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NAT2 GENE POLYMORPHISMS IS A RISK FACTOR IN PATIENTSCO-INFECTED WITH HIV AND TUBERCULOSIS

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Abstract

Actuality. The number of human immunodeficiency virus (HIV)-infected patients with active tuberculosis (TB) and multiple/extensive drug resistance has increased in recent years. Backup drugs with high hepatotoxicity are used in the treatment regimens of this category of patients. Identification of markers of adverse events in the form of hepatotoxicity by determining the type of acetylation in patients with HIV infection and TB when prescribing drugs metabolized by NAT2 enzyme may influence the selection of optimal doses and reduce the toxic effect on the liver.

The **aim** of the research was to determine the features of N-acetyl-transferase 2 (NAT2) gene polymorphisms in HIV-infected patients with active tuberculosis with advanced drug-induced liver damage.

Materials and methods. NAT2 genotype and haplotype determination was performed in 151 TB/HIV-infected patients receiving drugs with different types of acetylation. Determination of allele frequencies for six single nucleotide polymorphisms (SNPs) of the genotype was used by real-time PCR, allele verification was confirmed by pyrosequencing using the AmpliSense® Pyroskrin PHARMA-screen-2a reagent kit. Analysis was performed using IBM SPSS Statistics 29.0.2.0. significance of differences in frequency distribution of variables was used for Pearson's Chi-square criterion. It was used as a measure of relative risk with a regression confidence interval of 95% and a significance level of 5% ($p < 0.05$). Reliability of differences in median values was determined based on the Mann-Whitney criterion for two groups, the Kraskell-Wallis criterion for multiple comparison of groups ($p < 0.05$).

Results. A larger proportion of patients were found to have a slow type of acetylation (57.6%). Among the haplotypes causing the slow type of acetylation the most frequent were haplotypes NAT2*5B (48.3%) and NAT2*6A (40.2%). The observation showed more frequent increase of aminotransaminase activity from 3 to 10 norms from the upper limit in patients with slow type of acetylation of the NAT2 enzyme gene. They accounted for 43.7% of the total number of identified cases of the slow type of acetylation. The median value of aminotransaminase levels was significantly higher in patients with slow type of acetylation compared to patients in the fast acetylation group ($p < 0.0001$).

Conclusions. Slow type of acetylation should be considered a high risk factor for hepatotoxicity in TB/HIV patients when prescribed anti-TB drugs. Data on isoniazid acetylation and NAT2 genotypes can be used to improve treatment quality and reduce the risk of isoniazid toxicity in TB treatment.

Keywords. *hepatotoxicity, tuberculosis, HIV, acetylation type, isoniazid, NAT2.*

Резюме

ПОЛИМОРФИЗМ ГЕНА NAT2 – ФАКТОР РИСКА У ПАЦИЕНТОВ С КО-ИНФЕКЦИЕЙ ВИЧ И ТУБЕРКУЛЕЗОМ

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Актуальность. В последние годы увеличилось число инфицированных вирусом иммунодефицита человека (ВИЧ) пациентов с активным туберкулезом (ТБ) и множественной/широкой лекарственной устойчивостью. В схемах лечения данной категории больных используются резервные препараты с высокой гепатотоксичностью. Выявление маркеров нежелательных явлений в виде гепатотоксичности путем определения типа ацетилирования у больных ВИЧ-инфекцией и туберкулезом при назначении препаратов, метаболизирующихся ферментом NAT2, может повлиять на подбор оптимальных доз и снизить токсическое действие на печень.

Цель исследования - определить особенности полиморфизма гена N-ацетилтрансферазы 2 (NAT2) у ВИЧ-инфицированных больных активным туберкулезом с распространенным лекарственным поражением печени.

Материалы и методы. Определение генотипа и гаплотипа NAT2 проведено у 151 больного ТБ/ВИЧ, получавшего препараты с различными типами ацетилирования. Для определения частот аллелей шести однонуклеотидных полиморфизмов (SNP) генотипа использовали метод ПЦР в реальном времени, верификацию аллелей подтверждали методом пиросеквенирования с использованием набора реагентов AmpliSense® Pyroskrin PHARMA-screen-2a. Анализ проводился с использованием IBM SPSSStatistics 29.0.2.0. Достоверность различий в частотном распределении переменных использовалась для критерия хи-квадрат Пирсона. Его использовали в качестве меры относительного риска с доверительным интервалом регрессии 95% и уровнем значимости 5% ($p < 0,05$). Достоверность различий медианных значений определяли на основе критерия Манна-Уитни для двух групп, критерия Краскела-Уоллиса для множественного сравнения групп ($p<0,05$).

