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POLYMORPHISM OF *CYP3A4*1G GENE* AS A PREDICTOR OF THE HEPATOTOXICITY OF ANTITUBERCULOSIS THERAPY

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Ключевые слова: *цитохром Р-4503А4, полиморфизм гена, гепатотоксичность, туберкулез*

Abstract. Polymorphism of CYP3A4*1G gene as a predictor of the hepatotoxicity of antituberculosis therapy. Poludenko H.O., Antonenko P.B., Antonenko K.O., Makarenko O.V. *The risk of anti-tuberculosis (ATB) drug-induced liver injury could be determined by genotype polymorphism of the xenobiotic-metabolizing enzymes. The aim of presented research was the investigation of an impact of CYP3A4*1G polymorphism on liver function in patients with TB during anti-tuberculosis therapy. There were analyzed case histories of 105 patients with newly diagnosed pulmonary TB at Odessa Regional TB Hospital in 2012-2014. We have considered their medical records at the beginning and at the end of inpatient treatment including activity of biochemical indices such as total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutathione transferase (GGT). The genotype CYP3A4*1G, 20230G>A was detected by PCR. At the beginning of the treatment the level of studied biochemical indices was almost the same regardless of CYP3A4*1G genotype. After the conducted in-patient treatment the biochemical indices in fast metabolizers insignificantly increased, while the level of bilirubin dropped by 10.4% ($p<0.05$). In slow metabolizers after in-patient treatment the serum total bilirubin level increased by 8.0% ($p<0.05$), the activity of ALT raised by 67.2% ($p<0.05$), AST – by 37.4% ($p>0.05$), also the number of the patients with ALT and AST level beyond normal almost doubled. After completion of in-patient treatment in moderate and slow metabolizers serum GGT activity increased by 2.5 times ($p<0.05$) and 1.3 times ($p>0.05$) correspondently, among fast metabolizers – on the contrary, the number of the individuals with increased GGT level dropped ($p<0.05$). Thus in slow metabolizers according to CYP3A4*1G genotype after completion of in-patient stage of anti-TB treatment the level of cytolysis and toxicity indexes was much higher than in fast metabolizers. That is why detection of CYP3A4*1G 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.*

Реферат. Полиморфизм гена CYP3A4*1G как предиктор гепатоксичности противотуберкулезной терапии. Полуденко А.А., Антоненко П.Б., Антоненко Е.А., Макаренко О.В. *Риск лекарственного повреждения печени противотуберкулезными препаратами зависит от полиморфизма ферментов, метаболизирующих ксенобиотики. Целью данного исследования было изучение влияния полиморфизма CYP3A4*1G на функциональное состояние печени у больных туберкулезом (ТБ) легких во время противотуберкулезной терапии. Был проведен анализ медицинских карт 105 больных с впервые выявленным ТБ легких в Одесском областном противотуберкулезном диспансере в 2012-2014 гг. Учитывали биохимические показатели, такие как билирубин, аланинаминотрансфераза (АлТ), аспартатаминотрансфераза (АсТ) и гамма-глутатионтрансфераза (ГТФ) в начале и при завершении стационарного лечения. Генотип CYP3A4*1G, 20230G>A определяли с помощью ПЦР. В начале лечения уровень исследованных биохимических показателей практически не отличался у носителей разного генотипа CYP3A4*1G. После проведенного лечения биохимические показатели у быстрых метаболизаторов незначительно выросли, однако уровень билирубина, наоборот, снизился на 10,4% ($p<0,05$). У медленных метаболизаторов после стационарной фазы лечения уровень общего билирубина в крови увеличился на 8,0%, активность АлТ выросла на 67,2% ($p<0,05$), АсТ – на 37,4% ($p>0,05$); также количество пациентов с превышением*

