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Clinical trial

ASSOCIATION OF SPECIFIC GENETIC MARKERS OF THE HOST WITH THE RESPONSE TO ANTIRETROVIVAL THERAPY IN HIV POSITIVE PATIENTS

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Abstract

The aim of our study was to investigate the influence of various host genetic markers on the efficacy and safety of antiretroviral drugs in the Macedonian population. The analyzed cohort consisted of 361 HIV patients, 333(92.2%) male and 28(7.8%) female, with mean age at diagnosis of 33.6 years (range 4-65 years). The median follow-up after diagnosis was 4.3 years (0-29.5 years), within which 12 patients died. As expected, the antiretroviral treatment had a dramatic effect on reducing the viral load, from $4.702x10^4$ (range $0-10^9$) to 0 between their first and last PCR test ($p=2.2x10^{-16}$).

We determined the allele frequencies of CYP2D6 and stratified them between HIV patients who never achieved undetectable viral load (UVL) and those who demonstrated UVL at least at one PCR test. Based on the CYP2D6 status, patients were categorized as Extensive Metabolizers (EM) and 16 as Poor Metabolizers (PM). We found that neither CYP2D6 allele or allele combination, nor the EM/PM status were significantly associated with achieving UVL (p=0.2). When adjusted for age at diagnosis and sex in a logistic regression model, neither CYP2D6 alleles nor EM/PM phenotype reached a statistically significant level of association with achieving UVL, except for the trend for CYP2D6 allele *6 to lower the odds of reaching UVL, OR=0.16 (95CI 0.02-0.974).

The results from our preliminary analysis indicate much higher frequency of CYP2D6 EM *vs.* PM phenotype in the Macedonian population and no significant influence on the treatment efficacy in terms of achieving UVL. Further analyses of our cohort are underway to elucidate the effects of CYP2D6 alleles and phenotypes on the safety of the antiretroviral treatment.

Keywords: HIV infection, highly active antiretroviral therapy

Introduction

The world pandemic with acquired immune deficiency syndrome (AIDS) which has been going on for forty years and still represents a very significant health, psychosocial and socioeconomic problem. Although tremendous progress has been made in recent decades in the monitoring, diagnosis and treatment of the infection and disease, the world is still far from its cure and eradication. From the beginning of the pandemic in 1981 to the end of 2020, a total of 77.5 million people were infected with HIV, and almost half of them, or 34.5 million, died of AIDS-related disease[1-3].

Current protocols and recommendations for the treatment of HIV infection recommend early initiation of highly active antiretroviral therapy (HAART) for all people

living with HIV. Guidelines for the treatment of HIV infection provide key recommendations on how to combine drugs, monitor therapy success and side effects and toxicities, assess immune reconstitution, warn of drug interactions, and the emergence of HIV-related comorbidities and diseases. These guidelines pay special attention to aging with HIV infection, with separate chapters addressing special populations-children and pregnant women[4-9].

The revolution in the treatment of HIV infection with antiretroviral therapy drugs (ART) has changed the natural course of the infection from being almost certainly fatal to becoming a chronic disease that does not significantly affect patients' quality of life. During the last three decades, an evolution of antiretroviral treatment modalities took place, so the need for research in the field of optimal selection of therapy protocols for groups of patients with certain clinical characteristics was imposed. It was quickly realized that the success of treating HIV infection depends on the pathogen's ability to adapt, but also on the optimal choice of antiretroviral drugs both in terms of achieving optimal efficiency and in terms of reducing side effects, which indirectly, through improvement persistence with treatment positively affects therapeutic response. The parallel development of pharmacogenomics allowed insight into human biological mechanisms that are involved in the metabolism of antiretroviral drugs and whose interindividual genetic specificity affects therapeutic success and the occurrence of side effects. In addition to this, research related to host biological characteristics of HIV infection has identified markers that have evolved into diagnostic markers of contraindication to treatment with certain antiretroviral drugs[10-16].

In the pharmacogenomics of ART, until now, certain biological systems in the patient's (host) organism are known, which determine the exposure to ART and increase the risk of certain side effects. Polymorphisms in CYP2D6 and MDR1 are associated with the pharmacogenetic profile of ART and determine exposure to these drugs, and polymorphisms in the HLA system are associated with side effects mediated by allergic reactions[17-19].

