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Histopathologic identification of *Trypanosoma cruzi* (Chagas') encephalitis in an AIDS patient

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ABSTRACT

Trypanosoma cruzi (Chagas') encephalitis is an uncommon manifestation of *T. cruzi* infection, typically seen in immunocompromised patients. Encephalitis results from the reactivation of chronic infection predominately in individuals from endemic areas. Increased awareness of this complication is essential especially with increased migration of patients from endemic areas with concomitant HIV infection. Here we report a case of Chagas' encephalitis in an AIDS patient from Mexico in which there was no evidence of acute serologic, CSF, or blood infection by *T. cruzi* trypomastigotes.

1. Introduction

Chagas' disease is a zoonosis caused by infection with the flagellated protozoan *Trypanosoma cruzi*. Chagas' disease is most common in southeastern Mexico, Central America, and South America. Cases reported in the United States are most often among immigrants from endemic countries, although vector-borne transmission in North America has been described. Infection is primarily acquired via contact with the feces of the triatomine insect. The insect nests in cracks and holes inside of homes [1]. Transmission occurs when an infected vector contaminates the skin or mucosa of a human with feces containing the trypomastigotes [2]. The parasite enters the bloodstream when the host scratches the skin area containing the insect feces. Less common means of transmission of the parasite include blood transfusion, organ transplantation, and vertical transmission from mother to infant [3]. In endemic areas, infection can be prevented by assuring proper housing structure, avoiding cracks in house structures, use of repellant, or insecticide-treated bed nets, and screening of tissue and blood products for the parasite [2].

Acute *T. cruzi* infection may be associated with fever or swelling at the inoculation site (chagoma). Early infection may be asymptomatic. Acute infection is associated with a robust parasitemia. Most infected individuals then proceed to a phase of illness defined as the "chronic indeterminate" phase. During this phase, patients are asymptomatic with little or no evidence of parasitemia. The organisms can remain dormant for many years. Only 20–30% of infected individuals suffer long-term sequelae of infection [1]. The most common manifestations of chronic infection include gastric motility disorders such as megaesophagus and megacolon, and cardiac conditions such as dilated cardiomyopathy and conduction abnormalities [1,2].

The reactivation of chronic stages of disease is uncommon. Patients at greatest risk for reactivation include those with cellular immunity disorders such as acquired immunodeficiency syndrome (AIDS), leukemia, and transplantation [3]. Cerebral tumors or chagomas caused by *T. cruzi* is an uncommon complication of Chagas' disease, observed only in immunosuppressed patients [3]. Individuals with advanced HIV disease who have spent significant time living in areas of high endemicity of *T. cruzi* are at risk for the reactivation of Chagas' disease. Those with CD4+ cell counts <200 cells/mm^3 are also at increased risk of reactivation [3]. Such individuals may present with meningitis or focal cerebral chagoma, which may be indistinguishable from toxoplasmosis, tuberculosis, or a neoplastic process such as lymphoma.

2. Case report

A 47-year-old Mexican male with a history of alcohol and intravenous drug abuse presented to an outside hospital with right arm and

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bilateral lower extremity weakness. He was found to be HIV positive with a CD4 count of 18 cells/mm^3 and HIV viral load of 224,586 copies/mL. This low CD4 count together with evidence of HIV infection qualified him for a diagnosis of AIDS. He had not received HAART therapy. The neurological symptoms were thought to represent HIV neuropathy. During his hospitalization, his weakness progressed rapidly with strength of 0/5 in the right upper extremity and 3/5 in bilateral lower extremities. Initial MRI performed at an outside institution demonstrated a left frontal craniotomy with a small resection cavity in the left frontal lobe. There was a dominant mass in the left frontal lobe white matter demonstrating ill-defined nodular and peripheral enhancement with surrounding vasogenic edema. There were small areas of diffusion restriction. A smaller lesion was present in the left aspect of the midbrain–pons junction. The patient stated the craniotomy was the result of an accident that necessitated a neurosurgical procedure 8 years prior to admission.

Based on the imaging findings and the patient's CD4 count, he was empirically treated for *Toxoplasmosis* with pyrimethamine and clindamycin. However, after approximately 9 days of empiric therapy for presumed toxoplasmosis, he did not show any clinical improvement and subsequent brain MRI showed progression in size and the number of supratentorial lesions. At this point, he was transferred to Thomas Jefferson University for brain biopsy and further management.

