Correlation between inflammatory mediators and biochemical markers in patients with active pulmonary tuberculosis

Danyelle Cristina Pereira Santos[1], Amanda Caroline de Souza Sales[1], Douglas Henrique dos Santos Silva[1], Érika Alves da Fonseca Amorim[1], Silvana Jozie Assunção Braga Bacelar Lobato[1], Eduardo Martins de Sousa[2], Luís Cláudio Nascimento da Silva[1], Adrielle Zagmignan[1]

ABSTRACT

Objective: Correlate inflammatory mediators and biochemical parameters in patients with active pulmonary tuberculosis (TB) treated at a public hospital in São Luís, MA. **Methods**: This is a case-control study of patients with a positive diagnosis of active pulmonary TB. Serum samples from patients and the control group were collected for the clinical trials, and epidemiological data were collected through medical records and interviews. The control group consisted of healthy volunteers with no previous contact with TB cases, matched by age and sex to the clinical group. To measure inflammatory cytokines, we used the Human IL-6 ELISA Set and Human IFN-γ ELISA Set kits. Oxidative stress was measured by quantification of thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO). In biochemistry, the levels of uric acid, anti-streptolysin "O" (AEO), alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), calcium, total cholesterol, gamma-glutamyl transferase (Gamma GT), glucose, alkaline phosphatase, high-density lipoprotein (HDL), C-reactive protein (CRP) and triglycerides were measured. **Results**: The clinical group consisted of 53 patients. There was a substantial decrease in IFN-γ ($p<0.0001$) and a significant increase in IL-6 ($p<0.0001$). TBARS production increased significantly ($p= 0.0414$). There was no significant difference in NO production ($p= 0.3194$). In biochemistry, there was a significant increase in ALT ($p= 0.0072$), AST ($p= 0.0016$), Gamma GT ($p= 0.0011$), alkaline phosphatase ($p<0.0001$), CRP ($p<0. .0001$) and triglycerides ($p= 0.0343$), and a significant decrease in calcium ($p<0.0001$). A significant positive correlation was found between IL-6 and IFN-γ ($p= 0.0448$), as well as AST and ALT ($p<0.0001$); CRP and gamma GT ($p<0.0001$); Gamma GT and ALT ($p= 0.0016$); Gamma GT and AST ($p=0.0004$); triglycerides and cholesterol ($p= 0.0002$); alkaline phosphatase and gamma GT ($p<0.0001$); CRP and alkaline phosphatase ($p<0.0001$); triglycerides and calcium ($p= 0.0121$); cholesterol and calcium ($p= 0.0261$); glucose and cholesterol ($p= 0.0373$); and triglycerides and glucose ($p= 0.0127$) in biochemistry, with a significant negative correlation between glucose and uric acid ($p= 0.0092$); and CRP and HDL ($p=0.0037$). The correlation between inflammatory mediators and biochemical markers was positive between IL-6 and gamma GT ($p= 0.0011$); IL-6 and CRP ($p<0.0001$); IL-6 and alkaline phosphatase ($p=0.0076$); and NO and triglycerides ($p= 0.0016$), and significant negative correlation between IFN-γ and cholesterol ($p= 0.0171$) and TBARS and cholesterol ($p= 0.0138$). **Conclusion**: Immunosuppression of IFN-γ activity was observed. A correlation was found between IL-6 and inflammatory biochemical markers, indicating damage and injury caused by *M. tuberculosis*.

Keywords: Biochemistry, Cytokines, Inflammation mediators, Pulmonary tuberculosis**.**

INTRODUCTION

Tuberculosis (TB) is an infectious and contagious disease that is the focus of concern for world health authorities. It is caused by bacteria of the genus *Mycobacterium* spp., mainly *Mycobacterium tuberculosis*, and, despite all the advances in terms of improvements in health systems and wide implementation of control programs, TB remains one of the greatest threats to public health[1-2].

According to the World Health Organization (WHO), every year, about 10 million people are affected by TB, leading to approximately 1.6 million deaths. A quarter of the world's population is estimated to be infected with *M. tuberculosis*, and 5-15% of this estimate will develop active TB. In Brazil, in 2021, 88 thousand cases of TB

[1] Universidade Ceuma.Laboratório de Patogenicidade Microbiana, São Luís, (MA), Brasil.
[2] Universidade Ceuma. Laboratório Virologia, São Luís, (MA), Brasil.

https://doi.org/10.11606/issn.2176-7262.rmrp.2023.208110

were reported, of which 29% were women and 68% were men aged over 15 years. The incidence rate is 48 people per 100 thousand inhabitants, with mortality in 2.8 cases in 100 thousand inhabitants. Universal health coverage and social protection for TB treatment in 2021 was 76% of cases[2]. In Maranhão, 2,885 cases were registered in the same period, with 1,364 notifications only in the capital São Luís[3].

Belonging to a group called *Mycobacterium tuberculosis* Complex (MTBC), the mycobacteria that cause TB are bacteria of the Mycobacteriaceae family, non-spore-forming, aerobic, non-motile bacilli with a slightly curved shape, which presents ramifications in their complex cell wall of mycolic acids and makes it difficult to its permeability. This decrease in permeability characterizes these microorganisms as acid-alcohol resistant, identified by specific stainings, such as *Ziehl-Neelsen* since they cannot be classified according to Gram staining[4].

The pathophysiology of human TB caused by MTBC, especially *M. tuberculosis*, is a complex interaction process between the host's immune system and bacterial factors. The pathogenesis of *M. tuberculosis* derives from its ability to manipulate macrophages, granulomas, and host cell metabolism, with evolutionary adaptations of transmissibility and evasion of the immune system[5].

Pathogenesis begins with inhaling airborne infectious particles containing droplets of *Mycobacterium* sp. bacilli from an individual with active TB. Once in the lung alveoli, mycobacteria are phagocytosed by phagocytes, which serve as a primary niche favorable for the multiplication of *M. tuberculosis*, which has immunoevasion mechanisms to resist the action of microbicidal agents produced in the phagolysosome, such as reactive oxygen species (ROS)[6].

The innate and adaptive immune systems work together to contain and eliminate mycobacteria. In the innate response, different signaling pathways, such as the STING pathway (stimulator of IFN genes), induce the production of signaling interleukins from Natural Killer (NK) cells such as interleukin 12 (IL-12) and interleukin 23 (IL-23). Activated NK cells produce interferon-gamma (IFN-γ), which induces the superactivation of phagocytes for the killing of phagocytosed mycobacteria. In adaptive immunity, the cellular response mediated by T cells acts by inducing: a) bacterial killing by the secretion of IFN-γ by CD4+ T cells differentiated into Th1 effectors, under the influence of IL-12/IL-23, resulting in the activation of phagocytes; b) cell killing by cytotoxic T CD8+ cells, eliminating mycobacteria that escape phagocytic vesicle[5-7].

Intracellular bacteria resistant to phagocytic killing can cause chronic and persistent activation of macrophages and T cells, leading to tissue damage and the formation of granulomas surrounding the mycobacteria. This granuloma can contain the infection but can cause loss of function associated with tissue necrosis and fibrosis, clinical characteristics of TB. A failure in this containment system, mainly associated with immunosuppression, can cause the release of *M. tuberculosis* into the extracellular environment with primary activation/reactivation of TB[8-9].

