Abstract Human T-cell lymphotropic virus type I (HTLV-I) is a human retrovirus and the aetiologial agent of a progressive neurological disease called tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM), as confirmed by evidence accumulated in HTLV-I seroprevalence studies. TSP/HAM is rarely diagnosed in Italy, given the low prevalence of HTLV-I in the population. TSP/HAM begins insidiously in the fourth decade, mainly with spastic paraparesis of the lower extremities and positive Babinski reflex, as well as interfering with bowel and bladder functions. In this study we report the clinical, virological and haematological data of a 54-year-old woman, born in the Ivory Coast, with symptoms suggestive of TSP. The presence of HTLV-I infection was demonstrated by the detection of antibodies in serum and in cerebrospinal fluid by immunoenzymatic assay and Western blot analysis. In addition, viral isolation was carried out in peripheral blood cells, and the presence of HTLV-I proviral DNA was confirmed by polymerase chain reaction/Southern blot and sequencing analysis. According to our results, HTLV-I testing might be useful when TSP/HAM is suspected.

Key words HTLV • HAM/TSP • Cerebrospinal fluid • PCR • Southern blot • Sequence analysis

Introduction

Human T-cell lymphotropic virus type I (HTLV-I) is a retrovirus with a tropism for mature T-lymphocytes that has been linked to two clinical conditions: adult T-cell leukaemia lymphoma (ATL) and a neuromyelopathy called tropical spastic paraparesis (TSP) or HTLV-I-associated myelopathy (HAM) [1–4]. TSP was first described by Strachan in the late 19th century and by Cruickshank in 1956, while HAM was described by Vernant et al. and Gessain et al. [5, 6], followed by Osame et al. [7] in Japan in 1986. Soon after, TSP and HAM were recognised to be one and the same syndrome. The causative virus has long been known to be HTLV-I, although case reports [8, 9] indicate that HTLV-II is likely to be responsible as well.

The association between HTLV-I and TSP/HAM varies across geographical regions. They both occur in south-western Japan and the Caribbean area. HTLV-I infection may also be prevalent in some areas of Africa, including the Ivory Coast. Outside these established endemic area, the rates of HTLV-I infection are unknown but appear to be comparatively low.

This study describes a case of HTLV-I positive TSP/HAM in a woman born in the Ivory Coast, who has been living in Italy for a number of years. The analysis was conducted on cerebrospinal fluid and serum specimens and the ELISA test identified the presence of HTLV-I antibodies, confirmed also by Western blot analysis. The virus was isolated in a long-term culture of peripheral blood cells and polymerase chain reaction (PCR) on the DNA extracted from this culture revealed the presence of HTLV-I sequences, confirmed by sequence analysis.

Case report

A 54-year-old woman born in the Ivory Coast and resident in Brescia for 15 years was admitted to the Department of Neurology of Brescia’s Spedali Civili Hospital in August
2004, with no family history of neurological disease nor a personal record of drug abuse or blood transfusion. On admission, she complained of difficulty in walking, limb dysesthesia and sphincter disorders lasting for 5 days. In 2001 she experienced a similar episode with paraparesis followed 3 months later by a complete spontaneous recovery without conventional therapy. Neurological examination showed the presence of spastic paraparesis with increased tendon reflexes and bilateral Babinski signs. Weakness was more prominent in the proximal muscles than in the distal ones. Abdominal reflexes were absent; exteroceptive and proprioceptive perceptions were normal. She had urinary frequency and urgency. Muscle atrophy was absent and cranial nerve examination was normal.

Cranial and spinal cord MRI, performed at the same time, did not show lesions. In particular spinal cord MRI did not reveal atrophy or abnormal increased signal (Fig. 1). Motor evoked potential (MEP) and somatosensory evoked potential (SEP) of lower extremities showed significant bilateral increase of central conduction time. During hospitalisation there was a moderate improvement in paraplegia, with rehabilitation and after methylprednisolone therapy (1 g/day for 5 days).

Analysis of cerebrospinal fluid revealed a mild pleocytosis (20 cells/µl, 90% lymphocytes) and increased protein content (56 mg/dl, normal value ≤45 mg).

Laboratory data carried out on whole blood showed a haemoglobin value of 9.5 g/l and a MCV of 71 fl. Tests performed on the cerebrospinal fluid and/or serum revealed 44.3 mg/dl of C-reactive protein and serum calcium levels in the normal range; protein electrophoresis showed α2-microglobulin at 5.6% (6%–11%), γ-globulin at 23.7% (12%–19%), a thickening in the γ-zone and IgG lambda monoclonal component; oligoclonal antibody-bands were present. Thyroid function tests revealed a moderate reduction in FTH3 (1.6 pg/ml).

The results of serological tests for syphilis, hepatitis B virus, hepatitis C virus and HIV-1 were negative. By contrast, serum and cerebrospinal fluid were positive for HTLV-I/II antibody by enzyme-linked immunosorbent assay (ELISA) performed using a commercial HTLV-I/II kit (Nuclear Laser Medicine, Milan, Italy). Western blot analysis was used to confirm results obtained by ELISA, using a commercial INNO-LIA™ HTLV-I/II kit (Innogenetics, Ghent, Belgium), which identified a strong reactivity to all HTLV-I gag and env proteins.

The presence of proviral DNA was determined by molecular methods. DNA was extracted from cultured peripheral blood cells, using a High Pure PCR template preparation kit (Roche, Indianapolis, IN) according to the manufacturer’s instructions. Amplification was carried out in a DNA thermal cycler (GeneAmp 2400 PE), as described by Manca et al. [10, 11]. The oligonucleotide primers used were: SK43 and SK44, designed to amplify a 159-bp fragment of DNA from the tax region of both HTLV-I and HTLV-II genomes; SK54 and SK55, designed to amplify a 119-bp fragment of DNA from the pol region of the HTLV-I genome; and SK58 and SK59, designed to amplify a 103-bp fragment of DNA from the pol region of the HTLV-II genome. The amplified products were detected also by Southern blot DNA hybridisation, using a previously described procedure [10] with minor modifications. The primers and the specific probes (tax gene: SK45; pol-I gene: SK56; pol-II gene: SK60) were synthesised by MWG Biotech (MWG Biotech S.r.l., Ebersberg, Germany). The probes were labelled with Biotin-16-dUTP (Boehringer-Roche, Mannheim, Germany) in 3'-OH, using a TdT enzyme Kit (Boehringer-Roche) according to the