Projects related to the 13 fields of occupational injuries and illnesses
Field: “Mental health of workers”

“Objective Indicators for Depression and Fatigue in Workers”

Research Report

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Table of Contents

Introduction

Mental health disorders are initiated by psychological stresses, including changes in industrial structure and deteriorating economic conditions. Many workplaces have difficulty dealing with workers with adjustment disorder caused by subjective stress, exhaustion due to overwork, and poor performance and development of depression due to chronic exhaustion. In addition, approximately 30,000 people commit suicide annually and most of these suicides are caused by psychiatric disorders and anxiety related to health problems. These problems have gradually increased, mainly in young workers.

Professional medical organizations offer core resources outside the workplace (Guidelines for Mental Health) and play a major role in providing opinions and advice to both workers and workplaces for (1) prevention of health problems and early detection of diseases, (2) diagnosis and treatment, and (3) judgment of eligibility and support for job searching and return to work (balance between treatment and work). Our project on occupational injuries and illnesses uses medical findings to establish standards for labor health that are disseminated to workplaces. This project has been underway for 10 years.

Mental health problems and disorders require approaches from biological, psychological, and social perspectives. First, a study entitled "Development of objective indicators of depression using cerebral blood flow (CBF) measured by ^{99m}Tc-ECD SPECT" (2004-2008) was started to examine biological issues. In this study, imaging analysis of CBF was performed in a longitudinal study of frontal lobe hypoperfusion and normalization of frontal cerebral perfusion associated with remission of workers with depression. The results suggested a tendency for a decrease in CBF in the dorsal frontal lobes that was correlated with severity of fatigue and serious insomnia, and a possible relationship of chronic insomnia with fatigue and depression. The second study (2009-2013) focused on these findings and examined correlations of hypothalamo-pituitary-adrenal (HPA) axis endocrine activities, which may be related to dysfunction in the frontal lobes and limbic system, with depression, fatigue, QOL, and CBF changes (Study 1: Depressive state, fatigue, sleep and HPA-related endocrine kinetics in workers; Study 2: Depressive state, fatigue, QOL and HPA-related endocrine kinetics in workers (cortisol, DHEA, DHEA-S in saliva)). These studies detected hormone activities that were significantly correlated with hypofrontality and possibly related to depression, fatigue, insomnia, and interview items related to QOL and sleep.

This report describes the two studies summarized above. Based on these results, further work is planned to develop questionnaires for (1) prevention and early detection of mental health problems, (2) diagnosis and treatment, and (3) support for job searching and return to work (balance between treatment and work).

Chief Investigator: Fumihiko Koyama

Study 1: Depressive state, fatigue, sleep and HPA-related endocrine kinetics in workers

I. Objective and Summary

Strong correlations of depression, stress, and fatigue with variation in HPA axis hormones, especially cortisol, are well known, but associations between depression and fatigue scores and the corticosteroids dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEA-S) are unclear. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) enables simultaneous determination of small amounts of hormones in saliva, which can be collected noninvasively, as an innovative endocrine test. In this study, cortisol, DHEA and DHEA-S levels in saliva were determined using LC-MS/MS to examine associations with depression, fatigue, quality of sleep and HPA-related endocrine functions in workers. The study was approved by the ethical review board certified by the Japan Labour Health and Welfare Organization (December 2009).

II. Subjects and Methods

1. Subjects

The subjects were 96 day-shift workers (56 males, mean age 42.79 ± 10.72 years; 40 females, mean age 42.98 ± 11.24 years) who provided written informed consent after receiving oral and written explanations of the purpose of the study. The absence of fever, trauma, cancer, pregnancy, psychotropic drugs and corticosteroids was confirmed in an interview with each subject.

2. Methods

(1) Depression, fatigue, and quality of sleep were assessed using the following scales.

(i) SIGH-D (Structured Interview Guide for the Hamilton-Depression Rating Scale): A structured interview was used to assess 17 items of the Hamilton Depression Rating Scale to rate severity of depression based on the total score. Quality of sleep was evaluated using items related to sleep disorder (insomnia score: IS).

(ii) SDS (Self-rating Depression Scale): Subjective severity of depression was evaluated using the self-rating scale.

(iii) Self-assessment checklist for chronic fatigue in workers: I. Subjective symptoms, II. Work situation, and III. Total burden on workers were self-assessed.

(iv) Brief Job Stress Questionnaire: Nine items on fatigue and depression were assessed based on the frequency of self-reported fatigue, depressed mood, poor concentration, and grief over the past month. Scores were divided into 4 grades.

(2) Saliva was collected after obtaining written informed consent based on ethical considerations

• Amount of saliva: 2 mL/collection

• Storage: ≤ -20°C

• Collection time: 5 time points: 9 a.m., 12 a.m., 3 p.m., 6 p.m., and 9 p.m.

(3) Determination of cortisol, DHEA, and DHEA-S levels in saliva by LC-MS/MS

An aliquot of the test compound as a deuterated internal standard was added to 1 mL of saliva and the

mixture was extracted with organic solvent, separated using an ionic cartridge column, and purified. Cortisol and DHEA fractions were converted to picolinic acid derivatives, and each fraction was purified again using the cartridge column. The resulting purified products were simultaneously analyzed by LC-MS/MS. DHEA-S fractions were immediately determined by LC-MS/MS.

(4) Data presentation and statistical analysis

(i) Measurements and data analysis for hormone levels were performed as follows.

- Lower limit of quantitation (LLOQ): Cortisol (10 pg/assay), DHEA (4 pg/assay), DHEA-S (10 pg/assay)
- Below the lower limit of quantification (BLQ) was defined as 50% of LLOQ.
- Cortisol, DHEA, and DHEA-S levels in saliva are expressed as pg/mL (significant figures: 3 digits)

(ii) Correlations of hormone levels with depression, fatigue, and quality of sleep.

Multiple regression analysis was performed using scores on the 5 scales (including the IS) used to assess depression, fatigue, and quality of sleep as dependent variables and age, gender, hormone levels, and hormone ratios (10 items) as explanatory variables. JMP® 10 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis, with a significance level of less than 5%.

III. Results

1. Correlations of gender and age with hormone levels in saliva

Cortisol, DHEA, and DHEA-S levels at 9 a.m. and DHEA and DHEA-S levels at 9 p.m. were significantly higher in males, whereas cortisol/DHEA-S ratios at 9 a.m. and 9 p.m. were significantly greater in females (Table 1).

Table 1. Gender differences in hormone levels in saliva

	male (n = 56)		female (n = 40)		p
F 9 a.m.(pg/mL)	1176.3	2.0	1111.0	1.9	0.024[a]
F9 p.m. (pg/mL)	308.3	2.7	286.8	2.5	0.675[a]
D9 a.m. (pg/mL)	60.0	1.8	44.2	1.7	0.012[b]
D 9 p.m. (pg/mL)	45.0	1.8	33.8	1.8	0.021[a]
D-S 9 a.m.(ng/mL)	2.7	2.2	1.2	2.5	<0.001[b]
D-S 9 p.m. (ng/mL)	2.4	2.2	1.1	2.8	<0.001[b]
F/D9 a.m.	19.6	2.3	25.1	2.1	0.336[a]
F/D 9 p.m.	6.8	2.4	8.5	2.4	0.275[a]
F/DS9 a.m.	434.8	2.7	890.5	3.3	0.002[a]
F/DS 9 p.m.	131.0	3.4	271.4	3.9	0.014[b]

F: cortisol, D: DHEA, D-S: DHEA-S, a: willcoxon's test, b: t-test

DHEA levels at 9 a.m. and 9 p.m. were negative 56 rrelated with age (regardless of gender), DHEA-S levels tended to have a negative correlation with age, and cortisol levels had no correlation with age. The cortisol/DHEA ratio at 9 a.m. was positively correlated with age in males (Table 2).