Cytochrome P450 (CYP) is an enzyme system that forms a polymorphic superfamily of enzymes involved in the metabolism of various exogenous and endogenous substances in the body. There are 57 known CYP genes in the human genome that encode functional enzymes, grouped into 18 protein families and designated as CYP1-18. There are literature data on the association between metabolically more and less active variants of CYP2D6 and the concentration of antiretroviral drugs (efavirenz and nelfinavir) showing that rapid CYP2D6 metabolizers have lower mean values than slow metabolizers[20-23].

The multidrug resistance gene MDR1 carries information for the synthesis of the transporter P-glycoprotein (Pgp), which has an important role in the cellular transport of many different substrates, including some antiretroviral drugs. The degree of expression and functionality of P-glycoprotein directly affect the therapeutic efficacy of several drugs, and are particularly significant determinants of resistance to various types of drugs, such as chemotherapeutics, and could also affect the metabolism of antiretroviral drugs.

Sometimes the treatment of HIV infection can be limited due to adverse reactions and/or the development of resistance to treatment because of certain individual biological characteristics of the host (patient). One such example is the significant association between abacavir hypersensitivity reaction and the presence of HLA-B*5701 allele in HIV-positive patients. Abacavir is an effective nucleoside reverse transcriptase inhibitor, and is one of the first-line drugs of choice in the treatment of treatment-naïve patients, in combination with other drugs. The use of abacavir has been associated with side effects, such as immunologically induced hypersensitivity in about 5 to 8% of HIV-positive patients during the first six weeks of use[25-26].

In the Republic of North Macedonia, three studies have been done so far on the continuum of treatment and care in people living with HIV (PLHIV) infection, but there is a

lack of data regarding the biological characteristics of patients living with HIV that are related to the success of antiretroviral therapy treatment. The purpose of this paper was to investigate the association of certain biological characteristics (gene polymorphisms) of the host with HIV infection and the natural course of HIV infection among PLHIV in our country.

Material and methods

Patients

In this study, patients living with HIV, diagnosed, treated and monitored at the Clinic for Infectious Diseases and Febrile Conditions in Skopje were analyzed retrospectively and prospectively. The study included patients over the age of 18 who gave informed consent to participate in a scientific research study.

Efficacy analyses included achievement of viral neutralization status (percentage and rate) and normalization of CD4+ T lymphocyte counts (percentage and rate).

Methods

DNA samples. DNA was isolated from leukocytes from peripheral venous blood with the automated MagCore apparatus, RBC Bioscience on the principle of magnetic microparticles.
HLA DNA typing. HLA alleles at the HLA-B locus were determined by the SSO (Sequence Specific Oligonucleotides) hybridization method using LABType SSO Typing Tests from ONE LAMBDA, INC, USA. Further typing of B*57 allele was done by high resolution SSP (Sequence Specific Priming) typing using HLA-B*57 kit from Olerup, CareDx, Sweden using the SCORE software program.

Genotyping of polymorphic alleles in MDR1 and CYP2D6. To determine allelic variants in MDR1 and CYP2D6 we used the reverse hybridization method with the PGX-HIV Strip Assay kit from Vienna Lab, Austria.

Determination of the number of peripheral CD4+ T-lymphocytes. The absolute and percentage number of peripheral CD4+ T-lymphocytes was determined in the immunological laboratory of the University Clinic for Infectious Diseases and Febrile Conditions in Skopje, using the method of fluorescence photomicroscopy and light absorption detection on a multicolor platform.

Determination of HIV viral load. Viral load was determined by real-time polymerase chain reaction for the quantitative detection of HIV type 1 RNA in plasma with a standardized system COBAS AMPLIPREP, COBAS TAQ/MAN.

Statistical analysis. Analysis of demographic data, response to ART treatment, determination of statistical significance of differences in ART efficacy and safety in subgroups of patients included in the study was conducted using the SSPS software program. To determine the frequency of HLA-B*5701 and polymorphisms in CYP2D6 and MDR1 we used the statistical package Arlequin.

