CXCR4$^+$ and CCR5$^+$ expression on blood lymphocytes and its changes in AIDS development in HIV/HCV coinfected patients

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ABSTRACT

Purpose: To measure the CCR5$^+$ and CXCR4$^+$ expression on blood lymphocytes, its changes in AIDS development, and correlation of CCR5$^+$ and CXCR4$^+$ expression with a number of T-cells, T-helpers, cytotoxic T-cells, activated T-cells (CD3$^+$ HLA-DR$^+$), T-regs cells, natural killer (NK)-cells, B cells, in order to determine the type of immune response activation in HIV/HCV coinfected patients.

Materials and methods: The patients were divided into four groups. The 1st group included 51 patients with HIV/HCV coinfection; the 2nd group consisted of 23 patients with HIV-infection; the 3rd group was composed of 10 patients with HCV-infection. Control group consisted of 16 healthy individuals. In the current investigation, the monoclonal antibodies were used. The cells were analyzed using flow cytometer.

Results: In patients with HIV/HCV coinfection CXCR4 expression on blood lymphocytes was reduced in comparison with control. CCR5 expression on blood lymphocytes, HLA-DR expression on CD3+lymphocytes and cytotoxic T-cells, numbers of CD8 T-lymphocytes were increased and in the same time the number of CD4+CD25+ was reduced in HIV/HCV coinfected patients in comparison with the HCV-infected patients and control group.

Conclusions: In chronic HIV/HCV coinfected patients CCR5$^+$ expression on blood lymphocytes was increased and CCR5$^+$ expression on CD4+ T-lymphocytes was decreased in comparison with healthy individuals and HCV-infected patients. With the development of AIDS, CXCR4$^+$ and CCR5$^+$ expressions on CD4$^+$T-lymphocytes, CXCR4$^+$ expressions on blood lymphocytes were decreased and CCR5$^+$ expression on blood lymphocytes was increased. Increased CCR5$^+$ expression on blood lymphocytes in HIV/HCV coinfected patients was associated with Th1 type immune response activation.

Key words: HIV, HCV, coinfection, immune response, chemokine receptors, CCR5, CXCR4.

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INTRODUCTION

Hepatitis C virus (HCV)-infection is common in human immune deficiency virus (HIV)-infected individuals because of a similar way of transmission. According to the investigation performed in 2009 about 50% of total population of HIV-infected patients in different regions of Belarus was coinfected with HCV [0].

Chemokine receptors CXCR4 (CD184) and CCR5 (CD195) are predominant coreceptors for HIV-1 entry into human's cells. Depending on the character of the used coreceptors three variants of HIV are distinguished: CCR5/M (R5)-tropic virus, CXCR4/T(X4)-tropic virus, R5/X4 dual-tropic virus. R5-tropic virus uses CD4 and CCR5 for HIV-1 entry into the cell, while X4-tropic HIV uses CD4 and CXCR4. Individuals homozygous for the CCR5/Δ32 alleles are not susceptible for HIV-infection because they are not able to express CCR5 on cell-surface [1, 2].

It was established that GB virus C coinfection in advanced HIV -1 disease was associated with low CCR5 and CXCR4 surface expression on $CD4^+$ T-cells. It can serve as a molecular mechanism for the clinical benefit of GB virus C/HIV coinfection in AIDS [3]. Immature dendritic cells, on monocytes/macrophages, on memory $CD4^+$T-cells, on Th1 cells, on $CD8^+$T-cells. On $CD4^+$T-cells 4000 to 24 000 CCR5 molecules may be numerated per cell. Density of CCR5 on surface of $CD4^+$ T-cell is a surface over time in individual but the percentage of $CD4^+$ T-cells expressed CCR5 may drive according to circumstances. $CCR5^+$ expression on the surface of $CD4^+$ T-cell increases in HIV infection and stays stable for a long time in a given patient [2, 4].

