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Original Article

# Liver function in children with human immunodeficiency virus infection before and after 6 months of highly active antiretroviral therapy

**Eva Jacomina Jemima Sapulete, I Gusti Ngurah Sanjaya Putra, Ketut Dewi Kumara Wati, Hendra Santoso, Komang Ayu Witarini, I Putu Gede Karyana, Ni Nyoman Metriani Nesa**

## Abstract

***Background*** Highly active antiretroviral therapy (HAART) has resulted in dramatic decreases in morbidity and improved survival rate in human immunodeficiency virus (HIV)-infected patients. Although the risk of morbidity has decreased, it has been replaced by other long-term complications, such as hepatotoxicity. Hepatotoxicity is often reflected in biochemical abnormalities of liver function, such as elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and aspartate aminotransferase-to-platelet ratio index (APRI).

***Objective*** To compare liver function spectrum (AST, ALT, and APRI) in HIV-infected children before and after at least 6 months of HAART.

***Methods*** This observational study (before and after) was conducted in pediatric patients with HIV infection who received HAART for at least 6 months at Sanglah Hospital, Denpasar. Data were collected from medical records.

***Results*** Forty-nine patients were observed in this study. The mean AST, ALT, and APRI levels before HAART were higher than after at least 6 months of HAART. Anti-tuberculosis treatment and fluconazole therapy were not confounding factors for AST, ALT, and APRI.

***Conclusion*** Liver function spectrum enzyme levels of AST, ALT, and APRI are improved after at least 6 months of HAART. **[Paediatr Indones. 2018;58:159-64; doi: http://dx.doi.org/10.14238/pi58.4.2018.159-64 ]**.

***Keywords:*** *liver function; pediatric; human immunodeficiency virus; antiretroviral*

Human immunodeficiency virus (HIV) infection is a major cause of mortality worldwide. Human immunodeficiency virus-infected patients have increased dramatically during the last decade.[1] Highly active antiretroviral therapy (HAART) has resulted in dramatic decreases in morbidity and improved survival rate in HIV infection.[2] Although the morbidity risk has decreased in the era of HAART, it has been replaced by longer-term, non-traditional morbidity and mortality risks.[3]

The name HAART was introduced in the late 1990s to report the usefulness of combination drug therapies to treat HIV.[4] The HAART recommendations in Indonesia are based on the 2014 Ministry of

From the Department of Child Health, Universitas Udayana Medical School/Sanglah Hospital, Denpasar, Bali, Indonesia.

**Corresponding author:** Eva Jacomina Jemima Sapulete/Department of Child Health, Medical School, Udayana University, Sanglah Hospital/Jalan Pulau Nias, Denpasar 80114, Bali, Indonesia. Tel. +62-361-244038/+62-361-244038. E-mail: evasapulete@gmail.com.

Health decree and are as follows: (1) the first line treatment includes 2 nucleoside reverse transcriptase inhibitors (NRTI) + 1 non-nucleoside reverse transcriptase inhibitor (NNRTI), (2) the first line for children undergoing tuberculosis (TB) treatment are a combination of zidovudine (AZT) or stavudine (d4T) + lamivudine (3TC) + efafirenz (EFV) or other alternatives that are a combination of AZT or d4T + 3TC + abacavir (ABC) or AZT or d4T + 3TC + Nevirapine (NVP), combination AZT/d4T + 3TC + ABC or AZT/d4T + 3TC + EFV or AZT/d4T + 3TC + NVP for children who will start TB treatment, and (3) the first line alternative is tenovofir disoproxil fumarate (TDF) + 3TC/FTC + EFV/NVP.[5]

Though HAART has the potential to slow disease progression, patients may experience several side effects, which include liver toxicity, hematuria, decreased bone density, cardiovascular disease, gastrointestinal tract infection, hypersensitivity reaction, lactic acidosis, and Stevens-Johnson syndrome. Of these complications, the most common is hepatotoxicity.[4] Hepatotoxicity induced by HAART is mostly due to NNRTI, but it can caused by NRTI and protease inhibitor (PI).[5] Mechanisms of drug-induced hepatotoxicity include direct toxicity, hypersensitivity reactions, mitochondrial toxicity, and metabolic abnormalities.[6]

