Antonenko P.B., Kresyun V.I., Antonenko K.O. Results of intensive phase tretament of tuberculosis treatment according to *CYP2E1* genotype. Journal of Health Sciences. 2014;04(03):027-038. ISSN 1429-9623 / 2300-665X.

The journal has had 5 points in Ministry of Science and Higher Education of Poland parametric evaluation. Part B item 1107. (17.12.2013).

Conflict of interest: None declared. Received: 05.01.2014. Revised 15.03.2014. Accepted: 25.03.2014.

RESULTS OF INTENSIVE PHASE TRETAMENT OF TUBERCULOSIS TREATMENT ACCORDING TO *CYP2E1* GENOTYPE

Antonenko P.B., Kresyun V.I., Antonenko K.O.

Odessa National Medical University, Odessa, Ukraine

Nowadays, tuberculosis (TB) remains a major cause of death among infectious diseases in post-soviet countries, including Ukraine. Despite certain positive changes, for example a reducing the TB incidence during the period 2006-2011from 83.2 to 67.2 per 100 000 population [1,2], it is still a high level of multidrug-resistant tuberculosis, for example primary resistance vary from 7 to 25 %, which significantly reduces the effectiveness of treatment [3,4].

It is known that the efficiency of treatment of numerous diseases, the severity of their course and their outcome largely depends on the genetic characteristics of a person, for example from the polymorphism of genes of xenobiotics detoxification [5,6]. Among the last there is an important gene of cytochrome P-450 2E1 (*CYP2E1*) - a putative enzyme that participate in metabolism of the most effective antituberculosis drug isoniazid [6]. That is why the polymorphism of that gene may affect the concentration of isoniazid and consequently an effectiveness of TB treatment. In previous studies it was

shown that the polymorphism of N-acetyltransferase 2, which determine the concentration of isoniazid, has an influence on outcome of treatment in [7,8]. So the next step was to study the effectiveness of treatment of TB-patients considering *CYP2E1* genotype.

Aim of research: to detect the peculiarities of pulmonary TB course and outcome after in-patient treatment according to *CYP2E1* genotype of the patients with primary TB.

Materials and methods. An analysis of medical cards from 86 patients with primary pulmonary tuberculosis at the end of in-patient treatment in Odessa regional antituberculosal dispensary was conducted in 2012 year. Among enrolled patients 40 (46.5%) were women, others – 46 (53.5%) – were men. The age varied from 18 to 73 years (average – 35.9 years). All TB patients were receiving a standard therapy according to Order of Ministry of Healthcare № 384 from 9.06.2006 that implemented principles of DOTS-strategy [10]. We have considered medical diagnosis at the beginning and at the end of in-patients treatment including TB-form, characteristics of TB-lesions, bacterial excretion etc.

At the beginning of provided treatment it was detected a *CYP2E1* genotype in TB-patients. DNA material was extracted from the blood of donors using a DNA sorbB kit (AmpliSens, Russian Federation). A *CYP2E1* genotype was detected with the help of polymerase chain reaction (PCR) and endonuclease analysis [11,12]. It has been studied presence of mutation in intron 6 and flanking ´5 region with the help of corresponding enzymes *Dra*I and *Rsa*I. Processing of obtained statistical data was performed using Microsoft Excel and «Primer Biostatistica» program.

Results. According to *CYP2E1* genotype out of 86 patients 84 (97.7%) had no mutation in ´5-flanking region (*c1/c1* genotype), others – 2 (2.3%) had one mutated allele (*c1/c2* genotype). Concerning intron 6 the majority of patients 74 (86.0%) had no mutation in this region (*CC* genotype), 11 patients (12.8%) had

