High Prevalence of Herpes Simplex Virus DNA in Temporal Arteritis Biopsy Specimens

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Key Words: Herpes simplex; Temporal arteritis; Giant cell arteritis; Polymerase chain reaction; PCR

Abstract

Giant cell arteritis (GCA) affecting the cranial arteries is a disease of unknown cause that causes blindness, stroke, and other morbidity. Its sudden onset and segmental distribution are suggestive of diseases that involve viral reactivation, and cranial arteries are known to be innervated by ganglia that harbor herpes simplex virus (HSV). We used a high-sensitivity polymerase chain reaction assay to test for HSV DNA in specimens from 39 consecutive temporal artery biopsies performed for suspected GCA. HSV DNA was detected in 21 (88%) of 24 histologically positive and 8 (53%) of 15 histologically negative specimens \( (P = .027; \text{Fisher exact test}) \). Analysis of 10 renal artery samples from age-matched control subjects using the same assay showed no detectable HSV DNA. We conclude that detectable HSV DNA is correlated with histologically confirmed GCA in this patient population.

Giant cell arteritis (GCA) is an idiopathic cranial vasculitis that is a significant cause of blindness, stroke, and other morbidity. Immunologic and infectious causes have been proposed.\(^1\) The disease frequently involves the temporal arteries, which are biopsied routinely to establish a histologic diagnosis. The sudden onset and segmental distribution that characterize GCA are reminiscent of recurrent herpetic lesions. In addition, some factors postulated to contribute to reactivation of latent herpes simplex virus (HSV), eg, sunlight\(^2\) and hormonal disturbances, are possible triggering factors for GCA. The temporal arteries receive a portion of their innervation from the trigeminal ganglia,\(^3\) which are a major repository of latent HSV.\(^4\) Therefore, we hypothesized that reactivation of latent HSV might contribute to the pathogenesis of GCA involving the temporal arteries (temporal arteritis \( \text{TA} \)).

The present study sought to determine whether histologically confirmed GCA is associated with the presence of HSV DNA sequences detectable by polymerase chain reaction (PCR) in archival temporal artery biopsy specimens.

Materials and Methods

Formalin-fixed, paraffin-embedded specimens from 39 consecutive temporal artery biopsies for suspected GCA were analyzed. The series represented 28 women (72%) and 11 men (28%) ranging in age from 52 to 88 years (mean, 74.9 years). Clinical criteria leading to biopsy were those established by the American College of Rheumatology in 1990 and recently reviewed.\(^1\) A positive histologic diagnosis of GCA was defined by the standard criteria of granulomatous inflammation centered at the internal elastic lamina or panarteritis with a mixed inflammatory infiltrate.\(^1\)
DNA was extracted from unstained paraffin sections consecutive with sections examined histologically, using a DNeasy Tissue Kit (QIAGEN, Hilden, Germany) according to the manufacturer’s instructions. Only arterial tissue was extracted, without adjacent soft tissue. Extract of a cell culture of MRC-5 human lung cells infected with HSV-1 was included in every run as a positive control sample. Paraffin sections of a cervical biopsy or brain tissue with classic histologic HSV changes were extracted identically to the temporal artery sections as additional control samples for the effects of tissue processing on the ability to detect the HSV sequence. To control for the possibility that HSV DNA detected in arterial tissue was a false-positive, we also identically extracted and assayed a series of 10 renal artery samples from nephrectomies performed for renal cell carcinoma. The patients undergoing nephrectomy ranged in age from 45 to 80 years (5 women, 5 men) and had no known symptoms of temporal arteritis.

PCR primers designed to generate an amplicon of 92 base pairs from a conserved region of the HSV DNA polymerase gene were from Espy et al:5 forward, CAT CAC CGA CCC GGA GAG GGA; reverse, G GGC CAG GCG CTT GTT GGT GTA.

Amplification was performed with an initial DNA denaturation step at 96°C for 10 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. The run was concluded with a final extension at 72°C for 7 minutes. To test the general integrity of DNA extracted from our archival samples, we tested for the presence of β-actin using primers designed to generate a 202-base-pair product based on the published sequence of human β-actin: forward, CCT TCC TGG GCA TGG AGT CCT G; reverse, GGA GCA ATG ATC TTG ATC TTC.

PCR products were separated on a 2% agarose gel, with β-actin bands detected by ethidium bromide staining. To detect the HSV amplicon, products were transferred to positively charged nylon membranes (Nytran, Schleicher and Schuell, Keane, NH) by capillary transfer. Membranes were probed with a 3’ end digoxigenin-labeled oligonucleotide (TCC AAA GAC AGC AGA AAA C) complementary to a portion of the amplified region using the Genius nonradioactive detection kit (Roche, Mannheim, Germany) according to the manufacturer’s instructions. The identity of amplified HSV bands also was confirmed by sequencing the PCR product. Ethidium bromide–stained bands from 4 samples (3 histologically positive for GCA and 1 negative) were excised from agarose gels, purified by phenol-chloroform extraction, amplified by a second round of PCR, purified again by phenol-chloroform extraction, and sequenced at the Tufts University School of Medicine sequencing facility.