Results

Demographics

A total of 361 people living with HIV (PLHIV) were recruited into the study, labeled for 159 variables. Of them, 333 were men (92.2%) and 28 were women (7.8%). The mean age at diagnosis was 33.6 years (range 4-65 years, SD 8.9). Age at diagnosis did not differ between men (mean 33.8 years, SD 8.7) and women (mean 31.3 years, SD 12.0), two-tailed Wilcoxon test $p = 0.281$.

Disease status

The mean follow-up time after the diagnosis of CKD was 4.3 years (range 0-29.5 years with SD 4.8). During this period, 12 HCVs died (3%), 9 were lost to follow-up (2.5%), and 4 discontinued treatment (1.1%). The median time to death among the 12 CKD who died was 3.04 years (range 0-17.5 years with SD of 5.9). Of the 12 CKD who died, 11 were male and the median age at diagnosis was 45.9 years (range 24-63 years with SD 12.5). Antiretroviral treatment had a significant effect on reducing viral load, regardless of belonging to the CYP2D6 phenotype of extensive or slow metabolizers (Figure 1). Patients had a median viral load of 4.7 x 104 at their first PCR test result (range 0-109, SD 5.3 x 107), which decreased to a mean viral load of 0 at their last test result (range 0-107, SD 5.5 x 105), two-tailed Wilcoxon test p < 2.2 x 10-16. During the follow-up period, 331 patients (91.7%) achieved an undetectable viral load in at least one test result. However, 13 patients (3.6%) had increased virus levels after reaching undetectability, leaving 318 patients (88.1%) with PCR-undetectable virus at the end of the follow-up period. This was followed by an overall increase in circulating CD4+ cells, which had a median count of 347 CD4+ cells/μL at the first visit (range 0-1934 CD4+ cells/μL, SD 244.8) and increase molecular status and impact on viral load.

CYP2D6 allele frequencies are shown in Table 1, stratified for patients who never reached an undetectable viral load and those who reached undetectability on at least one PCR test. Based on the detected CYP2D6 alleles, the phenotype status could be determined for 345 patients, of which 329(91.1%) were categorized as extensive headaches (EM) and 16(4.4%) as poor metabolizers (SM); 8(2.2%) have a repeat test awaiting results, and 8(2.2%) have an unknown phenotype status. The rate of achieving viral non-susceptibility according to extensive and slow metabolizers group is shown in Figure 2. Neither CYP2D6 allele combinations nor defined phenotype status were associated with achieving viral non-susceptible status (Fisher's test, p = 0.540 and p = 0.629, respectively).

Table 1. CYP2D6 allele frequency in the Macedonian cohort with PLHIV

Allele 1/ Allele2	All PLHIV	%	Not achieved ND HIV status	%	Achieved ND HIV status	%
*1/*1	209	57.895	18	60.000	191	57.704
*1/*3	7	1.939	1	3.333	6	1.813
*1/*4	109	30.194	8	26.667	101	30.514
*1/*6	4	1.108	1	3.333	3	0.906
*3/*4	3	0.831	0	0.000	3	0.906
*4/*4	12	3.324	0	0.000	12	3.625
*4/*6	1	0.277	0	0.000	1	0.302
Unknown	16	4.432	2	6.667	14	4.230

Adjusting for age at diagnosis and gender in a logistic regression model, none of the molecular status variables showed an association with the odds of achieving visceral undetectability at one time point during follow-up (p>0.05), including status of the phenotype and different combinations of CYP2D6 alleles observed in CKD. Adjusting for age at diagnosis and sex, the presence of CYP2D6 allele *6 had a trend towards a decreased odds of achieving viral undetectability (odds ratio OR=0.376), but the findings were not significant (95% confidence intervals (CI): 0.052 -7.544). A similar trend was observed when adjusting for age at diagnosis, sex, HLA B*5701 status, and presence of CYP2D6 allele *6, taking into account the time required to reach the first finding of viral undetectability in regression models (Cox hazard ratio). Men were more likely to achieve viral non-susceptibility over time than women (Hazard ratio, HR=1.848, 95% CI: 1.211-2.820), PLWHA with a positive status of

