Original Article

Association between Effectiveness of Tuberculosis Treatment and cytochrome P-4502E1 Polymorphism of the Patients

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Abstract

Context: The risk of antituberculosis (TB) drug-induced liver injury could be determined by patients' genotype polymorphism of the xenobiotic-metabolizing enzymes. To find the meaning of cytochrome P-4502E1 (*CYP2E1*) polymorphism in TB patients. Corresponding of *CYP2E1* polymorphism in TB patients with the level of isoniazid and rifampicin as well as for the outcome and toxicity development during inpatient TB treatment. **Methods:** *CYP2E1* genotype was detected with the help of polymerase chain reaction and endonuclease analysis. The level of rifampicin, isoniazid, diene conjugates (DC), and catalase activity in the blood was determined spectrophotometrically. We have considered medical records at the beginning and at the end of inpatient treatment. Statistical Analysis Used: Kruskal–Wallis, ANOVA, and Chi-square tests were used in this study. **Results:** The concentration of rifampicin 6 h after its intake was 17.6% higher in carriers of slow metabolizer (SM) *CYP2E1* genotype than in patients with rapid metabolizer (RM) genotype that proved a participation of hepatic enzyme CYP2E1 in metabolism of rifampicin. According to obtained results in TB patients with RM genotype, the indexes of cytolysis (alanine aminotransferase, aspartate aminotransferase) and bile stasis (gamma-glutathione transferase) were higher comparatively to SM genotype both before and after inpatient treatment. This correlated with a higher concentration of DC in the blood (+8.6%) and lower plasma catalase activity (-50.0%) in the patients with RM genotype comparatively with the patients with SM genotypes. **Conclusion:** Polymorphism of *CYP2E1* genotype is an important criterion for the development of hepatotoxicity before and during TB treatment while increased rifampicin level has no influence on it.

Keywords: Cytochrome P-4502E1, gene polymorphism, hepatotoxicity, rifampicin, tuberculosis

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB) that annually kills about 2 million people worldwide.[1-4] Adverse effects of anti-TB agents, which include anti-TB drug-induced liver injury observed in 10%–26% of TB patients who were receiving standard short-course chemotherapy, are the important obstacles for successful TB treatment.[5-9] The risk of anti-TB drug-induced liver injury could be determined by patients' genotype polymorphism of the xenobiotic-metabolizing enzymes such as cytochrome-4502E1 (*CYP2E1*), N-acetyltransferase 2, and glutathione S-transferase.[10,11] Numerous literature data prove an association between *CYP2E1* polymorphism and liver injury.[10-12] However, Tang *et al*. 2013 did not find such association.[13] In addition, the aforementioned enzymes play an important role in biotransformation of the anti-TB drugs. Thus, patients' genotype polymorphism has certain impact on the drugs' concentration in the blood and finally on the effectiveness of TB treatment. It is clear that in the individuals with "rapid metabolizer" (RM) genotype, serum drug's concentration would be lower and treatment outcome would be worse than in the carriers of "slow metabolizer" (SM) genotype. For example, TB patients with SM genotype of *CYP2C9* gene had the highest serum isoniazid and rifampicin level and the most favorable treatment outcome comparatively to RM genotype group.[14] The aim of this research was to find the meaning of *CYP2E1* polymorphism in TB patients for the

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level of isoniazid and rifampicin as well as for the outcome and toxicity development during inpatient TB treatment.

METHODS

Patients

Blood samples were obtained from 86 patients with newly diagnosed pulmonary TB at Odessa Regional TB Hospital in 2015. Blood samples from 122 healthy volunteers were used as control. The study was approved by Ethical Commission of Odessa National Medical University. DNA material was extracted from the blood using a kit of DNA-Sorb-B (AmpliSens, Russian Federation). A *CYP2E1* genotype was detected with the help of polymerase chain reaction (PCR) and endonuclease analysis.[15] The PCR primers were 5'-CTGCTGCTAATGGTCACTFG-3' and 5'-GGAGTFCAAGACCAGCCTAC-3', which produced a 686-bp product. The presence of mutation in intron 6 has been studied with the help of restriction with *Dra*I enzyme – only the *D* allele was sensitive to enzyme cleavage, resulting in fragments of 335 and 351 bp [Figure 1]. All TB patients were receiving complex therapy including rifampicin and isoniazid orally around 8–12 and 4–6 mg/kg of body weight per day (totally 450–600 and 300–400 mg), respectively, according to the order of Ministry of Health of Ukraine № 384 and recommended by the World Health Organization DOTS strategy. We have considered medical records at the beginning and at the end of inpatient treatment including TB form, characteristics of TB lesions, smear status, activity of biochemical indices such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutathione transferase (GGT).

