RELATÓRIO FINAL

VARIABILIDADE GENÉTICA DO VÍRUS DA IMUNODEFICIÊNCIA HUMANA TIPO I: INFECÇÃO EM PACIENTES HEMOFÍLICOS, DOADORES DE SANGUE E PORTADORES DE AIDS EM MINAS GERAIS

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I. RESUMO

Neste trabalho foi estudada a variabilidade genética do HIV-1 isolado de indivíduos do Estado de Minas Gerais (doadores soropositivos assintomáticos, pacientes soropositivos com coagulopatias congênitas, pacientes portadores de AIDS tratados no Centro de Referência Orestes Diniz). Inicialmente foi feito um estudo transversal da soroprevalência do HIV-1 em pacientes hemofílicos tratados na Fundação Hemominas e avaliados os fatores de risco para soropositividade (sorologia para outros patógenos transmissíveis pelo sangue, história transfusional e outros). Em seguida, para o estudo da variabilidade genética em pacientes hemofílicos, doadores e portadores de AIDS, foi feita a subtipagem dos isolados de HIV-1 através da técnica de mobilidade de heteroduplexes (“heteroduplex mobility assay”, HMA) e sequenciamento de nucleotídeos de amostras selecionadas (gene da protease e região C2V3 do gene env). Foi analisada a relação filogenética dos isolados caracterizados com isolados de referência dos diversos subtipos conhecidos do HIV-1. Para se verificar a soroprevalência do HIV-1 em pacientes hemofílicos, foram testadas amostras de 226 pacientes, dos quais 36 (15,9%) eram soropositivos (ELISA, Abbott, USA confirmado pelo WB, Cambridge Biotech, USA e/ou imunofluorescência, IF-R37-3, Fiocruz, Brasil). O teste para HTLV-I/II (ELISA, Ortho, USA) mostrou que 14/148 (9,5%) eram positivos. A prevalência de anticorpos contra HIV-1 foi aproximadamente o dobro da do HTLV-I/II e o paciente com um resultado positivo para HIV-1 tinha um risco significativamente aumentado de ser positivo para HTLV-I/II, HCV e Treponema pallidum. A PCR (reação em cadeia da polimerase), seguida pelo HMA foi realizada em 8 pacientes hemofílicos, a fim de subtipar o vírus, sendo encontrado que todos eles pertenciam ao subtipo B do HIV-1, que é o mais prevalente no Brasil, Estados Unidos e Europa. A PCR foi positiva em 12/50 doadores (24%). O HMA mostrou que os isolados de HIV-1 de 11 doadores pertenciam ao subtipo B e um ao subtipo F. Os resultados da análise das seqüências de nucleotídeos dos pacientes hemofílicos, doadores e pacientes com AIDS foram compatíveis com os resultados de HMA, com exceção do doador com o subtipo F, que se
agrupou com o subtipo B, mostrando que provavelmente se trata de recombinação B+F. Os pacientes hemofílicos foram provavelmente infectados por concentrados importados de São Paulo e Rio de Janeiro (Brasil), Europa e Estados Unidos, usados antes do conhecimento do HIV e portanto feitos com plasma sem triagem ou tratamento para inativar o vírus. O teste de HMA dos produtos amplificados por PCR mostrou ser um meio relativamente simples e rápido de subtipar HIV-1 em indivíduos infectados por diversos meios, inclusive transfusão, dando informação sobre o(s) tipo(s) de vírus que estão sendo transmitidos em determinada população.
2. METODOLOGIA E RESULTADOS:

manuscritos submetidos para publicação, aceitos ou no prelo.
HIV-1 detection and subtyping by PCR and heteroduplex mobility assay in blood donors: can these tests help to elucidate conflicting serological results?

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Running head: HIV-1 PCR and HMA in blood donors
ABSTRACT

Testing blood donors for the human immunodeficiency viruses (HIV-1 and 2), requires serological tests that are frequently inconclusive. Peripheral blood mononuclear cells of 50 blood donors with the screening test (ELISA) reactive to HIV-1, but with indeterminate results in the first Western Blot (WB) performed, were submitted to the polymerase chain reaction (PCR) and heteroduplex mobility assay (HMA), a nonsequencing method that can distinguish between HIV-1 subtypes (A to I). PCR amplification of HIV-1 env gene regions has been obtained in 12 (24.0%) samples. HMA of the amplified DNA showed that 12 belonged to HIV-1 B subtype and one to F subtype. PCR testing of the amplified DNA helped to elucidate doubtful serological results and HMA proved to be a relatively simple and rapid way of subtyping HIV-1 for epidemiological purposes.

Key words: HIV-1 subtypes, blood donors, PCR, Heteroduplex Mobility Assay (HMA), Brazil.
INTRODUCTION

The sensitivity and specificity of available screening tests for HIV-1 and HIV-2 have improved substantially over the years since they first became available in 1985. However, the occurrence of even a small number of false results by these tests can have profound implications when testing a population at low risk for infection\(^1\). Blood donor screening tests generate a substantial number of conflicting results. In most cases, the blood center attempts to elucidate the results of screening test by the performance of additional assays, that may include detection of HIV-1 inserted provirus in PBMCs by the polymerase chain reaction (PCR). The PCR technique has proved useful in confirming the diagnosis of HIV-1 infection in seropositive adults, infants of seropositive mothers and in helping to clarify inconclusive results in low-risk individuals like blood donors\(^2\).

One remarkable feature of the HIV genome is the degree of variability that exists among independent virus isolates. Within the HIV envelope gene, sequence divergence may reach 30\% or more among viruses of the nine different subtypes (A-I) described so far\(^3,4\), which may have an impact on the ability of serological tests to detect specific subtypes\(^5\).

A method was recently described for HIV-1 genetic screening, the heteroduplex mobility assay (HMA)\(^6\). This method is able to distinguish between individual strains and may also provide reliable information for phylogenetic analysis. HMA is based on the observation that the structural deformations in double stranded DNA that result from mismatches and nucleotide insertions or deletions cause a
reduction in the electrophoretic mobility of these fragments in polyacrilamyde gel electrophoresis. Heteroduplexes are formed between strands from the unknown and the reference viral DNA by heat denaturation and subsequent cooling to permit reannealing. The reduction in mobility is proportional to the relative genetic distances determined by the number of insertions/deletions and mismatches and the results are in agreement with the complete or partial HIV envelope sequencing.

