

Physiological Stress Response Based on Salivary α -Amylase Activity and Secretory Immunoglobulin A Levels in Female Care Workers and General Workers

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ABSTRACT

Secretory immunoglobulin A (s-IgA)/total protein ratio and α -amylase are components of saliva used as indices of physiological stress; the levels of these components fluctuate with stress.

This study aimed to compare the stress levels in female care workers with general workers based on two indices in the saliva.

The study included 22 (50.2 ± 11.4 years) female care workers with 20 female general workers served as a control group (53.9 ± 8.6 years).

Saliva samples were collected eight times a day from each participant: after waking up, after breakfast, before lunch, after lunch, at 15:00 h, before dinner, after dinner and at bedtime.

The s-IgA/total protein ratio and α -amylase activity were examined by two-way analysis of variance (ANOVA) (care workers \times general workers) with pairing only one way (the collection time).

By two-way ANOVA, s-IgA/total protein decreased in the afternoon and slightly increased at night in care workers and gradually increased from the morning to the night in general workers, showing comparatively lower values in care workers than in general workers. However, the result of ANOVA showed no statistically significant difference for the interaction or the main factors between the groups.

The α -amylase activity in both groups of workers increased throughout the day from waking up to afternoon and then decreased at bedtime. However, statistically insignificant difference was shown for the interaction of both factors (care workers \times general workers and the collection time).

There was insignificant difference in stress based on physiological stress indicators (s-IgA and α -amylase activity) during their days off work between female care workers and female general workers. This could be owing to the fact that the care workers in our study were in their 50s, had developed skills to deal with stress through their life and working experience, and did not experience work stress on their days off.

Keywords

Female care workers, Female General Workers, Stress, Salivary α -Amylase, Secretory Immunoglobulin A.

Introduction

In the 21st century, Japanese society is undergoing a drastic change caused by a low birth-rate on one hand and longevity on the other. In short, Japan is experiencing a rapid increase in its

population aged >65 years combined with a rapid decrease in juvenile population. Hence, the Health and Welfare Services for the Persons with Disabilities based on the Long-term Care Insurance Act [1] and the Services and Support for Persons with Disabilities Act [2] have been established in Japan; additionally, the need for care services is increasing.

However, in spite of the need for their services, care workers have a high turnover and low retention rate [3]. It has been frequently reported that care workers are frustrated with certain aspects of their work such as environment, conditions, and content [4,5].

Few studies have been performed on the mental stress of physical care workers in Japan. In an attempt to objectively analyse care workers' physical and mental work burden, Wakui [6] investigated the daily physical activity, energy consumption, number of steps, heart rate and metabolic rate, and reported that the physical and mental burden depends on the job type. Particularly the care receiver's state of mind and family relationships during home visits may affect the care worker's mental tensions; hence, they often have greater mental burden than physical one.

Kawahara et al. [7] developed a remote system that can measure the elderly person's respiratory and heart rate without the need for any physical restraint or body contact and confirm their wellbeing automatically. This system enables monitoring of vital signs during night time. With increased use of this system, the workload related to night shift is expected to decrease.

Hayashi [8] reported that irregular labour circumstances, such as working hours of care workers, cause major mental stress and form an important factor in their high job separation rate.

Kawano et al. [9] established the following: regardless of differences in sex, age, the care environment (home care or home visits) and the employment form (full-time or part-time), many care workers experience occupational stress. In addition, stress levels tend to be higher in care workers with longer work experience than in those with shorter experience, and in certified care workers than in home visiting care workers for persons with severe disabilities.

Furthermore, Kawano et al. [10] examined the four stressors (physical, mental, wages and human relationships) in care workers and how they are related to sex, generation, years of experience, care environment, employment form and care qualification. They found that higher levels of stress are experienced by care workers in their 30s-50s than those in their 60s. Workers in institutional services experience more physical stressors than those providing home visit services, and workers in their 30s and 40s experience more mental stressors than those in other age groups.

As described above, studies on the stress levels of care workers in Japan have mainly addressed psychological stress using questionnaires for assessing fatigue or stress. Conversely, the physiological stress of care workers has been primarily evaluated based on the physical burden during work such as energy

consumption, number of steps and heart rate. A few studies have quantitatively evaluated care workers' physiological stress using blood, urine or bioactive substances (biomarkers) in saliva as indicators.

There are at least two systems regulating physiological stress responses: the limbic-hypothalamic-pituitary-adrenal axis and the sympathetic-adrenal-medullary axis [11,12]. Components of saliva and blood have been used as indicators for evaluation [13,14].

Different from blood, collection of saliva to investigate physiological stress can be performed non-invasively, without causing pain or burden to the subjects and by non-medical people; hence, many studies have been performed using saliva [15-20].

