Efavirenz does not cause false-positive urine cannabis test in HIV-infected patients on Highly Active Anti-Retroviral Therapy

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SUMMARY
Efavirenz is a non-nucleoside reverse transcriptase inhibitor used in combination with other drugs for the treatment of patients with HIV infection. Efavirenz has been reported to cause a positive urine cannabis test reaction which may create problems between HIV-infected patients on Efavirenz and law enforcement agencies. Doctors are at loss whether to issue documents certifying the potential false positive urine cannabis test with Efavirenz to patients. We investigated if the urine of HIV-infected patients on Efavirenz caused a positive urine cannabis test using the Axsym Cannabinoids Assay®. Urine samples from 51 eligible patients on Efavirenz were tested for cannabis. All tested negative except for one who had used cannabis the day before. Efavirenz does not cause false positive urine cannabis test with the Axsym Cannabinoids Assay®. Certification documents from doctors are therefore unnecessary.

KEY WORDS: HIV, Efavirenz, Urine Cannabis test

INTRODUCTION
Human immunodeficiency virus (HIV) is the causative agent for Acquired Immuno-Deficiency Syndrome (AIDS). By the end of 2011, Malaysia had a cumulative figure of 94,841 HIV, 17,686 AIDS and 14,986 deaths, with 79,855 People-Living-With-HIV (PLHIV). The epidemic in Malaysia is still concentrated within most-at-risk populations (MARPS) especially among intravenous drug users (IDU), sex workers and transgender population.1

Efavirenz, an oral non-nucleoside reverse transcriptase inhibitor (NNRTI), is commonly used as part of a 3-drug-cocktail first-line therapy in the treatment of HIV/AIDS in Malaysia. It binds directly to a site on reverse transcriptase, which causes the disruption of the enzyme's active site, thereby blocking RNA-dependent and DNA-dependent DNA polymerase activities.2 Efavirenz is marketed as Stocrin® (Merck Sharp & Dohme Corp) in most parts of Asia and as Sustiva® (Bristol-Myers-Squibb) in other parts of the world.

Cannabis, also known as marijuana, is a derivative from a variety of flowering plants (Cannabis sativa, C. indica and C. ruderalis) indigenous to South Asia and Central Asia. When consumed via inhalation, cannabis has psychoactive and physiological effects. The major psychoactive compound in cannabis is delta-9-tetrahydrocannabinol (THC) which produces a state of relaxation and euphoria. THC can be measured in the blood, urine, saliva and sweat and is used as a surrogate marker in screening tests for cannabis use. Urine is commonly used for the purpose of testing as it is convenient and non-invasive. The detection of THC in urine suggests the use of cannabis.

Efavirenz has been reported to potentially cause false positives for cannabis on urine drug testing4-6. This finding has caused difficulties for HIV-infected current or previous illicit drug users who are on Efavirenz as part of their Highly Active Anti-Retroviral Therapy (HAART) regimen, and for the law-enforcement agencies if the former test positive for urine cannabis with onsite urine drug testing kits used by the latter during law enforcement exercises.

In such situations, the former may deny using illicit drug use and may attribute the positive urine cannabis test to the Efavirenz they are taking while the latter, because of the positive urine test, would be compelled to detain the former pending further confirmatory testing results. In order to avoid detention, some were forced to divulge their medication history and in so doing revealed their HIV status. In addition, at times the former may still be detained based on strong suspicion of drug use regardless of the onsite urine cannabis test results. During detention, they may be deprived of HAART. Scenarios like these may have grave consequences as HIV-infected patients who missed their medications, especially for an extended period, would increase the risk of resistance and treatment failure.

Consequently, in order to avoid these potentially tricky situations, some HIV-infected patients on Efavirenz-containing HAART regimen have resorted to requesting a written document from their health-care providers, certifying that the Efavirenz in their medications may cause positive urine test for cannabis. Unfortunately this, in turn presents a dilemma to the health-care providers as they cannot ascertain if the positive urine cannabis test was indeed caused by Efavirenz or the patient had actually been using cannabis.
Very few studies have looked into whether HIV-infected patients on Efavirenz-containing HAART would indeed test positive for urine cannabis which formed the basis of our study which was to determine if the urine of HIV-infected patients on Efavirenz would test positive for cannabis. This study was a collaborative study between the Department of Medicine, International Medical University, Malaysia and the Department of Pathology, Hospital Tuanku Jaafar, Seremban, Negeri Sembilan, Malaysia and has been approved by the National Medical Research Register of Malaysia.

MATERIALS AND METHODS

Study Inclusion and Exclusion Criteria
This was a cross sectional study conducted between December 2011 and February 2012 in the infectious diseases clinic of the Hospital Tuanku Jaafar, Seremban, Negeri Sembilan, Malaysia. Fifty-one eligible patients who presented for their regular follow-up at the clinic and who fulfilled the inclusion and exclusion criteria in this study were recruited.