In this study we report PCR and HMA results of HIV-1 env gene of 12 individuals from a group of 50 blood donors from Fundação Hemominas, Minas Gerais State Center of Hemotherapy and Hematology, Brazil, who tested positive in the screening test (ELISA), but had inconclusive results on the WB.
MATERIALS AND METHODS

Population and collection of specimens

Samples of peripheral blood mononuclear cell (PBMC) were obtained from a group of 50 blood donors at Fundação Hemominas, Belo Horizonte, Brazil, who presented reactive screening tests to HIV-1 (ELISA, Abbott, USA) and initial indeterminate Western blot (Cambridge Biotech, USA). Western blots (WB) of subsequent samples presented varied results, with 3 donors becoming positive, 18 becoming negative and 29 remaining with indeterminate pattern.

PBMC separation and DNA extraction

PBMC were separated from blood samples by Ficoll-Hypaque (Pharmacia, Sweden) gradient centrifugation and lysed by addition of 200µl of lysis solution (TE pH 8.1; Triton X-100, 0.001%; SDS, 0.0001%) and then digested by addition of 50µg of proteinase K. Alternatively, genomic DNA from samples was isolated using the Isoquick Nucleic Acid Extraction Kit (Microprobe, CA, USA).

PCR

PCR was performed on the 50 samples obtained from blood donors, at least three times in each sample. All donors had blood collected at different times and in some cases they were used to double check the results. Appropriate positive and negative controls were included in each PCR run. Three separate areas were used to prepare the DNA samples, the stock solutions and handle the amplicons. For amplification of env gene regions, crude lysate (10% proportion in reaction) or 1µg of purified DNA was used as
template as described\(^9\). The primers were based on the HIV-1 HXB2 (GenBank accession number: K03455), as described in the World Health Organization (WHO) Guidelines for Standard HIV Isolation Procedures (1995). The first round reactions were conducted using ED3/ED14 corresponding to positions 5956-5985 and 7960-7931, amplifying a 2.0 Kbp fragment. The second round reactions included three sets of primers including ED5/ED12, positions 6556-6581 and 7822-7792, fragment of 1.2 Kbp (region V1-V5 of gp120); ES7/ES8, positions 7001-7020 and 7667-7647, 0.7 Kbp (region V3-V5); ED31/ED33, positions 6816-6844 and 7359-7380, 0.5 Kbp (region V2-V4). Briefly, the substrate was added to a first round reaction of 20\(\mu l\) containing 50mM KCl, 10mM Tris pH 8.3, 1.25 - 1.8 mM MgCl\(_2\), 1% glicerol, 1% DMSO, 5pM of each primer and 0.2mM of each dNTP. Second round reactions (nested PCR) were formulated with 2.0 \(\mu l\) of first round PCR in 100\(\mu l\) of reaction. Standard conditions were three initial cycles of 94°C / 1 min; 55°C/ 1 min ; 72°C / 1 min and 32 cycles of 94°C / 15 sec; 55°C/45 sec; 72°C/1 min, with final extension 72°C/5 min (DNA thermal cycler, Perkin Elmer Cetus, CA, USA). After PCR, the DNA samples were analyzed on 2% agarose gel in TAE 1x (0.04M Tris-acetate, 10mM EDTA), stained with ethidium bromide, visualized and photographed under UV light.

**HMA**

PCR amplified DNA from 12 individuals was tested by HMA. For heteroduplex formation, 5.0\(\mu l\) of the second round PCR product from each sample was mixed with 5.0\(\mu l\) of homologous product from a reference subtype provided by WHO (WHO
Guidelines for Standard HIV Isolation Procedures, 1995) and 1.0μl of 10x annealing buffer (1M NaCl, 100mM Tris pH 7.8, 20mM EDTA). It was heated to 94°C for 2 minutes, cooled rapidly on ice, mixed with loading dye and loaded onto a 5% polyacrilamide gel (30:0.8 acrylamide/bisacrylamide). The gels were stained with ethidium bromide (0.5μg/ml in water) and DNA was detected by UV light or alternatively the gels were stained by silver and exposed to electrophoresis duplicating film (EDF, Kodak, USA).
RESULTS

Blood donors (n=50) were selected based on HIV-1 repeated reactivity on screening test (ELISA, Abbott, USA) and initial indeterminate Western blot results (Cambridge Biotech, USA). Table I shows that the follow-up WB results could be positive (n=3), negative (n=18), or indeterminate (n=29). PBMCs were separated from blood samples of the 50 individuals, DNA was submitted to PCR, which showed amplification of the HIV-1 provirus DNA in 12 samples (tables I and II). All donors with positive WB (n=3) had also a positive PCR. Four of 18 donors (22.2%) with reactive ELISA but negative WB and 5/29 (17.2%) with indeterminate WB were positive in the HIV-1 PCR. The age and gender of the twelve blood donors with positive PCR results were not significantly different from the remaining donors.

HMA results (Table II) showed that 11 individuals had infection with B subtype of HIV-1 and one showed mobility of F subtype. Results of PCR of this donor were confirmed using different primers (gag, pol) and in two distinct samples. Nucleotide sequence obtained directly from PCR amplification products of the C2-V3 region of donor GM19 (F subtype) confirmed the HMA results (data not shown). Figure 1 shows results from donor GM24, with HMA mobility of HIV-1 B subtype. A modification introduced in the original technique, that is, staining the gel with silver and copying it to a positive film (Electrophoresis Duplicating Film, EDF, Kodak, USA), provided an image of the original gel that can be kept indefinitely. First lane is the DNA amplified from the patient's sample
(unknown - Uk). Lanes 2-9 show mixtures of the patient’s amplified DNA with DNA amplified from HIV-1 standards of subtypes B (B1, B2, B3), C (C1, C2, C3, C4) and F (F1, F2), supplied by WHO. The heteroduplexes (HE) in lanes 2, 3 and 4 (HIV-1 B subtype standards) are formed below the single stranded DNA (SS) and above the homoduplex (HO), thereby being classified as B subtype.
DISCUSSION

Technological developments combined with improved donor screening techniques have significantly reduced the risk of post-transfusion retroviral infections. On the other hand, testing all units donated generate an enormous number of inconclusive results that need further clarification\textsuperscript{10}. This represents a constant challenge, since unclear results are a constant source of concern for the blood bank, donors and recipients of blood and require elucidation using supplementary tests\textsuperscript{11}.

