Assessment of Salivary Human Herpesvirus-6 and Immunoglobulin A Levels in Nurses Working Shifts

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Purpose  The purpose of the present study was to assess whether salivary human herpesvirus (HHV)-6 acted as a new sensitive stress marker, providing a reliable indicator of stress among shift work nurses. Salivary HHV-6, immunoglobulin (Ig) A and mood states were compared among nurses who worked day shifts only and nurses who worked different numbers of night shifts.

Methods  Participants included 56 female nurses working in Japanese general hospitals. The 56 subjects were categorized into three groups: Group A (n = 7), which consisted of nurses who only worked day shifts; group B (n = 29), which consisted of nurses working shifts with ≤8 night shifts per month; and group C (n = 20), which consisted of nurses working shifts with ≥9 night shifts per month. Mood was assessed using the Profile of Mood States Short Form Japanese version (POMS-SFJ). Salivary biomarker levels and mood were compared among the three groups.

Results  Although the salivary HHV-6 level was significantly higher in group C than in group A (p < .05), salivary IgA and mood levels were not significantly different among the three groups.

Conclusion  Salivary HHV-6 level may be a more sensitive stress marker than salivary IgA or mood for assessing chronic fatigue in nurses working shifts. Improvement to shift assignments using assessment by salivary HHV-6 is required. [Asian Nursing Research 2008;2(3):159–165]

Key Words  chronic fatigue assessment, nurses, salivary human herpesvirus-6, shift work

INTRODUCTION

Night shift work is common in nursing. Previous studies have reported a significant positive correlation between chronic fatigue and depression in nurses (Muecke, 2005; Ruggiero, 2003). Therefore, depressed mood has been considered an index of chronic fatigue (Muecke; Ruggiero). Nurses working night shifts report more fatigue and depressed mood than nurses working day shifts (Ruggiero). It has been reported...
that chronic fatigue in nurses not only decreases the quality of patient care, but also increases the nurses’ risk of health problems, such as depression (Lee, Colditz, Berkman, & Kawachi, 2004; McVicar, 2003). However, the physical and psychological response to shift work among hospital nurses is not well understood. It is necessary to develop more accurate indicators for the assessment of chronic fatigue. Therefore, this investigation compares groups of healthy nurses working three shifts and a control group of nurses working only day shifts.

Human herpesvirus (HHV)-6, which belongs to the beta herpesvirus group, is a common human pathogen. At least 90% of the adult population has been infected with HHV-6 (Abdel-Haq & Asmar, 2004). HHV-6 has a life-long latency (which is a hallmark of herpes viruses), reactivates frequently, and is shed in saliva. Reactivation occurs due to stress and is also associated with serious diseases and their complications, such as chronic fatigue syndrome, hepatitis, and encephalitis (Cohrs & Gilden, 2001).

Kondo (2005) studied the association between HHV-6 reactivation, as determined by salivary HHV-6 levels, and work-induced fatigue in healthy office workers. Reactivated HHV-6 was detected in the saliva of 80% of subjects who worked overtime for 1 week. However, HHV-6 was detected in only 23% of the subjects following a 1-week holiday. Therefore, salivary HHV-6 levels appear to be useful as an accurate indicator of chronic fatigue. Salivary secretory immunoglobulin A (sIgA) is known to be involved in the immunological defense of mucosal surfaces, and salivary sIgA levels are decreased in patients with chronic fatigue (Yang et al., 2002). Since saliva specimens can be collected noninvasively and painlessly, salivary stress biomarkers are easy to obtain. However, only a few studies have used salivary HHV-6 and IgA levels to assess chronic fatigue in nurses working shifts.

Our purpose was to assess whether salivary HHV-6 could be used as a new sensitive stress marker to provide a reliable indicator of stress among shift work nurses. Salivary HHV-6, IgA and mood states were compared among nurses working different numbers of night shifts per month.

**METHODS**

**Study design**

A cross-sectional research design was used because it is appropriate for describing relationships between phenomena at a fixed point in time.

**Subjects**

Three general hospitals (Hospital A, public; Hospitals B and C, private) located in Oita prefecture in Japan participated in the study. The capacity of the hospitals ranged from 340 to 500 beds and there were 280–400 nursing staff in each hospital. Registered nurses from these three hospitals participated in the study. The investigators visited each of these hospitals’ wards to explain the purpose of the study, and then recruited female volunteers as participants. Inclusion criteria for participation were healthy female nurses working three shifts or only days. None of the participants reported a history or current diagnosis of liver or kidney dysfunction, chronic disease affecting the immune system, or any other medical condition. No participants had any symptoms of acute illness (i.e., fever, sore throat, myalgia, diarrhea) or upper respiratory tract infections at the time of the study or in the preceding week. No participants were taking oral contraceptives, prescription drugs, antibiotics, immunosuppressant medications, aspirin, or vitamin supplements. All participants were non-smokers and were not pregnant. Although 65 female nurses agreed to participate in this study, nine nurses were excluded by the exclusion criteria. Thus, the final convenience sample consisted of 56 female registered nurses. The participants were categorized into three groups: Group A (n=7) included nurses working only days (no night shifts); group B (n=29) included nurses working three shifts that were irregularly and rapidly rotated, with up to eight night shifts per month; group C (n=20) included nurses working three shifts that were irregularly and rapidly rotated and included nine or more night shifts per month.