Laboratory evaluation

The blood samples were collected from TB patients during the first 2 weeks of inpatient treatment 2, 4, 6, and 24 h after administration of rifampicin and isoniazid. The level of rifampicin was determined according to Chubaryan, 1994.[16] The method is based on the extraction of rifampicin from the blood using chloroform and KON with further spectrophotometric analysis of the extract at 470 nm. The content of isoniazid was determined according to the method of Wallenberg in modification of Shenderova, 1975.[17] Briefly, isoniazid forms a colored complex with vanadium-acidic ammonia in the acidic medium; the

Figure 1: The restriction fragment length polymorphisms of polymerase chain reaction-amplified fragments obtained using *Dra* I and subjected to agarose gel electrophoresis. Lanes 1, 2, 4–9, and 11–13 presented *DD* homozygote genotype; lanes 10, 14–16 presented *CD* heterozygote genotype; and lane 3 presented *CC* homozygote genotype

intensity of coloring can be measured at a wavelength of 400 nm. The concentration of diene conjugates (DC), which characterizes rate of lipid peroxidation process in studied patients, was measured spectrophotometrically by intensive light absorption at 220 nm by conjugated lipid hydroperoxide structures.[18] The enzyme activity of catalase, which reflects activity of antioxidant serum system, was determined by Beutler, 1984 that measures the rate of decomposition of hydrogen peroxide by catalase spectrophotometrically at 240 nm.[19]

Ethics

The project was approved by the Ethics Committee of Odessa National Medical University, Odessa, Ukraine. It was conducted according to the Declaration of Helsinki standards. All of the patients gave written informed consent and explicitly provided permission for treatment and blood analyses, as well as for the collection of relevant clinical data.

Statistical evaluation

Statistical analysis was performed using the "Primer Biostatistic" package (Kruskal–Wallis and ANOVA tests). The Chi-square test was used to determine whether there is a significant difference concerning frequency of studied criteria between two groups. Statistical significance was assumed at the $P < 0.05$.

RESULTS

CYP2E1 genotyping of 86 patients has shown that 86.0% had no mutation in this region (*DD* genotype), 12.8% had one mutated allele (*CD* genotype), and 1.2% had both mutated alleles (*CC* genotype) [Figure 1]. The group of patients with *DD* genotype was termed as "rapid metabolizers" and the group of patients with *CD*, *CC* genotypes was termed as "slow metabolizers". Among healthy donors, *CD* genotype was observed more frequently (17.2%) and *DD* and *CC* genotypes were more rare (82.0% and 0.8%, respectively) than in TB patients. Hence, we did not find significant difference in *CYP2E1* polymorphism between TB patient and healthy volunteers. The frequency of RM genotype in Ukrainians was close to those found in other European countries similar to the UK, France, and Russian Federation where it ranged from 81.0% to 84.9%[20-22] and was significantly higher than in Malaysia, China, and the USA (62.3%–67.7%).[23-25]

There were no significant differences of isoniazid concentration between different *CYP2E1* genotypes [Table 1]. In TB patients with RM genotype, the level of rifampicin was lower than in patients with SM genotypes. For example, in SM, carriers of the concentration of rifampicin 6 h after intake and overall were 17.6% ($P = 0.036$; confidence interval [CI] = −3.37−−0.43) and 14.9% ($P = 0.047$; CI = −2.90−−0.02) higher than in the patients with RM genotype.

According to literature data, a recommended interval of rifampicin concentration in blood during treatment should range from 8 to 24 mcg/ml.[26] Approximately 71.2% of the
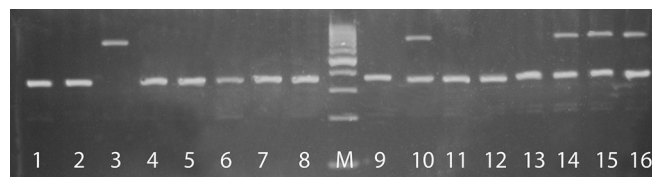
individuals with RM genotype and 33.3% of those with SM genotype had subeffective concentration 24 h after rifampicin intake [Figure 2]. Thus, in carriers of RM genotype, cases of subeffective level of rifampicin were 2.1 times more often than in patients with SM genotype ($P < 0.05$; $\chi^2 = 5.21$ at the critical value here and below of $I^2 = 3.84$).

