Polish Annals of Medicine

Journal homepage: https://www.paom.pl

Research paper

Serum isoniazid concentration in the patients as an indicator of the effectiveness and toxicity of tuberculosis treatment

Petro B Antonenko[1], *Kateryna OO Antonenko*[1], *Valentin I Kresyun*[1], *Olga Żuk*[2], *Kateryna G Lobashova*[1]

[1] *Department of General and Clinical Pharmacology, Odessa National Medical University, Ukraine*
[2] *Department of Biotechnology and Molecular Biology, Opole University, Poland*

Article info

Article history
Received 24 September 2018
Accepted 29 November 2018
Available online 11 December 2019

Keywords
Treatment
Tuberculosis
Concentration
Toxicity
Isoniazid

Doi
https://doi.org/10.29089/2019.19.00080

Abstract

Introduction: The low serum concentration of the anti-tuberculosis (TB) drugs in TB patients is observed quite often, but the significance of this phenomenon remains controversial.

Aim: The aim of the presented research was to clarify the association between isoniazid concentration and TB-treatment outcomes.

Material and methods: Blood samples were obtained from 86 patients with newly diagnosed pulmonary TB at Odessa Regional TB Hospital in 2015. The level of isoniazid in blood was measured 2 h, 4 h, 6 h, and 24 h after the administration of isoniazid. The medical records of the enrolled patients at the beginning and at the end of the in-patient treatment, and the activity of the biochemical indices were considered.

Results and discussion: At the end of the in-patient treatment in patients with a serum isoniazid concentration of more than 2 μg/mL (HIC), 4 h after the drug administration, the resorption of TB infiltrates in lungs occurred 1.3 times more frequently than in patients with an isoniazid concentration of less than 2 μg/mL (LIC). According to the culture method, the smear conversion occurred 3.1 times more often in patients with HIC than in the LIC ones. A positive correlation was found between the isoniazid serum level with the bilirubin level, thymol probe, and the AsAT activity, which proved the higher risk of hepatotoxicity in patients with HIC.

Conclusions: Thus, the measurement of the isoniazid concentration after 4 h of isoniazid administration can predict the outcome of the TB-treatment. It is postulated that the recommended concentration of isoniazid in blood 4 h after its administration > 2 μg/mL.

Corresponding author: Petro B. Antonenko, Department of General and Clinical Pharmacology, Odessa National Medical University, Valichovsky lane, 2, 65023 Odessa, Ukraine. Tel.: +38 0975875636.
E-mail address: petrosantonenko@gmail.com.

1. INTRODUCTION

Pulmonary tuberculosis (TB) is an important medical and social problem worldwide. The currently used directly observed treatment short-course (DOTS) strategy of TB-treatment has shown high effectiveness for countries with a low frequency of new TB cases (less than 10 cases per 100 000 people) and a low rate of primary multi-drug resistant (MDR) TB (3%–5%). At the same time, in the developing countries including almost all post-soviet states such as Ukraine, there is still a high-incidence of TB.[1,2] According to the Ukrainian national report, an estimated 71.3 and 70.5 people per 100 000 people developed TB in 2014 and 2015, respectively. This characterize Ukraine as a high-incidence TB country. The peculiarity of the TB incidence in Ukraine is the intensive spreading of the *Mycobacterium tuberculosis* strains of the Beijing family, associated with MDR, from 39.6% in 2003 to 54.8% in 2012.[3] One of the reasons for the development of MDR of *M. tuberculosis* could be the insufficient (under-effective) serum concentration of anti-TB drugs. According to Chideya et al., the low serum concentrations of isoniazid and rifampicin were observed in 37% and 84% of TB patients, respectively;[4] these numbers according to Um et al. were 15% and 24%, respectively.[5] Thus, the low serum concentration of the anti-TB drugs in TB patients is observed quite often; however, the clinical significance of this phenomenon remains controversial. On the one hand, the good therapeutic effect of anti-TB therapy was observed even at a low serum concentration of isoniazid.[6] On the other hand, the low level of isoniazid led to the drug resistance of *M. tuberculosis* and poor outcomes of TB-treatment.[7,8] Thus, scanty and controversy information is available on the association between isoniazid serum concentrations, treatment efficacy and toxicity.[9]

2. AIM

The aim of the presented research was to clarify the association between isoniazid concentration and TB-treatment outcomes on the basis of clinical data and serum biochemical indexes after initial phase of therapy.

