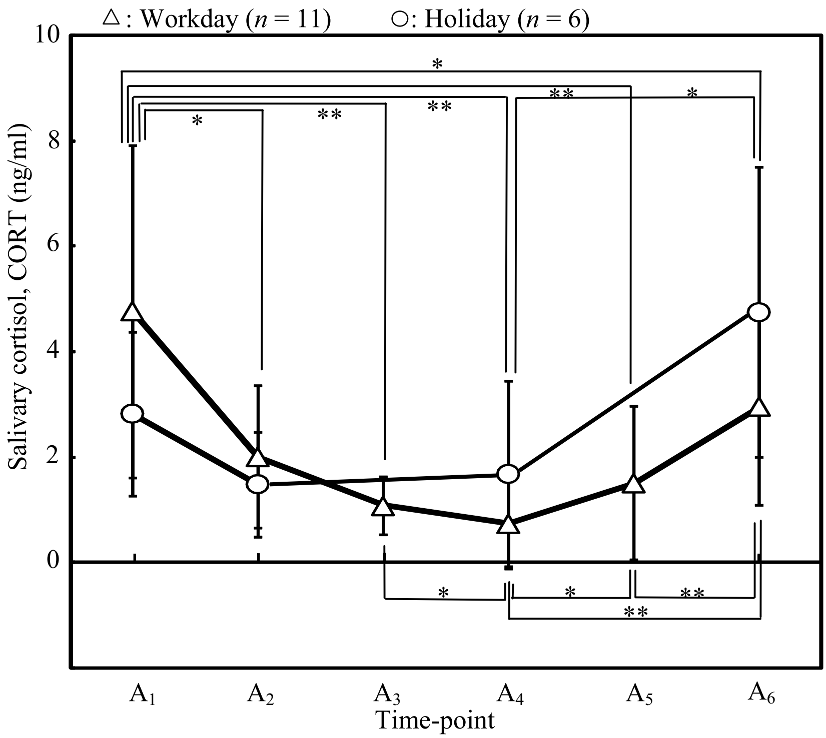

Figure 2: Mean diurnal variation of CORT on both workdays and holidays. All values were expressed as mean ± SD; *: $p < 0.05$, **: $p < 0.01$.

Table 2: Calculation results for the similarity of diurnal variations using dynamic programming matching.

Condition	Similarity
Workday vs. Holiday	
$CORT_w$ vs. $CORT_h$	0.57
SAA_w vs. SAA_h	0.94
CORT vs. SAA	
$CORT_w$ vs. SAA_w	-0.36
$CORT_h$ vs. SAA_h	-0.62

3.2 Diurnal variation of SAA

The mean diurnal variation of SAA is shown in Fig. 3. The mean SAA of workday (SAA_w) showed the lowest value of 59.3 ± 31.5 kU/l during daytime duty and the highest value of 112.7 ± 90.1 kU/l at the start of night-time duty. The mean SAA_w at the start of night-time duty was statistically higher than during a daytime duty and after the examination of critical patients during a night-time duty ($p < 0.05$). The mean SAA of holiday (SAA_h) showed a higher value than the mean SAA_w, but a significant difference was not observed.

The similarity between the mean diurnal variation of SAA_w and that of SAA_h was 0.94 (Table 2). The similarity of the individuals' diurnal variation of SAA_w was 0.30 ± 0.51 and SAA_h was 0.39 ± 0.54.