Table 2. Correlations of hormone levels in saliva with age by gender

	male (n=56)		female(n=40)	
	r	p	r	p
F 9 a.m. (pg/mL)	-0.05	0.73	-0.01	0.95
F 9 p.m. (pg/mL)	-0.06	0.66	-0.16	0.32
D 9 a.m. (pg/mL)	-0.36	0.01	-0.36	0.02
D 9 p.m. (pg/mL)	-0.29	0.03	-0.39	0.01
D-S 9 a.m. (ng/mL)	-0.19	0.16	-0.28	0.08
D-S 9 p.m. (ng/mL)	-0.10	0.47	-0.26	0.10
F/D 9 a.m.	0.29	0.03	0.31	0.05
F/D 9 p.m.	0.11	0.40	0.09	0.59
F/DS 9 a.m.	0.13	0.36	0.19	0.23
F/DS 9 p.m.	0.05	0.72	0.12	0.47

r: pearson's correlation coefficient

2. Work situation and hormone levels in saliva

Based on answers to questions about work situation in the self-assessment checklist for chronic fatigue in workers, total scores (0-15) were calculated for (i) overtime work/month, (ii) irregular work hours, (iii) burden of business trips, (iv) burden of midnight shift, (v) appropriate rest/short sleep, (vi) emotional strain, and (vii) physical strain. Correlations of these scores with hormone levels in saliva were then examined. In the female subjects, there were significant negative correlations with the cortisol level and cortisol/DHEA ratio at 9 a.m. and a tendency for a negative correlation with the cortisol/DHEA-S ratio at 9 a.m. There were no significant correlations in male subjects (Table 3).

Table 3. Correlations of work situation with hormone levels in saliva

	male (n=56)		female (n=40)	
	r	p	r	p
F 9 a.m.	0.12	0.39	-0.37	0.02
F 9 p.m.	0.00	0.97	0.04	0.80
D 9 a.m.	0.08	0.55	0.24	0.13
D 9 p.m.	0.07	0.61	0.25	0.12
D-S 9 a.m.	0.07	0.63	0.09	0.57
D-S 9 p.m.	-0.18	0.17	0.28	0.09
F/D 9 a.m.	0.01	0.96	-0.55	0.00
F/D 9 p.m.	-0.05	0.69	-0.19	0.23
F/DS 9 a.m.	0.01	0.96	-0.30	0.06
F/DS 9 p.m.	0.09	0.52	-0.23	0.16

r: spearman's rank correlation coefficient

3. Association of depression, fatigue, and quality of sleep with hormones (multiple regression analysis)

(1) Work situation not included in the explanatory variables (adjusted for gender and age)

DHEA-S levels at 9 p.m. were significantly associated with severity of depression (SIGH-D) and quality of sleep (insomnia score, IS), with standardized partial regression coefficients (β) of 0.257 and 0.261, respectively (Tables 4 and 5). DHEA-S levels at 9 p.m. were significantly associated with quality of sleep (IS), with an adjusted R^2 of 0.051. There was no significant association of hormone levels in saliva with subjective depression (SDS) (Table 6). Total work burden (chronic fatigue) was significantly related to the cortisol/DHEA ratio at 9 a.m., with β of -0.216 (Tables 7 and 8).

Table 4. Association of SIGH-D scores with hormone levels in saliva (n=96)

	β	p	R^2	p
F 9 a.m.	0.074	0.477	-0.005	0.469
F 9 p.m.	0.074	0.473	-0.005	0.467
D 9 a.m.	0.213	0.057	0.029	0.128
D 9 p.m.	0.110	0.321	0.001	0.388
D-S 9 a.m.	0.120	0.297	0.002	0.373
D-S 9 p.m.	0.257	0.023	0.045	0.064
F/D 9 a.m.	-0.079	0.465	-0.004	0.464
F/D 9 p.m.	0.017	0.875	-0.010	0.562
F/DS 9a.m.	-0.039	0.721	-0.009	0.541
F/DS 9p.m.	-0.108	0.314	0.001	0.384

*adjusted for gender and age, β: standardized partial regression coefficient, R^2: adjusted R square

Table 5. Association of insomnia score with hormone levels in saliva (n = 96)

	β	p	R^2	p
F 9 a.m.	-0.062	0.549	-0.002	0.421
F 9 p.m.	0.016	0.879	-0.005	0.480
D 9 a.m.	0.063	0.576	-0.002	0.429
D 9 p.m.	0.088	0.424	0.001	0.377
D-S 9 a.m.	0.046	0.692	-0.004	0.456
D-S 9 p.m.	0.261	0.021	0.051	0.049
F/D 9 a.m.	-0.101	0.349	0.004	0.342
F/D 9 p.m.	0.067	0.525	-0.001	0.414
F/DS 9 a.m.	-0.074	0.497	-0.001	0.405
F/DS 9 p.m.	-0.085	0.430	0.001	0.380

*adjusted for gender and age, β: standardized partial regression coefficient, R^2: adjusted R square

Table 6. Association of total SDS with hormone levels in saliva (n = 96)

	β	p	R^2	p
F 9 a.m.	0.060	0.547	0.069	0.023
F 9 p.m.	0.094	0.345	0.074	0.018
D 9 a.m.	0.100	0.358	0.074	0.018
D 9 p.m.	0.061	0.571	0.069	0.023
D-S 9 a.m.	0.037	0.741	0.066	0.025
D-S 9 p.m.	0.141	0.199	0.082	0.012
F/D 9 a.m.	-0.013	0.897	0.065	0.026
F/D 9 p.m.	0.069	0.496	0.070	0.022
F/DS 9 a.m.	0.013	0.904	0.065	0.024
F/DS 9 p.m.	-0.018	0.865	0.066	0.026

*adjusted for gender and age, β: standardized partial regression coefficient, R^2: adjusted R square

Table 7. Association of total work burden with hormone levels in saliva (n = 96)

	β	p	R^2	p
F 9 a.m.	-0.131	0.718	0.021	0.175
F 9 p.m.	-0.045	0.662	0.006	0.324
D 9 a.m.	0.136	0.222	0.020	0.188
D 9 p.m.	0.123	0.264	0.017	0.208
D-S 9 a.m.	-0.013	0.908	0.004	0.348
D-S 9 p.m.	0.103	0.364	0.012	0.249
F/D 9 a.m.	-0.216	0.042	0.047	0.058
F/D 9 p.m.	-0.125	0.228	0.019	0.191
F/DS 9 a.m.	-0.077	0.479	0.009	0.285
F/DS 9 p.m.	-0.102	0.339	0.013	0.240

*adjusted for gender and age, β: standardized partial regression coefficient, R^2: adjusted R square

Table 8. Association of 9 items on fatigue and depression with hormone levels in saliva (n = 96)

	β	p	R^2	p
F 9 a.m.	0.014	0.892	-0.014	0.641
F 9 p.m.	0.024	0.814	-0.014	0.633
D 9 a.m.	0.047	0.677	-0.012	0.607
D 9 p.m.	-0.039	0.729	-0.017	0.619
D-S 9 a.m.	-0.093	0.423	-0.013	0.511
D-S 9 p.m.	0.029	0.803	-0.007	0.632
F/D 9 a.m.	-0.020	0.857	-0.014	0.638
F/D 9 p.m.	0.051	0.628	-0.012	0.594
F/DS 9 a.m.	0.077	0.484	-0.009	0.540
F/DS 9 p.m.	0.001	0.995	-0.014	0.646

*adjusted for gender and age, β: standardized partial regression coefficient, R^2: adjusted R square

(2) Work situation included in explanatory variables (adjusted for gender, age, and work situation)

As in (1) above, DHEA-S levels at 9 p.m. were significantly associated with severity of depression (SIGH-D) and quality of sleep (IS), with β of 0.23 for both and adjusted R^2 values of 0.10 and 0.13, respectively (Tables 9 and 10). Hormone levels in saliva were not significantly correlated with the total score for the 9 items, including subjective depression (SDS), total work burden, fatigue, and depression.