one mutated allele (*CD* genotype), 1 patient (1.2%) had both mutated alleles (*DD* genotype). Both patients with mutations in ´5-flanking region, also had a mutation in 6^{th} intron. Further for convenience we formed a group of patients without mutations in 6^{th} intron (*CC* genotype) and a group of patients that had mutation(s) in mentioned locus (*CD*, *DD* genotypes). After completion of in-patient treatment in two patients with *CD*, *DD* genotype (16.7%) a diagnosis of TB was cancelled after the proving pulmonary cancer. In patients with *CC* genotype it was no cases of detected pulmonary cancer, thus,*CD*, *DD* genotype more often associated with pulmonary cancer development that *CC* genotype ($P<0.05$; $\chi^2=14,63$ at a critical value here and after 3.84). In addition in every group it was proved one case of pneumonia that at the beginning was diagnosed as TB. So, in the end hospital stage treatment a diagnosis of pulmonary TB was supported in 73 patients with *CC* genotype and in 9 patients *CD*, *DD* genotype. The medical cards of patients with proved pulmonary TB were enrolled in further statistic processing.

At the beginning of in-patient department treatment the destruction processes in lungs were observed approximately in two-third of the patients with *CD*, *DD* genotype and in 42.5% with *CC* genotype. Approximately 38.4% of the carriers of *CC* genotype and 44.4% of *CD, DD* genotype had bilateral pulmonary TB. Two-third of the patients in both groups had infiltrative TB, others – had disseminative and focal TB.

Among the individuals with *CC* genotype the processes of disintegration and dissemination in lungs occurred in 23.3 and 13.7% respectively. At the same time among the patients with *CD, DD* genotype mentioned above processes were observed in 11.1% and 33.3% correspondently. So, among the patients with *CD, DD* genotype the processes of dissemination were somewhat more frequent than in carriers of *CC* genotype.

At the beginning of in-patient treatment regardless of genotype and according to the microscopy approximately half of patients with genotype *CC*

and one-third of individuals with genotype *CD, DD* were smear positive. According to the cultural method the majority of patients – 67.1% of *CC* genotype carriers and half of *CD, DD* genotype carriers were smear-positive. Also vast majority of the patients with genotype of *CC* and *CD, DD* belonged to I (primary pulmonary tuberculosis) – 72.6% and 77.8% respectively. Other patients belongs either to III category (smear-negative primary tuberculosis) – *CC* genotype or to II category *CD, DD* genotype. Thus, at the beginning of treatment there was no significant differences between TB-patients according to *CYP2E1* polymorphism, however the patients with genotype *CD, DD* had the manifestations of dissemination and destruction in pulmonary tissues somewhat more often than carriers of *CC* genotype.

Duration of in-patient treatment was almost the same in both groups - 93,1±8,0 days for patients with *CC* genotype and 96,2±3,4 days with *CD, DD* genotype.

At the end of in-patient treatment the processes of destruction remained in 20.5% of the patients with *CC* genotype and in 44.4% with *CD, DD* genotype. Termination of destruction was observed in half of the persons with *CC* genotype, in one-third of the *CD, DD* genotype carriers. The longest conversion of destruction was in *CD, DD* patients – 59.7 days; somewhat faster it was CC carriers – 53.5 days.

At the end as well as at the beginning of in-patient treatment in majority of the individuals with *CC* and *CD, DD* genotype (58.9% and 66.7%, respectively) an infiltrative form of a tuberculosis process was observed. The frequency of other TB forms also did not change during treatment.

As a result of the conducted treatment the number of the *CC* genotype carriers with tuberculosis infiltration has decreased at 11.5 times ($P<0.05$ $\chi^2=53.66$), while decreasing of TB-patients with infiltration among *CD, DD* genotype carriers was insignificant. In addition at the time of completion of TB-treatment the signs of pulmonary infiltration among patients with *CD, DD*

genotype have been observed in 6 times more often than in patients with *CC* genotype (P<0,05; χ^2=7,96). In patients with *CC* genotype the number of patients with pulmonary destruction dropped in 2.4 times (P<0,05; χ^2=4,99), with signs of dissemination - in 9,8 times (P<0,05; χ^2=9,08). The symptoms of destruction and dissemination aborted in carriers with *CD, DD* genotype. At the same time, the signs of resorption and consolidation in the pulmonary tissue were observed in 83.5% of the *CC* group and 66.7% of the *CD, DD* group.