In this study, we have shown that PCR was helpful to elucidate a number (5/29) of HIV-1 indeterminate results in blood donors, as reported previously in the literature\textsuperscript{11,12}. Although it may not shorten the follow-up period of donors who may or may not become definitely seropositive, it adds evidence that the donor should be followed closer, perhaps in a reference center.

We could amplify HIV-1 proviral DNA in four blood donors with repeatedly reactive HIV-1 ELISA, but with a negative WB result. The WB used (Cambridge Biotech) has been established to be very sensitive, both for detection of early seroconversion and in end-point dilution studies. Validation of infection in these WB negative and indeterminate subjects requires testing of follow-up samples to confirm positive PCR results and non-positive WB\textsuperscript{12}. PCR targeting of other genomic regions (gag, pol) in six samples confirmed the initial PCR results (data not shown). Sequencing of the amplified PCR products (env and other regions) is also important to confirm that these represent unique sequences and thereby rule out contamination during nested PCR, which was conducted in
three separate areas, with appropriate controls included in each run. Preliminary sequencing of the env region of the F subtype (donor GM19) demonstrated that this had unique sequence, different from the positive control provided by WHO (Barbosa EF, manuscript in preparation). To our knowledge, there is no precedent for Brazilian F subtype individuals giving indeterminate WB results, but the numbers of F subtype detected are still limited and further studies are needed.

HMA testing of PCR amplified DNA does not add to PCR in sorting out inconclusive serologic results, but is a relatively simple and rapid procedure to obtain molecular data of HIV infection in epidemiological studies in blood banks. The use of HMA in a blood donor with indeterminate HIV results allowed the detection of HIV-1 F subtype for the first time in Minas Gerais State, Brazil.
REFERENCES


Table I - HIV-1 Western blot and PCR results of blood donors with reactive ELISA tests

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* HIV-1 env gene
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pos = positive  neg = negative  ind = indeterminate
Figure 1

Heteroduplex Mobility Assay of HIV-1 B subtype (GM24), stained by silver. First lane is the DNA amplified from patient’s sample (unknown - Uk). Lanes 2-9 show runs of mixtures of patients’ amplified DNA with DNA amplified from HIV-1 standards of subtypes B (B1, B2, B3), C (C1, C2, C3, C4) and F (F1, F2). Heteroduplexes (HE) were formed below the single stranded DNA (SS) and above the homoduplex (HO) in lanes 2, 3 and 4.
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Dear Dr. Proietti,

Thank you for submitting your manuscript entitled "HIV-1 detection and subtyping by PCR and heteroduplex mobility assay in blood donors" for possible publication. Please refer to manuscript #97-11 in all communications with our office regarding your manuscript. We anticipate the review process to be completed in about 6 weeks.

We appreciate your interest in Transfusion Science in submitting this paper for our consideration.

Sincerely,

Gail A. Rock, PhD, MD, FRCP
Editor-in-Chief

GR:hb

Dr. Anna Proietti
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By Fax: 55-31-226-2002

Dear Dr. Proietti,

The review of your manuscript #97-11 has been completed and we are pleased to inform you that it has been accepted for publication in our journal and will appear in the next available issue which is Volume 19.1. When the proofs are sent to you we would appreciate their prompt return to ensure timely publication.

Please note that publication is subject to copyright. Therefore I would be grateful if you would sign and return the attached Copyright Transfer Agreement. If possible, it would be greatly appreciated if you could also send us a computer disk containing the manuscript. Please label the disk specifying what type of computer was used (IBM compatible PC or Apple Macintosh) and what software was used, including which release, eg. WordPerfect 5.1.

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GR:hb/Encl.
Genetic Variability of HIV-1 isolates from Minas Gerais, Brazil: Phylogenetic Analysis of the C2-V3 env Regions

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To the Editor: The objective of this work was to examine the genetic variation and phylogenetic relationships the env gene from HIV-1 isolates of seropositive patients with history of use of clotting factor concentrates (n=6), multiple transfusions (n=1), or sexually transmitted infection (n=4) in Belo Horizonte, Brazil. Belo Horizonte is the third largest city in Brazil, with over 3 million inhabitants in the metropolitan area and the fourth in absolute number of AIDS cases in Brazil, as of March 1997, with over 2,400 cumulative cases (1).

PBMCs were separated from peripheral blood of eleven HIV-1 seropositive patients. Crude lysates were obtained and PCR was performed using primers encompassing the C2-C3 region of the env gene of HIV-1 (nested reaction), using primers based on isolate HXB2 genome (GenBank accession number KO3455), provided by WHO. The first round reactions of the env gene were performed using primers ED3/ED14, corresponding to positions 5956-5985 and 7960-7931, which amplified a 2.0 Kb fragment. The nested reactions were done with primers ED31/ED33, corresponding to positions 6816-6844 and 7359-7380, producing a 0.5 Kb fragment (region C2-C3). DNA amplified from 11 patient’s PBMCs was sequenced directly from PCR material (Sequenase®, USB, USA), using the primers ED31, ED33 and ES7 (C2-V3 region). Nucleotide sequences were obtained from both strands and each was done at least twice. Sequences were aligned using Clustal W. Pairwise distance methods, using the Jukes and Cantor correction and bootstrap analysis were performed for phylogenetic inference, with the package Treecon 3.1 for Windows (2).

HIV-1 env sequences obtained from Belo Horizonte isolates clustered with isolates of subtype B (figure 1), with reliable clusters and branches (8/11 branches of subtype B cluster had 70% or higher bootstrap values). These results are in concordance with env heteroduplex mobility assays (HMA) (4) results of the same
samples, except for an isolate from an asymptomatic blood donor (BHGM19, figure 1), whose HMA results showed F subtype mobility, but whose C2V3 env sequences clustered consistently with subtype B. This discordant branching has been reported before in an isolate from São Paulo, Brazil (93BR019, ), which was a mosaic of subtypes B and F (5). Env sequences (figure 1) presented variation in the C2V3 nucleotide sequence of up to 53% from HIV-1 B prototype HXB2. Using the Jukes and Cantor correction to compute the distances, the distance values are greater than the actual percentage of differences between two sequences, because it takes into account multiple mutations per site (2). In nine of the eleven env amino acid sequences, the tetrameric subdomain at the crown of V3 loop had the sequence GPGR, including the F subtype (BHGM19), whose crown had the sequence HIGPGGRAF, identical to previously described and independently collected F subtype isolates from other cities in Brazil (6,7). One isolate had the GPGQ sequence, which is common in subtypes A, C and E isolates and the one left had the sequence GPGK, not reported previously in subtype B, to our knowledge. The five cysteine residues of the C2-V3 region were conserved in all isolates, including the ones at the base of V3 loop (C1 and C35).

