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Influence of Shift-work on Selected Immune Variables in Nurses

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Abstract: Shift-work, particularly night-work, interferes with the physiological circadian rhythm and has the potential to induce psycho-physiological disturbances. A nurse population was investigated to establish whether shift-work can induce changes in a number of immune variables. Lymphocyte immunophenotype and proliferative response, NK cytotoxicity, cytokines and cortisol were determined in 68 shift-working and 28 daytime nurses at baseline and at 12 months. None of the variables studied differed significantly between shift and daytime workers, either at baseline or at 12 months, except IL-1β and TNF-α, which were significantly higher among daytime nurses at baseline, but not at follow-up. No effect of shift-work on immune variable and cortisol levels was seen at 12 months after adjustment for baseline values and job seniority. The specific work schedule as well as job type likely influenced our results, suggesting that rotational shift-work does not necessarily affect the immune system adversely. The immune changes reported by other studies in shift-workers should not be generalized.

Key words: Shift-work, Immune system, Nurses, Chronobiology disorders, Work schedule tolerance

Introduction

The cross-talk between the nervous, endocrine and immune systems is based on the fact that they share a common biochemical language involving shared ligands and receptors, including neurotransmitters, growth factors, neuroendocrine hormones and cytokines[1–3]. Brain function and some immune-regulating organs therefore participate in the immune response[4]. Adequate communication between these systems is considered essential for maintaining homeostasis and good health[5]. Shift-work, particularly night-work, interferes with the physiological circadian rhythm and has the potential to induce psycho-physiological disturbances and neuro-immuno-endocrine homeostasis alterations[6–11]. Sleep deprivation is related to substantial immune system changes[12, 13] and to increased production of pro-inflammatory cytokines[14]. Several pathological conditions and chronic stress are related to immune changes[15–19]. In particular, chronic stressors have been correlated with suppression of both cellular and humoral measures[20, 21]. Studies of work in high-stress situations have demonstrated that job type and job strain can induce immune changes[22–26] such as increases in CD3+CD16CD56+ cells, increased IL-6 plasma levels, and reduction in NK CD57+ and CD8+CD11b+ cells.

There are few studies on the immune changes induced

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by shift-work in individuals in good psycho-physical conditions[27–30], these investigations have found reduced T-lymphocyte function[27, 28] and NK activity[29, 30] in shift-workers.

We present the data of a longitudinal study performed in a population of nurses ostensibly not suffering from job stress to evaluate whether the circadian desynchronization induced by rotational shift-work may interfere with some immune parameters.

Subjects and Methods

Participants and procedures

The study was performed in Ancona, central Italy, from June 2004 to June 2005. Subjects were 96 consecutively recruited National Health Service nurses, 68 were shift-workers (SW) and 28 were daytime nurses (DT); 34 nurses worked in a resuscitation ward (24 SW/10 DT), 29 in the operating room (20 SW/9 DT), 21 in a long-term ward for elderly people (15 SW/6 DT), and 12 in a ward providing care and assistance to terminally ill patients (9 SW/3 DT). The study was performed as part of the periodic occupational health surveillance and needed no formal approval by the local ethics committee, which was nevertheless consulted and granted an informal authorization. Subjects were informed of the study aims and procedures, they were offered the opportunity to ask questions, and gave their written informed consent to participate. Inclusion criteria were age<60 yr, at least 2 yr in the current job without changes in ward type or between shift- and daytime work, no infectious disorders or chronic medical diseases, no history of major psychiatric disorders, and no current or recent treatment with drugs affecting the immune system (e.g., corticosteroids, cytostatics, immunosuppressors, immunomodulators).

Participants were scheduled for a baseline assessment (T0) and for a follow-up session at 12 months (T1). They were seen between 08:30 h and 09:30 h in a quiet, comfortable room on a day off from work. None of the shift nurses had just worked a nightshift. Subjects were asked to refrain from exercising, smoking, eating, drinking alcohol, and taking medications for at least 12 h before evaluations. After a rest of at least 5 min in sitting position, a blood sample was taken from a forearm vein using Vacutainer tubes containing ethylenediaminetetraacetic acid (Becton Dickinson, San Jose, CA). Samples were processed as described below, or stored at –20°C for subsequent analysis. Participants then had breakfast and were administered a standardized form to collect information about health-related habits.