Regardless of genotype the percentage of patients with bi- and unilateral TB-lesions of the lungs, almost did not change during in-patient treatment. During conducted treatment the number of patients with genotype *CC*, which belonged to the 4 category (drug-resistant tuberculosis), rose up approximately in 16 times (P<0.05; χ^2=14.98), with genotype CD, DD – on 22.2% (P>0.05). In the end of in-patient treatment in both groups around 22% of patients belong to 4 category. Approximately 23.8% *M.tuberculosis* strains that were obtained from patients *CC* genotype, were multi-resistant (simultaneous resistance to isoniazid and rifampicin); for patients with *CD, DD* genotype it was 25,0% of multi-resistant strains.

According to the bacterioscopy all patients with *CD, DD* genotype, as well as 98.6% of the patients with *CC* genotype at the time of discharge from the hospital were smear-negative. In addition smear conversion has happened in 97.3% individuals with *CC* genotype and 100% - with *CD, DD* genotype. It took 56-62 days for the conversion of smear positive with no significant difference between two groups.

According to the data of the culture method at the end of in-patient treatment 41.1% of the individuals with *CC* genotype as well as 28.6 of individuals with *CD, DD* genotype remained smear-positive. In the group with *CC* genotype number of smear-positive patients decreaed in 1.6 times (P<0,05; χ^2=9,96). Conversion of smear-positive into smear-negative have happened in 38.8% of the patients with *CC* genotype and in half (2 out of 4) patients with

CD, DD genotype. In the same time the conversion of smear-positive patients took 67.1 days for *CC* genotype patients and 95.0 days for *CD, DD* genotype. Thus conversion of the patients with *CD, DD* genotype was in 1.4 times longer than in patients with *CC* genotype (P<0,001; CI=-40,36…-15,42).

The obtained data has proved that at the beginning of treatment there was no significant difference between TB patients concerning the signs of destruction, infiltration and disintegration according to *CYP2E1* polymorphism, however the patients with *CD, DD* genotype more frequently had sings of dissemination and destruction in pulmonary tissues than the patients with *CC* genotype.

At the end of in-patient treatment the processes of resorption and abortion of pulmonary infiltration more often associated with *CC* genotype, than with *CD, DD* genotype. It could be related with difference in metabolism of certain antituberculosis drugs by CYP2E1. In the same time differences in *CYP2E1* genotype almost had no effect on spreading of multiresistant *M.tuberculosis* strains. It will be interesting to check an influence of *CYP2E1* polymorphism in TB-patients on toxicity of antituberculosis therapy. It will be important to check in coming researches an influence of *CYP2E1* polymorphism in TB-patients on toxicity of antituberculosis therapy.

Conclusion.

1. At the beginning of treatment a polymorphism of *CYP2E1* in TB-patients almost has no influence on severity and forms of pulmonary tuberculosis.

2. At the end of in-patient treatment the patients with *CD, DD* genotype more often exhibited signs of infiltration of pulmonary tissues and longer remained smear-positive according to cultural method, than the patients with *CC* genotype.

3. Despite of *CYP2E1* genotype around 25% of tuberculosis patients had multidrug resistant strains of M.tuberculosis in the end of in-patient treatment.

References

1. Feshchenko YuI, Melnyk VM, Matusevych VG et al. Otsenka controlyu za tuberculozom v Ukraine za period 2006-2010 roky [Assessment of tuberculosis control in Ukraine for period 2006-2010]. Ukrainskiy Pulmonologichnyy Zhurnal [Ukrainian Pulmonology Journal]. 2011; 4: 5-10 (Ukr).

2. Falzon D, Jaramillo E, Schünemann HJ, Arentz M. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. Eur. Respir. J. 2011; 38(3): 516-528.

3. Melnyk VM, Novozhylova IO, Matusevych VG, Linnyk MI. Analitychnyy poglyad na problemu chimioresistentnogo tuberculoza: nyneshniy status, dosyagnenya ta deyaki nevyrisheni pytanya [Analytical view on a problem of drug-resistant tuberculosis: current status, achievements and unsolved issues]. Ukrainskiy Pulmonologichnyy Zhurnal [Ukrainian Pulmonology Journal]. 2012; 1: 5-7 (Ukr).