*нормального уровня практически удвоилось. После стационарного лечения у умеренных и медленных метаболизаторов активность ГТФ увеличилась в 2,5 (p<0,05) и 1,3 раза (p>0,05) соответственно, среди быстрых метаболизаторов – наоборот, количество с повышенным уровнем ГТФ уменьшилось (p<0,05). Таким образом, у медленных метаболизаторов согласно генотипу CYP3A4*1G после завершения стационарной фазы противотуберкулезной фазы уровень маркеров цитолиза и интоксикации был значительно выше, чем у быстрых метаболизаторов. Поэтому определение генотипа CYP3A4*1G у ТБ-больных в начале противотуберкулезной терапии позволит определить группы больных с повышенным риском лекарственного поражения печени.*

Tuberculosis (TB) remains an important problem for Eastern European countries, including Ukraine. Unfortunately, there is a spread of multidrug-resistant (MR TB) and extensively drug-resistant (DR TB) tuberculosis, TB/HIV co-infections [3]. Frequent reasons for treatment interruptions are an increase in the number of adverse reactions at the end of the main course of chemotherapy [2]. Among the measures that can prevent the development of side effects of anti-tuberculosis therapy, an important place is the personalization of treatment, that is, the correction of pharmacotherapy depending on the genetic characteristics of patients [1]. It is known that in tuberculosis patients who are fast metabolizers according to the *CYP2E1* genotype, slow acetylators according to the *NAT2* genotype, or slow metabolizers *CYP3A4*1B*, the risk of liver damage is higher [5, 6, 10]. According to the literature, the enzyme cytochrome (CYP) 3A4/5 is involved in the metabolism of more than a third of drugs [9]. The activity of the enzyme is largely determined by the polymorphism of the corresponding *CYP3A* genes [9]. It is known that the presence of the polymorphic allele **1G* is accompanied by a slowdown in the metabolism of the opioid fentanyl, which is associated with a decrease in the expression of CYP3A4 mRNA; with an increase in the risk of ischemic stroke [7, 11], an increase in the hypolipidemic effectiveness of atorvastatin and the hypotensive effect of amlodipine [8, 13]. At the same time, in the literature there are no studies on the significance of the *CYP3A4*1G* polymorphism in tuberculosis patients.

The aim of this study was to study the significance of the *CYP3A4*1G* polymorphism for the functional state of the liver in patients with pulmonary TB receiving anti-tuberculosis therapy.

MATERIALS AND METHODS OF RESEARCH

An analysis of the medical records of 105 patients with pulmonary tuberculosis, diagnosed for the first time at the end of inpatient treatment at Odesa Regional TB Hospital in 2012-2014 was carried out.

The study was conducted in accordance with the principles of bioethics set forth in the Declaration of Helsinki "Ethical Principles of Medical Research Involving Humans" and the "Universal Declaration of Bioethics and Human Rights (UNESCO)".

All tuberculosis patients received standard therapy, in accordance with the order of the Ministry of Health of Ukraine No. 384 dated 06.9.2006. Biochemical parameters were taken into account: total bilirubin, thymol test for alanine aminotransferase (ALT), aspart-tataminotransferase (AST), gamma-glutamyltransferase (GGT), which were measured on the HumaStar300 automatic analyzer ("Human GmbH," Germany). To maintain quality, daily Serodos and monthly Prevecal international control were carried out, as well as annual verification at the state institution "Odesa Regional Center for Standardization, Metrology and Certification". In the first week of treatment, the *CYP3A4*1G*, 20230G>A genotype using PCR was determined in patients [13]. Statistical data were calculated using Statistica 10.0 software (Dell Software, Austin, TX, USA; Serial number: STA999K347150-W). If necessary, both parametric methods (t-test) and non-parametric methods (Mahn-Whitney, Sign test, $\chi 2$-test) of statistical data processing were used. The Shapiro-Wilk test was used to determine the normality of the distribution.

