Does salivary alpha-amylase physiologically fluctuate in patients with Parkinson's disease?

Yui Mukaiuama[#1], Mariko Inoue[#1], Takao Mitsui[#2].

#1. Shikoku Nerve and Muscle Center, Tokushima National Hospital Organization, 1354 Shikiji, Kamojima, Yoshinogawa, Tokushima 776-0031, Japan

#2. Department of Neurology, Tokushima National Hospital, National Hospital Organization, 1354 Shikiji, Kamojima, Yoshinogawa, Tokushima 776-8585 Japan

Abstract

We investigated whether salivary alpha-amylase (sAA) shows diurnal and/or interday fluctuations in patients with Parkinson's disease (PD). The subjects included 50 patients with PD (28 males and 22 females). Salivary samples were collected from PD patients on a single weekday for five weeks at 8:30 am, 12:30 pm, and 6:30 pm. Subjective stress was assessed concurrently using Numerical Rating Scale (NRS). The diurnal fluctuations of sAA was significantly different between 8:30am and 12:30pm (p=0.002) and between 12:30pm and 6:30pm (p=0.009). On the other hand, NRS showed a significant difference between 8:30am and 6:30pm (p=0.003). sAA and NRS were strictly correlated in all time periods (p<0.05). The sAA levels and NRS scores for each time period during weeks 1-5 showed that interday fluctuations in sAA at 8:30am was different between weeks 1 and 4 (p=0.05) and between weeks 3 and 4 (p=0.007). There was a significant difference in the diurnal fluctuations of NRS at 8:30am in weeks 2 and 5 (p=0.04). Both diurnal and interday fluctuations were partially significant, but the dynamic range of sAA levels obtained in this study was narrow. At all times during the day, sAA and NRS were always well correlated, but NRS did not show the same diurnal and interday fluctuations as sAA. Further studies are needed to determine if these sAA fluctuations are physiological fluctuations in PD patients.

Introduction

Patients with Parkinson's disease (PD) suffer from a neurodegenerative disorder in which a variety of motor and non-motor symptoms worsen with progression. Since psychiatric symptoms such as depression and anxiety are common in PD [1], PD patients continue to be exposed to chronic stress. Various biomarkers have been used to assess the diagnosis of PD [2], but the assessment of psychiatric symptoms and chronic stress is mainly based on subjective indicators. However, since many PD patients develop dementia [3], it is difficult to assess them solely by subjective assessment. We focused on salivary alpha-amylase (sAA) as an objective indicator of mental stress in PD. Although sAA has been widely used in psychology and medicine as a surrogate marker of the sympathetic nervous system, its usefulness is controversial [4]. This is because it may be affected by other factors including age, gender, and diurnal fluctuations [5-7]. It is also not clear how sAA is involved in the pathogenesis of patients with neurological intractable diseases. Therefore, we planned to investigate the clinical significance of sAA in PD patients. In the present study, we followed sAA and subjective stress indices in PD patients and examined the diurnal and interday fluctuations.

Correspondence to: Yui Mukaiyama., Tokushima National Hospital, National Hospital Organization, 1354 Shikiji, Kamojima, Yoshinogawa, Tokushima 776-8585 Japan   Phone: +81-883-24-2161   Fax : +81-883-24-8661
e-mail: mukaiyama.yui.cr@mail.hosp.go.jp

Materials and Methods

The subjects were 50 PD patients (28 males and 22 females, mean age 71.1±7.1, Hoehn-Yahr 1-4) admitted to Tokushima National Hospital. We used the Salivary Amylase Monitor (Nipro Co., Japan). The saliva collection paper of the test strip is inserted into the oral cavity and saliva is collected directly from the lower part of the tongue over a period of 30 seconds [8]. The manufacturing guide indicated the following criteria: 0-30 KU/L is "unstressed"; 31-45 KU/L is "somewhat stressed"; 46-60 KU/L is "stressed"; and 61 KU/L and above is "quite stressed" (Nipro Co., Japan).

The present study employed the Numerical Rating Scale (NRS) as subjective stress assessments in PD. The scale ranges from 0 to 10; 0 representing no stress and 10 representing the worst possible stress [9].

Samples were collected from PD patients on a single weekday (Monday-Wednesday, for five weeks), and PD patients were asked to provide three saliva samples over the course of the day: 8:30 am, 12:30 pm, and 6:30 pm. Each sample was taken approximately one hour after eating. Along with the taking of sAA, the subjective stress level was assessed by NRS.

Corresponding one-way analysis of variance was performed to investigate the diurnal and interday fluctuations of sAA and NRS. Then, Spearman's rank correlation coefficient was calculated to examine the relationship between sAA and NRS for diurnal fluctuations.

