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Kawaguchi, Yoshichika Department of Health Sciences, School of Medicine, Kyushu University

Toyomatsu, Kouji Institute of Health and Sports Sciences, Kurume University

Yoshida, Noriko Institute of Health and Sports Sciences, Kurume University

Baba, Kaori Department of Health Sciences, School of Medicine, Kyushu University

他

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Original Article

Measuring Job Stress among Hospital Nurses : An Attempt to Identify Biological Markers

Yoshichika Kawaguchi¹⁾, Kouji Toyomasu²⁾, Noriko Yoshida²⁾, Kaori Baba¹⁾, Masaharu Uemoto³⁾ and Shoichi Minota³⁾

1) Department of Health Sciences, School of Medicine, Kyushu University ²⁾ Institute of Health and Sports Sciences, Kurume University 3) Kobe City College of Nursing

Abstract The purpose of this study was to identify biological markers corresponding to job stress among hospital nurses. The subjects of this study were 128 nurses working at a university hospital. The NIOSH job stress questionnaire and the Miki Nurse Stressor 35-item Scale measured their job stress levels. The GHQ28 was also used to measure the subjects' general mental health status. Blood analyses for neuroendocrine function and immunity reaction were performed in order to identify biological markers of job stress. Stress is related to the plasma levels of catecholamine, cortisol, adrenocorticotrophic hormone, and natural killer cell activity, therefore these factors were measured accordingly. In consideration to circadian rhythms, blood was collected from the subjects prior to the start of the day shift.

The nurses filled out the questionnaires on the day of the blood tests. In order to investigate the correlation between job stress reactions indicated by the questionnaires and the results of the blood tests, we utilized Pearson's correlation coefficient and partial correlation coefficient for which other affected items were controlled. In this study, significant correlations were found between job stress and biological factors; however, the correlations were not strong.

Thus, it can be said that the biological markers associated with a specific kind of job stress remain unclear. In the future, rather than implementing a simple cross-sectional study, a longitudinal study including follow-up research will be more effective in establishing biological markers for job stress.

Key words: biological markers, job stress, the NIOSH job stress questionnaire, the GHQ, nurse

Introduction

In recent years, a number of occupational health studies have focused in the objective ratings of reactions to psychological stress¹⁾. The majority of the studies have been reports on acute stress reactions to a specific life event and its correlation between

these reactions and biological markers²⁾³⁾. There are also several reports on the biological effects of chronic job stress⁴⁾. However, few studies on this subject manner have been reported on health care workers, and the development of objective parameters or markers to rate job stress reactions has not been greatly successful. Among healthcare workers, nurses are known to have high levels of job stress⁵⁾. This leads to a high turnover rate⁶⁾ which in turn nega-

Editorial correspondence: Yoshichika KAWAGUCHI, R.N., Ph.D.

Telephone number: +81-92-642-6754 Fax number: +81-92-642-6754

E-mail: kawaguchi@shs.kyushu-u.ac.jp

tively affects the quality of patient care. For these reasons, this study was implemented to establish biological markers for job stress among hospital nurses. Job stress was measured using a combination of questionnaires and blood tests, and the correlation between job stress levels compared to neuroendocrine function and immunity reaction was investigated.

Our research group has previously studied stress and its related factors^{7)~9)} among medical health care workers, especially in nurses. Our prior study on 3-methoxy-4-hydroxyphenylglycol levels (MHPG)¹⁰⁾, one of the metabolites of catecholamine, attempted to develop a biological index, but there was no significant difference between the levels of MHPG in subjects' saliva and the results of a questionnaire taken at that time. This current study uses an increased number of questionnaires and biological markers in order to measure job stress among nurses.

Subjects and Methods

This study took place in August of 2000. The population targeted was registered female nurses employed at a university hospital in Fukuoka Prefecture, Japan. Male nurses and nurses' aides were excluded from this study. The nursing department approved of the intent and content of this research proposal and assisted in the recruitment of subjects. A total of 131 eligible nurses, all of who understood the purpose of the study, agreed to participate on their own will. Written consent was obtained from each subject.

Data was collected in the following manner. The first part consisted of demographic and general background questions and information. The subjects then completed 3 separate questionnaires related to

job stress and health. Lastly, blood tests were conducted in order to identify the biological markers of stress. Subjects with incomplete data from any part of the study were eliminated, resulting in a final total of 128 subjects who were used for analysis.