RESULTS AND DISCUSSION

Homozygous individuals with a wild type for the studied CYP3A4*1G gene were defined as fast metabolizers *(*1/*1)*; individuals who had one mutated allele were defined as moderate metabolizers *(*1/*1G)*; and individuals who were homozygous for the mutant gene were defined as slow metabolizers *(*1G/*1G)*. It was established that among 105 tuberculosis patients, 96 (91.4%) individuals belonged to fast metabolizers, 5 (4.8%) and 4 (3.8%) individuals belonged to moderate and slow metabolizers. At the beginning of inpatient treatment, the highest level of bilirubin was observed in carriers of the fast metabolizer genotype, the level was slightly lower in moderate and slow metabolizers, and in the latter it was 30.2% lower than in fast metabolizers (p=0.007) (Table 1).

Hyperbilirubinemia was observed in almost a third of carriers of the fast metabolizer genotype; among moderate metabolizers there were 20% of such patients, among slow metabolizers – none (Fig. 1A).

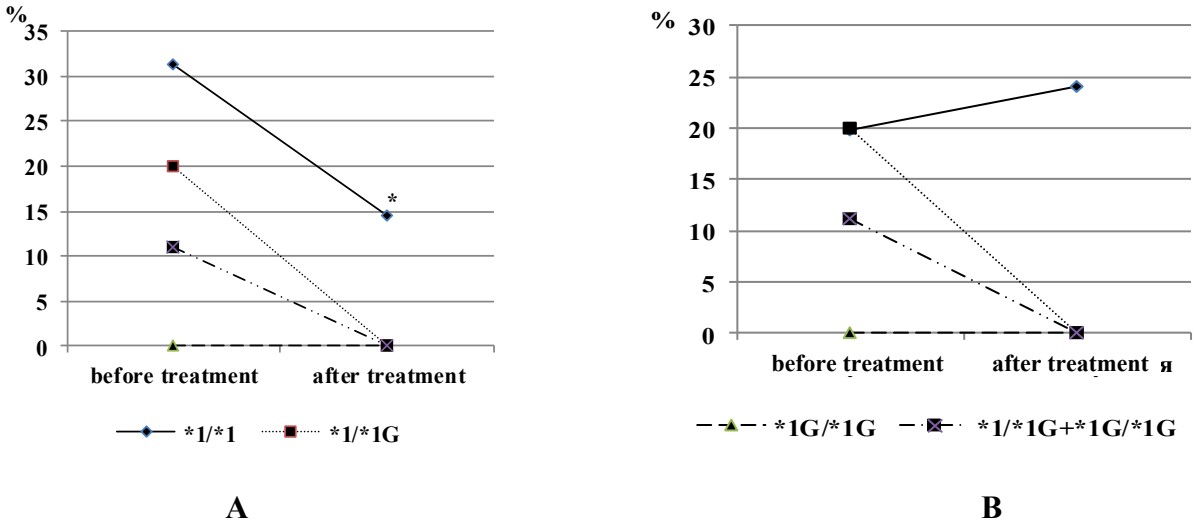
Table 1

Biochemical indices at the onset of treatment depending on genotype *CYP3A4*1G* (M±SEM)

	Genotype *CYP3A4*1G*			
	****1/1***	****1/1G***	****1G/1G***	****1/1G+*1G/1G***
Total bilirubin	**14.67±0.53**	**11.93±1.40**	**8.70±1.42 P_1=0.007**	**10.24±0.82**
Thymol test	**2.28±0.19**	**1.85±0.79**	**2.60±0.42**	**2.23±0.43**
ALT	**23.99±1.57**	**22.67±4.91**	**22.00±3.16**	**22.34±1.39**
AST	**28.47±1.52**	**26.20±6.68**	**19.00±3.92**	**23.00±3.93**
GGT	**29.44±2.30**	**33.33±4.23**	**28.67±4.99**	**29.00±2.93**

Note: p_1 – compared with the group **1/*1*.

At the onset of the treatment, the highest activity of cytolysis indices – enzymes ALT and AST was observed in fast metabolizers, the lowest – in slow metabolizers, at the same time the difference was unreliable. About a fifth part of patients had an increase in the activity of AST and about a quarter of patients had an increase in the activity of ALT (Fig. 2A, 2B).