Results

The sAA levels and NRS scores for PD at each time point are summarized in Table 1. The mean sAA of PD patients at 8:30 am was 53.4 (48.1-58.7) (mean (95%CI)), at 12:30 pm was 45.4 (41.0-49.7), and at 6:30 pm was 51.4 (46.9-55.9). The mean NRS of PD patients at 8:30 am was 4.7 (4.4-5.0), at 12:30 pm was 4.5 (4.3-4.8), and at 6:30 pm was 4.5 (4.0-4.6). One-way analysis of variance showed that the diurnal variation of sAA was significantly different between 8:30am and 12:30pm (difference in means) (9.7) (p=0.002) and between 12:30pm and 6:30pm (-7.7) (p=0.009). On the other hand, NRS showed a significant difference between 8:30am and 6:30pm (0.4) (p=0.003). Spearman's rank correlation coefficient showed that sAA and NRS were strictly correlated in all time periods (8:30am; r_s=0.30, p=0.000009, 12:30pm; r_s=0.18, p=0.01, 6:30pm; r_s=0.17, p=0.02) (Figure1-3).

Table 1. Descriptive data of salivary alpha-amylase (sAA) and Numerical rating scale (NRS) for each time period (8:30am, 12:30pm, 6:30pm) in PD

	8:30 am	12:30 pm	6:30 pm
N	228	231	230
sAA (mean) (95%CI)	53.4 (48.1-58.7)	45.4 (41.0-49.7)	51.4 (46.9-55.9)
p	0.002[#1]	0.009[#2]	
NRS (mean) (95%CI)	4.7 (4.4-5.0)	4.5 (4.3-4.8)	4.5 (4.0-4.6)
p		0.003[#3]	

#1 8:30am vs 12:30pm   #2 12:30pm vs 6:30pm   #3 8:30am vs 6:30pm
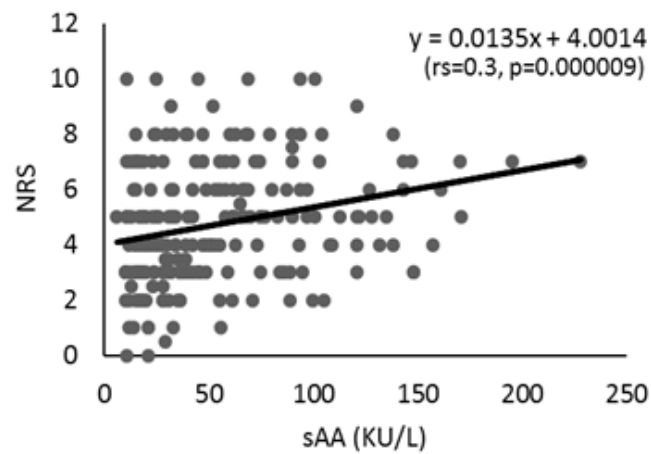
Figure 1. Correlation between sAA and NRS at 8:30am
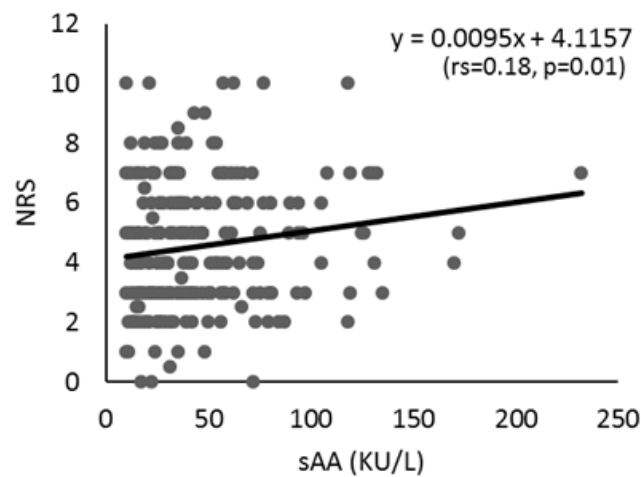   sAA at 8:30am was strictly correlated with the NRS (rs=0.3, p=0.000009).