Results

A statistically significant association (P = .027; Fisher exact test) was found between histologic evidence of GCA and the presence of HSV DNA sequence detected by PCR in temporal artery biopsy specimens Image 1, Image 2, and Table 1. The predicted PCR product was detected by Southern blot hybridization in 21 (88%) of 24 biopsy specimens with histologic evidence of GCA, including granulomatous and polymorphous types of inflammation. These included 5 of 5 histologically positive specimens from men and 16 of 19 histologically positive specimens from women. The product also was detected in 7 (47%) of 15 temporal artery specimens that showed no histologic evidence of GCA but not in any of the 10 approximately age-matched renal artery samples Image 3. β-actin bands were detectable in all specimens.
The specificity of positive PCR results was verified by sequencing, which showed that all sequenced samples matched a predicted conserved sequence of HSV DNA polymerase in the GenBank database (bases 66339-66430).7

Discussion

TA is a manifestation of GCA, a systemic vasculitis of unknown cause. GCA affects elastic and muscular arteries, with a predilection for the cranial arteries. It predominantly affects elderly patients, especially postmenopausal women. Almost any medium or large-sized artery may be involved. Involvement of the carotid artery or its branches produces the majority of symptoms. The temporal artery most often is biopsied because of its accessibility and its frequent involvement.3 Polymyalgia rheumatica, a clinical syndrome characterized by bilateral and symmetric pain and stiffness, fever, weight loss, and increased erythrocyte sedimentation rate (ESR), is considered a closely related condition. Immunologic studies have strongly suggested that GCA is antigen driven,2 and immune reactivity against arterial elastic fibers has been hypothesized.2

Several lines of evidence suggest that infectious agents might be involved in the pathogenesis of TA. These include accompanying or preceding clinical symptoms such as fever and malaise and high ESR. Seasonal fluctuation in the incidence of the disease suggests an exogenous etiologic factor. Recently, a 40-year longitudinal study of the epidemiology of GCA in Olmsted County, MN, demonstrated epidemic-like, cyclic fluctuation in the incidence of the disease, peaking every 6 to 7 years.10 Several reports describe the simultaneous occurrence of biopsy-proven TA in conjugal pairs,11,12 consistent with common exposure to an infectious agent. Chlamydia pneumoniae13 and parvovirus B1914 DNA have been detected by PCR in temporal artery biopsy specimens from patients with GCA. Onset of TA has been reported after vaccination for influenza, tetanus, and hepatitis B.15

We have demonstrated a significant correlation between biopsy-proven TA and HSV DNA sequence detected by PCR. However, we also detected HSV DNA in 8 specimens that were negative histologically, and 3 PCR-negative specimens were histologically positive. The high overall prevalence of HSV in temporal artery biopsy specimens in this series is unlikely to represent a false-positive artifact related to arterial tissue because HSV was not detectable in identically analyzed renal arteries from patients without symptoms of arteritis. Therefore, it seems to be a true reflection of the prevalence of HSV in temporal arteries biopsied because of clinical symptoms of TA. If the clinical symptoms were caused by HSV, the presence of HSV DNA in a histologically negative biopsy specimen might be explained by healing, by sampling error, or by biopsy during an early phase or forme fruste of TA. Alternatively, a high background prevalence of HSV might be characteristic of all temporal arteries. That possibility is consistent with recent studies suggesting that the prevalence of occult herpes family virus infections is greater than previously believed and might approach 50% in some populations.16 The difference between temporal and renal arteries in that case might be explained by relationships of the temporal arteries to ganglia that are HSV repositories.4

Despite the aforementioned considerations, our detection of HSV DNA in a high percentage of histologically negative temporal artery biopsy specimens was somewhat surprising. Therefore, we attempted to determine whether discordance between PCR findings and histologic features in this study could be accounted for by the pattern or severity of inflammation or by the nature of clinical signs or symptoms. PCR-positive or PCR-negative specimens exhibited granulomatous and polymorphous inflammation that varied from mild to very severe. Detailed clinical information was not available in most cases. In addition, variable periods of steroid treatment before biopsy might have hampered clinicopathologic correlations. However, clinical data were intriguing in several cases. Two PCR-positive specimens from patients with severe clinical signs and symptoms, including high ESR, headache, temporal artery tenderness, fever, and retinal ischemia, were only weakly positive for TA histologically.

We do not know whether HSV is a causative agent of TA or merely a bystander. If HSV is a major cause of TA, temporal arteries with histologically confirmed arteritis would be expected...
to have a higher prevalence of HSV than those with an unconfirmed diagnosis, despite a high background prevalence. Our findings are consistent with the possibility that activation of latent HSV delivered to arterial walls by retrograde axonal transport causes local inflammation and sensitizes susceptible individuals to constituents of the vessel wall. This could account for the presence of granulomas, which are not typically associated with viral infections, and the segmental distribution of lesions, the latter being determined by the distribution of axonal fields from infected neurons. Granulomatous inflammation, including granulomatous vasculitis, has been associated with herpesvirus infections, and the granulomas in some cases have been associated with viral antigens.

A previous PCR study failed to detect HSV DNA in histologically positive temporal artery biopsy specimens. The discrepancy with our positive results might reflect greater sensitivity of our method. Compared with the previous study, we tested for a different and shorter PCR product, used more sensitive Southern blotting detection of PCR products, and analyzed sections of artery immediately consecutive to those in which histologic lesions were observed, rather than adjacent tissue. Southern blotting of PCR products generally is considered to increase sensitivity by at least 10-fold compared with direct Southern blotting of PCR products.

In future studies, it would be of interest to determine the prevalence of HSV detectable prospectively by the sensitive technique that we used in autopsy specimens of temporal arteries without clinical suspicion of GCA. Although this determination was beyond the scope of the present study, the results of such studies might suggest an association of HSV with clinical signs and symptoms of GCA, even in cases in which low-grade or burned-out lesions cannot be diagnosed histologically. Confirmation and expansion of our findings in larger series could lead to new approaches to the treatment of GCA and related disorders.

**References**