Int Arch Occup Environ Health (2010) 83:779–789
DOI 10.1007/s00420-010-0544-0

ORIGINAL ARTICLE

A pilot study on the association between job stress and repeated measures of immunological biomarkers in female nurses

Kyoung-Mu Lee · Daehee Kang · Kijung Yoon · Sun-Young Kim · Ho Kim · Hyung-Suk Yoon · Douglas B. Trout · Joseph J. Hurrell Jr

Received: 17 August 2009 / Accepted: 7 May 2010 / Published online: 23 May 2010

Abstract
Objective To evaluate the immunosuppressive effects of job stress in female nurses, an 8-month longitudinal study was conducted at a major university hospital.
Methods Four groups of ten subjects each were constructed to represent high versus low objective stress and high versus low subjective stress based on their responses to a job stress questionnaire and objective stress ratings of the hospital's work units. Number of white blood cells (i.e., T cells, B cells, and natural killer cells), and lymphocytic proliferation to mitogens (concanavalin A, phytohemagglutinin, and pokeweed) and toxoid (tetanus) were measured by flow cytometry and radioimmunoassay. Serum levels of hydrocortisol, IL-1β, IFN-γ, and TNF-α, and salivary IgA were measured by enzyme-linked immunosorbent assay. The data were analyzed by repeated measures analysis of variance controlling for age and smoking.
Results The level of white blood cells was lower among high objective stress group (median: 7,170/m^3; range: 5,386–10,057) compared with that among low objective stress group (8,063; 5,888–9,875) ($P = 0.03$), however, no other cellular blood variables were found to be significant. In terms of humoral immuno-biomarkers, the level of TNF-α was moderately lower among high objective stress group (1.7 ng/ml; 0.3–2.7) compared with that among low objective stress group (2.2; 0.5–3.5) ($P = 0.07$), whereas the level of total sIgA was significantly higher among higher objective stress group (72.9 end-point titer/mg/ml/min; 14.4–153.4) compared with that among low objective stress group (44.8; 9.9–123.8) ($P = 0.02$).
Conclusion The results of the study suggest that psychological job stress affects the levels of some immunological biomarkers in female nurses.

Keywords Job stress · Immune · Biomarker · Nurse · Repeated measures

K.-M. Lee · D. Kang (✉) · H.-S. Yoon
Department of Preventive Medicine,
Seoul National University College of Medicine,
28 Yongon-Dong Chongno-Gu, Seoul 110-799, Korea
e-mail: dhkang@snu.ac.kr

K. Yoon
Department of Occupational Medicine,
Kangbuk Samsung Hospital, School of Medicine,
Sungkyunkwan University, Seoul, Korea

S.-Y. Kim
Department of Environment and Occupational Health Sciences,
School of Public Health and Community Medicine,
University of Washington, Seattle, WA, USA

H. Kim
Graduate School of Public Health,
Seoul National University, Seoul, Korea

D. B. Trout
DSHEFS, NIOSH, Cincinnati, OH, USA

J. J. Hurrell Jr
Private consultant, Cincinnati, OH, USA

Abbreviations

ANOVA	Analysis of variance
HIV	Human immunodeficiency virus
HPA	Hypothalamic–pituitary–adrenal
Ig	Immunoglobulin
IL	Interleukin
INF	Interferon
LPS	Lipopolysaccharide
NIOSH	National Institute for Occupational Safety and Health

NK	Natural killer
TNF	Tumor necrosis factor
WBC	White blood cells
SAM	Sympathetic–adrenal–medullary
sIgA	Secretory immunoglobulin A

Introduction

While we still do not have an adequate conceptual model for the relationships between stress and immune system changes (Segerstrom and Miller 2004), there is an increasing acceptance of the inference that immune suppression mediates the often-observed occurrence of illness following commonplace stress such as bereavement (Goodkin et al. 1996), marital discord (Kiecolt-Glaser et al. 1988), examination (Jemmott and Magloire 1988), unemployment (Arnetz et al. 1991), and care-giving for patient (Irwin et al. 1991; Pariante et al. 1997) by affecting the susceptibility to infectious and malignant diseases. In addition to commonplace stress, work-related stress has also been reported to alter immunological parameters in nurses (Endresen et al. 1987; Henningsen et al. 1992; De Gucht et al. 1999; Ng et al. 1999; Yang et al. 2002; Morikawa et al. 2005; Amati et al. 2010), male shift workers (Meijiman et al. 1995), blue-collar workers (Kawakami et al. 1997; Bosch et al. 2009), electric power plant workers (Nakata et al. 2000; Nakata et al. 2002), athletes (Gleeson 2000), police officers (Piercecchi-Marti et al. 1999), and air traffic controllers (Zeier et al. 1996).

However, the relationships between job stressors and the level of immune-biomarkers are not consistent. For example, the associations reported between role-stress and nonparticipation in decision making among nurses and increased level of IgA and IgG, respectively (Endresen et al. 1987), was not replicated in the following studies that reported inverse association between self-reported levels of stress and the level of both salivary IgA (Ng et al. 1999; Yang et al. 2002) or lysozyme (Yang et al. 2002). Although the relationship between stress level (i.e., quantitative work load and a conflict with physicians) and decreased natural killer (NK) cell activity among nurses has been reported by Morikawa et al. (2005), another study could not find strong association (Ng et al. 1999). A recent longitudinal study conducted for nurses (Amati et al. 2010) has suggested the association between job satisfaction and decreased low cell numbers of CD8(+) suppressor T cells, CD8(+)–CD57(+) activated T cells, CD56(+) NK cells, and low IL-6 levels.

