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Abstract: Effects of Fatigue on Immune Function in Nurses Performing Shift Work: Makie NAGAI, *et al.* Department of Epidemiology and Public Health, Kanazawa Medical University—Objectives: We investigated the effects of fatigue on NK cell function and lymphocyte subpopulations in nurses performing shift work using a longitudinal design. **Methods:** Fifty-seven female nurses engaged in shift work at a hospital in Japan were selected for our study cohort. The hospital used a counterclockwise rotating three-shift system. Night shifts followed day shifts after a seven-hour interval. Immune parameters measured at the beginning of the day shift through to the end of the night shift were compared between two groups stratified by their level of fatigue. Statistical differences were evaluated after adjusting for baseline immune values and other demographic features. **Results:** Subjective feelings of fatigue increased progressively from the beginning of day shifts to the end of night shifts. From the beginning of day shifts to the end of night shifts, NK cell activity and $CD16^+CD56^+$ lymphocytes decreased, while $CD3^+$ and $CD4^+$ lymphocytes increased. The group with the greater increase in fatigue showed a larger decrease in NK cell activity and a larger increase in $CD4^+$lymphocytes when compared with the group reporting less fatigue. These findings did not change after adjusting for demographic factors and sleep hours. **Conclusion:** Our data suggest that shift work has deleterious effects on NK cell function and that the effects depend on the degree of fatigue. Proper management of shift work may lessen fatigue in workers and also ameliorate many health problems experienced by shift workers.
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The relationship between shift work and cancer has been a recent subject of interest. The International Agency for Research on Cancer (IARC) concluded that shift work that involves circadian disruption is a probable carcinogenic risk factor for humans (group 2A) for hormone-dependent cancers, such as breast cancer[1]. Among the IARC findings is that cancer risk increases due to the disturbance of biological circadian rhythms, which results in decreased secretion of melatonin and changes in hormonal secretion patterns and immunological function[2]. A reduction in melatonin production leads to immune suppression, including a reduction in the number of natural killer (NK) cells and cytotoxic lymphocytes and a decrease in proinflammatory cytokines[3].

There have been quite a few studies examining the effects of shift work on immunological function. Nakano *et al.*[4] reported that lymphocyte proliferation in shift workers was decreased compared with that of fixed-hour daytime workers in a wholesale market and manufacturing company. Okamoto *et al.*[5] also reported that natural killer (NK) cell activity in rotating shift workers was lower than in workers with fixed daytime schedules in a study of medical doctors. However, these studies utilized cross-sectional designs and a direct, causal relationship between shift work and immune function has not been demonstrated.

There are several laboratory studies that have used simulated night work to investigate the effects of partial or total sleep deprivation on immune function[6–11]. Both enhancing and suppressive effects on NK cell activity and lymphocyte subsets have been reported. It has been suggested that human subjects exposed to laboratory stressors exhibit transient increases in immune function, but that more intense and/or long-lived stressors impair the same measures of immune function[12]. Furthermore, both natural and specific immunity are impacted by chronic and acute stressors[13]. We hypothesized that

Table 1. Demographic characteristics of the nurses working in a Japanese psychiatric hospital

	n=57
Age (yr)	42.1 (8.4)
Years working as a nurse	20.5 (8.7)
Years of shift work	19.0 (8.4)
Position, manager/subordinate	15.8/84.2
Wards for patients with schizophrenia and schizophreniform disorder/dementia	50.9/49.1
Marital status, single/married	12.3/87.7
Cigarette smoking habits, current/former or never	5.3/94.7

Values are expressed as means (standard deviation) or percentages of participants in the category.

greater levels of sleep deprivation or circadian rhythm disruption will have a greater impact on immunity/numerous immune variables.

Based on this hypothesis, we used a longitudinal study design to investigate the effects of shift work-related fatigue level on immune function among nurses engaged in shift work. The impact of shift work as a stressor was measured by subjective feelings of fatigue. The relationship between increased levels of fatigue and changes in immune function during a 24-hour observational period including a night shift was examined. Immune function was evaluated by assessing NK cell activity and various T lymphocyte subpopulations that have been demonstrated to be important for anticancer immunity.

