Orofacial Pain of Muscular Origin Is Not Associated with Herpes Virus-6 Infection: A Pilot Study

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Aims: To carry out a pilot study to test the hypothesis that human herpes virus-6 (HHV-6) infection or reactivation plays a role in the pathogenesis of temporomandibular disorders (TMD) of muscular origin (ie, localized myalgia). **Methods:** Sixteen patients with localized myalgia participated in this pilot study. Thirty-six healthy individuals served as controls. The participants were examined clinically for the presence of the TMD according to the Research Diagnostic Criteria for TMD, and the salivary levels of HHV-6 were measured by quantitative polymerase chain reaction (qPCR). The Z test, Student *t* test, and Mann-Whitney *U* test were used as appropriate. **Results:** The results demonstrated that 77.8% of healthy individuals were HHV-6 positive, but a significantly lower proportion (43.8%) of the TMD patients with localized myalgia were positive for HHV-6 ($P < .05$, Fisher exact test). The levels of HHV-6B DNA were lower in the saliva of HHV-6–positive TMD patients with localized myalgia (median: 564 genome/mL; range: 184 to 5,835 genome/mL) than in that of healthy individuals (median: 1,081 genome/mL; range: 193 to 8,807 genome/mL), but the difference was not statistically significant ($P > .05$, Mann-Whitney *U* test). **Conclusion:** The results of this pilot study indicate that HHV-6 infection or reactivation does not appear to play a role in the pathogenesis of TMD reflecting a localized myalgia. *J Oral Facial Pain Headache 2014;28:3346–349. doi: 10.11607/ofph.1095*

Key words: *chronic fatigue syndrome, herpes virus 6, myalgia, saliva, temporomandibular disorders*

Human herpes virus 6 (HHV-6) levels have been considered as markers for various diseases. Recent studies have linked HHV-6 levels with diverse disorders, including hepatitis, encephalitis, mononucleosis syndrome, chronic fatigue syndrome (CFS), fatal disseminated infection, and, more recently, multiple sclerosis (MS).[1–7] HHV-6 has been isolated from saliva,[8,9] and its DNA has been detected in saliva of healthy persons.[10,11]

It has been reported that there are overlapping symptoms between patients with CFS, fibromyalgia (FM), and temporomandibular disorders (TMD), such as myalgia, fatigue, sleep disturbances, and impairment in the ability to perform activities of daily living.[12] Furthermore, previous studies using molecular analysis have reported a higher prevalence of HHV-6 in CFS patients.[13,14]

Human herpes virus 6 (HHV-6) is a pathogen that was first isolated from patients with lymphoproliferative disorders and acquired immunodeficiency syndrome (AIDS).[15] HHV-6 is classified in the Betaherpesvirinae subfamily, along with human cytomegalovirus (HCMV) and HHV-7. There are two distinct HHV-6 variants, HHV-6A and HHV-6B.[16,17] Both variants have displayed > 90% identical DNA sequences with the exception of a few genes or regions.[18,19] Despite the high degree of similarity in their DNA content, HHV-6A and HHV-6B have distinct biologic properties and association with specific pathologic conditions.[20]

To the authors' knowledge, there have been no studies comparing the level of HHV-6 in patients with TMD of muscular origin (ie, localized myalgia) with that of healthy individuals. The aim of this study was, therefore, to carry out a pilot study to test the hypothesis that HHV-6 infection or reactivation plays a role in the pathogenesis of TMD of muscular origin (ie, localized myalgia).

Materials and Methods

The study was conducted over a 14-month period from September 2010 to November 2011. The study was approved by the International Medical University (IMU) Institutional Review Board, and all subjects provided written informed consent as part of the study protocol.

Subjects

Sixteen patients with localized myalgia, 7 men and 9 women, aged 15 to 69 years (median 25 years), who had been referred to the Oral Health Center, IMU in Malaysia because of orofacial pain of muscular origin, participated in the study. In addition, 36 healthy students of IMU, 15 men and 21 women, aged 19 to 23 years (median 21 years), who underwent the same clinical evaluation in order to exclude the presence of TMD, were included as controls. The case and control groups were gender-matched ($P < .05$; Z test) but not age-matched ($P > .05$; Student *t* test) due to the demographics of the patients visiting the oral health clinic. All cases were diagnosed by a trained specialist who made a clinical judgment that the pain was primarily of muscular origin (in accordance with a diagnosis of Group I of the Research Diagnostic Criteria for TMD [RDC/TMD][21]).

