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Brief Report

Potential objective biomarkers for fatigue among working women

Chie Ebata[1,2], Hitomi Tatsuta[1] and Masayuki Tatemichi[3]

[1]Wakayama-Rosai Hospital, [2]Ebata Occupational Health Research Institute and [3]Department of Preventive Medicine, Tokai University, School of Medicine

Abstract: Objective: The prediction of health impairment due to work overload is subjectively assessed based on recognized symptoms; however, objective evaluation is primarily ideal in the field of occupational health. Recently, some biomarkers of autonomic function and/or oxidative stress were reported to be associated with fatigue. This study aimed to preliminarily investigate whether these biomarkers could be objective indicators for fatigue and stress among working women. **Method:** Participants included 118 full-time female workers (mean age 37.8 years), including 55 shift workers. Self-administered questionnaires, such as visual analog scale (VAS) for general health, a lifestyle questionnaire, SF-8 for health-related quality of life, and K6 for mental health screening, were used. In addition, biomarkers such as acceleration plethysmogram (APG), reactive oxygen metabolites-derived compounds (d-ROMs), and biological antioxidant potential (BAP) were measured. **Results:** A significant association was observed between BAP and VAS (r=0.482, p<0.01) among shift workers. However, other biomarkers such as APG and d-ROMs were not significantly associated with symptoms. d-ROMs were significantly correlated with age and body mass index. There was a significant negative correlation between BAP and smoking. Results of the APG (low-frequency (LF)/high-frequency (HF) ratio) were significantly correlated with BAP, but not with d-ROMs. The LF/HF ratio and BAP for shift workers were significantly higher than those for day-time workers. **Conclusions:** Our results suggest that APG and BAP are potential objective biomarkers for fatigue among working women, although further follow-up studies are needed to clarify the scope of usefulness of the biomarkers for fatigue.
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Correspondence to: M. Tatemichi, M.D., Ph.D., Department of Preventive Medicine, Tokai University, School of Medicine, 143 Shimokasuya, Isehara, Kanagawa 259-1193, JAPAN (e-mail: tatemichi@tokai-u.jp)

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Introduction

In Japan, the law "promotion for activity of women on work life" (Woman Activity Promotion Law) was established in November 2015[1)]. Japanese women are influenced by various external environmental factors such as housework and child care, in addition to social labor. Furthermore, internal environmental factors, such as dramatic changes in sex hormones, could lead to alterations in their physical and mental states. Thus, work-related fatigue and stress among working women is complicated due to the added work related to housekeeping[2)].

As a current counterplan against "Karoshi," fatigue is subjectively estimated using an "occupational workplace stress simple questionnaire." However, fatigue is considered to be related not only to recognized symptoms but also to unrecognized physical reactions. Fatigue is a unique discomfort and a decline in the state of physical activity for ability with the desire to rest because of excessive physical and mental activities or illness. Although "fatigue" and the "sensation of fatigue" are considered to be the same, the conception of "fatigue" is distinguished from the "sensation of fatigue." "Fatigue" means a decrease in physical ability for activity that occurs due to an overload to mind and body, and "sensation of fatigue" is the sense of noticing that fatigue exists, with discomfort and a decrease in activity being recognized. Therefore, objective biomarkers are required for the precise evaluation of fatigue.

Recent studies on fatigue have reported that autonomic nerve balance and oxidative stress makers in serum can potentially estimate mental and physical fatigue[3,4)]. Guidelines for fatigue evaluation have recently been published by the Japanese Society of Fatigue Science. These guidelines established methods for objectively evaluating fatigue as well as to measure biomarkers, medications, or services for combating fatigue[5)].

More recently, Fukuda et al.[6] reported that oxidative stress and anti-oxidative activity were potential biomarkers for fatigue in patients with chronic fatigue syndrome. Oxidative stress and anti-oxidative activity are associated with a risk of atherosclerosis or coronary heart disease[7,8]. Thus, we hypothesized that biomarkers of autonomic function and oxidative stress are potential objective indicators for fatigue among working women. This study preliminarily investigated the association between these biomarkers and results of the self-administered questionnaires for "sensation of fatigue." Furthermore, we examined the differences between shift workers and daytime workers as shift workers are considered to have greater exposure to mental and/or physical stress than office or daytime workers.

Methods

This study was approved by the ethics review board of the Japan Organization of Occupational Health and Society. All participants provided written informed consent.