Methods

Subjects

This study was conducted in a public hospital for psychiatric disorders (400 beds, 150 nurses) in Ishikawa, Japan. One hundred and five nurses (28 males and 77 females) aged 22–59 participated in this study. Among them, 61 female nurses engaged in shift work were our study subjects. Four subjects who took medications known to affect the immune system (medications for allergy or rheumatism) were excluded, leaving 57 subjects remaining. All subjects were physically healthy and not suffering from overt infections. Table 1 presents demographic features of our study participants. The mean age was 42.1 yr (range of 23–59 yr, standard deviation (SD) of 8.4 yr), and the mean number of years of shift work was 19.0 (range of 0.5–39 yr, SD of 8.4 yr). The percentages of participants engaged in a managerial position, married and a current smoker were 15.8, 87.7 and 5.3%, respectively. This study was approved by the Ethics Committee for Epidemiologic Research at Kanazawa Medical University (Ishikawa Prefecture, Japan). All participants provided informed consent in order to participate in our study.

Survey

The target hospital used a counterclockwise rotating three-shift system. The ordinal order of each shift was day (08:30–17:15), night (00:30–09:15), evening (16:30–01:15) and off. The interval between day shifts and night shifts was approximately seven hours. Taking a nap during night shifts was not officially permitted.

Blood samples were collected from subjects on two consecutive mornings. Sampling was carried out at the beginning of the daytime shift on the first day (8:00–9:00), and the second day's sample was collected at the end of the night shift (9:00–10:00). The participants were not allowed to smoke, eat or move vigorously within 30 minutes of blood sampling. Twelve-hour presampling sleep diaries and questionnaires exploring each subject's fatigue immediately before and immediately after each shift were completed by each study participant.

NK cell activity and lymphocyte surface antigens were assayed by BML Inc. (Tokyo, Japan). NK cell activity was measured by a standard chromium release assay[14]. The effector: target (E : T) ratios were 10 : 1 and 20 : 1. Percent cytotoxicity was calculated for each E:T ratio. NK cells were measured by two-color flow cytometry using anti-CD16 and anti-CD56. Single-color flowcytometry was used to assay for lymphocytes expressing CD3, CD4, and CD8 to measure mature T cells, helper T cells, and cytotoxic T cells, respectively.

Fatigue was evaluated at four time points (the beginning of day shift, the end of day shift, the beginning of night shift and the end of night shift) by a questionnaire for work-related fatigue feelings, the "Jikaku-sho shirabe" designed by the Industrial Fatigue Research Committee of the Japan Society of Occupational Health[15, 16]. This questionnaire was developed to evaluate trends in feelings of fatigue due to work, and factorial validity and internal consistency have been verified[17]. The questionnaire consists of 25 subjective fatigue symptom items that are categorized into five factors with five items in each: "drowsiness" indicating sleepiness, "instability" indicating mental fatigue, "uneasiness" indicating symptoms like autonomic imbalance, "local pain or dullness" measuring physical complaints, and "eyestrain" indicating asthenopia. For each item, respondents were requested to estimate the

Table 2. Changes in subjective feelings of fatigue due to shift work, from the beginning of day shifts to the end of night shifts

Fatigue	Beginning of day shift[a]		End of day shift[b]		Beginning of night shift[c]		End of night shift[d]		a/b	b/c	c/d
Drowsiness	6.0	5.0–18.0	8.0	5.0–25.0	12.0	5.0–24.0	13.0	5.0–25.0	**	**	**
Instability	6.0	5.0–14.0	7.0	5.0–21.0	7.0	5.0–17.0	9.0	5.0–23.0	**		**
Uneasiness	6.0	5.0–16.0	6.0	5.0–17.0	7.0	5.0–23.0	8.0	5.0–23.0	*	**	**
Local pain or dullness	6.0	5.0–19.0	8.0	5.0–25.0	7.0	5.0–20.0	9.0	5.0–25.0	**	**	**
Eyestrain	5.0	5.0–19.0	7.0	5.0–22.0	8.0	5.0–20.0	11.0	5.0–25.0	**		**

Fatigue feelings are evaluated by a questionnaire for work-related fatigue feelings, the "Jikaku-sho shirabe" of the Industrial Fatigue Research Committee of the Japan Society of Occupational Health. Values are expressed as medians (range). Statistical differences were evaluated by the Wilcoxon signed-rank sum test. * and **: $p<0.05$ and $p<0.01$, respectively.

intensity of their feelings as "disagree completely," "agree slightly," "agree somewhat," "agree considerably" and "agree strongly." These five intensities were assigned values of 1 to 5 points, respectively, and summed within each factor. Therefore, the range of possible scores for each factor is 5 to 25 points, and a higher score means greater fatigue. Cronbach's alpha for the five factors was greater than 0.7, except for "instability" (0.67), indicating a high degree of internal consistency in our study.