* p<0.05 (relative to initial state)

Fig. 1. Number of patients with the increased level of total bilirubin (A) and thymol test (B) in blood before and after treatment

Every fifth patient among carriers of the genotype of fast and moderate metabolizers had increased activity of the cholestasis index of glutathione-transferase and thymol test, while among carriers of the genotype of slow metabolizers there were no such patients. Also, there were no significant differences in the average level of GGT activity and thymol test in carriers of different **1G* genotypes. Among carriers of the genotype of fast and moderate metabolizers, approximately 20% of patients had indicess that exceeded the normal level, while among slow metabolizers there were no such patients (Fig. 3).

After the end of the inpatient stage of treatment in fast metabolizers, a decrease in the content of total bilirubin in the blood by 10.4% (p=0.023; CI=-2.85...-0.21) was observed; a certain decrease was also observed in moderate metabolizers (Table 2). Also, the number of patients with hyperbilirubinemia among fast and moderate metabolizers decreased relative to the initial index – from 31.3% to 14.6% in fast metabolizers (p=0.010) and from 20% to 0 in moderate metabolizers (p>0.05) (Fig. 1A).

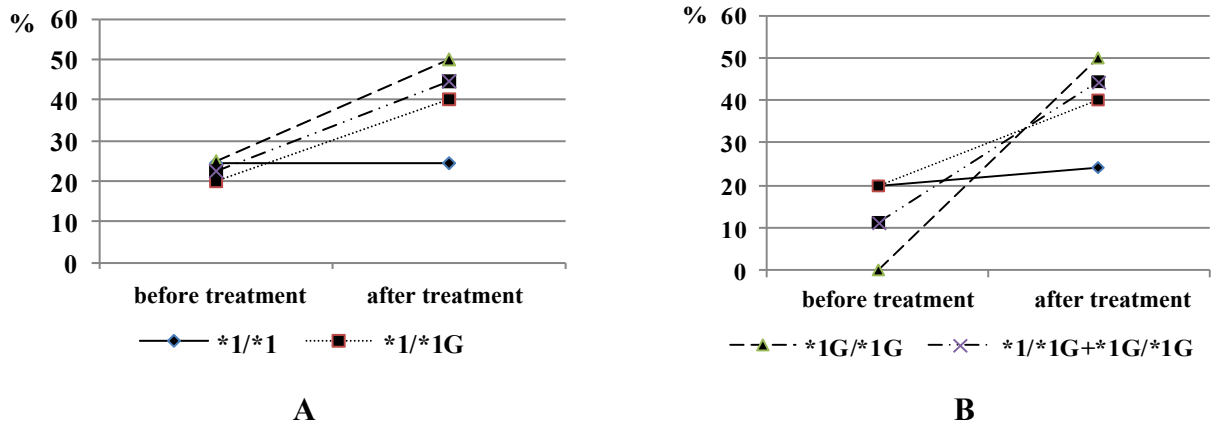
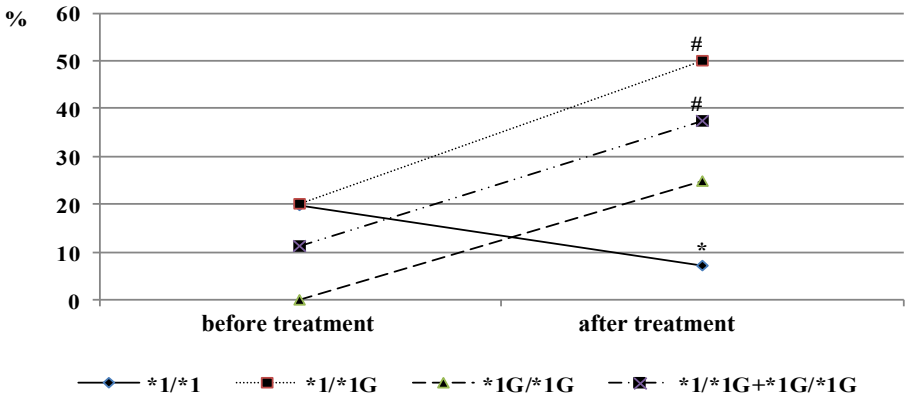


Fig. 2. Number of patients with the increased activity of ALT (A) and AST ACT (B) in blood before and after treatment

At the end of inpatient treatment, there was an incredible decrease in thymol test indices in moderate and slow metabolizers, while in fast metabolizers this index remained almost unchanged both in terms of the average level and the number of patients with an excess of the normal thymol test index – 19.8% before treatment and 24.0% after completion of treatment (p>0.05) (Table 2; Fig. 1B).

