Viral-Associated Trichodysplasia

Characterization of a Novel Polyomavirus Infection With Therapeutic Insights

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Background: Viral-associated trichodysplasia of immunosuppression is a rare cutaneous eruption that is characterized by follicularly based shiny papules and alopecia with characteristic histopathologic findings of abnormally anagen follicles with excessive inner root sheath differentiation. Prior reports have described the histopathologic characteristics on vertical sections; however, to our knowledge, immunohistochemical analysis of polyomavirus proteins has not been previously performed.

Observations: We discuss the thorough diagnostic evaluation and therapy of an unusual case of viral-associated trichodysplasia due to a newly described human polyomavirus that occurred in a patient with post-treatment chronic lymphocytic leukemia and an abnormal white blood cell count. Unique to our study is the immunohistochemical staining for the polyomavirus middle T antigen, which demonstrated positive staining of cellular inclusions within keratinocytes that compose the inner root sheath. Further evaluation with scanning electron microscopy and polymerase chain reaction analysis of viral DNA confirmed the presence of the virus. Treatment with topical cidofovir resulted in dramatic clinical improvement and hair regrowth.

Conclusions: Several tools, including immunohistochemical staining for the polyomavirus middle T antigen, can be used to identify the pathogenic virus associated with viral-associated trichodysplasia. This case highlights the utility of multiple diagnostic modalities and a robust response to a topical therapeutic agent, cidofovir.

ent on her nose, forehead, cheeks, and chin, with background erythema and nonscarring alopecia. She also had small white spicules protruding from some papules on her nose, madarosis of the eyebrows (Figure 1A and B), and small skin-colored papules on her arms, thighs, chest, neck, and ears as well as alopecia within the affected areas. Laboratory tests were remarkable for a white blood cell count of 3600 µ/L (to convert to \( \times 10^9/L \), multiply by 0.001) (reference range, 4000-10 900 µ/L), with an otherwise normal complete blood cell count and comprehensive metabolic panel.

Skin biopsy specimens from her eyebrow and arm were examined in both vertical and horizontal cross sections. Merkel cell carcinoma samples and scar tissue from re-excisions served as controls. Formalin-fixed, paraffin-embedded human skin sections, 8 to 10 µm thick, were obtained from the Penn Skin Disease Research Center Tissue Bank according to protocols approved by the institutional review board of the University of Pennsylvania, Philadelphia.

Routine hematoxylin-eosin staining and immunohistochemical analysis for the polyomavirus middle T antigen were performed on sections (Lifespan Biosciences Inc.). Immunohistochemical analysis was performed using a mouse anti-SV-40 antibody (EMD Chemicals), which recognized the NH\(_2\) terminus within amino acids 83-128 of a large tumor antigen of SV-40 and JCV.\(^1\) Unstained slides were deparaffinized, rehydrated, quenched with endogenous peroxidase, and immunostained using the biotin-peroxidase technique (Spring Bioscience). Antigen retrieval was done with 10mM citrate buffer at subboiling temperature. Negative and positive controls were run at the same time.

Histopathologically, aberrant keratinization of the inner root sheath hair shaft cells was observed, with numerous enlarged, bulbous anagen hairs and a thin layer of basophilic, germinative cells transitioning to inner root sheath-type cells containing several enlarged bluish gray inclusions. There also were vacuolated keratinocytes with pyknotic nuclei and coarse keratohyaline granules. (Figure 2A and D) Immunohistochemical analysis for the polyomavirus middle T antigen demonstrated positive staining of cellular inclusions within keratinocytes composing the inner root sheath in all of the follicles examined in the patient’s samples (Figure 2E and F). Follicles of control samples (Merkel cell carcinoma, representing another poly-

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**Figure 1.** Photographs of the patient before (A and B) and after (C and D) treatment. A and B, The patient presented with skin-colored papules that were nearly confluent on her nose, forehead, cheeks, and chin, with background erythema, nonscarring alopecia, and small, white spicules. C and D, Dramatic improvement is seen after 4 months of treatment with topical cidofovir ointment, 1%, once daily. Note the flattening of the papules, absence of white spicules, and improvement in erythema.
omavirus cutaneous infection, and normal skin) demonstrated negative staining. An additional skin biopsy specimen was obtained for electron microscopy and revealed small (35.6-nm), icosahedral, regularly spaced, intracellular viral particles within these inclusions, consistent with polyomavirus (Figure 2G and H).

