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ПОВНОГЕНОМНИЙ СИКВЕНС УКРАЇНСЬКОГО ІЗОЛЯТУ ЦИРКОВІРУСУ СВИНЕЙ 2-ГО ТИПУ

Свинячий цирковірус 2 (PCV2) асоціюється з різними синдромами і хворобами свиней, які відомі під загальною назвою свинячих цирковірус-асоційованих захворювань (PCVAD), які включають синдром мультисистемного розладу (PMWS) PCV2-асоційовані пневмонії (PRDC), PCV2, асоційований з ентеритом, PCV2, асоційований з репродуктивною функцією, а також свинячі дерматит і синдром нефропатії (PDNS) (1-3). PCV2-інфекція широко поширена і по суті всі свині стада заражені PCV2. Свинячий цирковірус 2 (PCV2), член роду* Circovirus *родини Circoviridae. Має ол- ДНК вірусу приблизно 1,7 кб (4). Геном PCV2 кодує три основних відкритих рамок зчитування (ORF,), які кодують репліказу (ORF1), вірусний білок капсида (ORF2), і білок, із запропонованою апоптичною активністю (ORF3) (5). Попередні дані показали, що існує п'ять генотипів PCV2, в тому числі PCV2a, PCV2b, PCV2c, PCV2d і PCV2e (6-9). В нашій роботі ми провели секвенування повного геному генома українськогих ізолятів PCV2з різних регіонів України.

Ключові слова: свинячий цирковірус 2, свинячий цирковірус-асоційованих захворювань, мультисистемний розлад.

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ПОЛНОГЕНОМНЫЙ СИКВЕНС УКРАИНСКОГО ИЗОЛЯТА ЦИРКОВИРУСА СВИНЕЙ 2-ГО ТИПА

Свиной цирковирус 2 (PCV2) ассоциируется с различными синдромами и болезнями свиней которые известны под общим названием свиных цирковирус-ассоциированных заболеваний (PCVAD), которые включают синдром мультисистемных нарушений (PMWS) PCV2-ассоциированной пневмонии, PCV2, ассоциированный с энтеритом, PCV2, ассоциированных с репродуктивной функцией, а также свиные дерматит и синдром нефропатии (PDNS) (1-3). PCV2-инфекция широко распространена и по существу все свиньи стада заражены PCV2. Свиной цирковирус 2 (PCV2), член рода* Circovirus *семейства Circoviridae Геном – очень маленькая оц- ДНК вируса примерно 1,7 кб (4). Геном PCV2 кодирует три основных открытых рамок считывания (ORF,), кодирующие репликазуи (ORF1), вирусный белок капсида (ORF2), и белок, с предложенной апоптотической активностью (ORF 3) (5). Предыдущие данные показали, что существует пять генотипов PCV2, в том числе PCV2a, PCV2b, PCV2c, PCV2d и PCV2e (6-9). Здесь мы сообщаем полную последовательность генома украинских изолятов PCV2из разных регионов Украины.

Ключевые слова: свиной цирковирус 2, свиной цирковирус-ассоциированных заболеваний, синдром мультисистемных нарушений.

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PREVALENCE OF DRUG RESISTANT HIV STRAINS IN HIV-INFECTED PATIENTS OF REPRODUCTIVE AGE

The prevalence of drug resistant HIV strains among HIV-positive reproductive aged persons with ineffective antiretroviral therapy (ART) was assessed. The prevalence of drug resistant strains of HIV was 73.8% in the group of women and 89.29% in the group of men (totally in 80.0% of patients). In the spectrum of drug resistance mutations (DRMs) the most prevalent mutation associated with high-level resistance to nucleoside reverse transcriptase inhibitors was substitution M184V (80.36%); in addition, the high prevalence of K65R (26.79%) was indicated. The most common mutations causing a high-level resistance to non-nucleoside reverse transcriptase inhibitors were G190S/A (57.14%), Y181C (37.50%), K101E (33.93%). The DRMs to protease inhibitors were indicated with significantly less frequent (5.36%).

Key words: HIV, drug resistant mutations, antiretroviral therapy.

