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THE PREVALENCE OF HIV-1 STRAINS RESISTANT TO ANTIRETROVIRAL DRUGS AMONG CHILDREN RECEIVING INEFFICIENT HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

Here we present the results of analysis of prevalence of HIV strains with drug resistance mutations among HIV-infected children receiving inefficient HAART. Blood samples from 60 HIV-infected children aged <15 years were taken to perform the viral resistance genotyping. The prevalence of HIV-1 strains characterized with high resistance to any drug constituted 65.0%. In total, 51.67% of children required a correction of HAART scheme. The majority of isolated HIV strains (96.67%) belonged to subtype A of HIV-1.

Key words: HIV-1, HAART.

Introduction. The drugs currently used to treat HIV-infection belong to six distinct classes: nucleoside-analog reverse transcriptase inhibitors (NRTIs), nonnucleoside reverse-transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors, fusion inhibitors and receptor antagonists [1]. Combinations of antiretroviral drugs are used now for the treatment of HIV infection – so-called highly active antiretroviral therapy (HAART). HAART regimens generally comprise three antiretroviral drugs, usually two nucleoside analogues and either a protease inhibitor or a nonnucleoside reverse-transcriptase inhibitor.

One of the major causes of treatment failure is the development of drug resistance to antiretroviral drugs (ARVs). Resistance testing is recommended for optimization antiretroviral therapy after treatment failure, for effective changing regimens of HAART. In this connection the research to identify resistant to ARVs HIV strains is necessary to achieve the efficiency of treatment. Given the fact that the vast majority of children in Ukraine and in the world at large infected by vertical HIV transmission from HIV-infected mothers, HIV-infected infants may acquire resistant HIV strains from the mother in utero or during the intrapartum period. Resistance may also emerge from exposure to antiretroviral drugs given to the infant for prophylaxis against HIV transmission. Resistant HIV strains may also emerge from exposure to antiretroviral drugs given to the infant for prophylaxis against HIV transmission [2]. On the other hand, receiving suboptimal doses of drugs during HAART in turn leads to the formation of resistant strains of HIV too.

Antiretroviral therapy is considered like virologic ineffective if the level of HIV viral load is not reduced to a level less than 1000 RNA-copies/ml blood after 24 weeks of HAART.

The aim of this work was to establish the prevalence of resistant strains of HIV in HIV-infected children with virologic failure of HAART.

Materials and methods. Samples of plasma HIV- infected children under 15 years of age receiving HAART were used for investigation. Whole blood samples were obtained by venipuncture into EDTA-containing tubes. After centrifugation, plasma was separated and stored at –70 °C for RNA viral extraction. Samples of plasma were pretested by PCR to determine the level of HIV-1 RNA using the Abbott RealTime HIV-1 (Abbott RT HIV-1) to assess the effectiveness of HAART. Samples in which the viral load of HIV-1 RNA exceeded 2000 copies VIL-1/ml plasma were selected for further sequencing. HIV genome sequencing was performed on the genetic analyzer ABI PRISM 3100 (Applied Biosystem) using the test system ViroSeqTM HIV-1 Genotyping System (Abbott, USA), which detects mutations in the reverse transcriptase (RT) and protease regions of the pol gene and provides the physician with a report indicating genetic evidence of viral resistance. The entire protease gene and two-thirds of the RT gene are amplified to generate a 1.8 kb amplicon. The amplicon is used as a sequencing template for seven primers that generate an approximately 1.3 kb consensus sequence. The Viroseq HIV-1 Genotyping System software assembles, edits, and identifies mutations within this 1.3kb sequence. The software compares the consensus sequence with a known reference, HXB-2, to determine mutations present in the sample. Analysis of primary nucleotide sequences was performed using BioEdit (v.7.0.0). Evaluation of nucleotide substitutions were performed using the database at Stanford University, USA (hivdb.stanford.edu). All HIV-1 pol sequences for genotyping were analyzed using the REGA HIV-1 Subtyping Tool, version 2.0.