3. MATERIAL AND METHODS

The enrolled TB patients were chosen using a cross-sectional method. As the exclusion criteria, the HIV-positive status of TB patients and the presence of viral hepatitis B or C were considered. The study was approved by the Ethical Commission of Odessa National Medical University and was conducted according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans. At the beginning of the research, we obtained written consent from all of the enrolled TB patients. In total, 86 patients with newly diagnosed TB were enrolled; there were 53 men (61.6%) and 33 women (38.4%). Age ranged 18–71 years.

All the TB patients received the complex therapy recommended for Category-I TB patients including oral isoniazid dose of approximately 4–6 mg/kg per day (in total, 300–400 mg), 20–35 mg/kg pyrazinamide, 15 mg/kg ethambutol, and 450–600 mg of rifampicin according to the recommendation of Ministry of Health of Ukraine nr 384 and of the World Health Organization DOTS strategy. The blood samples were collected from the TB patients at Odessa Regional TB Hospital in 2015 on the 7th day from the beginning of in-patient treatment, 2 h, 4 h, 6 h, and 24 h after the isoniazid administration. In general, it is recommended to measure the concentration of a drug 2 h after the drug's intake, when the drug's concentration reaches the peak level, and after 6 h, which allows us to distinguish delayed absorption from incomplete absorption.[10] The level of isoniazid was measured using high-performance liquid chromatography using UV detection.[11] Isoniazid was extracted from plasma using C18 extraction column with 1 mL 0.5 mM potassium phosphate buffer. Isoniazid was also quantitated on a C8 analytic reverse-phase column. The mobile phase included 3% acetonitrile and 0.06% trifluoroacetic acid in the ration 5 : 95. The UV detection wavelength was 270 nm. The standard curve provided a detection range of 0.2–20.0 μg/L.

The concentration of diene conjugates (DC; μmol/L), which characterized the rate of the lipid peroxidation process in the studied patients was measured spectrophotometrically by intensive light absorption at the wavelength 232 nm by conjugated lipid hydroperoxide structures.[12] To do that, 2 mL of serum (with 1 : 10 dilution) were placed in separate tube, where 5 mL of heptanes-isopropanol mixture (1 : 1) were added, and the contents were shaken for 10–15 minutes. Out of this mixture the upper heptanes layer (2 mL) were taken to measure the concentration of DC. The enzyme activity of catalase, which reflected the activity of the antioxidant serum system, was determined by Beutler in 1984, as a measure of the rate of decomposition of hydrogen peroxide by catalase spectrophotometrically at 240 nm.[13] In brief, 0.1 mL of blood serum was added to a test tube containing 2.975 mL of 0.05M sodium phosphate buffer and 0.4 mM EDTA. After that 25 μL of 3% H_2O_2 was added to start the reaction. Catalase activity was expressed as μkat/L.

We considered the medical records of the enrolled patients at the beginning and at the end of the in-patient treatment, including the TB form, characteristics of the TB lesions, bacterial excretion, results of the drug-susceptible tests for obtained *M. tuberculosis* strains, activity of biochemical indices such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutathione transferase (GGT) in the biochemical laboratory of Odessa Regional TB Hospital in 2015. The activity of ALT and AST was measured using the International Federation of Clinical Chemistry (IFCC) method, while the GGT activity was measured using the kinetic colorimetric method.

A statistical analysis was performed using the 'Primer Biostatistica' package (Kruskal–Wallis and ANOVA tests) and the Microsoft Excel program. A χ^2 test was used to determine whether there was a statistically significant dif-

ference in the frequency of the studied criteria between the two groups. Statistical significance was assumed to be at $P < 0.05$.

