A CASE REPORT OF 40 PATIENTS WITH HIV IN SÃO BERNARDO DO CAMPO WITH PREVIOUS ANTIVIRAL DRUG RESISTANCE, ENFUVIRTIDE ANTIRETROVIRAL TREATMENT AND TREATMENT FAILURE.

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ABSTRACT

Human immunodeficiency virus (HIV) infection leads to T CD4⁺ cell depletion with progressive immune dysfunction, and despite benefits of antiretroviral therapy, if not judiciously used, it leads to the emergence of drug resistance. In this study, we evaluated 40 patients with HIV that enrolled the Unique Health System-SUS and pharmacy of Medical Specialties Clinic of São Bernardo do Campo, São Paulo, Brazil. The enrolled patients were treated with enfuvirtide after treatment failure for HIV by the use of reverse transcriptase inhibitors (NRTIs), non-nucleoside inhibitors of transcriptase reverse (TTRNN) and protease inhibitors (PI). The effectiveness of enfuvirtide treatment was evaluated through viral RNA quantification, T CD4⁺ cell count and patient’s clinical manifestations. Our results show that all patients presented viral load reduction after treatment and the reduction seen during enfuvirtide treatment occurred under the first 6 months of treatment. All patients reported to be satisfied with enfuvirtide therapy, however discontinuation of enfuvirtide treatment once and more than once in the previous week was observed due to adverse reactions involving cutaneous signs and digestive symptoms, suggesting, in this case, the presence of a favorable situation towards the development of drug resistance.

Keywords: Enfuvirtide; Antiretroviral treatment; HIV; Antiviral drug resistance.

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INTRODUCTION

During the course of HIV, an intense viral replication occurs in different anatomical sites, resulting in T CD4⁺ lymphocytes destruction (Hoetelmans, 1998), this leads to quantitative and qualitative changes of the immune system causing the emergence of opportunistic infections and neoplasm development. Thus, antiretroviral therapy should be able to provide maximum suppression of viral replication and slow down the progression of immunodeficiency context and/or to restore immunity and quality of life of the infected individual (Agrawal et al. 2006). Despite all therapeutic advances, it is estimated that 10 to 20% of patients entering in treatment cannot suppress viremia satisfactorily after a few months of therapy (primary virological failure) and 20 to 50% of those with good initial response present failure of virological response after one year under treatment (secondary virological failure) (Bello et al. 2011). The main causes of discontinuation of therapy are side effects of antiretroviral treatment, drug toxicity and the need for high treatment adherence (Agrawal al. 2006). Our objective in this study was to evaluate the effectiveness of enfuvirtide (T20 or Fuzeon) treatment (Rimsky et al. 1998) in 40 HIV patients attended by Unique Health System-SUS and Clinical Pharmacy Specialities of São Bernardo do Campo-SBC, São Paulo, Brazil, after the detection of treatment failure by the use of reverse transcriptase inhibitors (NRTIs), non-nucleoside inhibitors of transcriptase reverse (TTRNN) and protease inhibitors (PI). We evaluated patient’s clinical manifestations, T CD4⁺ cell count, viral load, degree of patient’s satisfaction after treatment and failure in treatment adherence.

MATERIAL AND METHODS

Patients

We conducted a descriptive study with qualitative approach evaluating 40 patients (28 male, 75% and 12 female, 25%, aging 31 to 48 years old) with diagnosis of HIV attended by Unique Health System-SUS and that had enrolled the pharmacy of Medical Specialties Clinic of São Bernardo do Campo. All data
obtained for each patient were made by consulting medical records, SICLOM dispersion system reports and individual monthly interviews during a 1 year period. The previous antiretroviral therapy with NRTI, TTRNN and IP treatment that patients were under was abandoned after the detection of virological failure and drug resistance and replaced by enfuvirtide. All patients that we evaluated had started treatment with enfuvirtide (90 mg subcutaneously, twice a day) and were under care at Unique Health System-SUS and Pharmacy of Medical Specialties Clinic in the city of São Bernardo do Campo. They had been HIV genotyped during a period of one year by the genotyping Network RENAGENO of National Program DST/AIDS/Ministry of Health, Brazil. Patients enrolled in the present study were defined as treatment-experienced based on their treatment histories and/or current genotypic sensitivity score (GSS) or phenotypic sensitivity score (PSS). The Ethic Committee of the institution participant of this study approved the study protocol.