Полученные результаты. У большей части больных выявлен медленный тип ацетилирования (57,6%). Среди гаплотипов, вызывающих медленный тип ацетилирования, наиболее часто встречались гаплотипы NAT2*5B (48,3%) и NAT2*6A (40,2%). Наблюдение показало более частое повышение активности аминотрансаминаз от 3 до 10 норм от верхней границы у пациентов с медленным типом ацетилирования гена фермента NAT2. На их долю пришлось 43,7% от общего числа выявленных случаев медленного типа ацетилирования. Медианное значение уровня аминотрансаминаз было достоверно выше у пациентов с медленным типом ацетилирования по сравнению с пациентами в группе быстрого ацетилирования ($p < 0,0001$).

Выводы. Медленный тип ацетилирования следует рассматривать как высокий фактор риска гепатотоксичности у больных ТБ/ВИЧ при назначении противотуберкулезных препаратов. Данные об ацетилировании изониазида и генотипах NAT2 могут быть использованы для улучшения качества лечения и снижения риска токсичности изониазида при лечении туберкулеза.

Ключевые слова. *гепатотоксичность, туберкулез, ВИЧ, тип ацетилирования, изониазид, NAT2.*

Түйіндеме

NAT2 ГЕНІ ПОЛИМОРФИЗМІ АҚТҚ-ЖҰҚПАСЫ ЖӘНЕ ТУБЕРКУЛЗ МЕНЕН ҚОС ИНФЕКЦИЯЛАНҒАН НАУҚСТАРДАҒЫ ТӘУЕКЕЛ ФАКТОРЫ

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Өзектілігі. Соңғы жылдары адамның иммун тапшылығы вирусын (АИТВ) жұқтырған, белсенді туберкулезбен және бірнеше / экстенсивті дәріге төзімділігімен ауыратын науқастардың саны артты. Гепатотоксикалық әсері жоғары резервтік препараттар пациенттердің осы санатын емдеу режимдерінде қолданылады. NAT2 ферментімен метаболизденетін препараттарды тағайындау кезінде АИТВ-инфекциясы және туберкулезбен ауыратын науқастарда ацетилдену түрін анықтау арқылы гепатоуыттылық түріндегі жағымсыз құбылыстардың маркерлерін анықтау оңтайлы дозаларды таңдауға әсер етуі және бауырға уытты әсерін төмендетуі мүмкін..

Зерттеудің мақсаты белсенді туберкулезі бар АИТВ жұқтырған науқастарда n-ацетилтрансфераза 2 (NAT2) генінің полиморфизмдерінің ерекшеліктерін анықтау болды.есірткіден туындаған бауырдың зақымдануы).

Материалдар мен әдістер. NAT2 генотипі мен гаплотипін анықтау ацетилденудің әртүрлі түрлері бар препараттарды қабылдаған туберкулез / АИТВ жұқтырған 151 пациентте жүргізілді. Генотиптің алты бір нуклеотидті

полиморфизмі (SNPs) үшін аллель жиіліктерін анықтау нақты уақыттағы ПТР ӘДІСІМЕН қолданылды, Аллельді тексеру AmpliSense® Pyroskrin PHARMA-screen-2a реагенттер жинағы арқылы пиросеквенирлеу арқылы расталды. ТАЛДАУ IBM SPSS Statistics 29.0.2.0 көмегімен жүргізілді. Пирсонның Хи-квадрат критерийі үшін айнымалылардың жиіліктік таралуындағы айырмашылықтардың маңызы пайдаланылды. Ол регрессияға сенімділік аралығы 95% және маңыздылық деңгейі 5% (p <0,05) болатын салыстырмалы тәуекел өлшемі ретінде пайдаланылды. Медианалық мәндердегі айырмашылықтардың сенімділігі Екі топқа Арналған Манн-Уитни критерийі, топтарды бірнеше рет салыстыру Үшін Краскелл-Уоллис критерийі негізінде анықталды (p <0,05