4. RESULTS

At the first stage, we identified a group of patients who had a sub-effective concentration of isoniazid in 2 h (<3 μg/mL), 4 h (<1.5 μg/mL), 6 h and 24 h (<0.5 μg/ml).[9] But it was turned out that only about 20% of enrolled patients had a sub-effective concentration at a time interval of 2–6 h that complicated their statistical comparison with patients who had therapeutic concentrations. Consider relatively small number of enrolled patients, it was decided to divide the patients into two nearly equal groups according to the means of isoniazid concentration in the blood. The mean isoniazid concentration was 4 μg/mL after 2 h, 2 μg/mL after 4 h, 1 μg/mL after 6 h and 0.025 μg/mL after 24 h (Figure 1). Previously, a statistically significant association between the isoniazid serum concentrations and outcomes of TB-treatment in the enrolled patients was found only 4 h after anti-tuberculosis agents' administration. For further convenience, all the studied TB patients were divided into two groups according to the mean of the isoniazid concentration in the blood: 42 patients (48.8%) had an isoniazid level of less than 2 μg/mL (0.48–1.99 μg/mL) (low isoniazid concentration – LIC), while 44 patients (51.2%) had an isoniazid concentration of more than 2 μg/mL (2.02–6.54 μg/mL) (high isoniazid concentration – HIC). In both studied groups, at the beginning of the treatment, the pulmonary TB infiltration was observed in almost every 2nd patient, TB disintegration (decay) was observed in 16%–17% of the patients, and TB dissemination was verified in 33.3% of the LIC and 29.5% of the HIC patients (Figure 2).

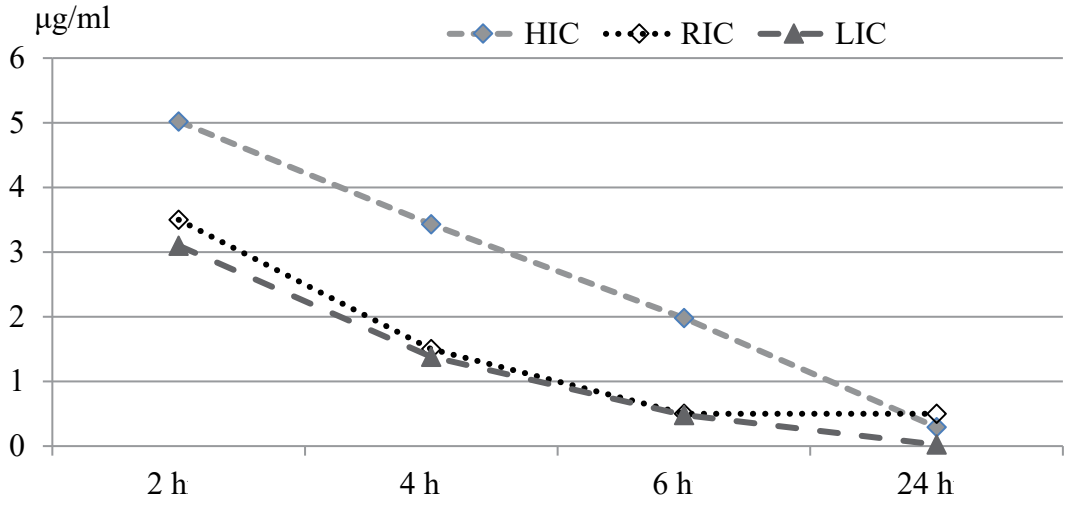

Figure 1. Mean isoniazid level in the blood (ordinate axis) in the patients with low or high serum isoniazid concentration (LIC or HIC) 2–24 h after administration (abscissa axis) comparatively to recommended (effective) isoniazid concentration (RIC).