Psychological stress was assessed with the Rapid Stress Assessment (RSA) scale[31], a 15-item multiple choice self-assessment tool with four possible answers rated from 0 to 3. The scale explores individual responses to stressful situations and divides them into five dimensions (clusters) to quantify stress: depression, anxiety, somatization, aggressiveness, and lack of social support. The total stress score is obtained from the sum of the 5 cluster scores and ranges from 0 to 45 points. The first 9 items explore perceived stress at the time of questionnaire administration; the following 6 items regard the stress levels experienced in the previous 6 months. The test proved to be reliable and valid (test-retest reliability: r between 0.7 and 0.92, $p<0.0001$; significant content validity, with RSA areas correlating with "Minnesota Multiphasic Personality Inventory" (MMPI) scales; Pearson's coefficients: depression r=0.61, anxiety r=0.6, somatization r=0.54, aggressiveness r=0.38, $p<0.0001$; lack of social support r=0.38, $p<0.005$. Satisfactory concurrent validity)[32].

The shift-work (68 nurses) was organized as follows: 1st day: 06:00–14:00 h; 2nd day: 14:00–22:00 h; 3rd day: 22:00–06:00 h; 48 h of rest; resumption of the cycle. The working hours of daytime workers (28 nurses) were 08:00–14:00 h (15 nurses) or 14:00–20:00 h (13 nurses), 6 days a week.

Assessments

Cortisol

Cortisol (which has an immunomodulatory function[33–35]), was determined with a chemiluminescent competitive binding immunoenzymatic method with paramagnetic particles in a Unicel DXI 800 (Beckman-Coulter, Fullerton, CA).

Immunophenotype analysis

Changes in the cellular component of the immune system (which may contribute to increased incidence and severity of infectious diseases and possibly cancer[36]) were assessed by immunophenotype analysis, performed on fresh whole blood within 2 h of collection using a direct immunofluorescence cytofluorimetric assay. Lymphocyte surface antigens were identified using the following monoclonal antibodies (mAbs): antihuman CD3 (APC), antihuman CD4 (FITC), antihuman CD8 (APC), antihuman CD57 (FITC), antihuman CD19 (PE), antihuman CD16 (PE), and antihuman CD56 (FITC), all from Becton Dickinson. After 30 min incubation at 4°C with mAbs, fresh whole blood was treated with lysing reagent (FACS Lyse, Becton Dickinson) for flow cytometry. Four-colour flow cytometric acquisition was performed on FACScalibur using MultiSet software (both from Becton Dickinson). At least 10,000 events/sample were acquired. The proportions of cells expressing CD3+CD4+ (T helper lymphocytes), CD8+

(cytotoxic/suppressor T lymphocytes), CD8+CD57+ (T lymphocytes with cytotoxic activity), CD19+ (B cells) and CD3-CD16+CD56+ (NK cells) were calculated. Absolute values were obtained based on lymphocyte counts provided by an automated Haematology Analyzer (Gen-S, Beckman-Coulter).

Lymphocyte proliferative response

The ability of lymphocytes to proliferate *in vitro* in response to mitogens or microbial antigens was assessed using phytohaemagglutinin (PHA) as the stimulus. Ficoll Hypaque-enriched peripheral blood mononuclear cells were used at 1×10^6/ml concentration in Roswell Park Memorial Institute medium containing 10% foetal calf serum (both from GIBCO, Grand Island, NY), penicillin (100 U/ml), and streptomycin (10 μg/ml). Aliquots of 0.1 ml were distributed in microwells (Nunc, Roskilde, Denmark). PHA (10 μl/well; Difco Laboratories, Detroit, MI) was added to reach final concentrations of 0.0125 μg/ml and 0.05 μg/ml. After 48 h incubation in a 5% CO_2-air environment at 37°C, 1 μCi/well H^3-Td (Amersham, UK; specific activity, 2 Ci/ml) was added. After additional 18 h incubation in CO_2 atmosphere, cultures were killed with a cell harvester (Skatron, Lier, Norway). Radioactivity was measured in a scintillation counter (TriCarb 2100TR, Packard Instruments, Meriden, CT). All cultures were performed in quadruplicate. The results, referring to the highest responses regardless of the mitogen concentration used, are expressed as counts per minute (cpm) per culture[37].