The high incidence, morbidity, and mortality rates portray the enormous impact of human TB infection. Thus, understanding the factors associated with the immune response against *M. tuberculosis* is essential for creating a patient profile and, consequently, developing control and treatment strategies. Therefore, this study aimed to correlate inflammatory mediators and biochemical parameters in patients with active pulmonary TB treated at a public hospital in São Luís, MA.

METHODS

Study population and data collection

This case-control study collects clinical samples from patients with a positive diagnosis of active pulmonary TB. The sampling was for convenience, collected at a referral hospital for the diagnosis of TB in São Luís, MA, from January 2021 to August 2022, upon approval and signing of the free informed consent form by the volunteers (CEP No. 4.657.164).

Clinical-epidemiological data were collected through interviews and patients' medical records. The variables analyzed to characterize the clinical group were sex, age, the form of diagnosis, signs, and symptoms, previous vaccination with BCG vaccine (*Bacillus Calmette-Guérin*),

comorbidities, co-infection with the human immunodeficiency virus (HIV), alcoholism, smoking, in addition to of weight and height to stipulate the body mass index (BMI). For serum assessments, serum samples were collected.

As inclusion criteria, patients diagnosed with active pulmonary TB, who had not yet started treatment, with characteristic symptoms, suggestive chest X-ray, positive bacilloscopy, and/or rapid molecular test for TB (TRM-TB) were selected. Patients under treatment with incomplete clinical-epidemiological data, who did not allow the collection of serum samples for analysis and/or refused to sign the free informed consent form, were excluded.

For the formation of the control group, healthy volunteers were recruited if they had no previous contact with TB cases, without any clinical manifestation, in addition to meeting the criteria of age and sex-matched to the clinical group. Volunteers who used immunosuppressants or pregnant women were excluded.

Serum processing of clinical samples and control group

The blood samples were collected from patients and healthy volunteers in vacuum blood collection tubes without anticoagulant and with separator gel VACUETTE® (Greiner Bio-one, Kremsmuster, Austria). The material was sent to the Laboratory of Microbial Pathogenicity at CEUMA University. The tubes with the coagulated samples were centrifuged at 1,500 rpm for 10 minutes to separate the serum. Aliquots of approximately 500 µL were prepared in microtubes (KASVI, PR, Brazil) and stored at -80 ºC for conservation and future analysis.

Analysis of serum levels of inflammatory cytokines (IFN-γ and IL-6)

To measure serum levels of inflammatory cytokines, the Human IFN-γ ELISA Set and Human IL-6 ELISA Set kits (BD Biosciences, Oxford, UK) were used, following the manufacturer's protocols.

Analysis of markers for oxidative stress

Quantification of thiobarbituric acid reactive species – TBARS

For the assessment of TBARS, the methodology by Chaves et al.[10] was used, with modifications. In microtubes of 2 mL (KASVI, PR, Brazil), 50 µL of serum from the clinical or control group, 500 µl of thiobarbituric acid 1% (v/v), 5 µl of sodium hydroxide 10 M (w/v), and 250 µl of phosphoric acid 20% (v/v) were added. The solutions were incubated in a boiling water bath for 15 minutes. After this period, the tubes were cooled to room temperature. 1 mL of absolute butyl alcohol was added, and the tubes were vortexed for 1 minute. Samples were centrifuged at 1,500 rpm for 15 to 20 minutes. Finally, 200 µL of the supernatant was removed and added to a 96-well microplate (KASVI, PR, Brazil) and read in a spectrophotometer at 535 nm. To measure the TBARS concentration value, the methodology according to Buege and Aust[11] was used, with an extinction coefficient (molar absorptivity) of 1.53 $mM^{-1}cm^{-1}$.

Quantification of nitric oxide (NO)

For the NO assessment, the methodology by Griess[12] and Wang et al.[13] was used with modifications. The Griess reagent was used, prepared in equal parts of 1% sulfanilamide (reagent A) (w/v) and 0.1% N-1-naphthyl-ethylenediamine (reagent B) (w/v), diluted in acid 2.5% phosphoric (v/v). In a 96-well plate (KASVI, PR, Brazil), 50 µL of clinical and control group serum and 50 µL of Griess reagent were added. The standard curve was created from the microdilution (1:2) of 1 nM/mL sodium nitrite. The microplate was left to rest for 15 minutes in a dark environment. After resting, the result was read in a spectrophotometer at 535 nm.

Analysis of biochemical markers

The programmed automatic analyzer LABMAX Plenno (Labtest, MG, Brazil) was used to evaluate all biochemical markers. Serum levels of uric acid, anti-streptolysin-O (AEO), alanine aminotransferase (ALT), amylase, aspartate aminotransferase (AST), calcium, total cholesterol, gamma-glutamyl transferase (Gamma GT),

glucose, alkaline phosphatase, high-density lipoprotein (HDL), C-reactive protein (CRP) and triglycerides were measured. The protocol followed the manufacturer's recommendations.

Statistical analysis

The GraphPad Prism 8 software (San Diego, CA, USA) was used for statistical analysis. The characterization of the standard curve and concentration of parameters was performed using linear regression. A comparison of study markers between the case group and the control group was performed using Student's t-test. The analysis of the correlation between the evaluated parameters was performed using the Pearson correlation r, classified as positive ($r > 0$), negative ($r < 0$), or null ($r = 0$), in addition to weak ($0 < r \leq 0.39$), moderate ($0.4 \leq r \leq 0.69$) and strong ($r \geq 0.7$), following the classification proposed by Baba, Vaz and da Costa[14], with adaptations. The result was considered statistically significant when $p<0.05$.

RESULTS

Clinical-epidemiological aspects of the study group

The clinical study group consisted of 53 patients, 40 male (75.47%), and 13 female (24.53%), with a mean age of 37±13.36 years. The most common diagnostic test was imaging, performed by 51 patients (96.23%). The main symptoms presented were cough [47 cases (88.68%)], weight loss [46 cases (86.79%)], and fever [42 cases (79.25%)]. Regarding the BCG vaccine, 45 patients (84.91%) had a positive sign for previous vaccination (Table 1).

Addressing the risk factors of the patients treated, 12 people had some comorbidity (22.64%), with diabetes mellitus being the most reported [9 cases (16.98%)]. None of the analyzed patients tested positive for HIV co-infection. It was that written 24 patients (45.28%) declared themselves alcoholics (active and frequent consumption of alcoholic beverages), while 18 patients (33.96%) reported the habit of smoking. Regarding BMI, an average of 20.42±3.54 kg/m² was found, with the majority being classified as having normal weight [34 cases (64.15%)], while 13 cases were underweighted (24 .53%) and 6 cases were overweight (11.32%) (Table 1).

Analysis of serum levels of inflammatory cytokines (IFN-γ and IL-6)

Regarding the levels of inflammatory mediators in the clinical group, when compared to the control group, there was a significant decrease ($p<0.0001$) in the production of the cytokine IFN-γ (Figure 1A). In contrast, the production of IL-6 increased significantly ($p<0.0001$) compared to healthy volunteers (Figure 1B).