4. Dymova MA, Liashenko OO, Poteiko PI, Krutko VS, Khrapov EA, Filipenko ML. Genetic variation of Mycobacterium tuberculosis circulating in Kharkiv Oblast, Ukraine. BMC Infect Dis. 2011; 11: 77.

5. Castillejos-López Mde J, García-Sancho MC, Quiñones-Falconi F, Pérez-Padilla JR. Cytochrome P450 and NAT2 polymorphisms and drug metabolism in DOTS. Rev. Invest. Clin. 2008; 60(1): 47-57.

6. Ramachandran Geetha, Swaminathan Soumya Role of pharmacogenomics in the treatment of tuberculosis: a review. Pharmacogenomics and Personalized Medicine. 2012; 5: 89–98.

7. Antonenko PB, Kresyun VI. Riven rifampicynu v krovi u hvoryh na tuberculoz z riznum genotypom cytochromu 2C19 [The level of rifampicin in blood of patients with tuberculosis with different cytochrome 2C19 genotype]. Odeskyy medychnyy zhurnal [Odessa Medical Journal]. 2013; 5:16-20 (Ukr.).

8. Antonenko PB, Kresyun VI. Osoblyvosti polymorphismu genu cytochromu-450 2C19 sered hvoryh na tuberculoz [Polymorphism pecularities of cytochrome-450 2C19 in patients with tuberculosis]. Visnuk naukovych doslidzen [Bulletin of scientific research] 2013; 2:32-35 (Ukr).

10. Order from Minisrty of Healthcare of Ukraine № 384 since 09.06.2006 y. *"Pro zatverdjenya protocolu nadanya medichnoy dopomogu chvorum na tuberculos"* ["About approval of the protocol of medical aid for tuberculosis patients"] [Text] : documents. - Kyiv, 2006. – 87 p (Ukr.).

11. Dong-Xin Lin, Yong-Ming Tang, Qiong Peng, Shi-Xin Lu [et al.] Susceptibility to esophageal cancer and genetic polymorphisms in glutathione-S-transferases T1, P1 and cytochrome P450 2E1. Cancer epidemiology, biomarkers and prevention 1998; 7: 1013-1018.

12. Shunji Kato, Peter G. Shields, Neil E. Caporaso, Robert N. Hoover et al. Cytochrome P4502E1 genetic polymorphisms, racial variation, and lung cancer risk. Cancer research 1992; 52: 6712-6715.

RESULTS OF INTENSIVE PHASE TRETAMENT OF TUBERCULOSIS TREATMENT ACCORDING TO *CYP2E1* GENOTYPE

Antonenko P.B., Kresyun V.I., Antonenko K.O.
Odessa National Medical University

Summary. The polymorphism of the gene of cytochrome P-450 2E1 (*CYP2E1*) that participate in metabolism of antituberculosis agent isoniazid may influence on effectiveness of tuberculosis (TB) treatment.

Aim of research: to detect the peculiarities of pulmonary TB course and outcome after in-patient treatment according to *CYP2E1* genotype of the patients with primary TB.

Materials and methods: analysis of medical cards from 86 patients with primary pulmonary tuberculosis at the end of hospital treatment in Odessa regional antituberculosal dispensary was conducted in 2012 year with consideration of *CYP2E1* genotype.

Results: the obtained data has proved that at the beginning of treatment there was no significant difference between TB patients concerning the signs of destruction, infiltration and disintegration according to *CYP2E1* polymorphism, however the patients with *CD, DD* genotype more frequently had sings of dissemination and destruction in pulmonary tissues than the patients with *CC* genotype. At the end of in-patient treatment the patients with *CD, DD* genotype more often exhibited signs of infiltration of pulmonary tissues and longer remained smear-positive according to cultural method, than the patients with *CC* genotype. Despite of *CYP2E1* genotype around 25% of tuberculosis patients had multidrug resistant strains of M.tuberculosis in the end of in-patient treatment. It will be important to check in coming researches an influence of *CYP2E1* polymorphism in TB-patients on toxicity of antituberculosis therapy.