Questionnaire Surveys

The Japanese version of the National Institute for Occupational Safety and Health (NIOSH) job stress questionnaire¹¹⁾, and the Miki Nurse Stressor 35-item Scale¹²⁾¹³⁾ were used as indicators of job stress. The NIOSH job stress questionnaire was originally developed by the National Institute for Occupational Safety and Health in the U.S, and the Japanese version used for this study was created by Haratani et al¹⁴). The contents of the NIOSH job stress questionnaire include job stressors, buffer factors, and acute reactions, along with their respective measurement scales. The Miki Scale includes items specifically related to nursing work, such as Difficult judgment in a job, Patient/ family-nurse relationships, Experience of patient's death and Psychological job reward. The 28-item General Health Questionnaire (GHQ28)15)16) Japanese version was used to measure general mental health status. The GHQ28 consists of measurements from four subscales— Somatic symptoms, Anxiety and insomnia, Social dysfunction, and Severe depression - along with the total sum score. All questionnaires were chosen for their reliability and validity.

Blood Analyses

Blood tests for neuroendocrine function and immunity reaction were performed in order to detect biological markers. Stress is related to levels of catecholamine (adorenaline, noradrenaline, and dopamine), cortisol, adrenocorticotrophic hormone (ACTH), and natural killer cell activity (NK cell activity). Therefore, these factors were tested. In addition, hemoglobin (Hb), hemoglobin A1c (HbA1c), hematocrit (Ht) and platelet (Plt) counts were taken. In consideration to circadian rhythms, blood was collected from the subjects prior to the start of the day shift.

The subjects filled out the questionnaires on the day of the blood tests.

The following methods were used to measure blood chemistry: High performance liquid chromatography (HPLC) for catecholamine, radioimmunoassay (RIA) for cortisol and ACTH, latex agglutination (LA) for HbA1c, 51Cr release assay for NK cell activity.

Data analysis

In order to investigate the correlation between job stress reactions measured through the questionnaires and the results of the blood analyses, we utilized Pearson's correlation coefficient and the partial correlation coefficient for which other affected items were controlled. Statistical analysis was carried out by SPSS version 11.0. The significance level was set at 5%.

Results

Table 1 shows main characteristics of the 128 subjects. The average age of the participating nurses was 30.0 yr (SD: 7.4).

Table 2 shows mean (\pm SD), kurtosis, skewness, maximum and minimum of the variables. The higher score of the variable indicated poorer mental health and mental health status, except in the variable Buffer Factors in the NIOSH job stress questionnaire and Psychological job reward in the Miki nurse stressor 35-item scale. Dates of dopamine, ACTH and HbA1c were not

appropriate to use for correlation analysis because their absolute values of kurtosis and skewness were over 2 points¹⁷⁾. Therefore, logarithmic transformations of these variables were performed as needed to improve normality. Table 3 shows the mean (±SD), kurtosis, skewness, and the maximum and minimum of dopamine, ACTH, and HbA1c after logarithmic transformation. The absolute values of kurtosis and skewness of their dates were less than 2 points.

Table 4 shows the relationship between the serological markers and job stress by partial correlation coefficient. At the time the partial correlation coefficient was estimated, the secondary influences of age, years of nursing experience, work schedule, hospital ward, and smoking habit were controlled, and outlying factors were eliminated. The results of the blood examination and the NIOSH questionnaire showed that the quantitative workload and variance in workload, under the category of job stressors, were negatively correlated with natural killer cell activity, whereas job control was positively correlated with noradrenaline levels. Depression, under the category of acute reactions, was positively correlated with HbA1c levels. Social support from family and friends, under the category of buffer factors, was positively correlated with adrenaline levels. Based on the results of the Miki Nurse Stressor Scale, Experience of patient's death was negatively correlated with dopamine levels.

Table 5 shows the relationship between the blood examinations and the GHQ28 by the partial correlation coefficient. There was no significant difference between the GHQ28 and the blood examinations.

Table 1 Characteristics of the subjects

N = 128

| | | N = 128 |
|-----------------------------|----------------|------------|
| Age (Mean±SD) | 30.0 ± 7.4 | |
| Years of Nursing Experience | \sim 5 | 55 (43.0) |
| | 5~10 | 38 (29.7) |
| | $10\sim$ | 35 (27.3) |
| Work Schedule | three shift | 109 (85.2) |
| | other types | 18 (14.1) |
| | | (%) |