Analysis of markers for oxidative stress

When analyzing the quantification of TBARS production, a significant increase in oxidative stress was observed in patients with active TB compared to the control group ($p= 0.0414$) (Figure 2A). However, when evaluated using the NO quantification method, there was no significant difference between groups ($p= 0.3194$) (Figure 2B).

Analysis of biochemical markers

When biochemical parameters were evaluated and compared to the control group (Figure 3), the markers that showed a significant increase in production were ALT ($p= 0.0072$) (Figure 3C), AST ($p= 0.0016$) (Figure 3E), gamma GT ($p= 0.0011$) (Figure 3H), alkaline phosphatase ($p<0.0001$) (Figure 3J), CRP ($p<0.0001$) (Figure 3L) and triglycerides ($p= 0.0343$) (Figure 3M). Furthermore, a significant decrease ($p<0.0001$) in serum calcium levels was observed in the clinical group (Figure 3F).

Correlation between the different groups of parameters analyzed

Correlation analysis allows checking the degree of linear statistical dependence between

Table 1

Clinical and epidemiological aspects of patients with active pulmonary tuberculosis treated at a public hospital, São Luís, Maranhão, Brazil (2022).

Variables	n	%	Mean±SD
Sex			
Male	40	75,47	
Female	13	24,53	
Age			37±13,36
18-25	11	20,75	
26-33	13	24,53	
34-41	9	16,98	
42-49	8	15,09	
≥50	12	22,64	
Form of diagnosis			
Imaging exam	51	96,23	
Baciloscopy	29	54,72	
TRM-TB	24	45,28	
Culture	1	1,89	
Signs and symptoms			
Cough	47	88,68	
Weight loss	46	86,79	
Fever	42	79,25	
Weakness	29	54,72	
Loss of appetite	27	50,94	
Malaise	23	43,40	
Inappetence	13	24,53	
Hemoptysis	12	22,64	
Allergy	2	3,77	
BCG vaccination			
Vaccinated	45	84,91	
Not vaccinated	8	15,09	
Comorbidities			
With comorbidity	12	22,64	
Diabetes mellitus	9	16,98	
COPD	1	1,89	
Hypertension	1	1,89	
Colitis	1	1,89	
Rhinitis	1	1,89	
Gastritis	1	1,89	
No comorbidity	41	77,36	
HIV			
Negative	53	100	
Positive	-	-	
Alcoholism			
Yes	24	45,28	
No	29	54,72	
Smoking			
Yes	18	33,96	
No	35	66,04	
BMI (kg/m²)			20,42±3,54
Normal weight	34	64,15	
Underweight	13	24,53	
Overweight	6	11,32	

Legend - TB: Tuberculosis; n: Absolute count; %: Percent count; BCG: Bacillus Calmette-Guérin; SD: Standard Deviation; TRM-TB: Rapid molecular test for tuberculosis; COPD: Chronic Obstructive Pulmonary Disease.

different variables, that is, the affinity between different types of parameters. Inflammatory, biochemical, and oxidative stress mediators were contrasted to assess this correlation of parameters in the clinical group, with the results shown below in Figure 4.

When analyzing the correlation between cytokine measurements, a weak positive correlation (r= 0.2767) is observed between IL-6 and IFN-γ, that is, the increase in IL-6 production may be related to the increase of IFN-γ production, being directly proportional, even though it is a weak but significant relationship (p= 0.0448).

Regarding the biochemical parameters, it is estimated that the correlation can be observed in a strong positive way between AST and ALT (r= 0.7608; p<0.0001); and CRP and gamma GT (r=0.7029; p<0.0001). The moderate positive correlations were: gamma GT and ALT (r= 0.4232, p= 0.0016); gamma GT and AST (r= 0.4698, p= 0.0004); triglycerides and cholesterol (r= 0.4838; p= 0.0002); alkaline phosphatase and gamma GT (r= 0.6737; p<0.0001); and CPR and alkaline phosphatase (r=0.5578, p<0.0001). Of the weak positive correlations, the significant ones were: triglycerides and calcium (r= 0.3422, p= 0.0121); cholesterol and

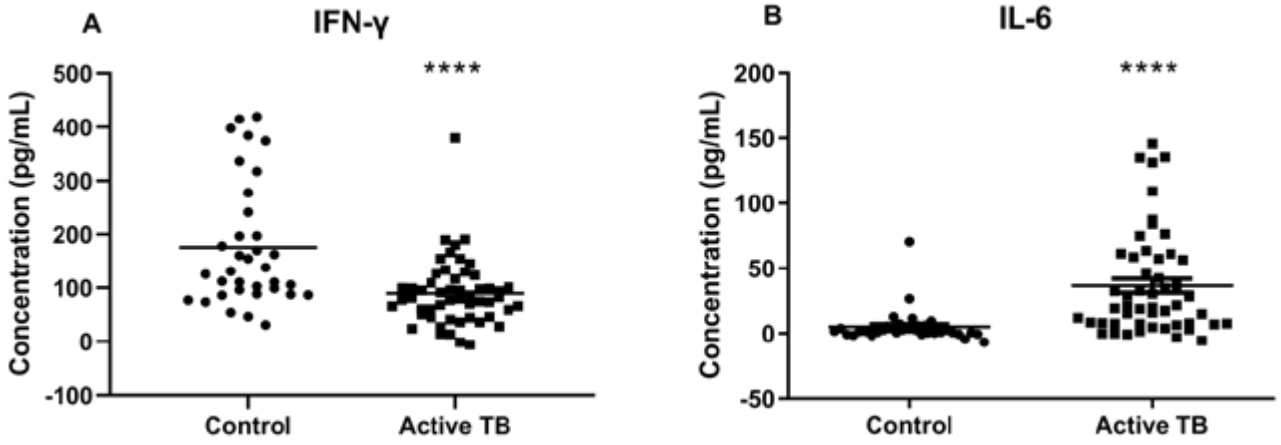

Figure 1: Analysis of inflammatory cytokines in patients with active pulmonary TB treated at a public hospital, São Luís, Maranhão, Brazil (2022). A - Comparison of IFN-γ serum levels between the active TB group and the control group (*p<0.0001); B - Comparison of IL-6 serum levels between the active TB group and the control group (*p<0.0001). Analysis performed using the Student's t-test. IFN-γ: Interferon gamma; IL-6: Interleukin 6; TB: Tuberculosis; *: Significance (p<0.05).