The stress-related substances in saliva include cortisol, dehydroepiandrosterone (DHEA), testosterone, chromogranin A (CgA), α -amylase and secretory immunoglobulin A (s-IgA), and consist of mainly two types: substances secreted in response to acute stress and those secreted in response to chronic stress. It is generally known that cortisol, DHEA and CgA [13] levels change in response to acute stress.

Recently, α -amylase and s-IgA have been used as indicators of chronic stress [21,22]. Rohleder et al. [23] reported that α -amylase activity reflects psycho-physiological stress better than cortisol.

Salivary s-IgA is considered to be affected by psycho-social factors [24,25] and by physical as well as mental health conditions [26].

It is assumed that care workers, due to their irregular work content and working hours [9,27], have higher stress levels and greater circadian rhythm fluctuation than general workers. In addition, they are considered to have more chronic stress. Hence, we assumed that care workers have physiological stress even on their days off.

This study aimed to compare the stress levels in female care workers and general workers based on s-IgA/total protein ratio and α -amylase activity in their saliva.

Methods

Participants

Participants included 22 female care workers (hereafter referred to as care workers). They all had a higher qualification than the 'Home Helper grade 2'.

Twenty female general workers were served as a control group (hereafter referred to as general workers). Their occupations were office workers, the occupation of practising medicine, university workers, restaurant workers, and full-time house wives. The mean age of women in the care worker group was 50.2 ± 11.4 years (range 29–72) and that in the control group was 53.9 ± 8.6 years (range 32–65) (Table 1 and 2).

We obtained informed consent from all participants after explaining the purpose and protocol of this study in detail. This study was approved by the Ethics Committee on Human Experimentation of the Faculty of Human Science, Kanazawa University (approval No. 2012-19).

Saliva Collection and Analysis Methods

Saliva samples for s-IgA and α -amylase activity analysis were collected eight times a day from each participant: after waking up, after breakfast, before lunch, after lunch, at 15:00 h, before dinner, after dinner and at bedtime. These samples were collected between December 2016 and January 2017 according to the methods described by Nakata et al. [28] Saliva is usually collected by placing a cotton ball in the mouth of the subject and asking them to chew for several minutes.

However, we collected their saliva directly into a sterile 50-ml centrifuge tube up to a level of approximately 1 ml. We collected the saliva on holidays. All saliva samples were immediately frozen. Prior to the measurement of s-IgA and α -amylase, saliva samples were defrosted, transferred to 1.5-ml tubes and centrifuged.

In addition, considering the difference in the daily routine on a day off for participants, instead of analysing the saliva collected eight times a day, we considered it adequate to compare only the three periods, i.e. morning (awaking, after breakfast or before lunch), afternoon (after lunch, at 15:00 or before dinner) and night (after dinner or bedtime).

We also recorded the wake-up time, bedtime and sleep duration because it has been reported [27-29] that the s-IgA ratio and α -amylase activity are affected by sleeping time.

Statistical Analysis

Physical characteristics, wake-up time, bedtime and sleeping duration in each group were examined using the unpaired t-test. S-IgA/total protein ratio and α -amylase activity were examined by two-way analysis of variance (ANOVA) (care workers \times general workers) with pairing only one way (the collection time). If a statistically significant difference was found in the interaction or main effect, multiple comparisons were made using Tukey's honestly significant difference test. Effect size (ES, η^2) was calculated to examine the size of mean values. The level of significance was set at $p < 0.05$.

Results

Physical characteristics (age, height, weight and BMI), wake-up time, bedtime and sleeping duration for both groups are summarised in tables 1 and 2. The means of age, height, body weight and BMI in care workers were 50.2 years, 159.0 cm, 57.3 kg and 22.7, respectively. The means of their wake-up time, bedtime and sleeping duration were 7:17, 23:42 and 7 h 35 min, respectively.

The means of age, height, body weight and BMI of general workers were 53.9 years, 156.5 cm, 53.5 kg and 21.9, respectively. The

means of their wake-up time, bedtime and sleeping duration were 6:50, 23:44 and 7 h 6 min, respectively. No statistically significant differences were found between the physical characteristics, wake-up time, bedtime or sleeping duration of care workers and general workers.

	Care Workers(n=22)				General Workers(n=20)				t	p	d
	M	SD	MAX	MIN	M	SD	MAX	MIN			
Age	50.2	11.4	72.0	29.0	53.9	8.6	65.0	32.0	1.181	0.245	0.36
Height	159.0	6.3	170.0	150.0	156.5	4.7	166.0	147.0	1.447	0.156	0.45
Weight	57.3	9.1	75.0	43.0	53.5	7.2	70.0	38.5	1.469	0.150	0.45
BMI	22.7	3.4	30.0	17.7	21.9	2.8	27.7	16.4	0.837	0.408	0.26

M: average value (median) SD: standard deviation s: straight Max: maximum value Min: minimum value
t: statistic t p: appearance probability d: effect size
*p<0.05

Table 1: Demographic and physical data of care workers and general workers (age, height, weight and BMI).