Results and discussion. The presence of mutations of HIV-1 resistance to antiretroviral drugs was determined in HIV-1 RNAs isolated from blood samples of 60 HIV-infected children under 15 years of age. All children received HAART for at least one year. All children included in the study have virological failure of HAART: indexes of viral load of HIV-1 in samples of their blood were higher than 2000 RNA-copies/ml after 6 months after initiation of HAART or after the last modification scheme of HAART and ranged from 2681 to 10 million RNA-copies/ml plasma. Among the children included in the study 30 (50.0 %) received the first scheme of therapy, in 30 (50%) children the scheme of therapy was changed: in 18 children – twice, in 7 – three times, and in 5 – four times.

HIV RNA was isolated from all 60 specimens of blood. Determination of subtypes of HIV-1 was based on the analysis of polymerase gene sequences (a protease and RT regions). According to an analysis of the database of Stanford University, two of the selected strains of viruses can be classified as subtype B HIV -1 (3.33 %), 29 – subtype A HIV -1 (48.33 %), 28 samples were CRF01-AE (46.67%), one – CRF02 – AG (1.67%). But all CRFs were classified as subtype A in additional analysis with REGA HIV-1 Automated Subtyping Tool (Version 2.0). Thus, most of the sequences belonged to subtype A (96.67%), and only two sequences belonged to subtype B (3.33%) (fig. 1).

The resistant strains of HIV-1 were detected in 40 (66.67 %) of 60 samples of plasma HIV-infected children, in 39 (65.0%) samples HIV strains with high resistance to at least one antiretroviral drug were found. In total group 31 (31/60, 51.67%) children needed for the correction of the scheme of HAART. Ten from 39 children (25.64 %) had levels of HIV viral load higher than 100 000 RNA copies/ml plasma. It is known that mutations of HIV resistance to antiretroviral drugs contribute to reducing its replicative capacity compared to the "wild" sensitive virus [3]. At the same time, the HIV genome can form spontaneously polymorphic compensatory mutations that contribute to the restoration of replicative capacity of the virus. Therefore, high HIV viral load (more than 100 000 RNA-copies/ml plasma) in the presence of resistance mutations may be explained by manifestation of polymorphism mutation.

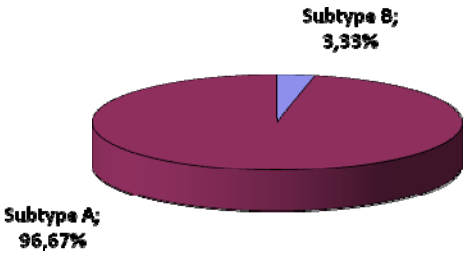

Fig.1. Distribution of HIV-1 subtypes among HIV-infected children

The prevalence of HIV drug resistance mutations was analyzed according to the drug class family. The most commonly detected mutations were mutations of resistance to NRTIs – in 33 from 60 patients were identified strains of HIV with at least one mutation from this group (Table 1).

***Table* 1.The prevalence of HIV strains with resistance mutations among children with virological failure of HAART**

Indicator	Quantity of samples/total group	Rate,%
High-level resistance to at least one antiretroviral drug	39/60	65.0
Resistance to the drug classes::		
NRTIs	33/60	55.0
NNRTIs	29/60	48.33
PIs	8/60	13.33
Resistance to two classes of drugs	24/60	40.0
Resistance to two classes of drugs	4/60	6.67

There are two main mechanisms of resistance to NRTIs. Both of these mechanisms are realized through the formation of mutations in the region of the gene pol, which encodes reverse transcriptase (RT) of HIV. The first – increasing the RT's phosphorolytic activity, which in the presence of a pyrophosphate donor (usually ATP) allows to remove chain-terminating inhibitors from the 3′ end of the primer. This mechanism is associated with mutations M41L, D67N, K70R, L210W, T215Y, T215F, K219Q, K219E – family of mutations known as thymidine analogue mutations (TAMs) because they are selected by thymidine analogs zidovudine (AZT) and stavudine (d4T) [4]. The development of resistance to thymidine analogues is a result of the gradual accumulation of specific mutations in 41, 67, 70, 210, 215 and 219 positions of HIV reverse transcriptase. Viruses acquire phenotypic resistance to thymidine analogues resulting combined mutations at positions 41 and 215, or storage of at least four of the six mutations. Another mechanism – the formation of conformational changes in the molecule reverse transcriptase of HIV that result in loss of the ability of the enzyme to bind to NRTIs, which makes it impossible to include them in the chain of provirus DNA of HIV- 1[5]. Among mutations that result in the inability to include NRTIs in chain of provirus DNA in HIV-1 there are M148V, L74V, M184I, K65R. Among them the most common is the M184V mutation. The RT mutation M184V confers high-level phenotypic resistance to the cytidine analogs lamivudine (3TC) and emtricitabine (FTC) and low-level cross-resistance to abacavir (ABC) and didanosine(DDI). Despite the high level of phenotypic 3TC and FTC resistance caused by M184V, there is often some benefit in including 3TC or FTC in a salvage therapy regimen because M184V increases susceptibility to AZT, d4T, and tenofovir (TDF) and causes a decrease in HIV-1 replication capacity [6].