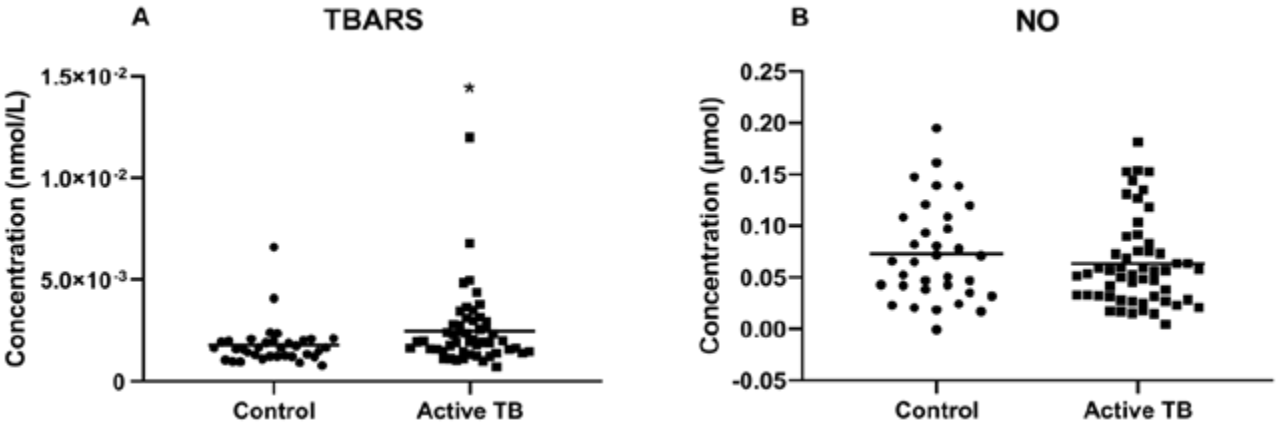

Figure 2: Oxidative stress analysis of patients with active pulmonary TB treated at a public hospital, São Luís, Maranhão, Brazil (2022). A - Comparison of TBARS serum levels between the active TB group and the control group (*p= 0.0414); B - Comparison of NO serum levels between the active TB group and the control group (p= 0.3194). Analysis performed using the Student's t-test. TBARS: Thiobarbituric acid reactive substances; TB: Tuberculosis; NO: Nitric Oxide. *: Significance (p<0.05).

calcium (r= 0.3055; p= 0.0261); glucose and cholesterol (r= 0.2868; p= 0.0373); and triglycerides and glucose (r= 0.3402; p= 0.0127). Regarding weak negative correlations, the following was detected: glucose and uric acid (r= -0.3543; p= 0.0092); and CRP and HDL (r= -0.3918; p= 0.0037).

Exposing the correlation between inflammatory mediators and biochemical markers evaluated, different results are found. Serum IL-6 levels demonstrate a significant moderate positive correlation with GT gamma (r= 0.4373; p= 0.0011) and CRP (r= 0.6893; p<0.0001), in addition of

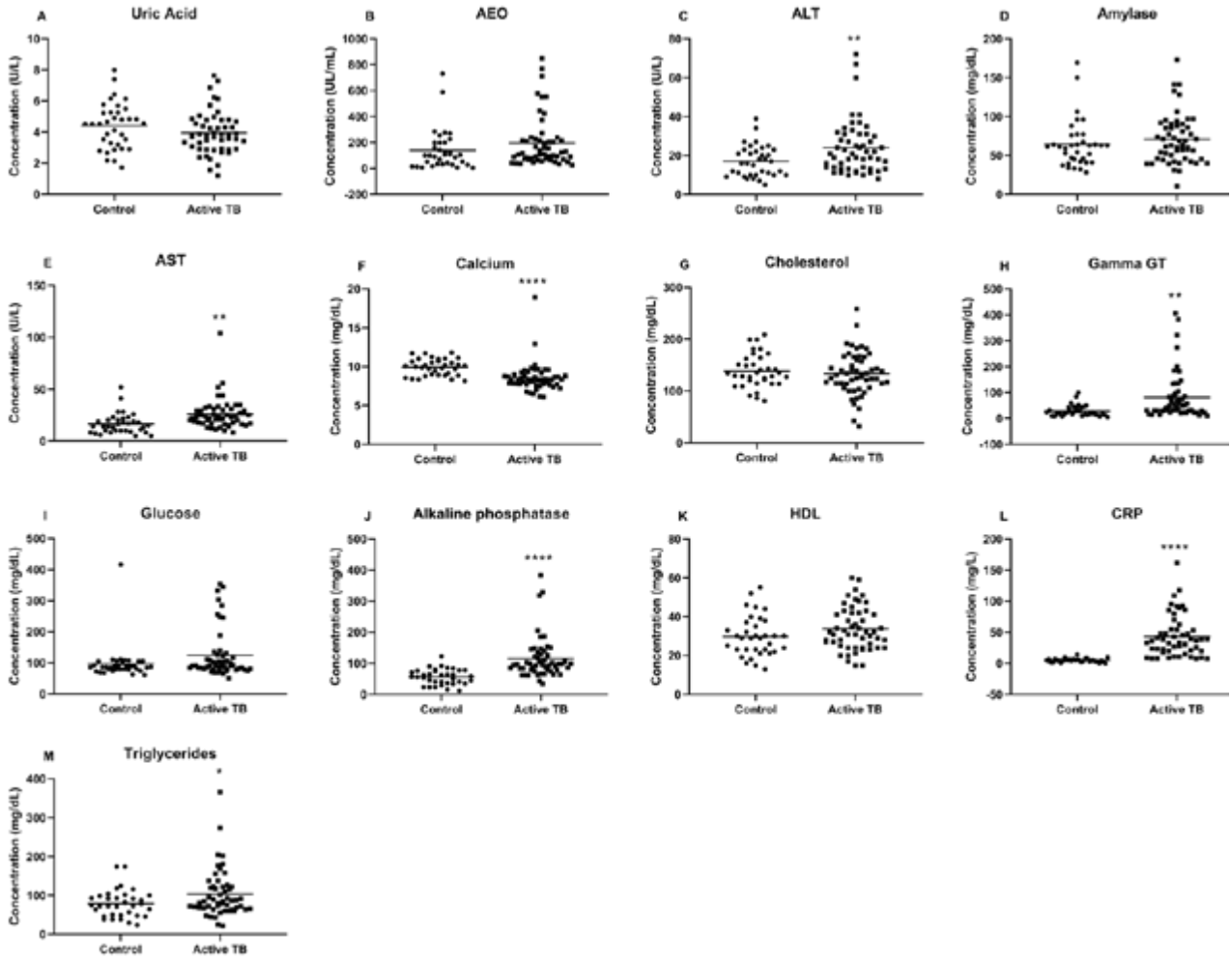

Figure 3: Analysis of biochemical markers of patients with active pulmonary TB treated at a public hospital, São Luís, Maranhão, Brazil (2022). A - Comparison of uric acid serum levels between the active TB group and the control group (p= 0.1382); B - Comparison of AEO serum levels between the active TB group and the control group (p= 0.1438); C - Comparison of ALT serum levels between the active TB group and the control group (*p= 0.0072); D - Comparison of amylase serum levels between the active TB group and the control group (p= 0.3542); E - Comparison of AST serum levels between the active TB group and the control group (*p= 0.0016); F - Comparison of calcium serum levels between the active TB group and the control group (*p<0.0001); G - Comparison of cholesterol serum levels between the active TB group and the control group (p= 0.5326); H - Comparison of GT gamma serum levels between the active TB group and the control group (*p= 0.0011); I - Comparison of glucose serum levels between the active TB group and the control group (p= 0.0761); J - Comparison of alkaline phosphatase serum levels between the active TB group and the control group (*p<0.0001); K - Comparison of HDL serum levels between the active TB group and the control group (p= 0.1057); L - Comparison of CRP serum levels between the active TB group and the control group (p<0.0001); M - Comparison of triglyceride serum levels between the active TB group and the control group (*p= 0.0343). Analysis performed using the Student's t-test. TB: Tuberculosis; AEO: Anti-streptolysin-O; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Gamma GT: Gamma glutamyl transferase; HDL: High Density Lipoprotein; CRP: C-reactive protein; *: Significance (p<0.05).