All the isolates in this study belonged to HIV-1 subtype B, with one exception of a "B/F" mosaic. This B/F recombinant from Belo Horizonte (BHGM19) is similar, but not identical in its nucleotide sequence to other B/F mosaics described in Brazil. Sequencing of additional regions of these viruses may help to understand the recombination events that took place to generate these chimeric genomes.

GeneBank accession numbers: AF025918 to AF025926; U46210; U46122
Acknowledgments

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References


**Figure 1.** Phylogenetic relationships of 11 newly characterized HIV-1 *env* sequences of the C2V3 region (asterisks) from Belo Horizonte, Minas Gerais, with representatives of subtypes A to G. Quotation marks indicate the mosaic nature of subtypes E and G envelope genes. Values at nodes represent the percentage of bootstraps in which the cluster to the right was found (only values above 70% are shown).
Presence of Human Immunodeficiency Virus (HIV) and T-Lymphotropic Virus type I and II (HTLV-I/II) in a haemophiliac population in Belo Horizonte, Brazil and correlation with additional serologic results.

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Running title: HIV-1 and HTLV-I/II infections in haemophiliacs
Key words: haemophilia, AIDS, blood transmittable diseases, HIV, HTLV, Brazil.
Summary
The aim of this study was to determine the prevalence of human immunodeficiency virus type 1 (HIV-1) and human T lymphotropic virus types I and II (HTLV-I/II) infections in 226 haemophiliac patients treated at Fundação Hemominas in Belo Horizonte, Minas Gerais State, Brazil, and to verify association with other serologic results. Patients positive for HTLV-I/II had also a neurological, haematological and ophthalmologic evaluation.
Fundação Hemominas offers comprehensive care for all haemophiliac patients in Minas Gerais. Thirty-six (15.9%) of the 226 patients showed reactive results to HIV-1 [ELISA, Abbott, USA confirmed by Western blot (WB), Cambridge Biotech, USA and/or Immunofluorescence, Fiocruz, Brazil] and 16 (7.1%) had reactive sera to HTLV-I/II (ELISA, Ortho, USA). Eleven of these 16 (4.9%) were positive, 3/16 (1.3%) were indeterminate and 2/16 (0.9%) were negative in the HTLV WB (Cambridge Biotech, USA). Neurological, haematological and ophthalmologic examination of 9/16 patients revealed no abnormality suggestive of HTLV disease. Of the 16 patients reactive to HTLV-I/II ELISA test, six (37.5%) were also positive evidence to HIV-1 ($\chi^2 = 5.92; p=0.01$).
Seropositivity for HTLV-I/II and HIV-1 was associated with advancing age and positive results for hepatitis C virus (HCV), Chagas’ disease (T. cruzi infection) and syphilis. No association was detected of HTLV presence with type and severity of haemophilia and hepatitis B results. The prevalence of antibodies against HIV-1 is approximately three times that of HTLV-I/II and a patient showing positive result for HTLV-I/II had a significantly increased risk of being positive for HIV-1, HCV and T. cruzi.
Introduction
The lives of persons with haemophilia have been transformed by advances in the
treatment during the past two decades. The programs of self-administration of
concentrates of clotting factors provided a means of preventive or early treatment,
minimising hospital admissions and severity complications among haemophiliac
patients. The availability of clotting factor concentrates has also allowed surgical
treatment of acute problems and severe joint deformities, improving life perspectives
for many persons with haemophilia [1]. The risk associated with exposure to plasma
from multiple donors, however, has long been a problem in the care of these patients,
primarily because of evidence of virus induced liver disease (hepatitis B virus and,
more recently, hepatitis C virus infection) [2,3]. With the advent of HIV-1 infection,
transmitted mainly by clotting concentrates in the late 1970s and mid 1980s, the
acquired immunodeficiency syndrome (AIDS) became a leading cause of morbidity
and mortality among haemophiliac patients [4]. The reported prevalence of antibodies
to HIV-1 varied, being as high as 90.0 % in some groups [5,6,7,8,9]. In 1987, 36 (22.0
%) of a group of 148 haemophiliac patients in Belo Horizonte, Brazil, were positive to
HIV-1 [10]. Human T-lymphotropic virus type I (HTLV-I), the causative agent of
adult T cell leukaemia/lymphoma (ATL), tropical spastic paraparesis (TSP/HAM) and
some types of uveitis can also be transmitted by cellular blood products, being a
potential risk to patients receiving these components of blood [11].
Haemophiliac patients from areas endemic for Trypanosoma cruzi (T. cruzi) infection
(Chagas' disease or American trypanosomiasis) have also the hazard of contracting this
infection due to exposure to contaminated blood [12].
In this study, we have evaluated the seroprevalence of HIV-1, HTLV-I/II, hepatitis B
and C viruses (HBV and HCV, respectively), Treponema pallidum and T. cruzi
infections in 226 haemophiliac patients treated at Fundação Hemominas, Belo Horizonte, Brazil during the period of 1983 to 1994.
Material and Methods

Study subjects. The individuals in this study were 226 haemophiliacs [197 (87.2 %) with haemophilia A and 29 (12.8%) with haemophilia B] from a total of 449 patients [372 (83.0%), haemophilia A and 77(17.0%), haemophilia B] who were enrolled in comprehensive treatment at Fundação Hemominas from 1983 to 1994. This government foundation is the only centre in Minas Gerais State that offers comprehensive care to patients with inherited coagulation diseases. Patients are offered routine and emergency care, factor concentrate and blood transfusion, periodic laboratory evaluation, dental treatment, psychological and orthopaedic assistance. There is also a team of social workers and voluntary teachers to deal with work, education and occupational aspects. The study was conducted in a confidential way and patients were identified with numbers.