Upon admission, the patient was in no apparent distress and communicative. His musculoskeletal and neurological examination revealed an alert, awake male, oriented only to person, unable to spell words frontward or backward, and somewhat inattentive. Cranial nerves II through XII were grossly intact. Examination revealed left upper extremity dysmetria on finger-to-nose test. Deep tendon reflexes were 2+ and symmetric in the upper and lower extremities bilaterally, with down-going toes bilaterally. Strength was worsening (0/5 strength in the right upper and lower extremities, both distally and proximally; 5/5 strength in the left upper extremity, both distally and proximally, and 0/5 on the left lower extremity, both distally and proximally). Sensory examination was abnormal to all modalities and decreased in the right upper and bilateral lower extremities throughout. A lumbar puncture revealed red blood cell count of 5 cells/μL, white blood cell count of 2 cells/μL, elevated CSF protein (141 mg/dL, normal range 15–55 mg/dL) with decreased glucose (29 mg/dL; normal range 40–70 mg/dL) with oligoclonal bands (>5 IgG), and elevated myelin basic protein (15.4 mcg/L, abnormal: > 6.0 mcg/L). He was found to be neutropenic during his hospital stay, attributable to HIV/AIDS. A CSF *Toxoplasma gondii* quantitative PCR test was negative. He had positive *Toxoplasma* IgG, and he continued to receive empiric therapy for presumed toxoplasma encephalitis, with a regimen of pyrimethamine, sulfadiazine, and leucovorin. Additionally, he was started on Vancomycin, ceftriaxone, and metronidazole for a presumptive intracranial abscess. Testing for the following infectious agents were negative in the CSF: cryptococcal antigen, EBV quantitative DNA PCR, herpes simplex virus, types I and II DNA, syphilis antibody, and JC polyoma virus DNA quantitative PCR. Serology was negative for active infection with syphilis, hepatitis A (IgM negative), or hepatitis C (negative HCV antibody, negative HCV RNA PCR). Hepatitis B core antibody and hepatitis B surface antibody were positive although HBV surface antigen was negative. On hospital day 7, he was started on antiretroviral therapy. After 15 days of antiretroviral therapy, HIV viral load declined to 806 copies/mL.

A follow-up MRI performed at our institution showed an increase in size and surrounding edema of the lesions, as well as interval hemorrhage (Fig. 1). A cervical spine MRI with contrast (not shown) was obtained and did not demonstrate any abnormality other than mild degenerative changes. There were multiple, irregularly enhancing masses with areas of diffusion restriction and hemorrhage involving the supratentorial and infratentorial compartments. The differential diagnosis before biopsy included *Toxoplasmosis* and CNS lymphoma.

The patient's neurologic condition continued to deteriorate to the point that he could not provide consent for brain biopsy. Due to the lack of any known relative or close contacts, emergent consent was ultimately obtained and an open biopsy was performed on the 17th day following admission to our hospital. The biopsy specimen demonstrated a confluent lymphohistiocytic infiltrate with plasma cells and some neutrophils within central nervous system tissue. There were areas of reactive gliosis and foci of necrosis. Scattered throughout were individual and clusters of small protozoal organisms (Fig. 2A). Individual organisms measured 1–3 μm in greatest dimension. The larger clusters resembled bradyzoites of *Toxoplasmosis* (Fig. 2B). Well-preserved organisms contained a round nucleus with an adjacent rectangular bar of chromatin consistent with a kinetoplast. There was minimal cytoplasm. Immunohistochemistry for toxoplasmosis (performed by Integrated Oncology, New York, New York) was negative.

Based on the histopathologic findings and the patient demographics (recently emigrated from Mexico), the differential diagnosis included kinetoplastids such as *T. cruzi* and *Leishmaniasis*. Therefore, formalin-fixed paraffin-embedded tissue was sent to the Centers for Disease Control (CDC) where PCR analysis positively confirmed the presence of *T. cruzi*. While awaiting the results, the patient was continued on therapy for possible *Toxoplasma* infection. He was briefly treated with intravenous amphotericin due to concern for possible *Leishmania* infection. Serum *T. cruzi* antibody testing sent to the CDC was negative. Following CDC Investigational New Drug (IND) Protocol no. 5765.0, benznidazole was provided by the CDC for treatment of Chagas' disease. He received a total course of 17-day therapy with benznidazole. Concurrent steroid therapy was used. His course was complicated by transaminitis, necessitating brief interruption of benznidazole therapy. Over the course of his treatment, the patient's respiratory and hemodynamic status greatly deteriorated, with concern for increased intracranial pressure and seizures, possibly secondary to the initiation of antiprotozoal treatment and possibly due to immune reconstitution syndrome secondary to initiation of HAART therapy. The patient remained in a minimally responsive state with a stable neurologic exam and expired on the 50th hospital day.