NK cytotoxicity assay

NK cytotoxicity (which plays an important immunological role in tumour surveillance and in bacterial and viral infection) was assayed using a fluorimetric method described previously[38]. Briefly, a stock solution (20 mg/ml acetone stored at –20°C) of carboxyfluorescein diacetate (c'FDA; Molecular Probes, Eugene, OR) was diluted in phosphate-buffered saline (PBS) to a final concentration of 75 μg/ml. The K562 tumour cell line was used as the target; K632 cells were washed twice with PBS and then labelled with c'FDA by incubation at 37°C in a humidified 5% CO_2 incubator for 30 min. They were then washed 3 times in PBS containing 1% bovine serum albumin and resuspended at a final concentration of 1×10^5 cells/ml. c'FDA-labelled K562 cells (1×10^4) were incubated with effector cells in 200 μl total volume per well of a round microtiter plate. Effector target cell ratios from 100:1 to 12.5:1 were tested in triplicate. The plates were kept at 37°C in a humidified 5% CO_2 incubator for 3 h and then centrifuged at 1,500 rpm for 5 min. The supernatant was separated by rapidly inverting the plate and flicking the supernatant out. Then, 100 ml 1% Triton X-100 in 0.05 M borate buffer, pH 9.0, was added to each well. The plate was kept for 20 h at 4°C to allow for solubilization and then read for fluorescence with a Titertek Fluoroskan II (Flow Laboratories, McLean, VA). The proportion of specific lysis was calculated as follows: % Specific Lysis = F med = F exp/F med, where F is the fluorescence of solubilized cells after the supernatant has been removed, med is F from target cells incubated in culture medium alone, and exp is F from target cells incubated with effector cells. Results were then normalized by conversion to lytic units, calculated as the number of effector cells required to lyse 20% of target cells, and reported as the number of lytic units contained in 10^7 cells[39].

Human inflammatory cytokine assays

Cytokines regulate host responses to infection, immune responses, inflammation, and trauma. Pro-inflammatory cytokine (IL-1β, IL-6, INF-γ, TNFα) arrays were analysed by multiplex sandwich ELISA (SearchLight, Pierce Biotechnology, Rockford, IL) according to the manufacturer's instructions. Briefly, each well of the microplate was pre-spotted with target protein-specific antibodies. The antibodies capture the specific target protein in standard and plasma samples added to the plate (50 μl of 1:5 diluted plasma). Unbound proteins were washed away and biotinylated detecting antibodies added. After washing, antibody streptavidin-horseradish peroxidase was used for detection. Each sample was tested in duplicate. Results are expressed as pg/ml.

Statistical Analysis

Between-groups comparisons at T0 and T1 were performed using the χ^2 test when categorical variables were analysed. Since quantitative variables were not normally distributed, a non-parametric approach was chosen. Data at baseline and at 12 months were compared using Wilcoxon's rank-sum.

Spearman correlation coefficients and 95% confidence intervals (95%CI) between the T0 and T1 value of each parameter were calculated to evaluate the association of the values of each inflammation measure at the two time points.

A multiple quantile regression analysis was performed to estimate the effect of shift-work on stress and each immune status indicator at T1, adjusted for job seniority, presence of children and for the value of the same variable at T0.