Statistical analysis

Fatigue scores from the beginning of day shifts to the end of night shifts were tested by Wilcoxon signed-rank sum tests. The values were compared between two groups of subject characteristics, age group (23–43 vs. 44–59), hours of sleep before night shift (0–2.0 h vs. 2.5–4.0 h), marital status (married vs. single) and ward assignment (wards for patients with schizophrenia and schizophreniform disorder vs. wards for patients with dementia), by *t*-test. Comparisons of immune parameters between the beginning of day shifts and the end of night shifts were carried out by paired *t*-test. Changes in immune parameters from the beginning of day shifts to the end of night shifts were compared between two groups divided by their increased level of fatigue (small vs. large). The comparison was carried out in each of the five fatigue factors described above. Statistical differences in immune parameters between two groups were evaluated by simple comparison (*t*-test) and by analysis of covariance using baseline immune values and other demographic features as covariates.

Statistical analyses were performed using SPSS version 18 (SPSS Inc, Chicago, IL, USA).

Results

Table 2 shows the changes in scores of subjective feelings of fatigue from the beginning of day shifts to the end of night shifts. All fatigue scores increased progressively from the beginning of day shifts to the end of night shifts. The scores at the beginning of night shifts were significantly higher than those at the end of day shifts for all factors except for "local pain or dullness" and "eyestrain," as determined by Wilcoxon signed-rank sum tests. This suggests that fatigue was not ameliorated by the interval between the day shift and night shift. Increased levels of fatigue from the beginning of day shifts to the end of night shifts were compared between two groups of demographic features such as age, marital status, ward assignment and hours of sleep during the interval (Table 3). The subjects who were older, married and experienced fewer sleep hours tended to show greater fatigue, although the observed differences did not reach statistical significance.

Table 4 shows changes in immune parameters from the beginning of day shifts to the end of night shifts. Since NK cell activity at the two different E:T ratios tested showed similar findings, only the results of the 20:1 E:T ratio are shown. For all groups examined, NK cell activity and $CD16^+CD56^+$ lymphocytes were significantly lower at the end of night shifts than at the beginning of day shifts. Conversely, $CD3^+$ and $CD4^+$ lymphocyte counts were significantly higher at the end of night shifts than at the beginning of day shifts. There were significant differences in some parameters between two groups of demographic features such as age, marital status, ward assignment and sleep hours (Table 5). The decrease in NK cell activity was larger in married subjects and in subjects assigned to wards for patients with dementia. The increases in $CD3^+$, $CD4^+$ and $CD8^+$ lymphocytes were larger in younger subjects than in older subjects.

Changes in immune parameters from the beginning of day shifts to the end of night shifts were compared between groups differentiated by the magnitude of the increase in fatigue (small vs. large). Table 6 shows the results for three of the five fatigue factors, drowsiness, instability, and local pain or dullness, which had significant differences in some immune parameters between the fatigue groups. Statistically significant differences in immune parameters between the different dichotomous fatigue groups were evaluated by simple comparison (*t*-test) and by analysis

Table 3. Changes in five fatigue factors, from the beginning of day shifts to the end of night shifts, grouped by age, marital status, ward assignment and hours of sleep before the night shift

