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HTLV-1 AND MYELOPATHY IN SALVADOR (NORTHEASTERN BRAZIL)

A CASE CONTROL STUDY

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SUMMARY — The principal aim of the study was to determine the degree of association between cerebrospinal fluid (CSF) that is positive for HTLV-1 and myelopathy in Salvador, Brazil. From the same hospital, twenty-eight cases of myelopathy and twenty-eight cases showing no neurological disorder were studied using blind selection matched 1:1 by age and sex. The twenty-eight pairs underwent HTLV-1 serology tests. In those with a positive result, anti-HTLV-1 antibodies were investigated in the CSF. The ELISA method was used, complemented by the Western-blot test. Myelopathy was considered associated with HTLV-1 only when the CSF was positive indicating neurotropism of the virus. The mean age of the cases was 44.6 ± 15.6 years and the control group was 43.5 ± 16.0 ($p > 0.05$). An OR of 9.0 was detected with a reability interval (95%) of 1.652-48.866 and chi-square significant at the 0.02 level. Despite a strong degree of association and considering the low level of precision, there is a need for analytical studies with larger samples which besides improving the precision will allow for greater control of the confounding variables.

KEY WORDS: HTLV-1, retrovirus, analytical studies, tropical spastic paraparesis, Brazil.

Estudo caso-controle de mielopatias por HTLV-1 em Salvador

RESUMO — Procurou-se determinar a magnitude da associação entre positividade do liquido cefalorraqueano (LCR) para HTLV-1 e mielopatias em Salvador. Foram estudados 28 casos de mielopatias e 28 casos sem doença neurológica, todos procedentes de um único hospital. A seleção foi de modo cego, com pareamento 1:1, por idade e sexo. Os 28 pares realizaram sorologia para HTLV-1 e, nos casos com mielopatia, foram pesquisados anticorpos para HTLV-1 no LCR. Na investigação dos anticorpos foram utilizados os métodos ELISA e Western-blot. Os casos tiveram média de idade de 44,6 ± 15,6 anos e os controles de 43,5 ± 16,0 ($p > 0,05$). Observamos um OR=9,0 com intervalo de confiança 95% de 1,652-48,866 e qui-quadrado significante a 0,02. Apesar do grande nível de associação encontrado, estudos analíticos com associações mais amplas são necessários com o objetivo de melhorar a precisão e o controle de variáveis intervenientes.

PALAVRAS-CHAVE: HTLV-1, retrovírus, estudo analítico, paraparesia espástica tropical, Brasil.

After Gessain and colleagues [7] published their findings on the association between positive serology for human T-lymphotropic virus type-1 (HTLV-1) and tropical myelopathy in Martinique, several other observations confirmed the association in different geographic areas [2,3,8,14,17-21,23]. In 1985 and 1986 the first cases were published regarding CSF positivity for HTLV-1 in patients with tropical spastic paraparesis [3,8,18] and in patients with myelopathy in temperate

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zones [14]. There is suggestive evidence that HAM may be autoimmune in nature. The findings of high levels of antibodies against HTLV-1 and oligoclonal IgG bands in CSF and serum [9,15], perivascular cuffing by lymphocytes observed in autopsy [1], and improvement of the clinical state with steroids and danazol [10,11,15] favor a role for autoimmunity in the pathogenesis of HAM. Several papers have described that HAM may be associated with other non-neurologic diseases as ichthyosis, uveits, arthropathy, polymiosytis, vasculitis, Sjogren's syndrome, alveolitis and adult T-cell leukemia/lymphoma [16]. These pleomorphic manifestations and the findings that less than 1% of seropositives subjects will manifest clinical disease, points to an immunological systemic disease whose clinical express is related to the immunogenetic relationship between the organism and the virus.

In Brazil, positive serology for HTLV-1 was observed in AIDS risk groups [5], among natives in the Amazon region [12] and among adult patients with T-cell leukemias or lymphomas [13]. The first cases of myelopathy associated with positive serology for HTLV-1 in Brazil were detected in São Paulo and later in Fortaleza and Salvador [4-6]; positive CSF was observed by Spina-França and colleagues in 24 of 56 cases of spastic myelopathies [22].

Out of two papers [2,21], all the information regarding the association between HTLV-1 and myelopathy are descriptive being necessary analytical studies whose features are more appropriate to establish associations. It is our proposal to establish the risk of infections due to HTLV-1 in patients with myelopathy in Salvador.

PATIENTS AND METHODS

The study was carried out in Salvador, the capital city of the State of Bahia, located in the Northeastern region of Brazil, at 12 degrees 59 minutes South latitude and 38 degrees 31 minutes West longitude. The city's population is predominantly mulatto due to miscegenation. The study was based on 28 cases of myelopathy out of 49 which were diagnosed between June 1990 and December 1991. Both the study and the control groups were chosen using the blind selection method and were matched 1:1. The matching criteria were age (margin of $\pm$ 3 years) and sex. All the patients were from the same hospital, a philantropic institution exclusively for lower income individuals. All participants in both the study and control groups underwent serology tests for HTLV-1 and HIV. The cases of myelopathy with positive results also underwent CSF tests for anti-HTLV-1 antibodies.