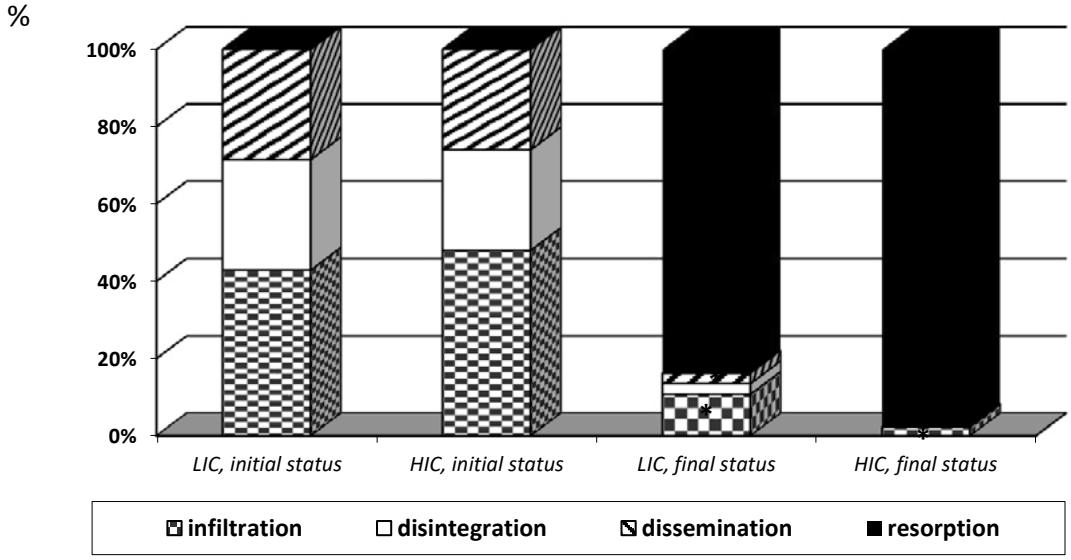

Figure 2. Description of pulmonary TB-lesions regarding to low or high serum isoniazid concentration (LIC or HIC) 4 h after isoniazid administration at the beginning (initial) or at the end (final) of in-patient treatment. Comments: * $P < 0.05$ in comparison with initial status; # $P < 0.05$ in comparison with LIC group.

At the end of the in-patient TB-treatment, the frequency of TB infiltration in the LIC group decreased by 5.3 times, while in the HIC group, it decreased by 23.7 times. Moreover, the signs of TB dissemination completely disappeared in the HIC group, but, in the LIC group, they decreased by 13.9 times from the initial status. In the HIC group, after the treatment, the symptoms of pulmonary TB disintegration completely disappeared, which reflected a considerably better status than the initial status and the status of the LIC group. In addition, the process of pulmonary TB resorption was observed in 73.8% of the LIC group and in 97.7% of the HIC group, which was significantly high as compared to the initial status. Thus, the TB resorption in the HIC group occurred 1.3 times more often than in the LIC group.

At the beginning of the TB-treatment, no one from the studied TB patients had the MDR (simultaneously resistant to isoniazid and rifampicin) strains of *M. tuberculosis*. However, at the end of the in-patient treatment, 28.6% out of the LIC and 9.1% out of the HIC patients developed MDR. Thus, at the end of the in-patient treatment, the frequency of MDR strains in the LIC group was 3.1 times higher than in the HIC group.

The microscopy results obtained at the end of the in-patient treatment showed that 100% of the HIC patients and 97.6% of the LIC patients were smear-negative (Figure 3). The conversion of the smear status was observed in 95.0% of the LIC patients and in 100% of the HIC group ($\chi^2 = 22.92$ and $\chi^2 = 27.58$ as compared to the initial status). Furthermore, the smear conversion, considering bacterioscopy, in both the groups took around 60 days.

According to the culture (medium) method used, approximately 40% of all the TB patients remained smear-positive irrespective of the isoniazid concentration. Moreover, only in the HIC group did the number of smear-positive patients decrease by 1.9 times as compared to the initial state. In general, the smear conversion during the in-patient treatment according to the culture method occurred in the HIC group patients 3.1 times more often than in the LIC group patients (46.9% vs. 15.0%). Finally, it took, on average, 65 days for the smear conversion considering the culture method irrespective of the serum isoniazid concentration.

At the beginning of the TB-treatment, the level of the thymol probe was 37.8% higher in the LIC group than in the HIC group (Table 1). In both the studied groups, we ob-