All of the participants were given a verbal explanation of the study procedures before giving their written informed consent. The study was approved.
Procedure
Salivary HHV-6 and Chronic Fatigue

Saliva samples (1 mL) were collected into 15 mL sterile plastic collection tubes between 11 am and 2 pm from the nurses during the morning shift. Subjects were asked not to eat and to only drink water 1 hour before saliva collection in order to minimize possible food debris and stimulation of salivation. The samples were distributed into tubes and then frozen at –80°C until analysis for HHV-6 DNA and IgA antibody levels. All samples were defrosted only once.

Between 11 am and 2 pm, after the saliva samples were collected, self-reports of mood were made using the Profile of Mood States Short Form Japanese version (POMS-SFJ) scale.

Instruments
Real-time polymerase chain reaction (PCR) was used to detect and quantify salivary HHV-6. HHV-6 DNA was isolated from 100 μL saliva samples using the SUMAITEST EX-R&D kit (G&G Science, Japan) according to the manufacturer’s instructions. The primers and probes for HHV-6 were derived from the major capsid protein as described by Kondo, Mukai, and Yamanishi (1992). Real-time PCR was performed on an ABI PRISM7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The PCR run contained negative controls and a 10-fold dilution series of a positive control.

Salivary IgA levels were measured using a salivary secretory IgA indirect enzyme immunoassay kit (Salimetrics, State College, PA, USA) according to the manufacturer’s instructions. Absorbance was measured spectrophotometrically using a microplate reader (Spectra Fluor, TECAN, Salzburg, Austria) at 450 nm.

POMS is a self-report scale consisting of 65 mood adjectives (McNair, Lorr, & Droppleman, 1971) rated on a 5-point scale from 0 (absence) to 4 (extremely), according to the subject’s mood in the previous week. POMS has six subscales: anxiety, depression, anger, vigor, fatigue, and confusion. The 30-item POMS-SFJ, which was previously validated by Yokoyama, Araki, Kawakami, and Tkakeshita (1990), was used here. The POMS-SFJ subscale scores correlate highly with the subscale scores in the POMS Japanese version (r = .92 to r = .98) (Yokoyama, 2005). Internal consistencies for the POMS-SFJ are equivalent to those of the original POMS, which has a Cronbach’s α coefficient around .88 (Yokoyama). The Cronbach’s α coefficient was .92.

Data analysis
Statistical analyses were performed using SPSS version 14.0J (SPSS Inc., Chicago, IL, USA). To reduce the negative influence of unequal allocation of subjects to each group on the results, χ² tests and one-way analyses of variance (ANOVAs) were used to establish equivalence at baseline among the three groups with respect to demographic variables. The Kruskal-Wallis test and follow-up post hoc tests were used to compare salivary HHV-6 and IgA levels among the three groups. ANOVAs were used to compare the POMS-SFJ scores among the three groups.

RESULTS
Participants’ characteristics
Table 1 shows the nurses’ demographic characteristics and work experience. Other than marital status, there were no obvious differences in either of these factors. Since only a small percentage of the nurses worked only day shifts in each hospital, the size of group A was much smaller than the other two groups. Although working night shift is obligatory for the nurses in each of these three hospitals, of the seven nurses in group A, five (20–29 years of age) were working only day shifts for various reasons such as child care.

Differences in salivary HHV-6 and IgA levels and the POMS-SFJ depression subscale score among the three groups
Table 2 shows the POMS-SFJ subscale scores for the three groups. There were no significant differences among the groups in the POMS-SFJ depression
Table 1