In terms of other occupational groups, low reward, high effort–reward imbalance, and low social support at work were affected with a significantly lower CD4:CD8 ratio among blue-collar workers (Bosch et al. 2009). The working sessions caused a marked increase in the concentration and secretion rate of salivary immunoglobulin A (sIgA), as well as in the concentration of salivary cortisol among air traffic controllers (Zeier et al. 1996). Higher job strain decreased the number of CD4+CD45RA+ T lymphocytes in young male Japanese workers aged 20–39, but increases serum IgG concentrations (Nakata et al. 2000), while lower job control is associated with a decrease in the number of CD4+CD45RO+ T lymphocytes in male middle-aged workers aged 40–60 (Nakata et al. 2002). Meijiman et al. (1995) reported inverse correlations between job demand and CD4+ T lymphocytes and between job control and CD4+ and CD8+ T lymphocytes in male shift workers.

The hypothalamic–pituitary–adrenal (HPA) and the sympathetic–adrenal–medullary (SAM) axes are the two major pathways through which immune function can be altered by stress (Padgett and Glaser 2003). Lymphocytes, monocytes or macrophages, and granulocytes have receptors for many neuroendocrine products of the HPA and SAM axes such as cortisol and catecholamine, respectively, which can induce changes in cellular trafficking, proliferation, cytokine secretion, antibody production (e.g., IgG, IgM, and IgA) and cytolytic activity. Recent studies suggest that chronic stress alters patterns of cytokine secretion to cause the Th1-to-Th2 shift in immune system (Iwakabe et al. 1998; Marshall et al. 1998; Salak-Johnson and McGlone 2007). According to this hypothesis, chronic stress suppress Th1 cytokines such as IL-1, IL-2, IFN-γ, and TNF activating cellular immunity to provide defense against many kinds of infection and some kinds of neoplastic disease, whereas enhance Th2 cytokines such as IL-4, IL-5, IL-10 and IL-13 activating humoral immunity and exacerbate allergy and many kinds of autoimmune disease. Thus, it is biologically plausible that job stress may change the number of lymphocyte subpopulations, lymphocytic proliferation, NK cell activity, and the level of neuroendocrine products, cytokines, and immunoglobulins.

To better understand the effect of job stress on the immune system, we designed and conducted a longitudinal study with repeated measures of multiple immuno-biomarkers. Most previous studies utilized cross-sectional design, too few data points to take temporal variability into account, and measured only several immuno-biomarkers. Thus, more well-designed studies with repeated measures and multiple biomarkers are required to elucidate the causal relationship between job stress and immunological response.

In this context, this exploratory longitudinal study has been designed to adapt multiple measures of extensive immune-biomarkers in the evaluation of the effect of job stress. Cellular and humoral immuno-biomarkers have been selected with consideration of biological mechanisms, previous studies reported, and experimental methods

Table 1 The criteria and data by which the nurses were grouped into high and low stress groups (Douglas 1992)

Variable	Objective (nursing unit based data)	Subjective (individual based data)
Death and dying	Actual count of patients death per nursing unit	Score from 14-item scale (Numerof and Abrams 1984; Grey-Toft and Anderson 1981) assessing stress associated with caring for terminal patients
Work load	Data on staffing patterns and the case mix index	Score from Caplan (1975) scale (4 items) assessing speeds, quantity, and pace of work
Decision and control	Median score of the Greenberger (1981) scale (16 items) from the head nurses and supervisors for that nursing unit	Score from Greenberger (1981) scale (16 items) assessing control over the variety of tasks performed, content of the work, performance of others, decision making in the work setting, etc.
Interpersonal relations	Median score of the Rahim (1983) scale (16 items) from the head nurses and supervisors for that nursing unit	Score from Rahim (1983) scale (16 items) assessing intragroup and intergroup conflict

available, and were evaluated for the association with job stress in nurses who are considered to be under particularly high stress. The choice of job stressors was based on research in the fields of occupational stress and nursing; Quantitative work load, quality of interpersonal relationship and control (job decision latitude) are supported in both fields of research as domains related to job stress (Celentano and Johnson 1987). The specific contribution from the field of nursing is the stressor of dealing with factors related to death and dying (Hay and Oken 1972; Grey-Toft and Anderson 1981; Numerof and Abrams 1984). Considering that several reviews of the workplace stress literature have called for studies which collect both objective and subjective measures (Jick and Burke 1982; Payne et al. 1982; Kasl 1986; Hurrell et al. 1998), selected job stressors were partitioned into objective and subjective components using a modified version of the model developed by the National Institute for Occupational Safety and Health (NIOSH) (Douglas 1992).

Methods

Subject Selection

Source population

We selected nurses as our study subjects considering that nurses' job stress level is relatively higher while job satisfaction is lower compared to other occupational group, and that nurses are easy to access for recruitment and to collect biological samples and questionnaire data. The source population consisted of nurses working at a major university hospital. A total of 46 in-patient nursing units (unit size: 8–83 nurses) were classified into high (≥median) and low (<median) objective stress work areas based on the sum of scores of the objective criteria (death/dying, work load, decision/control, and interpersonal relations) (Table 1). The scale of actual data for the four job stressor categories were adjusted to have the same maximum scores (i.e., 100) to have equal weight, and then summed up to generate sum of scores.

Detailed explanations for job stress scales

Detailed explanations for the indices and scales for each job stressor are available in Douglas (1992). Objective data were computer data on staffing, patient status, and the number of patient deaths maintained by the hospital and ratings from the nurse managers and shift coordinators. (1) Death/dying: Objective data for statistics related to death and dying was the actual count of patient deaths per nursing unit from January to September 1990. (2) Work load: The objective measure of work load was determined by two separate measures; staffing patterns and the case mix index. If a unit has the designated number of staff working during a shift, the staffing number for that period of time would be "0", indicating that the staffing requirement had been met. Case mix was a proxy measure for how much ill the patients were on each unit. Case mix represented the utilization of resources by the units' patient population. (3) Decision and control: The scale for control over the content of work developed by Greenberger (1981) was given to the nurse manager of each nursing unit and the day and evening supervisor responsible for each functional unit. The objective measure of control for each nursing unit was the median of the ratings from them. (4) Interpersonal relations: The objective measure for interpersonal relations was the median score from the Rahim (1983) scale. The objective score for each nursing unit was determined by the median score from the nurse managers and supervisors.