Нәтижелер. Пациенттердің көп бөлігінде ацетилденудің баяу түрі анықталды (57,6%). Ацетилденудің баяу түрін тудыратын гаплотиптердің ішінде ен жиі кездесетіні NAT2*5b (48,3%) және NAT2*6a (40,2%) гаплотиптері болды. Бақылау NAT2 ферменті генінің ацетилденуінің баяу түрі бар емделушілерде аминотрансаминаза белсенділігінің жоғарғы шектен 3-тен 10 нормаға дейін жиірек жоғарылағанын көрсетті. Олар ацетилденудің баяу түрінің анықталған жағдайларының жалпы санының 43,7% құрады. Аминотрансаминаза деңгейінің орташа мәні ацетилденудің баяу түрі бар емделушілерде жылдам ацетилдену тобындағы емделушілермен салыстырғанда айтарлықтай жоғары болды (p < 0,0001).

Қорытынды. Ацетилденудің баяу түрін туберкулезге қарсы препараттарды тағайындаған кезде туберкулез / АИТВ-мен ауыратын науқастарда гепатоуыттылықтың жоғары қауіп факторы ретінде қарастырған жөн. Изониазидті ацетилдеу және NAT2 генотиптері туралы деректерді емдеу сапасын жақсарту және туберкулезді емдеуде изониазидтің уыттылық қаупін азайту үшін пайдалануға болады.

***Түйінді сөздер**. гепатоуыттылық, туберкулез, АИТВ, ацетилдену түрі, изониазид, NAT2.*

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Introduction

According to the results of 2023, Russia recorded a historical minimum of TB morbidity and mortality, but the disease requires constant monitoring and control, as in some regions of Russia there is still a rather unfavorable situation [1]. And, despite the successes in the fight against TB in Russia, as well as worldwide, there have been certain shifts in the structure of the tuberculosis process that significantly affect the epidemiologic situation. First of all, this is due to the annual increase in the proportion of patients with active multidrug-resistant and extensively drug-resistant TB (MDR/XDR-TB) with an indicator of about 30% of all TB cases [2]. In addition, HIV patients are 21 times more likely to get TB than people without HIV and are at the highest risk of developing MDR/XDR-TB. At the same time, TB is the most common disease among those infected with HIV, including those receiving antiretroviral therapy (ART). This requires the combined administration of a large list of drugs with high hepatotoxicity [3]. The development of idiosyncratic hepatotoxicity is a complex process involving both parallel and sequential events that determine the direction of the pathways, the extent of liver damage and its outcome.

Among first-line antituberculosis drugs, isoniazid is a key drug and is widely used in the treatment and prevention of TB, showing selective antimicrobial activity against Mycobacterium tuberculosis. isoniazid is effective in reducing the incidence of TB disease. isoniazid is effective in reducing TB incidence both alone and in combination with ART [4]. Unfortunately, INH, which plays an important role in TB therapy, is associated with hepatotoxicity, Approximately 10-20% of patients taking INH experience a temporary increase in serum alanine aminotransferase (ALT) levels. Most patients can adapt to it and serum ALT levels return to normal without discontinuation of the drug, while some patients (1-3%) develop severe liver damage up to liver failure [3].

The cause of hepatotoxicity seems to be intermediate products of INH metabolism. Thus, the main pathway of INH metabolism includes acetylation to acetylisoniazid catalyzed by hepatic and intestinal enzyme N-acetyl-transferase 2 (NAT2) followed by hydrolysis to acetylhydrazine. Acetylhydrazine is oxidized by cytochrome P4502E1 (CYP2E1) to form hepatotoxic intermediates [5]. These active metabolites can destroy hepatocytes either by disrupting cellular homeostasis or by triggering immunologic reactions in which active metabolites that bind to hepatocyte plasma proteins can act as haptens. Another metabolic pathway involving NAT2 involves the formation of toxic metabolites by direct hydrolysis of INH to the potent hepatotoxic hydrazine. Differences in INH-induced toxicity have been attributed to genetic variability in several loci such as NAT2, CYP2E1, GSTM1, and GSTT1, which encode drug-metabolizing enzymes [6].

