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Human herpesvirus 6 and 7 are biomarkers for fatigue, which distinguish between physiological fatigue and pathological fatigue

Ryo Aoki [a, *], Nobuyuki Kobayashi [b], Go Suzuki [c], Hirohiko Kuratsune [d, e], Kazuya Shimada [b], Naomi Oka [b], Mayumi Takahashi [b], Wataru Yamadera [a], Masayuki Iwashita [a], Shinichi Tokuno [f], Masashi Nibuya [g], Masaaki Tanichi [g], Yasuo Mukai [h], Keiji Mitani [i], Kazuhiro Kondo [b], Hiroshi Ito [a], Kazuhiko Nakayama [a]

[a] Department of Psychiatry, The Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi, Minato-ku, Tokyo 105-8461, Japan
[b] Department of Virology, The Jikei University School of Medicine, 3-25-8 Nishi-Shimbashi, Minato-ku, Tokyo 105-8461, Japan
[c] Flight Crew Operations and Technology Unit, Japan Aerospace Exploration Agency Houston Office, 18050 Saturn Lane, Suite 310, Houston, TX 77058, USA
[d] Clinical Center for Fatigue Science, Osaka City University Graduate School of Medicine, Osaka, Japan
[e] Department of Health Science, Faculty of Health Science for Welfare, Kansai University of Welfare Sciences, Osaka, Japan
[f] Department of Defense Medicine, National Defense Medical College, Tokorozawa, Saitama, Japan
[g] Department of Psychiatry, National Defense Medical College, Tokorozawa, Saitama, Japan
[h] Military Medicine Research Unit, Test and Evaluation Command, Japan Ground Self Defense Force, Setagaya, Tokyo, Japan
[i] Department of Internal Medicine, Japan Self-Defense Force Central Hospital, Setagaya, Tokyo, Japan

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ABSTRACT

Fatigue reduces productivity and is a risk factor for lifestyle diseases and mental disorders. Everyone experiences physiological fatigue and recovers with rest. Pathological fatigue, however, greatly reduces quality of life and requires therapeutic interventions. It is therefore necessary to distinguish between the two but there has been no biomarker for this. We report on the measurement of salivary human herpesvirus (HHV-) 6 and HHV-7 as biomarkers for quantifying physiological fatigue. They increased with military training and work and rapidly decreased with rest. Our results suggested that macrophage activation and differentiation were necessary for virus reactivation. However, HHV-6 and HHV-7 did not increase in obstructive sleep apnea syndrome (OSAS), chronic fatigue syndrome (CFS) and major depressive disorder (MDD), which are thought to cause pathological fatigue. Thus, HHV-6 and HHV-7 would be useful biomarkers for distinguishing between physiological and pathological fatigue. Our findings suggest a fundamentally new approach to evaluating fatigue and preventing fatigue-related diseases.

1. Introduction

Resulting from work-related or other stress or insomnia, fatigue is something that everyone experiences. Long-term fatigue can cause cardiovascular dysfunction, mental disorders such as depression, and occupational sudden death (karoshi) [1–3]. Fatigue is therefore a major social problem.

People recover from physiological fatigue with rest. Pathological fatigue, however, persists for 3 months or more and greatly affects QOL [4]. As the latter requires therapeutic interventions, we must distinguish between physiological and pathological fatigue. In this study, we investigated potential biomarkers for objectively assessing fatigue.

Fatigue is frequently assessed by self-reporting using the Checklist Individual Strength, Profile of Mood States or visual analog scales [5,6]. However, its perception is influenced by negative or positive work events and compensation practices can motivate workers to distort self-reported fatigue levels [7], which therefore may not be a correct indicator.

We focused on human herpesvirus (HHV-) 6 and HHV-7, which are reactivated by fatigue. These viruses cause exanthem subitum and establish latency in almost all individuals [8]. Frequently reactivated and shed in saliva, they are potentially a useful fatigue biomarker [9]. HHV-6 is reportedly useful for monitoring cognitive

* Corresponding author.
E-mail address: ryo-fcb@jikei.ac.jp (R. Aoki).

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function, and adverse reactions in cancer chemotherapy [10,11] and HHV-7 for assessing fatigue in end-stage renal disease [12].

We assessed fatigue prior to, during and after Japan Self-Defense Force (JSDF) military training using HHV-6 and HHV-7 DNA quantities. The JSDF was considered to be a uniform population regarding sleep, diet and other characteristics, with few confounding factors. We also investigated the mechanism of HHV-6 and HHV-7 reactivation in a mouse fatigue model, focusing on changes in inflammatory cytokines and CD14 and CD16 expressions in spleens due to fatigue. Research has shown that HHV-6 latently infects monocytes/macrophages [13] and that inflammatory cytokines play an important role in its reactivation [14]. Inflammatory cytokines are reportedly involved in monocyte activation and differentiation [15] and mature macrophages are positive for CD14 and CD16 [16,17], markers of their differentiation.

We also measured salivary HHV-6 and HHV-7 DNA quantities in patients with obstructive sleep apnea syndrome (OSAS), chronic fatigue syndrome (CFS) and major depressive disorder (MDD), which are thought to cause pathological fatigue, and normal controls (NCs). In OSAS, repeated episodes of upper-airway obstruction during sleep and cause nocturnal awakening, impairment of sound sleep, and other forms of insomnia. Causing daytime sleepiness and fatigue [18,19], OSAS is a major societal problem because sufferers become less efficient at work. CFS is triggered by complex conditions, including infection, and its diagnosis requires 6 months of unexplained fatigue that is not alleviated by rest, with 4 of 8 additional symptoms (e.g. unrefreshing sleep, sore throat, muscle pain) [20]. Depression is a low mental state with loss of interest and feeling of joy, that is frequently accompanied by fatigue [21].

