



Article/Artigo

Antituberculosis drug-induced hepatotoxicity: a comparison between patients with and without human immunodeficiency virus seropositivity

Hepatotoxicidade induzida pelos tuberculostáticos: comparação em pacientes com e sem soropositividade para o vírus da imunodeficiência humana

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ABSTRACT

Introduction: The prevalence and risk factors for rifampin, isoniazid and pyrazinamide hepatotoxicity were evaluated in HIV-infected subjects and controls. **Methods:** Patients with tuberculosis (30 HIV positive and 132 HIV negative), aged between 18 and 80 years-old, admitted to hospital in Brazil, from 2005 to 2007, were selected for this investigation. Three definitions of hepatotoxicity were used: I) a 3-fold increase in the lower limit of normal for alanine-aminotransferase (ALT); II) a 3-fold increase in the upper limit of normal (ULN) for ALT, and III) a 3-fold increase in the ULN for ALT plus a 2-fold increase in the ULN of total bilirubin.

Results: In groups with and without HIV infection the frequency of hepatotoxicity I was 77% and 46%, respectively ($p < 0.01$). Using hepatotoxicity II and III definitions no difference was observed in the occurrence of antituberculosis drug-induced hepatitis. Of the 17 patients with hepatotoxicity by definition III, 3 presented no side effects and treatment was well tolerated. In 8 (36.4%) out of 22, symptoms emerged and treatment was suspended. Alcohol abuse was related to hepatotoxicity only for definition I. **Conclusions:** Depending on the definition of drug-induced hepatitis, HIV infection may or may not be associated with hepatotoxicity. The impact that minor alterations in the definition had on the results was impressive. No death was related to drug-induced hepatotoxicity. The emergence of new symptoms after initiating antituberculosis therapy could not be attributed to hepatotoxicity in over one third of the cases.

Key-words: Hepatotoxicity. Antituberculosis drugs. Human immunodeficiency virus. Antiretroviral drugs. Hepatitis. Tuberculosis.

RESUMO

Introdução: Avaliou-se a prevalência e os fatores de risco para hepatotoxicidade aos tuberculostáticos em pacientes HIV positivos e controles. **Métodos:** Selecionou-se 162 pacientes com tuberculose, tratados com rifampicina, isoniazida e pirazinamida, na faixa etária de 18 a 80 anos, internados em hospital público no Brasil, entre 2005 e 2007. Eles foram divididos em dois grupos: 30 infectados pelo HIV e 132 controles. Adotou-se três definições para hepatotoxicidade: I) aumento de três vezes no valor inferior normal da alanina-aminotransferase (ALT); II) aumento de três vezes no valor superior normal (VSN) da ALT; III) aumento de três vezes no VSN da ALT e duas vezes no VSN da bilirrubina total. **Resultados:** Nos grupos com e sem infecção pelo HIV, a frequência de hepatotoxicidade I foi de 77% e 46%, respectivamente ($p < 0,01$). Para as definições II e III a frequência de hepatotoxicidade não diferiu entre os grupos estudados. De 17 pacientes com hepatotoxicidade induzida por droga (definição III), três não apresentaram sintomas e o tratamento foi mantido sem intercorrências. Oito (36,4%) de 22 indivíduos apresentaram efeitos colaterais e interromperam o tratamento, mas não apresentavam hepatotoxicidade pela definição III. O abuso de álcool associou-se à hepatotoxicidade apenas para a definição I. **Conclusões:** Na dependência da definição escolhida, a infecção pelo HIV pode ou não associar-se à hepatotoxicidade. Foi grande o impacto que pequenas alterações na definição de hepatotoxicidade tiveram nos resultados. Nenhuma morte associou-se ao uso de tuberculostáticos. O surgimento de sintomas não pôde ser atribuído aos tuberculostáticos em um terço dos casos.

Palavras-chaves: Hepatotoxicidade. Tuberculostáticos. Vírus da imunodeficiência humana adquirida. Drogas antirretrovirais. Hepatite. Tuberculose.

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INTRODUCTION

Eighty five thousand cases of tuberculosis and 30,000 new cases of HIV infection were reported in Brazil in 2008¹. Tuberculosis is the most common opportunistic disease in HIV-infected patients²⁻⁸.

In Africa, tuberculosis has been reported to be the worst epidemic faced by local health authorities ever. The average incidence of tuberculosis increased from 149 to 343 cases per 100,000 inhabitants between 1990 and 2005⁹.

The usual drug regimens for treating tuberculosis are the same as those recommended for HIV coinfecting patients. However, therapeutic failures and recurrences have been more frequently reported among the latter⁹.