All descriptive statistics were performed using the SAS System software, vs 9.1 (SAS Institute, Cary, NC). A probability of 0.05 was chosen for significance; since

many hypothesis tests were performed on the same dataset, the multiple testing problem was addressed using Hommel *p*-value adjustment (SAS MultTest procedure). The R statistical software was applied in the quantile regression analysis. Results were expressed as point estimates and 95%CI of regression coefficients; when the 95%CI did not contain the zero value, the regression coefficients were considered significant.

Results

Data were available for all 96 nurses, none of whom was lost to follow-up. Their characteristics are reported in Table 1. Shift and daytime workers were comparable in terms of age and gender distribution, marital status, smoking and drinking habits, and psychological stress. Shift-working nurses had a significant lower job seniority and they less frequently had children.

Immune measures (lymphocyte number and immunophenotype, lymphocyte proliferative response and NK cytotoxicity) and serum cortisol levels were not significantly different between the two groups at T0 (Table 2). At this time point, the levels of cytokines IL-1β and TNF-α were significantly lower among shift-working nurses, IFN-γ was also lower in shift-workers, but the value was of borderline statistical significance.

Table 1. Socio-demographic characteristics of shift-working and daytime nurses

	Shift-workers (no. 68)	Daytime nurses (no. 28)	$p^{\#}$
Socio-demographic variables			
Age: median (25th–75th percentile)	35 (30–40)	40 (34–43.5)	0.239
Job seniority: median (25th–75th percentile)	6 (3–14)	17 (12–21.5)	0.003
Male: no. (%)	19 (28)	7 (25)	1.000*
Female: no. (%)	49 (72)	21 (75)	
Single: no. (%)	38 (56)	6 (21)	0.053*
With offspring: no. (%)	25 (37)	21 (75)	0.019*
Smokers: no. (%)	31 (45)	7 (25)	0.862*
Alcohol drinkers: no. (%)	19 (27)	14 (50)	0.704*
Rapid stress assessment score: median (25th–75th percentile)	14 (10–19)	15.5 (10.5–20)	1.000

Wilcoxon rank-sum test.
*χ^2 test
#Hommel-adjusted *p*-value.

Table 2. Baseline immune status data and cortisol of shift-working and daytime nurses

	Shift-workers (n=68)	Daytime nurses (n=28)	$p^{\#}$
Immune status variables [median (25th–75th percentile)]			
Lymphocytes (cells/mm^3)	2,112 (1,666–2,596)	2,144 (1,788–2,539)	1.000
T cells (cells/mm^3)	1,676 (1,277–1,904)	1,555 (1,367–1,993)	1.000
T helper lymphocytes (cells/mm^3)	966 (809–1,288)	979 (861–1,196)	1.000
Cytotoxic/suppressor T lymphocytes (cells/mm^3)	571 (435–746)	581 (470–670)	1.000
T lymphocytes with cytotoxic activity (cells/mm^3)	126 (83–179)	156 (84–238)	1.000
B cells (cells/mm^3)	258 (194–312)	241 (161–304)	1.000
NK cells (cells/mm^3)	204 (130–298)	208 (161–291)	1.000
Lymphocyte Proliferative Response (10^3 cpm/culture)	101,264 (72,236–129,565)	84,695 (40,561–111,172)	0.279
NK Cytotoxicity Assay ($LU_{20}/10^7$ cells)	41 (25–69)	48 (33–71)	1.000
IL-1β (pg/ml)	0.5 (0.2–2)	2 (1–4)	0.003
IL-6 (pg/ml)	2 (1.5–3)	4 (1–7)	1.000
IFN-γ (pg/ml)	2.1 (1.4–3.6)	3.8 (2.7–7.1)	0.062
TNF-α (pg/ml)	9.8 (6.5–16.5)	22.7 (9.8–39.5)	0.032
Cortisol (μg/dl)	18.1 (14.7–25.8)	17.7 (13.2–21.4)	1.000

Wilcoxon rank-sum test.
#Hommel-adjusted *p*-value.