	Care Workers(n=22)				General workers(n=20)				t	p	d
	M	SD	MAX	MIN	M	SD	MAX	MIN			
awakening time	7:17	56min	9:00	5:30	6:50	48min	9:30	6:00	1.717	0.094	0.544
bedtime	23:42	76min	1:30	21:30	23:44	72min	1:30	22:00	0.053	0.958	0.017
sleeping duration	7:35	69min	10	5.5	7:06	69min	9.5	5	1.354	0.184	0.429

M,SD,MAX,MIN is the same as table 1. *p<0.05

Table 2: Wake-up time, bedtime and sleeping duration of care workers and general workers.

Table 3 show basic statistics and the result of ANOVA for the s-IgA/total protein ratio and α -amylase levels of care workers and general workers. As mentioned before, we calculated the basic statistics of the three classifications according to saliva collection timing, which were morning (awakening, after breakfast and before lunch), afternoon (after lunch, at 15:00 h and before dinner) and night (after dinner and before bedtime), considering the difference of their routine on holidays.

s-IgA/total protein increased gradually in general workers; however, it decreased at afternoon and slightly increased at night in care workers, thus showing comparatively lower in care workers than in general workers (Table 3). However, the result of ANOVA showed no statistically significant difference for the interaction or the main factors. It was reported that the peak secretory activity time of α -amylase is between 16:00 and 17:00 [29]. Our study results show a tendency towards low activity levels in the morning, which increases in the afternoon and again lowers at night time in both groups, (Table 3), but result of ANOVA showed no statistically significant difference for the interaction or the factors themselves.

	Care Workers(n=22)			General Workers(n=20)			total average	F	p	η^2	post-hoc	
	morning	afternoon	night	morning	afternoon	night						
s-IgA/protein	M	18.6	9.9	12.1	15.1	17.9	20.0	15.6	F1	0.43	0.52	0.01
	SD	22.4	11.6	18.9	15.8	33.0	45.2	24.5	F2	0.29	0.75	0.01
	MAX	52.1	52.8	89.2	50.2	141.5	168.2		IN	1.12	0.30	0.03
	MIN	0.3	0.9	0.5	1.7	1.3	0.0					
α -amylase activity (unit/ml)	M	50.6	66.2	54.7	48.4	58.4	47.1	54.2	F1	0.27	0.61	0.01
	SD	27.6	37.5	33.6	47.7	60.7	52.6	43.3	F2	2.80	0.07	0.06
	MAX	117.4	133.8	116.3	157.9	187.5	199.1		IN	0.15	0.86	0.00
	MIN	4.6	36.4	16.0	-1.6	-2.2	-2.1					

M,SD,MAX,MIN is the same as table 1. F: student F-test p: appearance probability η^2 : quantity of effect of the analysis of variance
*p<0.05

Table 3: Basic statistics of care workers and general workers and the results of two-way analysis of variance of s-IgA/total protein ratio and α -amylase activity.

Discussion

Care workers tend to have irregular working hours [9,27] due to night shifts and overtime, which are typical in the care work environment. Consequently, they have a comparably irregular life style, and it is difficult for them to ensure sufficient sleeping time. In addition, they tend to suffer from both the physical and mental burden resulting from their heavy work load in delivering service to care receivers along with the mental burden of the maintaining the relationship with their superiors, co-workers and the care receivers.

It is considered that particularly female care workers suffer from severe stress on a daily basis because of women-specific life circumstances and managing the balance between work and family life. The resulting stress level sustains even on their days off. Therefore, in this study, we hypothesised that care workers have more stress on their days off than general workers, as demonstrated by the changes of s-IgA/total protein and α -amylase activity in the saliva.

Although there are various kinds of substances indicating a physiological stress response, the s-IgA and α -amylase activity in the saliva have been used in many studies as useful indicators of physiological stress because they can be easily collected and impose little burden on subjects [13,15-17,23,29]. The s-IgA in saliva is an indicator of the level of activity of the sympathetic nervous system, parasympathetic nervous system and immune system. It plays an essential role in body defence and local immune system function. It has been reported that s-IgA levels decrease with both psychological and physical stress [30,31].

Generally, as stress levels increase, s-IgA levels decrease, whereas as stress levels decrease, s-IgA levels increase [12,15]. It has been reported that, during normal daily life, s-IgA levels are high when waking up and decrease throughout the day, with no apparent sex differences [32-34]. In addition, the level of the s-IgA/protein ratio is also reflected by the sleeping time [30,35,36].