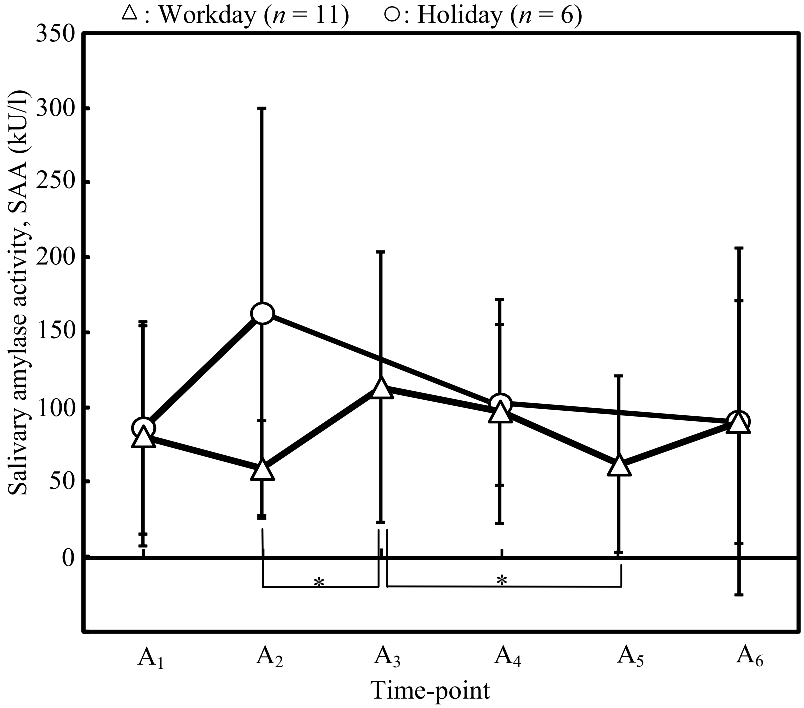

Figure 3: Mean diurnal variation of SAA on both workdays and holidays. All values were expressed as mean ± SD; *: $p < 0.05$.

3.3 Relationship between CORT and SAA

The diurnal variations in an individual's SAA showed more variability than that one of CORT. A significant difference was not observed between each time-point value of CORT and SAA ($R = 0.001$).

The similarity between mean $CORT_w$ and $CORT_h$ was 0.57, and was 0.94 for SAA_w and SAA_h (Table 2).

The mean similarity of individual $CORT_w$, $CORT_h$, SAA_w and SAA_h was 0.79, 0.59, 0.30 and 0.39, respectively (Fig. 4). Individual subject's $CORT_w$ showed higher similarity than $CORT_h$, a significant difference was not observed between the individual diurnal variation of $CORT_w$ and $CORT_h$ ($p = 0.39$).

Individual subject's SAA_h showed higher similarity than their SAA_w, but a significant difference was not observed between the diurnal variation of SAA_w and SAA_h ($p = 0.71$). The similarity between individual SAA_w and SAA_h showed a lower value than individual $CORT_w$ and $CORT_h$. A significant difference was observed between the individual diurnal variation of $CORT_w$ and SAA_w ($p < 0.01$).

The similarity between the individual's CORT and SAA in a workday ($CORT_w - SAA_w$) was –0.36, and for CORT- SAA in a holiday ($CORT_h - SAA_h$) was –0.62 (Fig. 5). The similarity of individual $CORT_h - SAA_h$ showed a higher negative value than the $CORT_w - SAA_w$.

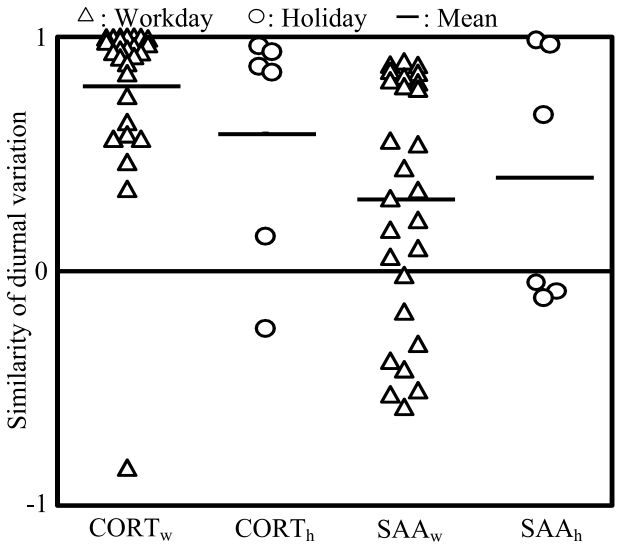

Figure 4: Similarity of diurnal variations between mean curve and individual curve. The horizontal black lines represent the mean value in each group.