Subjective data for the four domains were collected in a questionnaire given to all nurses in the population sample. (1) Death and dying: Numerof and Abrams (1984) and

Grey-Toft and Anderson (1981) developed scales assessing the frequency and stress associated with caring for terminal patients, discussing issues of death and dying with patients and families, and participation in emergency situations resulting in death. The internal constancy of the Numerof and Abrams (1984) scale was 0.89, and exceeded 0.70 for the Grey-Toft and Anderson (1981). (2) Work load: The subjective measures of work load was determined by Caplan's scale (1975) containing four items assessing speed, quantity, and pace of work rated on a five-point Likert scale. This scale has an internal consistency of 0.85. (3) Decision and control: This was determined using a sixteen-item scale developed by Greenberger (1981) assessing control over the variety of tasks performed, influence over the hours worked, content of the work, performance of others, decision making in the work setting, etc. This scale, measured on a five-point Likert scale ranging from "very little" to "very much", has an internal consistency of 0.90. (4) Interpersonal relations: The subjective measure was a sixteen-item scale of Conflict at Work (Rahim 1983) measured on a five-point Likert scale from "strongly disagree" to "strongly agree". The scale is partitioned into a measure of intra-group conflict with an internal consistency of 0.86, and intergroup conflict with an internal consistency of 0.85.

Classification of each job stress group and selection of subjects

Among registered nurses at the hospital ($n = 1{,}043$), 514 nurses (response rate: 49%) participated in a voluntary job stress survey providing the individual based data (Numerof and Abrams 1984; Grey-Toft and Anderson 1981; Caplan et al. 1975; Greenberger 1981; Rahim 1983) used for subjective job stress classification purposes (see Table 1). Based on the sum of scores of the four subjective criteria, the subjects were categorized into high (≥median) and low (<median) subjective stress group. Nurses were further classified based upon their agreement with the objective classification of the work unit (Greenberger 1981; Rahim 1983), for use in developing high objective low subjective and low objective high subjective groups. In all, four groups of nurses were identified: high objective/high subjective stress, low objective/high subjective stress, high objective/low subjective stress, and low objective/low subjective stress group. In this pilot study, we decided to select ten volunteers, approximately 10% from each group, as non-random sample. All the procedures of this study were reviewed and approved by Johns Hopkins University School of Medicine, and all subjects gave informed consents prior to participate in this study.

Subjects were asked not to volunteer if they had conditions which likely affect their immune systems such as rheumatoid arthritis, thyroid disorder, cancer, systemic lupus erythematosus (SLE), myasthenia gravis, Graves' disease, scleroderma, or infection with HIV. This initial self-screening was verified as part of the administration of health status questions in the initial questionnaire to the 40 accepted volunteers.

All but one of the forty female subjects remained in the study throughout the study period (December to August) and when she left her job, she was replaced by another nurse from the same group. Thus, data were actually collected on 41 subjects. Even though one participant dropped out and was replaced by another nurse, they were treated as two different subjects and analyzed as such.

All of the nurses in this study were premenopausal women aged between 22 and 43 (Table 2). The mean age was 29.9 and median age was 28.

Blood and saliva sampling

Blood and saliva samples were collected at least 1 h after meals for each of the nurses. Venipuncture was performed monthly at the hospital by a phlebotomist. Approximately 23 cc of blood was drawn from the antecubital site by vacutainer containing heparin anticoagulant. After centrifugation, cells and serum were separated and aliquots of lymphocytes and sera were evaluated with a panel of immunologic tests.

Whole saliva samples were collected weekly for 5 min into sterile 15 ml conical plastic tubes; the samples were stored at −80°C and shipped on dry ice in weekly batches to the NIOSH laboratory for analyses.

Enrolment of all subjects did not initially take place in the same week, but instead took place over the course of several weeks with the result that the first and successive weeks were not identical to the same calendar weeks for all subjects. It was decided, after the study had started, that it would be a stronger design to have data collection and calendar weeks as the same for all subjects, so at 6 weeks were made to conform.

Immunological measurements

All analyses were conducted blinded to stress group of each subject.

Natural killer (NK) cell cytolytic assay

An in vitro cytotoxicity assay using 51Cr-labeled Yac-1 cells was used. Splenocytes were adjusted to 1×10^7 cells/p ml in complete medium (RPMI, 10% fetal calf serum, 50 IU penicillin, and 50 μg streptomycin). After 4-h incubation at 37°C and 5% CO_2, 25 μl of supernatant was transferred to a 96-well plate containing

Table 2 Selected characteristics of subjects [*n* (%)]