Study participants consisted of 118 full-time-working women, including 54 public employees, 40 nurses, and 24 office workers. Mean age ± standard deviation was 37.8± 11.0 years. Of the 118 women, 55 were shift workers. Of the 55 local government prison officers who attended a workshop, 54 were accepted to participate in this study by informed consent. Of the 40 nurses who worked in a hospital, all were accepted. Of the 29 office workers in an organization for occupational health and safety, 24 were accepted.

Self-administered questionnaires including a visual analog scale (VAS) for general health: "sensation of fatigue," life-style questionnaire, SF-8 for health-related quality of life[9], and K6 for screening of mental health[10] were carried out two days prior to blood work. Shift workers completed the questionnaires before the start of the day shift.

Beat-to-beat variation in each subject was measured and analyzed using an acceleration plethysmography (APG) system (ARTETD; Umedica, Osaka, Japan)[11]. The APG test was performed before the start of the day shift. APG waves were recorded from one finger while subjects were in the sitting position and fully clothed. An APG wave is the second differential wave of a pulse wave, which stabilizes the baseline of the waves and allows for an accurate measurement of rate intervals (consecutive a-wave intervals). To assess autonomic function according to frequency domains, [low-frequency (LF) and high-frequency (HF) power and LF/HF ratios] were determined[11]. For the analyses of frequency domains, consecutive a-a intervals were analyzed using the maximum entropy method, where the power spectra at 0.02-0.15 and 0.15-0.5 Hz corresponded to LF and HF, respectively. The resolution of the spectrum was set at 0.001 Hz. From the power spectra, LF and HF components were presented as ms^2 [11].

Blood was collected from the shift workers at rest before the start of the day shift. Among non-shift workers, blood was collected on a holiday or after work and at rest. The serum level of d-ROMs and biological antioxidant potential (BAP) were evaluated[6]. Oxidation and anti-oxidation activities were measured simultaneously in the serum. Oxidative and anti-oxidative activities were assessed by measuring d-ROMs and BAP, respectively, using FREE Carrio Duo (Diacron International, Italy).

Statistical Analysis

First, the distribution of d-ROMs, BAP, and the LF/HF ratio was determined using a histogram. LF/HF distribution was considered abnormal, and a $\log_{10}$ conversion was performed. To find confounding factors among variables, the correlation was determined using Spearman's or Pearson's method. The significance of difference between the two groups was determined using the Mann-Whitney U test or Student's t-test. The significance of difference among three groups was determined using one-way analysis of variance and Bonferroni's method. Multiple adjustments were performed using analysis of covariance. A P value of <0.05 was considered statistically significant.

Results

Participant characteristics are shown in Table 1. Body mass index (BMI) was 21.9±3.5 kg/m^2. The means of d-ROMs, BAP, and the LF/HF ratio were 349 CARR U, 2206 μmol/*l*, and 1.89, respectively.

Correlations between the LF/HF ratio, d-ROMs, and BAP and background characteristics and lifestyles are examined (see supplementary Table). d-ROMs were significantly correlated with age (r=0.311, p<0.01), BMI (r= 0.201, p<0.05), and drinking habit (r=0.253, p<0.01), but not with other lifestyle factors. BAP showed significant negative correlation with smoking (r=-0.240, p<0.01). The means of BAPs of non-smokers, ex-smokers, and current smokers were 2265.9±329.0, 2271.5±346.5, and 1985.4±323.5 μmol/*l*, respectively, showing significantly low values in current smokers (p=0.005). However, BAP was not associated with the Brinckman index (r=0.073, p =0.781).

BAP showed a significant association with VAS (r= 0.482, p<0.01) and the $\log_{10}$ LF/HF ratio (r=0.395, p< 0.01) among shift workers. The $\log_{10}$ LF/HF ratio was significantly negatively associated with exercise habit (r=-0.200, p<0.05), and positively associated with commuting-time (r=0.186, p<0.05) (see supplement Table).

The results of a comparison between shift and daytime workers are shown in Table 2. Although there were no

Table 1. Characteristics of Participants

number		118
Age, year (mean±SD)		37.8±11.0
BMI kg/m2 (mean±SD)		21.9±3.5
Occupation	Official	54
	Hospital nurse	40
	Office	24
Commuting time (min) (mean±SD)		36.3±36.7
Sleep time		6.0±0.61
Style of Work	day-worker	63
	shift-worker	55
Smoking habit	current smoker	19 (16.1%)
	Ex-smoker	11 (9.3%)
	Never-smoker	88 (74.6%)
Drinking habit	>0 days/week	56 (47.5%)
	1-2 days/week	37 (31.4%)
	3-5 days>week	12 (10.2%)
	every day	13 (11.0%)
Exercise	0 time /Week	72 (61.0%)
	1 times/Week	24 (20.3 %)
	2-3 times/Week	17 (14.4 %)
	>=4 times/Week	5 (4.2%)
Present history of illness	non	87 (73.7%)

Supplement Table. Association between biomarkers and background factors or symptoms.