Introduction. Antibiotic and antiretroviral resistance has become a major clinical and public health problem nowadays. The selection of resistant forms of pathogens is based on natural processes of microbes adaptation to conditions of environment. Uncontrolled, inappropriate and overabundant using of antimicrobial, antiviral and antifungal drugs, absence of strong clinical protocols of there using, extensive applying of them in agriculture and animal husbandry, the

strong pressure of civilization as a whole lead to the activation of pathogens genetic variability, the emergence of new pathogens and fast evolution of existing ones.

The development of resistance HIV to antiretroviral drugs (ARVs) is the main cause of antiretroviral therapy (ART) virological inefficiency. Biological properties of HIV – fast reproduction of new viral particles, high level of genetic variability connected to features of the reverse transcription, archiving of all variants of HIV genoms as proviral DNAs, high probability of genetic recombination – are the factors, which determine fast evolution of viral population and replacement of wild viruses on resistant strains under selective pressure of suboptimal concentrations of ARVs.

Consequences of HIV resistance development are the growing of risk of drug resistant HIV strains spreading, the appearing of the necessity of switch to more expensive second- and third-line regimens, the loss of ART and prophylaxis effectiveness. During few last years the main HIV spreading way in Ukraine is sexual way, connected to growing risk of HIV vertical transmission. Considering that, the spreading of drug resistant HIV strains among reproductive aged people can lead to the growing of the HIV-infection incidence.

The aim of the work was the definition of level of prevalence drug resistant HIV strains among HIV-positive reproductive aged people with virological ineffective ART.

Materials and methods. A total of 70 HIV-infected individuals at reproductive age (a median age 36.4years) with virological failure of ART were enrolled: among them 42 were female and 28 were male. All men and 24 women (received ART scheme 2 nucleoside reverse transcriptase inhibitors (NRTIs)+non-nucleoside reverse transcriptase inhibitor (NNRTI), 18 (28.57±6.9%) women received scheme 2NRTIs+1 protease inhibitor (PI).

In 42 (60.0±5.86%) patients there were no substitutions of ART regimens in anamnesis, 10 (14.29±4.18%) patients had one replacement of therapy scheme, in 18 (25.71±5.22%) patients the scheme of therapy has been changed twice or more.

In the group of women indexes of HIV viral loud (VL) were from $2.4x10^3$ to $3.18\ x10^6$ HIV RNA copies/ml of plasma; in the group of men – from $4.7x10^3$ to $2.55x10^6$ HIV RNA copies/ml of plasma.

For the HIV genome sequencing was performed on the genetic anlizer ABI PRISM 3130 using the test-system ViroSeq®HIV-1 Genotyping System v.2.0 (Celera Corporation, USA). Data analysis and interpretation were performed using Stanford University Database (http://hivdb.stanford.edu). Data were analyzed by program R (version 2.13.1; 2015-06-18).

Results and discussion.

HIV resistance is developing as a result of forming of specific mutations in the HIV genome, which lead to changes of amino acids structure of viral proteins – targets of ARVs. The selection of drug resistant variants of HIV is carried under the selective pressure of sub-optimal doses of ARVs, usually as a result of low adherence of patients to therapy. The test-systems, what were used in that work, allow determining the DRMs to three classes of ARVs, which are used in Ukraine: to NRTIs, NNRTIs, and PIs.

Among 70 tested blood samples obtained from HIV-positive patients with ineffective ART, 56 (80.0±3.7%) were positive for the presence of drug resistant HIV strains. DMRs to NRTIs (in 71.43±5.4% of samples) and to NNRTIs (in 75.71±5.13% of samples) were most frequent, and in 68.57±5.55% of samples the DMRs to both these classes of ARVs have been detected. The DRMs to PIs were much rarely – in 4.29±2.42% of samples.