Inclusion criteria:
1. All HIV-infected patients on HAART containing Efavirenz as part of the combination therapy.
2. Age 18 years and above.

Exclusion criteria:
1. Age below 18 years old.
2. HIV-infected patients on HAART which did not contain Efavirenz.
3. HIV-infected patients not on HAART.
5. HIV-infected patients on HAART containing Efavirenz who defaulted medications (defined as non-adherence to medication dosing and timing within the last 48 hours).
6. Patients who are on Ketoconazole for fungal infection.

All the patients in our study were on Efavirenz (Stocrin®) which is manufactured and marketed by Merck Sharp & Dohme Corp (MSD).

Data Collection
Data for this study was collected from interviews with the selected patients in the language that the patients were most conversant in and from the patients’ case notes. Prior to each interview, the study objectives were explained to the patient and a signed informed consent was obtained from the patient. The following data were collated:
1. Socio-demographic data, which consisted of the patient’s age, gender, ethnicity, marital status and years of education.
2. Information on HIV infection, which included the year of diagnosis, the CD4 count at diagnosis, the year HAART was initiated and the latest CD4 count available within the last 4 months.
3. Adherence to Efavirenz, which included the time the patient usually took his or her Efavirenz tablet, and their adherence to the timing.
4. Additional information from patients who were either actively using or were former intravenous drug users, which included the type of drug used, route of administration, history of police detention, number of days in detention, the result of their urine cannabis test during detention and whether they were deprived of HAART during detention.

Definition for adherence and non-adherence
1. Adherence: Efavirenz was taken within 30 minutes before or after scheduled timing.
2. Non-adherence: Efavirenz was taken more than 30 minutes before or after scheduled timing.

At the conclusion of each interview, a urine sample (between 5 – 10 ml) was collected from each patient in clean urine bottles. The urine sample was then visually inspected before being stored in temperature between 2-8 C and then transferred to the laboratory to be tested for the presence of cannabis. The results of the screening test were conveyed back to the patients on their subsequent follow-up with strict confidentiality maintained.

Urine Cannabinoids Screening Test
The AxSYM Cannabinoids Assay®, which is marketed by Abbott, USA, was used in our study institution for the detection of cannabinoids in human urine. It is a semi-
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Table II: Details of 3 patients with history of police detention

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Gender</th>
<th>Age</th>
<th>Years since diagnosis of HIV</th>
<th>Year EFV* started</th>
<th>Drug habit at time of detention</th>
<th>Details of detention</th>
<th>Urine test for cannabis</th>
<th>Length of detention (days)</th>
<th>HAART** deprivation during detention</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>Male</td>
<td>47</td>
<td>5</td>
<td>2007</td>
<td>Active heroin user</td>
<td>Detained once in 2008</td>
<td>Negative</td>
<td>4 days</td>
<td>No</td>
</tr>
<tr>
<td>40</td>
<td>Male</td>
<td>40</td>
<td>3</td>
<td>Early 2010</td>
<td>Active cannabis user</td>
<td>Detained once in December 2010</td>
<td>Positive</td>
<td>7 days</td>
<td>Yes</td>
</tr>
<tr>
<td>48</td>
<td>Male</td>
<td>36</td>
<td>2</td>
<td>2010</td>
<td>Active heroin user</td>
<td>Detained once in 2011</td>
<td>Positive</td>
<td>Unable to recall</td>
<td>No</td>
</tr>
</tbody>
</table>

* EFV; Efavirenz  
** HAART; Highly Active Antiretroviral Therapy

quantitative reagent system and provides a preliminary analytical test result. The minimum volume of urine needed for each test is 150 µl. The sensitivity of the assay (defined as the lowest measurable concentration which can be distinguished from zero with at least 95% confidence) was calculated to be 13.00 ng/ml and may cross-react with a number of compounds (11-Nor-delta-8-THC-9-carboxylic acid, 11-OH-delta-9-THC, Cannabinol and Ketocanazole) at above the 13.00 ng/ml sensitivity. This test was utilized by law-enforcement agencies as the screening test for urine cannabinoids for the entire state of Negeri Sembilan. The laboratory in the institution where this study was conducted was the sole facility responsible for the urine cannabinoids screening tests. Gas chromatography/mass spectrometry was used as the confirmatory test if the screening test was positive.

Statistical Analyses

Descriptive analyses of the data in this study were performed using the Statistical Package for the Social Sciences (SPSS) version 17 for Windows.