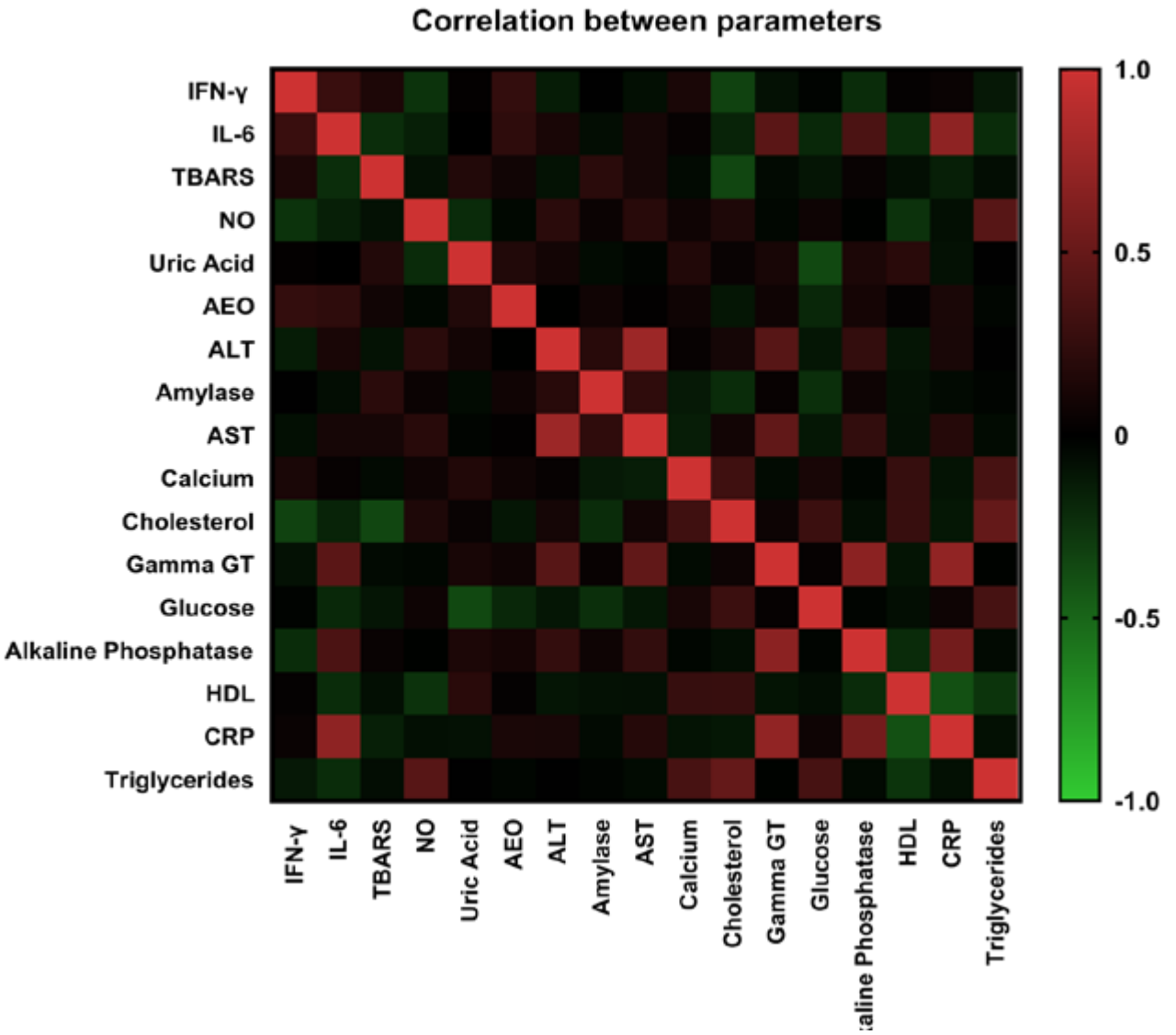

Figure 4: Analysis of the correlation between inflammatory mediators and biochemical markers in the group of patients with active pulmonary TB treated at a public hospital, São Luís, Maranhão, Brazil (2022). IL-6 and IFN-γ: weak positive (r= 0.2767; *p= 0.0448); TBARS and NO: weak negative (r= -0.0873; p= 0.5426); IL-6 and TBARS: weak negative (r= -0.2228; p= 0.1161); IFN-γ and TBARS: weak positive (r= 0.1378; p= 0.3350); IL-6 and NO: weak negative (r= -0.1533; p= 0.2730); IFN-γ and NO: weak negative (r= -0.2502; p= 0.0708); AST and ALT: strong positive (r= 0.7608; *p<0.0001); CRP and Gamma GT: strong positive (r= 0.7029; *p<0.0001); Gamma GT and ALT: moderate positive (r= 0.4232, *p= 0.0016); Gamma GT and AST: moderate positive (r= 0.4698, *p= 0.0004); Triglycerides and cholesterol: moderately positive (r= 0.4838; *p= 0.0002); Alkaline phosphatase and Gamma GT: moderate positive (r= 0.6737; *p<0.0001); CRP and alkaline phosphatase: moderate positive (r= 0.5578; *p<0.0001); Triglycerides and calcium: weak positive (r= 0.3422; *p= 0.0121); Cholesterol and calcium: weak positive (r= 0.3055; *p= 0.0261); Glucose and cholesterol: weak positive (r= 0.2868; *p= 0.0373); Triglycerides and glucose: weak positive (r= 0.3402; *p= 0.0127); Glucose and uric acid: weak negative (r= -0.3543; *p= 0.0092); CRP and HDL: weak negative (r= -0.3918; *p= 0.0037); IL-6 and Gamma GT: moderate positive (r= 0.4373; *p= 0.0011); IL-6 and CRP: moderately positive (r= 0.6893; *p<0.0001); IL-6 and alkaline phosphatase: weak positive (r= 0.3629; *p= 0.0076); IFN-γ and cholesterol: weak negative (r= -0.3262; *p= 0.0171); TBARS and cholesterol: weak negative (r= -0.3427; *p= 0.0138); NO and triglycerides: moderately positive (r= 0.4239; *p= 0.0016). Analysis performed using the Pearson Correlation r. IL-6: Interleukin 6; IFN-γ: Interferon-gamma; TBARS: Thiobarbituric acid reactive substances; NO: Nitric Oxide; AEO: Anti-streptolysin-O; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Gamma GT: Gamma glutamyl transferase; HDL: High Density Lipoprotein; CRP: C-Reactive Protein; *: significance (p<0.05).

a significant weak positive correlation with alkaline phosphatase (r= 0.3629; p= 0.0076).. Meanwhile, serum IFN-γ levels showed a weakly significant negative correlation with cholesterol levels (r= -0.3262; p= 0.0171), with no further inflammatory associations.