Among 42 blood samples of women 31(73.80±6.78%) were positive for the presence of HIV strains with resistance to ARVs (Table 1). DMRs to NRTIs and NNRTIs have been detected with approximately equal frequency – in 27 (64.28±7.39%) and 28 (66.67±7.27%) samples respectively. In 25 (59.52±7.57%) samples DRMs to both classes of drugs were detected. Drug resistance strains of HIV have been detected in samples women's blood, who received the therapy scheme with 2NRTIs+1NNRTI, and in 38.89±11.45% of women on regimen with PI (2NRTIs+1PI). Due to results of the analysis of DRMs combinations 28 women (66.67±7.27%) needed for therapy regimen correction; in all cases the presence of DRMs to NNRTIs determined necessity to change the therapy regimen. And in all cases the presence of DRMs to NNRTIs required for the choice of ART regimen with NNRTI to scheme with PI.

***Table* 1. The incidence of drug-resistant strains of HIV in persons of reproductive age**

Marker	The frequency of detection					
	Female (n=42)		Male (n=28)		Total (n=70)	
	Abs.	Rel., M±m, %	Abs.	Rel., M±m, %	Abs.	Abs, M±m, %
Any DRMs to ARVs	31	73.80±6.78	25	89.29±5.76	56	80.0±3.7
Necessity in correction of the therapy regimen	28	66.67± 7.27	24	85.71±6.57	52	74.29±5.22
DRMs to NRTIs	27	64.28±7.39	23	82.14±7.16	50	71.43±5.4
DRMs to NNRTIs	28	66.67± 7.27	25	89.29±5.76	53	75.71±5.13
DRMs to NRTIs+NNRTIs	25	59.52±7.57	23	82.14±7.16	48	68.57±5.55
DRMs to PIs	3	7.14±3.97	0	0	3	4.29±2.42

Among men the percentage of persons with resistant strains of HIV was higher: DRMs have been detected in 25 samples of men plasma (89.29±5.76%). The necessity for ART regimen correction was indicated in 24 cases (85.71±6.57%) (tab.1) DRMs associated with resistance to NRTIs, were detected in 23 samples (82.14±7.16%), to NNRTIs – in 25 samples (89.29±5.76%), to both classes of ARVs – in 23 samples (82.14±9.56%). In all cases the combination of DRMs led to ineffectiveness of all ARVs, concluded into ART regimen. In contrast to results, obtained in women's group, there were no DRMs to PIs in the men's group.

DRMs to NRTIs. The mechanism of action of NRTIs is termination of HIV proviral DNA synthesis by incorporating into the growing DNA chain [6]. The development of HIV resistance to this class of ARVs can be carried out in two ways:

1. The accumulation of mutations, which make it impossible to conclude NTRIs in DNA chain: substitutions M184V, non-thymidine analogue mutations K65R, K70E/G, L74V, Y115F and mutations of Q151M-complex.

2. The accumulation of mutations that facilitate excision of 3'-terminal chain-terminating inhibitors from blocked DNA chain through phosphorolysis mediated by ATP or pyrophosphate. These mutations, known as thymidine analogue resistance mutations (TAMs), include M41L, D67N, K70R, L210W, T215F/Y and K219E/Q [16].

There are two different ways of TAMs forming: first – by the accumulation of substitutions M41L, L210W, T215Y

(Type 1 TAMs), second – includes mutations D67N, K70R, T215F, K219Q/E (Type 2 TAMs). Factors that lead to selection of mutations by first or second way are unknown. Perhaps this is a random process, or it can be connected to genetic features of HIV, to immunologic characteristics of patient, to the list and sequence ART regimens and other reasons [16]. TAMs of 1 Type have a greater negative impact on virological response to an ABC-, ddI-, or TDF-containing regimen than do TAMs of Type 2 [11].

The frequency of detection of different DRMs was calculated with respect to the total quantity of samples with at least one DRM. Among mutations connected to first mechanism of NRTI-resistance development the mutation M184V was dominant in samples obtained from women and men. In some samples the substitution M184I was detected – mutation that usually appeared before M184V because it results from a more common HIV-1 nucleotide substitution. M184I has the similar effect on HIV resistance. Generally, the detection rates of M184V/I were 77.42±5.34% in a group of women and 80.0±4.74% in a group of men (Tab.2). The selection of this substitution occurs under the press of therapy with 3TC and FTC, and leads to 100-fold decrease of HIV sensitivity to these drugs. Besides, M184V/I appear at treating by ABC and ddI causing low-level HIV resistance to them. In the same time, M184V increases HIV susceptibility to AZT, d4T and TDF and slows down the development of resistance to them. M184V/I are associated with reduced HIV replication *in vitro* and *in vivo*. That is why M184V/I are not contraindications to continued treatment with 3TC or FTC. The combination of TDF, AZT or d4T + 3TC/FTC inhibits HIV with M184V [9].