Table 9. Association of SIGH-D scores with hormone levels in saliva (n = 96)

	β	p	R^2	p
F 9 a.m.	0.13	0.22	0.07	0.03
F 9 p.m.	0.09	0.35	0.06	0.04
D 9 a.m.	0.19	0.09	0.08	0.02
D 9 p.m.	0.07	0.51	0.06	0.05
D-S 9 a.m.	0.12	0.27	0.06	0.04
D-S 9 p.m.	0.23	0.04	0.10	0.01
F/D 9 a.m.	-0.02	0.89	0.05	0.06
F/D 9 p.m.	0.06	0.53	0.06	0.05
F/DS 9 a.m.	-0.01	0.93	0.05	0.06
F/DS 9 p.m.	-0.08	0.47	0.06	0.05

*adjusted for gender, age and work situation

Table 10. Association of insomnia score with hormone levels in saliva (n = 96)

	β	p	R^2	p
F 9 a.m.	-0.01	0.96	0.09	0.01
F 9 p.m.	0.13	0.18	0.11	0.01
D 9 a.m.	0.03	0.79	0.09	0.01
D 9 p.m.	0.04	0.70	0.09	0.01
D-S 9 a.m.	0.05	0.66	0.09	0.01
D-S 9 p.m.	0.23	0.03	0.13	0.00
F/D 9 a.m.	-0.03	0.81	0.09	0.01
F/D 9 p.m.	0.13	0.21	0.10	0.01
F/DS 9 a.m.	-0.04	0.71	0.09	0.01
F/DS 9 p.m.	-0.05	0.66	0.09	0.02

*adjusted for gender, age and work situation

IV. Discussion

Brain function changes (dysfunction of the frontal lobe and hippocampus) are clearly understood to be correlated with depression. These changes promote HPA axis endocrine activities. Prolonged stress may act on the stress management mechanism in the brain to cause excessive corticotropin-releasing factor (CRF) activity, which may stimulate GABAergic neurons in the dorsal raphe nucleus. This may inhibit the serotonin nervous system, which links the dorsal raphe nucleus with the prefrontal cortex (PFC). Volumetry morphological studies[1,2] indicate that an increase in blood cortisol in blood induces cell injury in the hippocampus, and there is strong evidence that dysfunction of the frontal lobe and limbic system is associated with increased activity of the HPA axis. Such an increase in blood cortisol due to increased HPA axis activity influences cerebral neural plasticity, which might weaken stress management and decrease the onset threshold for psychiatric disorders such as depression[3]. Thus, studies on brain function and neuroendocrine activities are critical for treatment and management of depression and fatigue.

The dexamethasone suppression test (DST) was first described by Carroll[4]. There have subsequently been many reports in Japan on the DST with regard to depression[5,6]. The American Psychiatric Association reported that the sensitivity of the DST in thousands of patients with major depression was 40-50% and the specificity in normal controls was >90%[7]. Holsboer et al[8] developed a combined dexamethasone suppression/CRH-challenge (DEX/CRH) test and showed that the sensitivity for major depression was >80%. Kunugi et al[9] found that the sensitivity of the DEX/CRH test in 20 patients with major depression was about 70%[10]. However, the standard test procedure[11] is relatively invasive because blood collection and intravenous administration of CRH are required at 3 p.m. with subsequent blood collection 4 times every 15 minutes on the day after oral administration of dexamethasone (at 11 p.m.).

ACTH loading theoretically produces stress and increases DHEA-S among hormones released from the adrenal cortex. DHEA-S in serum is more prolonged than cortisol, and this bioactivity might be associated with aging, but this is uncertain. Plasma DHEA is increased in premenopausal women with posttraumatic stress disorder (PTSD) associated with depressed mood[12] and DHEA-S in saliva is increased in depressed patients[13]. Thus, many studies have focused on DHEA and DHEA-S as indicators of major strain or stress. In the current study, cortisol, DHEA, and DHEA-S levels in saliva were measured by LC-MS/MS after saliva was collected in a noninvasive manner, and correlations between these levels and questionnaire scores were evaluated. The results are summarized in the following sections (i) to (v).

(i) Gender difference and hormone levels in saliva

Cortisol, DHEA, and DHEA-S levels at 9 a.m. and DHEA and DHEA-S levels at 9 p.m. were higher in males, whereas cortisol/DHEA-S ratios at 9 a.m. and 9 p.m. were greater in females (Table 1). Levels of DHEA and DHEA-S in blood and DHEA in saliva were greater in males, consistent with previous studies.

(ii) Age and hormone levels in saliva

The subjects ranged from 22 to 64 years old. DHEA levels at 9 a.m. and 9 p.m. were negatively correlated with age regardless of gender, and the cortisol/DHEA ratio at 9 a.m. in males was positively correlated with age (Table 2). These results for the relationships of age with DHEA and DHEA-S levels in saliva are consistent with those in previous studies.

(iii) Work situation and hormone levels in saliva

Cortisol levels at 9 a.m. and the cortisol/DHEA ratio were negatively correlated with work situation in females (Table 3) but not in males, which suggests that the influence of corticosteroids on chronic stress and fatigue is stronger in females. These results indicate that cortisol and DHEA levels in saliva and the cortisol/DHEA ratio are good parameters for measurement of fatigue. This agrees with a previous study showing lower morning salivary cortisol levels in females who worked the midnight shift[14)].

(iv) Depression, sleep, and fatigue, with work situation not included in the explanatory variables

The severity of depression (SIGH-D) and quality of sleep (IS) were significantly correlated with DHEA-S levels at 9 p.m., with β values of 0.257 and 0.261, respectively (Tables 4 and 5). DHEA-S levels at 9 p.m. were also significantly associated with quality of sleep (IS), with an adjusted R^2 of 0.051. In contrast, there was no significant correlation between subjective depression (SDS) and hormone levels in saliva (Table 6). The most important finding was that total work burden (chronic fatigue) was significantly correlated with the cortisol/DHEA ratio at 9 a.m., with β of -0.216 (Tables 7 and 8). This result supports that in (iii), and identifies an important factor for a workers' fatigue scale.

(v) Work situation included in the explanatory variables (adjusted for gender, age, and work situation)

As in (iv), severity of depression (SIGH-D) and quality of sleep (IS) were significantly correlated with DHEA-S levels at 9 p.m., with β of 0.23 and adjusted R^2 values of 0.10 and 0.13, respectively (Tables 9 and 10). Hormone levels in saliva were not significantly correlated with the total score for the 9 items of subjective depression (SDS), total work burden, fatigue, and depression.