RESULTS

A total of 51 patients were recruited for this study. The mean age was 44.1 years old (range: 23-73 years). The male to female ratio was 2:1. The majority of the patients were of Malay ethnic groups (N = 29, 56.9%) followed by the Chinese (N = 19, 37.3%). Slightly more than two-thirds of the patients received at least secondary level education (N = 36, 70.6%) while the rest received at least primary level education except for one patient who received tertiary level education. Two-thirds (N = 34, 66.7%) of them were married at the time of the survey. The majority of the patients were diagnosed with HIV (N = 24, 47.1%) and started on Efavirenz (N = 36, 70.6%) within 5 years from diagnosis. The socio-demographic parameters of the patients in this study are tabulated in Table I. The urine samples from all the patients tested negative for urine cannabis except for one patient who tested positive for urine cannabis. The patient who tested positive admitted to using cannabis a day prior to the survey.

Three patients in our study had history of detention by the police within a year after the initiation of HAART which contained Efavirenz. All three were active drug users at the time of detention. Two of them eventually tested positive for urine cannabis while in detention out of which one patient was deprived of HAART while in detention. The details of these three patients are tabulated in Table II.

DISCUSSION

Our study is the first study in Malaysia to investigate if patients on Efavirenz as part of their HAART regimen would test positive for urine cannabis. In our study, only 1 out of 51 patients who were on Efavirenz tested positive for urine cannabis using the AxSYM Cannabinoids Assay® (Abbott, USA) while the other 50 tested negative. The one patient who tested positive had used cannabis a day prior to sampling.

The result in our study is in contrast to other studies which reported that Efavirenz may cause false positives on urine cannabis tests. A study by Rossi et al reported that some commonly used immunoassays for urine cannabis were susceptible to cross-reaction errors resulting from the presence of Efavirenz metabolite in the urine sample. Efavirenz is excreted in the urine as an 8-hydroxy-efavirenz (EFV-8-OH) and/or 8-ether glucuronide (EFV-8-G). The cross-reactivity was attributed to EFV-8-G. The false-positive finding can be reversed by acid hydrolysis of the urine to convert EFV-8-G into EFV-8-OH before re-testing which necessitated an extra step in ruling out false-positives when testing for urine cannabis with test kits which also detect EFV-8-G.

In Rossi et al’s study, the urine samples from 8 individuals receiving HAART containing 600mg of Efavirenz once daily were randomised for analysis using 6 different immunoassay methods. The study showed that immunoassays performed with reagents from certain manufacturers were subject to interference attributable to urinary EFV-8-G while there were no occurrences of false-positive findings in immunoassays performed with reagents from other manufacturers which included the kit by Abbott Laboratories (AxSYM Cannabinoids Reagents). The study by Rossi et al demonstrated that false-positive urine cannabis test may be dependent on the immunoassay test kits used, namely some test kits are subjected to interference from EFV-8-G while others are not.

In our study, one of the patients alleged that he was deprived of HAART while he was incarcerated for 7 days for testing positive for urine cannabis. Missing HAART medications,
especially for a prolonged period of time have been linked to loss of viral load suppression and development of drug resistance. In the institution where our study was conducted, doctors were often requested by HIV-infected patients on HAART to provide written documents certifying that they are on Efavirenz and they may test positive for urine cannabis. The patients would then use the document to avoid detention during raids in case they tested positive for urine cannabis. Naturally, it would be difficult for the doctor to know if these patients were also using cannabis or otherwise while they are taking Efavirenz and the doctors would be hard-pressed to decide whether to issue the requested document or not. On the one hand, by not issuing the document, there is a very real risk of these patients being detained and deprived of HAART during detention while on the other hand; issuing the document may result in possible medico-legal challenges.

The results from our study have conclusively proven that the documents from doctors vouching for these patients are unnecessary, at least in the State of Negeri Sembilan; or in any other facility where the immunoassay test used is the AxSYM Cannabinoids Reagents (Abbott Laboratories). There does not appear to be any likelihood of false-positives when this test kit is used.

STUDY LIMITATIONS

The results from our study may be influences by several limitations. Firstly, this study was conducted on a relatively small sample size because there was limited number of patients in our institution who met the strict inclusion and exclusion criteria of the study. A larger sample size may yield different results although it is unlikely. Secondly, our findings were dependent on the history given by the patients which could not be verified. Finally, only one type of immunoassay reagent, the AxSYM Cannabinoids Assay® (Abbott, USA), was used in our study. Results may differ if other test kits were used instead. However, it is stated in the MSD drug information sheet that false positive urine cannabinoid test results have been reported in uninfected volunteers who received Stocrin® and these were only observed with the CEDIA DAU Multi-Level THC assay, and have not been observed with other cannabinoid assays including tests used for confirmation of positive results.

CONCLUSION AND RECOMMENDATION

Our study has conclusively shown that Efavirenz does not cause false-positives for urine cannabis when tested using the AxSYM Cannabinoids Assay® (Abbott, USA) in our institution. Hence, written document requested by patients to certify that their medications may cause false-positive urine test for cannabis is neither justifiable nor necessary. Further studies using different types of immunoassays on a larger population should be conducted.

REFERENCES

7. WHO. HIV Drug resistance Early W arning Indicators. 2010.