All patients underwent a complete medical and dental history. The history investigated pain, limitation of mandibular motion, and TMJ sounds. The clinical examination, which was performed according to the RDC/TMD,[21] included the evaluation of pain and tenderness to palpation of the masticatory muscles and TMJs and the range of mandibular movements. The degrees of behavioral, psychological, and psychosocial distress were evaluated by using the General Health Questionnaire (GHQ-12).[22] Scores ranged from 0 to 12, with a higher score indicating a higher level of distress. The inclusion criteria were complaints of orofacial pain of muscle origin for more than 3 months. The exclusion criteria were patients with TMD and a history of liver or kidney dysfunction, symptoms of acute illness (ie, fever, sore throat, body aches, and diarrhea), visible oral lesions at the time of enrollment, or a GHQ-12 score of more than 4 (indicative of having a probable nonpsychotic psychiatric disorder).[22] The pain intensity was not evaluated.

Collection of Saliva and Quantitative Polymerase Chain Reaction

Saliva samples (5 mL) were collected through mouth rinses with water. All samples were maintained on ice, divided into 1-mL aliquots, and stored at −80°C until use. DNA in the saliva was extracted from 0.5-mL samples by using the QIAampUltraSens Virus kit (Qiagen) according to the manufacturer's instructions. Variant-specific TaqMan quantitative real-time polymerase chain reaction (PCR; Applied Biosystem) was used to detect and quantify HHV-6 in saliva as described previously.[23–25] Briefly, the 25-μL reaction mixture contained 2 μL of purified DNA from saliva, 12.5 μL TaqMan PCR master mix, 200 nM of each primer, and a 100-nM probe specific for HHV-6 (Applied Biosystem). The sequence of the forward primer was 5'-GACAATCACATGCCTGGATAATG-3'. The sequences of the reverse primers specific for HHV-6A, HHV-6B, and both variants were: 5'-TGGTAATGGACTAATTGTGTGTTGTTTTA-3', 5'-TGGTAATGGACTAAGTGTGCGTTATTTTC-3', and 5'-TGTAAGCGTGTGGTAATGGACTAA-3', respectively. The TaqMan probe was 5'-FAM-AGCAGCTGGCGAAAAGTGCTGTGC-TAMRA-3'. All PCR reactions were performed using the iQ5 real-time PCR detector system (Bio-Rad), and data were analyzed using Bio-Rad iQ5 Optical System Software V1.0. The conditions for all PCR reactions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, and 45 cycles of 95°C for 15 seconds and 60°C for 1 minute. Purified DNAs from HHV-6 variant A (HHV-6A; GS strain) and HHV-6 variant B (HHV-6B; Z29 strain) (Source Bioscience) were used as standard controls. Determination of standard curves for both DNAs was based on triplicate tests over a range of 1 to 104 copies for each reaction. HHV-6 DNA quantitation in saliva was normalized per milliliter of saliva. The detection limit of the assay was 10 copies of viral genomes per milliliter of saliva for detection of HHV-6 and 40 copies of genomes per milliliter of saliva for detection of HHV-6A or HHV-6B without any cross-reactivity.

Statistical Analyses

The proportions of positive cases obtained from healthy individuals and patients with localized myalgia were compared using the Fisher exact test. The Z test and Student *t* test were used to check similarity between the groups' demographic data. Given that the data did not conform to a normal distribution, a Mann-Whitney *U* test was used to compare the HHV-6B DNA levels between healthy individuals and those with localized myalgia. Statistical significance was set at the $P < .05$ level (two-tailed). All data were analyzed with use of the SPSS statistical analysis software V18 (SPSS Inc).

Table 1 Summary of HHV-6 Status in Saliva of Healthy Individuals and TMD Patients with Localized Myalgia

	No. of cases (%)		Fisher exact test (*P* value)
	Healthy (n = 36)	Localized myalgia (n =16)	
HHV-6			
Positive	28 (77.8%)	7 (43.8%)	.019*
Negative	8 (22.2%)	9 (56.2%)	

*Statistical significance ($P < .05$).
Percentage (%) indicates the percentage within the subgroup.

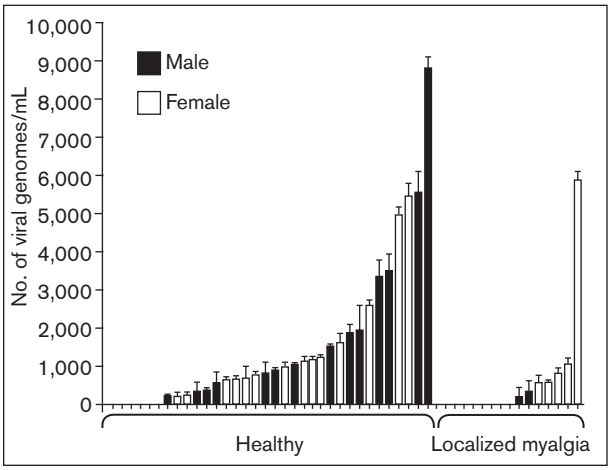

Fig 1 *(right)* The level of salivary HHV-6B DNA in each of the 28 healthy individuals and the 7 TMD patients with localized myalgia who tested positive for HHV-6B.