The rate of INH acetylation varies from patient to patient and is divided into three phenotypic groups: slow, intermediate, and fast acetylators. Certain alleles of genetic polymorphisms determine different degrees of NAT2 activity and individual acetylation profile. Several clinical studies have shown that slow acetylators cause the risk of adverse reactions due to the toxicity of high concentrations of isoniazid metabolites, which can lead to hepatotoxic effects caused by INH [7, 12]. On the contrary, when rapid acetylators are used, the concentration of active drug decreases rapidly and this affects the success of treatment in the form of lack of efficacy [9, 11]. NAT2 activity status is associated with haplotypes consisting of several single nucleotide polymorphisms (SNPs) in the NAT2 gene [8]. Generally, single nucleotide substitutions in NAT2 usually result in low N-acetylation activity, decreased NAT2 expression, or instability of the enzyme. The wild-type NAT2*4 allele, which has no single nucleotide substitutions in NAT2, is associated with a rapid acetylator phenotype [10].

Studies of the influence of NAT2 genotypes on INH metabolism allow to increase the effectiveness of treatment; at the same time, side effects associated with this drug can be minimized, which determines the relevance of further studies.

The aim of the research was to determine the peculiarities of NAT2 gene polymorphisms in HIV-infected patients with active TB with advanced drug-induced liver damage.

Materials and methods

The research conducted in the period 01.01.2021-12.12.2022 at the "National Medical Research Center of Phthisio-pulmonology and Infectious Diseases" of the Ministry of Health of the Russian Federation and in the Department of Insectology, head Dr. M.D. Kaminsky G.D., and Director of the Federal State Budgetary Institution "National Medical Research Center for Phthisiopulmonology and Infectious Diseases" of the Ministry of Health of the Russian Federation (FGBU "NMRC FPI" of the Ministry of Health of Russia), Dr. Professor Vasilieva I.A. on the basis of CINIE the development and approbation of methods for allele determination was carried out.

The Research was approved by the Research Ethics Committee of the Federal State University N.I. Pirogov RNIMU of the Ministry of Health of Russia in accordance with protocol No. 5 of "29" 12.2020. All Research participants signed an informed consent form as required by the ethics statement contained in the resolution dated 01.04.2016 N 200n of the Ministry of Health of the Russian Federation.

The Research took into account the results of 151 patients with TB/HIV infection who were prescribed drugs metabolized by the NAT2 enzyme.

Genotypes associated with the type of acetylation (fast, slow and intermediate) were determined in samples of biological material (venous blood). The data of genetic analysis were compared with clinical and laboratory parameters in patients.

When evaluating NAT2 haplotypes, a risk factor for toxicity in the form of transaminase levels above 3 norms from the upper limit (UL) was taken into account. At the beginning of therapy in all patients, aminotranaminase activity indices were within normal values.

To determine alleles of single nucleotide polymorphisms, real-time PCR method was used; the verification of the method was confirmed by pyrosequencing using the reagent kit "AmpliSense® Pyroscreen PHARMA-screen-2a" (RU No. FSR 2012/13246).

To determine haplotypes and acetylation type, the Internet resource "NAT2 Calculator" (http://shtest.evrogen.net/NAT2/) was used.

Statistical analysis

Data were stored in a database, for this study. All data were entered twice to determine possible outcomes. Analysis was performed using IBM SPSS Statistics 29.0.2.0. significance of differences in frequency distribution of variables was used for Pearson's Chi-square criterion. It was used as a measure of relative risk with a regression confidence interval of 95% and a significance level of 5% ($p < 0.05$).

Reliability of differences in median values was determined based on the Mann-Whitney criterion for two groups, the Kraskell-Wallis criterion for multiple comparison of groups ($p < 0.05$).

Results

Table 1 shows the frequency distribution of clinical parameters and genotypes in HIV-infected and TB patients on ART/PTT therapy with liver damage.

The patient group was represented by individuals aged 18 to 65 years (median 41.6 years), of whom 53 (35.1) were female and 98 (64.9%) were male.

According to the evaluation of acetylation type, slow type was found in 87 (57.6%) patients, intermediate type in 48 (31.8%) patients, and fast type in 12 (7.9%) patients. Acetylation type could not be determined for another 4 (2.7%) patients (Figure 1).

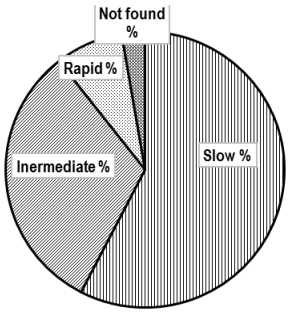

Figure 1. Proportion of patients according to the type of acetylation, %

The captions are poorly visible, it is better to give this figure before the table

The most frequent haplotypes were NAT2*5B, NAT2*5U, NAT2*6A, NAT2*5U, NAT2*5C, NAT2*5KA, NAT2*4. Among the haplotypes responsible for the slow type of acetylation (n=87), haplotypes NAT2*5B (48.3%) and NAT2*6A (40.2%) were the most frequent (Table 1).