Toxic hepatitis is the most severe adverse reaction to antituberculosis drugs. It usually installs in the first few weeks of treatment, in parallel with liver necrosis, which may evolve to encephalopathy and death. The risk factors most frequently associated with drug-induced hepatitis are: old age, undernourishment, chronic alcohol abuse, B and C viral hepatitis and the use of antiretroviral therapy^{10,11}.

Increased hepatotoxicity with antituberculosis drugs has been reported in HIV-infected patients by some authors^{12,13}; however, others do not corroborate this association¹⁴⁻¹⁸. In addition, a diversity of definitions for toxic hepatitis are currently in use and numerous drug regimens are involved in treating tuberculosis.

In this study, the prevalence and risk factors for hepatotoxicity to rifampin, isoniazid and pyrazinamide were evaluated in HIV-infected subjects.

METHODS

This is a case control study. Medical records of 450 adult patients admitted to the Eduardo de Menezes Hospital in Belo Horizonte, MG, Brazil, from 2005 to 2007, were examined. Of these, 288 were excluded: 14 with viral hepatitis and 274 with pulmonary diseases other than tuberculosis.

Thus, 162 patients with tuberculosis (30 HIV positive and 132 HIV negative), with ages ranging from 18 to 80 years, were selected for this investigation (Figure 1).

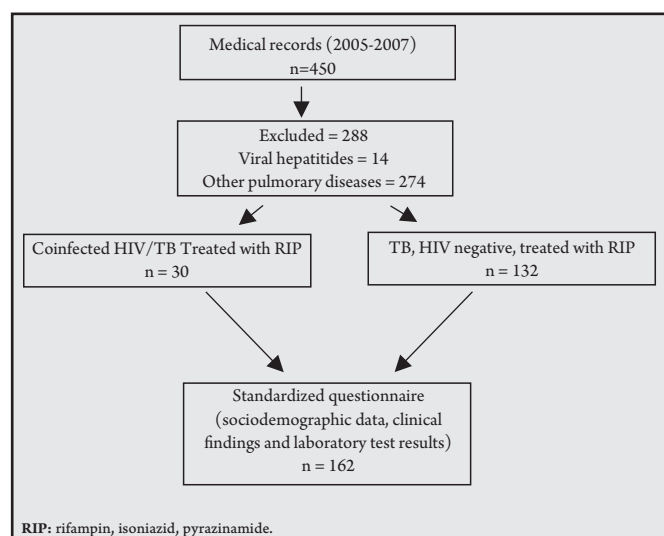


FIGURE 1 - Flow chart of patients with tuberculosis selected for the present study.

Definition of hepatotoxicity

Three definitions of hepatotoxicity were used: I, a 3-fold increase in the lower limit of normal for alanine-aminotransferase (ALT); II, a 3-fold increase in the upper limit of normal (ULN) for ALT; and III, a 3-fold increase in the ULN for ALT plus a 2-fold increase in the ULN of total bilirubin¹⁹⁻²¹.

Alcohol abuse

Excessive alcohol use was defined as the ingestion of more than 60g of alcohol per day, which is the equivalent to 140mL of a local distilled sugarcane derived drink, *pinga*, or two bottles of beer²².

Data collection

Information obtained from the medical records of patients admitted to the Hospital Eduardo de Menezes, Belo Horizonte, Brazil, from 2005 to 2007, were transferred to a data bank and analyzed by SPSS 12.0. For each patient, a standardized questionnaire was filled out with sociodemographic data, clinical findings and laboratory test results.

Laboratory and radiological tests

Hemogram and erythrocyte sedimentation rate; amylase, bilirubin, BUN, LDH, alkaline phosphatase, glucose, alanine aminotransferase (Merck-Selectra and Roche-Cobas-Mira), HBsAg, anti-HBcAg and anti-HCV were tested in sera samples (AXSYM HCV 3.0. Abbott Laboratories, Brasil) and chest x-rays were taken.

HIV serology and lymphocyte count

Enzyme-linked immunosorbent assay (ELISA) and western-blot were used according to Brazilian Ministry of Health recommendations. For the western-blot, a commercial kit was used (HBB 404 - HEMOBIO, HIV-1 Immunoblot, Westernblot, Empresa Brasileira de Biotecnologia SA). CD4+ and CD8+ counts in blood were quantified by flow cytometry (Coulter, Becton and Dickinson, Facscalibur flow cytometer, San Diego, California, USA).