| Table 2 Mean, kurtosis, skewness, maximum, minimum of variables | | | | | | | | | | |
|--|-------------------|----------|--------------|---------|---------|------------------|--|--|--|--|
| variables | Mean±SD | kurtosis | skewness | maximum | minimum | normal range | | | | |
| (biological parameters) | | | | | | | | | | |
| adrenaline (pg/ml) | 41.1 ± 18.7 | 1.85 | 1.12 | 114 | 10 | ~ 100 | | | | |
| noradrenaline (pg/ml) | 393.2 ± 132.7 | -0.18 | 0.72 | 781 | 173 | $100 \sim 450$ | | | | |
| dopamine (pg/ml) | 14.1 ± 7.4 | 5.98 | 1.90 | 52 | 5 | \sim 20 | | | | |
| cortisol (ug/dl) | 13.7 ± 5.1 | -0.34 | 0.56 | 28.2 | 3.9 | $4.0 \sim 18.3$ | | | | |
| ACTH (pg/ml) | 24.5 ± 14.7 | 3.94 | 1.78 | 88 | 5 | 9-52 | | | | |
| HbA1C (%) | 4.9 ± 0.3 | 3.33 | 0.94 | 6.1 | 4.4 | $4.3 \sim 5.8$ | | | | |
| Hb (g/dl) | 12.6 ± 1.2 | 1.86 | -0.64 | 15.3 | 7.8 | $11.3 \sim 15.2$ | | | | |
| Ht (%) | 37.4 ± 2.7 | 1.81 | -0.41 | 43.8 | 25.8 | $33.4 \sim 44.9$ | | | | |
| Plt ($\times 10^3/\text{ul}$) | 24.4 ± 4.8 | 0.15 | 0.51 | 40.4 | 14.8 | $13.0 \sim 36.9$ | | | | |
| RBC ($\times 10^6/\text{ul}$) | 428.4 ± 30.0 | 0.34 | 0.24 | 526 | 358 | $376 \sim 500$ | | | | |
| WBC ($\times 10^6/\text{ul}$) | 59.7 ± 16.1 | 1.93 | 1.12 | 124.9 | 31.2 | $35\sim 91$ | | | | |
| NK cell activity (%) | 38.3 ± 16.2 | -0.54 | 0.52 | 79 | 11 | 18~40 | | | | |
| (the NIOSH job stress question | naire) | | | | | | | | | |
| Job Stressors | , | | | | | | | | | |
| Job Control | 39.8 ± 10.5 | -0.66 | -0.06 | 65 | 17 | _ | | | | |
| Role Conflict | 26.4 ± 8.0 | 0.42 | 0.41 | 53 | 8 | _ | | | | |
| Role Ambiguity | 19.7 ± 5.1 | 0.08 | 0.27 | 34 | 9 | _ | | | | |
| Quantitative Workload | 15.2 ± 3.9 | -0.27 | -0.72 | 20 | 4 | _ | | | | |
| Variance in Workload | 10.3 ± 3.0 | -0.60 | -0.22 | 15 | 3 | _ | | | | |
| Skill Underutilization | 9.1 ± 2.9 | -0.80 | -0.08 | 15 | 3 | _ | | | | |
| Cognitive Demands | 16.5 ± 2.3 | 0.52 | -0.80 | 20 | 9 | _ | | | | |
| Buffer Factors | | | | | | | | | | |
| Social Support from Supervisor | 15.0 ± 3.1 | 1.01 | -0.92 | 20 | 4 | _ | | | | |
| Social Support from Coworkers | 17.1 ± 2.3 | 1.59 | -1.09 | 20 | 8 | _ | | | | |
| Social Support from Family/Friends | 17.5 ± 2.2 | 1.58 | -1.10 | 20 | 9 | _ | | | | |
| Acute Reactions (psychological | | | | | | | | | | |
| Job Satisfaction | 9.0 ± 1.7 | 0.40 | -0.31 | 13 | 4 | _ | | | | |
| Depression | 16.8 ± 8.5 | -0.27 | 0.59 | 38 | 1 | _ | | | | |
| (41 - M*1 *2 1 - f | | | | | | | | | | |
| (the Miki's stressors scale for n Difficult judgment in a job | 14.6±3.2 | 0.29 | 0.00 | 24 | 6 | _ | | | | |
| Responsibility for patient's life | | | 0.28 0.93 | | 5 | _ | | | | |
| 1 2 1 | | -0.17 | | 16 | 5 5 | _ | | | | |
| Patient/family-nurse relationships | 13.5 ± 3.1 | 0.41 | -0.51 | 20 | 5 5 | _ | | | | |
| Psychological job reward | 12.0 ± 2.3 | 0.63 | -0.31 | 16 | | _ | | | | |
| Experience of patient's death | 6.9 ± 2.5 | -0.48 | 0.61 | 14 | 4 | _ | | | | |
| Doctor-nurse relationships | 11.0 ± 2.6 | -0.41 | -0.33 | 16 | 4 | _ | | | | |
| Lack of contacts and communications | | -0.38 | 0.42 | 14 | 4 | | | | | |
| Technological innovation | 4.7 ± 1.7 | 1.44 | 1.13 | 12 | 3 | _ | | | | |
| (GHQ28) | | | | | | | | | | |
| GHQ total | 10.0 ± 5.1 | -0.33 | 0.45 | 24 | 1 | _ | | | | |
| Somatic Symptoms | 3.2 ± 2.0 | -1.21 | -0.03 | 7 | 0 | _ | | | | |
| Anxiety and Insomnia | 3.5 ± 2.0 | -0.98 | -0.11 | 7 | 0 | _ | | | | |
| Social Dysfunction | 1.67 ± 1.8 | -0.21 | 0.91 | 6 | 0 | _ | | | | |
| Severe Depression | 1.2 ± 1.9 | 1.32 | 1.53 | 7 | 0 | _ | | | | |

