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Original Article

Level function levels and oxidative stress markers in patients with multidrug-resistant tuberculosis in the Brazilian Amazon

Níveis da função hepática e marcadores de estresse oxidativo em pacientes com tuberculose multirresistente na Amazônia Brasileira

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Abstract

This study aimed to correlate the values of liver markers with oxidative stress markers in patients with multidrug-resistant tuberculosis in the Brazilian Amazon. A total of 30 patients from the Tuberculosis clinic of a referral hospital were admitted to the study. Whole blood samples were collected for analysis of liver enzyme values and oxidative stress markers by spectrophotometry. The prevalence was male (60%) and the 18-29 age group was the most affected. Patients with multidrug-resistant tuberculosis presented catalase values with a median equal to 6.94 U/gHb and for glutathione, the median was equal to 14.76 µg/ml. As for the values of liver enzymes (AST, ALT, Gamma-GT and Alkaline phosphatase) the patients had medians equal to 60.50 (U/L); 80 (U/L); 54 (U/L); and 100 (U/L) respectively ($p<0.0001$). The results suggest a hepatotoxic effect of the drug, which recommends further studies with a larger number of samples in order to investigate the predictors of liver damage in patients with multidrug-resistant tuberculosis.

Keywords: tuberculosis, multidrug-resistant tuberculosis, oxidation enzymes, liver markers and levofloxacin.

Resumo

Este estudo teve como objetivo correlacionar os valores de marcadores hepáticos com os marcadores de estresse oxidativo em pacientes com tuberculose multirresistente da Amazônia Brasileira. Um total de 30 pacientes do ambulatório de Tuberculose de um hospital de referência foram admitidos no estudo. Realizou-se a coleta de amostras de sangue total para análise dos valores das enzimas hepáticas e dos marcadores de estresse oxidativo por espectrofotometria. A prevalência foi do sexo masculino (60%) e a faixa de 18 a 29 anos foi a mais acometida. Os pacientes com tuberculose multirresistente, apresentaram valores da catalase, com mediana igual a 6,94 U/gHb e para a glutationa, a mediana foi igual a 14,76 µg/ml. Já para os valores das enzimas hepáticas (AST, ALT, Gama-GT e Fosfatase alacalina) os pacientes apresentaram medianas iguais a 60,50 (U/L); 80 (U/L); 54 (U/L); e 100 (U/L) respectivamente ($p<0,0001$). Os resultados sugerem um efeito hepatotóxico do fármaco, o que recomenda estudos adicionais com maior número de amostras a fim de investigar os preditores de dano hepático em pacientes com tuberculose multidroga resistente.

Palavras-chave: tuberculose, tuberculose multirresistente, enzimas oxidação, marcadores hepáticos e levofloxacina.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis*, a pathogenic aerobic bacterium that preferentially settles in the lungs and is basically transmitted through the upper respiratory tract (Ferreira et al., 2024). This microorganism can reach any organ by hematogenous dissemination and when the disease is established, its location, severity and evolution are highly variable (WHO, 2018).

TB is a serious global public health problem. In the world, in 2018, about ten million individuals fell ill and 1.5 million people died from the disease, being considered the main cause of death by a single infectious agent (Resende et al., 2019). The pathology mainly affects males, young adults and low-income countries, indicating a clear association between the incidence of TB and socioeconomic factors (Rodrigues and Mello, 2018). In Brazil, in 2019, 73,864 new cases were diagnosed, which corresponded to an incidence coefficient of 35.0 cases per 100,000 inhabitants. In this scenario, the country has been developing actions aimed at reducing morbidity and mortality from TB. Among them, the assistance provided by high BCG vaccination coverage and increased surveillance and recommendations for investigation and treatment of latent infection by *M. tuberculosis* stand out, with the aim of enhancing prevention actions and reducing illness caused by this pathogen (Santos et al., 2021).