CXCR4 receptors can be found at 41.6 ± 16.1% of T-lymphocytes ($CD3^+$), at 53.5 ± 18.4% of B-lymphocytes ($CD20^+$), 20.4 ± 9.4% of monocytes ($CD14^+$) and are almost absent at natural killer (NK) cells (0.2 ± 0.3), neutrophils and eosinophils. Expression of $CXCR4^+$ at $CD4^+$ и $CD8^+$ T-lymphocytes is approximately the same [5, 6]. Natural ligands for CCR5 are CCL3 (MIP-1a), CCL4, (MIP-1b), CCL5 (RANTES) [1, 2, 7]. Chemokine SDF-1 (stromal cell-derived factor-1) is a natural ligand for CXCR4; it blocks *X4-tropic viral cell entry* in vitro [8].

CCR5 is not only a chemokine receptor; it is also a coactivation receptor [2, 5]. CCR5 function is demonstrated in cellular immune response. CCR5 are expressed on Th1 cells, producing IL-2 and IFN-γ. IL-2 and IFN-γ up-regulate CCR5 expression, whereas the type 2 cytokine IL-10 and IL-4 down-regulates it. CCL3, CCL4 and CCL5-produced by Th1 cells favor differentiation of CD4T-cell into Th1-type, activate macrophages in synergism with IFN-γ, acting like cytokines of type 1. Mice with CCR5 deficit expression were defected with the type Th1 of IR [2].

HIV coreceptors $CXCR4^+$ and $CCR5^+$ are known to be expressed on mutually opposed subsets of freshly isolated peripheral blood lymphocytes. CXCR4 are expressed mostly on resting, unactivated naive T cells (CD26low CD45RA+ CD45RO–). While $CCR5^+$ T-cells had phenotype (CD26high CD45RA– CD45RO+), what corresponds to activated memory cells [5, 9, 10].

In this study, we measured the $CCR5^+$ and $CXCR4^+$ expression on blood lymphocytes, its changes in AIDS development, and correlation of $CCR5^+$ and $CXCR4^+$ expression with the number of T-cells, T-helpers, cytotoxic T-cells, activated T-cells ($CD3^+$ $HLA\text{-}DR^+$), T-regs cells, natural killer (NK)-cells [12], B cells, in order to determine the type of immune response activation in HIV/HCV coinfected patients.

MATERIALS AND METHODS

The patients were divided into four groups. The 1st group included 51 patients with HIV/HCV coinfection; the 2nd group consisted of 23 patients with HIV-infection; the 3rd group was composed of 10 patients with HCV-infection. Control group consisted of 16 healthy individuals (three men and 13 women negative for the markers of viral hepatitis type B and C and HIV-infection, aged 32.5±15.1 years).

To determine the clinical stage of HIV-infection, WHO classification was used (2006). All patients were tested for HBV and HCV serological markers using ELISA kits.RNA load and HCV genotype was studied using "Amplisens" kits (Russia). The diagnosis of HIV-infection was verified by detection of antibodies to HIV using ELISA and immunoblot.

Plasma viral loads (VL) of RNA HIV were detected using Ampisens monitor (Russia). Detectable level of RNA was defined as 500 copies/mL. In the current investigation the following monoclonal antibodies were used (Becton Dickenson, USA):

- CD3 (SK7, FITC) / CD16 (B73.1, PE) + CD56 (NCAM 16.2, PE) / CD45 (2D1, PerCP) / CD19 (SJ25C1, APC)
- CD4 (SK3, FITC) /CD8 (SK1, PE) /CD3 (SK7, PerCP)
- HLA-DR (L243, APC)
- CD4 (SK3, APC)
- CD184 (CXCR4) (12G5, PE)
- CD195 (CCR5) (2D7/CCR5, FITC)
- CD25 (M-A251, FITC).

The blood (100 μL) was incubated with monoclonal antibodies for 15 min at four °C, and then the erythrocytes were lysed by ammonium

chloride solution for 15 min at 18–25 °C. The cells were suspended in 300 μL of 1% paraformaldehyde solution and analyzed using (FACSCalibur) flow cytometer (Becton Dickenson, USA). Cell acquisition and analysis were performed using (CellQuest) version 3.3 and (Weasel) version 2.9 software (WEHI, Australia).