Variable	Category	All	Objective stress		Subjective stress	
			Low ($n = 20$)	High ($n = 21$)	Low ($n = 20$)	High ($n = 21$)
Age	22–29	21 (55)	9 (45)	12 (67)	9 (50)	12 (60)
	30–43	17 (45)	11 (55)	6 (33)	9 (50)	8 (40)
Smoking	No	27 (71)	13 (65)	14 (78)	12 (67)	15 (75)
	Yes	11 (29)	7 (35)	4 (22)	6 (33)	5 (25)
Marital status	Married	19 (50)	10 (50)	9 (50)	8 (44)	11 (55)
	Single/never married	16 (42)	8 (40)	8 (44)	9 (50)	7 (35)
	Single/divorced	1 (3)	1 (5)	–	–	1 (5)
	Living together	2 (5)	1 (5)	1 (6)	1 (6)	1 (5)
Currently taking medicines at baseline	No	14 (28)	8 (42)	6 (33)	12 (67)	2 (11)
	Yes	23 (62)	11 (58)	12 (67)	6 (33)	17 (89)
Oral contraceptive use	No	26 (72)	14 (74)	12 (71)	12 (71)	14 (74)
	Yes	10 (28)	5 (26)	5 (29)	5 (29)	5 (26)
Family history of immune system disease	No	36 (95)	20 (100)	16 (89)	17 (94)	19 (95)
	Yes	2 (5)	–	2 (11)	1 (6)	1 (5)
Job title	Graduate/clinical nurse	21 (57)	9 (47)	12 (67)	12 (67)	9 (47)
	Clinical nurse masters/senior clinical nurse	16 (43)	10 (53)	6 (33)	6 (33)	10 (53)
Work shift	Rotating, 8 h	14 (36)	10 (50)	4 (22)	4 (22)	10 (50)
	Rotating, 12 h	9 (24)	1 (5)	8 (44)	5 (28)	4 (20)
	Permanent day	4 (10)	3 (15)	1 (6)	3 (16)	1 (5)
	Permanent evening	1 (3)	1 (5)	–	1 (6)	–
	Permanent night	1 (3)	–	1 (6)	–	1 (5)
	Other	9 (24)	5 (25)	4 (22)	5 (28)	4 (20)
Hours worked per week	30–35	4 (11)	2 (11)	2 (11)	3 (17)	1 (5)
	36–40	31 (84)	15 (78)	16 (89)	14 (78)	17 (90)
	41–45	2 (5)	2 (11)	–	1 (5)	1 (5)
Hours worked overtime per week	0	16 (42)	6 (30)	10 (56)	11 (61)	5 (25)
	1–5	18 (47)	12 (60)	6 (33)	5 (28)	13 (65)
	6–10	4 (11)	2 (10)	2 (11)	2 (11)	2 (10)
Job continuity at current job (year)	≤2	11 (29)	7 (35)	4 (22)	7 (39)	4 (20)
	≤4	11 (29)	5 (25)	6 (33)	5 (28)	6 (30)
	≤10	11 (29)	4 (20)	7 (39)	4 (22)	7 (35)
	10>	5 (13)	4 (20)	1 (6)	2 (11)	3 (15)

Note: Total 38 participated in the first survey for subject characteristics

solid scintillant. Plates were air dried overnight, and then, after a 10-min dark delay on the Packard Top Count, counted for 5 min. The results are expressed in lytic units per 10^7 splenocytes using 10% lysis as the reference point.

Number of white blood cells (WBC)

Number of white blood cells including T lymphocytes (CD3, CD4, CD8, CD4/CD8 ratio), B lymphocytes (CD20) and natural killer cells (CD56) were measured by immunofluorescence staining and flow-cytometry analysis.

Lymphocytic proliferation to mitogens or toxoid

Lymphocytic proliferation to mitogens (concanavalin A, phytohemagglutinin, and pokeweed) and toxoid (tetanus) was measured by radioimmunoassay. Peripheral blood T lymphocyte cells were separated by Ficoll-Paque density-gradient centrifugation, and washed in RPMI 1640. [3H] TdR incorporated into cells was measured using a liquid scintillation counter (Packard, Meriden). The results were expressed as average cpm. Based on cpm numbers obtained, the delta-cpm was calculated as cpm incubated with mitogen or toxoid minus 3-day (phytohemagglutinin

and concanavalin A) or 5-day (pokeweed mitogen and tetanus toxoid) control cpm.

Serum levels of cytokines: hydrocortisol, IL-1β, IFN-γ, and TNF-α

Serum levels of hydrocortisol, IL-1β, IFN-γ, and TNF-α were measured by enzyme-linked immunosorbent assay (ELISA) according to manufacturer's manual (R&D Systems, Minneapolis, MN).

Salivery IgA

Saliva samples were analyzed in a "blind" fashion for concentrations of total secretory IgA (sIgA) and end-point titers of specific sIgA against five combined strains of *E. coli* cell wall antigens [lipopolysaccharides (LPS)] adjusted for total protein concentration (mg/ml), and salivary flow rate (ml/min). A modified ELISA method was used to measure both total and specific sIgA antibodies in the whole saliva samples. Detailed procedures were described in previous study (Henningsen et al. 1992).

Statistical analyses

Each individual had up to 9 data points for immunological measures of blood and up to 33 data points for immunological measures of saliva and weekly psychosocial variables. For salivary measures data, all measurements from week 1 to 5 were excluded from the analysis because of a change in procedure that was implemented after the 5th week of the study. However, all data were used for monthly immunological measures. Immunological data collected were analyzed by repeated measures analysis of variance (ANOVA) controlling for age and smoking to evaluate if mean values are different between low and high objective groups, or low and high subjective groups. In the model of repeated measures ANOVA, time has been included as one of explanatory variables (1, 2, 3, 4, 5, 6, 7, 8, and 9 for monthly measurement, 1, 2, 3,…, and 28 for weekly measurement). When the distribution of response variable deviates from normal distribution, transformation such as log transformation was performed to have normal distribution, and then tested for its statistical significance. Repeated measures ANOVA were conducted using Statistical Analysis Software (SAS v. 9.1) with the procedure of Proc Mixed with proper covariance matrix option. Selected results of repeated measures ANOVA were represented as graphs showing the variation over time with mean values and standard errors in the form of error bars. Additional adjustment for other covariates such as currently taking medicines at baseline was conducted as sensitivity analysis.

Given this was a pilot study, we considered the results with statistical significance of 0.1 as noteworthy, and decided not to consider the probability of false positive associations.

Results

Among the subjects, current smokers were 29%, and average number of cigarettes smoked per day was 2. Half of them were married, while most of the rest were single and had never been married. With regard to job characteristics, Table 2 shows that half of the subjects were clinical nurses, and most of the rest were senior clinical nurses. Only three were working part-time, while the others were all working full-time. The majority of the nurses worked some amount of overtime during a typical week. The mean time that the subjects had been at their current job was about four and a half years.