Among the intermediate acetylation haplotypes (n=48), NAT2*5U (83.3%) was the most frequent and the rapid acetylation haplotype (n=12) was NAT2*4 (91.6%). (Table 2). Therapy included isoniazid, rifampicin, ethambutol, pyrazinamide, bedaquiline, linezolid, clofazimine, cycloserine, ethionamide, prothionamide, levofloxacin, moxifloxacin, PASC, streptomycin, kanamycin, capreomycin, imipenem/cilastatin, meropenem, lamivudine, dolutegravir, tenofovir, emtricitabine, efavirenz, darunavir, ritonavir, raltegravir, etravirine at standard doses.

Table 1.

Frequency distribution of biological and clinical parameters in HIV-infected and TB patients on the background of therapy with ART/PTP with liver damage Hepatotoxicity.

N=151 HIV/TB, HIV, TB Person's chi-square criteria value P

Categories No % (n) Yes % (n), value P
Gender Male 68 (67) 31 (31) .35
Female 75 (40) 24 (13)
Age >40 years 70 (65) 29 (30) .88
<40 years 71 (42) 28 (14)
CD4+ > 200 cells/μL 77 (42) 22 (12) .01a
HIV RNA Not detected 75 (101) 24 (12) <.001*a
Detected 33 (6) 66 (32)
HCV RNA Not detected 77 (95) 22 (16) <.001*a
Detected 42 (12) 57 (28)
HBV DNA Not detected 70 (100) 29 (2) .68
NAT-2 phenotypes Intermediate 72 (37) 27 (17 .04a
Fast 73 (12) 26 (20)
Slow 56 (87) 43 (87)
NAT2 alleles NAT2*12C 50 (4) 50 (13) .82
NAT2*4 72 (3) 27 (6)
NAT2*5A 50 (4) 50 (14)
NAT2*5B 70 (21) 29 (3)
NAT2*5C 100 (20) 0 (1)
NAT2*5R 80 (34) 20 (1)
NAT2*5U 61 (6) 38 (4)
NAT2*6A 77 (8) 22 (5)
NAT2*6J 66 (4) 33 (5)
NAT2*7B 60 (2) 40 (2)
HIV/TB diagnosis 60 (39) 39 (25) .04*a
HIV 83 (26) 16 (5)
Type of liver lesion (Rucam-scale) None 72 (108) 27 (27) 0.48
Hepatocellular 75 (31) 25 (30)
Cholestatic 55 (43) 44 (34)
Mixed 80 (34) 20 (8)
RUCAM scale for assessing the likelihood of drug-induced liver injury DILI / HILI Excluded
Unlikely 100 (1) 0 (0)
Probable 50 (45) 50 (43)
Probable 75 (113) 25 (38)
Determined 63 (59) 36 (49)
Duration of ART Before hospitalization 75 (6) 25 (14) .11a
During hospitalization 72 (89) 28 (23)
Duration of PTP Before hospitalization 64 (43) 35 (34) .15a
During hospitalization 70 (%2) 29 (5)
ART combination 2 NRTI+ 1 NNRTI 76 (43) 23 (23) .03a,*
2 NIOT+ 1 IP/dr. 60 (42) 39 (14)
Others 28 (17) 71 (3)
PTP scheme HRZ 75 (26) 25 (5) .05a,*
HRZE 50 (45) 50 (15)
MLU 76 (9) 23 (9)
SCHLU 56 (16) 43 (5)
Emperic 40 (9) 60 (7) TB 75 (42) 25 (14)

Table 2.

Frequency of detection of haplotypes causing a certain type of acetylation.

Acetylation type NAT2*4 NAT2*5U NAT2*5B NAT2*5C NAT2*5KA NAT2*6A
Slow (n=87) - 5 (5.7%) 42 (48.3%) 2 (2.3%) 3 (3.5%) 35 (40.2%)
Intermediate (n=48) - 40 (83.3%) 4 (8.3%) 4 (8.3%) 4 (8.3%) - - - - -
Rapid (n=12) 11 (91.6%) 1 (8.4%) - - - - - - - -

The observation showed more frequent increase of aminotransaminase activity from 3 to 10 VGN in patients with slow type of acetylation of NAT2 enzyme gene, their share was 43.7% of the total number of detected cases of slow type of acetylation (Fig. 2).