HLA B*5701 variant were 2.5 times more likely to achieve undetectable status than those with negative status (HR=2.448, 95% CI: 1.254-4.779), and patients with the presence of CYP2D6 allele *6 had a trend towards a lower probability of achieving viral undetectability, although statistically insignificant (HR=0.723, 95 % CI: 0.268-1.955). Adjusted for age at diagnosis, sex, and HLA B*5701 variant status, SM phenotype had a trend towards a higher probability of achieving viral non-susceptibility compared to the extensive metabolizer phenotype, but this was also not statistically significant (HR=1.119, 95% CI: 0.674-1.858).

Viral load

Boxplot representation of the viral load analysis between the first point (PCR before the start of antiretroviral treatment) and the last control. The results showed a dramatic reduction in viral load regardless of belonging to the CYP2D6 extensive (orange) or poor metabolizer (green) phenotype (Figure 1).

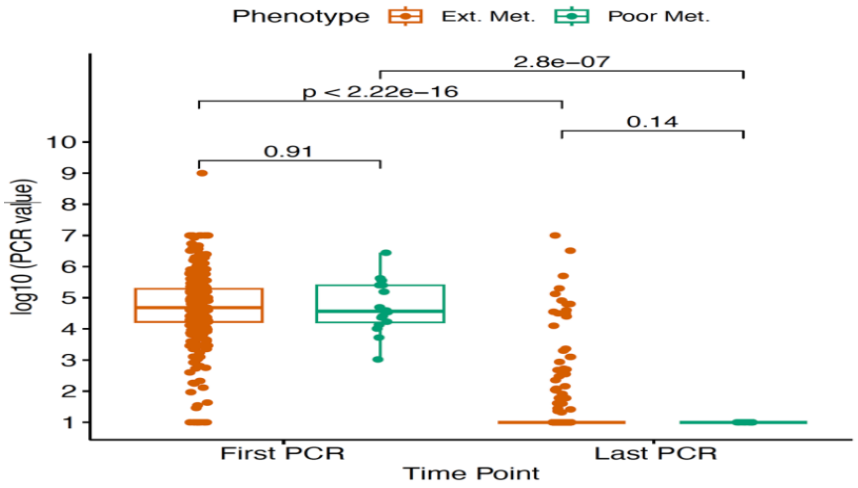

Fig. 1. Viral load during the study

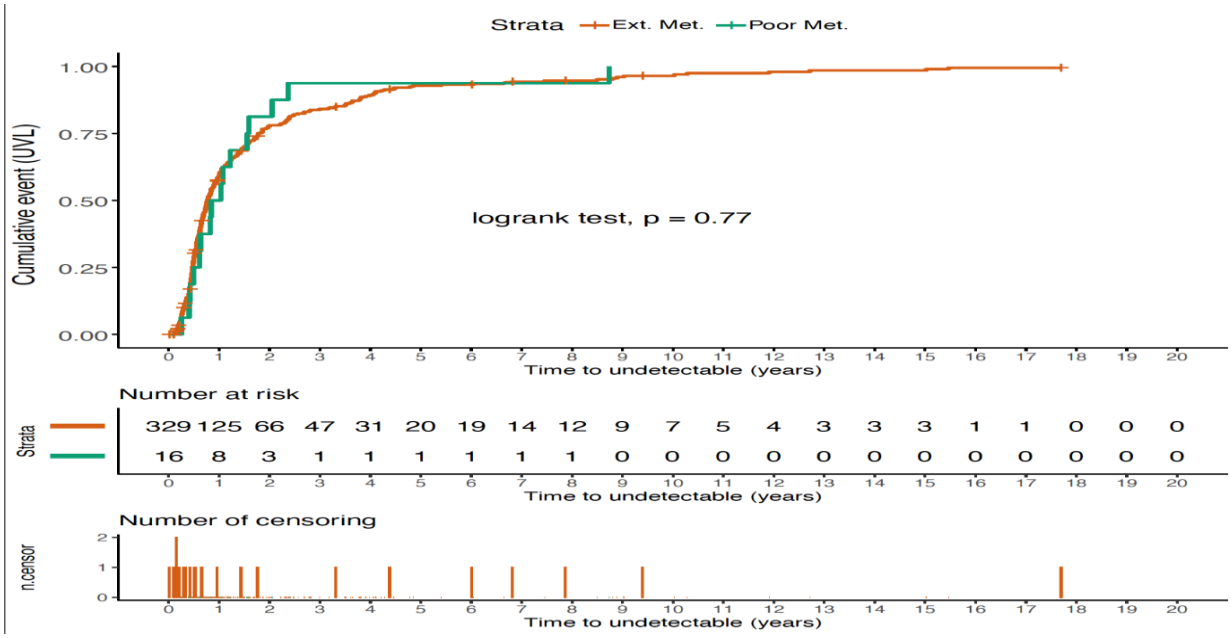

Fig. 2. Kaplan-Meier analysis of the rate of achieving viral neutrality status in patients

Kaplan-Meier analysis of the rate of achieving viral neutrality status in patients according to susceptibility to the CYP2D6 phenotype of extensive and poor metabolizers. The analysis showed that there was no statistically significant difference in achieving viral undetectability status between the two groups (Figure 2).

Discussion

CYP2D6 gene is of great interest in clinical practice because it is responsible for the metabolism of many commonly used drugs and its genetic polymorphism can have a strong effect on the substrate and lead to large inter-individual variations. Variation in CYP2D6 activity has important therapeutic consequences and may play a significant role in the development of toxicity or therapeutic failure in susceptible individuals[25-27].

Knowing how a drug is metabolized and which enzymes are involved helps predict drug interactions and how quickly an individual patient may metabolize a particular drug. CYP2D6 metabolizes at least 25.0% of marketed drugs, antidepressants, beta blockers, opiates, neuroleptics, antiarrhythmics as well as antiretroviral drugs; serious *in vivo* drug interactions of clinical significance are possible[27].