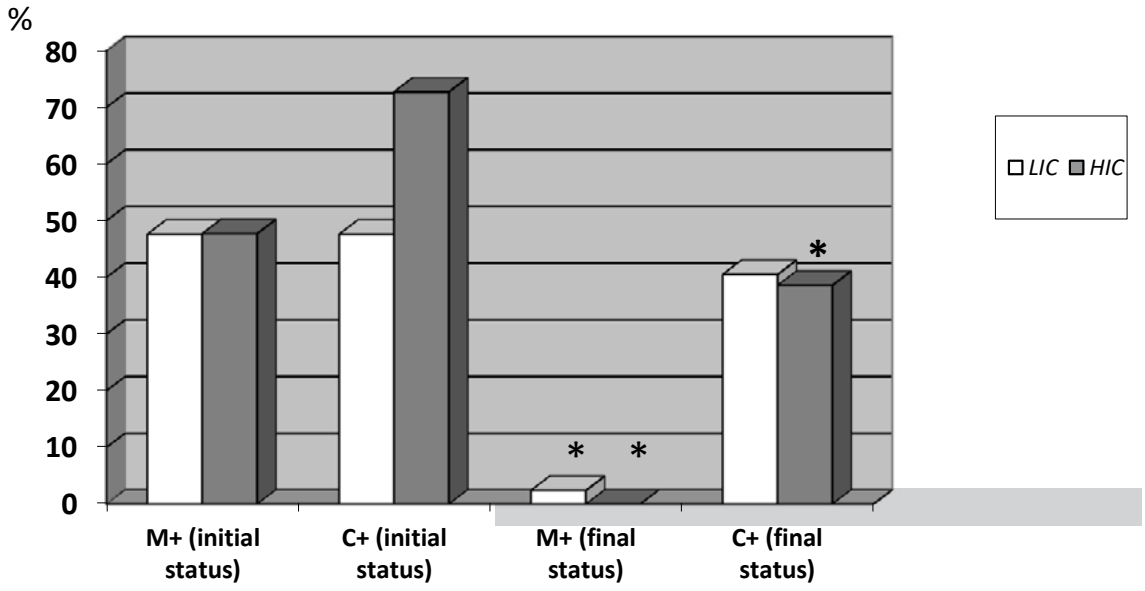

Figure 3. Frequency of smear-positive status according to bacterioscopy (B^+) or cultural (C^+) method and regarding to low (LIC) or high (HIC) serum isoniazid concentration at the beginning (initial status) or at the end (final status) of in-patient TB-treatment. Comments: * $P < 0.05$ comparatively to the initial level of the correspondent group.

Table 1. Biochemical serum indexes at the beginning and at the end of the in-patient TB-treatment with respect to the isoniazid level in the blood (mean ± SEM).

Groups	*N*	Total bilirubin, μmol/L	Thymol probe, U	L, U/L	GG , U/L
Initial state					
LIC	42	13.30 ± 0.79	1.56 ± 0.09	19.80 ± 2.32	29.42 ± 2.27
HIC	44	14.80 ± 0.47	2.15 ± 0.19*	22.46 ± 1.63	27.27 ± 1.47
Final state					
LIC	42	10.62 ± 0.23#	2.17 ± 0.15#	24.08 ± 1.67	31.50 ± 1.28
HIC	44	12.04 ± 0.28*#	2.50 ± 0.19	25.67 ± 1.28	32.11 ± 1.52#

Comments: * $P < 0.05$ (in comparison with LIC group); # $P < 0.05$ (in comparison with initial level).

Table 2. Laboratory indexes at the beginning and at the end of the in-patient treatment (mean±SEM) and their correlation with serum isoniazid concentration.

Laboratory indexes	Initial state	Final state
Erythrocytes, G/L	4.59 ± 0.06	4,54 ± 0.09*
Lymphocytes, %	29.87 ± 0.39*	36.05 ± 0.31
Monocytes, %	4.92 ± 0.20*	5.02 ± 0.33
Total bilirubin, mcM/L	14.07 ± 0.67	11.34 ± 0.27*
Thymole probe, U	1.86 ± 0.15*	2.34 ± 0.19*
AST, U/L	25.89 ± 1.08	27.96 ± 1.11*

Comments: * Correlation $P < 0.05$.

served a gradual increase in the thymol probe level during the in-patient treatment that showed the hepatotoxic influence of the TB agents. As a result of the provided TB-treatment, the level of total bilirubin decreased in the LIC group and in the HIC group by 25.2% and 22.9%. At the same time, the level of total bilirubin in the LIC group was 13.4% lower than in the HIC group.

Moreover, during the in-patient treatment, a certain increase in the cytolysis indexes, namely ALT and AST, as well as the bile stasis index, GGT, was observed. For instance, the activity of GGT in HIC patients increased by 17.7%. Moreover, in the HIC group, the levels of the abovementioned indexes at the end of the treatment were higher than in the LIC group.

Thus, in the LIC group, both at the beginning and at the end of the in-patient TB-treatment, we observed more significant abnormalities in the laboratory indexes and lower efficiency of the treatment than in the HIC group. This conclusion was proven by the positive correlation of isoniazid with the lymphocyte percentage and the erythrocyte count and the negative correlation with the monocyte percentage (Table 2). In contrast, we found a positive correlation between the isoniazid serum level with the bilirubin level, thymol probe, and AST activity.

With respect to the influence of the isoniazid serum concentration on the lipid peroxidation, we found that the maximal level of DCs (1.663 mol/L) was 2 h after the TB agent administration. In the following measurement, the DC level gradually decreased to 1.521 mol/L 24 h after the drug administration. With respect to the catalase activity, we found an opposite tendency – the minimal activity of catalase was observed 2 h after the TB agent administration 0.151 μkat/L with the following increase in the catalase activity that reached 0.168 μkat/L 24 h later. Thus, a gradual decrease in the DC level and an increase in the catalase activity in blood were observed in association with a decrease in the isoniazid serum level.