In addition to this mutation, in the spectrum of DRMs to NRTIs, detected in women's samples of plasma, non-TAMs mutations K65R (38.71±8.69%), Y115F (22.58±7.44%), L74V/I (19.35±7.02%) were prevalent. Among TAMs substitution K219Q/E was dominant (22.58±7.44%), it refers to Type 2 TAMs.

***Table* 2. The detection rate of major and accessory mutations in the pol gene of HIV-1 associated with resistance to NRTIs[1]**

Mutation	The frequency of detection in the spectrum of DRMs					
	Female (n=31)		Male (n=25)		Total (n=56)	
	Abs.	Rel.,M±m,%	Abs.	Rel.,M±m,%	Abs.	Rel.,M±m,%
M41L	0	0	2	8.0±5.33	2	3.57±2.22
A62V	3	9.68±3.53	3	12.0±6.42	6	10.71±4.01
K65R	12	38.71±5.82	3	12.0±6.42	15	26.79±5.29
D67N	2	6.45±2.94	5	20.0±7.94	7	12.50±3.95
T69N	2	6.45±2.94	1	4.0±3.78	3	5.36±2.84
K70E	3	9.68±3.53	5	20.0±7.94	8	14.29±4.18
K70R	1	3.23±2.11	2	8.0±5.33	3	5.36±2.84
L74V/I	6	19.35±4.72	6	24.0±8.48	12	21.43±4.9
V75I	0	0	4	16.0±7.26	4	7.14±3.29
Y115F	7	22.58±4.5	3	12.0±6.42	10	17.86±4.58
Q151M	0	0	0	0	0	0
M184V/I	24	77.42±5.34	20	80.0±7.94	45	80.36±4.74
L210W	0	0	2	8.0±5.33	2	3.57±2.27
T215F/Y	1	3.23±2.11	3	12.0±6.42	4	7.14±3.29
K219Q/E	7	22.58±4.5	2	8.0±5.33	9	16.07±4.80

It is known that mutation K65R leads to 2-fold decreasing of HIV susceptibility to ABC, TDF, d4T, ddI, and 5-10-fold – to 3TC and FTC, but increase HIV sensitivity to AZT (accept of cases of combination with Q151M substitution) [9]. It should be taken into account for ART regimen changing.

The L74V is selected by therapy of ABC and ddI. In combination with M184V it is the most common substitution for patients receiving ART with ABC/3TC; together these mutations lead to 5-fold decreasing of HIV susceptibility to ABC and 2-fold – to ddI. Mutation L74I is selected by therapy with the same ARVs and TDF; its effect on the resistance is less pronounced [9].

Y115F is selected by ABC and TDF. Alone, Y115F reduces ABC susceptibility about 3-fold but has a little phenotypic effect on TDF susceptibility. In combination with K65R or Q151M, Y115F synergistically reduces ABC and TDF susceptibility [14]. Mutation K219Q/E in combination with other TAMs reduces susceptibility about 3-fold to AZT and d4T.

In the samples of men, except of dominant M184V/I, the substitutions L74V/I, K70E and two TAMs (Type 2) – D67N and K70R were detected with higher frequency (Tab.2). D67N and K70R reduce of HIV susceptibility to AZT and d4T. In combination with other TAMs it leads to decreasing of HIV susceptibility to ABC, ddI and TDF. The frequency of K65R detection in the samples, obtained from men, was lower compared to group of women – 12.0±6.42%. Mutation K70E reduces HIV susceptibility to ARVs only in combination with other NRTI-resistance mutations.

Except of major DRMs to NRTIs, some accessory mutations have been detected (A62V, T69N, V75I) with low frequency. Without major mutations they don't influence on HIV resistance largely. For example, among mutations of Q151M complex, only additional substitutions were detected – A62V and V75I – with the frequency 10.71±4.01% and 7.14±3.29% respectively. Without main mutation of this complex – Q151M – additional substitutions do not affect the HIV resistance. Mutation A62V is a common polymorphic substitution for HIV strain circulating in Russian Federation which is entrenched in that area due to the "funder effect" [2]. The prevalence of A62V in HIV strains detecting in Russian Federation is about 13%.