No significant differences regarding to *CYP2E1* genotype were found concerning TB course, localization, and severity neither at the beginning nor at the completion of the inpatient stage. Furthermore, there was no difference in the development of multidrug-resistant strains, conversion of smear-positive status to smear-negative status.
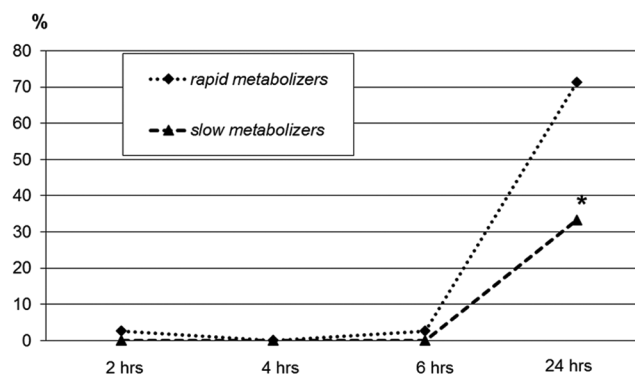

Figure 2: Number of tuberculosis patients that did not reach recommended rifampicin concentration in the blood after different time interval concerning cytochrome P-4502E1 polymorphism. *$P < 0.05$ (relatively to rapid metabolizer genotype)

At the beginning of treatment in TB patients with RM genotype, the level of bilirubin was on 33.2% higher than in carriers of SM genotypes ($P = 0.042$; $CI = 0.13–7.17$), ALT on 65.6% ($P = 0.006$; $CI = 2.79–15.97$), and GGT on 41.0% ($P = 0.020$; $CI = 1.48–16.72$), respectively [Table 2].

Besides that, at the beginning of treatment, the number of patients with biochemical indexes that exceeded normal level was slightly higher in RM genotype carriers than in SM carriers; however, the difference was insignificant [Figure 3a].

Indexes of pro- and anti-oxidant systems basically coincide in the TB patients with mentioned beyond biochemical indexes. For example, the level of DC in blood in the carriers of RM genotype was 8.6% higher ($P < 0.05$; $CI = 0.06–2.60$) while the serum catalase activity was in 2 times lower ($P < 0.001$; $CI = -1.78--1.04$) than in the carriers of SM genotype.

At the moment of discharge from the hospital, there was insignificant dropping of serum bilirubin level in both groups. Meanwhile, the number of patients with RM genotype with hyperbilirubinemia has decreased in 2.4 times ($P < 0.05$; $\chi^2 = 4.17$) comparatively to the initial level and totally disappeared among patients with SM genotypes [Figure 3b].

During treatment in TB clinic in the carriers of RM genotype, the activity of ALT has enhanced on 18.4% ($P = 0.009$; $CI = -7.58–1.12$) and in patients with SM genotype on 30.9% ($P = 0.018$; $CI = -7.98–0.86$). In the same time, the activity of AST and GGT has raised insignificantly in the carriers of RM genotype while in the carriers with SM genotype on 16.9% ($P = 0.036$; $CI = -6.59--0.25$) and 31.4% ($P = 0.016$;

Table 1: Isoniazid and rifampicin concentration in tuberculosis patients depending on cytochrome P-4502E1 polymorphism (mean±standard error mean)

CYP2E1 genotype	n	Time interval after drug's administration				
		2 h	4 h	6 h	24 h	Daily average
Isoniazid level in blood (mcg/ml)						
RM	77	4.16±0.15	2.48±0.14	1.23±0.13	0.17±0.05	2.01±0.10
SM	9	4.27±0.25	2.68±0.29	1.29±0.30	0.02±0.01	2.06±0.15
Rifampicin level in blood (mcg/ml)						
RM	77	11.66±0.28	15.67±0.41	10.80±0.22	7.23±0.18	11.16±0.22
SM	9	12.70±0.94	16.87±1.08	12.70±1.14*	7.90±0.78	12.82±0.97*

*Relatively to RM genotype group. RM: Rapid metabolizers, SM: Slow metabolizers, CYP2E1: Cytochrome P-4502E1

Table 2: Biochemical indexes in blood depending on cytochrome P-4502E1 polymorphism (mean±standard error mean)