Results

As shown in Table 1, 77.8% (28/36) of the healthy individuals tested positive for HHV-6 viral infection but a significantly lower proportion (43.8%, 7/16) of the TMD patients with localized myalgia were HHV-6 positive ($P < .05$). The DNA content of the saliva from the two study groups was also comparable (324.8 ± 15.2 ng in control group vs 361 ± 21.5 ng in TMD patients). Interestingly, all HHV-6–positive saliva samples were found to harbor only HHV-6B DNA but not HHV-6A DNA.

Analysis by qPCR of HHV-6B levels in saliva of the TMD patients with localized myalgia revealed that the levels of HHV-6B DNA were lower in the saliva of HHV-6–positive patients with localized myalgia (median: 564 genome/mL; range: 184 to 5,835 genome/mL) than of healthy individuals (median: 1,081 genome/mL; range: 193 to 8,807 genome/mL), but the difference was not statistically significant ($P > .05$, Mann-Whitney *U* test) (Fig 1).

Discussion

TMD often coexist with other musculoskeletal and non-musculoskeletal disorders. It has been reported that TMD patients frequently have symptoms similar to those of patients with CFS, FM, chronic headaches, panic disorder, gastroesophageal reflux disease, irritable bowel syndrome (IBS), multiple chemical sensitivity, posttraumatic stress disorder (PTSD), and interstitial cystitis.[26] For instance, Aaron et al reported that patients with TMD, CFS, and FM share key symptoms, including generalized pain sensitivity, sleep and concentration difficulties, bowel complaints, and headache.[12] In addition, CFS patients were predominantly female (female:male ratio of 3:1) and their average age at onset of approximately 30 years was similar to that of TMD patients.[26–28] Thus, it has been hypothesized that these conditions may share a similar pathophysiology. In spite of this coexistence of symptoms, there is a lack of studies exploring the possible mechanisms responsible for the overlap.[29] Numerous reports have demonstrated that HHV-6 infection or reactivation is strongly associated with CFS.[5,13,14,30] Therefore, the present pilot study aimed to explore the potential association between the levels of salivary HHV-6 and TMD of muscular origin (ie, localized myalgia).

The long duration in patient recruitment in this 14-month study was mainly due to the fact that the IMU Oral Health Center is located in a developing country that is generally lacking in TMD patients. In addition, most of the patients who visited the IMU Oral Health Center are diagnosed as Group I and II patients according to the RDC/TMD criteria, and not given a single diagnosis of myofascial pain.

The use of a highly sensitive and HHV-6 variant-specific qPCR revealed that the TMD patients with localized myalgia displayed a significantly lower rate of HHV-6 and had relatively lower HHV-6 DNA levels in their saliva compared with healthy controls, contrary to the authors' expectations. Unfortunately, a thorough analysis of the current literature has not provided evidence to suggest a plausible explanation of why the HHV-6 DNA was lower in the myogenous TMD patients. Also, the factors that affect HHV-6 levels and reactivation remain largely unclear. However, it is known that infection with HHV-6 is very common, approaching 100% in seroprevalence.[31] Following primary infection, HHV-6A and HHV-6B are thought to persist for life in a latent form in peripheral blood mononuclear cells, macrophages, and vascular endothelial cells, but also as a low-level chronic replicating form also in the salivary glands; therefore, HHV-6 is secreted in saliva (as HHV-6B) without inducing any obvious pathology in the oropharyngeal epithelial cells.[31,32] A large body of evidence suggests that

HHV-6 may act as an opportunistic agent in patients with immunodeficiencies, particularly those who have undergone bone marrow or organ transplantation and are infected with the human immunodeficiency virus (HIV).[33] It is tempting to speculate that the immune system of the TMD patients with localized myalgia might have been elevated. Alternatively, it is also plausible that the reduced HHV-6 levels observed in these patients could have been due to co-infection of other viruses or other microbials. The possibility that there was a selection bias due to the limited sample size and disparity in age matching also cannot be ruled out. Although the implications of these findings remain to be further elucidated, the results of this pilot study indicate that HHV-6 infection or reactivation does not appear to play a role in the pathogenesis of TMD reflecting a localized myalgia.

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