Interestingly, that analysis of DRMs prevalence has indicated some gender differences: TAMs (Type1 and Type2) were detected in men's samples of plasma more frequent. The connection between the gender and the prevalence of some DRMS was detected in other investigations [3, 15]. However, based on our data it is difficult to draw conclusions on the causes of this phenomenon, the additional study is necessary.

DRMs to NNRTIs. NNRTIs can block HIV reverse transcriptase by binding to special hydrophobic region of the enzyme (so-colled "pocket"). That binding leads to the change of the spatial configuration of reverse transcriptase

[1] Bold – high-level resistance mutations, plane – reduced HIV susceptibility in combination with other mutations, italics – accessory mutations

active center. The development of resistance to NNRTIs is caused by forming of mutations in the "pocket" region.

Among the most frequent major DRMs to NNRTIs in HIV isolates obtained from women's samples of plasma were G190S/A (54.84±8.88%), K101E (36.26±8.34%), Y181C/I (41.94±8.81%) (Tab.3). Mutation G190S is selected during the therapy with NVP and EFV. It 50-fold reduces HIV susceptibility to specified ARVs. Mutation G190A is selected by the selective pressure of the same ARVs and leads to 50-fold and more decreasing of HIV susceptibility to them. Substitution K101E is forming during the therapy with NNRTIs and leads to 3-10-fold decreasing of HIV sensitivity to NVP, 1-5- fold – to EFV, 2-fold – to ETR and RPV. Y181C/I are selected by the therapy with any NNRTIs and more-less decreases the susceptibility to every drug of that class [9].

Among HIV isolates obtained from men, in addition to aforecited mutations, accessory substitutions V90I (20.0±7.94%) and V106I (24.0±8.48%) have been indicated. Both of them increase HIV resistance to NNRTIs, but in a less degree.

DRMs to PIs. PIs can block viral protease by binding to the active center of enzyme [10]. Therefore the resistance to PIs develops as a result of forming amino acids substitutions changing spatial configuration of the active center. PIs have a high genetic barrier to resistance. It means that a significant level of resistance to PIs is forming after accumulation of 3-10 mutations in the HIV genome – major and minor. Major mutations have an effect on HIV resistance, but at the same time they decrease the HIV viability and replicative activity, because lead to structural changes in molecule of HIV enzyme. Minor mutations don't influence on HIV resistance, but they can restore the viral fitness.

***Table* 3. The detection rate of mutations in the pol gene of HIV-1 associated with resistance to NNRTIs**

Mutation	Frequency of detection					
	Female (n=31)		Male (n=25)		Total (n=56)	
	Abs.	Rel.,M±m, %	Abs.	Rel.,M±m, %	Abs.	Rel.,M±m, %
V90I	3	9.68±5.22	5	20.0±7.94	8	14.29±4.68
A98G	1	3.23±3.01	1	4.0±3.79	2	3.57±2.48
L100I	0	0	0	0	0	0
K101E	10	32.26±8.34	9	36.0±9.55	19	33.93±6.33
K103N	4	12.90±5.94	4	16.0±7.26	8	14.29±4.68
V106I	1	3.23±3.01	6	24.0±8.48	7	12.50±4.42
V108I	2	6.45±4.30	1	4.0±3.79	3	5.36±3.0
V179F	1	3.23±3.01	0	0	1	1.79±1.77
Y181C/I	13	41.94±8.81	8	32.0±9.27	21	37.50±6.47
G190S/A	17	54.84±8.88	15	60.0±9.75	32	57.14±6.61
H221Y	0	0	3	12.0±6.42	3	5.36±3.0
P225H	2	6.45±4.30	2	8.0±5.33	4	7.14±3.44

The major DRMs to PIs were found in three plasma samples (5.36±3.0%) obtained from women: M46I/L, V82F/A, I47A (Tab. 4). In all these samples combination of substitutions M46I/L and V82F/A was found (Tab.5). And in all cases the major DRMs to PIs was accompanied by various minor substitutions – L10F, L10I, L33F, Q58E, A71V.