In conclusion, this study provides the first evidence showing that sleep, fatigue, and depression are related to the physiological actions of DHEA-S and DHEA released from the adrenal cortex. The results indicate that DHEA or DHEA-S levels or ratios to cortisol can be used as scales for fatigue and sleep.

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Depressive state, fatigue, sleep and HPA-related endocrine kinetics in workers

To date, it has been clarified that depressive state, stress and fatigue correlate closely with changes in hypothalamic-pituitary-adrenal (HPA) hormones, especially cortisol. However, no relationships have been established between the scales of depressive state, fatigue, etc., and adrenal cortical hormones (DHEA, DHEA-S), other than cortisol. Recently, endocrine examination using liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been developed to enable concurrent measurement of multicomponents of hormones in minute amounts, using saliva samples, which can be collected easily and non-invasively. This study was performed to clarify the relationship between depressive state, fatigue and sleep and HPA-related endocrine function. In 96 day-shift workers (male: 56; mean age: 42.79 ± 10.72 years, female: 40; mean age: 42.98 ± 11.24 years), cortisol, DHEA and DHEA-S were measured in saliva using LC-MS/MS to examine the relationship with individual scales. In the analysis of the working situations and endocrine kinetics in the subjects, it was found that the levels of cortisol and the ratio of cortisol/DHEA, which were measured at 9 o'clock, in female subjects correlated negatively with the burdens of the working situations. Through multiple linear regression analysis, after adjusting for age, sex and working situations, DHEA-S level measured at 21 o'clock showed a significant beta coefficient (0.23) for both the severity of depressive state (SIGH-D) and sleep situation (insomnia score: IS), with R-square (R^2) values of 0.10 and 0.13, respectively. It was suggested that the high level of DHEA-S secretion at night was related to depressive state and sleep disorder.

Study 2: HPA-related endocrine kinetics correlate with cerebral blood flow changes measured by ^{99m}Tc-ECD SPECT and mental health-related markers

I. Objective and Summary

Correlations among clinical features, CBF distribution, and hormone levels in saliva were examined in workers by focusing on depression, fatigue, and QOL. Clinical features were evaluated using the SIGH-D (Structured Interview Guide for the Hamilton-Depression rating scale), SDS (Self-rating Depression Scale), a self-assessment checklist for chronic fatigue in workers, SF-36 (MOS Short-Form 36-item Health Survey), SPM (Statistical Parametric Mapping), and vbSEE (voxel-based Stereotactic Extraction Estimation). Correlations of these results with the CBF distribution obtained by ^{99m}Tc-ECD SPECT and with hormone levels in saliva and those between changes in CBF and hormone levels in saliva were evaluated. Potential associations of depression, fatigue and QOL with changes in CBF and HPA axis-related endocrine kinetics were examined with the goal of establishing useful indicators for mental health (interview items on a questionnaire) and test parameters for early detection of stress, exhaustion, and depression. The study was approved by the ethical review board certified by the Japan Labour Health and Welfare Organization (December 21, 2009).

II. Subjects and Methods

1. Subjects

Of workers aged ≥20 to ≤ 60 years who were right-handed and suffered from stress caused by work and personal relationships, 42 who provided written informed consent after receiving a full explanation of the study were included as the subjects. None of the subjects had an organic brain disorder.

2. Methods

(1) Depression, fatigue, and QOL were assessed using the following scales.

(i) SIGH-D (Structured Interview Guide for the Hamilton-Depression Rating Scale): A structured interview was used to assess 17 items of the Hamilton Depression Rating Scale to rate severity of depression based on the total score. Quality of sleep was evaluated using items related to sleep disorder (insomnia score: IS). To minimize inter-examiner deviations, interviews were conducted after training using a video.

(ii) SDS (Self-rating Depression Scale): Subjective severity of depression was evaluated using the self-rating scale.

(iii) Self-assessment checklist for chronic fatigue in workers: I. Subjective symptoms, II. Work situation, and III. Total burden on workers were self-assessed.

(iv) SF-36 v2 standard Japanese: Comprehensive health status, 8 health concepts, and summary scales (physical and mental health) were self-assessed.

(v) Brief Job Stress Questionnaire: Nine items on fatigue and depression were assessed based on the frequency of self-reported fatigue, depressed mood, poor concentration, and grief over the past month. Scores were divided into 4 grades.

(2) Correlations of depression, fatigue, and QOL with changes in CBF

CBF was determined using ^{99m}Tc-ECD SPECT after obtaining written informed consent based on ethical considerations. Imaging analysis using SPM and vbSEE was used to evaluate correlations of CBF with total scores and scores for each scale obtained in (1) (i) - (v).

Procedures for measurement of regional CBF using SPECT (^{99m}Tc-ECD)

Pretreatment

Subjects rested in the supine position with eyes closed for 10 min from 5 min before intravenous administration of ^{99m}Tc-ECD to avoid an increase of blood flow in the vision center of the brain by photic stimulation.

Examination method

i) Injection site of radioisotope (RI) (^{99m}Tc-ECD) (Patlak plot method)

The subjects adopted a supine position to allow the region from the parietal region to aortic arch to be viewed with a scintillation camera. A three-way stopcock with an extension tube (contents: 2 mL) was attached to a 20-G winged needle. A syringe containing 20 mL of physiological saline for RI flush was connected to the three-way stopcock. Cubital venous access was gained through the right median cubital vein. RI was slowly pooled in the extension tube and the three-way stopcock was switched to the position of physiological saline. Simultaneously with rapid intravenous administration of RI, 100 to 120 frames of dynamic data were acquired with a 128×128 matrix and 1 frame/s to improve the IV bolus of RI, with a 90° angle maintained between the body and right arm. The dose of ^{99m}Tc-ECD (Neutrolite® Injection Daiichi; [N,N'-ethylene-L-cysteinate (3-)]oxotechnetium (^{99m}Tc), diethylester) was approximately 600 MBq, and divided doses were given if the entire dose could not be put in the extension tube.

ii) SPECT imaging

SPECT imaging (64×64 or 128×128 matrix size, 60-72 angulations for 360°, 15 s per angulation) was performed 10 min after intravenous administration of RI.

iii) SPECT image reconstruction

SPECT images were reconstructed using the projection data obtained in ii) to obtain qualitative images.

iv) Data processing

• Determination of mean CBF (mCBF) using the Patlak plots

ROIs were set in areas of the aortic arch and bilateral hemispheres in dynamic data obtained in i) for analysis using Patlak plots and determination of mCBF in bilateral hemispheres using the Brain Perfusion Index (BPI).

• Determination of regional CBF (rCBF)

rCBF was calculated based on mCBF using Lassen's correction algorithm. The unaffected hemisphere was used as the reference region for the correction. If both hemispheres were affected, the hemisphere with greater mCBF was used as the reference region. The correction coefficient α was 2.59. Quantitative images were obtained using these procedures.

(3) Correlation of depression, fatigue, and QOL with hormone levels in saliva

Correlations were evaluated for total scores and scores for each indicator obtained in (1) with hormone levels in saliva. The subjects collected saliva after providing written informed consent based on ethical considerations.