Hepatotoxicity in children with HIV infection is often reflected in biochemical abnormalities of liver function. Biochemical markers commonly used are aspartate aminotransferase (AST) and alanine aminotransferase (ALT) involved in breakdown of amino acids, higher levels of which reflect liver cell injury.[7] Hepatotoxicity can be graded according to the toxicity tables of the AIDS Division of Adults and Pediatrics Adverse Event, and is considered to be present when ALT and AST levels rise above the upper limits of normality (ULN).[8] The scoring system is based on ALT and AST increased as follows: grade 1 (mild) 1.25 to <2.5 x ULN; grade 2 (moderate) 2.5 to <5.0 xfrom ULN; grade 3 (severe) 5.0 to <10.0 x ULN; and grade 4 (potentially life-threatening) ≥10.0 x ULN.[9]

Liver function spectrum studies in children with HIV infection who received HAART have yielded conflicting results. The use of HAART was shown to induce hepatotoxicity after 3 months and even more significantly after 6 months.[6] The HAART may cause liver damage (hepatotoxicity) or have a protective effect on the liver, so it is important to monitor liver function in patients taking HAART. This study was conducted to compare liver function in children with HIV infection before and after receiving HAART for at least 6 months.

## Methods

This observational study (before and after) was done to determine liver function (ALT, AST, and APRI score) in children with HIV infection before and after receiving HAART for at least 6 months. Data were collected from medical records in Sanglah Hospital, Denpasar. The target population of this study was children with HIV infection who received HAART for at least 6 months at Sanglah Hospital, Denpasar, Bali. We recruited subjects by consecutive sampling during the year 2012. Patients with incomplete medical records were excluded. This study was approved by the Ethics Committee of Universitas Udayana Medical School.

Collected data were subject characteristics, as well as ALT, AST, and APRI score before HAART and at least 6 months after HAART. Demographic data included address, age, sex, body weight, body height, nutritional status, clinical stage of HIV, and confounding factors which would be controlled by analysis. The confounding factors were hepatitis infection, tuberculosis treatment, and fluconazole therapy.

Subjects aged 18 months to 18 years. Diagnosis of HIV was based on clinical and physical examination, as well as serology markers. The World Health Organization (WHO) classified clinical stage of HIV into stages 1 (asymptomatic), 2 (mild), 3 (moderate), and 4 (severe). The first line antiretroviral therapy for HIV-infected children consisted of NRTI, 3TC, and NNRTI. Liver enzyme levels were assessed by ALT and AST blood tests. Aspartate aminotransferase-to-platelet ratio index (APRI) was calculated by the formula (AST/ULN/platelet count [$10^9$]) x 100. An APRI threshold of 0.5 was considered to indicate significant fibrosis. Hepatotoxicity was defined as AST and ALT ≥2.5 times above the ULN after 6 months of HAART.

## Results

A total of 49 HIV-infected pediatric patients received HAART for at least 6 months at Sanglah Hospital, Denpasar, comprised 29 boys and 20 girls. Most study participants aged 1-5 years. Thirty-six subjects were malnourished and 13 were well nourished. The majority of subjects were in the clinical HIV stage 3-4 group. Subjects' characteristics are shown in **Table 1**.

The mean AST, ALT, and APRI levels before HAART were higher than after at least 6 months of HAART. The mean AST concentration before HAART was 3.4 times higher than the ULN, while that after HAART was only 1.4 times higher than the upper limit normal (ULN), a statistically significant difference (P<0.0001) (**Table 2**). The mean ALT level before HAART was increased 2.03 times from the ULN, but after HAART was within normal limits, however, the difference was not statistically significant (P=0.08) (**Table 3**). The mean APRI before HAART was more than 0.5, but after HAART was within normal limits and significantly different (P<0.0001) (**Table 4**).

Logistic regression was done to analyze for confounding factors of AST, ALT, and APRI levels. The possible confounding factors were hepatitis infection, tuberculosis treatment, and fluconazole therapy. However, there were no subjects with

**Table 1**. Subject characteristics

| Characteristics | (N=49) |
|---|---|
| Sex, n (%) | |
| Male | 29 (59.2) |
| Female | 20 (40.8) |
| Age, n (%) | |
| 1-5 years | 38 (77.6) |
| 6-18 years | 11 (22.4) |
| Nutritional status, n (%) | |
| Severe malnutrition | 12 (24.5) |
| Moderate malnutrition | 24 (49.0) |
| Well-nourished | 13 (26.5) |
| Clinical stage of HIV, n (%) | |
| 1-2 | 17 (34.7) |
| 3-4 | 32 (65.3) |
| Hepatitis infection, n (%) | |
| Yes | 0 (0) |
| No | 49 (100) |
| Tuberculosis treatment, n (%) | |
| Yes | 14 (28.6) |
| No | 35 (71.4) |
| Fluconazole therapy | |
| Yes | 15 (30.6) |
| No | 34 (69.4) |