Figure 2. Correlation between sAA and NRS at 12:30pm
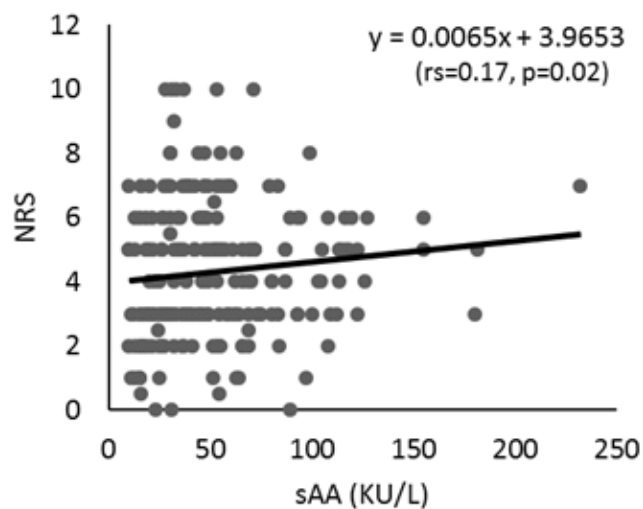   sAA at 12:30 pm was correlated with NRS (rs=0.18, p=0.01).

Figure 3. Correlation between sAA and NRS at 6:30pm
        sAA at 6:30 pm was correlated with NRS (rs=0.17, p=0.02).

The sAA levels and NRS scores for each time period during weeks 1-5 are shown in Table 2-4. One-way analysis of variance showed that interday fluctuations in sAA at 8:30am was different between weeks 1 and 4 (17.8) (p=0.05) and between weeks 3 and 4 (16.7) (p=0.007). There was no clear difference between 12:30 pm and 6:30 pm. There was a significant difference in the diurnal fluctuations of NRS at 8:30am in weeks 2 and 5 (0.8) (p=0.04). There were no differences at other time points.

Discussion

Neuroendocrine markers are known to play an important role in the reaction to stress. Stress responsiveness is primarily regulated by two neuroendocrine axes: the hypothalamic-pituitary-adrenocortical (HPA) and sympathetic adrenomedullary (SAM) systems [10-12]. A role for HPA axis activity in mediating stress responses has been intensively investigated for decades. The HPA axis is a complex neuroendocrine stress system involved in bio-behavioral adjustments to confrontational stimuli and change. Cortisol is an essential hormone in the regulation of stress responsiveness. Recently, salivary cortisol has been used as a simple, noninvasive index of free circulating cortisol levels. Salivary cortisol sampling has been used as a measure of HPA axis activity for quite some time [13]. Salivary cortisol levels increase several fold within a short time period after the onset of psychological stress [14] and physical stress including exercise [15] and cold pressor stress [16]. Salivary α-amylase (sAA), which is secreted by the parotid gland in response to adrenergic activity, has also become established as another biomarker of the psychosocial stress response within the SAM system [17]. Currently, cortisol and SAA are the most representative salivary stress markers. It is well known that cortisol is a hormone that exhibits diurnal variation. Cortisol is high upon waking, rises sharply in the first 30-40 minutes after waking, drops sharply a few hours later, and then declines slowly until bedtime [18-19]. On the other hand, not much is known about the diurnal fluctuations of amylase.

The relationship between PD and mental stress has been reported before, and it has been suggested that chronic stress exacerbates motor symptoms of PD [20]. Therefore, the development of mental stress markers in PD is considered an important issue. In this study, we investigated whether sAA shows diurnal and/or interday fluctuation in patients with PD.

Table 2. Descriptive data for sAA and NRS at 8:30am for weeks 1-5

	1st week	2nd week	3rd week	4th week	5th week
N	45	44	49	48	41
sAA **(mean)** **(95%CI)**	59.8 (46.3-73.4)	50.9 (38.5-63.2)	58.4 (46.1-70.8)	48.1 (36.8-59.5)	50.1 (41.6-58.7)
p		0.05[#1]		0.007[#2]	
NRS **(mean)** **(95%CI)**	5.1 (4.3-5.8)	5.0 (4.3-5.7)	4.4 (3.8-5.0)	4.8 (4.2-5.4)	4.4 (3.9-4.9)
p				0.04[#3]	

#1 1st week vs 4th week   #2 3rd week vs   4th week   #3 2nd week vs 5th week

Table 3. Descriptive data of sAA and NRS at 12:30 pm for weeks 1-5

	1st week	2nd week	3rd week	4th week	5th week
N	46	47	48	46	44
sAA **(mean)** **(95%CI)**	42.4 (32.3-52.5)	51.5 (42.2-60.8)	49.7 (38.8-60.6)	39.0 (31.2-46.8)	43.8 (33.8-53.7)
NRS **(mean)** **(95%CI)**	4.6 (3.9-5.3)	4.7 (4.1-5.2)	4.5 (3.9-5.2)	4.5 (3.9-5.1)	4.4 (3.8-5.0)