After inpatient treatment, the activity of cytolysis markers ALT and AST in tuberculosis patients with the genotype of fast metabolizers increased unreliably by 6.0% and 2.2% (p>0.05). In slow metabolizers, the activity of ALT and AST increased by 67.2% (p<0.05) and by 37.4% (p>0.05); the number of patients with ALT and AST activity that exceeded the normal level also doubled, but, given the relatively small number of patients with a polymorphic allele and a significant margin of error, the difference was unreliable (Table 2; Figs. 2A and 2B). Among carriers of the genotype of slow metabolizers, the activity of ALT and AST during treatment increased by 72.7% (P=0.033; CI=-30.14...-1.86) and 110.5% (P=0.049); every second patient had an excess of the normal level of ALT and AST, although before the onset of treatment, these were 25% and 0%, respectively. GGT activity during treatment in fast metabolizers practically did not change, although the number of patients with exceeding the limit indices decreased from 19.8% to 7.3% (p=0.02). In moderate and slow metabolizers GGT activity increased by 2.5 (p=0.001; CI=-60.85...-19.49) and 1.3 times (p>0.05), respectively; the number of patients with the genotype of moderate metabolizers increased by 2.5 times relative to the initial level (p>0.05) (Table 2; Fig. 3). Among moderate metabolizers, the average GGT activity at the end of inpatient treatment decreased by 2.4 times compared to fast metabolizers (p1<0.001; CI=-53.79...-32.35).



* – p<0.05 (relative to initial state); # – p<0.05 (relative to the group with genotype **1/*1*)

Fig. 3. Number of patients with the increased activity of GGT in blood before and after treatment

Table 2

Biochemical indices at completion of inpatient treatment depending on genotype *CYP3A4*1G* (M±SEM)

	Genoytpe *CYP3A4*1G*			
	****1/1***	****1/1G***	****1G/1G***	****1/1G+*1G/1G***
Total bilirubin	**13.14±0.41 p_2=0.023 (CI=-2.85…-0.21)**	**10.80±0.89**	**12.88±0.67 p_2<0.001 (CI=2.33…6.03)**	**11.99±0.61**
Thymol test	**2.38±0.18**	**1.08±0.29**	**1.88±0.18**	**1.48±0.21**
ALT	**25.43±1.62**	**36.67±10.78**	**38.00±4.84 p_2=0.033 (CI=-30.14…-1.86)**	**37.29±8.23 p_1=0.045 (CI=-23.42…-0.30) p_2=0.005 (CI=-25.17…-4.73)**
AST	**29.10±1.42**	**37.00±6.15**	**41.00±8.24 p_2=0.049**	**38.78±4.41 p_2=0.017 CI=-28.30…-3.26 p_1=0.048 CI=-19.26…-0.10**
GGT	**30.43±1.17**	**73.50±7.91 p_1<0.001 (CI=-53.79..-32.35) p_2=0.002 (CI=-60.85..-19.49)**	**36.00±5.04**	**53.20±6.59 p_2=0.004 CI=-39.49…-8.91 p_1<0.001 CI=-31.28…-14.26**

Notes: p_1 – compared with the group **1/*1;* p_2 – compared with the state before treatment.

Also, among carriers of the genotype of moderate metabolizers, cases of exceeding the limit indices of GGT occurred 6.8 times more often than among fast metabolizers (p=0.042).