	Total (n=118)			Day-time workers (n=63)			Shift workers (n=55)		
	Log10LF/HF	d-ROM	BAP	Log10LF/HF	d-ROM	BAP	Log10LF/HF	d-ROM	BAP
Age	.130	.311**	.117	–.026	.416**	.070	.351**	.208	.265
BMI	–.149	.201*	–.123	–.154	.155	–.060	–.144	.252	–.207
exercise	–.200*	–.086	–.123	–.233	.011	–.031	–.138	–.172	–.112
smoking	–.022	.033	–.240**	–.075	–.060	–.273*	.064	.140	–.204
drinking	–.015	.253**	–.147	.038	.238	.112	.008	.225	–.215
sleep time	.085	–.014	–.009	.089	.100	–.032	.097	–.127	.080
Commuting time	.186*	.101	.132	.206	.037	.221	.199	.147	.047
VDT time	.163	.082	.018	.255*	.118	.271*	.201	–.151	–.068
VAS	.095	.038	.303**	.063	.138	.048	.078	–.045	.482**
K6	–.031	–.145	.067	–.157	–.192	.201	.009	–.103	–.082
PCS	.039	–.068	–.050	.132	–.025	–.051	–.053	–.105	–.056
MCS	–.027	.181*	–.122	.013	.219	–.119	–.022	.139	–.068
Log10LF/HF	-	.165	.372**	-	.164	.246	-	.201	.395**
d-ROM	.165	-	.087	.164	-	.074	.201	-	.148
BAP	.372**	.087	-	.246	.074	-	.395**	.148	-

Date represent correlation coefficient by Pearson for Age, BMI, VAS, K6, PCS, and MCS
Date represent correlation coefficient by Spearman for smoking, drinking, sleep time, communicate time and VDT time
VAS: Visual analog scale
PCS: Physical component summary
MCS: Mental component summary
*p<0.05
**p<0.01

Table 2. Comparison between day-time worker and shift worker

		Day-time worker n=63	Shift worker n=55	*p*
Age, year	(mean±SD)	39.2±10.7	36.3±11.1	0.152
BMI kg/m2	(mean±SD)	21.9±3.6	22.0±3.4	0.883
Commuting time (min)	(mean±SD)	40.8±45.1	30.6±22.9	0.134
Sleep time		6.1±1.0	5.9±0.9	0.261
Smoking habit				
	Never-smoker	46	42	
	EX-smoker	5	6	
	current smoker	12	7	0.591
Drinking habit				
	>0 days/week	24	32	
	1-2 days/week	21	16	
	3-5 days>week	10	2	
	every day	8	5	0.062
Exercise	0 time /Week	35	37	0.316
	1 times/Week	16	8	
	2-3 times/Week	10	7	
	>=4 times/Week	2	3	0.318
VDT time (hr.)		5.9 ± 3.6	3.8 ± 2.0	<0.001
Sensation of Fatigue				
VAS	(mean±SD)	61.7±19.1	66.4±17.7	0.17
PCS	(mean±SD)	46.8±7.2	46.8±7.9	0.985
MCS	(mean±SD)	45.6±8.2	45.6±9.1	0.235
K6	(mean±SD)	11.2±4.2	12.7±6.0	0.131
Biomarkers for fatigue				
Log LF/HF		0.003±0.42	0.173±0.42	0.032
d-ROM		341.5±44.9	335.4±54.8	0.504
BAP		2102.2±260.1	2357±390.4	<0.0001
d-ROM/BAP		0.165±0.03	0.146±0.032	0.001

VAS: Visual analog scale for general health
PCS: Physical component summary calculated by SF-8
MCS: Mental component summary calculated by SF-8

significant differences in background factors, such as age, BMI, communication time, duration of sleep, and drinking/smoking, the BAP and $\log_{10}$ LF/HF ratio were significantly higher in shift workers than in daytime workers (Table 2). BAP was more widely distributed in shift workers than daytime workers. In addition, BAP and $\log_{10}$ LF/HF ratio were adjusted for age, BMI, communication time, duration of sleep, habit of drinking/smoking, and VDT time. After adjustment, the results also show significant difference between daytime and shift workers in BAP (2088.1 (standard error=38.2) vs. 2348.8 (42.4), p< 0.0001) and $\log_{10}$ LF/HF ratio (-0.034 (0.054) vs. 0.201 (0.059), p=0.005).