To determine the usefulness of salivary HHV-6 and HHV-7 as fatigue biomarkers, we used subjects with physiological fatigue and those with pathological fatigue.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Ethics Committees of the Jikei University School of Medicine, National Defense Medical College, Osaka City University, Soiken Inc. and Soiken Clinic. Written informed consent was obtained from each subject. Animal experiments were approved by the Institutional Animal Care and Use Committee of the Jikei University.

2.2. Participants

Members of the Japan Self Defense Force (JSDF) 1st Airborne Brigade participating in 9-week ranger training were recruited for the study [22]. It was confirmed that participants had no serious physical diseases or previous psychiatric illnesses from the results of annual physical examinations and pre-training selection examinations. Those who dropped out during the training were excluded. All were male and their average age was 26.6 ± 0.4 [mean ± s.e.m.] years (n = 55). The training program basically consisted of self-training for a week, a day's rest, base training for 4 weeks, a day's rest and then 4 week's of field training. Subjects rated their fatigue level on a visual analog scale (VAS) whose total score ranged from 0 (no fatigue) to 100 (total exhaustion) at four time points: before training (2 days before training), during base-training (2 weeks into training), during field-training (3 weeks into training), and after training (3–5 days after end of the training) [22]. Saliva samples were all collected at the same time.

We also recruited 113 NCs (49 females, 64 males; age 43.5 ± 0.8 [mean ± s.e.m.]) using an advertisement. Exclusion criteria were history of medical illness, taking chronic medication or supplemental vitamins, body weight less than 40 kg, and blood donation within one month before the study or blood hemoglobin level less than 12.0 g/dl.

Regarding pathological fatigue, 42 patients aged 20–64 years who were diagnosed with OSAS at Jikei University Hospital (Tokyo) (6 females, 36 males; age 49.8 ± 1.7 [mean ± s.e.m.]) were enrolled. Diagnosis was by overnight polysomnography (PSG) in accordance with the International Classification of Sleep Disorders (ICSD-2) [23]. Also enrolled were 97 CFS patients (68 females, 29 males; age 37.8 ± 0.8 [mean ± s.e.m.]) aged between 20 and 64 years who were diagnosed at the Osaka City University Hospital based on the 1994 revised working case definition of Centers for Disease Control and Prevention (CDC) [20]. Symptoms were assessed using the Chalder Fatigue Scale [24]. We additionally enrolled 33 MDD patients (10 females, 23 males; age 45.3 ± 1.9 [mean ± s.e.m.]) aged between 20 and 64 years who were diagnosed at the Jikei University Hospital or the Jikei University Kashiwa Hospital based on the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) [21]. Depressive symptoms were assessed using the Montgomery-Asberg depression rating scale (MADRS) [25,26].

Saliva samples of less than 200 μL were omitted from the analysis.

2.3. Measurement of salivary HHV-6, HHV-7

Saliva samples were collected in a test tube (Salivette; Sarstedt AG & Co.). Following centrifugation, the flow-through was stored at −80 °C until analyzed. Viral DNA was extracted from 400 μl saliva or phosphate-buffered saline-diluted samples by automatic isolation with the BioRobot EZ1 workstation and EZ1 virus mini kit v2.0 (QIAGEN, Inc.), according to the manufacturer's protocol. DNA was eluted in 90 μl of elution buffer. Copies of HHV-6 and HHV-7 DNA in the saliva samples were quantified by real-time PCR with an Applied Biosystems 7300 real-time PCR System (Applied Biosystems). Amplifications were performed in duplicate in a total volume of 50 μl containing 25 μl of Premix Ex Taq (Perfect Real Time) (Takara Bio Inc.), 0.45 μl of PCR forward primer (100 μM), 0.45 μl of PCR reverse primer (100 μM), 1.25 μl of TaqMan probe (10 μM), 1 μl of Rox reference dye, 5 μl of the viral DNA, and 16.85 μl of PCR-grade water. The primers used for real-time PCR were as follows: HHV-6 forward primer, 5′-GACAATCACATGCCTGGATAATG-3′; HHV-6 reverse primer, 5′-TGTAAGCGTGTGGTAATGGACTAA-3′; HHV-6 probe, 5′-FAM-AGCAGCTGGCGAAAAGTGCTGTGCTAMRA-3′; HHV-7 forward primer, 5′-CGGAAGTCACTGGAGTAATGAC-3′; HHV-7 reverse primer, 5′-CCAATCCTTCCGAAACCGAT-3′; and HHV-7 probe, 5′-FAM-CCTCGCAGATTGCTTGTTGGCCATGTAMRA-3′ [27,28]. The thermal profile was 95 °C for 30 s, followed by 50 cycles of 95 °C for 5 s and 60 °C for 31 s. Data analysis used Sequence Detection Software version 1.4 (Applied Biosystems).

2.4. Animals

Six-week-old C57BL/6NCrSlc male mice were purchased from SLC Japan. They were housed in standard cages in a temperature- and humidity-controlled room with a 12-h light/dark cycle (lights on at 8:00) and free access to standard lab chow and water. We modified a rat model of fatigue for application to mice [29]. Briefly, the mice were divided into two groups: control group (Control) with no stress load and 24-h group (Fatigue) in which the mice were placed for 24 h in a cage filled with water to a height of 1 cm. The mouse spleens were harvested immediately after stress loading and preserved at −80 °C until analysis.