Blood collection and PBMC isolation

Peripheral blood of all HIV patients evaluated in this study was collected twice. Peripheral Blood Mononuclear Cells (PBMC) were isolated from heparinized venous blood by Isolymph gradient (Gallard-Schlesinger Industries, Inc, Norway) and resuspended in DMEM (Sigma Laboratories, St. Louis, MO) supplemented with gentamicin (40 µg/ml, Sigma Laboratories, St. Louis, MO) and 5% pooled AB normal human serum (Human serum type AB-Biocell Laboratories, INC., Rancho Dominguez, C.A., USA) for T CD4+ lymphocyte cell count.

Viral load count

The viral load count was performed before the treatment and repeated every 3-4 months, and after therapy modification with enfuvirtide, after 4 weeks and then at every 6 months. The quantification of plasma HIV RNA was performed using commercial kit Quantiplex HIV-1 RNA 3.0 Assay (bDNA, Bayer Diagnostic, Walpole, Massachusetts) with a lower limit of quantification of 50 copies of RNA/ mm3 blood, according to manufacture instructions.

T CD4+ lymphocyte count

To evaluate the expression of cell surface markers on PBMC ex vivo of HIV patients, T CD4+ lymphocyte count was performed before the treatment and repeated every 3-4 months, and after modification with enfuvirtide retroviral therapy, after 4 weeks and then every 6 months, after T CD4+ lymphocyte count up more 500 cells/mL of blood. Fluorescein isothiocyanate (FITC)-conjugated anti-human CD4 and CyChrome-conjugated anti-CD3 (Becton-Dickinson, Mountain View, CA) mAb were used in this study. Saturating concentrations of fluorochrome-conjugated murine anti-human mAb were added to resuspended pellet of 0.5-1.0x10^6 cells for a 30-min incubation at 4°C, followed by two washes in phosphate-buffered saline (PBS), pH 7.4, containing 0.5% AB normal human serum. The cells were analyzed on FACS-Scan (Becton-Dickinson, Sunnyvale, CA) and total blood counts were performed in an automated hematology analyzer (Cell Dyn 3000 and Cell Dyn 3500, Abbott).

RESULTS

Our descriptive study with qualitative approach evaluated 40 patients that had been diagnosed with HIV for a period between 12-16 years (80%) and for a period over then 16 years (20%). Of all patients evaluated, at the time of study was made, 50% of them had from 39 to 48 years old and 50% of them were older than 50 years of age. Among all enrolled patients, 72.5% of them had been receiving antiretroviral therapy for 11 to 15 years and 27.5% of all patients were under treatment for more than 16 years (Table 1).

Table 1. Time of HIV diagnosis, antiretroviral therapy and enfuvirtide use. Time of diagnosis of 40 patients with HIV under care in São Bernardo do Campo and attended by Sistema Único de Saúde-SUS.

<table>
<thead>
<tr>
<th>Time of Therapy</th>
<th>HIV diagnosis</th>
<th>ARV therapy use</th>
<th>Enfuvirtide use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Years of Therapy</td>
<td>12-16</td>
<td>&gt;16</td>
<td>11-15</td>
</tr>
<tr>
<td>Number of patients</td>
<td>32</td>
<td>8</td>
<td>29</td>
</tr>
</tbody>
</table>

For our study propose, patients were separated into the following categories: less than 1 year (6 months, n=40) of treatment (22.5%); between 1 to 2 years (n=25) of treatment (62.5%); and over 5 years (n=6) of enfuvirtide treatment (15%). Our results show that all patients presented viral load reduction after treatment and, the reduction seen during enfuvirtide treatment occurred under the first 6 months of treatment (Fig. 1).
number of viral copies was preserved lower during the time of study for all patients (n=40).