Table 3. Immune status and cortisol levels in shift-working and daytime nurses at 12 months

	Shift-workers (n=68)	Daytime nurses (n=28)	$p^{\#}$
Immune status variables [median (25th–75th percentile)]			
Lymphocytes (cells/mm^3)	2,310 (1,964–2,794)	2,064 (1,853–2,400)	0.640
T cells (cells/mm^3)	1,800 (1,468–2,090)	1,636 (1,342–1,839)	0.963
T helper lymphocytes (cells/mm^3)	1,206 (933–1,465)	1,009 (888–1,198)	0.322
Cytotoxic/suppressor T lymphocytes (cells/mm^3)	574 (466–687)	524 (440–671)	1.000
T lymphocytes with cytotoxic activity (cells/mm^3)	84 (54–128)	121 (61–177)	0.889
B cells (cells/mm^3)	285 (203–411)	229 (187–302)	0.963
NK cells (cells/mm^3)	181 (140–226)	181 (146–263)	1.000
Lymphocyte Proliferative Response (10^3 cpm/culture)	91,341 (69,789–125,541)	78,186 (47,163–106,183)	0.429
NK Cytotoxicity Assay ($LU_{20}/10^7$ cells)	41 (30–61)	45 (31–69)	1.000
IL-1β (pg/ml)	0.45 (0.2–1.1)	0.9 (0.3–1.85)	0.922
IL-6 (pg/ml)	2.85 (1.5–5.5)	1.28 (0.8–3.47)	1.000
IFN-γ (pg/ml)	3.35 (1.5–6.8)	2.6 (2.05–3.89)	1.000
TNF-α (pg/ml)	11 (7–20)	14 (9–39)	1.000
Cortisol (μg/dl)	16.9 (10.55–21.4)	16.9 (13.8–20.9)	1.000

Wilcoxon rank-sum test.
$^{\#}$Hommel-adjusted p-value.

Table 4. Correlations of the T0 and T1 values of each variable

Variables	rho	95%CI
Lymphocytes (cells/mm^3)	0.44	0.26–0.59
T cells (cells/mm^3)	0.53	0.37–0.66
T helper lymphocytes (cells/mm^3)	0.67	0.54–0.77
Cytotoxic/suppressor T lymphocytes (cells/mm^3)	0.64	0.51–0.75
T lymphocytes with cytotoxic activity (cells/mm^3)	0.78	0.68–0.85
B cells (cells/mm^3)	0.77	0.67–0.84
NK cells (cells/mm^3)	0.45	0.27–0.60
Lymphocyte Proliferative Response (10^3 cpm/culture)	0.39	0.19–0.56
NK Cytotoxicity Assay ($LU_{20}/10^7$ cells)	0.36	0.12–0.57
IL-1β (pg/ml)	0.45	0.27–0.60
IL-6 (pg/ml)	0.47	0.29–0.62
IFN-γ (pg/ml)	0.17	–0.04–0.36
TNF-α (pg/ml)	0.29	0.09–0.45
Cortisol (μg/dl)	0.26	0.05–0.44

No significant differences between the two groups were found at T1 (Table 3). Evaluation of the association of the T1 and T0 values of each inflammation measure (Table 4) showed that all variables except IFN-γ were significantly correlated over time. A good level of correlation (coefficients>0.60) was found for T helper lymphocytes, cytotoxic/suppressor T lymphocytes, T lymphocytes with cytotoxic activity and B cells.

Adjustment of each variable for its T0 value, for job seniority and for presence of offspring highlighted no significant effect of shift-work on immune variables or cortisol levels at T1 (Table 5).

Discussion

Shift-work, especially night-work, can alter the physiological sleep/wake cycle induced by the dark/light rhythm. This rhythm regulates the secretion of cortisol, which among other actions has an immunomodulatory function[33–35]. Pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 are produced in response to immune/inflammatory insults as well as psychophysical stress, acting on the immune system directly as modulators and indirectly by inducing secretion of glucocorticoid hormones[40]. In the long term rotating shift-work may give rise to chronic adaptive stress, with possible repercussions on the neuro-endocrine-immune systems[41].