Table 3 Mean, kurtosis, skewness, maximum, minimum of variables after logarithmic transformation

| variables | Mean±SD | kurtosis | skewness | maximum | minimum |
|----------------------|-----------------|----------|----------|---------|---------|
| (biological markers) | | | | | |
| dopamine | 1.10 ± 0.20 | -0.12 | -0.14 | 1.6 | 0.7 |
| ACTH | 1.33 ± 0.23 | -0.05 | 0.25 | 1.9 | 0.7 |
| HbA1C | 0.69 ± 0.02 | 0.46 | 0.15 | 0.8 | 0.6 |

Table 4 Relationship between the serological markers and job stress by partial correlation coefficient

| variables | adr | norad | dopa | cort | • | HbA1C | Hb | Ht | Plt | RBC | WBC | NK |
|--------------------------------------|-----------|--------|--------|----------|--------|-----------------|--------|--------|--------|--------|--------|---------|
| (the NIOSH job stress questionnaire) | | | | | | | | | | | | |
| Job Stressors | | | | | | | | | | | | |
| Job Control | 0.060 | 0.232* | -0.023 | 0.028 | -0.078 | 0.103 | -0.084 | -0.073 | -0.007 | 0.058 | -0.056 | -0.052 |
| Role Conflict | -0.024 | -0.100 | -0.076 | -0.064 | -0.102 | -0.045 | -0.018 | -0.036 | 0.052 | -0.101 | 0.017 | -0.129 |
| Role Ambiguity | -0.061 | -0.001 | 0.062 | -0.066 | -0.017 | -0.054 | -0.123 | -0.130 | 0.010 | -0.095 | -0.085 | -0.041 |
| Quantitative Workload | 0.048 | -0.164 | 0.134 | 0.070 | 0.218 | -0.074 | -0.101 | -0.116 | 0.147 | -0.174 | 0.125 | -0.220* |
| Variance in Workload | 0.122 | -0.129 | 0.127 | 0.073 | 0.116 | -0.043 | -0.092 | -0.118 | 0.092 | -0.176 | 0.016 | -0.201* |
| Skill Underutilization | -0.113 | 0.064 | 0.144 | -0.050 | -0.153 | 0.125 | -0.037 | -0.006 | -0.017 | -0.015 | 0.101 | 0.105 |
| Cognitive Demands | -0.014 | -0.067 | 0.119 | -0.065 | 0.056 | -0.019 | -0.072 | -0.037 | 0.082 | -0.016 | -0.006 | 0.015 |
| Buffer Factors | | | | | | | | | | | | |
| Social Support from Supervisor | 0.128 | 0.038 | 0.070 | 0.025 | 0.069 | -0.041 | 0.046 | 0.200 | -0.039 | 0.017 | 0.053 | 0.051 |
| Social Support from Coworkers | -0.020 | -0.080 | -0.004 | 0.093 | -0.022 | 0.078 | -0.001 | 0.028 | 0.056 | 0.052 | -0.029 | 0.044 |
| Social Support from Family/Friends | 0.202 | 0.174 | 0.102 | 0.124 | 0.073 | 0.063 | -0.058 | -0.059 | 0.177 | -0.041 | -0.078 | 0.027 |
| Acute Reactions (psychological) | | | | | | | | | | | | |
| Job Satisfaction | -0.061 | 0.066 | -0.029 | 0.042 | -0.029 | 0.057 | -0.008 | -0.027 | -0.001 | 0.017 | 0.053 | -0.053 |
| Depression | 0.044 | 0.013 | 0.057 | -0.059 | -0.015 | 0.186° | -0.150 | -0.124 | 0.109 | -0.042 | -0.003 | -0.106 |
| (the Miki's stressors scale for nurs | se) | | | | | | | | | | | |
| Difficult judgment in a job | -0.002 | 0.069 | -0.042 | 0.071 | 0.028 | 0.059 | -0.054 | -0.062 | -0.078 | 0.016 | -0.021 | 0.051 |
| Responsibility for patient's life | 0.011 | 0.062 | -0.057 | 0.039 | 0.051 | 0.152 | -0.003 | 0.023 | 0.016 | 0.114 | -0.048 | -0.090 |
| Patient/family-nurse relationship | s - 0.093 | 0.007 | -0.042 | 0.028 | -0.105 | 0.132 | 0.057 | 0.068 | -0.020 | 0.132 | 0.101 | 0.054 |
| Psychological job reward | -0.039 | -0.104 | -0.009 | 0.091 | -0.027 | -0.028 | 0.075 | 0.057 | -0.137 | 0.022 | 0.087 | 0.140 |
| Experience of patient's death | -0.044 | -0.105 | -0.264 | ** 0.031 | 0.046 | 0.180 | 0.029 | 0.046 | -0.078 | 0.036 | 0.113 | 0.010 |
| Doctor-nurse relationships | -0.085 | -0.037 | 0.014 | 0.193 | 0.049 | 0.086 | -0.014 | -0.008 | 0.127 | 0.070 | -0.053 | 0.001 |
| Lack of contacts and communications | 0.051 | 0.097 | -0.030 | 0.101 | 0.000 | 0.105 | -0.043 | -0.041 | 0.082 | -0.098 | -0.058 | 0.008 |
| Technological innovation | 0.019 | 0.059 | -0.082 | -0.002 | -0.032 | -0.007 | -0.007 | -0.005 | 0.008 | 0.018 | -0.046 | -0.123 |