Furthermore, people who experience more stress in interpersonal relationships have lower s-IgA levels [37]. Particularly in women, it has been reported that IgA secretion decreases when they experience negative psychological and societal factors, and vice versa [38]. In this study, care workers and general workers showed insignificant differences in their average age, physical characteristics, wake-up time, bedtime and sleeping duration.

Hence, it is assumed that both groups had similar ages, physical characteristics and sleep cycles. They were all women in their 50s; most of them had mastered the common life challenges for female workers such as marriage, pregnancy and maintaining a balance between work and family life. They had established routines in their daily life such as regular wake-up and sleeping times.

In this study, we collected saliva from care workers and general workers during their days off to examine circadian rhythms. Considering the personal differences in wake-up time, mealtimes,

and sleeping duration, the basic statistics were calculated in the three categories of morning, afternoon and night.

Although the results show that the s-IgA/total protein ratio in care workers tends to be lower in the afternoon and at night than that in general workers, insignificant interaction or main effects were confirmed.

There are many reports on the psychological stress of care workers on workdays [28,39,40]; however, reports focusing on physical stress on their days off are very limited. Matsuura et al. [15] examined the circadian changes in s-IgA/total protein ratio and α -amylase activity on days off in wheelchair-dependent individuals with congenital physical disabilities and in persons without disability on days off. Our study employed the same saliva collection and analysis method as described by them.

Although the s-IgA/total protein ratio in our study is higher than that observed in Matsuura et al. [15] dealing healthy population, the circadian rhythm shows a similar pattern.

Participants of our study were in 50s. According to Takao et al. [41], care workers aged >50 years tend to have gained social skills through the rich working experience in care centres, those include dealing with problems with customers (care receivers), workers of other occupations and co-workers, in addition to coping with psychological stress.

According to a research report of the Ministry of Health, Labour and Welfare [42], in the general population, mental stress levels are high among both men and women in their 30s and 40s.

Kawano et al. [9] found that care workers in their 30s and 40s have higher mental stress levels than those in their 60s; those in their 50s have the same stress level as those under 40s or in their 60s, implying that the mental stress of younger care workers' is related to difficulties in balancing work and family life as well as in the relationships with their superiors, co-workers and care receivers.

As Takao et al. [41] suggested, well-experienced care workers aged >50 years may have developed specific social skills for decreasing their mental stress and may not experience work stress on their days off compared with care workers aged <40 years. Furthermore, it is considered that care workers aged >50 years have mostly done with parenting, know how to release stress and do not perform activities that stimulate the sympathetic nervous system on work-off days.

Conversely, salivary α -amylase activity is an indicator of stress caused by sympathetic nerve activity, and is reflected by both physical and mental stress [29,43]. In addition, salivary α -amylase is one of the digestive enzymes and is therefore affected by meal times [44,45]; its levels are low in the morning until before noon, increase over time to reach a peak in the afternoon and decrease at bedtime.

In both groups of workers in our study, α -amylase activity increased throughout the day from waking up to afternoon before it lowered at bedtime, showing a circadian rhythm.

Karibe et al. [46] examined daily and inter-day variations of salivary α -amylase activity hourly (seven times/day) from 10:00 to 16:00 on workdays in six healthy adult men and women. They reported no significant difference between the mean values of α -amylase activity.

Ueda et al. [47] measured the α -amylase activity of care workers at a nursing facility and suggested that there is no clear reference value for α -amylase activity. The values differ from individual to individual and are affected by the individual's specific environment.

The α -amylase activity and s-IgA/total protein ratio in our study showed insignificant interaction or main effect (group and period factors). On days off work, when care workers were free from work stress, they did not feel physical or mental stress that stimulates the sympathetic nerve, and may have been in a normal state similar to what Karibe et al. described [46].

Our study subjects were care workers with an average age of 50 years. These care workers have developed high social skills through their life and working experience at care centres. As a result, it is considered that they have also developed the ability to adequately deal with physical and mental stress, smoothly solve various problems with care receivers (customers) and care centres, and do not experience work stress on their days off. The result of ANOVA showed no statistically significant difference for the interaction or both s-IgA/ total protein and α -amylase factors.

As a further step, stress on their days off should be studied in care workers in their 20s to 40s.

Conclusion

There was insignificant difference in stress based on physiological stress indicators (s-IgA and α -amylase activity) during their days off work between female care workers and general workers. This could be because the care workers in our study were in their 50s, had developed skills to deal with stress through their life and working experience, and did not experience work stress on their days off.

In future, similar studies with care workers in their 40s are required.

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