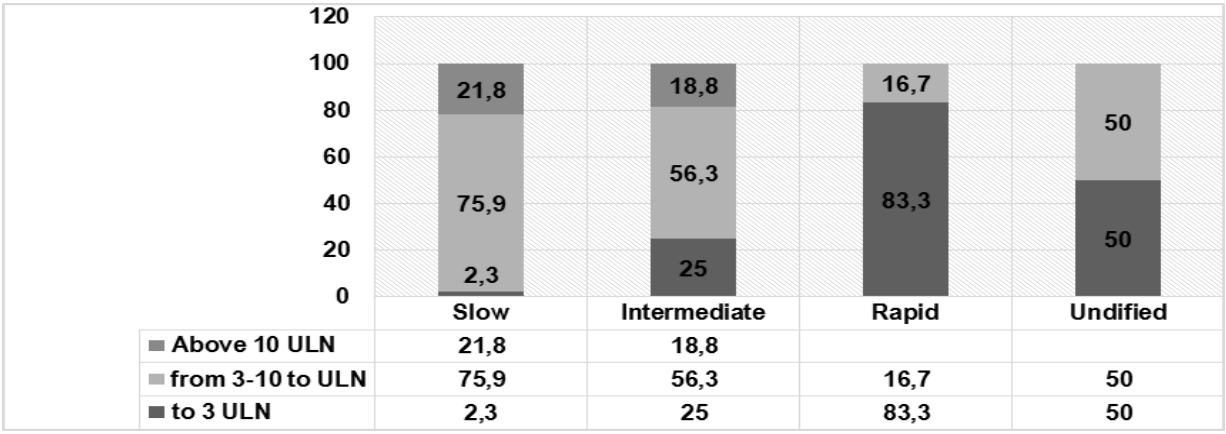

Figure 2: Distribution of patients depending on the ratio of acetylation type and aminotransaminase level, %.

A high VGN (more than 10) was detected in 22% of patients with the slow type of acetylation of the NAT2 enzyme gene.

Among patients with the intermediate type of acetylation (n=48), an increase in aminotransaminase levels from 3 to 10 VGN was also most often registered - in 56.3% of the total number of detected cases of the intermediate type of acetylation. High aminotransaminase activity (more than 10 VGN) was detected in 18.8% of patients with intermediate type of NAT2 enzyme gene acetylation, low (up to 3 VGN) - in 25%.

Among the patients with rapid type of acetylation (n=12) the level of aminotransaminases within the limits of up to 3 VGN was registered most often - in 83,3% of the total number of detected cases of rapid type of acetylation. There was no high index (more than 10 VGN) in patients with the rapid type of acetylation of the NAT2 enzyme gene; the median index (from 3 to 10 VGN) was registered in 16.7% of patients in this group.

The median value of aminotransaminase activity was statistically significantly higher in patients with slow type of acetylation in comparison with this index in the group of fast acetylation (U=76, p=0.00002) (Fig. 3). What does the U stand for?

There was also a difference between the groups of patients with slow and intermediate acetylation types with a predominance of marked hyperenzymemia in patients with slow acetylation type (Median ALT value 237 U/L in slow type and 85 U/L in intermediate type, U=1580, p=0.009). Accordingly, between the groups of patients with intermediate and fast types of acetylation, a significant predominance of ALT level was found in patients with intermediate type of acetylation (U=110, p=0.001).

Isoniazid is highly effective in the treatment of tuberculosis. However, it can cause liver damage and even hepatic failure. Isoniazid metabolism is thought to be associated with hepatocellular-type liver damage, which is characterized by a marked increase in ALT levels (more than 10-fold HGN). NAT2 is the predominant enzyme that catalyzes the acetylation of isoniazid. The presence of slow acetylator alleles has a higher risk of hepatotoxicity of the drug.

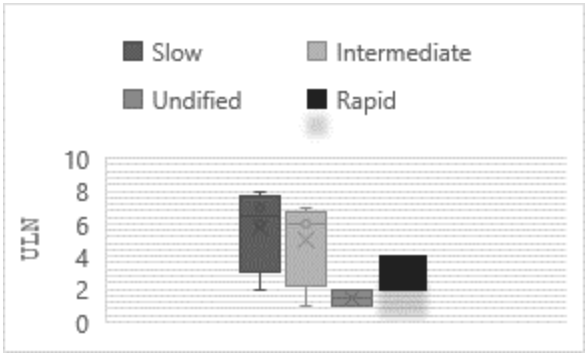

Figure 3. Aminotransaminase levels (Me) depending on the type of acetylation.