• Amount of saliva: 2 mL/collection • Storage: ≤ -20°C

• Collection time: 2 time points, at 9 p.m. on the day before SPECT and 9 a.m. on the day of SPECT

In determination of DHEA-S, cortisol, cortisone, and DHEA in saliva by LC-MS/MS, an aliquot of the test compound as a deuterated internal standard was added to 1 mL of saliva and the mixture was extracted by organic solvent, separated using an ionic cartridge column, and purified. DHEA-S fractions were analyzed immediately by LC-MS/MS. Cortisol, cortisone, and DHEA fractions were converted to picolinic acid derivatives and each fraction was purified again using the cartridge column. The resulting purified products were simultaneously analyzed by LC-MS/MS.

The lower limits of quantitation (LLOQ) for cortisol, DHEA, and DHEA-S were 10 pg, 4 pg, and 10 pg, respectively, and a value below the lower limit of quantification (BLQ) was defined as 50% of LLOQ. Cortisol, DHEA, and DHEA-S levels are expressed as pg/mL (significant figures: 3 digits).

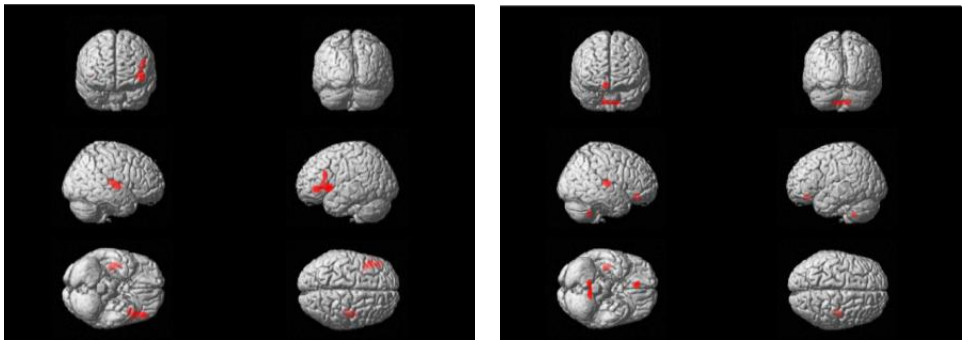
(4) Imaging analysis of correlations of hormone levels in saliva with changes in CBF

Correlations of cortisol, DHEA, and DHEA-S levels in saliva with CBF obtained by ^{99m}Tc-ECD SPECT were evaluated using eZIS (easy Z-score imaging system) images. Brain regions and local areas with significant correlations were analyzed by SPM and vbSEE.

III. Results

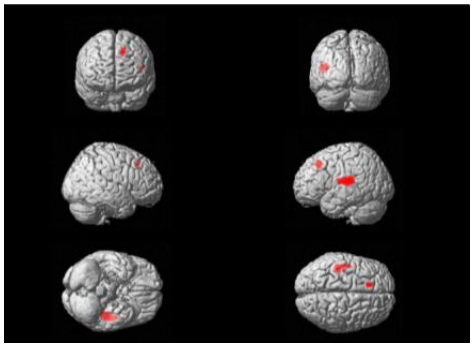
(1) Depression, fatigue, QOL, and hormone levels in saliva, and changes in CBF

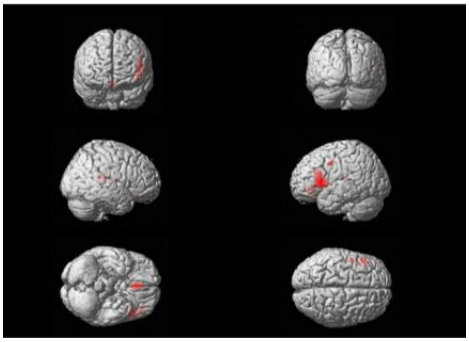
Regions showing changes in CBF that were positively correlated with scores on each questionnaire and hormone levels or ratios in saliva were calculated using SPM ver. 8, with a height threshold T=2.429 and P<0.01. The brain images are as follows (n=42, P<0.0, t-value images).



(ⅰ) (ⅱ)

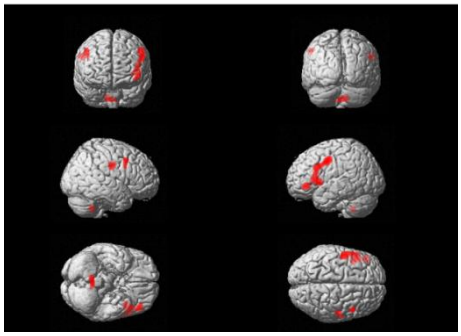
(i) Depression (SIGH-D): Decreases in CBF in the left inferior frontal gyrus and dorsolateral PFC were more common in persons with severe depression. (ii) Insomnia (IS): Decreases in CBF in the polus frontalis and ventroanterior PFC were more common in persons with severe insomnia.





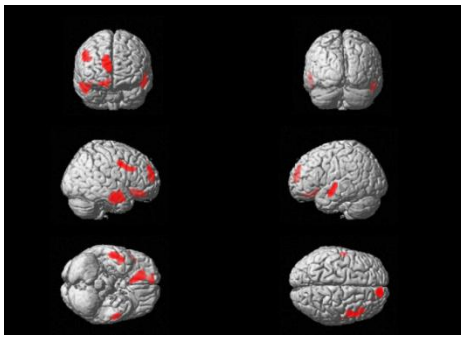
(iii) (iv)

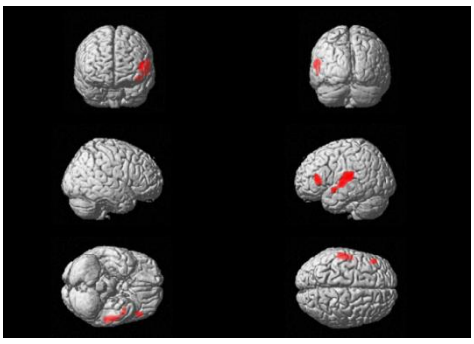
(iii) Chronic fatigue due to hard work: Decreases in CBF in the dorsal PFC and supramarginal gyrus were more common in persons with chronic fatigue. (iv) Role physical: Decreases in CBF in the polus frontalis, dorsolateral PFC, and subgenual PFC were more common in persons with more difficult daily activities.



(v)

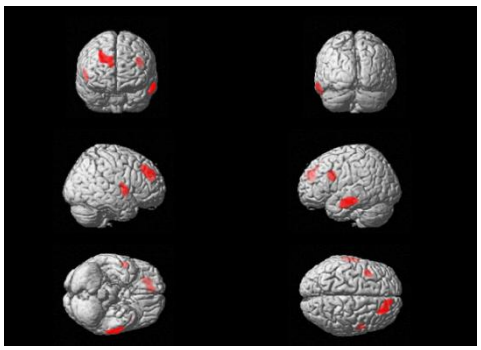
(v) Role emotional: Decrease in CBF in the dorsolateral PFC was more common in persons with more difficult daily activities.





(vi) (vii)

(vi) F9 (cortisol levels at 7 a.m. to 9 a.m.): Decreases in CBF in the ventral/dorsolateral PFC and basal temporal lobe were more common in persons with lower F9. (vii) F/D9 (cortisol/DHEA ratio at 7 a.m. to 9 a.m.): Decreases in CBF in the dorsolateral PFC and insula were more common in persons with a smaller F/D9.