The levels of cellular (Table 3) and humoral immuno-biomarkers (Table 4) were compared by objective and subjective stress groups. In terms of cellular immuno-biomarkers, the level of white blood cells was lower among high objective stress group (median, 7,170/m^3; range, 5,386–10,057) compared with that among low objective stress group (8,063; 5,888–9,875) ($P = 0.03$), however, no other cellular blood variables were found to be significant. In terms of humoral immuno-biomarkers, the level of TNF-α was moderately lower among high objective stress group (1.7 ng/ml; 0.3–2.7) compared with that among low objective stress group (2.2; 0.5–3.5) ($P = 0.07$), whereas the level of total sIgA was significantly higher among higher objective stress group (72.9 end-point titer/mg/ml/min; 14.4–153.4) compared with that among low objective stress group (44.8; 9.9–123.8) ($P = 0.02$). However, no significant association was found with regard to subject stress. Significant results were represented graphically in Fig. 1.

Additional adjustment for other covariates such as currently taking medicines at baseline tended to attenuate the statistical significances, however, the results remained similar.

Discussion

The results of this study suggest that psychological job stress is related to several immuno-biomarkers such as WBC, TNF-α, and total salivary IgA.

In the present study, the number of WBC was reduced in objectively high stress group compared to low stress group, while there was no effect on the level of lymphocytes (i.e., T cells, B cells, and NK cells). Thus, lower levels of WBC

Table 3 Summary statistics of cellular immuno-biomarkers by objective and subjective stress group

		Objective stress				P^a	Subjective stress				P^a
		Low ($n = 20$)		High ($n = 21$)			Low ($n = 20$)		High ($n = 21$)		
		Median	Range	Median	Range		Median	Range	Median	Range	
White blood cells	n^b	8,063	5,888–9,875	7,170	5,386–10,057	0.03	7,725	6,500–10,057	7,346	5,386–9,875	0.65
Lymphocytes	n^b	2,438	1,863–3,523	2,508	1,701–3,848	0.84	2,522	1,907–3,848	2,438	1,701–3,523	0.82
T cells (CD3)	n^b	1,678	1,085–2,526	1,817	1,203–3,214	0.91	1,752	1,085–3,214	1,778	1,160–2,526	0.92
Helper T–cell (CD4)	n^b	1,326	676–1,867	1,241	726–2,352	0.87	1,241	680–2,352	1,326	676–1,806	0.86
Suppressor T–cell (CD8)	n^b	512	240–794	514	324–805	0.72	541	304–805	497	240–687	0.33
CD4/CD8 ratio		2.4	1.1–5.6	2.5	1.5–4.6	0.93	2.5	1.1–5.1	2.6	1.4–5.6	0.46
B cell (CD20)	n^b	383	223–665	353	236–489	0.51	371	239–546	364	223–665	0.59
Natural killer cell (CD56)	n^b	179	63–326	147	67–271	0.31	171	63–326	140	67–274	0.68
NK cell activity	Lytic units[c]	31.7	4.9–157.5	49.1	25.2–104.3	0.91	52.3	4.9–157.5	39.1	16.1–100.1	0.31
Lymphocytic proliferation to mitogens or toxoids											
Concanavalin A	Delta-cpm[d]	6,112	2,016–18,363	7,493	2,362–12,153	0.96	6,190	2,296–12,293	6,861	2,016–18,363	0.93
Phytoheamagglutinin	Delta-cpm[d]	13,980	6,475–21,966	13,605	5,565–29,935	0.99	13,417	5,565–21,966	15,122	6,475–29,935	0.92
Pokeweed	Delta-cpm[d]	5,700	3,370–11,673	6,419	2,473–12,472	0.53	6,271	2,473–12,472	5,554	3,504–11,673	0.11
Tetanus toxoid	Delta-cpm[d]	2,682	365–9,392	3,462	357–20,056	0.12	3,170	563–20,056	2,583	357–12,870	0.26

[a] Calculated by repeated measures ANOVA (analysis of variance) adjusting for age and smoking

[b] Number of cells per mm^3

[c] Lytic units per 10^7 splenocytes using 10% lysis as the reference point

[d] Delta-cpm was calculated as cpm incubated with mitogen or toxoid minus 3-day (phytohemagglutinin and concanavalin A) or 5-day (pokeweed mitogen and tetanus toxoid) control cpm

Table 4 Summary statistics of humoral immuno-biomarkers by objective and subjective stress group

		Objective stress				P^a	Subjective stress				P^a
		Low ($n = 20$)		High ($n = 21$)			Low ($n = 20$)		High ($n = 21$)		
		Median	Range	Median	Range		Median	Range	Median	Range	
Hydrocortisol	ng/ml	25.2	16.9–52.3	27.0	15.2–52.3	0.98	27.9	16.9–52.3	23.8	15.2–37.1	0.18
IL1β	ng/ml	427.3	100–2,403	446.9	116.7–1,254	0.12	411.6	100–1,743.1	486.6	200–2,403	0.50
IFN–γ	ng/ml	19.2	5.1–60.6	11.4	2.4–48.8	0.12	15	2.6–48.8	13.4	2.4–60.6	0.74
TNF–α	ng/ml	2.2	0.5–3.5	1.7	0.3–2.7	0.07	2	0.3–3.2	2	0.5–3.5	0.45
Total sIgA	EP/mg/ml/min[b]	44.8	9.9–123.8	72.9	14.4–153.4	0.02	56.7	14.4–153.4	42.2	9.9–145.8	0.58
Specific sIgA	EP/mg/ml/min[b]	58.2	8.8–372.1	54.7	23.3–212.2	0.31	49.8	8.8–209.7	67.6	23.7–372.1	0.16

[a] Calculated by repeated measures ANOVA (analysis of variance) adjusting for age and smoking

[b] Endpoint titers of IgA corrected by protein amount (mg/ml) and volume (ml/min)