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Chemotherapy is the main way to eliminate the bacillus and cure the patient, which contributes to the control and eradication of the disease. However, there are obstacles in the treatment, which can reduce the cure rates of the disease, as well as provide the emergence of resistant strains, among these, the following stand out: the prolonged treatment time, the lack of information of patients about the disease, adverse drug reactions, social and economic conditions that hinder access to health services, among others (Brasil, 2019). Thus, these factors help to reduce treatment adherence rates. Finally, chemotherapy does not guarantee the total elimination of the bacteria, and it is possible that, despite clinical cure, the bacillus remains in a latent state within macrophages, leading to the emergence of multidrug-resistant strains (Stott et al., 2018).

The quinolone group, especially the fluoroquinolones, has been increasingly studied in the treatment of TB, such drugs have high oral bioavailability and excellent bactericidal activity, since they inhibit bacterial DNA gyrase, which is an enzyme involved in replication processes and transcription of the bacteria's DNA, from the formation of hydrogen bonds with the acceptors present in this molecule. After oral administration, they are widely distributed throughout the body, where they reach the interior of cells, including macrophages, which explains their great activity on intracellular mycobacteria (Alsultan and Peloquin, 2014; Cardona, 2018).

The pharmacological use of levofloxacin, a third-generation quinolone, can cause several adverse reactions during treatment, such as hepatotoxicity, which usually manifests itself in a few cases, however, severe cases are potentially fatal. Risk factors associated with levofloxacin hepatotoxicity are genetic predisposition, age, nutritional status, comorbidities and concomitant exposure to other hepatotoxic agents (Babalik et al., 2013; Jové et al., 2021).

In Brazil, there are few studies referring to liver alterations resulting from the use of levofloxacin in the treatment of TB. Therefore, the present study aims to investigate the levels of liver function markers in patients undergoing treatment for multidrug-resistant tuberculosis (MRTB). In addition, we intend to investigate the consumption of reduced glutathione and catalase activity in these patients, in order to infer about the probable redox imbalance caused by multidrug therapy.

2. Material and Methods

2.1. Casuistry

A prospective study of MRTB patients was carried out from July 2019 to December 2019 at the TB outpatient clinic of the João de Barros Barreto Hospital (HUJBB) of the Federal University of Pará, where 30 patients were being treated for MDR-TB. The HUJBB is a state reference in the treatment of infectious diseases.

The inclusion criteria were patients of both sexes, adults (>18 years old), with a clinical, laboratory and radiological diagnosis of pulmonary TB caused by *Mycobacterium tuberculosis*. Patients who did not have MRTB, reported glucose-6-phosphate dehydrogenase deficiency, positive HIV test, hepatitis A, B, C or E, diabetes mellitus, drug abuse or dependence, including regular use of ethanol and tobacco and who did not use first-line drugs.

A control group formed by individuals with a negative diagnosis for MRTB, matched by gender and age, and who met the same study exclusion criteria, was constituted.

2.2. Treatment and clinical-laboratory follow-up

Patients were treated with a combined daily dose of rifampicin (600 mg), isoniazid (300 mg), streptomycin (500 mg), capreomycin (1000 mg), ethambutol (1100 mg); pyrazinamide (1600 mg), levofloxacin (750 mg), terizidone (750 mg) and ethionamide (750 mg). As determined by the Ministry of Health (Brasil, 2019). Doses were adjusted for the patient's body weight and dispensed in a fixed combined dose in both phases of treatment.

2.3. Ethical statement

The research protocol was reviewed and approved by the Research Ethics Committee of the Institute of Health Sciences at Federal University of Pará, under number 1,591.019. All patients agreed to participate in the study.

2.4. Blood sampling and laboratory analysis

Approximately 10 mL of total blood was collected from the patients by venipuncture. Blood samples were obtained during the intensive phase of treatment and divided into two tubes with a volume of 5 mL, respectively. The first tube, without anticoagulant, was used for biochemical analyzes and the second tube, with anticoagulant (heparin), was used for catalysis and reduced glutathione analyzes (Rivera et al., 2021).