The brain autopsy showed an area of extensive necrosis involving the left cerebral hemisphere, left basal ganglia, pons, and medulla measuring 8 × 4 × 4 cm. There were numerous macrophages with rare degenerated amastigotes. No viable organisms were identified. The right basal ganglia/thalamic lesion seen on MRI showed similar findings and accounts clinically for the patient's left-sided motor and sensory deficits. The systemic autopsy revealed no evidence of Chaga's disease in any other organ. There was acute bronchopneumonia positive for Cytomegalovirus.

3. Discussion

The definitive diagnosis of *T. cruzi* meningoencephalitis or chagomas in HIV patients is often challenging. Trypomastigotes can sometimes be visualized on the microscopic examination of cerebral spinal fluid [3,4]. Serologic testing is available, although cases of confirmed central nervous system Chagas' disease among individuals with negative serologic testing for *T. cruzi* have been described [3,4]. Negative serology for *T. cruzi* does not exclude the possibility of Chagas' central nervous system disease. Indeed, our patient did not have evidence of trypanosomes in the blood and was serologically negative for *T. cruzi*.

Brain lesions caused by the reactivation of *T. cruzi* can have a variable radiographic appearance and have been described as single or multiple lesions, with edema and contrast-enhancement [3]. In two series comprising a total of 25 patients with HIV/AIDS, most of the lesions were supratentorial in location [4,7]. The majority of lesions in one of these series demonstrated enhancement with white matter involvement [7]. Enhancing intramedullary spinal cord lesions have also been reported [8]. The MRI spectroscopy of Chagas' lesions consists of a non-specific pattern of increased choline, decreased or absent *N*-acetylaspartate, and a lactate peak [6]. In summary, the MRI findings of cerebral Chagas'

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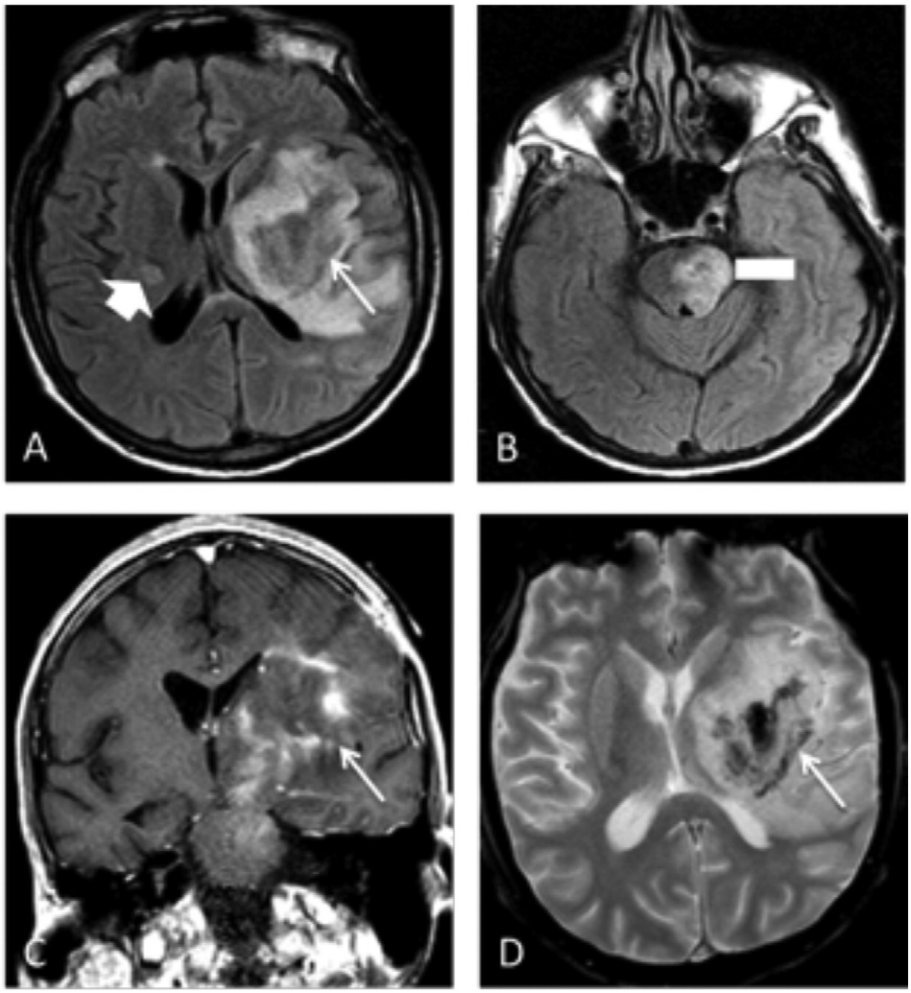