adr: adrenaline norad: noradrenaline dopa: dopamine cort: cortisol NK: natural killer cell activity

p < 0.05 p < 0.01

Table 5 Relationship between blood examinations and GHQ28 by partial correlation coefficient

| variables | adr | norad | dopa | cort | АСТН | HbA1C | Hb | Ht | Plt | RBC | WBC | NK |
|----------------------|-------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| (GHQ28) | | | | | | | | | | | | |
| GHQ total | 0.055 | 0.028 | 0.120 | -0.092 | 0.071 | -0.030 | 0.096 | 0.091 | -0.034 | 0.038 | 0.026 | -0.019 |
| Somatic Symptoms | 0.022 | -0.033 | 0.032 | -0.090 | 0.108 | -0.078 | 0.083 | 0.054 | -0.102 | 0.010 | 0.050 | -0.055 |
| Anxiety and Insomnia | 0.051 | 0.067 | 0.086 | -0.109 | -0.015 | -0.020 | 0.133 | 0.141 | 0.001 | 0.055 | 0.000 | 0.114 |
| Social Dysfunction | 0.001 | -0.007 | 0.055 | -0.092 | -0.018 | 0.050 | -0.024 | -0.018 | -0.024 | 0.030 | -0.057 | -0.118 |
| Severe Depression | 0.037 | 0.001 | -0.129 | -0.004 | 0.092 | 0.007 | 0.010 | 0.008 | -0.025 | -0.040 | -0.015 | -0.070 |

adr: adrenaline norad: noradrenaline dopa: dopamine cort: cortisol NK: natural killer cell activity

Discussion

This study was implemented to establish biological markers for job stress among hospital nurses. Job stress was measured using a combination of questionnaires and blood examinations, and the correlation between job stress levels compared to neuroendocrine function and immunity reaction was investigated.

In this study, the GHQ mean scores of above 10.0 indicate a higher stress level in comparison to the results of our previous studies¹⁸⁾. In addition to these GHQ mean scores, the NIOSH mean scores were higher than those of other occupations, suggesting that this particular group was a high stress group.