It was also possible to detect an increased TCD4+ cell number after treatment and 6 months after the substitution of antiretroviral therapy for enfuvirtide. Most patients presented increased T CD4+ cell count (less than 1 year, 6 months, n=40) (Fig. 2).

![Graph showing TCD4+ cell count recovery during enfuvirtide treatment](image)

**Fig. 2** - Recovery of T CD4+ cell count during enfuvirtide treatment. After 6 months of enfuvirtide treatment (n=40), 1-2 years (n=25) and more 5 years (n=6), patients present recovered the absolute number of T CD4+ cells to normal levels under FACS-Scan analysis (Becton-Dickinson, Sunnyvale, CA) after treatment. The complete blood count was performed in a automated hematology analyzer (Cell Dyn 3000 and Cell Dyn 3500, Abbott).

The same effect was observed in two other time categories, between 1 to 2 years (n=25), and over 5 years (n=6) of enfuvirtide treatment. The great majority of patients (75%) reported to be very satisfied while 25% were satisfied with enfuvirtide therapy. Regarding the reason responsible for the discontinuation of enfuvirtide treatment, we found out in our study group that the main reasons reported were collateral side effects related to the drug, the use of injectable pharmaceutical form of administration and the twice a day dosage. Due to the reported adverse effects, 32.5% of patients failed to take a dose of enfuvirtide once during the previous week, and 67.5% failed to take the drug more than once in the previous week. Among the observed adverse reactions in our study group to enfuvirtide, cutaneous signs and digestive symptoms were detected by individual physical evaluation and patient reports and 25% of our court reported a very intense, 50% reported an intense and 25% reported less prominent adverse effects to enfuvirtide (Table 2).

<table>
<thead>
<tr>
<th>Level of Satisfation of Enfuvirtide Treatment</th>
<th>Not satisfied</th>
<th>Satisfied</th>
<th>Very Satisfied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>10</td>
<td>30</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of Failure Events in the Administration of Enfuvirtide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Once in last week</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Level of Collateral Side-Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
</tr>
<tr>
<td>10</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Collateral Side-Effects Reactions</th>
<th>Skin Symptoms</th>
<th>Digestive Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodules</td>
<td>Edema</td>
<td>Erythema</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nodules</th>
<th>Hematoma</th>
<th>Nausea</th>
<th>Constipation</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

**Table 2.** Degree of Satisfaction, profile of adhesion to treatment and adverse reactions events. Description of degree of satisfaction of enfuvirtide treatment, number of failure of enfuvirtide drug administration, and report of adverse side effects intensities and signs.