Statistical analysis. Data are presented as median and ranges (minimum and max). Normality was tested by Shapiro-Wilk's test. The majority of obtained data were significantly different from a normal distribution; therefore, nonparametric tests were applied. Differences between groups were analyzed via Kruskal-Wallis test and two-tailed unpaired nonparametric Mann–Whitney's test. The correlation between variables was evaluated using Spearman's rank coefficient (r). A value of $p \leq 0.05$ was considered significant. All calculations were performed using the Statistica (StatSoft, USA) and StatPlus (AnalystSoft, USA) software.

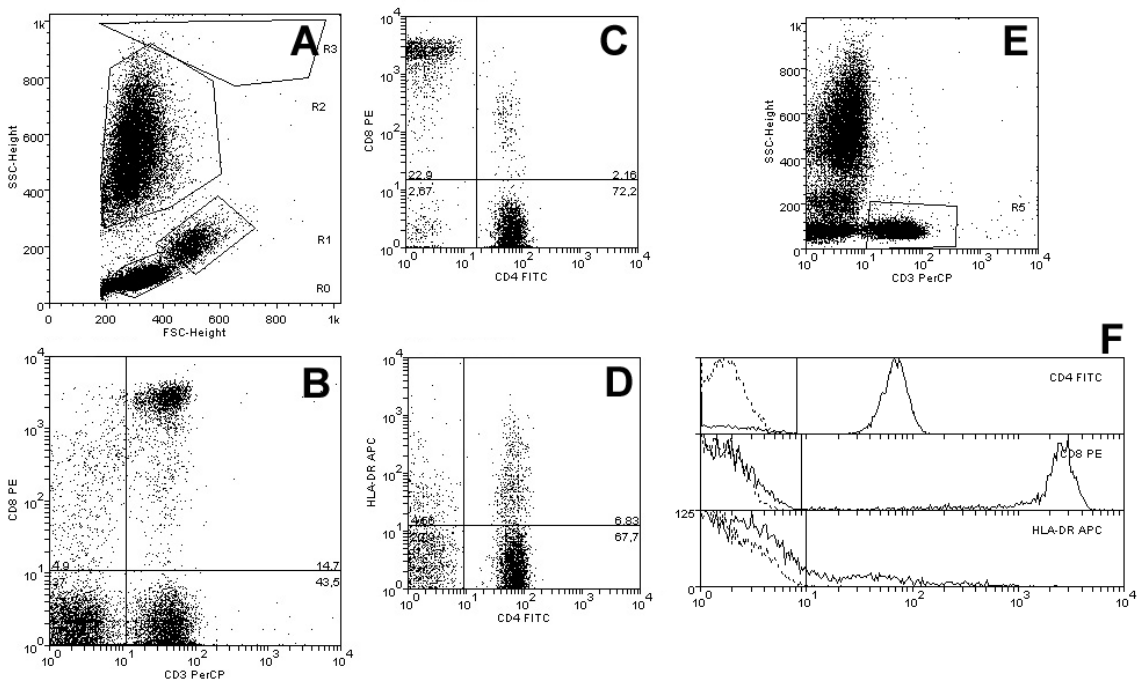

Figure 1. Immunophenotypic analysis of peripheral blood lymphocytes (control group)

A –FSC/SSC dot plot; region R0 includes lymphocytes. B –FL3/FL2 dot plot. C –FL1/FL2 dot plot. D –FL1/FL4 dot plot. E –FL3/SSC dot plot; region R5 was created to select CD3$^+$. F – fluorescence histograms for FL1, FL2, FL4 channels; solid line shows expression of molecules by cells after incubation with appropriate antibodies, dotted line represents negative controls.

RESULTS

Clinical characteristics of patients are presented in table 1.

