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EPIDEMIOLOGY

Factors associated with anti-TB drug-induced hepatotoxicity and genetic polymorphisms in indigenous and non-indigenous populations in Brazil

Melissa M. Heinrich [a, 1], Verônica M. Zembrzuski [b, 1], Marcos M. Ota [c], Flavia P. Sacchi [d], Raquel L.F. Teixeira [e], Pedro H. Cabello Acero [b, f], Geraldo Marcelo Cunha [a], Reinaldo Souza-Santos [a], Julio Croda [c, g], Paulo C. Basta [a, *]

[a] Escola Nacional de Saúde Pública, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil
[b] Laboratório de Genética Humana, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil
[c] Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brazil
[d] Faculdade de Medicina, Universidade Federal de Mato Grosso do Sul, Campo Grande, MS, Brazil
[e] Laboratório de Biologia Molecular Aplicada a Micobactérias, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz, Rio de Janeiro, RJ, Brazil
[f] Escola de Ciências da Saúde, Universidade do Grande Rio, Duque de Caxias, RJ, Brazil
[g] Fundação Oswaldo Cruz, Mato Grosso do Sul, Campo Grande, MS, Brazil

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SUMMARY

Anti-tuberculosis (TB) drugs are responsible for the occurrence of several adverse drug reactions (ADRs), including hepatotoxicity.

The aim was to estimate the incidence of hepatotoxicity and its association with genetic polymorphisms and clinical-epidemiological factors by comparing indigenous and non-indigenous TB patients.

We investigated clinical-epidemiological variables, serum levels of liver enzymes and *NAT2*, *CYP2E1* and *GSTM1* polymorphisms. A non-conditional logistic regression was used to identify the factors associated with hepatotoxicity. Odds ratios were used as the association measures.

The incidence of hepatotoxicity was 19.7% for all patients. The risk of hepatotoxicity was almost four times higher in indigenous patients, comparing to non-indigenous. We identified a new nonsynonymous single nucleotide polymorphism of *NAT2* in indigenous patients. In total, 54.6% of the patients expressed a slow acetylation phenotype profile. The frequency of the null genotype of *GSTM1* was higher in non-indigenous patients ($p = 0.002$), whereas no significant differences in relation to polymorphisms of *CYP2E1* were observed between the groups. Hepatotoxicity was associated with patients older than 60 and indigenous (OR = 26.0; 95%CI:3.1–217.6; OR = 3.8; 95%CI:1.3–11.1, respectively). Furthermore, hepatotoxicity was associated with a slow acetylation profile in indigenous patients (OR = 10.7; 95% CI:1.2–97.2).

Our findings suggest that there are distinct acetylation profiles in the Brazilian population, emphasizing the importance of pharmacogenetic analyses for achieving personalized therapeutic schemes and better outcomes.

1. Introduction

During the last two decades, there have been significant advances in the control of tuberculosis (TB) in Brazil. These advances can be partially attributed to the reduction in mortality rates, the expansion of directly observed treatment (DOT) [1], and more recently, the increase in new cases detected using rapid molecular diagnostic tests [2]. Despite these important healthcare victories, TB remains one of the principal challenges for Brazilian public health.

Currently, the main challenges in controlling the disease worldwide include a deficit in TB control programs, particularly in developing countries; the concentration of cases in vulnerable

* Corresponding author. Rua Leopoldo Bulhões, 1480, Sala 608, Manguinhos, Rio de Janeiro/RJ, CEP: 21041-210, Brazil. Fax: +55 21 25982610.
E-mail addresses: paulobasta@gmail.com, pcbasta@ensp.fiocruz.br (P.C. Basta).
[1] These authors contributed equally to this work.

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populations, such as the homeless, immigrants, refugees, prisoners, and indigenous populations; and more recently, the growing association with diabetes mellitus [3–10].

Recent studies have shown that the incidence of TB among indigenous populations in various parts of the world is higher than that reported for non-indigenous populations in the same countries [11–15]. In Brazil, the situation is even more critical, due to higher incidence rates of latent and active diseases, elevated concentrations of cases along international borders, emergence of drug resistance and reported patterns of recent and ongoing transmission in the investigated villages [16–21]. Despite the increase of knowledge about TB in vulnerable groups, there is no information available about the development of hepatotoxicity during treatment, acetylation profile and genetic polymorphisms related in Brazilian indigenous populations.

First-line anti-TB drugs are responsible for the occurrence of several adverse drug reactions (ADRs). Generally, hepatotoxicity is the most serious ADR. It occurs during the first 30 days of treatment and can be responsible for the interruption of the therapy and for changes in the therapeutic scheme [22–24]; moreover, it is potentially fatal if diagnosed in an advanced stage [25]. Hepatotoxicity can be detected by monitoring the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) either alone or in association with the manifestation of clinical symptoms such as persistent vomiting, fever, jaundice, and abdominal pain during treatment [25–27].