Serology. The tests for known blood transmittable diseases are requested regularly for all patients in treatment at Fundação Hemominas, ideally every 6 months. For this research, we used the results of the routine screening. Serum was tested for evidence of contact with hepatitis B virus (HBV), T. pallidum, T.cruzi, human immunodeficiency virus (HIV) and, more recently, hepatitis C virus (HCV) and human T-lymphotropic virus I and II (HTLV-I/II). The tests were performed according to the manufacturer s’ instructions. The tests and companies were as follows: HBV surface antigen: HBsAg (ELISA monoclonal, Abbott, USA); T. pallidum: VDRL (Hoescht-Behring, Germany), FTA-Abs (Biolab, Brazil); T.cruzi: Indirect Immunofluorescence (IF-K37-3, Biolab, Brazil), indirect haemagglutination (HAI, Biolab, Brazil); HIV-1/2:
ELISA (Abbott, USA), Western blot (WB, Cambridge Biotech, USA); HCV: ELISA (Ortho, USA); HTLV-I/II: ELISA (Ortho, USA), WB (Cambridge Biotech, USA).

**Clinical evaluation.** Nine patients seropositive to HTLV-I/II had history taken and physical examinations performed by a neurologist, a haematologist and an ophthalmologist.

**Statistical analysis.** All data were stored in D-Base software. Simple frequency distribution, parametric (Student’s t test) and non-parametric (chi-square and Fisher’s test) tests were used to evaluate the data. We calculated p values by comparing two binomial proportions. Values of 0.05 or less were considered significant. Data were analysed with the software Epi-info 6.02 (Centers of Disease Control, USA).
Results and Discussion

In the group of 226 patients studied (all males), age was not significantly different between haemophilia A and B (p=0.17). The means were 20.20 ± 11.98 years for haemophilia A and 16.93 ±12.28 years for haemophilia B patients. According to the level of factor VIII or IX in the plasma, they belonged to three categories: 107 (47.3%) had severe haemophilia (< 2 U/dl of factor VIII or IX), 92 (40.7%) had moderate condition (2-5 U/dl) and 27 (11.9%) had mild disease (5-25 U/dl).

The serological results (Table 1) showed that 36/226 (15.9%) had positive HIV-1 ELISA results, all confirmed by Western blot (WB) or immunofluorescence (IF). Twenty-two/36 (61.0%) had severe haemophilia (Table 2). Thirty-four/36 (94.4%) had haemophilia A and 2/36 (5.6%) haemophilia B. The HIV seroprevalence found in this study reflects a decline in the prevalence found in other cross-sectional study conducted previously, in 1986, which was of 36/148 (22.0%) [10]. Although the absolute number of HIV seropositive patients was the same, the population changed due to deaths (n=27) and new detections of seropositive individuals. Moreover, the number of seronegatives increased as new diagnosis of haemophilia were done, and the patients started treatment in the era of testing blood for HIV and treating the concentrates to eliminate virus(es). Nevertheless, we have still had one relatively recent seroconversion (three years ago, in 1994), which may be due to use of contaminated blood or cryoprecipitate tested as HIV negative, considering that treated concentrates seem to have reached a satisfactory level of safety. This has also been reported in the literature [7,11,13], one of the reasons being the seronegative “window period” in which the donor has been recently infected with HIV, but has not yet detectable antibodies in the serum.
Human T-lymphotropic virus (HTLV-I/II) is solidly present in the group studied, with a prevalence of 4.9% (11/226) of patients with sera reactive in the ELISA test and confirmed by WB. All of them had haemophilia A (Table 3). Five other patients had positive ELISA tests, but negative (2/226) or indeterminate (3/226) WB results. This prevalence is considerably higher than that of eligible blood donors in the same area, which is 0.32% [14]. These results differ from data of other centres [15,16,17], where HTLV infection was not found in haemophilic patients treated exclusively with concentrates, although the virus was detected in the donors when testing for HTLV was implemented. This absence indicated that this retrovirus, in contrast to HIV, has not been transmitted through factor VIII or IX [15]. The divergence of our data from these previously published reports may be due not only to the higher prevalence of HTLV in local blood donors, but also to greater use of cellular products, due to suboptimal use of concentrate factors.

Nine of the 16 haemophilic patients positive in the HTLV-I/II ELISA test had a complete neurological, haematological and ophthalmologic evaluation which revealed no abnormalities suggestive of HTLV-I/II disease. This was expected, since the majority of individuals infected with HTLV-I do not develop disease and even when they do, it is usually after decades of contact with the virus and HTLV-II is not known to be associated with disease [11].

Of the 16 patients reactive to HTLV-I/II ELISA test, six (37.5 %) were also positive to HIV-1 (Fisher’s; p=0.026) (Table 3). Coinfection of HTLV-I/II and HIV in haemophilic patients is reported in areas where both viruses are endemic and cellular products were used in the treatment of haemophilia before testing for HTLV-I/II was implemented, but not in patients HIV-1 positive treated exclusively with noncellular products [15].
The results for hepatitis C were unexpected, with 138/226 (61.1%) patients reactive with the test used (ELISA, Ortho, USA). Most of them (127/138) had haemophilia A, which was statistically significant ($\chi^2 = 7.38, p=0.0065$). On the other hand, HBsAg, as a marker of chronic hepatitis B, was positive in only 3/226 (1.3%) of the patients. This probably reflects the longer period of blood screening of this virus in the donated blood and the results of vaccination efforts in this group of individuals with haemophilia. On the other hand, hepatitis B core and surface antibodies were detected in ... and ... patients respectively, probably as a result of contact with blood products and/or vaccination. ... patients presented signs of liver disease (evidenced by elevated ...) associated with HCV or HBV positive results (data not shown).

VDRL was reactive in 11/226 (4.9%) and FTA-Abs in 4 of the 11 reactive in the VDRL (data not shown). Indirect immunofluorescence and indirect haemagglutination for Chagas’ disease (T. cruzi infection) was positive in 5/226 (2.2%) of the haemophiliac patients. These cases could be due to the use of contaminated blood [12] or direct contact with the insect vector (Triatominae) in endemic areas.

Seropositivities for HTLV-I/II, hepatitis C, HIV and T. cruzi infections were associated with advancing age ($p=0.0001, 0.000006, 0.025$ and $0.02$ respectively), probably reflecting increased cumulative exposure to blood products. HIV-1 and HTLV-I/II positive results were also associated with positivity for hepatitis C virus (HCV) and T. cruzi (Table 2). No association was detected of HIV-1 and HTLV-I/II presence with type and severity of haemophilia, syphilis and hepatitis B results.