It is known that the level of bilirubin and thymol in the blood characterizes the detoxifying function of the liver. Therefore, at the onset of treatment, the highest content of bilirubin was observed in carriers of the genotype of fast metabolizers, the lowest – in slow metabolizers. After the inpatient phase of anti-tuberculosis therapy, the content of bilirubin decreased in fast metabolizers and to some extent – in moderate metabolizers. Perhaps this is due to the property of some anti-tuberculosis drugs, in particular rifampicin to induce the enzymatic function of the liver with a gradual decrease in the content of rifampicin and the number of patients with hyperbilirubinemia [12]. At the same time, the bilirubin content probably increased only in carriers of the genotype of slow metabolizers, which is probably related to the lower ability of rifampicin to induce liver enzymes and the deterioration of the detoxifying function of the liver in this group of patients. At the beginning, the activity of cytolysis indices ALT and AST probably did not differ between the groups, however, a certain tendency was observed for a higher activity of AST in fast metabolizers than in moderate and especially slow metabolizers – the lowest activity of AST was observed in the latter. The inpatient stage of treatment was accompanied by a slight increase in the activity of cytolysis indices in carriers of the fast metabolizer genotype and a significant increase in both average activity indices and an increase in the number of patients with hyperfermentemia among carriers of polymorphic alleles (moderate and slow metabolizers). At the onset of treatment, the highest level of GGT in the blood plasma, which is considered as a marker of cholestasis, was observed in moderate metabolizers, slightly lower in fast and slow metabolizers. During inpatient treatment, GGT activity in fast metabolizers practically did not change, while the number of patients with hyperenzymemia even decreased relative to the initial level. Among carriers of the genotype of slow and especially moderate metabolizers, an increase in GGT activity was observed, as well as an increase in the number of patients with hyperenzymemia.

The given data indicate that at the onset of treatment significant differences in liver function were not observed in carriers of different *CYP3A4*1G* genotypes, although carriers of the fast metabolizers genotype had the highest indicators of cytolysis markers, higher bilirubin content; in carriers of the genotype of slow metabolizers – on the contrary, the indicated indicators were the lowest. After the inpatient treatment, the bilirubin content decreased in carriers of the fast metabolizer

genotype, which is probably related to the induction of liver enzymatic systems under the influence of rifampicin, the indicators of cytolysis and cholestasis did not change significantly. The presence of a polymorphic allele was associated with a significant increase in the activity of cytolysis markers ALT and AST, especially in the case of a homozygous state of the polymorphic allele (slow metabolizers); increase in the activity of the cholestasis marker GGT (mostly in the heterozygous state of the allele - moderate metabolizers). In the literature, there are certain contradictions regarding the impact of the studied polymorphism on metabolic activity – according to some data, the presence of the variant allele *1G is associated with a decrease in enzyme activity and an increase in the content of medicinal preparations (fentanyl, atorvastatin, and amlodipine) [8, 11, 13], according to others – on the contrary, with an increase in enzyme activity and a decrease in the content of drugs (cyclosporine) [4]. According to our data, the presence of the variant allele was accompanied by a slowdown in the biosynthetic (second) phase of biotransformation in the liver, as well as by intensive cytolysis, including in the liver, which is probably associated with a slowdown in biotransformation of antituberculosis drugs and the accumulation of toxic compounds.

CONCLUSIONS

1. Polymorphism of the CYP3A4*1G gene does not have no significance for the initial functional state of the liver in patients with tuberculosis.
2. The presence of the genotype of slow metabolizers is an unfavorable factor in terms of the probability of liver damage, deterioration of the detoxifying function of the liver during antituberculosis therapy.
3. Determination of the CYP3A4*1G genotype in tuberculosis patients makes it possible to identify risk groups for liver damage, which will allow timely correction of pharmacotherapy.

Contributors:

Poludenko H.O. – methodology, validation, research, writing – reviewing and editing, visualization;

Antonenko P.B. – conceptualization, formal analysis, project administration, management;

Antonenko K.O. – methodology, validation, research;

Makarenko O.V. – conceptualization, formal analysis, project administration.

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