Discussion

This study shows that "fatigue" and the "sensation of fatigue" could be not correlated with each other. When high values of biomarkers for the physical fatigue state are continuous, they may cause an increased risk in the onset of cardiovascular disease[7,8]. When symptoms of the sensation of fatigue are high, the worker can take action to notify their supervisor and/or occupational health staff about their condition, who will then take countermeasures to reduce the risk. However, fatigue is currently assessed based on recognized symptoms alone. Thus, when symptoms are absent and biomarkers are high, the worker would be at high risk in the future because no action to reduce the risk would be taken by the supervisor and/or occupational health staff. Therefore, measuring these biomarkers might be helpful to reduce the risk of health impairment caused by work overload.

Literature on d-ROMs and BAP reports that confounding factors include sex differences, age, obesity, smoking, drinking, excessive exercise, ultraviolet rays, pregnancy, VDT time, and corpus luteum hormone[12,13]. However, in the present study, d-ROMs and BAP were not found to be consistently associated with these factors. According to correlation analysis, the same differences were seen between shift workers and daytime workers. Correlations between oxidative biomarkers and lifestyles may depend on the study subjects. As our study population consisted of healthy full-time workers, the appropriate adaptation for oxidative stress could be concurred. If d-ROMs are chronically high, BAP would be induced, resulting in the lowering of d-ROMs by neutralization.

In the present study, BAP was negatively associated with smoking, consistent with the finding of a previous report[14]. This result indicates that smoking is a strong inhibitory factor against oxidative stress among women. A decrease in BAP is frequently observed in patients with chronic fatigue syndrome[6], whereas an increase in BAP is considered to be the result of adaptation for oxidative stress. When oxidative stress is continued by chronic fatigue and the capacity of neutralization is over, BAP will be decreased by consumption for neutralization. Therefore, BAP may depend on the balance between oxidative stress and neutralization capacity.

In our study, BAP was found to be higher in shift workers than in daytime workers. Shift workers have disturbed circadian rhythms and imbalances in the level of melatonin, the hormone involved in the regulation of sleep, resulting in increased risk of health impairment[15]. In this study, the individual value of BAP was widely distributed among shift workers compared with daytime workers. The effects of working in shifts seem to vary based on individuals. Some shift workers showed a high value of BAP, suggesting that BAP may be induced by shift work.

LF/HF ratio was also higher in shift workers than in daytime workers. Literature on APG reports that confounding factors include strain, obesity, arrhythmia, sleep time, and exercise habits[11]. A high LF/HF ratio indicates that the sympathetic nerve is predominant in autonomic function, suggesting the possibility of a future health impairment[11]. The LF/HF ratio was negatively associated with exercise, and positively associated with commuting-time, which are particularly reasonable results. Thus, the LF/HF ratio may be indicative of fatigue and stress. In this study, the LF/HF ratio was associated with BAP. The association between and mechanisms of the autonomic nervous system and oxidative stress, however, are largely unknown. Further studies are required to better understand this relationship.

In this study, we examined the efficacy of indicators: oxidative stress and APG for women. Thus, the effects of the menstrual cycle were examined. According to a result of our preliminary investigation, information on the days from the first day of menstruation was obtained by an interview, and divided into four periods; the follicle period (menstruation 0-13 days), luteal phase (menstruation 14^{th}~), menopause, and other days. We then examined the associations between menstrual cycles and LF/HF ratio, d-ROMs, and BAP. Consequently, no associations were seen (Ebata's unpublished data).

There are several limitations to this study. First, it is a small-scale study, with a small sample size obtained from different workplaces. Second, it is a cross-sectional study. Third, uncontrolled confounding factors may be present. In addition, blood sampling time was not controlled. Thus, further controlled, prospective studies with consideration of the circadian rhythm are needed.

In this study, there were workers who had fewer recognized symptoms and higher values of d-ROMs and LF/HF ratio. In the future, these workers may have health impairments. Thus, future prospective studies should be conducted. These studies are particularly important because the current counterplan against work overload is based on self-reported questions. Objective biomarkers to estimate fatigue and stress should be established in the field of occupational health as soon as possible. We believe that the LF/HF ratio, d-ROMs and BAP are potential candidates for this purpose.

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