Isoniazid (INH) is mainly responsible for the occurrence of drug-induced hepatic adverse effects, and the metabolic intermediates of INH appear to be the cause of hepatotoxicity. In the liver, isoniazid is metabolized into acetylisoniazid via the enzyme *N*-acetyltransferase 2 (NAT2) [28–32]. Single nucleotide polymorphisms (SNPs) in the gene that codes for NAT2 are capable of altering the acetylation of this enzyme in the process of isoniazid metabolism and expressing three distinct phenotypes: slow, intermediate, and rapid acetylators [33]. Patients with the slow acetylation phenotype have a higher risk of hepatotoxicity because of their higher blood concentrations of isoniazid and its metabolic intermediates [27]. However, patients with the rapid acetylation phenotype may show plasma concentrations of isoniazid below the minimum inhibitory concentration, which may cause treatment failure [34]. In turn, the effect of the *CYP2E1* and *GSTM1* polymorphisms on the formation of hepatotoxins is still not fully understood.

Although the main mechanisms involved in the development of hepatotoxicity are known, this field has not been significantly explored in Brazil [35,36], particularly in vulnerable populations. Therefore, the objective of this study was to estimate the incidence of hepatotoxicity during TB treatment and to investigate the associations with genetic polymorphisms and clinical and epidemiological factors by comparing indigenous and non-indigenous patients.

2. Materials and methods

2.1. Geographical area and study population

This cross-sectional cohort study was conducted in the municipality of Dourados in Mato Grosso do Sul (MS), a state in the west-central region of Brazil.

According to the last national census, Dourados/MS has a population of 196,035 and covers an area of 4086.235 km^2, including 12,602 indigenous people (Guarani-Kaiowá ethnicity) who reside in the indigenous reserves of Bororó and Jaguapiru, which is the second largest indigenous population in the country [37,38].

The study population included all cases of TB that were included in the Information System for Notifiable Diseases (*Sistema de Informação de Agravos de Notificação*, SINAN) between March 1, 2010, and December 31, 2013, in Dourados/MS.

2.2. Exclusion criteria

We excluded patients who dropped out of treatment within the first 15 days, patients who were transferred to other municipalities, and patients in whom there were changes in diagnosis during the treatment.

2.3. Study design

This study was designed as a longitudinal epidemiological study in which a cohort of patients who received treatment for TB was followed during the period outlined above.

2.4. Data collection and variables

A semi-structured standardized questionnaire was previously used for TB research in Dourados/MS [39,40]. To estimate the abuse of alcohol, we included four questions from the CAGE questionnaire (Cut Down, Annoyed, Guilty and Eye Opener), which was validated in Brazil by Masur and Monteiro [41].

In addition to the primary data collected directly from the study subjects, we consulted the SINAN database to collect clinical and operational information about the cases receiving treatment during the study period.

The following variables were analysed: i) sex; ii) age group (0–19, 20–59, ≥60 years); iii) clinical form of TB (pulmonary, extrapulmonary, mixed); iv) sputum smear and sputum culture (positive, negative, not performed); v) HIV test (positive, negative, not performed); vi) alcohol abuse (yes or no); and vii) race or colour (indigenous versus non-indigenous).

2.5. Sample collection and analysis

At the moment of first contact between the team and the patients, a 5-mL blood sample was collected for the later extraction of genomic DNA. In addition, blood samples were collected at four times during the treatment (0–15, 30, 60, and 180 days) to analyse the levels of the hepatic enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, alkaline phosphatase, gamma GT and albumin, using the Kit Roche Diagnostics (Switzerland) AG COBAS INTEGRA® 400 plus/800. The analyses were performed at the Laboratório de Análises Clínicas do Hospital Universitário da Universidade Federal da Grande Dourados (Clinical Analysis Laboratory of the University Hospital of the Federal University of Grande Dourados).

2.6. Operational classifications

In accordance with national and international criteria [42,43], hepatotoxicity was defined as an increase in liver transaminases (AST and ALT) more than twice the maximum limit of the reference value. The reference values adopted here were up to 40 U/L for both AST and ALT. Individuals who presented with AST and ALT levels more than three times the maximum limit, accompanied by symptoms such as nausea, vomiting, abdominal pain, or jaundice, and individuals presenting with AST and ALT levels more than five times higher than the maximum limit, with or without clinical symptoms, were considered to have drug-induced hepatitis.