**Table 2**. AST level before and after HAART

| Variables | Mean (SD) | Mean difference | P value |
|---|---|---|---|
| AST, U/L | | | |
| Before HAART | 120.28 (171.23) | 71.37 | <0.0001 |
| After HAART | 48.91 (46.14) | | |

*analysis by Wilcoxon signed-rank test, SD=standard deviation

**Table 3.** ALT level before and after HAART

| Variables | Mean (SD) | Mean difference | P value |
|---|---|---|---|
| ALT, U/L | | | |
| Before HAART | 70.89 (85.25) | 38.77 | 0.08 |
| After HAART | 32.13(29.58) | | |

*analysis by Wilcoxon signed-rank test, SD=standard deviation

**Table 4**. APRI before and after HAART

| Variables | Mean (SD) | Mean difference | P value |
|---|---|---|---|
| APRI | | | |
| Before HAART | 3.23 (15.14) | 2.90 | <0.0001 |
| After HAART | 0.33 (0.19) | | |

*analysis by Wilcoxon signed-rank test, SD=standard deviation

**Table 5**. Factors associated with elevated liver enzymes were controlled by analysis

| Dependent variables | Independent variables | B | 95%CI | P value |
|---|---|---|---|---|
| AST | TB treatment | -1.172 | 0.034 to 2.793 | 0.296 |
| | Fluconazole | 0.355 | 0.287 to 7.081 | 0.664 |
| ALT | TB treatment | 0.405 | 0.098 to 23.069 | 0.771 |
| | Fluconazole | 19.832 | 0.000 to 0.998 | 0.998 |
| APRI | TB treatment | 1.500 | 0.833 to 24.086 | 0.081 |
| | Fluconazole | 0.730 | 0.372 to 11.567 | 0.405 |

*Logistic regression test; B=beta coefficient; 95%CI = 95% confidence interval

hepatitis infection. Anti-tuberculosis treatment and fluconazole therapy had no significant correlation to AST, ALT, and APRI, as shown in **Table 5**.

## Discussion

In children with HIV infection, HAART may lead to significant hepatotoxicity. Approximately 6 to 30% of treated HIV patients had significantly increased serum liver enzyme levels, which may require discontinuation of treatment.[8] Hepatotoxicity due to HAART may be related to agents from a number of classes, including NRTI, NNRTIs, and PI. The severity of hepatotoxicity may range from transient elevations in transaminase levels to hepatic failure and death, via a variety of mechanisms.[10]

We found that children with HIV infection had liver enzyme elevations up to 3.5 times from the ULN at the time of diagnosis. Both AST and ALT clinically decreased after 6 months of HAART, but the difference was not statistically significant for ALT. This result was in contrast to past studies that suggested the HAART can cause hepatotoxicity.10 Our finding of decreased liver enzymes was consistent with a study in HIV-infected patients in Uganda, which showed few subjects with clinically significant AST elevation during the first three years of HAART. Other studies reported an overall fall in liver enzymes in patients in rural Uganda.[12] Pryce *et al.* also noted that elevations of bilirubin, AST, and ALT prior to commencing HAART could be due to the severity of HIV disease, and these elevations did not necessarily lead to hepatotoxicity after the initiation of HAART.[13] Since HIV is a hepatotrophic virus, the improvement of liver function after HAART initiation could have been due to reduced viral loads, which we did not measure in our study.

Some factors must be considered when comparing our study results to the theory of hepatotoxicity in HAART. Risk factors associated with elevated ALT are high HIV RNA, prolonged HAART exposure, high body mass index (BMI), and increasing age.[14] Aspartate aminotransferase can be transiently elevated, especially in the first 12 weeks of HAART.[11] Most subjects in our study had moderate malnutrition and we evaluated liver enzymes after 6 months of therapy, therefore, overall liver enzyme may have declined by that point.