Group	n	Bilirubin (total), (mM/l)	ALT (units)	AST (units)	GGT (units)
At the beginning of inpatient treatment					
RM	77	14.63±0.64	23.67±1.20	26.65±1.12	31.27±1.39
SM	9	10.98±0.77*	14.29±1.30*	20.29±1.07	22.17±1.44*
After inpatient treatment					
RM	77	13.12±0.48	28.02±1.11#	29.37±1.15	33.43±1.26
SM	9	11.96±0.51	18.71±1.09#,*	23.71±1.07#,*	29.14±2.18#

*Relatively to RM genotype, #Relatively to the same group at the beginning of treatment, RM: Rapid metabolizers, SM: Slow metabolizers, GGT: Gamma-glutathione transferase, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase
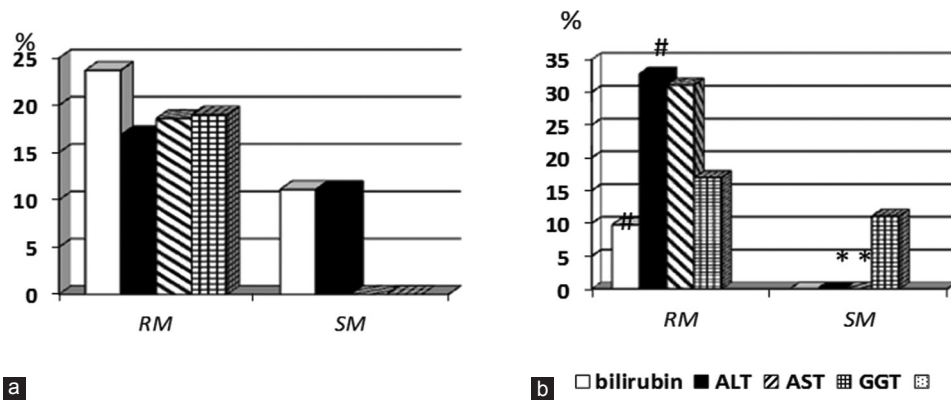
Figure 3: Number of the patients with biochemical indexes that exceeded normal level regarding cytochrome P-4502E1 polymorphism at the beginning (a) and after completion of inpatient treatment (b). *$P < 0.05$ relatively to RM genotype; #$P < 0.05$ relatively to the same group at the beginning of treatment

CI = −12.46–−1.48) correspondently relatively to the initial level. Moreover, in the end of inpatient treatment, the rate of ALT and AST was on 49.5% ($P < 0.001$; CI = 9.37–21.95) and 23.9% ($P = 0.003$; CI = 3.21–15.41) correspondently higher in patients with RM genotype than in patients with SM genotype. Furthermore, after inpatient treatment, the number of TB patients with RM genotype with abnormally elevated ALT activity has increased by almost 2 times ($P < 0.05$; $\chi^2 = 4.01$). In conclusion, among patients with *SM* genotypes, there were no patients with elevated activity of ALT and AST, while in individuals with RM genotype, it was 32.8% ($P < 0.05$; $\chi^2 = 4.13$) and 31.1% ($P < 0.05$; $\chi^2 = 3.85$), respectively.

DISCUSSION

It was revealed that carriers of SM genotype, which are associated with decreasing of enzyme activity, had higher level rifampicin than carriers of RM genotype.[27] Hence, we can conclude that hepatic enzyme CYP2E1 directly or indirectly takes part in metabolism of rifampicin. Then, we expected that patients with SM genotype will succeed more in TB treatment, but it was not proved. Hence, there was no correlation between treatment outcome of TB patients and rifampicin concentration.

According to the obtained results in TB patients with RM genotype, the indexes of cytolysis (ALT, AST) and bile stasis (GGT) were higher comparatively to SM genotype both before and after inpatient treatment. This correlated with a higher concentration of DC in the blood and lower plasma catalase activity in the patients with RM genotype comparatively with the patients with SM genotypes. Thus, we can conclude that polymorphism of *CYP2E1* genotype is an important criterion for the development of hepatotoxicity before and during TB treatment while increased rifampicin level has no influence on it. The higher level of hepatotoxicity markers in carriers of RM genotype comparatively to SM individuals could be explained by expressive capability of RM to produce toxic metabolites in the liver.[28] Numerous literature data have proved an association between *CYP2E1* polymorphism and liver injury.[10-12] Thus, detection of *CYP2E1*

genotype of TB patients at the beginning of TB treatment could help to recognize a group of the individuals with increased risk of liver injury during therapy.

CONCLUSION

Thus, detection of *CYP2E1* genotype of TB patients at the beginning of TB treatment could help to recognize a group of the individuals with increased risk of liver injury during therapy.

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