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DETECTION OF HTLV-I ANTIBODIES AND DNA IN BLOOD SAMPLE OF A PATIENT WITH MYELOPATHY IN NIGERIA

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SUMMARY

We describe a case of human T-lymphotropic virus type I associated myelopathy in a 50-year old woman in Nigeria. The patient presented with progressive loss of tone to the two lower limbs and later inability to walk. The HTLV-I antibody presence in the plasma collected from the patient was repeatedly detected by enzyme immunoassays (Abbott HTLV-I EIA and Coulter SELECT-HTLV I/II) and confirmed by Western blot technique. In addition, HTLV-I DNA was amplified from the genomic DNA isolated from the peripheral blood mononuclear cells of the patient by the polymerase chain reaction technique. This finding is significant being the first report of association of HTLV-I with myelopathy in Nigeria.

KEYWORDS: HTLV-I; Myelopathy; Nigeria.

INTRODUCTION

The human T-cell lymphotropic virus type I (HTLV-I) has been epidemiologically and aetiologically associated with a syndrome of progressive spinal cord dysfunction without any evidence of spinal cord compression in many tropical and sub-tropical countries[1-4]. The commonly associated neurological disorders in HTLV-I endemic regions include tropical spastic paraparesis (TSP)/myelopathy and tropical ataxic neuropathies[2].

Several studies shown that the prevalence of HTLV-I is high in Africa[3, 7, 9]. Seropositivity for HTLV-I in the region range from 0.6% in Morocco[5] to 3.5% in South Africa[1] and 16.9% in Tanzania[4]. However, there have been limited reports of HTLV-I infection among patients with TSP and other neurological disorders from Africa. In Nigeria, antibodies to HTLV-I were previously reported in association with different forms of leukaemia/lymphoma[7] as well as among relatives of patients with these blood disorders. Recently, we showed that both HTLV-I and HTLV-II are endemic in Nigeria[9]. Since most of the seropositive individuals identified in that study were assymptomatic as of the time of blood sample collection, the full clinical implications of infection with these viruses can not be fully determined yet in this country.

Herein we present report of a case of HTLV-I associated myelopathy in a 50-year old woman referred to the neurology clinic of the Medical Outpatient Department of the University College Hospital, Ibadan, Nigeria.

SUBJECTS AND METHODS

Subjects

Approximately 10 ml of blood was collected into tubes containing ACD as anticoagulant from each of five patients with various neurological disorders at the Medical Outpatient Department, University College Hospital, Ibadan in September, 1993. Plasma was separated from each sample within one hour of collection and kept frozen at –20°C in 2 ml aliquots. In addition, peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-hypaque density gradient centrifugation and stored at –80°C in freezing medium containing 80% foetal calf serum, 10% RPMI-1640 medium and 10% DMSO. Frozen plasma and PBMC from each patient were later shipped on dry ice to the Laboratory of Viral Oncology and AIDS Research of the University of Southern California School of Medicine, Los Angeles, California, U.S.A. for serological and molecular analysis.

Two of the patients (a 36-year old male and a 50-year old female) had history of sudden inability to walk with loss of sensation and muscle tone to the lower limbs, antoher two (a 35-year old male and a 50-year old female) had history of gradual loss of tone to both lower limbs for about 5 years and progressive inability to walk shortly before presenting at the U.C.H. The 50-year old woman was referred from the Baptist Medical Centre, Ogbomoso (100 km north of Ibadan). All the other patients were normally residents of Ibadan. The other patient (a 25-year old female) fell and lost consciousness about 6 hours before blood sample was collected from her.

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LABORATORY TESTS

Serology

The presence or absence of HTLV-I/II antibodies in the plasma of the five patients was carried out as described previously[9]. Briefly, all the plasma samples were initially screened in duplicate using Abbott's HTLV-I enzyme immunoassay (EIA) kits (Abbott Laboratories, North Chicago, IL. USA). This test detects presence of both HTLV-I and HTLV-II antibodies. Initially reactive samples were then retested in duplicate using the SELECT HTLV-1/2® (Coulter, Hialeah, FL, USA). The test uses synthetic peptides to differentiate between HTLV-I and HTLV-II antibodies[8]. For this study, only one plasma sample was positive for HTLV-I antibodies in the Abbott's HTLV-I SELECT HTLV-1/2 assays. The presence of HTLV-I antibodies in that plasma sample was further confirmed by HTLV-I protein immunoblot (Western blot technique) as earlier described[9]. Western blot HTLV-I positivity was based on presence of reactive bands with virus – specific gag – P24, P19 and env – gp46 or gp 61/68 antigens. Each assay included known positive and negative HTLV-I or HTLV-II plasma samples. Molecular detection of HTLV-I genome:

Confirmation of HTLV-I genome in the PBMC of the patient with reactive plasma was carried out by polymerase chain reaction according to the method of EHRLICH et al.[6] and modified as previously described[10]. Briefly, genomic DNA was extracted from the PBMC collected from the five patients by phenol/chloroform and purified according to the method of SAMBROOK et al.[13].

HTLV-I/II proviral DNA amplification was performed initially using primer pairs specific for both viruses (SK43/SK44) in a Perkin-Elmer Cetus DNA Thermal Cycler. Similarly, probes (SK45) that are specific for the amplified fragments were used for detection using the liquid hybridization techniques[13]. Later, HTLV-I (SK 110 I/SK 111 I, probe SK112 and SG 166/SG 296, probe SG 242) or HTLV-II (SK 58/SK 69, probe SK 60 and SK 110 II/SK 111 II, probe SK 188) specific primers and probes were used for amplification and detection of the provirus of either virus from the genomic DNA of each of the five patients[6]. PCR product from each sample was electrophoresed in 4% NuSieve agarose 3:1 and stained with ethidium bromide before hybridization with specific probes. In addition, appropriate positive and negative controls were included in each reaction.