Demographic Data and Work Experience of Female Hospital Nurses

|                      | Group A\textsuperscript{a} \textit{(n = 7)} | Group B\textsuperscript{b} \textit{(n = 29)} | Group C\textsuperscript{c} \textit{(n = 20)} | \(p\)  \\
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<tr>
<td><strong>Age group ((n))</strong></td>
<td></td>
<td></td>
<td></td>
<td>.06\textsuperscript{d}</td>
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<tr>
<td>20–29</td>
<td>5</td>
<td>10</td>
<td>13</td>
<td></td>
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<tr>
<td>30–39</td>
<td>0</td>
<td>10</td>
<td>6</td>
<td></td>
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<tr>
<td>40–60</td>
<td>2</td>
<td>9</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Marital status ((n))</strong></td>
<td></td>
<td></td>
<td></td>
<td>.01\textsuperscript{d}</td>
</tr>
<tr>
<td>Single</td>
<td>2</td>
<td>17</td>
<td>16</td>
<td></td>
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<tr>
<td>Married</td>
<td>4</td>
<td>11</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No answer</td>
<td>0</td>
<td>1</td>
<td>1</td>
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<tr>
<td><strong>Child(ren) aged under 6 years ((n))</strong></td>
<td></td>
<td></td>
<td></td>
<td>.52\textsuperscript{d}</td>
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<tr>
<td>Absent</td>
<td>5</td>
<td>23</td>
<td>17</td>
<td></td>
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<tr>
<td>Present</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td></td>
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<tr>
<td>No answer</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td></td>
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<tr>
<td><strong>Hospital ((n))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>3</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>9</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td><strong>Work experience (\text{mean} \pm \text{SD})</strong></td>
<td>4.80 \pm 7.08</td>
<td>9.66 \pm 8.72</td>
<td>5.96 \pm 4.79</td>
<td>.13\textsuperscript{e}</td>
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Note. \textsuperscript{a}Group A included nurses who do not work night shifts; \textsuperscript{b}Group B included nurses who worked three shifts and worked night shifts \(\leq 8\) times per month; \textsuperscript{c}Group C included nurses who worked three shifts and worked night shifts \(\geq 9\) times per month; \textsuperscript{d}\(p\) value was calculated using \(\chi^2\) test; \textsuperscript{e}\(p\) value was calculated using ANOVA.

Table 2

POMS-SFJ Subscale Mean Scores in Nurses Who Work Three Irregularly and Rapidly Rotating Shifts and Day Shift Nurses

|                     | Group A\textsuperscript{a} \textit{(n = 7)} \textsuperscript{f} | Group B\textsuperscript{b} \textit{(n = 29)} \textsuperscript{f} | Group C\textsuperscript{c} \textit{(n = 20)} \textsuperscript{f} | \(p\) \textsuperscript{d}  \\
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<tr>
<td>Depression</td>
<td>4.85 \pm 2.79</td>
<td>6.79 \pm 3.91</td>
<td>7.40 \pm 4.59</td>
<td>.37</td>
</tr>
<tr>
<td>Fatigue</td>
<td>11.86 \pm 5.01</td>
<td>11.97 \pm 5.64</td>
<td>10.50 \pm 3.94</td>
<td>.59</td>
</tr>
<tr>
<td>Vigor</td>
<td>5.71 \pm 4.15</td>
<td>5.00 \pm 4.05</td>
<td>5.15 \pm 3.31</td>
<td>.91</td>
</tr>
<tr>
<td>Anxiety</td>
<td>11.71 \pm 4.31</td>
<td>9.31 \pm 4.77</td>
<td>8.65 \pm 3.18</td>
<td>.26</td>
</tr>
<tr>
<td>Confusion</td>
<td>7.86 \pm 3.48</td>
<td>8.72 \pm 3.78</td>
<td>8.50 \pm 3.41</td>
<td>.85</td>
</tr>
<tr>
<td>Anger</td>
<td>5.00 \pm 3.65</td>
<td>7.14 \pm 5.34</td>
<td>7.00 \pm 3.91</td>
<td>.55</td>
</tr>
</tbody>
</table>

Note. \textsuperscript{a}Group A included nurses who do not work night shifts; \textsuperscript{b}Group B included nurses who worked three shifts and worked night shifts \(\leq 8\) times per month; \textsuperscript{c}Group C included nurses who worked three shifts and worked night shifts \(\geq 9\) times per month; \textsuperscript{d}\(p\) value was calculated using ANOVA. POMS=Profile of Mood States Scale (McNair, Lorr, & Droppleman, 1971; Yokoyama et al., 1990; Yokoyama, 2005).
subscale scores ($F_{(2, 53)} = 1.02$, $p = .37$). The other POMS-SFJ subscales scores were not significantly different among the three groups.

HHV-6 DNA was detected in 29% of group A, 55% of group B, and 80% of group C. Figure 1 shows the salivary HHV-6 levels among the three groups. There were significant differences in the mean salivary HHV-6 levels among the three groups ($H = 6.18$, $df = 2$, $p < .05$). Mean ± SE salivary HHV-6 levels were $585.71 ± 485.79$, $2,378.96 ± 628.84$, and $5,753.00 ± 2,119.44$ in groups A, B, and C, respectively. The salivary HHV-6 level was significantly higher in group C than in group A (post hoc Wilcoxon’s rank sum test, $p < .05$).

Figure 2 shows the salivary IgA levels of the three groups. Mean ± SE salivary IgA levels were $439.86 ± 67.86$, $517.51 ± 47.43$, and $403.44 ± 65.43$ in groups A, B, and C, respectively. Although salivary IgA level was lowest in group C, there were no significant differences in the salivary IgA level among the three groups.