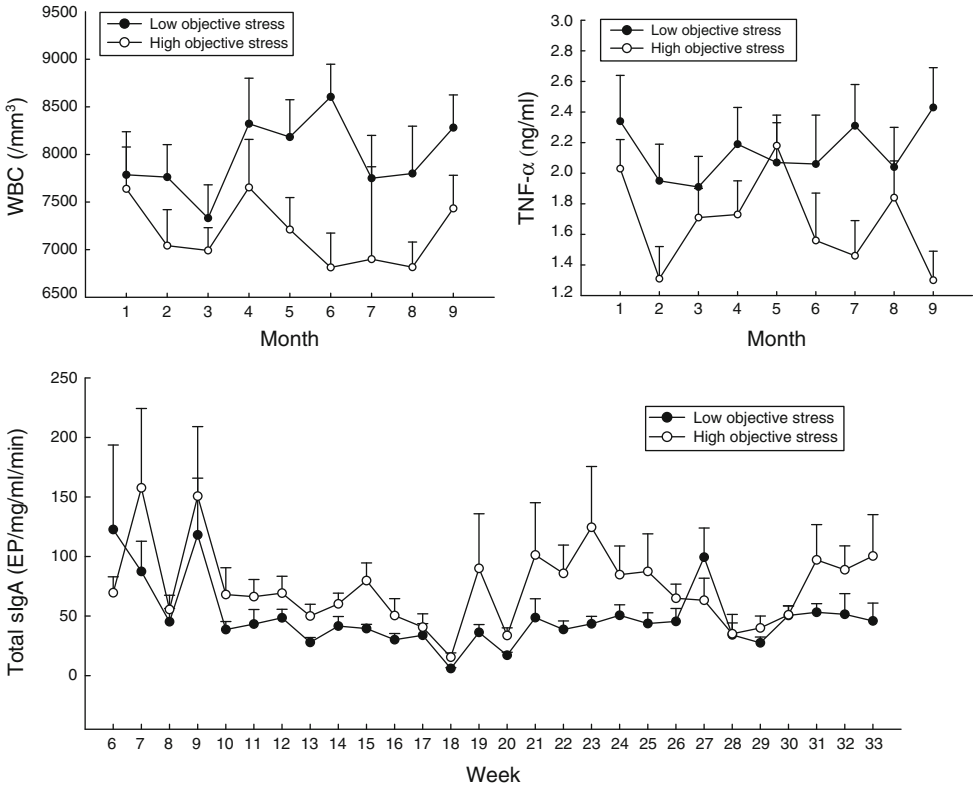

Fig. 1 Selected immuno-biomarkers and job stress in nurses were represented with *error bars* showing the standard errors at each data points. Repeated measures ANOVA adjusting for age and smoking showed that high objective stress was associated with lower level of white blood cells ($P = 0.03$), TNF-α ($P = 0.07$), and total salivary IgA ($P = 0.02$)

might be largely due to reduction of granulocytes, which are the most common white blood cells and participate in innate immunity. Reduction of granulocytes in this study may be possibly interpreted as Th1-to-Th2 shift by chronic stress repressing the production of proinflammatory cytokines. However, further study is warranted given the contrasting results that cutting whiskers of mice increased the granulocytes/lymphocytes ratio (Wang et al. 2007) and that the average WBCs were associated with driving time in taxi drivers (Chen et al. 2005).

In terms of T lymphocytes, Scanlan et al. (1998) reported that caregiver men had fewer CD4+ and CD8+ cell counts than did control men, but not in women, suggesting that gender difference might be considered in interpreting the results of cellular immuno-biomarkers. Given that the difference in subpopulations of CD4+ or

CD8+ have been reported by the level of job stress among nurses, 72% of whom were women (De Gucht et al. 1999), subpopulations also need to be considered.

No association was found between job stress and NK cell population among nurses, or activity in this study was inconsistent with the result of previous studies in which professional stress in nurses reduced the population of NK cells (De Gucht et al. 1999) or NK cell activity (Morikawa et al. 2005). Meta-analysis has also showed a decrease in NK cell activity with chronic life stress (Herbert and Cohen 1993; Zorrilla et al. 2001). There were no significant results for a number of subpopulations of lymphocytes including NK cells in this study, which could be partly explained by small number of subjects. Thus, lager study is needed to elucidate whether the effect of job stress is immediate on the population of immune cells or not.

Our finding that the level of TNF-α, a major pro-inflammatory mediator (Bazzoni and Beutler 1996), was lower among high objective stress group compared with low stress group in this study might be interpreted according to the Th1-to-Th2 model. TNF-α is known to play a role as Th1 cytokine (Marshall et al. 1998), thus reduced level of TNF-α may favor the humoral immune function. Consistently, Amati et al. (2010) has recently reported that the levels of TNF-α best reflected the change of psychological stress among nurses. We note that the same pattern was found for IFN-γ, i.e., another Th1 cytokine, though it was not statistically significant in our study, which was also consistent with Amati et al. (2010). However, further investigations are needed to evaluate whether TNF-α best reflects job stress among nurses or not, and to elucidate the underlying molecular mechanisms.

The results of this study show that sIgA might be a useful immuno-biomarker for job stress. The humoral components of the immune system, which defend the body against invading bacteria and other toxic substances, involves the production and release by lymphocytes of five types of immunoglobulins (IgA, IgG, IgD, IgE, and IgM). Among these, IgA plays a particularly important role in protecting the body against upper respiratory infections. The collection of saliva in a field setting as a basis for examining immune functioning effects seems particularly workable and relatively non-invasive, encouraging its greater use in such studies, although the variation was relatively large. The result of salivary IgA in this study is consistent with Henningsen et al. (1992) which reported that as the job stress in nurse increase, secretion of salivary IgA also increased. Endresen et al. (1987) also found the positive relationship between nurses' job stress (i.e., role-stress and nonparticipation in decision making) and the level of serum IgA. However, other studies conducted by Yang et al. (2002) and Ng et al. (1999) reported contrasting results: i.e., among the nurses in emergency department or surgical wards/operating theaters, the levels of salivary IgA and lysozyme were lower compared with those of the nurses in general department. Given that chronic stress may cause Th1-to-Th2 model shift in immune system (Iwakabe et al. 1998; Marshall et al. 1998; Salak-Johnson and McGlone 2007), increased level of salivary IgA among high objective stress group could be explained by enhanced Th2 cytokines activating humoral immunity. In addition, the level of salivary IgA was corrected for total protein concentration, and salivary flow rate in our study. The effect of job stress on the level of IgA needs to be investigated with the consideration of accurate measurement of IgA and exact assessment of job stress.