Culture for *Mycobacterium tuberculosis*

The samples were decontaminated using monosodium phosphate and trisodium phosphate (modified Corper & Stoner method)^{23,24} and were concentrated by centrifugation. Subsequently, smears prepared from an aliquot of the sediment were submitted to staining

(Ziehl-Neelsen and auramine)^{25,26} after which they were examined under microscopy. A quantity (0.1mL) of the sediment was added to at least two tubes containing Löwenstein-Jensen (LJ) medium, which were incubated at 37°C for up to eight weeks. The results were described using a semiquantitative scale. The strains isolated were identified by means of the growth inhibition test using p-nitro-alpha-acetylamino-beta-hydroxypropylphenone (NAP) in the BACTEC 460 system (Becton Dickinson Microbiology Systems, Sparks, MD, USA) and by means of other phenotyping tests (morphological analysis of colonies and classical biochemical tests)²⁷.

Diagnosing tuberculosis

The diagnosis of tuberculosis was accepted when the agent was identified in smear/culture of biological material (blood, tissue, sputum) or based on clinical and radiological evidence associated with a definitive response to antituberculosis treatment.

Tuberculin test

Purified protein derivative (PPD) test was administered by injecting a 0.1mL volume containing 5 TU (tuberculin units) under the skin of the forearm. The test was read between 48 and 72h after injection. A positive induration from 0 to 4mm was considered negative; 5 to 9mm of induration was considered a positive weak skin test result and above 9mm a strong response.

Statistical analysis

Data from the questionnaires were transferred to a data bank using EpiData 3.1 (EpiData Association, Odense, Denmark) and analyzed by the *Statistical Package for Social Sciences* (SPSS) 12.0 (SPSS inc, IBM Company, Chicago, Illinois). As a first step, descriptive analysis (frequency tables and exploratory data analysis for qualitative and quantitative variables, respectively) was conducted. Cases and controls were compared using the Chi square or Fisher exact (qualitative variables) and Mann-Whitney (quantitative variables) tests. Pre and posttreatment information was analyzed by the McNemar (qualitative) and Wilcoxon tests. Comparison of the time until hepatotoxicity between the two groups was done using Kaplan-Meier curves and log-rank test. Logistic regression models were performed to identify risk factors associated with hepatotoxicity. For variables to enter the model, a *p* value of 0.2 was used; to remain in the final model, a *p* value of 0.05 was adopted. Odds ratios (OR) and respective 95% confidence intervals (95%CI) were used to estimate hepatotoxicity risk. Model adjustment was tested by the Hosmer & Lemeshow technique²⁸.

Ethical

The present investigation was approved by the Human Research Ethical Boards of the Eduardo de Menezes Hospital and the School of Medicine of the Federal University of Minas Gerais.

RESULTS

Sociodemographic and clinical data

The mean age was 35.7±0.6 and 43.8±3.9 years-old for the HIV positive and negative groups, respectively (*p* < 0.003). For weight, skin color, sex and alcohol abuse, no significant difference was observed between the groups studied (Table 1).

Pulmonary or extrapulmonary tuberculosis

Sixteen (53.3%) out of 30 patients presented pulmonary tuberculosis in the HIV group and 122 (92.4%) out of 132 in the HIV negative (*p* = 0.003). The other organs affected were the central nervous system, intestines, spine and lymph nodes.

TABLE 1 - Sex, age, skin color, alcohol abuse and body weight of 162 patients (HIV positive and negative) with tuberculosis admitted to the Eduardo de Menezes Hospital in Belo Horizonte, Minas Gerais, Brazil, from 2005 to 2007.

Characteristics	Study groups				p
	HIV+ (n=30)		HIV- (n=132)		
	n	%	n	%	
Sex (male)	23	76.7	94	71.2	0.55
Age (median, yr)	36	29-41*	42	33-54	0.03
Skin color					
white	3	10	18	14	
black	2	6.7	11	8	0.56
other	2	7	20	15	
ignored	23	77	83	63	
Alcohol abuse	15	68	68	64	0.99
Body weight (median)	54	48-58*	53	48-60*	0.52

*First and third quartiles

Comorbidities

Twenty one patients presented diabetes mellitus, 17 presented systemic arterial hypertension, 2 presented heart failure and 1 presented chronic renal failure. No relation was observed between these comorbidities and hepatotoxicity.

Hepatotoxicity

Hepatotoxicity I occurred in 23 (77%) out of 30 HIV positive patients and in 60 (45.5%; $p < 0.01$) out of 132 HIV negative patients. Hepatotoxicity II occurred in 6 (20%) out of 30 HIV positive patients and in 12 (9%; $p = 0.107$) out of 132 HIV negative patients. Hepatotoxicity III occurred in 6 (20%) out of 30 HIV positive patients and in 11 (8.3%; $p = 0.09$) out of 132 HIV negative patients.