A non-significant weak negative relationship (r= -0.0873, p= 0.5426) between TBARS production and NO levels was observed when evaluating the correlation between oxidative stress markers. Contrasting with biochemical mediators, a significant weak negative correlation was found between TBARS and cholesterol levels (r= -0.3427; p= 0.0138). Compared to NO levels, only triglyceride serum levels showed a moderately significant positive correlation (r= 0.4239; p= 0.0016).

Evaluating a correlation between inflammatory mediators and markers of oxidative stress, no significant correlation was observed between IL-6 and TBARS quantification (r= -0.2228; p= 0.1161), being classified as weak negative, whereas, contrasted with IFN-γ, showed a non-significant weak positive correlation (r= 0.1378; p= 0.3350). The same non-significant weak negative correlation was found between IL-6 and NO (r= -0.1533; p= 0.2730) and IFN-γ and NO (r= -0.2502; p= 0.0708).

DISCUSSION

The IFN-γ and interleukin 6 (IL-6) are examples of cytokines active during establishing the immune response against TB. The IFN-γ, belonging to the type II interferon class, is one of the mediators of macrophage activation for the bacterial killing of phagocytosed mycobacteria and the fight against intracellular infections. Meanwhile, IL-6 is an essential acute-phase immune inducer, produced by tissue stimuli in response to damage or stress, such as irradiation, ROS, infections, and other pro-inflammatory cytokines[7].

During the bacterial killing process, cytokines, especially IFN-γ, induce increased production of ROS by phagocytes, such as NO, for microbicidal activity within the phagolysosome and consequent inhibition of respiration and growth of *M. tuberculosis*. This increase in the production of free radicals, if uncontrolled, favors cellular damage both in the mycobacteria and the host cell through different processes of oxidative stress, promoting tissue injury, inflammation, increased intracellular calcium ions, and lipid peroxidation of the membranes, which can be measured by quantification of TBARS[15-18].

By understanding this cycle of cytokine production and the promotion of oxidative stress, it is possible to evaluate the results of this study on the expression of each marker in patients with active pulmonary TB, in addition to the correlation between them.

The patients evaluated with active pulmonary TB had low production of IFN-γ and high production of IL-6, with a weak positive correlation between these cytokines. Furthermore, the results on oxidative stress were divergent: there was an increase in the estimated quantification of TBARS, indicating lipid peroxidation of membranes, while NO levels remained at the baseline level, contradicting the expected increase during infection by mycobacteria.

No significant correlations were observed between the measurement of cytokines and oxidative stress as an effect of the low level of IFN-γ production, the main cytokine active in the induction of ROS production. These results may be associated with immunosuppression of the IFN-γ-mediated response, an essential factor for establishing TB in its active form.

It has already been reported that IFN-γ plasma levels are higher in patients with advanced TB, with a higher intracellular bacterial load and recurrent macrophage activation, and that they decrease after starting treatment. Furthermore, a high burden of IFN-γ is associated with an increased risk of progression to active TB due to the exacerbated inflammatory process. A failure in mediation by this cytokine may favor resistance to infection by *M. tuberculosis*[19-20].

Studies indicate that the susceptibility to the development of TB in its active form is linked to genetic predisposition. Mendelian susceptibility to mycobacterial disease (MSMD) is a rare immunodeficiency of the response mediated by IFN-γ or complementary factors, leading to increased susceptibility to infections caused by mycobacteria with different degrees of virulence – from

non-tuberculous mycobacteria (BCG, for example) to the classic tuberculosis agent, *M. tuberculosis*. MSMD patients may be predisposed to infections by other intracellular agents such as *Salmonella* spp. and virus[20-21].

The severity of the disease and the onset of clinical signs depend on the degree of impairment in the IFN-γ pathway, which can be classified as complete when the patient has disseminated infections in early childhood; or partial, when only mild to moderate infections may occur in adolescence or adulthood[22].

Of the nine genetic factors already associated with MSMD, seven are autosomal hereditary, in the genes for IFN-γ receptor chain 1 (IFNGR1), IFN-γ receptor chain 2 (IFNGR2), signal transducers and transcription activation 1 (STAT1), p40 subunit common to IL-12 and IL-23 (IL12B), β1 chain common to IL-12 and IL-23 receptors (IL-12RB1), IFN-γ inducing molecule in synergism with IL -12 (ISG15), and IFN-γ-inducible transcription factor (IRF8). The other two mutations are linked to the X chromosome, being in the nuclear factor kappa B (NEMO) and cytochrome B (CYBB) modulator gene[20,23].

In addition to predisposition, the pathophysiology of *M. tuberculosis* is mainly characterized by immunosuppression of the IFN-γ response based on the genetic factors of MSMD[5]. There is evidence that *M. tuberculosis* can hinder the response of macrophages to IFN-γ by increasing the secretion of IL-6 by infected macrophages, which inhibits the expression of a subset of IFN-γ-responsive genes, at the transcriptional level, without inhibiting activation or function of STAT1. The IL-6 is a cytokine responsible for suppressing the Th1 response, which IFN-γ is associated with. This process results in the inability of the immune system to eradicate the infection[24-25].

Thus, it is possible to notice several mechanisms linked directly or indirectly to the activity of IFN-γ, such as the production of NO, which can hinder the immune response against intracellular bacteria, favoring immunosuppression and corroborating the low expression of IFN-γ by patients of the study.

Furthermore, this study aims to evaluate the correlation between these inflammatory markers and the biochemical markers of patients with active pulmonary TB. A positive correlation was found between IL-6 levels and inflammatory biochemical markers, particularly gamma GT, CRP, and alkaline phosphatase. IFN-γ immunosuppression did not allow a correlation of this cytokine with biochemical markers of interest for the research.

In the literature, few studies assess the correlation of immunological and biochemical markers in patients with TB. *In vitro* studies indicate that mononuclear phagocytes potentiate IL-6 secretion in the face of *M. tuberculosis* infection and its virulence components. This increase in the pro-inflammatory cytokine directly influences many inflammatory biochemical markers, especially those in the acute phase of the liver, such as CRP, gamma GT, and alkaline phosphatase[26-27].

It was possible to observe that the group of patients with active pulmonary TB showed significant alterations in inflammatory markers, in the case of ALT, AST, gamma GT, alkaline phosphatase, and, mainly, CRP, in addition to significant alterations of a nutritional nature, demonstrated by the increase in the triglycerides level and decrease in calcium level.

It is common for tuberculous inflammation to cause biochemical changes in biological fluids, and it is expected that the immune response would trigger an increase in biochemical markers of inflammation, such as CRP. Alteration in liver markers may show pre-existing pathologies, even considering the possibility of having alcoholic and smoking habits by the patients treated[28-29].

Hepatotoxicity alters important biochemical indicators before an injury, such as ALT, AST, gamma GT, and alkaline phosphatase, indicating various clinical effects and directly reflecting on patient adherence to drug treatment and its side effects. Many studies have evaluated the hepatotoxic effects of available therapies for TB, such as isoniazid, rifampicin, and pyrazinamide. However, few perform this assessment in patients before treatment and generally do not show significant changes[30-31].

The serum CRP level is a marker for several inflammatory diseases, such as active TB. The increase in this index in this study can be associated with the condition of the pathophysiology

of *Mycobacterium* spp., together with the action of the immune system to fight the infection[5]. High CRP concentrations can be associated with the severity of TB and poor prognosis, especially among patients who have cavitation compared to patients who do not[32].