Key words: tuberculosis, CYP2E1, treatment outcome

Table 1

The characteristics of TB-processes according to *CYP2E1* genotype

The characteristics of TB-processes		At the beginning of treatment, (%)		At the end of in-patient treatment, (%)	
		CC, n=73	*CC, CD*, n=12	*CC*, n=73	*CC, CD*, n=12
Spreading	bi-lateral	28 (38,4)	4 (44,4)	27 (37,0)	4 (44,4)
	unila-teral	45 (61,6)	5 (55,6)	46 (63,0)	5 (55,6)
Destruction	yes	31 (42,5)	6 (66,7)	15 (20,5)	4 (44,4)
	no	42 (57,5)	3 (33,3)	58 (79,5)	5 (55,6)
Stage of TB-process	infil-tration	46 (63,0)	5 (55,6)	4 (5,5)#	3 (33,3)*
	disinteg ration	17 (23,3)	1 (11,1)	7 (9,6)#	-
	dissemi nation	10 (13,7)	3 (33,3)	1 (1,4)#	-
	resorp-tion	-	-	61 (83,5)	6 (66,7)
Category of patients	1	53 (72,6)	7 (77,8)	46 (62,7)	6 (66,7)
	2	4 (5,5)	2 (22,2)	3 (4,5)	1 (11,1)
	3	15 (20,5)	-	8 (10,4)	-
	4	1 (1,4)	-	16 (22,4)	2 (22,2)

Footnote: # - $P<0.05$ (relatively to the initial level of the correspondent group);

* - $P<0.05$ (relatively to the patients with *CC* genotype)

Table 2

Conversion of destruction and smear positive depending on *CYP2E1* genotype

Group of patients	Conversion of destruction		Conversion of smear positive according to			
			bacterioscopy		culture	
	number of patients (%)	duration (days) ± SEM	number of patients (%)	duration (days) ± SEM	number of patients (%)	duration (days) ± SEM
CC	16/31 (51,6)	53,50±1,41	36/37 (97,3)	56,83±1,80	19/49 (38,8)	67,11±1,21
CC, CD	2/6 (33,3)	59,71±1,87	4/4 (100)	62,00±1,05	2/4 (50,0)	95,00±15,56*

Footnote: * - P<0.05 (relatively to the patients with *CC* genotype)