We conclude that the prevalence of antibodies against HIV-1 is approximately three times that of HTLV-I/II and the patient showing a positive result for HIV and HTLV-I/II has a significantly increased risk of being positive for HCV and T. cruzi.
Testing for HCV and HTLV-I/II recently introduced will help to reduce the prevalence of these viruses in haemophiliac patients. The exclusive use of treated or recombinant concentrates in adequate amounts should minimise from now on the need of cellular blood, domestic cryoprecipitate and fresh frozen plasma transfusions, thereby reducing even more seroconversions for all these pathogens in this population.
Acknowledgements

The authors would like to thank Drs. Henrique Guerra, Mitiko Murao, Edel F. Barbosa and Erna G. Kroon for their helpful suggestions and support.
References


12 Schmunis GA. Trypanosoma cruzi, the etiologic agent of Chagas’ Disease: Status in the blood supply in endemic and non-endemic countries. Transfusion, 1991; 31:547-577.


Table 1. Serological results for several blood transmittable diseases in 226 haemophilic patients. Fundação Hemominas, Belo Horizonte, Brazil, 1994.

<table>
<thead>
<tr>
<th>Results</th>
<th>HIV-1</th>
<th>HTLV-I/II</th>
<th>HCV</th>
<th>HBV</th>
<th>Syphilis</th>
<th>T. cruzi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ELISA</td>
<td>ELISA</td>
<td>WB</td>
<td>ELISA</td>
<td>HBsAg</td>
<td>VDRL</td>
</tr>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Positive</td>
<td>36 (15.8)</td>
<td>16 (7.1)</td>
<td>11 (4.9)</td>
<td>138 (61.1)</td>
<td>3 (1.3)</td>
<td>11 (4.9)</td>
</tr>
<tr>
<td>Negative</td>
<td>189 (83.6)</td>
<td>210 (92.9)</td>
<td>2 (0.9)</td>
<td>77 (34.0)</td>
<td>222 (98.3)</td>
<td>212 (93.8)</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>3 (1.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Not done</td>
<td>1 (0.4)</td>
<td>0 (0.0)</td>
<td>210 (92.9)</td>
<td>11 (4.9)</td>
<td>1 (0.4)</td>
<td>3 (1.3)</td>
</tr>
</tbody>
</table>

† All HIV-1 reactive ELISA results were confirmed by WB and/or IFI. ‡ WB was done only in the sera reactive with the ELISA test. § FTA-Abs was positive in 4/11 (36.4%) patients with positive VDRL.

Abbreviations: ELISA, Enzyme-linked immunosorbent assay; WB, Western blot; HCV, Hepatitis C Virus; HBV, Hepatitis B Virus; HBsAg, Hepatitis B surface antigen; IFI = indirect immunofluorescence; FTA-Abs, Fluorescence Treponema Antibodies - Absorbed.
Table 2. Univariate analysis of HIV-1 serostatus according to type, severity of haemophilia and several transfusion transmittable diseases for 226 haemophilic patients.
Fundação Hemominas, Belo Horizonte, Brazil, 1994.

<table>
<thead>
<tr>
<th>HIV-1 serostatus</th>
<th>HIV-1 ELISA Positive (n=36)</th>
<th>HIV-1 ELISA Negative (n=189)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Haemophilia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>34 (94.4)</td>
<td>162 (85.7)</td>
<td>NS</td>
</tr>
<tr>
<td>B</td>
<td>2 (5.6)</td>
<td>27 (14.3)</td>
<td></td>
</tr>
<tr>
<td>Severity of haemophilia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>3 (8.3)</td>
<td>23 (12.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Moderate</td>
<td>11 (30.6)</td>
<td>81 (42.9)</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>22 (61.1)</td>
<td>85 (45.0)</td>
<td></td>
</tr>
<tr>
<td>Age in years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.97 ± 10.05 (mean ± SD)</td>
<td>18.32 ± 12.10 (mean ± SD)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>(Student t = 2.77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg Positive</td>
<td>2 (5.6)</td>
<td>1 (0.5)</td>
<td>0.067 (Fisher’s exact test)</td>
</tr>
<tr>
<td>HCV Positive</td>
<td>31 (86.1)</td>
<td>107 (56.6)</td>
<td>0.0002</td>
</tr>
<tr>
<td>VDRL(^1) Positive</td>
<td>5 (13.9)</td>
<td>6 (3.2)</td>
<td>0.014 (Fisher’s exact test)</td>
</tr>
<tr>
<td>T.cruzi- IFA Positive</td>
<td>2 (5.6)</td>
<td>3 (1.6)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = not significant \(^1\) Data not available for FTA-Abs
Dear Dr Carneiro-Proietti

re: E163/97

Presence of human immunodeficiency virus and human T-lymphocyte virus type I and II in a haemophiliac population in Belo Horizonte, Brazil and correlation with additional serologic results

Your paper has now been accepted for publication. Could you please fill out and return the enclosed copyright form to me as soon as possible.

Yours sincerely

Christine A Lee
EDITOR

Fax No: 0055 31 2262 002
HIV-1 Subtyping by Heteroduplex Mobility Assay in Patients with Bleeding Disorders

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Supported by FAPEMIG, CNPq and Capes

Running head: HIV subtyping by HMA in hemophiliac patients
Abstract

Human immunodeficiency virus type 1 (HIV-1) establishes persistent infections in humans, in most cases leading to AIDS. HIV-1 has been genetically classified into major (M) and outlier (O) groups. The M group is further divided into nine subtypes (A-I), based on sequency diversity. HIV-1 infection was transmitted to hemophiliac individuals mainly by clotting concentrates in the late 1970s and mid 1980s and AIDS became a leading cause of morbidity and death among these patients. The aim of this study was to determine the subtype(s) of HIV-1 in eight seropositive patients with bleeding disorders from Belo Horizonte, Brazil, using heteroduplex mobility assay (HMA), a nonsequencing method that can distinguish between individual HIV-1 subtypes. We have performed polymerase chain reaction (PCR) followed by HMA to subtype the virus and have found all of them to belong to HIV-1 subtype B, which is the one most prevalent in United States, Europe and Brazil. The hemophiliac patients have probably been infected by concentrates imported from São Paulo and Rio de Janeiro (Brazil), Europe and United States, used prior to the knowledge of HIV and thereby with plasma not tested or treated to inactivate the virus. HMA testing of PCR amplified DNA proved to be a relatively simple and rapid way to subtype HIV-1 in individuals infected by transfusion and to gain insights as to the type(s) of viruses being transmitted.