Discussion of results

According to the data obtained, the majority of patients with an increase in aminotransaminase activity from 3 to 10 VGN were found to have a slow or intermediate type of acetylation with a predominance of NAT2*5B, NAT2*6A (slow type) NAT2*5U (intermediate type) haplotypes. Among patients with the fast type of acetylation, aminotransaminase levels of up to 3 VGN were more frequently recorded. All the differences found between hyperenzymemia and acetylation types were significantly significant ($p<0.05$). Also the study showed that hypotoxicity was most common on ART and PTV therapy with HIV infection with and tuberculosis, low CD4+ kL, active HIV viruses, active HCV RNA, combination of ART with NNRTIs and PIs, PTP regimen energizing drugs.

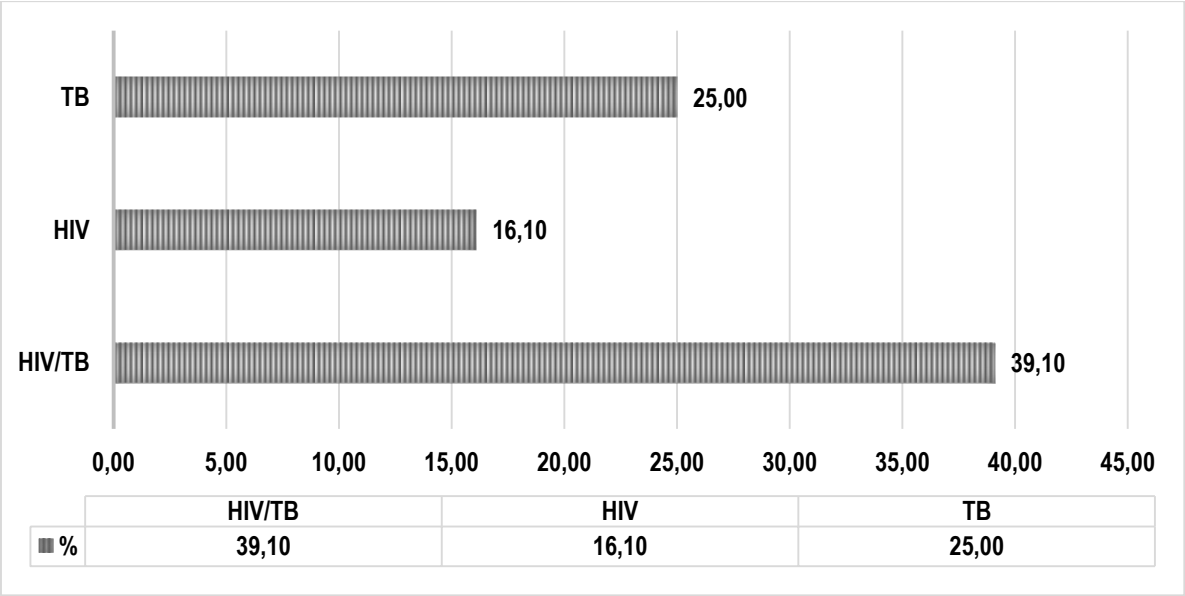

Figure 4: Proportion of patients according to the diagnosis of hepatotoxicity, % (from 3-10 to ULN Aminotransaminase)

Conclusion

Slow acetylation type is an important risk factor for hepatotoxicity in patients with TB/HIV infection. Data on INH acetylation and NAT2 genotypes can be used to improve the quality of treatment, because by taking into account the acetylation type depending on the NAT2 haplotype, the optimal drug dose can be selected, which will have a direct impact on the reduction of hepatotoxicity.

Ethical approval and consent to participate

This study was approved by Federal State Educational Institution of Higher Education "Russian National Research Medical University named after N.I. Pirogov" of the Ministry of Health of the Russian Federation (Protocol no. 5 from December 20, 2020). All participants provided written informed consent before conducting the interviews.

Availability of data and material

Original data can be provided by Federal State Educational Institution of Higher Education "Russian National Research Medical University named after N.I. Pirogov" of the Ministry of Health of the Russian Federation.

Conflict of interest

The authors declare no conflicts of interest.

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