***Table* 4.The detection rate of major mutations in the pol gene of HIV-1 associated with resistance to PI**

Mutation	Frequency of detection (n=56)	
	Abs.	Rel., M±m, %
M46I/L	3	5,36±3,0
V82F/A	3	5,36±3,0
I47A	1	1,79±1,77

M46I/L is selected by therapy with IDV, NFV, FPV, ATV, LPV; it is often associated with V82A; in combination with other PI-resistance mutations M46I/L decrease HIV susceptibility to ATV, FPV, IDV, LPV and NFV. Substitution V82F/A appears during the treating with IDV and LPV; it causes the HIV resistance to ATV, FPV, IDV, LPV and NFV, accept of that in combination with other DRMs to PIs – to SQV and FPV. I47A is selected by the therapy with LPV or DRV and decrease HIV susceptibility to all PIs accept of ATV and SQV [9]. HIV isolate obtained from the sample 320304TroAP has two major and two minor mutations; as a result the virus has high level resistance to NFV and IDV/r and reduced susceptibility to three other drugs from that class (Tab. 5). In the sample DidKY the HIV strain was detected what has three major and three minor mutations, and, as a result, was resistant to four PIs and has a reduced susceptibility to other drugs from that class. Interestingly, that in all cases HIV strains, accept of DRMs to PIs, have the DRMs to NRTIs included in to the therapy regimen. It confirms the fact, that the development of resistance to PIs occurs after the forming of resistance to NRTIs and subsequent selection of other mutations in the HIV genome connected to decreasing susceptibility to PIs.

It should be emphasized, that in sample DidKY the HIV strain with DRMs to NNRTIs (K101E, V106I, Y181C) was detected, although the therapy regimen of that patient doesn't include that drugs. During analysis of anamnesis data it was found, that patient DikKY previously received NNRTI – until 2011 the therapy scheme was TDF/3TC+EFV (that is 2NRTI+1NNRTI). In 2011 the scheme has been changed due to virologic ineffectiveness. Thus, during four years of therapy without NNRTIs the mutations of resistance to drugs of that class were saved in the HIV genome. According to results of different investigations, some mutations disappear quickly after therapy regimen replacement – when the selective pressure of appropriate drug terminate. For example, mutation M184V disappears during few weeks after termination of therapy with 3TC or FTC, the less-fitness HIV strain comprising the mutation is replaced by the high-fitness wild strain [8]. In the same time, mutations that don't affect the HIV fitness can be saved in the viral genome during a period from few months to few years after therapy regimen change. In the plasma samples of men the DRMs to PIs were not detected because men included into investigation have not received the ART with PIs.

***Table* 5. Mutations of resistance to PIs**

№	Patient	Current treatment regimen	DRMSs to PIs		DRMs to NRTIs	DRMs to NNRTIs	Drug resistance interpretation
			Major	Minor			
1	826567 PopOV	ABC+3TC+Lpv/r (2НІЗТ+1ІП)	M46IM	L10I	M184V	V90IV	High-level resistance to 3TC, low level resistance to ABC, potential low-level resistance LPV/r
2	320304 TroAP	TDF/FTC/Lpv/r 2НІЗТ+1ІП	M46I, V82A	L10F, A71AV	D67DG, L74IL, M184V	none	**NRTI:** high-level resistance 3TC, ABC, ddI, FTC (but hypersusceptibility to TDF coused by M184V). **PI:** high-level resistance to NFV, IDV/r, middle-level resistance to FPV/r, LPV/r, ATV/r
3	DidKY	ABC/3TC/Lpv/r (2НІЗТ+1ІП)	M46LM V82A I47A	L10I, L33F, Q58EQ	K70E, M184V	K101E, V106I, Y181C	**NRTI:** low-level resistance to ddI, d4T, TDF, middle-level resistance to ABC, high-level resistance to 3TC and FTC (but hypersusceptibility to ZDV caused by M184V) **NNRTI:** high-level resistance to NVP, RPV, middle-level resistance to EFV, ETR. **PI:** high-level resistance to LPV/r, NFV, IDV/r, FPV/r, middle level resistance to ATV/r, low level resistance to SQV/r, DRV/r

In women (12 persons, 35.71±7.33%) and men (3 persons, 10.71±5.76%), who have not resistant HIV isolates, the therapy ineffectiveness, obviously, has developed as a result of low adherence to ART.