	Number of subjects	Increase of feelings of fatigue				
		Drowsiness	Instability	Uneasiness	Local pain or dullness	Eyestrain
All	57	7.1 (5.7)	3.6 (4.6)	3.5 (4.5)	3.4 (4.1)	5.6 (4.9)
Age (yr)						
23–43	29	7.2 (6.2)	3.4 (4.6)	2.8 (4.2)	3.6 (3.5)	5.3 (4.6)
44–59	28	6.9 (5.3)	3.9 (4.8)	4.4 (4.8)	3.3 (4.7)	5.9 (5.2)
Marital status						
Single	10	4.4 (5.1)	1.7 (2.9)	1.4 (3.7)	2.7 (2.1)	3.8 (3.6)
Married	47	7.6 (5.7)	4.0 (4.9)	4.0 (4.6)	3.6 (4.4)	6.0 (5.0)
Wards for patients with:						
Dementia	28	6.7 (6.7)	3.9 (5.5)	3.0 (5.1)	2.9 (3.6)	5.3 (5.5)
Schizophrenia and schizophreniform disorder	29	7.4 (4.7)	3.3 (3.6)	4.1 (3.8)	3.9 (4.5)	5.9 (4.2)
Hours of sleep before the night shift						
0–2.0 h	34	7.7 (5.4)	4.0 (5.0)	4.2 (3.9)	3.8 (4.0)	6.1 (5.2)
2.5–4.0 h	23	6.1 (6.2)	3.0 (4.1)	2.6 (5.2)	2.9 (4.4)	4.8 (4.3)

Values are expressed as means (standard deviation) in the category. Statistical differences were evaluated by *t*-test.

Table 4. Comparison of immunological parameters between the beginning of day shifts and the end of night shifts (n=57)

	Baseline	Endpoint	t value	
NK cell activity (E:T=20:1, %)	17.0 (10.1)	13.7 (9.7)	3.9	**
$CD3^+$ lymphocytes (number/μl)	1,208.4 (397.8)	1,413.7 (365.8)	6.6	**
$CD4^+$ lymphocytes (number/μl)	736.7 (291.3)	860.7 (282.1)	6.4	**
$CD8^+$ lymphocytes (number/μl)	542.3 (196.8)	565.2 (172.3)	1.2	
$CD16^+CD56^+$ lymphocytes (number/μl)	292.0 (196.4)	222.3 (123.1)	3.6	**

Values are expressed as means (standard deviation). Statistical differences were evaluated by paired *t*-test. * and **: $p<0.05$ and $p<0.01$, respectively.

of covariance using baseline immune values and other demographic features. Model 1 shows the simple comparison. Model 2 shows the results after adjustment for each baseline (at the beginning of day shifts) immune variable. Model 3 shows the results after adjustment for baseline parameters, age, marital status, ward assignment and hours of sleep before the night shift. We found statistically significant decreases in NK cell activity in the group experiencing a large increase in "drowsiness" and "local pain or dullness." Although $CD16^+CD56^+$ lymphocytes tended to decrease in the group that experienced a large increase in fatigue, it did not reach statistical significance. Additionally, we found significant increases in $CD4^+$ lymphocytes in the group that experienced a large increase in "drowsiness," "instability" and "local pain or dullness."

Discussion

We investigated the effect of fatigue due to shift work on immune parameters such as NK cell function and lymphocyte subsets. Our subjects were engaged in a shift work system that is typical for hospital nurses in Japan. Night shifts followed day shifts after only a seven-hour interval. Since most subjects had to deal with housework during that interval, they actually slept 0–4 h prior to starting the night shift. Perceived fatigue was not alleviated during that interval and accumulated until the end of the night shift. Similar results have been reported in Japan previously[18,19].

NK cell function decreased from the beginning of day shifts to the end of night shifts. These changes were larger among subjects reporting greater fatigue, particularly those experiencing drowsiness, local pain and dullness. These

Table 5. Changes in immunological parameters between the beginning of day shifts and the end of night shifts grouped by age, marital status, ward assignment and hours of sleep before the night shift