Table 1. Characteristic of patients in groups of observation

Parameters	group 1 HIV/HCV n=51	group 2 HIV n=23	group 3 HCV n=10
Age, years	34.1±5.9	33.4±6.3	40.3±11.7
Men, n.(%)	39 (76.5)	11(47.8)	7 (70)
Women, n. (%)	12 (23.5)	12 (52.2)	3 (30)
Injection drugs users (IDUs) n (%)	28 (54.9)***	-	1(10)
HCV genotype 1, n (%)	17 (51.5)****	-	7 (70)**
HCV genotype not 1, n (%)	16 (48.5)	-	3 (30)

1 clinical category, n (%)	22 (43.1)	16 (69.6)	
2 clinical category, n (%)	23 (25.1) ***	2 (8.7) **	
3 clinical category, n (%)	4 (7.8)	4 (17.4)	
4 clinical category, n (%)	2 (3.9)	1 (4.4)	
AIDS, n (%)	11 (21.6)	6 (26.1)	
Antiretroviral therapy ART, n (%)	17 (33.3)	6 (26.1)	

Note: ** - p<0.05 in comparison with the patients from group 1, *** - p<0.05 in comparison with the patients from group 2, test χ^2.

According to table 1, the age doesn't significantly differ in the studied groups. There are more men in groups 1 and 3, while in a group, two distributions of patients according to gender were approximately equal. Parenteral route of HIV infection due to intravenous drug usage was revealed in 55% of cases in group 1, 10% - in group 3 and was absent in group 2. Seventeen patients (33.3%) in group 1 and 6 (26.1%) ones in group 2 received highly active antiretroviral therapy (HAART). The majority of patients in groups 1 and 2 had a clinical category 1 (asymptomatic infection). AIDS incidence was similar in groups 1 and 2.

Immunological parameters investigated in patients are presented in table 2.

Table 2. Immunological parameters of patients in the studied groups

Parameter Median (ranges)	**Control group n=16**	**group 1 HIV/HCV =51**	**group 2 HIV n=23**	**group 3 HCV n=10**
$CD3^-CD16^+ CD56^+$ (%)	15.10 (6.3-27.0)	7.92 (0.8-9.0)*.****	8.99 (0.5-40.9)*	12.83 (5.6-23.6)
$CD3^-CD16^+CD56^+$ (cells/μL)	328.69 (125.7-856.0)	119.54 (13.0-672.4) *.****	144.67 (8.3-733.6)*	198.79 (135.3-693.7)
$CD19^+$ (%)	8.20 (2.5-18.9)	5.33 (0.7-15.5)*	4.89 (0.6-11.9)*	7.32 (3.07-11.7)
$CD19^+$ (cells/μL)	182.18 (36.6-635.2)	95.73 (14.5-342.7)*	97.23 (9.2-434.8)*	148.09 (34.4-377.9)
$CD3^+$ (%)	70.92 (61.8-80.1)	80.70* (59.4-92.9)	82.37 (47.9-95.0) *.****	77.40 (65.8-85.6)***
$CD3^+$ (cells/μL)	1679.51 (875.7-2433.5)	1507.56 (227.4-3181.8)	1508.75 (510.0-3009.0)	1694.44 (828.8-2890.6)
$CD3^+CD4^+$ (%)	41.54 (27.2-55.6)	21.32 (4.2-43.2)*.****	25.39 (0.92-49.8) *.****	37.75 (28.5-54.1)**.***
$CD3^+CD4^+$ (cells/μL)	934.38 (467.3-1876.1)	394.28 (13.1-58.8)*.****	508.39 (13.8-1228.2)*	882.81 (346.0-1314.6)**
$CD3^+CD8^+$ (%)	24.12 (14.9-37.2)	54.60 (32.0-81.8)*.****	48.89 (15.9-81.3)*.****	31.73 (22.3-46.8)*.**.***
$CD3^+CD8^+$ (cells/μL)	551.01 (304.4-874.5)	957.74 (196.7-2299.2)*.****	912.64 (254.1-2145.4)*.****	577.80 (341.0-1689.3)**.***
CD4/CD8	1.69 (0.7-3.7)	0.44 (0.1-1.3)*.****	0.49 (0.0-2.0)*.****	1.15 (0.7-2.3)*.**.***

Note: * - p<0.05 if compared with control, ** - p<0.05 in comparison with the patients from group 1, *** - p<0.05 in comparison with the patients from group 2, **** - p<0.05 in comparison with the patients from group 3, Mann–Whitney U test.