A correlation between the lipid peroxidation and the isoniazid level in the blood of TB patients has also been studied. After 4 h of isoniazid administration, we observed an inverse correlation between the isoniazid concentration and the DCs in blood; however, after 6 h of isoniazid administration, we observed a direct correlation between the isoniazid concentration and the catalase activity in blood. Thus, for the serum isoniazid concentration, we found an inverse correlation with the DC level and a direct correlation with the catalase activity in blood.

5. DISCUSSION

At the end of the in-patient treatment in the patients with a serum isoniazid concentration of more than 2 μg/mL (HIC) 4 h after the drug administration, one could see better outcomes of TB-treatment – more frequent resorption of TB infiltrates in lungs and smear conversion, less frequent appearance of MDR strains than in patients with an isoniazid concentration of less than 2 μg/mL (LIC). Thus, the measurement of the isoniazid concentration 4 h after isoniazid administration could help in the prediction of the effectiveness of the TB-treatment with the following individual adjustment of the isoniazid dosage. According to literature data the recommended serum isoniazid concentration 4 h after its administration is above 1.5 μg /mL.[9] However, our results showed that the better effectiveness of TB-treatment associated with the serum isoniazid concentration of more than 2 μg/mL. In fact, according to Augustynowicz-Kopeć E, Zwolska Z the isoniazid concentration in blood as 2 μg/mL 4 h after its administration could help to distinguish the patients with slow acetylator phenotype from the patients with rapid acetylator phenotype.[14] Thus we can consider the HIC group as slow acetylator phenotype, while LIC group as rapid acetylator phenotype. It is wise to mention that the mean isoniazid concentration in the HIC group remains beyond recommended (effective) isoniazid concentration almost 24 h after its administration (Figure 1). However, the mean isoniazid concentration in the LIC group was sub-effective during studied time interval.

A positive correlation was observed between the isoniazid level and the lymphocyte percentage and the erythrocyte count, and a negative correlation with the monocyte percentage. TB patients have blood abnormalities such as anemia, lymphocytopenia, and monocytosis, and a successful TB-treatment is associated with the correction of these blood abnormalities (an increase in the erythrocyte and lymphocyte count and a decrease in the monocytes count). In contrast, a positive correlation was found between the isoniazid serum level and the bilirubin level, thymol probe and AST activity, which im-

plied a higher risk of hepatotoxicity and hepatocytes-lysis in the HIC patients. Thus, the isoniazid level in blood during the TB-treatment is an important predictor of its toxicity.

The obtained results demonstrated that the decrease in the isoniazid serum concentration is associated with a decrease in the lipid peroxidation marker acting as the DC and an increase in the antioxidant system components such as the serum catalase activity. These data coincide with the literature data regarding isoniazid's hepato- and nephrotoxicity[15] and the relatively high level of hepatotoxicity indexes in the HIC patients.[16] However, the current research failed to show significant increasing of the hepatotoxicity risk in the HIC group comparatively to the LIC group.

In contrast, we unexpectedly found that the isoniazid serum concentration had a direct correlation with the serum catalase activity and an inverse correlation with the serum DC level. This implied that the relatively high serum isoniazid concentration was associated with the increased serum antioxidant activity (catalase) and the decreased activity of the serum lipid peroxidation processes (DC). The controversial influence of isoniazid on the antioxidant and pro-oxidant systems could be linked with the peculiarities of isoniazid's metabolite actions such as acetylisoniazid and hydrazine. According to the literature data, the main toxicity of isoniazid administration was connected to the formation of toxic metabolites such as acetylhydrazine and hydrazine.[16–18] Therefore, the rapid decrease in the isoniazid serum level (in individuals with rapid acetylator phenotype) with the predominant formation of the low-toxicity acetylisoniazid led to a lower risk of isoniazid toxicity. In contrast, the slow decrease in the serum isoniazid concentration (in individuals with slow acetylators) could cause an intensive formation of hydrazine, which explained the higher risk of isoniazid toxicity in this category of individuals.

6. CONCLUSIONS

Conflict of interest

The authors declare no conflict of interests.

Funding

None.

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