**DISCUSSION**

The emergence of new antiretroviral forms of drugs has increased the number of treatment options and has improved the durability, tolerability and long-term efficacy of cART, even among patients with extensive treatment experience and high levels of drug resistance (Yazdanpanah et al. 2009). The human immunodeficiency virus type 1 fusion inhibitor Enfuvirtide (T-20, Fuzeon) is the first fusion inhibitor antiretroviral drug for HIV, it blocks the fusion of the virus with the T CD4 receptor cell membrane (Cooper & Lange 2004) and started to be used in Brazil in 2005 as a rescue therapy for patients that had presented resistance to reverse transcriptase inhibitors (NRTIs), non-nucleoside inhibitors of reverse transcription (TTRNN) and protease inhibitors (PI) (Santos, 2006). However resistance to this type of drug can be quickly developed in patients receiving enfuvirtide rescue therapy and also the number of confirmed resistant-associated mutations have been growing over the past years according to some reports (Lu et al. 2006, Morozov et al. 2007). One of the gold standard criteria in the evaluation of treatment response is the reduction of viral load and the detection of increase (or break of fall) in the number of T CD4+ cells. A major goal of antiretroviral therapy is to achieve undetectable viral load (below 50 copies/mm3 blood) within a period of 6 months, while the recovery of TCD4+ cell count to normal levels is normally observed in a slower rate than the reduction of viral load (Bunupuradah et al. 2011). In our study, we were capable to demonstrate a reduction of viral load and an increase of T CD4+ cell number in peripheral blood after a 6 months period. Previous reports show that enfuvirtide is well tolerated by the majority of HIV patients that are under treatment (Cohen et al. 2002) and can be highly effective in many clinical conditions.
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(Polk et al. 2007) even though with the necessity to be administered by subcutaneous injection twice a day. This necessity occurs due to it’s characteristic of being a rapidly degraded peptide following oral administration. We were also capable in identifying failure of adherence to enfuvirtide treatment, besides the observation that all patients’ were satisfied with enfuvirtide therapy. In our court most patients had been using antiretroviral therapy for a period higher than 11 years and the treatment had to be interrupted because failure of the initial treatment was identified. Complications in adherence to treatment are the most frequent cause of treatment failure. This occurs because the use of drugs under suboptimal doses or in an irregular administration scheme accelerates the selection process of resistant viral strains (Raboud et al. 2002). Some patients report uneasiness in self-injecting the medication or lower adherence rates, suggesting that self-injecting may be avoided through the administration of the drug by a relative or in an outpatient service and that patient should have easy access to psychiatric and medical care (Avelino-Silva et al. 2011). Adherence problems motivated by factors related to lifestyle, toxicity and/or drug interactions may interfere with the maintenance of adequate levels of antiretroviral drugs in blood and other body compartments (Garvie et al. 2011, Henderson et al. 2011). The presence of cutaneous signs, and digestive symptoms together with distinct groups of intensity of adverse effect seen in our work are very similar to what is commonly reported (Moyle & Carr 2002). Common reactions of patients associated with enfuvirtide therapy includes pain, swelling and darkening of the skin at the injection site, particularly in first week, peripheral neuropathy, insomnia, depression, cough, dyspnea, anorexia, arthralgia, infections (including bacterial pneumonia) and eosinophilia can also be observed. Hypersensitivities reactions may occur in 0.1 to 1.0% of patients, including rash, fever, vomiting, hypotension, and more severe reactions including respiratory stress, glomerulonephritis and anaphylaxis (Emerson et al. 2009), symptoms that were not observed in our study. Failure in administration resulting in discontinuation of therapy seen in the present report also corroborates with other reported data (Pessoa et al. 2011) indicating that enfuvirtide failure may also be associated with low adherence for a large number of cases. Injection site reactions (ISRs) are the most frequently reported adverse events related to enfuvirtide use, which has been reported to occur in about 98% of patients over 48 weeks of treatment (Lalezari et al. 2003; Lazzarin et al. 2003). Frequent ISRs of enfuvirtide administration include tenderness, pain, erythema, induration, nodules, cysts, ecchymosis, and pruritis (Trottier et al. 2005, Reynes et al. 2007). The development of disseminated granuloma annulare due to an abnormal immune reaction in HIV-positive patients (Ball & Kinchelow 2003) and also the development of an induced granulomatous reaction at an enfuvirtide injection site has also been described in the literature. Other than the granuloma annulare and granuloma annulare-like reaction (Ghadially et al. 1989, McGregor & McGibbon 1992, Ball & Kinchelow 2003), a different from of granuloma that is rich in multinucleated giant cells engulfing altered collagen (collagenophagic granuloma) (Sidhu et al. 2010) has also been reported as ISR to enfuvirtide injection. However, besides all side effects the number of Brazilian patients using enfuvirtide reached 21,946 in 2008 and 23,035 in 2009 (www.aids.gov.br). Thus, we were able to verify that enfuvirtide was beneficial as a medical approach in situations related to previous treatment failure for HIV according to the decreased in number of viral copies and increased T CD4⁺ cell number detected after enfuvirtide treatment. However, in our work, despite high degree of patient’s satisfaction we detected a failure in treatment adherence caused by the side effects of injectable pharmaceutical form, which makes possible the emergence of resistance to this drug.

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