	Number of subjects	NK cell activity (E:T=20:1, %)	$CD3^+$ lymphocytes (number/μl)	$CD4^+$ lymphocytes (number/μl)	$CD8^+$ lymphocytes (number/μl)	$CD16^+CD56^+$ lymphocytes (number/μl)
All	57	−3.3 (6.3)	205.3 (234.9)	124.1 (144.3)	22.8 (143.7)	−69.7 (147.8)
Age (yr)						
23–43	29	−4.6 (6.0)	290.2 (213.1)*	173.6 (125.4)*	68.6 (130.4)*	−46.9 (126.4)
44–59	28	−1.9 (6.5)	117.4 (227.1)	72.7 (146.6)	−24.5 (143.7)	−93.4 (166.1)
Marital status						
Single	10	−6.9 (7.6)*	150.8 (219.3)	68.7 (154.0)	7.9 (159.5)	−74.6 (165.0)
Married	47	−2.5 (5.9)	216.9 (238.7)	135.8 (141.0)	26.0 (141.8)	−68.7 (145.8)
Wards for patients with:						
Dementia	28	−5.2 (6.8)*	220.9 (247.7)	142.1 (141.8)	15.9 (167.2)	−98.3 (185.2)
Schizophrenia and schizophreniform disorder	29	−1.4 (5.4)	190.2 (225.1)	106.7 (146.9)	29.5 (119.5)	−42.1 (94.9)
Hours of sleep before the night shift						
0–2.0 h	34	−4.7 (5.7)*	237.2 (226.6)	144.3 (167.1)	35.6 (124.7)	−65.7 (117.8)
2.5–4.0 h	23	−1.1 (6.7)	158.2 (243.9)	94.1 (97.5)	4.0 (169.3)	−75.7 (186.3)

Values are expressed as means (standard deviation) in the category. Statistical differences were evaluated by t-test. *: $p<0.05$.

findings did not change after adjustment for other demographic factors or hours of sleep prior to starting the night shift. Our results demonstrate that shift work results in an accumulation of fatigue and that fatigue impacts important aspects of innate immunity, such as NK cell function. Chronic suppression of NK cell activity due to repetitive rounds of shift work could be one of the variables influencing the relationship between shift work and cancer[20]. It is possible that improving the shift system to reduce fatigue could reduce the negative impact on innate immunity. Interestingly, Kobayashi *et al.*[21] evaluated the effects of improvements of a shift system on fatigue and immune parameters among nurses. The subjects in their report engaged in a similar pattern of shift work as the one in use at the hospital in our study. Lengthening the interval between day shifts and night shifts by introducing a half-day shift reduced fatigue; however, NK cell function was virtually identical to that seen with the former shift work pattern. Several studies have examined the relationship of fatigue and immune function, although these studies did not target shift workers. Shakhar *et al.*[22] investigated the relationship between the level of fatigue and changes in immune parameters over a one-month interval among female workers engaged in day work and found that decreased fatigue and increased sleep hours resulted in an increase in NK cell activity. Studies that have investigated the impact of long work hours on immune function have identified fatigue as one of the most important factors[23–25]. These studies consistently identified suppressed NK cell activity in subjects with longer work hours.

We found an increase in the number of $CD4^+$ lymphocytes among subjects experiencing greater fatigue. The results of several laboratory studies were not consistent for T lymphocytes. Born *et al.*[11] examined the diurnal rhythms of immune parameters, including lymphocyte subsets, between groups receiving either normal nocturnal sleep or total sleep deprivation. They reported that the number of T lymphocytes reached peak levels at 23:00 and nadir levels at 8:00 in both groups; however, the peak levels in the sleep-deprived group were higher than in those receiving normal nocturnal sleep. Conversely, there are also reports showing decreases in $CD4^+$ T cell numbers due to mental stress[26, 27]. Although not currently a topic of study, lymphocyte proliferation might be influenced by acute and chronic stress or by mental and physical stress. Stressors impact the normal functioning of natural and specific immunity in proportion to the magnitude and duration of the stressors[13]. The fatigue levels of our subjects likely reflect both acute stress due to sleep deprivation during night shift and chronic stress due to persistent perturbations in their work/sleep schedules. The increase in T cell populations might be a defensive response to counter the risks of acute stress experienced during sleep deprivation. However, the increase in T cell populations may also have detrimental effects, since it has been suggested that some cytokines, such as IL-6 secreted by $CD4^+$ lymphocytes, can reactivate latent herpes virus[28].