The HIV viral loud levels in patients with ineffective therapy. Due to results of Wilcoxon rank sum test, there is a statistically significant difference ($p<0.05$) between indexes of HIV viral loud (VL) in women, who have HIV drug resistant strains in the blood (mean VL 75,25x103 RNA copies/ml of plasma), and women who don't have drug resistant strains (mean VL 494,25x103 RNA copies/ml of plasma). It could be explained by the effect of DRMs on the level of HIV reproduction. DRMs usually lead to conformational changes of the viral enzymes molecules, that is why drug resistant strains of HIV are less viable and have less level of reproduction than wild strains.

In the group of men high level of HIV VL was associated with the absence of drug resistant strains. Indexes of HIV VL in the samples without DRMs were from 1.45 to 2.55 $x10^6$ RNA copies/ml of plasma, mean level 2.18 $x10^6$ RNA copies. In samples were DRMs were indicated – from 5.8 $x10^3$ to 1.38 $x10^6$ RNA copies/ml of plasma, mean level – 185.0 x 10^3 RNA copies. It should be noted the high levels of HIV VL in the presence of drug resistant strains could be explained to prolonged using of ineffective therapy, that leads to accumulation of the accessory resistance mutations in the HIV genome, which recover HIV replication capacity.

We have compared the spectrum of dominant mutations to different classes of ARVs with the data of other investigations. Due to the results of A.Rakhmanova with colleagues, the most often DRMs in the HIV isolates, obtained from the HIV-positive people in Russia, were M184V, L74V, D67N (associated with resistance to NRTI), G190S/A and K103N(associated with resistance to NNRTI) [5]. During the survey of cohort of Indian HIV-positive people, it was indicated that the most often DRVs to NRTI were M184V, T215Y, D67N, K70R, to the NNRTI – Y181C, G190A, V108I [13].

In the countries of Central America among the patients with virologic ineffective ART the high prevalence of HIV strains with mutations M184V, T215Y, M41L, K103N, V108I were the most dominant. Substitution G190S was detected less frequently [6].

In aforecited investigations the frequency of mutation K65R indication was low, whereas it was present in 26.79% of HIV strains, examined in this work, (in women's samples – 38.71%, in men's samples –12.0%). It could be explained by the fact that the most of the patients included in this investigation (73.81% of women, 67.86% of men) either were obtaining ATR regimens with TDF, or have obtained of TDF-comprising regimen in anamnesis. Interestingly, mutation K65R is the antagonist of TAMs: they never appear together in one HIV genome [7]. Really, the results of the work confirm this fact. Thus, the spectrum of DRMs depends on primarily from the ARVs included in the ART regimens of examined cohort of patients.

The high prevalence of HIV strains with DRMs associated with resistance to two classes of ARVs simultaneously – to NRTIs and NNRTIs, is connected to the pharmacokinetics of reverse transcriptase inhibitors, the duration of their activity period, low genetic barrier of NRTIs and NNRTIs, due to which only one-two DRMs can lead to the loss of HIV susceptibility to that drugs. The period of half-life of NRTIs is significantly shorter compared to NNRTIs. That is why, if the patient messes the doses of ARVs, from time to time in his blood only one active drug could be – it is, practically, the monotherapy with NNRTI. Consequently, the HIV replication is continued during ART leads to the forming of HIV strains resistant to NNRTIs. Further remaining NRTIs quickly become ineffective due to the fast accumulation of DRMs associated with resistance to them.

Thus, the most cases of virologic ineffectiveness of ART in reproductive aged persons were connected to development of HIV resistance to ARVs: drug-resistant HIV strains were indicated in 73.8% women and 89.29% (generally – in 80,0% patients). It should be emphasized, that the most of indicated DRMs are related to the transmissible mutations by the WHO; it means, that the HIV strains with that mutations can be transmitted to other people, leads to grow the prevalence of primer resistance [10]. That is why, the monitoring of drug-resistance HIV strains prevalence among the reproductive aged people is the one of the main areas of the fight against the spread of HIV by the sexual and vertical ways.