Liver enzymes

A significant increase in liver enzymes after initiating treatment (ALT, AST, GGT, alkaline phosphatase and total bilirubin) was reported for both groups ($p < 0.01$).

Factors associated to hepatotoxicity

Hepatotoxicity was more frequent in men. Using definition I, alcohol abuse was equally associated with hepatotoxicity in both groups.

Treatment interruption

Antituberculosis treatment was suspended in 22 patients; 7 (23.3%) out 30 were HIV positive and 15 (11.4%) out of 132 were HIV negative ($p = 0.08$). Fourteen (63.6%) out of 22 fulfilled the criteria for toxic hepatitis by definition III. The remaining 8 (36.4%), who did not have hepatotoxicity by definition III, stopped treatment for tuberculosis because they presented symptoms (nausea, vomiting, abdominal pain and hyporexia) which precluded further use of antituberculosis therapy. They presented comorbidities (4 with toxoplasmosis, 1 with cor pulmonale and 3 with CMV infection) and prescribed drugs for these diseases may be the explanation for their complaints.

Table 2 lists the 17 patients with hepatotoxicity III. In 14, treatment was suspended because they developed symptomatic toxic hepatitis, while in 3 asymptomatic individuals, treatment with antituberculosis drugs was maintained and well tolerated.

TABLE 2 - Patients with hepatotoxicity according to definition III*, 3 weeks after treatment for tuberculosis with rifampin, isoniazid and pyrazinamide (HIV positive/negative) at the Eduardo de Menezes Hospital, Belo Horizonte, Minas Gerais, Brazil, 2005-2007.

Patients	ALT (UI/L)	Total bilirubin (mg/dL)	HIV serological status
1	333	2.7	-
2	279	4.8	-
3	205	4.4	+
4	199	3.2	-
5	910	2.9	-
6	260	2.5	-
7	307	3.6	+
8	313	6.3	+
9	382	5.2	-
10	235	2.3	-
11	200	3.6	-
12	882	4.5	-
13	306	2.5	-
14	214	2.2	+
15	277	3.5	+
16	516	4.9	-
17	1,270	5.7	+

*definition III: a 3-fold increase in the upper limit of normal for ALT (65UI/L) plus a 2-fold increase in the upper limit of normal for total bilirubin (2mg/dL).

ALT: alanine-aminotransferase.

DISCUSSION

The frequency of hepatotoxicity caused by rifampin, isoniazid and pyrazinamide varied, in the present study, according to the definition used. For hepatotoxicity I, 77% (23 out of 30) of the HIV-infected group and 45.5% (60 out of 132) of the noninfected group developed toxic hepatitis ($p = 0.002$). For definitions II and III, no statistical difference in toxic hepatitis was observed between HIV-infected and noninfected patients.

The definition of drug induced hepatitis differs in the literature, making comparisons between studies difficult. The World Health Organization (WHO)¹, for example, divides the intensity of hepatitis into four grades: I (slight), ALT ≤ 2.5 times the ULN; II (mild), ALT 2.6-5.0 the ULN; III (moderate), 5-10 times the ULN; and IV (severe), >10 times the ULN. In this study, three patients (cases 5, 12 and 17) presented severe hepatitis according to the WHO definitions (**Table 2**). The American Society for the Study of the Liver states that a three-fold increase in ALT above the ULN plus a two-fold increase in the ULN of total bilirubin and verification of clinical data are necessary to define toxic hepatitis²⁹. For the Brazilian Ministry of Health³⁰, a three-fold increase in ALT and AST, with symptoms or jaundice would be necessary to define hepatotoxicity.

In the recommendations published by the Ministry of Health of Brazil³⁰ prior to 2009, the normal value to be used in the definition of toxic hepatitis were not clearly defined. It simply stated, "... when liver enzymes are 3 times over the normal value". In the present study, when the lower limit of normal for ALT is considered, naturally, the number of patients with hepatotoxicity increases compared to the ULN and an association with HIV infection and alcohol abuse was observed. When using the lower limit of normal, sensitivity increases and specificity decreases.

In the new directives published by the Ministry of Health of Brazil in 2009³¹, hepatotoxicity that occurs after the onset of antituberculosis therapy was defined as an increase in ALT serum levels > 5 times the upper limit of normal (ULN) (with or without jaundice) or jaundice (with or without ALT increase). In Table 2 it is possible to see that only 6 (35.3%) out of the 17 patients listed would fulfill the criterion of ALT > 5 times the ULN.