4 Discussion

The objective of this research is to reveal the influence of chronic fatigue on the diurnal variations of salivary CORT and SAA. The concentration of CORT decreases from morning until before sleeping and increases until the next morning. This diurnal variation of CORT can be described as a bath-tub curve.

The $CORT_w$ level was lower than the $CORT_h$ not only before sleep (the lowest) but also the next morning. Based on the activity records of the subjects, it was assumed that there were no specific psychosomatic stressors on the holiday. As a result, differences in the diurnal variations of $CORT_h$ and $CORT_w$ could arise. In the night-time duty ($A_3 - A_6$), the diurnal variation (bath-tub) curve of CORT showed a lower level on the workday than during the same time on the holiday. The result indicated that the subjects were in a stressed state on the workday.

The diurnal variation of SAA showed a lower value in the morning and then increased during the daytime. This result well agreed with previous reports [13, 14]. Since SAA_w showed lower levels than SAA_h at all times, sympathetic nervous activity on the workday was lower than that on the holiday.

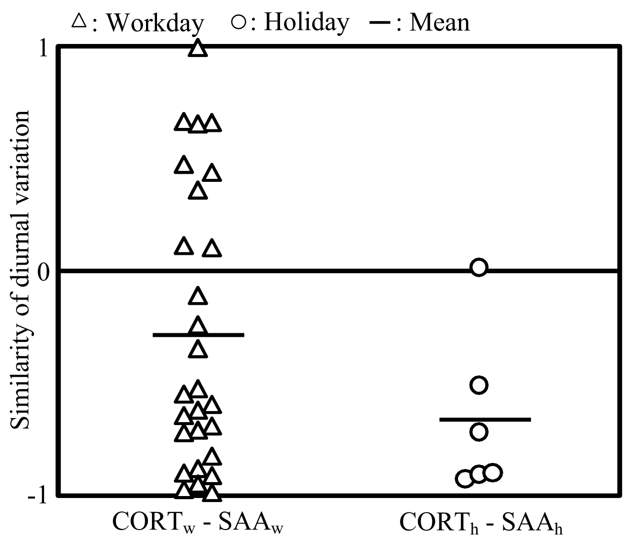

Figure 5: Similarity of individual diurnal variation between CORT and SAA. The horizontal black lines represent the mean value in each group.

It was considered that (i) $CORT_w$ showed lower values than $CORT_h$ not only before sleep but also in the next morning, (ii) based on the difference between that bath-tub curves of $CORT_w$ and $CORT_h$, the night-time duty can cause the subjects stress, and (iii) SAA indicated that sympathetic nervous activity on the workday was lower than that on the holiday. All of these results revealed that the night-time duty caused psychosomatic fatigue to the emergency critical medical doctors. These results revealed that the fatigued state of humans is possibly reflected in the diurnal variation of CORT and SAA.

In order to elucidate the regularity in the profiles with time-course changes, the similarity in the diurnal variations was calculated using the dynamic programming method. In the similarity of the mean diurnal variations on a workday and a holiday, SAA (0.94) exhibited a higher value than CORT (0.57). In comparison with the overall mean and individual values, CORT showed a higher value than SAA. In particular, the value on the workday exhibited a higher similarity of 0.79. These results suggested that the time profiles of CORT are common behaviours in humans.

Furthermore, a negative correlation was indicated between the diurnal variations of individual CORT and SAA under the same conditions. The negative similarity on the holiday was higher than the workday. It was considered that the psychosomatic fatigue by night labour caused a decrease in the concentration of CORT, whilst suppressing the secretion of SAA. Thus, levels of these biomarkers were shown to be influenced by workday stress conditions.

5 Conclusion

Emergency critical medical doctors were suitable as subjects for investigation of fatigue state because they had psychosomatic fatigue due to 24h-shifts and psychological fatigue due to managing critical patients. Our results revealed the diurnal variation of the salivary biomarkers CORT and SAA, both on workdays and holidays. In particular, the diurnal variation of CORT showed the bath-tub shaped curve. It was suggested that the fatigued state of humans is possibly reflected in the diurnal variations of salivary biomarkers such as CORT and SAA.

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