It is noteworthy that the differences were observed when comparing objective groups instead of subjective groups. Due to the variation in subjective feeling, objective grouping could be more indicative as the chronic status of job stress. The stress can be caused by an environmental condition which can be objectively defined and measured, but a subjective perception or appraisal of such an objective environmental condition may be different. Previous studies have favored the operational definition of stress as a subjectively appraised condition. However, several reviews of the workplace stress literature have called for collecting both objective and subjective measures, with the pairing of subjective measures with objectively established environmental conditions (Jick and Burke 1982; Payne et al. 1982; Kasl 1986; Hurrell et al. 1998). To elucidate the chronic effect of job stress, sufficient sample size, repeated measures, comprehensive immuno-biomarkers, and more accurate measurement of job stress is required to be conducted.

The strengths of this study are that the number of data points over time is larger than previous studies, and a relatively large number of assays were conducted on the multiple specimens of blood obtained from subjects. However, the result of this study should be interpreted cautiously. Limitations of our study include small number of subjects, not measuring the immunoglobulins other than IgA, missing data points, etc. Our study may have not found significant difference due to the lack of statistical power, either. Potential bias in terms of subject selection and confounding by uncontrolled variables should also be taken into account. Even though we collected samples within 1 h after meals, measurement bias may have occurred due to uncontrolled exact sampling time. Also, our subjects may not represent any larger group of people, due to a convenient sampling and low response rate. Therefore, it is important to attempt to replicate the findings in other studies of job stress and immunological biomarkers.

In conclusion, this exploratory longitudinal study with repeated measures of multiple immunological biomarkers

suggests that job stress of nurse is related to different levels of WBC, TNF-α, and total salivary IgA. However, more well-designed study with large sample size, multiple data points in time, and multiple immuno-biomarkers need to be conducted.

Acknowledgments The study was actually designed by NIOSH (Joe Hurrell). Data for this study were collected under contract with Johns Hopkins University. Analyses of the study data for the current manuscript were conducted by investigators at the Seoul National University (first six authors) in collaboration with NIOSH. The authors would like to thank Drs. Charlene Douglas, Jerry Henningsen and Frank Baker for their invaluable contributions to this manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

References

Amati M, Tomasetti M, Ciuccarelli M, Mariotti L, Tarquini LM, Bracci M, Baldassari M, Balducci C, Alleva R, Borghi B, Mocchegiani E, Copertaro A, Santarelli L (2010) Relationship of job satisfaction, psychological distress and stress-related biological parameters among healthy nurses: a longitudinal study. J Occup Health 52(1):31–38

Arnetz BB, Brenner SO, Levi L, Hjelm R, Petterson IL, Wasserman J, Petrini B, Eneroth P, Kallner A, Kvetnansky R (1991) Neuroendocrine and immunologic effects of unemployment and job insecurity. Psychother Psychosom 55(2–4):76–80

Bazzoni B, Beutler B (1996) Seminars in medicine of the Beth Isreal Hospital, Boston: The tumor necrosis factor ligand and receptor families. N Engl J Med 334(26):1717–1725

Bosch JA, Fischer JE, Fischer JC (2009) Psychologically adverse work conditions are associated with CD8+ T cell differentiation indicative of immune senescence. Brain Behav Immun 23(4):527–534 (Epub 2009 Feb 13)

Caplan RD, Cobb S, French JP, Van Harrison R, Pinneau SR (1975) Job demands and worker health. Contract No. HSM-99-72-61, Institute for Social Research, University of Michigan

Celentano DD, Johnson JV (1987) Stress in health care workers. Occup Med 2(3):593–606

Chen JC, Chen YJ, Chang WP, Christiani DC (2005) Long driving time is associated with haematological markers of increased cardiovascular risk in taxi drivers. Occup Environ Med 62(12):890–894

De Gucht V, Fishler B, Dmanet C (1999) Immune dysfunction associated with chronic professional stress in nurses. Psychiatry Res 85:105–111

Douglas CY (1992) Work environment, cognitive appraisal, social support and occupational stress. Unpublished doctoral dissertation, Johns Hopkins University School of Hygiene and Public Health, Baltimore

Endresen IM, Værnes R, Ursin H, Tønder O (1987) Psychological stress factors and concentration of immunoglobulins and complement components in Norwegian nurses. Work Stress 1:365–375

Gleeson M (2000) The scientific basis of practical strategies to maintain immunocompetence in elite athletes. Exerc Immunol Rev 6:75–101

Goodkin K, Feaster DJ, Tuttle R, Blaney NT, Kumar M, Baum MK, Shapshak P, Fletcher MA (1996) Bereavement is associated with time-dependent decrements in cellular immune function in asymptomatic human immunodeficiency virus type 1-seropositive homosexual men. Clin Diagn Lab Immunol 3(1):109–118

Greenberger DB (1981) Personal control at work: its conceptualization and measurement. Technical report 1–1–4, NR170-892, Office of Naval Research, University of Wisconsin-Madison, Madison, WI, USA

Grey-Toft P, Anderson J (1981) The nursing stress scale: development of an instrument. J Behav Assess 3:11–23

Hay D, Oken D (1972) The psychological stresses of intensive care unit nursing. Psychosom Med 34(2):109–118

Henningsen GM, JJ Hurrell Jr, Baker F, Douglas C, MacKenzie BA, Robertson SK, Phipps FC (1992) Measurement of salivary immunoglobulin A as an immunologic biomarker of job stress. Scand J Work Environ Health 18(Suppl 1):133–136