Detection of the polyomavirus genome was performed by PCR on genomic DNA isolated from paraffin sections (Qiagen). The PCR assay was performed with primers TSV288-F 5'-TATGTTTGCACTGGGTG-3' and TSV295-R CTTTGAACTTGGATGTGC-3', multiple primer pairs that are specific for the human polyomavirus that is associated with trichodysplasia spinulosa.2 The PCR conditions were as follows: 95°C for 5 minutes, 95°C for 1 minute, 48°C for 1 minute, and 72°C for 1½ minutes for 40 cycles; elongation at 72°C for 10 minutes; and then incubation at 4°C. The PCR products were run on 0.8% agarose gel at 100 V for 1 hour. Sequencing of PCR products confirmed the presence of TSV.2,3

All of the clinicohistopathologic findings supported a diagnosis of VAT. We treated our patient with topical cidofovir ointment, 1%, once daily based on prior reports of successful use.2,4 She had dramatic improvement in the papules and white spicules, with slow improvement in overall alopecia over a period of 6 months. Her renal function remained normal, and her white blood cell count remained stable throughout her course. To spread more diffusely, topical cidofovir was compounded into a lotion with increased ability to expand over large surfaces, with continued clinical response. Attempts to wean therapy in clinically improved areas resulted in a recurrence of the papules, and untreated areas remained affected, strongly supporting the efficacy of the topical treatment (Figure 1C and D).

COMMENT

Viral-associated trichodysplasia was originally described in 1999 as a folliculocentric viral infection in a patient who was receiving cyclosporine after kidney and pancreas transplantation.5 Since that time, it has been reported under a variety of names (eg, cyclosporine-induced folliculodystrophy, trichodysplasia spinulosa, pilomatrix dysplasia) and in association with other immunosuppressant agents that are used in cases of organ transplantation or as part of chemotherapy regimens, including tacrolimus, azathioprine, prednisone, mycophenolate mofetil, cyclophosphamide, methotrexate, rituximab, fludarabine, intravenous immunoglobulin, and vincristine. Both children and adults have been affected by VAT, and it has been reported to occur after lung, heart, and kidney transplantations and in the setting of acute lymphocytic leukemia.2,3,6-15 In 1 case, the diagnosis of VAT preceded a relapse of non-Hodgkin lymphoma in a 68-year-old man.4 Similar to our case, there is 1 report of VAT that occurred 2 months after completion of chemotherapy in a 70-year-old man with acute lymphocytic leukemia.16 Screening for this virus by PCR assay in unaffected immunosuppressed patients demonstrated a 4% prevalence, suggesting that active viral infection may be underreported or that the virus may lead only to overt clinical disease in a subset of immunocompromised patients.2
Our case highlights all of the previously reported findings, including the classic histopathologic appearance of large granules and the electron microscopic findings of intranuclear, icosahedral viral particles between 35 and 36 nm in diameter (reported virion diameters range from 28 to 46 nm). Also, sequencing of PCR products confirmed the presence of the human polyomavirus TSV, as reported previously. Unique to this report is the immunohistochemical identification of the human polyomavirus middle T antigen corresponding to large eosinophilic cellular inclusions in keratinocytes of the inner root sheath, which were visualized on both vertical and horizontal sections. In the initial description of VAT, immunohistochemical stains demonstrated increased Ki-67 protein expression and negative staining for both papillomavirus and the large T antigen of the BK polyomavirus. At that time, the initial virus was assumed to be part of the Papovaviridae family, which has subsequently been split into the Papillomaviridae and Polyomaviridae families. The PCR and sequencing confirmation of the human polyomavirus DNA, as well as the results of immunohistochemical staining for specific polyomavirus middle T antigen, supports this as a polyomavirus and not a papillomavirus. In our study, the positive staining for the polyomavirus middle T antigen in the trichohyaline granules suggests that this target may be a useful tool for the identification of this disease process and further supports the existing data that the human polyomavirus is responsible for the characteristic findings of VAT.