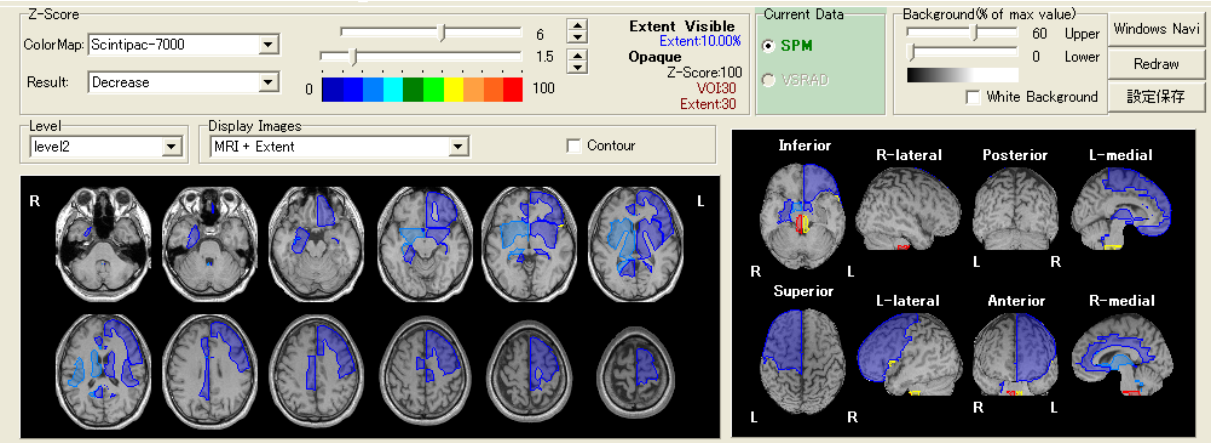


(viii)

(viii) F/D21 (cortisol/DHEA ratio at 8 p.m. to 10 p.m.): Decreases in CBF in the dorsolateral PFC and insula were more common in persons with smaller F/D21.

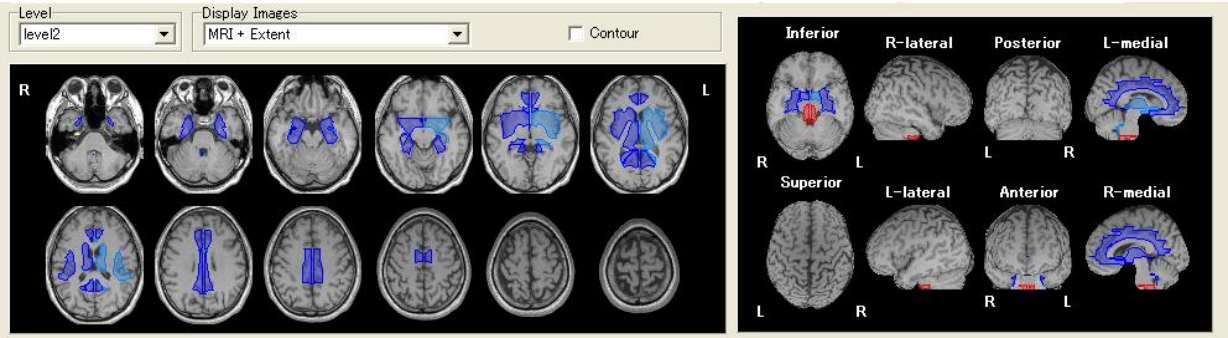
The significant changes in CBF in (i) - (viii) were then analyzed at levels 2 & 3 of vbSEE, with the following results.

(i) Depression (SIGH-D) (EXTENT MAP > 10%, t-score > 1.5)



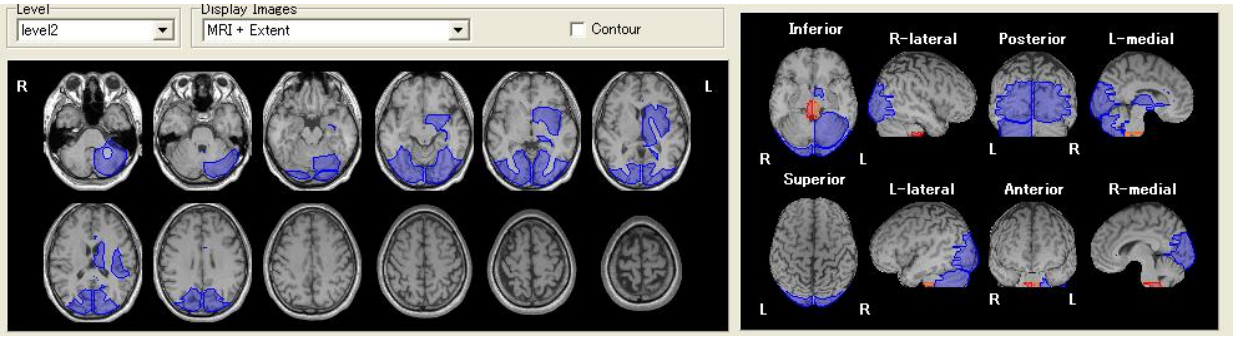
Level 2: lt. frontal-temporal space (64.29%), lt. frontal lobe (16.17%), rt. limbic lobe (13.78%), Level 3: lt. inferior frontal gyrus (53.78%), insula (lt.33.17%, rt.35.44%), lt. cingulate gyrus (16.59%), anterior cingulate (lt. 8.76%, rt.11.82%)

(ii) Insomnia (IS) (SIGH-D) (EXTENT MAP > 10%, t-score > 1.5)



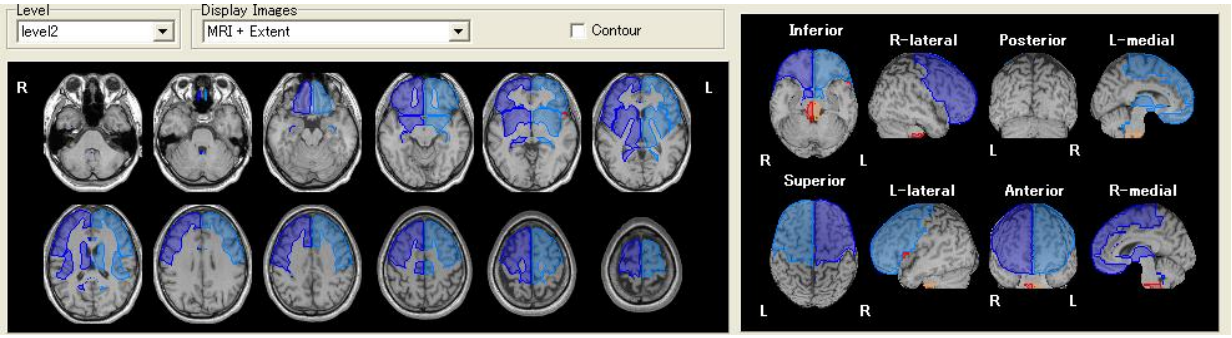
Level 2: limbic lobe (lt.16.64%, rt.15.09%), **Level 3:** cingulate gyrus (lt.41.20%, rt.10.02%), insula (lt.40.12%, rt.27.93%), culmen of vermis (lt.35.48%, rt.15.38%)