The socio-demographic characteristics showed a significantly greater job seniority among DT workers. However this finding was expected, since the hospital Management tends to allocate nurses with greater job seniority to daytime work[42, 43]. Shift-workers less frequently have children, probably due to the difficulty of conciliating family life and shift-work[44–56]. Job seniority and offspring were included in the statistical analysis and treated as covariates in estimating the effect of shift-work on the dependent variables.

In our study lower levels of IL-1β and TNF-α in shift-workers were the sole significant differences found between the groups at T0, whereas the lower levels of

Table 5. Effect of shift-work on immune variables and cortisol levels at T1, adjusted for the respective T0 value, job seniority and offspring. Results of quantile regression analysis

Dependent Variables [at T1]	Shift work	
	β	95%CI
Lymphocytes (cells/mm^3)	115.3	–152.7 ; 383.4
T cells (cells/mm^3)	78.9	–148.2 ; 305.9
T helper lymphocytes (cells/mm^3)	71.2	–100.6 ; 242.9
Cytotoxic/suppressor T lymphocytes (cells/mm^3)	14.0	–70.7 ; 98.7
T lymphocytes with cytotoxic activity (cells/mm^3)	–19.3	–54.9 ; 16.3
B cells (cells/mm^3)	12.3	–47.7 ; 72.3
NK cells (cells/mm^3)	–14.2	–77.1 ; 48.7
Lymphocyte Proliferative Response (10^3 cpm/culture)	–3,760.4	–26,541.8 ; 19,021.1
NK Cytotoxicity Assay ($LU_{20}/10^7$ cells)	5.5	–11.6 ; 22.5
IL-1β (pg/ml)	0.1	–0.7 ; 0.8
IL-6 (pg/ml)	0.7	–1.7 ; 3
IFN-γ (pg/ml)	0.7	–1.3 ; 2.7
TNF-α (pg/ml)	2.3	–15.1 ; 19.7
Cortisol (μg/dl)	0.5	–2.5 ; 3.4

INF-γ in shift-workers was of borderline statistical significance, probably due to insufficient statistical power of the study. All variables except IFN-γ were longitudinally stable and no differences between the groups was found at T1. It cannot be excluded that the findings at T0 were affected by contingent factors not related to the interference from shift-work.

Increased production of pro-inflammatory cytokines was expected in shift-workers, but was not found; however cytokines related to sleep debt[14] may have been minimized by the fact that samples were collected on a day off, rather than at the end of the night-shift.

The end of the night-shift is a time point associated with immune changes[30, 47]. Specifically, increased cortisol levels and a reduction in NK activity have been reported in nurses[47]. However, the facts that our investigation was performed on a day off and that these measures were not significantly different between groups neither at T0 nor at T1 suggest that the changes described by Kobayashi and coworkers may not be stable over time.

The immune response to shift-work is probably highly variable and is associated with several confounding factors (in particular job-related stress) that can make a job tolerable or otherwise, thus influencing the immune response. A study of Japanese emergency department physicians doing shift-work demonstrated immune changes at the start of the night-shift[26]. Such changes, suggesting immune alterations unrelated to night-work, can be ascribed to the high-stress job (a condition that does not apply to our population) or to the working hours (which were also different from those of our sample). Other studies have shown reduced T-lymphocyte function[27, 28], NK activity[29, 30] and production of anti-peroxidase thyroid autoantibodies in shift-workers[48].

Not only job stress, but also individual characteristics, schedule and type of work have been associated with tolerance of shift-work[49, 50], and probably influence the immune response to shift-work. Our study shows no changes in the variables tested between time points, suggesting that this type of shift-work is well tolerated by our nurses. This finding may be affected by a schedule characterized by an acceptable amount of working hours/week and adequate intervals between shifts. Our data agree with those of recent studies in finding that shift-work is overall well tolerated by nurses and does not impair health or sleep quality[51, 52].

Despite some limitations, especially the small sample and the large number of variables, our study indicates that rotational shift-work does not necessarily affect the immune system adversely. Further longitudinal studies are required to assess the influence of different shift-work schedules on the immune system.

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