Although there are limited data available regarding the treatment of VAT, clinical improvement has been reported with various antiviral therapies and with reductions in immunosuppression. Our case demonstrates an impressive, persistent response to topical cidofovir therapy without any adverse effects (Figure 1A). A mixture of topical doxycycline, imiquimod, topical retinoids, and oral minocycline have been tried, with limited success. Improvement in immune status also has led to resolution of symptoms, and there is a report of spontaneous remission. Notably, our patient had a recurrence of her lesions with cessation of topical cidofovir therapy and experienced improvement only in areas in which the topical cidofovir was directly applied, and while she has not been treated with immunosuppressive medications for more than 6 months, it is reasonable to presume that she may have some lingering immunodeficiency. Our case of VAT is important because it provides a comprehensive evaluation of the disease, including the immunohistochemical staining for the middle T antigen or other viral proteins as a potentially more useful and practical way to establish the diagnosis compared with PCR assay or electron microscopy. Routine testing with PCR assay, electron microscopy, or immunohistochemical stains is not necessary in all cases, as routine histologic staining can be sufficient to make the diagnosis in cases of classic clinical presentations. Because the true incidence and clinical spectrum of this recently recognized disease is not known, these tools may be valuable in confirming the diagnosis in cases involving more subtle or atypical presentations. It is important that clinicians are aware of all of the potential evaluations that can be performed and that they recognize the newly described human polyomavirus as the infectious agent that is responsible for VAT, which may become significant if new viruses emerge with similar clinical findings. Further cases are required to verify the utility of immunohistochemical staining, because a negative result would not exclude the diagnosis. Also, we report a dramatic response to topical cidofovir therapy and confirm its efficacy as an important treatment for this cosmetically disfiguring disease.

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The Clap Heard Round the World

Gonorrhea is an ancient disease that was transmitted by Chinese tradesman bartering their goods, Greek soldiers fighting for new territory, and international business men traveling across the world. The earliest written description dates from 2600 BC, in which Chinese Emperor Huang Ti describes a disease resembling gonorrhea in his medical textbook. Leviticus 15:2 refers to men with urethral discharge as follows: “[W]hen any man hath a running issue out of his flesh, because of his issue he is unclean.” Galen, a Greek physician (AD 130-200), is credited with naming gonorrhea after the Greek words γόνος (semen) and ροιος (to flow). He mistakenly attributed the disease to an involuntary ejaculation of semen.¹ By the 1300s, gonorrhea’s association with prostitution and sexual activity was noted. The evolution of gonorrhea’s slang name, the clap, comes from the French words les clapiers. A literal translation of les clapiers is rabbit huts, referring to the small huts where prostitutes often lived and serviced their customers.

Gonorrhea continued to spread from Europe to the New World through seafaring sailors. With the expansion of the United States into uncharted Western territories, gonorrhea and other sexually transmitted infections became a major concern for exploratory expeditions. Meriwether Lewis and William Clark included a surprisingly substantial amount of medical supplies—approximately 15%—in their expedition inventory. Most likely, they rightly expected to encounter venereal disease among Native American tribes and their own corps members. A review of their medical supplies revealed the following purchases: “2 lbs Sal Nitri, for treatment of fevers and gonorrhea, 6 oz Sugar of Lead/Lead Acetate, for treatment of eye problems and gonorrhea, ¼ lbs Balsam of Copaiba, for treatment of rheumatism and gonorrhea, and 4 penis syringes, for injection of gonorrhea medications.”²

Despite medical advances and the effective treatment of gonorrhea with present-day antibiotics, the clap continues to spread, affecting all ages, ethnicities, and social classes. An investigation into its history reveals its long coexistence with humans and predicts that the clap will continue to persist for many more centuries.

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