According to table 2, NK-cells ($CD3^-$ $CD16^+CD56^+$) (percentage and absolute values) were significantly reduced in the groups of HIV-infected patients in comparison with the control group. Moreover, in a group one value of NK-cells was decreased in comparison with group 3. Reduction of B-lymphocytes (percentage and absolute values) was marked in groups of HIV-infected patients (group 1 and 2) if compared with the control group. The percentage of $CD3^+$-lymphocytes was significantly higher in the patients of groups 1, 2 and 3 in comparison with the control group. However, the absolute number of $CD3^+$-cells did not differ significantly in the studied groups. Significant reduction of CD3+CD4+ percentage was detected in groups 1 and 2 in comparison with the control group and group 3. Absolute number of $CD3^+CD4^+$ cells in groups 1 and 2 was significantly

lower as compared to the control group, and in group 1, it was lower than in group 3.

Table 2 shows that $CD3^+CD8^+$ indicator was significantly higher in relative as well as absolute values in HIV-infected patients (groups 1 and 2) in comparison with control and group 3. The reduction of $CD4^+/CD8^+$ (immune-regulatory index - IRI) was established in all patient groups in comparison with the control group. Though, more evident reduction of IRI value was detected in the groups 1 and 2 in comparison with the group 3.

HLA-DR expression on T-lymphocytes and their subpopulations is presented in table 3.

Table 3. Parameters of HLA-DR expression on blood lymphocytes and T- lymphocytes in the studied groups

Parameters Median (ranges)	**Control group n=16**	**group 1 HIV/HCV =51**	**group 2 HIV n=23**	**group 3 HCV n=10**
HLA-DR (%)	21.20 (15.6-36.3)	45.79 (17.3-71.6)$^{*.****}$	39.14 (14.7-70.6)$^{*.****}$	17.17 (11.3-42.5)
HLA-DR cells/μL	574.96 (225.9-1269.2)	789.61 (140.3-1669.7)$^{*.****}$	624.12 (215.3 -2136.6)	378.99 (120.5-1534.3)
$CD3^+HLA\text{-}DR^+$ (%)	8.60 (2.2-18.7)	30.64 (10.8-67.6)$^{*.****}$	29.45 (8.0-60.5)$^{*.****}$	7.66 (4.3-22.73)
$CD3^+HLA\text{-}DR^+$ cells/μL	176.81 (60.9-652.8)	549.63 (74.3-1626.5)$^{*.****}$	418.74 (143.0-1581.4)$^{*.****}$	138.82 (77.2-796.6)
$CD4^+HLA\text{-}DR^+$ (%)	4.95 (2.9-9.9)	4.09 (1.6-10.0)	4.07 (1.2-11.6)	3.72 (2.4-9.1)
$CD4^+HLA\text{-}DR^+$ cells/μL	101.46 (64.5-345.4)	66.64 (10.0-274.5)*	70.03 (18.4-153.5)*	81.83 (29.0-186.1)
$CD8^+HLA\text{-}DR^+$ (%)	5.43 (2.3-17.0)	30.88 (11.0-60.3)$^{*.****}$	27.93 (8.5-61.6)$^{*.****}$	6.72 (3.6-18.4)
$CD8^+HLA\text{-}DR^+$ cells/μL	114.74 (63.9-577.9)	506.07 (100.0-1655.3)$^{*.****}$	454.47 (170.2 - 1568.9)$^{*.****}$	124.0 (62.8-664.7)

Note: * - p<0.05 if compared with control, ** - p<0.05 in comparison with the patients from group 1, *** - p<0.05 in comparison with the patients from group 2, **** - p<0.05 in comparison with the patients from group 3, Mann–Whitney U test**.**

Table 3 shows the increased expression of HLA-DR on blood lymphocytes, $CD3^+$ and $CD8^+$T-cells in HIV-infected patients (1 and two groups) in comparison with controls as well as with group 3 (mono-HCV). In the group 1 (HIV/HCV coinfected patients) there were no differences in percentage of HLA-DR expression on $CD4^+$ cells between the control group and group 3. In the groups 1 and two reduction of the absolute index, $CD4^+HLA\text{-}DR^+$ was detected in comparison with the control group. Expression of $CD25^+$ on the peripheral blood lymphocytes and $CD4^+$ T- lymphocytes is presented in table 4.