Fig. 1. MRI of brain 34 days after initial presentation. (A and B) FLAIR exhibits increased mass effect and edema in the left frontotemporal region, involvement of the left basal ganglia (*arrow*) and right basal ganglia (*arrowhead*). Increased edema surrounding the midbrain/pons lesion (*minus*). (C) Contrast-enhanced coronal T1-weighted image demonstrates nodular and irregular enhancement in the left frontal lobe white matter, left basal ganglia, midbrain and pons (*arrow*). (D) Gradient echo image shows areas of low signal corresponding to hemorrhage (*arrow*).

disease are non-specific and demonstrate features of both lymphoma and *Toxoplasmosis*.

Macroscopic brain autopsy findings take the form of an extensive necrotizing encephalitis. The majority of CNS Chagas' patients have the pseudotumoral form, characterized by the presence of single or multiple necrotic-hemorrhagic nodular lesions that are predominantly located in the cerebral lobes. In some cases, the lesions involve the brain stem and cerebellum. The lesions are poorly demarcated, measuring several centimeters in diameter, preferentially involving the white matter [9].

The histologic picture of CNS Chagas' is an acute necro-hemorrhagic encephalitis, characterized by microglial nodules, with associated hemorrhage, necrosis, and exudates of lymphocytes, macrophages, plasma cells, and occasionally neutrophilic granulocytes within the nervous tissue and perivascular spaces [9]. The parasites exist in abundance within macrophages and astroglia in the amastigote forms but are not found in neurons.

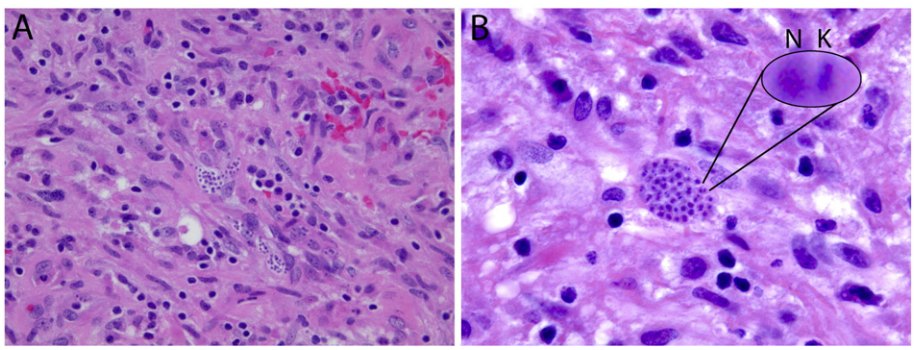

Fig. 2. Biopsy of Brain lesion. (A) Small clusters of protozoal organisms within a background lymphohistiocytic infiltrate (hematoxylin and eosin, original magnification 400×). (B) Bradyzoite-like cluster of protozoans with kinetoplasts (enlarged inset). Hematoxylin and eosin, original magnification 1000×. N, nucleus; K, kinetoplast.

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The identity of the parasite can be confirmed immunohistochemically or by PCR. Lymphoplasmacytic leptomeningitis is a constant finding in CNS Chagas' and thought to represent an extension of the subjacent necrotic-inflammatory lesions [9]. Histologically, the differential diagnosis of *T. cruzi* includes other protozoal infections such as *Toxoplasmosis*, *Leishmania*, and other trypanosome species. The key feature to recognize is the presence of the kinetoplast. Kinetoplasts are mitochondrial DNA that appears as a bar just above the nucleus (Fig. 2B) and can only be appreciated in well-preserved amastigotes at high magnification (400–1,000×). The kinetoplast resembles an eyebrow over the nucleus.

Primary prophylaxis with antiparasitic medication may prevent reactivation in immunocompromised individuals. HIV-infected individuals with epidemiologic risk factors for *T. cruzi* exposure should be tested for latent infection via serology. Such patients who test positive and have no symptoms of Chagas' disease may benefit from a single course of primary prophylaxis with an antiparasitic agent (benznidazole or nifurtimox) [5]. Additionally, initiation of antiretroviral therapy may prevent the reactivation of Chagas' disease [5].

The reactivation of Chagas' disease is severe in AIDS patients. While there are diagnostic challenges in regard to cerebral *T. cruzi* in AIDS patients, it is important to consider the diagnosis in individuals who are failing to respond to therapy for toxoplasmosis and who have epidemiologic risk for Chagas' disease. If cerebral Chagas' is not considered in the differential diagnosis, the diagnosis is often made late in the clinical course. Delay in accurate diagnosis can lead to life threatening events and significant mortality [3].

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