In addition, TB is a disease mainly affecting underprivileged individuals, often without access to basic health and good nutrition. It was also noted that several patients treated in this study had symptoms of loss of appetite and, consequently, weight loss because of the infection. Therefore, it is necessary to observe that these aspects influence biochemical parameters directly or indirectly linked to the nutritional status of patients with active pulmonary TB[33].

The lipid profile is related to the metabolism of lipoproteins, which can be evaluated in the laboratory by measuring total serum cholesterol, HDL, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), and triglycerides[34]. Some evaluations do not indicate significant differences in the levels of these parameters in patients with active TB, while other studies show low concentrations, associating them with nutritional deficiencies[35-36]. It is suggested that this deficiency may be related to the severity of the disease and the promotion of immunosuppression of the immune system[37].

Calcium is a mineral that performs several functions in the body as a component of bone formation, transmission of nerve impulses, muscle contraction, and blood clotting reactions, among others[38]. Abnormalities involving calcium levels have already been reported in several studies associated with TB, including hypocalcemia, considered a predisposing cause of the disease with a significant decrease in its concentration[33], corroborating the results found in this study.

This decrease in calcium levels can be caused by factors such as nutritional deficiency and the malabsorption associated with TB. Studies also indicate that hypocalcemia can be attributed to impaired intestinal absorption or poor intake due to eating disorders, in addition to decreased albumin serum levels or active vitamin D metabolites, which are essential for calcium absorption[39-40].

Because of the results of the biochemical analysis and the correlation with the inflammatory markers, it is highlighted how the immunosuppression of the response by IFN-γ and, mainly, the increase in the production of IL-6 interfere with different factors, which can exacerbate the inflammatory process and favor the infection.

The need to monitor these parameters before, during, and after the establishment of therapy to know the inflammatory profile of patients, together with all the associated factors, allows for a more specific targeting of treatment, better guidance for patients, and a reduction in evasion of them to therapy.

FINAL CONSIDERATIONS

When evaluating the inflammation markers, a low expression of IFN-γ was observed. At the same time, there was an increase in the production of IL-6, indicating a process of immunosuppression with possible genetic susceptibility of patients treated in the public health network of the city of São Luís. Thus, NO levels, essential for combating mycobacteria, were also low due to IFN-γ suppression. Even so, lipid peroxidation of cell membranes by TBARS levels was observed. The alteration of biochemical markers, especially those of inflammation and liver damage, allows knowing the inflammatory profile of patients before starting treatment and, thus, directing more specific therapies that aim to avoid the evasion of patients due to the adverse effects of medication throughout the treatment.

The correlation between these factors points to the simultaneous activity of inflammatory markers and biochemical markers to damage and injury stimuli caused by *M. tuberculosis*, although not very evident due to the immunosuppression of the main response by IFN-γ. The relatively small sample size of the case-control study, as they are convenience samples, limits the discussion on the correlation between inflammatory and biochemical markers. However, the results found in this study serve as a basis for further evaluation and development of control and treatment strategies for these patients.

REFERENCES

1. Houben RMGJ, Dodd, PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med. 2016;13(10):e1002152. DOI: https://doi.org/10.1371/journal.pmed.1002152.
2. World Health Organization (WHO). Tuberculosis [internet]. 2023. Disponível em: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022.
3. Ministério da Saúde (Brasil). Sistema de Informação de Agravos de Notificação - Sinan Net. Tuberculose - casos confirmados notificados no sistema de informação de agravos de notificação – Maranhão. Brasília: Ministério da Saúde; 2023.
4. Koch A, Mizrahi V. Mycobacterium tuberculosis. Trends Microbiol. 2018;26(6):555–6. DOI: https://doi.org/10.1016/j.tim.2018.02.012.
5. Miggiano R, Rizzi M, Ferraris DM. Mycobacterium tuberculosis Pathogenesis, Infection Prevention and Treatment. Pathogens. 2020;9(5):385. DOI: https://doi.org/10.3390/pathogens9050385.
6. Dannenberg Jr AM. Immune mechanisms in the pathogenesis of pulmonary tuberculosis. Rev Infect Dis. 1989;11(Suppl 2):S369-78. DOI: https://doi.org/10.1093/clinids/11.supplement_2.s369.
7. Abbas AK, Lichtman AH, Pillai S. Imunologia Celular e Molecular. 9a. ed. Rio de Janeiro: Elsevier; 2019.
8. Domingo-Gonzalez R, Prince O, Cooper A, Khader AS. Cytokines and chemokines in Mycobacterium tuberculosis infection. Microbiol Spectr. 2016;4(5): 10.1128/microbiolspec.TBTB2-0018-2016. DOI: https://doi.org/10.1128/microbiolspec.tbtb2-0018-2016.
9. Palucci I, Delogu G. Host directed therapies for tuberculosis: futures strategies for an ancient disease. Chemotherapy. 2018;63(3):172-180. DOI: https://doi.org/10.1159/000490478.
10. Chaves LB, Souza TF, Silva MVC, Oliveira CF, Lipp MEN, Pinto ML. Estresse em universitários: análise sanguínea e qualidade de vida. Rev Bras Ter Cogn. 2016;12(1):20-6. DOI: http://dx.doi.org/10.5935/1808-5687.20160004.
11. Buege JA, AUST SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-10. DOI: https://doi.org/10.1016/s0076-6879(78)52032-6.
12. Griess P. Bemerkungen zu der abhandlung der H.H. Weselsky und Benedikt "Ueber einige azoverbindungen". Ber Dtsch Chem Ges. 1879;12(1):426-8. DOI: https://doi.org/10.1002/cber.187901201117.
13. Wang Y, Xie J, Wang N, Li Y, Sun X, Zhang Y, et al. Lactobacillus casei Zhang modulate cytokine and toll-like receptor expression and beneficially regulate poly I:C-induced immune responses in RAW264.7 macrophages. Microbiol Immunol. 2013;57(1):54-62. DOI: https://doi.org/10.1111/j.1348-0421.516.x
14. Baba RK, Vaz MSMG, da Costa J. Correlação de dados agrometeorológicos utilizando métodos estatísticos. Rev Bras Meteorol. 2014;29(4):515-26. DOI: https://doi.org/10.1590/0102-778620130611.
15. Van Acker H, Coenye T. The Role of Reactive Oxygen Species in Antibiotic-Mediated Killing of Bacteria. Trends Microbiol. 2017;25(6):456-66. DOI: https://doi.org/10.1016/j.tim.2016.12.008.
16. Shastri MD, Shukla SD, Chong WC, Dua K, Peterson GM, Patel RP, et al. Role of Oxidative Stress in the Pathology and Management of Human Tuberculosis. Oxid Med Cell Longev. 2018;2018:7695364. DOI: https://doi.org/10.1155/2018/7695364.
17. Bolajoko EB, Arinola OG, Odaibo GN, Maiga M. Plasma levels of tumor necrosis factor-alpha, interferon-gamma, inducible nitric oxide synthase, and 3-nitrotyrosine in drug-resistant and drug-sensitive pulmonary tuberculosis patients, Ibadan, Nigeria. Int J Mycobacteriol. 2020;9(2):185-9. DOI: https://doi.org/10.4103/ijmy.ijmy_63_20.
18. Han F, Li S, Yang Y, Bai Z. Interleukin-6 promotes ferroptosis in bronchial epithelial cells by inducing reactive oxygen species-dependent lipid peroxidation and disrupting iron homeostasis. Bioengineered. 2021;12(1):5279-88. DOI: https://doi.org/10.1080/21655979.2021.1964158.
19. Ledesma JR, Ma J, Zheng P, Ross JM, Vos T, Kyu HH. Interferon-gamma release assay levels and risk of progression to active tuberculosis: a systematic review and dose-response meta-regression analysis. BMC Infect Dis. 2021;21(1):467. DOI: https://doi.org/10.1186/s12879-021-06141-4.
20. Casanova JL, Abel L. Genetic dissection of immunity to mycobacteria: the human model. Annu Rev Immunol. 2002;20:581-620. DOI: https://doi.org/10.1146/annurev.immunol.20.081501.125851.
21. Sologuren I, Boisson-Dupuis S, Pestano J, Vincent QB, Fernández-Pérez L, Chapgier A, et al. Partial recessive IFN-γR1 deficiency: genetic, immunological and clinical features of 14 patients from 11 kindreds. Hum Mol Genet. 2011;20(8):1509-23. DOI: https://doi.org/10.1093/hmg/ddr029.
22. Ramirez-Alejo N, Santos-Argumedo L. Innate defects of the IL-12/IFN-γ axis in susceptibility to infections by mycobacteria and salmonella. J Interferon Cytokine Res. 2014;34(5):307-17. DOI: https://doi.org/10.1089/jir.2013.0050.
23. Bustamante J, Boisson-Dupuis S, Abel L, Casanova JL. Mendelian susceptibility to mycobacterial disease: genetic, immunological, and clinica features of inborn errors of IFN-γ immunity. Semin Immunol. 2014;26(6):454-70. DOI: https://doi.org/10.1016/j.smim.2014.09.008.
24. Nagabhushanam V, Solache A, Ting LM, Escaron CJ, Zhang JY, Ernst JD. Innate inhibition of adaptive immunity: Mycobacterium tuberculosis-induced IL-6 inhibits macrophage responses to IFN-gamma. J Immunol. 2003;171(9):4750-7. DOI: https://doi.org/10.4049/jimmunol.171.9.4750.