In the spectrum of detected mutations of HIV resistance to NRTIs in the investigated samples at the mutation M184V (M184MV) was dominant by the frequency of detection – it was found in 32 samples from 60 (53.33 %). Other mutations of HIV resistance to NRTIs, were found with less frequently, but their range was wide. TAMs were found in 20 samples (20/60, 33.33%): in 4 samples M41L mutation were found, in 11- strains with substitution at position D67 (D67N, D67DN), in 7 – at position K70 (K70R, K70KR), in 7 – at position T215 (T215Y, T215 F),in 8 – at position K219 (K219E, K219Q, K219EQO). In samples of blood of 3 children HIV strains with four TAMs were found, in one sample – HIV strain with five TAMs. Also other mutations were found: in 6 samples – mutations at position L74 (L74V, L74LV), in two samples – L210W (L210 LW), V75M – in one.

The second group by the frequency of detection was the group of mutations that provide the resistance to the non-nucleoside reverse transcriptase inhibitors of HIV-1. These mutations were found in HIV strains isolated from 29 samples of blood (29/60, 48.33%). Drugs of this class trigger conformational changes in the HIV reverse transcriptase molecule with the formation of the so-called hydrophobic "pocket" – this region is NNRTI-binding site. The binding of NNRTIs, in turn, causes changes in the conformation of the active center of the enzyme and leads to loss its inability to synthesis of provirus DNA chain of HIV-1. Mutations that cause changes in the amino acid sequence in hydrophobic "pocket", lead to the formation of the stability of the virus to several or all drugs from this class [7]. These mutations include nucleotide substitutions in positions 100-110, 180-190 and 225-235. We found some mutation of that class, most frequently were following: K101E – in 8 samples, G190S – in 16 samples, K103N – in 10 samples, P225H (met in combination with K103N) – in 4 samples, Y181C – in 2 samples. Mutations G190S and K103N are causing high level of HIV resistance to nevirapine (NVP) and efavirenz (EFV). K103 reduces NVP and EFV susceptibility by about 50 and 20-fold, respectively [8]. G190S is mutation that accumulates during prolonged ineffective therapy with most NNRTIs. The nucleotide substitution K101E causes the average level of resistance of HIV to these drugs. P225H is a nonpolymorphic accessory mutation which in combination with K103N causes >50-fold reduced susceptibility to NVP and EFV [9]. Y181C causes >25-fold reduced susceptibility to NVP [10].

The mechanism of action of protease inhibitors is to block the activity of the enzyme, causing it to lose the ability to cleavage of precursor viral proteins (gag and gag–pol) permitting the final assembly of the inner core of viral particles [11]. Resistance to this class of drugs is caused by a complex of mutations that are divided into major (that cause reduced sensitivity of the virus to specific drugs of this class) and minor (that do not affect the stability of the virus to the PIs, but in the presence of major mutations can

enhance the level of HIV resistance to these drugs). Mutations of resistance to PIs were detected in 11 samples, but mutations associated with high levels of resistance to PIs were detected in 8 of them (8/60, 13.33%). Among the major mutations of resistance to PIs nucleotide substitutions at position 46 (M46L, M46LI) were detected – in 7 of the investigated sequences, at position 82 (V82A, V82F) – in 5 samples. M46I/L contribute reduced susceptibility to few PIs (indinavir (IDV), nelfinavir (NFV), fosamprenavir (FPV), atazanavir (ATV), and lopinavir (LPV)) [10]. V82A decreases susceptibility to IDV and LPV and confers cross-resistance to ATV and NFV [12]. The mutation I54V and L76V were detected in 3 and 2 samples, respectively. Among the minor mutations substitutions at position L10I dominated, which were found in 13 of the samples.