The strength of our study was that we utilized a longitudinal design to show that the effects of shift work on NK cell function and lymphocyte subsets depended on

Table 6. Comparison of changes in immunological parameters between groups stratified by different fatigue factors, from the beginning of day shifts to the end of night shifts

	Drowsiness[a)]		Instability[a)]		Local pain or dullness[a)]	
	Small (n=27)	Large (n=30)	Small (n=24)	Large (n=33)	Small (n=31)	Large (n=26)
Model 1						
NK cell activity (E:T=20:1, %)	−1.6 (7.0)	−4.8 (5.4)	−3.5 (8.3)	−3.1 (4.6)	−1.7 (6.4)	−5.2 (5.8)*
$CD3^+$ lymphocytes (number/μl)	135 (235.7)	268 (219.2)*	136 (246.5)	255 (216.1)	140 (215.7)	284 (236.7)*
$CD4^+$ lymphocytes (number/μl)	67 (135.3)	175 (134.3)*	59 (137.7)	171 (131.5)*	69 (117.5)	190 (147.5)*
$CD8^+$ lymphocytes (number/μl)	11 (149.4)	33 (140.2)	4 (160.8)	37 (130.8)	5 (140.8)	44 (147.0)
$CD16^+CD56^+$ lymphocytes (number/μl)	−73 (165.5)	−67 (132.6)	−78 (146.6)	−64 (150.6)	−74 (165.1)	−65 (127.1)
Model 2						
NK cell activity (E:T=20:1, %)	−1.6 (1.1)	−4.8 (1.0)*	−2.5 (1.3)	−3.9 (1.1)	−1.4 (1.0)	−5.5 (1.1)*
$CD3^+$ lymphocytes (number/μl)	149 (40.5)	256 (38.4)	128 (42.0)	262 (35.8)*	155 (37.9)	265 (41.5)
$CD4^+$ lymphocytes (number/μl)	70 (25.0)	172 (23.7)*	52 (25.6)	176 (21.8)*	75 (23.4)	182 (25.6)*
$CD8^+$ lymphocytes (number/μl)	28 (24.2)	18 (23.0)	16 (25.6)	28 (21.8)	16 (22.5)	31 (24.6)
$CD16^+CD56^+$ lymphocytes (number/μl)	−47 (17.9)	−90 (16.9)	−52 (19.2)	−82 (16.3)	−56 (16.8)	−86 (18.4)
Model 3						
NK cell activity (E:T=20:1, %)	−1.3 (1.0)	−5.1 (0.9)**	−1.9 (1.2)	−4.3 (1.0)	−1.8 (1.0)	−5.1 (1.0)*
$CD3^+$ lymphocytes (number/μl)	154 (4.0)	251 (37.9)	133 (42.1)	258 (35.7)*	157 (37.5)	262 (41.1)
$CD4^+$ lymphocytes (number/μl)	74 (23.7)	169 (22.4)**	57 (24.5)	173 (20.8)**	76 (22.0)	182 (24.2)**
$CD8^+$ lymphocytes (number/μl)	30 (24.4)	17 (23.1)	15 (26.3)	28 (22.2)	18 (22.9)	29 (25.1)
$CD16^+CD56^+$ lymphocytes (number/μl)	−47 (18.6)	−91 (17.6)	−51 (20.4)	−83 (17.2)	−56 (17.7)	−86 (19.4)

Values are mean (standard deviation or standard error) changes in immune parameters from the beginning of day shifts to the end of night shifts. a): Subjects were categorized into two groups, small and large, by dichotomy of the increased level of fatigue from day shifts to the end of night shifts. Model 1: simple comparison between two groups of increased level of fatigue feeling using *t*-test. Model 2: adjusted for the baseline (at the beginning of day shift) values of corresponding variables using analysis of covariance. Model 3: adjusted for the baseline values of corresponding variables and age group, marital status, ward assignment and hours of sleep before the night shift using analysis of covariance. * and **: $p<0.05$ and $p<0.01$, respectively.

the level of fatigue caused by shift work. However, our study had some limitations. First, we studied female nurses in one hospital, although the shift system employed by this hospital is in use in other Japanese hospitals. Studies on other shift systems and other workplace settings should be conducted. Secondly, we did not evaluate the effects of chronic mental stress, although there have been many studies on the relationship between mental stress and immune function. Finally, we did not evaluate the menstrual cycles of subjects, which might influence immune function.

In conclusion, our data show that the degree of fatigue due to shift work impacts immune function. Proper management of shift systems to reduce the level of fatigue in workers is important to diminish health problems including cancer risk.

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