Key words: HIV-1 subtype, AIDS, hemophiliacs, PCR, Heteroduplex Mobility Assay (HMA)
Material and Methods

Patients

Six individuals with hemophilia A, one with hemophilia B and one with hereditary hemorrhagic teleangiectasy (HHT) were studied. All were seropositive to HIV-1, tested by ELISA (Abbott, USA) and confirmed with Western blot (Cambridge Biotech, USA) and/or immunofluorescence (Fiocruz, Brazil). All hemophiliac patients reported previous use of factor concentrates in the seventies and early eighties. The patient with HHT reported continued use of locally prepared red cell units in large amounts (two units/week, average).

PBMC separation and DNA extraction

Venous blood (5ml in EDTA) was collected from patients after informed consent. PBMC were separated from blood samples by Ficoll-Hyphaque gradient centrifugation (Pharmacia, Sweden), lysed by addition of 200μl of lysis solution (TE pH 8.1; Triton X-100, 0.001%; SDS, 0.0001%) and then digested with 50μg of proteinase K. The crude lysates of PBMC obtained were used directly for PCR or, alternatively, genomic DNA was purified from samples using the Isoquick Nucleic Acid Extraction Kit (Microprobe, CA, USA).

PCR

For PCR amplification of env gene regions, crude lysate (10% proportion in reaction) or 1μg of purified DNA was used as template. The primers were based on the HIV-1 HXB2 (GenBank accession number: K03455), as described in the World Health Organization (WHO) Guidelines for Standard HIV Isolation Procedures (1995). The first round reactions were conducted using ED3/ED14 corresponding to positions 5956-5985 and 7960-7931, amplifying a 2.0 Kb fragment. The second round reactions included three sets
Introduction

The hemophiliac population has been much affected by HIV-1 contamination of clotting factor concentrates. With the advent of this virus, transmitted mainly in the late 1970s and mid-1980s, the acquired immunodeficiency syndrome (AIDS) became a leading cause of morbidity and mortality among hemophiliac patients (1). The reported prevalence of antibodies to HIV-1 varied, being as high as 90.0% in some groups (2,3,4,5). In 1994, 36 (24.32%) of a group of 148 hemophiliac patients in Belo Horizonte, Minas Gerais State, Brazil, were seropositive to HIV-1 (6).

HIV-1 has been genetically classified into major (M) and outlier (O) groups. The M group is further divided into nine subtypes (A-I) based in sequence diversity where members of the same subtype differ by less than 10%, and those of different subtypes by 15% or more (7, 8). Variation of HIV-1 can result in a spectrum of viruses exhibiting differences in cell tropism, replication and transmission rates and cytopathicity (9). At present, there is considerable effort in genotyping viruses recovered from recently infected individuals to gain insights as to the types of viruses being transmitted.

A non-sequencing method has been described for HIV-1 genetic screening, the heteroduplex mobility assay (HMA) (10). This method is able to distinguish between individual strains and may also provide reliable information for phylogenetic analysis. Heteroduplexes are formed between strands from the unknown and the reference viral DNA by heat denaturation and subsequent cooling to permit reannealing. HMA is based on the reduction in the electrophoretic mobility in polyacrilamide gel caused by structural deformations in double stranded DNA resulting from mismatches and nucleotide insertions or deletions. The reduction in mobility is proportional to the relative genetic distances
determined by the number of insertions/deletions and mismatches (11). HMA results are in agreement with the complete or partial HIV envelope sequencing (10, 12,13).

In this study we report PCR and HMA results of HIV-1 \textit{env} gene of eight patients with bleeding disorders treated at Fundaç\~{a}o Hemominas, Belo Horizonte, Brazil.
of primers including ED5/ED12, positions 6556-6581 and 7822-7792, fragment of 1.2 Kb (region V1-V5 of gp120); ES7/ES8, positions 7001-7020 and 7667-7647, 0.7 Kb (region V3-V5); ED31/ED33, positions 6816-6844 and 7359-7380, 0.5 Kb (region V2-V4). The substrate was added to a first round reaction of 20µl containing 50mM KCl, 10mM Tris pH 8.3, 1.25 - 1.8 mM MgCl₂, 1% glycerol, 1% DMSO, 5pM of each primer and 0.2mM of each dNTP. Second round reactions (nested PCR) were performed with 2.0 µl of first round PCR in 100µl of reaction. Conditions for PCR were three initial cycles of 94°C (1 min), 55°C (1 min), 72°C (1 min) and 32 cycles of 94°C (15 sec), 55°C (45 sec), 72°C (1 min), with final extension of 72°C (5 min) using a DNA thermal cycler (Perkin Elmer Cetus, CA, USA). After PCR, the DNA samples were analyzed on 2% agarose gel in TAE 1x (0.04M Tris-acetate, 10mM EDTA), stained with ethidium bromide, visualized and photographed under ultraviolet light.

HMA

PCR amplified DNA was next tested by HMA. For heteroduplex formation, 5.0µl of the second round PCR product (nested) from each sample was mixed with 5.0µl of homologous product from a reference subtype provided by WHO (WHO Guidelines for Standard HIV Isolation Procedures, 1995) and 1.0µl of 10x annealing buffer (1M NaCl, 100mM Tris pH 7.8, 20mM EDTA). It was heated to 94°C for 2 minutes, cooled rapidly on ice, mixed with loading dye and loaded onto a 5% polyacrylamide gel (30:0.8 acrylamide/bisacrylamide). The gels were stained with ethidium bromide (0.5µg/ml in water) and DNA was detected by UV light or, alternatively, the gels were stained by silver and exposed to electrophoresis duplicating film (EDF, Kodak, USA), which allows a permanent record to be kept.
Results

The eight patients in this study (Table 1) were selected based on HIV-1 repeated reactivity and all had AIDS. Their age ranged from 13 to 53 years (mean 27.87). Six had hemophilia A, one hemophilia B and one had hereditary hemorrhagic teleangiectasia (HHT). All hemophilic patients (males) reported use of clotting factor concentrates prior to 1985. The patient with HHT (female) presented recurrent bleeding in the digestive tract, needing weekly transfusions of red packed cells (2 units/week, average). She had never used clotting factor concentrates and was transfused exclusively in Minas Gerais State, with locally prepared blood.

We have obtained PCR amplification of the HIV env gene in samples from all patients. HMA results showed that all individuals had infection with B subtype of HIV-1.