Table 4. Descriptive data of sAA and NRS at 6:30 pm for weeks 1-5

	1st week	2nd week	3rd week	4th week	5th week
N	47	46	47	49	41
sAA **(mean)** **(95%CI)**	52.8 (43.9-61.7)	56.6 (43.2-70.0)	56.6 (46.8-66.3)	44.4 (37.9-50.9)	46.4 (35.7-57.1)
NRS **(mean)** **(95%CI)**	4.4 (3.7-5.1)	4.2 (3.6-4.8)	4.6 (3.9-5.2)	4.2 (3.7-4.7)	4.1 (3.6-4.7)

The diurnal variation of sAA in PD was significantly decreased from 8:30am to 12:30pm and increased from 12:30pm to 6:30pm. It has been reported that sAA in normal subjects shows a characteristic diurnal profile, with a sharp decrease after waking and a stable increase during the day [5]. This result was different from previous studies. The interday variability of sAA was different only between weeks 1 and 4, and between weeks 3 and 4 at 8:30am. However, the dynamic range of sAA levels obtained in this study is narrow. At all times during the day, sAA and NRS were always well correlated. However, the NRS did not show similar diurnal and daily fluctuations to sAA. Further study is needed to determine whether these fluctuations in sAA are physiological fluctuations in PD patients.

References

1. Sveinbjornsdottir S. The clinical symptoms of Parkinson's disease. J Neurochem. 2016; 139 Suppl 1:318–324.
2. Lotankar S, Prabhavalkar KS, Bhatt LK. Biomarkers for Parkinson's Disease: Recent Advancement. Neurosci Bull. 2017;33(5):585–597.
3. Gratwicke J, Jahanshahi M, Foltynie T. Parkinson's disease dementia: a neural networks perspective. Brain. 2015;138(Pt 6):1454–76.
4. Rosel JF, Jara P, Machancoses FH, Pallarés J, et al. Intensive longitudinal modelling predicts diurnal activity of salivary alpha-amylase. PLoS One. 2019 Jan 23;14(1):e0209475.

5. Nater UM, Rohleder N, Schlotz W, Ehlert U, Kirschbaum C. Determinants of the diurnal course of salivary alpha-amylase. Psychoneuroendocrinology. 2007;32(4):392–401.

6. Strahler J, Berndt C, Kirschbaum C, Rohleder N. Aging diurnal rhythms and chronic stress: Distinct alteration of diurnal rhythmicity of salivary alpha-amylase and cortisol. Biol Psychol. 2010;84(2):248–56.

7. Obayashi K. Salivary mental stress proteins. Clin Chimica Acta. 2013;425:196 –201.

8. Nakano A, Yamaguchi M. Evaluation of Human Stress using Salivary Amylase. Japanese society of biofeedback research. 2011;38(1):3–9. (in Japanese)

9. Karvounides D, Simpson P, Davies WH, Khan KA, Weisman S, Hainsworth K. Three studies supporting the initial validation of the stress numerical rating scale-11 (Stress NRS-11): A single item measure of momentary stress for adolescents and adults. Pediatr Dimens. 2016;1:105–109.

10. Herman J, Cullinan W. Neurocircuitry of stress: control of the hypothlamo-pituitary-adrenocortical axis. Trends Neuroscience 1997;20: 78–84.

11. Isogawa K, Tsuru J, Tanaka Y, Ishitobi Y, Ando T, et al. Association between salivary amylase, cortisol and stress. Handbook of Neuropsychiatry Research. 2010;113–123.

12. Tasker JG, Herman JP. Mechanisms of rapid glucocorticoid feedback inhibition of the hypothalamic-pituitary-adrenal axis. Stress. 2011;14: 398–406.

13. Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology. 1994;19: 313–333.

14. Stahl F, Dorner G. Responses of salivary cortisol levels to stress-situations. Endokrinologie. 1982;80: 158–162.

15. Perroni F, Tessitore A, Gibelli G, Lupo E, D'Aribale E, et al. Effects of simulated firefighting on the responses of salivary cortisol, alpha-amylase and psychological variables. Ergonomics. 2009;52: 484–491.

16. van Stegeren AH, Wolf OT, Kindt M. Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. Int J Psychophysiol. 2008;69: 33–40.

17. Granger DA, Kivlighan KT, el-Sheikh M, Gordis EB, Stroud LR. Salivary alpha-amylase in biobehavioral research: recent developments and applications. Ann NY Acad Sci. 2007;1098: 122–144.

18. Pruessner JC, Wolf OT, Hellhammer DH, Buske-Kirschbaum A, von Auer K, Jobst S, et al. Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity. Life Sciences. 1997;61:2539–2549.

19. Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology. 2009;34:1423–1436.

20. Djamshidian A, Lees AJ. Can stress trigger Parkinson's disease? J Neurol Neurosurg Psychiatry. 2014;85(8):878–81.