Several significant findings and correlations were found between job stress and biological factors in the present study. Other studies have shown that NK cell activity is inversely correlated to fatigue and chronic stress19), and that job stress resulting from increased workload is related to decreased NK cell activity²⁰⁾. Similarly, several reports²¹⁾²²⁾ find that increased HbA1c levels are associated with job stress in male workers. The result of this study indicates a similar pattern; however the correlations were not strong. The significant findings indicated in variables adrenaline, noradrenaline and dopamine were different to what the researcher had expected. It can be speculated that general knowledge about catecholamines are based on acute forms of stress and that the biological markers of chronic forms of stress need to be further examined.

Much care is needed in measuring stress and its effect on the human body. Stress affects each individual differently. Although the researchers attempted to standardize the times of the blood tests, shift work and individual biorhythm, especially of that in females, play a significant role in stress reactions. Adding to these, lifestyle habits such as smoking are known to decrease NK cell activity²³.

There are a number of limitations to this study. Measuring job stress or stress in general is a complicated task. As this study used a simple blood test, other experimental specimens, such as urine and saliva, may have been useful. Repeat tests may have been useful in establishing a pattern of stress reactions instead of a one-time evaluation. Other medical procedures such as MRI and cardiac monitors are possibilities, but extensive medical procedures are exhausting on the subjects, time consuming, and costly. In this study, expenses were a particular consideration because these experiments are costly.

The design and approach of this study are to be taken into consideration. In the future, instead of implementing a simple cross-sectional study, we will undertake a longitudinal study by conducting follow-up research in hopes of establishing biological markers of job stress among nurses. Although the results of this study do not clearly indicate the biological markers of job stress among nurses, this it shows promise that if biological markers are identified, stress levels can not only be measured accurately but efficiently as well. Further job stress research in the area of the health care is necessary for the improvement in occupa-

tional health and the delivery of better health care.

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(和文抄録)

生物学的指標による看護師の職業性ストレス測定の試み

1) 九州大学医学部保健学科 2) 久留米大学健康・スポーツ科学センター 3) 神戸市看護大学

川口貞親1),豊増功次2),吉田典子2),馬場香織1),植本雅治3),簑田昇一3)

【目的】看護師の職業性ストレスに反応する生物学的指標について検討した。

【対象および方法】対象は某大学病院に勤務する看護師である。看護部を通して被験者を募り、被験者に対し研究の目的、内容について直接十分な説明を行った。最終的に承諾の得られた者については承諾書を交わした上で調査を実施した。調査内容は個人属性、質問紙によるストレス評価および血液検査(生物学的指標)である。質問紙による職業性ストレス評価には、NIOSH職業性ストレス調査票、三木らの看護師のストレッサー35項目尺度を使用し、加えて一般的精神健康度を把握する目的でGHQ28項目版も用いた。生物学的指標としてcatecholamine、cortisol、ACTH、hemoglobin A1C、hemoglobin、hematocrit、natural killer cell activity などについて血液検査を実施した。調査実施日に質問紙の回答と血液検査をあわせて行った。血液検査の項目の中には、日内変動する項目も含まれているため、採血の時間をあらかじめ定め一斉に実施した。質問紙により得られた職業性ストレス反応と血液検査結果との関連性の検討には、Pearsonの積率相関係数および他の項目の影響をコントロールした偏相関係数の統計学的手法を用い評価を試みた。

【結果】 131 名の被験者の承諾が得られたが,主要な項目に記入漏れのある者等を除き,最終的に 128 名を分析対象とした。平均年齢は 30.0 ± 7.4 であった。dopamine,ACTH,hemoglobin A1C は結果数値が正規分布していなかったため,対数変換後のデータを分析に用いた。NIOSH 職業性ストレス調査票では仕事のストレッサーの量的労働負荷,労働負荷の変動が NK cell activity と負の相関を,仕事のコントロールが noradrenaline と正の相関を示した。ストレス反応の抑うつは hemoglobin A1C と正の相関を示した。緩衝要因の家族・友人からの社会的支援は adrenaline と正の相関(サポートが少ないと感じている者ほど adrenaline の値が高い)を示した。三木らの看護婦ストレッサー尺度では,患者の死との直面が dopamine と負の相関を示した。しかし,いずれも強い相関ではなかった。GHQ28 においては血液検査結果との相関は認められなかった。

【結論】職業性ストレスの下位尺度と血液検査との間に若干の有意な相関が認められたものの,いずれもそれほど強い相関ではなく,職業性ストレスに特異的に反応する生物学的指標を明らかにすることはできなかった。今後は生物学的指標に関してより具体的な吟味が必要であり,また単なる横断調査ではなく,対象者を経時的に追跡し縦断調査を行いながら生物学的指標の検討を進めていく必要があるものと考えられた。