Conclusions. Thus, HIV strains with mutations of high level resistance to ARVs were found in the majority (in 39 from 60) of blood samples obtained from children with virological inefficiency HAART. The frequency of detection of resistance mutations to NRTIs was 55.0 %, to NNRTIs – 48.33%, to PIs – 13.33 %. In total group 21 children (35.0 %) had treatment failure, probably related to their low adherence to therapy, abnormalities in the mode of taking the drugs. These results indicate the high relevance of problem of the formation and spread of resistant strains of HIV among HIV- positive children in Ukraine and the necessity for further study of that problem.

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ПОШИРЕННІСТЬ ШТАМІВ ВІЛ-1, СТІЙКИХ ДО АНТИРЕТРОВІРУСНИХ ПРЕПАРАТІВ, СЕРЕД ДІТЕЙ, ЯКІ ОТРИМУЮТЬ НЕЕФЕКТИВНУ ВИСОКОАКТИВНУ АНТИРЕТРОВІРУСНУ ТЕРАПІЮ

Представлені результати аналізу поширеності резистентних до АРВ – препаратів штамів ВІЛ- 1 серед дітей з неефективною ВААРТ. Для проведення досліджень з виявлення резистентних до АРВ – препаратів штамів ВІЛ були відібрані зразки крові 60 ВІЛ-інфікованих дітей у віці до 15 років. Частота виявлення штамів ВІЛ з мутаціями, що забезпечують стійкість високого рівня хоча б до одного препарату, включеному в схему лікування, склала 65,0 %, 51,67 % дітей потребували корекції схеми терапії. Більшість проаналізованих послідовностей РНК ВІЛ (96,67%) належали до субтипу А ВІЛ-1.

Ключові слова: АРВ прапарати штамів ВІЛ-1, ВААРТ.

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РАСПРЕДЕЛЕНИЕ ШТАММОВ ВИЧ-1, УСТОЙЧИВЫХ К АНТИРЕТРОВИРУСНЫМ ПРЕПАРАТАМ, СРЕДИ ДЕТЕЙ, КОТОРЫЕ ПОЛУЧАЮТ НЕЭФФЕКТИВНУЮ ВЫСОКОАКТИВНУЮ АНТИРЕТРОВИРУСНУЮ ТЕРАПИЮ

Представлены результаты анализа распространенности резистентных к АРВ-препаратам штаммов ВИЧ-1 среди детей с неэффективной ВААРТ. Для проведения исследований по выявлению резистентных к АРВ-препаратам штаммов ВИЧ были отобраны образцы крови 60 ВИЧ-инфицированных детей в возрасте до 15 лет. Частота выявления штаммов ВИЧ с мутациями, обеспечивающими устойчивость высокого уровня хотя бы к одному препарату, включенному в схему лечения, составила 65,0%, 51,67% детей нуждались в коррекции схемы терапии. Большинство проанализированных последовательностей РНК ВИЧ (96,67%) принадлежали к субтипу А ВИЧ-1.

Ключевіе слова: АРВ препараты штаммов ВИЧ-1, ВААРТ.

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ADVANCED APPROACHES TO IDENTIFICATION OF VIRUSES INFECTING OF WILD HERBACEOUS PLANTS

Recently, interest in studying viruses in wild flora was gradually increasing. This is connected with necessity of better understanding plant virus evolution, ecology, virulence, and even to avoid economic losses due to crop-wild hybridization, followed by introgression of pathogen-resistant transgenes to wild populations. In this review brief information about last contributions in development of wild plant virology is given. Different approaches to the researches are present here.

Key words: viruses in wild flora, transgenes to wild populations.

Introduction. Viruses commonly infect wild plants. However, virus infection is easily overlooked in wild plant populations. Although infections can be visually unapparent, it is frequently assumed that absence of visual symptoms (such as leaf mottling or malformation) indicates lack of virus infection. Moreover, symptoms of virus infection are sometimes difficult to distinguish from environmental stresses. For these reasons, in part, virus ecology in natu-