Herbert TB, Cohen S (1993) Stress and immunity in humans: a meta-analytic review. Psychosom Med 55(4):364–379

Hurrell JJ Jr, Nelson DL, Simmons BL (1998) Measuring job stressors and strains: where we have been, where we are, and where we need to go. J Occup Health Psychol 3:368–389

Irwin M, Brown M, Patterson T, Hauger R, Mascovich A, Grant I (1991) Neuropeptide Y and natural killer cell activity: findings in depression and alzheimer caregiver stress. FASEB J 5:3100–3170

Iwakabe K, Shimada M, Ohta A, Yahata T, Ohmi Y, Habu S, Nishimura T (1998) The restraint stress drives a shift in Th1/Th2 balance toward Th2-dominant immunity in mice. Immunol Lett 62(1):39–43

Jemmott JB III, Magloire K (1988) Academic stress, social support, and secretory immunoglobulin A. J Pers Soc Psychol 55(5):803–810

Jick TD, Burke RJ (1982) Occupational stress: recent findings and new directions. J Occup Behavior 3:1–3

Kasl SV (1986) Stress and disease in the workplace: a methodological commentary on the accumulated evidence. In: Cataldo MF, Coastes TJ (eds) Health and industry: a behavioral medicine perspective. Wiley, New York, pp 52–85

Kawakami N, Tanigawa T, Araki S, Nakata A, Sakurai S, Yokoyama K, Morita Y (1997) Effects of job strain on helper-inducer (CD4+CD29+) and suppressor-inducer (CD4+CD45RA+) T cells in Japanese blue-collar workers. Psychother Psychosom 66(4):192–198

Kiecolt-Glaser JK, Kennedy S, Malkoff S, Fisher L, Speicher CE, Glaser R (1988) Marital discord and immunity in males. Psychosom Med 50(3):213–229

Marshall GD Jr, Agarwal SK, Lloyd C, Cohen L, Henninger EM, Morris GJ (1998) Cytokine dysregulation associated with exam stress in healthy medical students. Brain Behav Immun 12(4):297–307

Meijiman TF, van Dormolen M, Herber RFM, Kuiper Symen (1995) Job strain, neuroendocrine activation, and immune status. In: Sauter SL, Murphy LR (eds) Organizational risk factors for job stress. American Psychological Association, Washington, DC, pp 113–126

Morikawa Y, Kitaoka-Higashiguchi K, Tanimoto C, Hayashi M, Oketani R, Miura K, Nishijo M, Nakagawa H (2005) A cross-sectional study on the relationship of job stress with natural killer cell activity and natural killer cell subsets among healthy nurses. J Occup Health 47(5):378–383

Nakata A, Araki S, Tanigawa T, Miki A, Sakurai S, Kawakami N, Yokoyama K, Yokoyama M (2000) Decrease of suppressor-inducer (CD4+ CD45RA) T lymphocytes and increase of serum immunoglobulin G due to perceived job stress in Japanese nuclear electric power plant workers. J Occup Environ Med 42(2):143–150

Nakata A, Tanigawa T, Fujioka Y, Kitamura F, Iso H, Shimamoto T (2002) Association of low job control with a decrease in memory (CD4+CD45RO+) T lymphocytes in Japanese middle-aged

male workers in an electric power plant. Ind Health 40(2):142–148

Ng V, Koh D, Chan G, Ong HY, Chia SE, Ong CN (1999) Are salivary immunoglobulin A and lysozyme biomarkers of stress among nurses? J Occup Environ Med 41(10):920–927

Numerof RE, Abrams MN (1984) Sources of stress among nurses: an empirical investigation. J Human Stress 10(2):88–100

Padgett DA, Glaser R (2003) How stress influences the immune response. Trends Immunol 24(8):444–448

Pariante CM, Carpiniello B, Orru MG, Sitzia R, Piras A, Farci AM, Del Giacco GS, Piludu G, Miller AH (1997) Chronic caregiving stress alters peripheral blood immune parameters: the role of age and severity of stress. Psychother Psychosom 66(4):199–207

Payne R, Jick TD, Burke RJ (1982) Whither stress research? An agenda for the 1980's. J Occup Behavior 3:131–145

Piercecchi-Marti MD, Leonetti G, Pelissier AL, Conrath J, Cianfarani F, Valli M (1999) Evaluation of biological stress markers in police officers. Med Law 18:125–144

Rahim MA (1983) Measurement of organizational conflict. J Gen Psychol 109:189–199

Salak-Johnson JL, McGlone JJ (2007) Making sense of apparently conflicting data: stress and immunity in swine and cattle. J Anim Sci 85(13 Suppl):E81–E88

Scanlan JM, Vitaliano PP, Ochs H, Savage MV, Borson S (1998) CD4 and CD8 counts are associated with interactions of gender and psychosocial stress. Psychosom Med 60(5):644–653

Segerstrom SC, Miller GE (2004) Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull 130(4):601–630

Wang L, Muxin G, Nishida H, Shirakawa C, Sato S, Konishi T (2007) Psychological stress-induced oxidative stress as a model of sub-healthy condition and the effect of TCM. Evid Based Complement Alternat Med 4(2):195–202

Yang Y, Koh D, Ng V, Lee CY, Chan G, Dong F, Goh SH, Anantharaman V, Chia SE (2002) Self perceived work related stress and the relation with salivary IgA and lysozyme among emergency department nurses. Occup Environ Med 59(12):836–841

Zeier H, Brauchli P, Joller-Jemelka HI (1996) Effects of work demands on immunoglobulin A and cortisol in air traffic controllers. Biol Psychol 42(3):413–423

Zorrilla EP, Luborsky L, McKay JR, Rosenthal R, Houldin A, Tax A, McCorkle R, Seligman DA, Schmidt K (2001) The relationship of depression and stressors to immunological assays: a meta-analytic review. Brain Behav Immun 15(3):199–226