(iii) Chronic fatigue due to hard work



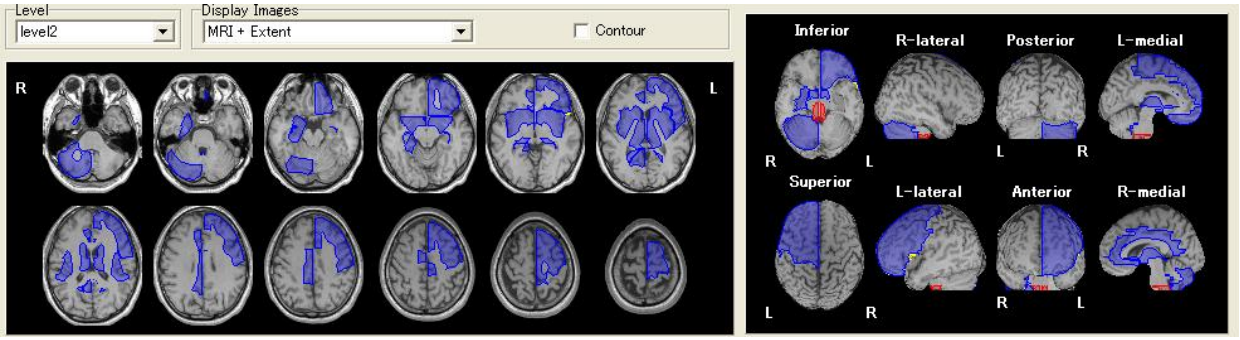
Level 2: occipital lobe (lt.13.69%,rt.14.58%), lt. posterior lobe (13.77%), **Level 3:** transverse temporal gyrus (lt.55.28%,rt.23.20%), lt. fastigium (37.25%), rt. post. cingulate (37.80%), declive of vermis (lt. 33.33%, rt. 27.91%), lt. insula (33.32%)

(iv) Role physical (sf36)



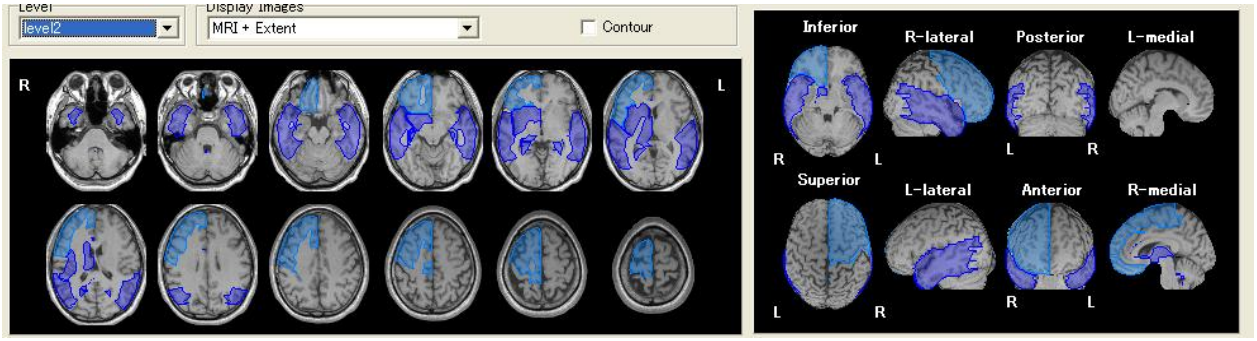
Level 2: lt. frontal-temporal space (95.71%), lt. frontal lobe (24.99%), rt. frontal lobe (10.46%), **Level 3:** lt. inferior frontal gyrus (59.49%), insula (lt.56.95%, rt.37.46%), lt. middle frontal gyrus (37.03%), anterior cingulate (lt. 5.84%, rt.13.13%)

(v) Role mental (sf36)



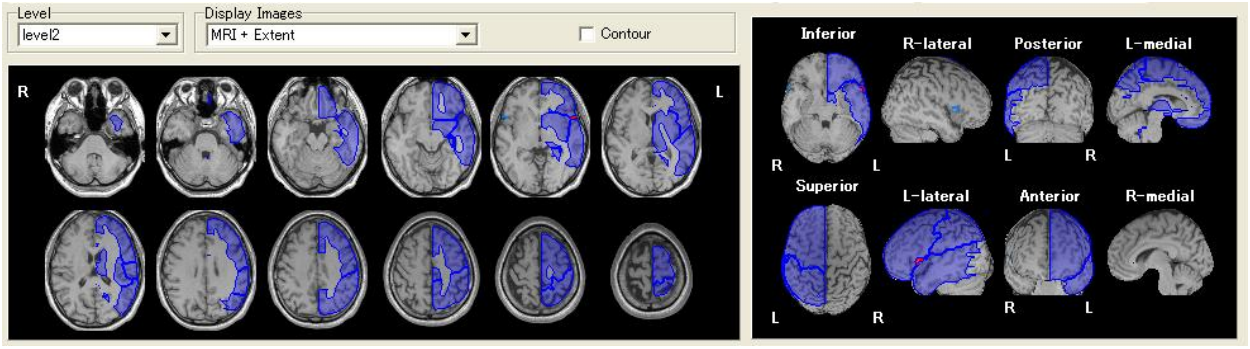
Level 2: lt. frontal-temporal space (67.14%), lt. frontal lobe (13.97%), rt. limbic lobe (13.39%), rt. post. Lobe (13.22%), **Level 3**: transverse temporal gyrus (lt.29.15%,rt.41.44%), culmen of vermis (lt.50.00%, rt.61.29%) lt. inferior frontal gyrus (52.00%), insula (lt.33.94%, rt.28.13%)

(vi) F_9 (cortisol levels at 7 a.m. to 9 a.m.)



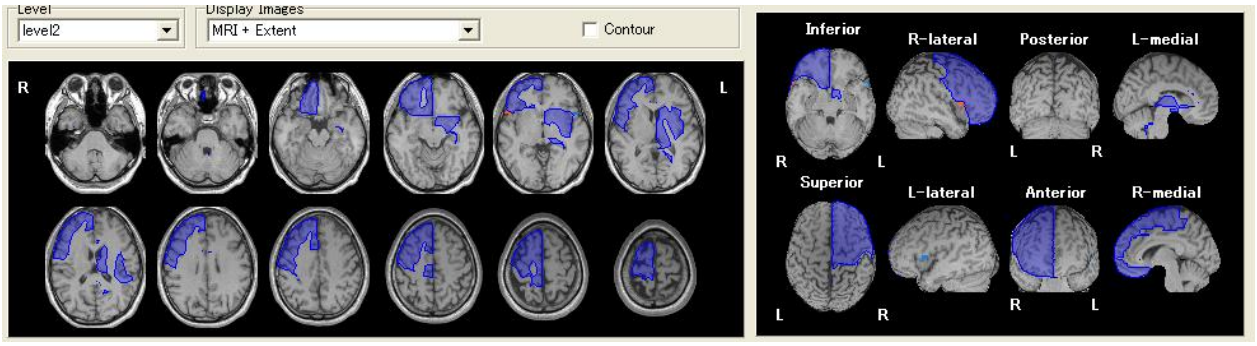
Level 2: rt. frontal-temporal space (73.61%), frontal lobe (lt. 9.54%, rt. 25.19%), temporal lobe (lt. 18.58%, rt. 16.98%), **Level 3:** subcallosal gyrus (lt. 64.67%, rt. 48.40%), rectal gyrus (lt. 45.16%, rt. 58.82%), caudate (lt. 30.00%, rt. 46.00%), lt. inf. occipital gyrus (39.26%), rt. inf. frontal gyrus (35.16%)

(vii) F/D_9 (cortisol/DHEA ratio at 7 a.m. to 9 a.m.)



Level 2: frontal-temporal space (lt. 91.43%, rt. 25.00%), lt. frontal lobe (11.95%), lt. temporal lobe (19.46%), lt. parietal lobe (12.08%), **Level 3:** lt. inferior frontal gyrus (43.01%), transverse temporal gyrus (lt. 41.71%, rt. 21.55%), lt. sup. temporal gyrus (34.53%), lt. supramarginal gyrus (31.47%)

(viii) F/D21 (cortisol/DHEA ratio at 8 p.m. to 10 p.m.)