The CSF from all participants was tested for cells, proteins, protein electrophoresis and infectious or parasitic diseases (syphilis, toxoplasmosis, cysticercosis, and schistosomosis). These exams were carried out in a single laboratory by the same researcher using identical techniques. All participants also underwent either magnetic resonance of the spinal cord or myelography.

Study group criteria:

a. Clinical criteria. Patients with myelopathy diagnosed clinically by: 1. progressive paraparesis, with a duration of between 6 and 36 months; 2. pyramidal syndrome, characterised by spasticity, hyperreflexy predominantly of the lower limbs, Babinski sign, clonus, spinal automatisms, sincinesis; 3. neurogenic spastic bladder, reduction in libido or masculine sexual impotence; 4. lower motor neuron syndrome, characterised by atrophy of the quadriceps and, in some cases, bilateral arreflexy of the achilleus; 5. sensitive syndrome, characterised by paresthesia and dysesthesia in lower limbs in some cases.

b. Laboratory criteria: sera and CSF negative for syphillis, toxoplasmosis, schistosomosis and cysticercosis.

c. Radiological criteria: absence of compressive, expansive or traumatic lesions of the spinal cord diagnosed by myelography or magnetic resonance imaging.

Criteria for the control group: patients from the same hospital, with no neurologic disorders selected blindly with respect to their HTLV-1 serology, taking into account the above mentioned age and sex factors.

Criteria for myelopathy associated with HTLV-1 (HAM): CSF positivity HTLV-1.

Serum and CSF exam for HTLV-1: antibodies to HTLV-1 were detected with a commercially available enzyme immunoassay (EIA). EIA repeatedly reactive samples were further examined using a new dot blot confirmatory immunoassay with highly purified HTLV-1 viral and recombinant proteins as an antigen source. Samples were considered positive if antibodies against both the gag (p24) and env (p21E) gene products were present.

The odds ratio (OR) was calculated for analysis of case-control studies matched 1:1 and the respective 95% confidence intervals. A statistical significancy test was carried out using the McNemar chi-square test for studies matched 1:1. The mean age was compared by t-student.

RESULTS

Of the 28 pairs, 14 were male and 14 were female; 17 of the study group and 17 from the control group (60.7%) have always resided in the State capital; the others are from other parts of the State and currently reside in Salvador. The patients were predominantly mulatto. The participants from both the study and control groups were blue collar workers (construction workers, masons, farm workers, maids, etc.) in the study group there were three people from a high risk group for AIDS (one homosexual, one prostitute and one who had received a blood transfusion in the past).

Study and control groups had mean ages of respectively 44.6 ± 15.6 years and 43.5 ± 16 years, $p > 0.05$ (Table 1).

Of the 28 study cases, 12 (42.8%) had positive serology and CSF for HTLV-1 and 4 controls (14.3%) had positive serologies. Their pairs, according to their test results, appear in Table 2, with an estimated relative risk (odds ratio, OR) of 9.0, $p < 0.02$.

No patient showed suggestive images of spinal cord compression using MRI or myelography. All patients had a CSF cellularity from 1 to 40, with a predominance of lymphocytes. In 100% of the patients the total proteins were above 40mg% and gamaglobulin in the CSF was above 14%. No patient showed positive tests for infectious or parasitic diseases; none were HIV positive.

COMMENTS

Despite the fact that there were 49 patients with myelopathy who fulfilled the methodological study criteria, it was possible to find only one adequate pair for 28 among those who fulfilled the criteria for the control group and who had already undergone serology tests for HTLV-1. As patients with myelopathy

Table 1. Age and positivity for HTLV-1 for study and control groups.

Age	Study Group	HTLV-1	%	Control Group	HTLV-1	%
15-19	1	—	0.0	1	—	0.0
20-29	5	2	40.0	6	2	33.3
30-39	5	2	40.0	5	—	0.0
40-49	6	3	50.0	6	1	16.7
50-59	6	3	50.0	5	—	0.0
60-69	3	1	33.3	3	—	0.0
70-79	2	1	50.0	2	1	50.0
Total	28	12	42.8	28	4	14.3

Mean age: of study group = 44.6 ± 15.6 years; of the control group = 43.5 ± 16.0 years; $t54 = 0.256$, $p > 0.05$.

Table 2. Distribuition of the pairs according to HTLV-1 positivity.

Study Group	Control Group HTLV-1 +	HTLV-1 —	Total
HTLV-1 +	3	9	12
HTLV-1 —	1	15	16
Total	4	24	28

OR=9, CI (95%) 1.652-48.866; x2=6.4, p<0.02.

with other aetiologies can show positive serologies for HTLV-1, only those patients with positive antibodies in their CSF were considered. This was an attempt to guarantee that the cases considered positive had HTLV-1 as the aetiology of their myelopathy.

The magnitude of the OR (9.0) confirms the association discussed in the scientific literature. The wide range of the reliability interval (1.652 - 48.866) suggests a low level of precision. However, this does not invalidate the association. Despite the fact that the OR (9.0) was high, it was four times lower than the forty obtained by Roman and colleagues in a non-matched study that was based on a study group of 20 and a control group of 16 [21]. If the association criteria had been HTLV-1 seropositivity the OR would have been higher.

Since this is the third analytical study on the association between HTLV-1 and myelopathy available to the scientific community, this model should be encouraged and more detailed case studies should be carried out, thus allowing greater control of the confounding variables.

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