Table 4. Parameters of CD25 expression on blood lymphocytes and CD4 T- lymphocytes in studied groups

Parameters Median (ranges)	**Control group n=16**	**group1 HIV/HCV n=51**	**group 2 HIV n=23**	**group 3 HCV n=10**
CD25+ (%)	5.50 (3.5-8.5)	3.82 (0.5-9.5)*	3.94 (1.4-18.9)*	5.14 (2.8-14.0)
CD25+ cells/μL	128.30 (54.8-243.1)	64.93 (7.6-260.5)$^{*.****}$	77.75 (13.3-283.0)*	108.82 (35.3-170.2)**
CD4+ CD25+ (%)	3.55 (2.2-6.0)	1.80 (0.3-6.7)$^{*.****}$	2.29 (0.3-4.4)$^{*.****}$	3.89 (1.4-8.1)$^{**.***}$
CD4+ CD25+ cells/μL	87.79 (40.9-162.1)	34.4 (1.0-224.0)$^{*.****}$	41.75 (5.2 -112.0)$^{*.****}$	87.69 (22.2-126.4)$^{**.***}$

Note: * - p<0.05 if compared with control, ** - p<0.05 in comparison with the patients from group 1, *** - p<0.05 in comparison with the patients from group 2, **** - p<0.05 in comparison with the patients from group 3, Mann–Whitney U test.

According to table 4, in groups 1 and 2 the reduction of percentage and absolute number of $CD25^+$ expression on blood lymphocytes was detected in comparison with the control group. Absolute value of this parameter in group 1 was also significantly lower than in group 3. The reduction of percentage and absolute number of $CD4^+CD25^+$ regulatory cells was observed in groups 1 and 2 in comparison with groups 3 and the control group. The expression of $CXCR4^+$ and $CCR5^+$ on blood lymphocytes in studied groups is presented in table 5.

Table 5. Parameters of CXCR4 and CCR5 expression on blood lymphocytes and CD4T- lymphocytes in studied groups

Parameters Median (ranges)	Control group n=16	group 1 HIV/HCV n=51	group 2 HIV n=23	group 3 HCV n=10
$CXCR4^+$ (%)	15.51 (8.9-24.8)	13.0 (3.7-33.0)	18.65(0.04-35.6)	15.60 (9.1-35.5)
$CXCR4^+$ cells/μL	345.56 (172.1-812.1)	226.39 (28.7-973.9)*	274.28 (0.7-863.0)	268.22 (113.1-1043.5)
$CD4^+CXCR4^+$ (%)	4.15 (1.1-8.1)	4.22 (0.6-17.9)	6.16 (0-15.3)	6.34 (3.4-18.4)
$CD4^+CXCR4^+$ cells/μL	82.42 (33.0-276.8)	64.55 (4.1-510.0)	93.05 (0-439.9)	92.82 (41.0-540.3)
$CCR5^+$ (%)	20.67 (12.3-35.8)	32.74 (5.9-61.3)*,****	27.98 (8.9-49.0)****	18.69 (9.8-30.1)****
$CCR5^+$ cells/μL	434.47 (225.9-975.1)	579.47 (132.7-1555.7)****	505.18 (110.2-1586.9)	337.04 (175.8-730.1)**
$CD4^+CCR5^+$ (%)	6.11 (0.1-1.2)	2.78 (0.6-11.7)*,****	2.8 (0.7-7.2)*,****	4.97 (3.0-12.6)**,***
$CD4^+CCR5^+$ cells/μL	119.25 (1.0-41.9)	50.17 (3.1-10.0)****	51.95 (8.5-29.0)****	128.91 (36.7-205.6)****
$CD4^+CXCR4^+CCR5^+$ (%)	0.24 (1.1-8.1)	0.24 (0.0-1.7)****	0.26 (0-2.1)****	0.5 (0.1-1.5)**,***
$CD4^+CXCR4^+CCR5^+$ cells/μL	6.66 (33.0-276.8)	3.78 (0.0-22.02)****	3.39 (0-21.1)****	9.02 (1.3-43.8)**,***