The lack of APRI elevation risk associated with HAART duration or specific HAART regimen.[15] Nevirapine-based HAART showed AST elevation in few subjects. Other clinical studies reported that NVP was effective and have a good tolerability, but caused an occurrence of characteristic liver injury.[3,11] However, Zidovudine can cause severe hepatotoxicity, and 3TC can lead to hepatotoxic conditions, if they were for long term, especially in patients with chronic hepatitis B.[3] Our subjects used AZT, 3TC, and NVP, and none were infected with hepatitis B.

Human immunodeficiency virus-infected patients are up to 30 times more likely to develop active TB and have a higher risk of dying from TB, than those who are not infected with HIV.[16] Antituberculosis drugs such as rifampicin (RMP), isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB), and streptomycin have good efficacy, but also many adverse effects. Antituberculosis drugs alone can induce hepatotoxicity, which is one of the adverse effects that can cause increased transaminase, bilirubin, icterus, anorexia, nausea, and vomiting.[17] The effect of HAART on TB treatment is not clear.[16] Our study showed that anti-tuberculosis therapy in HIV-infected children did not aggravate hepatotoxicity. A study conducted in both hospitalized patients and outpatients in Jimma, Ethiopia, found that TB treatment induced hepatotoxicity accounted

for a considerable number of cases in TB/HIV co-infected patients.[18] Several studies have shown that anti-TB drugs induced hepatotoxicity between 2-8 weeks.[18,19] We evaluated liver enzymes after 6 months of HAART, so it is possible that we did not detect TB treatment-induced hepatotoxicity in our subjects, because of the time frame.

Malnutrition and low CD4+ level are factors associated with increased risk of developing anti-TB drug-induced hepatotoxicity in HIV-infected children. Malnutrition may be related to depletion of glutathione stores, which makes patients more vulnerable to oxidative injury and slows the pace of liver drug metabolism. The risk of liver toxicity was higher in patients with low baseline CD4+ cell counts, especially those with 50-100 cells/mm$^3$, than in patients with high CD4+ cell counts. But patients with baseline CD4+ cell counts of <50 cells/mm$^3$ were not found to have risk of liver toxicity. The absence of an association between CD4+ cell count <50 cells/mm$^3$ and liver toxicity may be related to the fact that one of the mechanisms involved in the development of hepatotoxicity, the hypersensitivity reaction, which is immunologically mediated, may be less frequently observed in heavily immunosuppressed patients. However, this finding needs to be confirmed and further investigated.[18,19]

Fungal infections are a common cause of morbidity and mortality in HIV-infected patients.20 Fluconazole is commonly used for prophylaxis and treatment of cryptococcosis.[21] Also, HIV-infected patients are particularly at risk of developing local or systemic *Candida albicans* infections. Fluconazole is well established as a first-line management option for both localized and systemic *C. albicans*.[22] Fluconazole is a potent inhibitor of cytochrome P450 isoenzymes and increases the plasma concentrations of a number of co-administered drugs, potentially causing toxicity. One of the first-line drugs for HIV-infected children is NVP. Nevirapine undergoes extensive hepatic metabolism into inactive compounds via P450 isoenzymes, CYP3A4 and CYP2B6. Nevirapine has been associated with severe hepatotoxic reactions and, in some cases, hepatic failure and death.[5,20] Fluconazole significantly raises plasma NVP levels and may cause serious hepatotoxicity.[21]

Our study showed that HAART (containing NVP) and fluconazole, did not increase risk of hepatotoxicity. Wakeham *et al*.[20] and Manosuthi *et al*.[21] showed similar results, as the lack of hepatotoxicity may have been due to serum NVP concentrations of less than 6,000-8,000 ng/mL.[20] We did not measure NVP concentration in our study.

Limitations of this study were that we collected data from medical records and only had single evaluations of liver enzymes. Future studies should be prospective in design, and compared to a gold standard technique such as liver biopsy. More frequent and long-term evaluations, as well as larger sample size, are also recommended for future study.

In conclusion, liver function spectrum (AST, ALT, and APRI) results are improved after at least 6 months of HAART. Antiretroviral therapy effects on TB treatment-induced hepatotoxicity could not be conclusively determined after 6 months of HAART, since anti-TB drugs induce hepatotoxicity between 2-8 weeks. Antiretroviral therapy (containing NVP) and fluconazole do not increase the risk of hepatotoxicity.

## Conflict of Interest

None declared.

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