25. Dutta RK, Kathania M, Raje M, Majumdar S. IL-6 inhibits IFN-γ induced autophagy in Mycobacterium tuberculosis H37Rv infected macrophages. Int J Biochem Cell Biol. 2012;44(6):942-54. DOI: https://doi.org/10.1016/j.biocel.2012.02.021.

26. Rath S, Narasimhan R, Lumsden C. C-reactive protein (CRP) responses in neonates with hypoxic ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed. 2014;99(2):F172. DOI: https://doi.org/10.1136/archdischild-2013-304367.

27. Rajopadhye SH, Mukherjee SR, Chowdhary AS, Dandekar SP. Oxidative Stress Markers in Tuberculosis and HIV/TB Co-Infection. J Clin Diagn Res. 2017;11(8):BC24-BC28. DOI: https://doi.org/10.7860/jcdr/2017/28478.10473.

28. Soares AMSS. Tuberculose no Centro Hospitalar Cova da Beira e a sua relação com a imunodepressão. [dissertação]. Covilhão: Universidade da Beira Interior; 2014. 43 f.

29. Pinho L, Oliveira S, Serino J, Febra T, Ramos S, Silva C, et al. Tuberculose miliar no século XXI–a propósito de um caso clínico. Nascer Crescer. 2014;21(2):151-4.

30. Enoh JE, Cho FN, Manfo FP, Ako SE, Akum EA. Abnormal Levels of Liver Enzymes and Hepatotoxicity in HIV-Positive, TB, and HIV/TB-Coinfected Patients on Treatment in Fako Division, Southwest Region of Cameroon. Biomed Res Int. 2020;2020:9631731. DOI: https://doi.org/10.1155/2020/9631731.

31. Su Q, Liu Q, Liu J, Fu L, Liu T, Liang J, et al. Study on the associations between liver damage and antituberculosis drug rifampicin and relative metabolic enzyme gene polymorphisms. Bioengineered. 2021;12(2):11700-8. DOI: https://doi.org/10.1080/21655979.2021.2003930.

32. Brown J, Clark K, Smith C, Hopwood J, Lynard O, Toolan M, et al. Variation in C - reactive protein response according to host and mycobacterial characteristics in active tuberculosis. BMC Infect Dis. 2016;16:265. DOI: https://doi.org/10.1186/s12879-016-1612-1.

33. Ufoaroh CU, Onwurah CA, Mbanuzuru VA, Mmaju CI, Chukwurah SN, Umenzekwe CC, et al. Biochemical changes in tuberculosis. Pan Afr Med J. 2021;38:66. DOI: https://doi.org/10.11604/pamj.2021.38.66.21707.

34. Nordestgaard BG, Langsted A, Mora S, Kolovou G, Baum H, Bruckert E, et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. Eur Heart J. 2016;37(25):1944-58. DOI: https://doi.org/10.1093/eurheartj/ehw152.

35. Metwally MM, Raheem H. Lipid profile in tuberculous patients: a preliminar report. Life Sci. 2012;9(1):719-22.

36. Gebremicael G, Amare Y, Challa F, Gebreegziabxier A, Medhin G, Wolde M, et al. Lipid Profile in Tuberculosis Patients with and without Human Immunodeficiency Virus Infection. Int J Chronic Dis. 2017;2017:3843291. DOI: https://doi.org/10.1155/2017/3843291.

37. Chidambaram V, Zhou L, Ruelas Castillo J, Kumar A, Ayeh SK, Gupte A, et al. Higher Serum Cholesterol Levels Are Associated With Reduced Systemic Inflammation and Mortality During Tuberculosis Treatment Independent of Body Mass Index. Front Cardiovasc Med. 2021;8:696517. DOI: https://doi.org/10.3389/fcvm.2021.696517.

38. Hall JE, Hall ME. Guyton & Hall – Tratado de Fisiologia Médica. 14a. ed. Barueri: Editora Gen – Grupo Editorial Nacional Part S/A. 2021.

39. Hafiez AA, Abdel-Hafez MA, Salem D, Abdou MA, Helaly AA, Aarag AH. Calcium homeostasis in untreated pulmonary tuberculosis. I--Basic study. Kekkaku. 1990;65(5):309-16.

40. Mehto S, Antony C, Khan N, Arya R, Selvakumar A, Tiwari BK, et al. Mycobacterium tuberculosis and Human Immunodeficiency Virus Type 1 Cooperatively Modulate Macrophage Apoptosis via Toll Like Receptor 2 and Calcium Homeostasis. PLoS One. 2015;10(7):e0131767. DOI: https://doi.org/10.1371/journal.pone.0131767.

Financial support
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

Corresponding Author:
Adrielle Zagmignan
adrielle004602@ceuma.com.br

Editor:
Prof. Dr. Felipe Villela Gomes

Received: feb 13, 2023
Approved: may 17, 2023