RESULT

Out of the five patients' samples examined (four from hitherto unexplained myelopathy) in this study, one was positive for HTLV-I antibodies using the EIA manufacturers' criteria and stringent Western blot positivity criteria. In addition, HTLV-I provirus was detected by PCR in the genomic DNA of the same patient (Fig. 1). The remaining four patients were serologically and PCR negative for evidence of HTLV-I or HTLV-II infection.

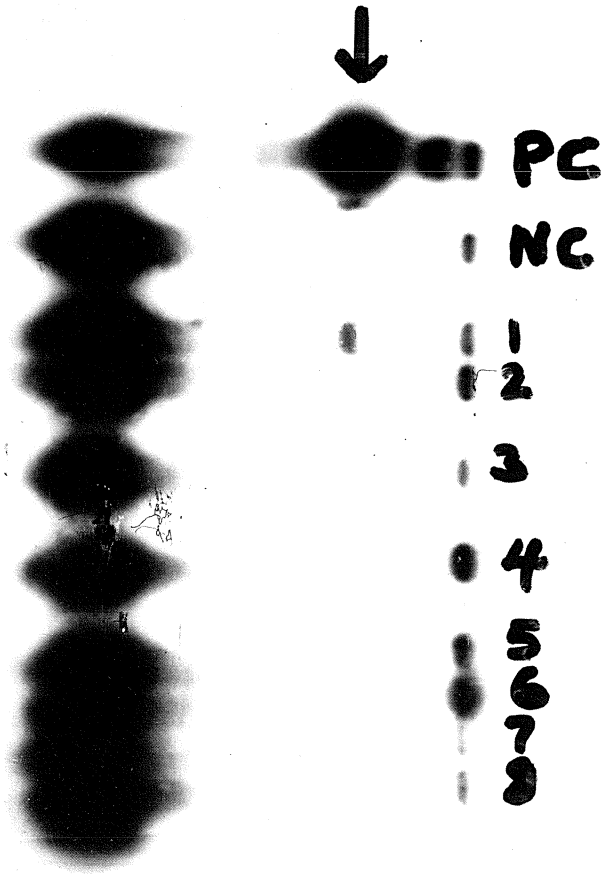

Fig. 1 – Showing HTLV-I proviral DNA amplified product (arrow) by PCR from PBMC of a patient with myelopathy in Nigeria (marked 1), known positive control (PC) and healthy HTLV-I/II seronegative donor (NC). Lanes marked 2 to 8 represent PBMC samples from other patients tested (negative) for HTLV-I/II infection as described in the text. PCR amplification and detection were performed as described under methods.

The positive sample was collected from the 50-year old woman with history of over 5 years progressive loss of tone to the two lower limbs and inability to walk shortly before presentation. She was born and resided in Ogbomoso, Nigeria and had never travelled to or lived in any other country.

DISCUSSION

The major aetiological factor associated with myelopathy, especially tropical ataxic neuropathies in Nigeria to date is excessive consumption of cassava containing cyanogenic glycosides[11]. The results of this investigation indicate that HTLV-I, previously shown to be prevalent in this country[9] is related to the common syndrome of TSP/unexplained myelopathy in Africa[12].

Although the number of samples tested in this study is relatively small compared with the overall prevalence of over 5% for HTLV-I/II infection in the general population in Nigeria[9], our

finding is significant because this is the first report of association of any infectious agent with unexplained myelopathy in this country. Specifically, as far as we can ascertain, this is the first report of HTLV-I related neurological disorder in Nigeria. HTLV-I infection in the patient was proved by reliable and specific serological tests[8]. In addition, the presence of the HTLV-I genome in the patient's lymphocytes was shown by PCR technique[6].

Our finding is in agreement with reports from other parts of Africa that many cases of previously unexplained spastic myelopathy can be linked with HTLV-I infection[2, 12]. Similarly, reports from various tropical and subtropical countries indicate that many cases of spastic paraparesis/myelopathies which are common in these areas[14] are caused by HTLV-I infection. It is also known that many cases of unexplained myelopathies have been linked with HTLV-I infection in other parts of the world with high population of Africans[14]. These reports suggest endemicity of this human retrovirus in Africa.

Although the precise role of HTLV-I in the pathogenesis of TSP/myelopathies is not yet known, specific HTLV-I antibodies were found in the cerebrospinal fluid of 18 of 20 (90%) seropositive patients by BHIGJEE et al.[2] indicating neuroinvasiveness of the virus. Further, that observation shows a strong association between the virus and myelopathy. It is therefore important to include HTLV-I in the differential diagnostic consideration of unexplained neurological disorders in Nigeria. Further studies are in progress to determine the HTLV-I status of a large number of cases of myelopathies and their full clinical manifestations in Nigeria.

RESUMO

Detecção de DNA e anticorpos anti-HTLV-I em amostra de sangue de paciente com mielopatia na Nigéria

Descrevemos um caso de infecção por HTLV-I associado a mielopatia, em mulher de 50 anos, na Nigéria. A paciente apresentou fraqueza progressiva dos membros inferiores e posteriormente incapacidade para andar. A presença de anticorpo HTLV-I no plasma coletado da paciente foi repetidamente detectada pelos ensaios imunoenzimáticos (Abbott HTLV-I EIA e Coulter SELECT-HTLV I/II) e confirmada pela técnica de Western Blot. Adicionalmente amplificou-se o DNA do HTLV-I a partir do DNA genômico isolado das células mononucleares do sangue periférico da paciente através da técnica PCR. Este achado é significativo sendo o primeiro relato de associação de HTLV-I com mielopatia, na Nigéria.

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