2.5. Determination of liver functions

Samples were centrifuged at 2500 rpm for 5 minutes. Subsequently, the serum was separated and liver function markers were analyzed by UV-visible spectrophotometry following the manufacturer's techniques (Katao®) for aspartate aminotransferase (AST), alanine aminotransferase (ALT), Gamma-GT and alkaline phosphatase. The parameters of normality adopted in the studies followed the Brazilian Society of Clinical Analysis, which stipulates as reference values for Aspartate aminotransferase (AST/TGO) for men up to 37 U/L and for women up to 31 U/L. While for Alanine aminotransferase (ALT/TGP), for men it is up to 41 U/L and for women up to 31 U/L. For Gamma-GT, the reference values for men are from 07 to 60 U/L and for women it is from 05 to 43 U/L. For alkaline phosphatase, the reference values are: men from 40 to 129 U/L and for women from 35 to 104 U/L (Jové et al., 2021)

2.6. Catalase-CAT dosage

The enzymatic activity of catalase was determined by the method of Beutler and West (1984) from the measurement of the decomposition rate of hydrogen peroxide by catalase by spectrophotometry at 240 nm.

The red blood cells were washed three times and, after the third wash, the supernatant was removed and transferred to another tube and 100 µl of red blood cell mash and 300 µl of ice water were added to it and waited for 5 minutes. Subsequently, the hemolysate was transferred to another tube, 3992 µl of Tris buffer solution was added and taken for reading in the spectrophotometer. CAT values were expressed in units per g of hemoglobin (U/gHb). One CAT unit corresponds to the enzyme activity necessary for the consumption of 1µmol in H2O2 in 1 minute.

2.7. Reduced Glutathione Dosage (RGD)

For the measurement of RGD, the Beutler method (1984) was followed, which consists of determining the intracellular values of the reduced form of GSH and is based on the ability of RGD to reduce 5,5-dithiobis-2-nitrobenzoic acid (DTNB) for nitro benzoic acid, which was quantified by spectrophotometry at 412 nm. Initially, a sensitivity curve of the DTNB for known concentrations of RGD (0 to 15 µg/mL) was produced, since the linear line observed in this concentration range allows us to determine the parameters of the calibration curve necessary for the calculation of levels of RGD in the samples.

2.8. Statistical analysis

Data are presented as median (range) and frequency of distribution. For comparisons of qualitative variables, the chi-square test was used and, when necessary, Fisher's exact test. Comparison between markers of liver function and those related to oxidative stress was performed using the Mann-Whitney U test. The accepted significance level was 5%. Biostat 5.0 and Excell software were used.

3. Resuls

A total of 30 patients were included in the study. Males were the most prevalent (60%), with the most affected age group being 18 to 29 years old. A similar gender and age group distribution was adopted for healthy volunteers in the control group. The patients came from the city of Belém and were referred by the Basic Health Units of primary reference or when they needed diagnosis and/or treatment of the disease in its resistant form.

Levels of catalase enzymatic activity were similar between study groups ($p>0.05$). A similar result was observed for reduced glutathione levels between the study groups ($p>0.05$) (Table 1).

All individuals in the control group had levels of liver function markers within the range of normal values. Patients with MRTB had minimum and maximum values equal to 52 to 88; 70 to 89; 44 to 68 and 60 to 109, with a median equal to 60,5; 80; 54; and 100 respectively (Table 2). When the Mann-Whitney test was performed, for two independent samples, we verified that the values of the control groups with the group of MRTB patients for the liver enzymes AST, ALT and Gamma-GT showed significant p values ($p<0.0001$) and for the alkaline phosphatase enzyme the value of $p=0.12$, making it non-significant (Table 2).

Table 1. Catalase and glutathione values in control and MRTB patients.