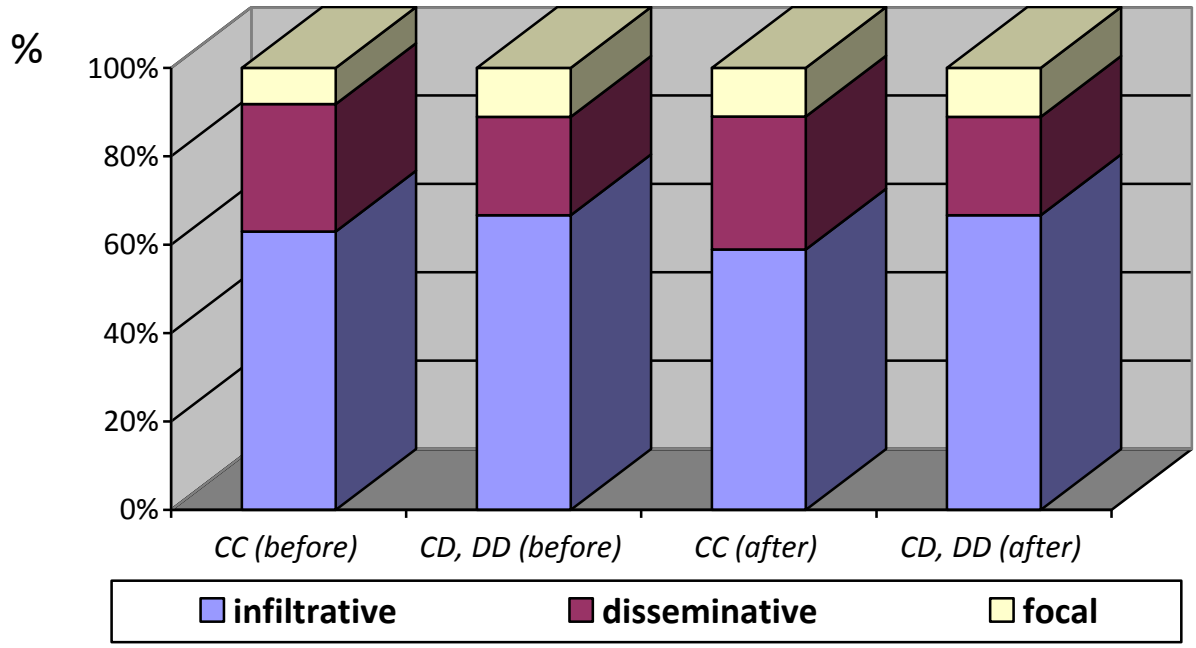

Fig. 1 Forms of pulmonary TB taken into consideration *CYP2E1* genotype at the beginning (before) and at the end (after) of in-patient treatment

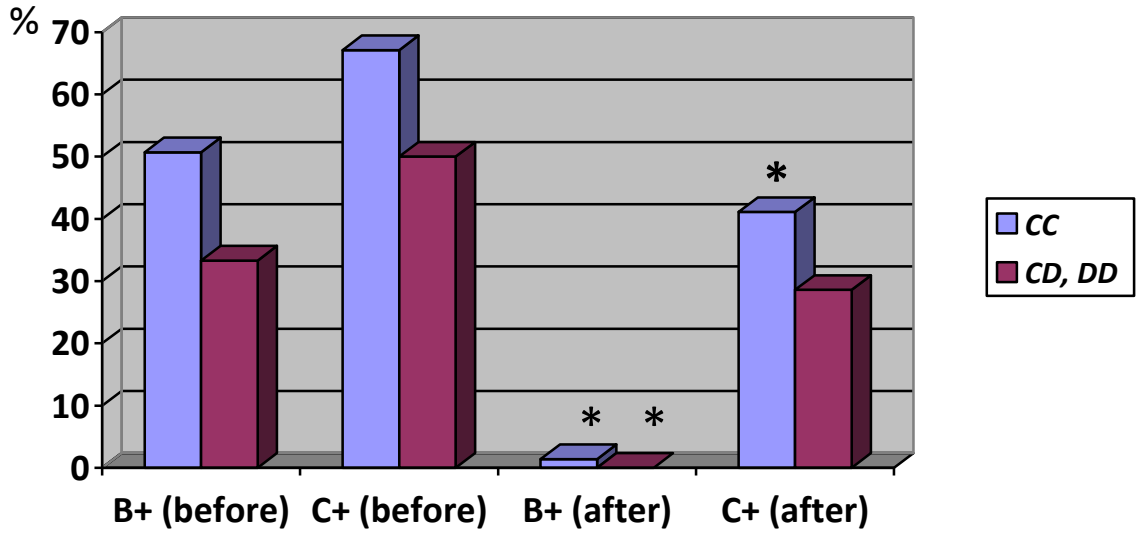

Fig. 2 Number of the patients with different *CYP2E1* genotype that were smear positive according to bacterioscopy (B+) or culture method (C+) at the beginning (before) and at the end (after) in-patient treatment

Footnote: *-P<0.05 relatively to the initial level of the correspondent group

AUTHOR'S INFORMATION

Antonenko Petro Borisovich – candidate of medical science (PhD), associative professor (docent) of department of general and clinical pharmacology ONMedU
W. tel.: 048-7173545
Mob. tel.: 097-5875636
E-mail: peterantonenko@yandex.ru
Address: Department of general and clinical pharmacology ONMedU, Valichovsky lane,2, Odessa, 65082

Kresyun Valentin Iosipovich – first pro-rector Odessa National Medical University (ONMedU), corr.-member. NAMS Ukraine, doctor of medical science, professor, head of department of general and clinical pharmacology ONMedU

Antonenko Kateryna Oleksiivna – candidate of biological science (PhD), senior laborant of department of general and clinical pharmacology ONMedU