Figure 1 shows HMA results of patient AAF, with electrophoretic mobility of HIV-1 B subtype. First lane is the DNA amplified from the patient’s sample (unknown - UK). Lanes 2-8 show mixtures of the patient’s amplified DNA with DNA amplified from HIV-1 standards of subtypes B (B1,B2,B3), C (C1,C2) and F (F1,F2), supplied by WHO. The heteroduplexes (HE) in lanes 2, 3 and 4 (HIV-1 B subtype standards) are formed bellow the single stranded DNA (SS) and above the homoduplex (HO), thereby being classified as B subtype.


<table>
<thead>
<tr>
<th>Patient Number</th>
<th>Patient Initials</th>
<th>Bleeding disorder</th>
<th>Age</th>
<th>Sex</th>
<th>HIV-1 HMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AAF</td>
<td>HHT</td>
<td>53</td>
<td>female</td>
<td>B</td>
</tr>
<tr>
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</table>

HMA = Heteroduplex mobility assay  
HA = Hemophilia A  
HB = Hemophilia B  
HHT = Hereditary Hemorrhagic Telangiectasia
Figure 1

Heteroduplex mobility assay of patient AAF stained by ethidium bromide, showing HIV-1 B subtype. First lane is the DNA amplified from patient’s sample (unknown - UK). Lanes 2-8 show runs of mixtures of patient’s amplified DNA with DNA amplified from HIV-1 standards of subtypes B (B1, B2, B3), C (C1, C2) and F (F1, F2). Heteroduplexes (HE) were formed above the homoduplex (HO) in lanes 2, 3 and 4.
3. DISCUSSÃO

(vide também manuscritos)

A infecção pelo vírus HIV-1 em Minas Gerais está presente em diversos segmentos da população e se deve quase exclusivamente ao subtipo B. Nos grupos estudados, as formas de transmissão detectadas foram a sexual, por concentrados de fatores da coagulação e por transfusão sanguínea.

Os pacientes hemofílicos foram provavelmente infectados por concentrados importados de São Paulo e Rio de Janeiro (Brasil), Europa e Estados Unidos (PROIETTI et al, 1992), usados antes do conhecimento do HIV e portanto feitos com plasma sem triagem ou tratamento para inativar o vírus.

Os resultados da análise das sequências de nucleotídeos dos pacientes hemofílicos, doadores e pacientes com AIDS foram compatíveis com os resultados de HMA, conforme relatado na literatura (DELWART & MULLINS, 1994). A única exceção foi o doador com o subtipo F (GM19), que se agrupou com o subtipo B, mostrando que provavelmente se trata de recombinação B+F, cuja presença já foi relatada no Brasil (SABINO et al, 1994).

A reação de PCR para o HIV-1 foi útil para complementar os resultados sorológicos em 5 de 29 casos de doadores de sangue com resultados indeterminados no Western blot. Embora a PCR talvez não diminua o tempo de acompanhamento do doador indeterminado, ela pode indicar quais doadores necessitam ser acompanhados mais de perto pelos serviços médicos de referência.

O teste de HMA dos produtos amplificados por PCR mostrou ser um meio relativamente simples e rápido de subtipar HIV-1 em indivíduos infectados por diversos meios, inclusive transfusão, dando informação sobre o(s) tipo(s) de vírus que estão sendo transmitidos em determinada população, sendo, a nosso ver, adequados para estudos de epidemiologia molecular e outros, que incluem a análise de variantes em indivíduos infectados ao longo do tempo e a determinação de pares de transmissão perinatal ou sexual.
4. CONCLUSÕES E PERSPECTIVAS

Concluímos com o presente estudo que o vírus HIV-1 está presente em Minas Gerais em diversos segmentos da população. Há predominância do subtipo B nos 20 indivíduos deste estudo que fizeram HMA e/ou sequenciamento de nucleotídeos; sendo encontrado um indivíduo com subtipo F.

As vias de transmissão do HIV detectadas neste estudo foram a sexual, o uso de concentrados de fatores da coagulação e a transfusão sanguínea. A positividade para HIV-1 em pacientes hemofílicos estava associada com a idade, infecção por HTLV-I/II, HCV e sifilis.

Os esforços conjuntos para elucidar o impacto da variação genética na patogênese, progressão da doença e transmissão do vírus devem prosseguir, aliados à caracterização genética e biológica de outros lentivírus de primatas, para que se possa ainda compreender a origem do HIV e os mecanismos responsáveis pela recente epidemia.

A questão de quais respostas imunes são essenciais para a proteção contra a infecção pelo HIV-1 é fundamental. O desenvolvimento de reagentes diagnósticos e vacinas capazes de provocar respostas imunes efetivas contra cepas clinicamente relevantes do vírus é, em última análise, o objetivo final da literatura consultada e do trabalho de pesquisa aqui apresentado, demandando um completo entendimento das consequências imunológicas da diversidade do HIV.

Para a execução deste trabalho mais amplo é necessário um trabalho conjunto, em nível nacional e internacional. A curto prazo, o trabalho deve continuar na forma de subtipagem de outros segmentos da população com HMA e sequenciamento de nucleotídeos e pode ainda continuar em projetos específicos como, por exemplo, o estudo de mutações associadas ao uso de inibidores do gene da protease pelos indivíduos deste estudo. Além disso, o sequenciamento de outras partes do genoma do subtipo F encontrado (BHGM19) será importante para se determinar os possíveis locais de recombinação (B+F).
Discussion

Subtyping HIV-1 is useful for determining transmission patterns and for molecular epidemiologic studies. All patients in this study presented results compatible with HIV-1 B subtype, which agrees with the epidemiological survey done before (6) that showed that HIV-1 infection in hemophiliac patients in Minas Gerais State was associated with the use of concentrates imported from São Paulo and Rio de Janeiro (Brazil), Europe and United States, where subtype B is prevalent (14, 15, 16). The patient with HHT denied use of factor concentrates and has never used blood products out of Minas Gerais State, thereby pointing out to local transmission of HIV-1 subtype B, probably prior to the implementation of HIV screening in the donated blood.

The exclusive use of treated or recombinant clotting concentrates in adequate amounts should minimise from now on the need of cellular blood, domestic cryoprecipitate and fresh frozen plasma transfusions, thereby eliminating seroconversions for HIV-1 in this population.

HMA testing of PCR amplified DNA proved to be a relatively simple and rapid way of subtyping HIV-1 in infected transfused individuals and to gain insights as to the type(s) of viruses transmitted to this population.
References