Level 2: frontal-temporal space (lt. 25.71%, rt. 87.50%), frontal lobe (lt. 7.19%, rt. 19.34%), **Level 3:** lt. claustrum (34.68%), rt. medial frontal gyrus (29.94%), rt. sup. frontal gyrus (26.87%), lt. inf. temporal gyrus (21.32%), rt. middle frontal gyrus (20.04%)

(ix) Areas with increased CBF correlated with conditions and hormone levels; Each area had increased CBF at vbSEE level 3 (Extent%)

	paracentral lobule		**parahippocampal gyrus**		**postcentral gyrus**	
	lt.	rt.	lt.	rt.	lt.	rt.
(i) Depression (SIGHD)	***53.04***	***36.71***	***25.08***	7.71	***29.95***	6.07
(ii) Insomnia (IS)	***53.70***	***22.32***	0.00	8.65	***27.80***	6.56
(iv) Role physical	***65.48***	***28.42***	***44.32***	0.94	***18.03***	5.56
(v) Role mental	***25.13***	9.09	***30.73***	2.44	***11.05***	3.39
(vii) F/D_9	4.23	0.23	***10.68***	***22.37***	0.93	3.31

IV. Discussion

5-HT uptake in the frontal lobe is decreased in the depressive phase[1)] and hypofrontality associated with frontal lobe hypoperfusion and a decrease in glucose metabolism indicates depression on PET/SPECT images[2,3)]. Drevits proposed that mood state might be associated with decreased CBF and reduced glucose metabolism in the subgenual and dorsal PFC, increased CBF and elevated glucose metabolism in the ventral PFC, and structural abnormalities in all these regions[4,5)]. In our previous study using SPECT eZIS analysis of 25 workers with moderate depression (ICD-10)[6)], frontal lobe hypoperfusion was found in 18 patients and blood flow returned to normal in 75% of those with remission. SPM analysis of correlations of fatigue with CBF performed in 45 subjects, including 20 healthy controls, indicated correlations between subjective fatigue severity and decreased CBF in the dorsal frontal lobe and between total work burden and decreased CBF in the inferotemporal cortex, using the "Self-assessment checklist of chronic fatigue in workers" (Ministry of Health, Labour and Welfare in Japan). The study also suggested that persons with severe sleep disorder had a greater decrease in blood flow in the dorsal part of the frontal lobe. These findings suggest that fatigue and sleep deficit might induce psychological fatigue, similar to hypofrontality during the depressive phase. Measurements of brain functions using fMRI during neuropsychological tasks[7,8)] also suggest that hypofrontality is a mood state-dependent finding that might be correlated with psychomotor inhibition[3)] and poor concentration. Mental fatigue of workers due to overtime work or stress decreases motor and cognitive functions due to decreased CBF in the PFC and cingulate gyrus on fMRI, consistent with fMRI findings for mental fatigue in healthy individuals and patients with chronic fatigue syndrome[9,10)].

This study confirmed the relationship between severity of depression and hypofrontality using vbSEE analysis of CBF. Future studies on industrial and occupational mental health are required to establish useful interview items for early detection of stress, exhaustion, and depression. The vbSEE analysis indicated an association between each condition and hormone levels or ratios. A pattern of decreased CBF from the left frontal lobe to the left frontal-temporal space (areas with dominant decreased blood flow) was observed for severity of depression (SIGH-D), role physical (sf36), role mental (sf36), and F/D_9 (cortisol/DHEA ratio at 7 a.m. to 9 a.m.). Assuming that decreased blood flow in the left frontal

lobe and temporal lobe is mood state-dependent, these findings suggest that depression is strongly associated with role physical (sf36), role mental (sf36), and F/D_9. Furthermore, based on the areas of dominant increased CBF that were correlated with each condition and hormone levels, the severity of depression (SIGH-D), insomnia (IS), role physical (sf36), role mental (sf36), and F/D_9 (cortisol/DHEA ratio at 7 a.m. to 9 a.m.) produced increased CBF near the paracentral lobule, parahippocampal gyrus, and postcentral gyrus.

In conclusion, data for role physical and mental, insomnia (IS), and F/D_9 may be useful for prevention of depression. A further study of these items is planned in clinical and industrial health settings.

Symptoms that may be associated with areas of decreased CBF.

(i) Depression (SIGH-D)
(iv) Role physical (sf36)
(v) Role mental (sf36)
(vii) F/D_9 (cortisol/DHEA ratio at 7 a.m. to 9 a.m.)

(i)-(vii) refer to the numbering in section III in the text.

Symptoms that may be associated with areas of increased CBF.

(i) Depression (SIGH-D)
(ii) Insomnia (IS)
(iv) Role physical (sf36)
(v) Role mental (sf36)
(vii) F/D_9 (cortisol/DHEA ratio at 7 a.m. to 9 a.m.)

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inhibition and impulsiveness: a fMRI study. Eur Arch Psychiatry Clin Neurosci. 2004;254:245-251

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HPA-related endocrine kinetics correlate with cerebral blood flow changes measured by ^{99m}Tc-ECD SPECT and mental health-related markers

In our preceding study, we demonstrated that the tendency of blood flow to decrease in the frontal lobe (dorsolateral prefrontal area, anterior cingulate cortex) was related to medical interview items for insomnia (insomnia score, IS), subjective fatigue, etc. This study was performed to select important clinical test items and questionnaire items for the early detection of depression in workers, by examining the relationship between cerebral blood flow changes in the frontal lobe, etc. and HPA-related endocrine kinetics and the items related to depressive state, fatigue and QOL. The subjects were 42 right-handed day-shift workers, aged between 20 and 60 years who provided informed consent prior to their participation in the study. Using ^{99m}Tc-ECD SPECT, cerebral blood flow was measured to examine the correlation of cerebral blood flow distribution with the interview results regarding depressive state, fatigue and QOL, and hormone levels in saliva (collected at 9 and 21 o'clock and measured by LCMS/MS). In the statistical analysis of images performed using SPM 8, the cerebral areas that correlated significantly with individual interview scores and the levels/ratio of hormones in saliva were determined (height threshold $T=2.429$, $P<0.01$). It was confirmed that the severity of depressive state (SIGH-D) correlated with decreased blood flow in the left inferior frontal gyrus; in the dorsolateral prefrontal area. Insomnia (IS) correlated with the frontal pole and the ventral prefrontal area, while the level of fatigue accumulation correlated with decreased blood flow in the dorsal prefrontal area. In addition, low F_9 (cortisol measured at 9 o'clock) correlated with decreased blood flow in the ventral/dorsolateral prefrontal area, while low F/D_9 (ratio of cortiso1/DHEA measured at 9 o'clock) correlated with decreased blood flow in the dorsolateral prefrontal area and the insular cortex. Furthermore, as a result of voxel-based Stereotactic Extraction Estimation (vbSEE) analysis, relatedness was estimated between the decreased blood flow pattern, which correlated with depressive state (SIGH-D), role functioning (body), role functioning (mind) and F/D_9, and the dominant sites of increased blood flow.

List of researchers (April 2009 to March 2014)

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This research is a part of the project on research, development and dissemination related to the 13 fields of occupational injuries and illnesses of the Japan Labor Health and Welfare Organization.