Conclusions.

1. It was indicated, that the prevalence of drug resistant HIV strains was 73.80% in the group of women and 89.29% in the group of men (80.0% in total group) with virologic ineffective ART. In 74.29% of incidence of DRMs the ART regimen correction was needed.

2. Among indicated mutations the DRMs to NRTIs and NNRTIs were dominant. HIV strains with DRMs to NRTIs were found in 64.28% of women's plasma samples and 82.14% men's plasma samples; HIV strains with DRMs to NNRTIs – in 66.67% and 89.29% of samples respectively. In most cases mutations of resistance to both ARVs classes were found simultaneously. The DRMs to PIs were indicated with significantly less frequent – totally in 4.29% of all tested plasma samples.

3. In the spectrum of drug resistance mutations (DRMs) the most prevalent mutation associated with high-level resistance to NRTIs was substitution M184V (80.36%); in addition, the high prevalence of K65R (26.79%) was indicated. The most common mutations causing a high-level resistance to NNRTIs were G190S/A (57.14%), Y181C (37.50%), K101E (33.93%). The percentage of most preva-

lent DRMs to PIs (M46L/I and V82F/A) was much lower and amounted 5.36% of all detected mutations.

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ПОШИРЕННІСТЬ ЛІКАРСЬКО СТІЙКИХ ШТАМІВ ВІЛ ІНФЕКЦІЇ У ВІЛ – ІНФІКОВАНИХ ПАЦІЄНТІВ

Визначено рівень поширеності штамів ВІЛ, резистентних до антиретровірусних препаратів, у ВІЛ-позитивних осіб репродуктивного віку з вірусологічно неефективною антиретровірусною терапію. Частота виявлення резистентних штамів ВІЛ становила 73,0% в групі жінок та 89,29% в групі чоловіків (80,0% серед всіх обстежених пацієнтів). У спектрі мутацій, асоційованих з високим рівнем резистентності ВІЛ до препаратів класу нуклеозидних інгібіторів зворотної транскриптази, найбільш поширеною була заміна M184V (80.36%); крім того, визначено високий рівень поширеності мутації K65R (26.79%). Найпоширенішими серед мутацій, що спричиняють резистентність високого рівня до ненуклеозидних інгібіторів зворотної транскриптази, були заміни G190S/A (57.14%), Y181C (37.5%), K101E (33.93%). Мутації резистентності до інгібіторів протеази виявлялися з нижчою частотою (5,36%).

Ключові слова: ВІЛ, мутації медикаментозної резистентності, антиретровірусна терапія.

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РАСПРОСТРАННЕНОСТЬ ЛЕКАРСТВЕННО УСТОЙЧИВЫХ ШТАММОВ ВИЧ ИНФЕКЦИИ У ВИЧ-ИНФЕЦИРОВАННЫХ ПАЦИЕНТОВ

Определен уровень распространенности штаммов ВИЧ, резистентных к антиретровирусным препаратам, у ВИЧ-положительных лиц репродуктивного возраста с вирусологически неэффективной антиретровирусной терапией. Частота выявления резистентных штаммов составила 73,0% в группе женщин и 89,29% в группе мужчин (80,0% среди всех обследованных пациентов). В спектре мутаций, ассоциированных с высоким уровнем резистентности ВИЧ к препаратам класса ненуклеозидных ингибиторов обратной транскриптазы, наиболее распространенной была замена M184V (80.36%); кроме того, был установлен высокий уровень распространенности мутации K65R (26.79%). Наиболее частыми среди мутаций, обуславливающих высокий уровень резистентности к ненуклеозидным ингибиторам обратной транскриптазы, были замены G190S/A (57.14%), Y181C (37.5%), K101E (33.93%). Мутации резистентности к ингибиторам протеазы определялись реже(5,36%).

Ключевые слова: ВИЧ, мутации лекарственной резистентности, антиретровирусная терапия.