DISCUSSION

This is the first study to investigate depressed mood and salivary HHV-6 levels in Japanese nurses working shifts. Although salivary IgA and mood levels were not significantly different among the three groups (Table 2, Figure 2), salivary HHV-6 levels were significantly higher in group C than in group A (Figure 1), indicating its usefulness as a new biomarker.

Group C had the highest POMS-SFJ depression subscale score, and the POMS-SFJ depression subscale scores were 34% and 39% higher in groups B and C, respectively, than the standard levels of the general Japanese healthy population (mean ± SD = 4.5 ± 4.2).
(Yokoyama, 2005). However, the POMS-SFJ vigor subscale scores were 43% and 41% lower in groups B and C, respectively (Table 2), than the standard level of the general Japanese healthy population (mean ± SD = 8.7 ± 4.2) (Yokoyama). Therefore, group B and C nurses appeared to suffer chronic fatigue, which is similar to the results of previous studies that reported that nurses working shifts had chronic fatigue and depression (Ruggiero, 2003; Tao & Kubo, 1996).

The salivary HHV-6 DNA level was significantly higher in group C than in the other groups (Figure 1). These results are consistent with those of a previous study by Kondo (2005), which showed that salivary HHV-6 levels were increased in office workers due to chronic stress associated with overtime work. Group C had a high POMS depression score, as well as a high salivary HHV-6 level. The high salivary HHV-6 level may reflect the presence of chronic fatigue in nurses working shifts. Future work is necessary to study the relationship between salivary HHV-6 and chronic fatigue, including other factors which might be related to fatigue and depression.

There were no significant differences in salivary IgA levels among the three groups (Figure 2). However, the salivary IgA level was 9% lower in group C than in group A. These results are consistent with a previous study that reported that salivary IgA levels were suppressed in nurses due to chronic stress (Yang et al., 2002). Gleeson et al. (2002) investigated salivary Epstein-Barr virus (EBV) DNA and IgA levels in swimmers. They found that after intensive training, salivary IgA levels were low and salivary EBV DNA levels were high. Here, group C had not only lower salivary IgA levels but also higher HHV-6 DNA levels than the other groups (Figures 1 and 2). Thus, the chronic fatigue that accompanies shift work appears to suppress IgA levels and promote shedding of HHV-6 in the saliva. Research is still needed to elucidate the mechanisms that increase HHV-6 production relating to chronic fatigue.

Normally, nurses working three shifts are expected to work less than nine night shifts per month in Japanese hospitals (The Japan Ministry of Health, Labour and Welfare, 2006). However, the frequency of working night shifts is often increased for various reasons, including nurse shortages (Ohotu, 2005). Our results suggest that working three shifts with nine or more night shifts per month may have negative effects on nurses. To relieve chronic fatigue associated with shift work in nurses, control of night shift frequency is important.

In recent years, prolonged shifts, such as 14-hour and 16-hour night shifts, have been introduced into nurses’ work schedules in Japan (Takahashi et al., 1999; The Japan Ministry of Health, Labour and Welfare, 2006). These prolonged night shifts are usually scheduled with rapidly and irregularly rotating shifts, which are often changed every 2 or 3 days. This scheduling pattern has led to negative consequences, such as an increased number of health problems, complaints of fatigue, and decreased quality of nursing care (Takahashi et al.). Nurses who work prolonged shifts, especially night shifts, are exposed to certain health risks (McVicar, 2003; Scott, 2000; van Mark, Spallek, Kessel, & Brinkmann, 2006). It is also necessary to investigate salivary HHV-6 levels of nurses working prolonged night shifts.

One limitation of this study is the cross-sectional design, because it cannot establish a cause-and-effect relationship between HHV-6 and shifts. Future research should investigate whether shift work induces changes in salivary HHV-6 through a longitudinal study design to evaluate causal relationships among the study variables. Another limitation was the small sample size, including the unequal allocation of subjects and convenience sampling methods (participants were all volunteers). Larger samples and randomization are necessary in future research. Finally, other potential confounding variables were not evaluated. Therefore, a more comprehensive study evaluating stress levels both at the workplace and in daily life, using salivary HHV-6 levels as a biomarker, should be carried out in the future.

CONCLUSION

To prevent health problems in nurses, nursing leaders must work to improve shift assignments based on
evidence. Leaders should acknowledge that shift work impacts on the health and performance of nurses working shifts. Reliable measures should be used to guide the development of intervention strategies, which might include increasing the number of nurses in the department assigned to permanent shifts, accommodating flexible scheduling, and designating a physical space where nurses working shifts can retreat during their shift break. Assessment by salivary HHV-6 to identify the effectiveness of these interventions is required in the future.

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