Eighty-three (51.2%) out of 162 patients in both groups (HIV positive and negative) presented hepatotoxicity when definition I was used. After 15 days, 17 (20.5%) of the 83 developed hepatotoxicity by definition III. This finding is worth mentioning because, once a patient presents hepatotoxicity by definition I, given enough time, 1/5 will develop toxic hepatitis by definition III and, in most cases, treatment for tuberculosis would be suspended.

In the literature, the prevalence of hepatotoxicity to antituberculosis drugs ranges widely from 5% to 27%^{12,32-36}. One possible explanation is that different definitions were used for drug-induced hepatitis³⁷⁻³⁹. Here, hepatotoxicity ranged from 8.3% to 45.5% in the HIV-negative group and from 20% to 77% in the HIV-positive group, depending on the definition applied.

Therefore, depending on the definition of hepatotoxicity, this potentially severe adverse reaction to antituberculosis drugs, may or may not be identified³⁴. Evidence both in favor of^{12,13,15} and against a role for HIV infection in the frequency of toxic hepatitis caused by antituberculosis therapy has been reported^{18,32,38}.

Treatment for tuberculosis was suspended for 22 patients, 14 (63.6%) of whom fulfilled the criteria for toxic hepatitis by definition III. In the remaining 8 (36.4%), who did not have hepatotoxicity according to definition III, treatment for tuberculosis was suspended because they presented symptoms (nausea, vomiting, abdominal pain and hyporexia) that precluded the continued use of the drugs. They presented comorbidities (4 with toxoplasmosis, 1 with *cor pulmonale* and 3 with CMV infection) and the drugs prescribed for these conditions may partially account for their complaints.

Seventeen out of 136 patients had toxic hepatitis according to definition III and in 14, RIP was suspended; however, in three asymptomatic patients, treatment for tuberculosis was maintained (ALT of 205, 199 and 235U/L) and no complications evolved. This confirms the importance of using associated clinical findings (e.g., vomiting, jaundice, nausea, fever) to suspend treatment.

While investigating hepatotoxicity caused by antiretroviral drugs in 868 HIV-infected patients in Africa, Hoffman et al¹³ observed that the frequency of toxic hepatitis was highest in patients concomitantly treated for tuberculosis. Information concerning the concomitant use of antiretroviral drugs and antituberculosis therapy was not possible in the present investigation, because data obtained from the medical records regarding drug interaction were scarce and unreliable.

Seventeen (10.5%) out of 162 patients died during treatment for tuberculosis: two in the HIV-infected group and 15 in the controls ($p = 0.45$). Five (22.7%) out of the 22 patients who did interrupt treatment died thereafter: seven (28.6%) were HIV positive and 15 (20%), HIV negative ($p = 0.66$). Based on previous reports, our hypothesis was that death would be more frequent in HIV-infected individuals¹². Two explanations for our incorrect prediction may be offered: 1) following the introduction of highly active antiretroviral therapy, mortality decreased in HIV-infected patients with tuberculosis, and 2) since tuberculosis is more common in

the HIV-infected group, physicians tend to consider the diagnosis of tuberculosis earlier and treat their patients at once, therefore, resulting in better survival.

It has been well documented that alcohol abuse is associated with higher toxic hepatitis in patients treated with antituberculosis drugs⁴⁰. In this work, alcohol abuse was confirmed as associated with tuberculosis treatment, although toxicity was not increased in the HIV-infected group.

The frequency of toxic hepatitis was not different for definitions II and III (only one patient had hepatotoxicity according to definition II and not III). The difference between definitions II and III is the presence of jaundice in definition III. This kind of result raises the question of whether jaundice is really important in the diagnosis of toxic hepatitis caused by antituberculosis drugs.

To our knowledge, this is the first investigation comparing three distinct definitions of antituberculosis drug-induced hepatotoxicity. Analysis of the findings obtained suggest that the definition of hepatotoxicity outlined by the Brazilian Ministry of Health should be updated and standardized⁴¹.

In conclusion, depending on the definition of toxic hepatitis, HIV infection may or may not be associated with hepatotoxicity. The impact that minor alterations in the definition; i.e., upper or lower limits of normal for ALT and how many times these normal values (2X, 3X, 5X), had on the results is impressive. No death was related to hepatotoxicity. Alcohol abuse is associated with toxic hepatitis, but not with HIV infection and mortality was not higher in the HIV-infected group. Symptoms are important in the definition of toxic hepatitis; however, the emergence of new symptoms following the onset of antituberculosis therapy could not be attributed to hepatotoxicity in over one third of the cases studied.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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