CYP2D6*3, CYP2D6*4, CYP2D6*6 are the most important non-functional alleles that are predominantly responsible for poor metabolic capacity. These alleles are found in 90.0-95.0% of PM in Europe. The prevalence of CYP2D6*4 allele frequency in the Republic of North Macedonia is consistent with that of other European populations, varying between 12.0 and 21.0%. Predictive genotyping of CYP2D6 is estimated to be useful for the treatment of about 30.0-40.0% of drug substrates of CYP2D6, which is about 7-10% of all clinically used drugs[26].

CYP2D6 testing in routine practice could allow individualization of treatment, thus increasing treatment efficacy.

Although statistical analyses in this study did not show a significant association with the odds and probability of reaching HIV undetectable status between EM and SM, it is worth pointing out that only 16 patients had SM phenotype and all of them reached HIV undetectable status over a period of time. From 329 patients with EM phenotype, 301 reached HIV undetectable status (91.489%). Given the small number of patients with SM phenotype and the small difference in the percentage achieving HIV undetectable status (8.511%), it is likely that the analysis was underpowered to detect a difference. we imagine that by following a larger sample size of the group over time, the effect might be noticeable. Following the current scenario, a preliminary sample size calculation suggests that a cohort including 85 patients of SM phenotype should detect the effect, which would require a total cohort sample of 1833 patients, given the observed distribution of the current phenotype.

Overall, the Macedonian cohort of HIV-infected patients has a much higher frequency of CYP2D6 alleles associated with EM phenotype than the metabolizer of SM. However, this showed no impact on the odds or hazard of achieving/not achieving HIV undetectability.

Following world trends in pharmacogenetics and FDA recommendations, we decided to conduct this study in order to help introduce pharmacogenetic testing for certain drugs into clinical practice, thereby avoiding adverse drug reactions, facilitating improved drug efficacy and the treatment is further individualized. Additional and broader data analysis, we believe, will provide useful data for clinical practice.

Conclusion

Our study confirmed that variation in CYP2D6 activity has important therapeutic consequences. The prevalence of CYP2D6*4 allele frequency in PLHIV in the Republic of

North Macedonia is consistent with that of other European populations, varying between 12.0 and 21.0%.

Conflict of interest statement. None declared.

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