Control patients	Values of catalase U/gHb	Glutathione values µg/ml	MRTB patients	Catalase values U/gHb	Glutathione values µg/ml
n	30	30	n	30	30
Minimum	8.24	12.01	Minimum	6.50	12.13
Maximum	8.94	18.31	Maximum	8.28	15.77
Median	8.69	15.91	Median	6.94	14.76

Table 2. Values of liver enzymes in control and MRTB patients.

Control patients	AST U/L	ALT U/L	Gamma-GT U/L	Phosph. Alcaline U/L	Patients MRTB	AST U/L	ALT U/L	Gamma-GT U/L	Phosph. Alcaline U/L
n	30	30	30	30	**n**	30	30	30	30
Minimum	18	23	28	54	**Minimum**	57	70	44	60
Maximum	39	36	38	89	**Maximum**	88	69	68	109
Median	28.5	29.5	33.5	73	**Median**	60.5	80	54	100

4. Discussion

MRTB represents a major threat to efforts to control the disease worldwide. The World Health Organization (WHO) has been dedicating itself to maintaining a systematized file of MRTB cases in the world, in addition to outlining objectives to face these problems, such as, for example, adequate treatment of sensitive forms, expansion of networks of rapid testing for timely identification of resistance, ensuring prompt access to treatment for cases of resistance, and preventing transmission along with securing political and financial commitment to TB (Kassa et al., 2016).

In the world, there are reports that show the occurrence of TB unevenly between genders, with men and those of working age (16 to 65 years) being the most affected by the disease (Margarit et al., 2017). In our study, it was possible to observe these same characteristics. These data also corroborate studies by Arbex et al. (2010), David et al. (2018) and Tveden-Nyborg et al. (2021). This fact is justified because this population has a greater exposure to the disease, since the vast majority are the family providers. Linked to this, there is the fact that women have a greater habit of looking for UBS, which would lead to early identification and treatment for the female population (Lima et al., 2019).

The oxidative imbalance of erythrocytes induces lipid peroxidation and membrane instability, and serves as a marker of cellular and tissue damage in the organism, for infectious and non-infectious agents. In tuberculosis, oxidative imbalance can be caused both by the host's immune response and by the use of drugs (Hermann et al., 2016).

Reduced glutathione (RGD) is present in high concentrations in erythrocytes and acts alone or through glutathione peroxidase as the main reducing source to maintain cell integrity (Ketata et al., 2015). RGD levels have been considered useful indicators of oxidative stress in vivo in several diseases. In the present study, RGD levels were slightly lower in patients when compared to the control group, indicating higher consumption of this compound, which can be credited to the greater oxidative imbalance in patients with tuberculosis (Schito et al., 2015). This result was corroborated by the higher catalase activity in the patients. This enzyme is associated with the removal of excess hydrogen peroxide, thus representing a defense mechanism in response to increased oxidative stress (Prasanthi et al., 2015).

Hepatotoxicity is considered the main adverse reaction during the treatment of TB. It is characterized by an increase of more than three times the normal value of alanine aminotransferase and aspartate aminotransferase (Lucena et al., 2019) Concomitantly administered drugs can interact with each other and with other drugs, which increases the risk of liver damage (Uchoa et al., 2019). Most patients undergoing treatment had a temporary and asymptomatic increase in AST and ALT levels, without any clinical manifestation, as well as without the need to interrupt or change the therapeutic regimen (Galvão et al., 2020) drug treatment will occur when the enzyme values reach three times the normal value, with the onset of symptoms, or as soon as jaundice appears, referring the patient to a secondary reference unit for clinical and laboratory follow-up, in addition to adjusting the treatment, if necessary. necessary (Uchoa et al., 2019; Galvão et al., 2020).

In summary, the study showed a predominance of males, aged between 18 and 29 years old. There was a significant increase in liver function markers (AST, ALT and Gamma-GT) in patients being treated for TB, however, oxidative stress markers indicated a non-significant oxidative imbalance in patients with multidrug-resistant tuberculosis.

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