Note: * - p<0.05 if compared with control, ** - p<0.05 in comparison with the patients from group 1, *** - p<0.05 in comparison with the patients from group 2, **** - p<0.05 in comparison with the patients from group 3, Mann–Whitney U test.

The absolute number of $CXCR4^+$ on blood lymphocytes was significantly lower in the 1st group in comparison with the control group (Tab.5). The expression of $CXCR4^+$ on $CD4^+$ T-lymphocytes did not differ statistically in studied groups. The percentage and absolute parameter of $CCR5^+$ on blood lymphocytes was significantly higher in the 1st group in comparison with the control group and group 3. The percentage of $CCR5^+$ in group 2 was significantly higher in comparison with controls and group 3. The percentage and absolute parameters of $CD4^+$ $CCR5^+$ in groups 1 and 2 were significantly lower in comparison with groups 3 and controls. The percentage and absolute parameters of $CD4^+CXCR4^+CCR5^+$ cells in groups 1 and 2 were significantly lower in comparison with groups 3 and did not differ significantly with controls. We have compared the expression of $CCR5^+$ and $CXCR4^+$ on blood lymphocytes and $CD4^+$ T-lymphocytes in patents of group 1 (HIV/HCV coinfection) according to presence or absence of AIDS (Tab.6).

Table 6. Parameters of CXCR4 and CCR5 expression on blood lymphocytes and CD4T- lymphocytes in AIDS and non AIDS patients with HIV/HCV

Parameters Median (ranges)	Non AIDS n= 40	AIDS n= 11	P
$CXCR4^+$ (%)	14.4 (3.7 –30.6)	9.9 (6.0 – 33.0)	NS
$CXCR4^+$ cells/μL	241.0 (56.7 – 715.0)	134.7 (53.9 – 973.9)	<0.002
$CD4^+CXCR4^+$ (%)	4.8 (1.0 – 17.9)	1.3 (0.8 – 17.3)	<0.004
$CD4^+CXCR4^+$ cells/μL	92.2 (16.6 – 407.3)	18.0 (7.1 –510.0)	NS
$CCR5^+$ (%)	29.3 (5.9 –61.2)	46.5 (35.5 – 61.3)	<0.03
$CCR5^+$ cells/μL	595.5 (132.7 – 1555.7)	512.4 (322.4 –1099.8)	<0.002
$CD4^+CCR5^+$ (%)	3.1 (0.6 –11.7)	2.3 (2.0 – 6.8)	NS
$CD4^+CCR5^+$ cells/μL	59.6 (13.3 –210.0)	31.4 (22.8 – 62.2)	<0.001

Note: * – NS – not significant (Mann-Whitney U Test).

AIDS was established if a number of $CD4^+$ T-lymphocytes were less than 200 cells/mkl and/or if a patient had the fourth stage of HIV-infection according to WHO classification, 2006. Expression of $CXCR4^+$ on blood lymphocytes was decreased in AIDS group in comparison with non AIDS. On the other hand expression of $CCR5^+$ on blood lymphocytes was increased in AIDS group in comparison with non-AIDS group. A relative parameter of $CXCR4^+$ and an absolute parameter of $CCR5^+$ expressions on CD4+ T-lymphocytes were decreased significantly in AIDS group in comparison with non AIDS. Besides expression of $CCR5^+$ on blood lymphocytes was positively correlated with AIDS (R=0.50, p=0.0002) and with plasma viral load of HIV (R=0.37; p=0.02) in HIV/HCV coinfected patients.

Relative and absolute parameter of the $CCR5^+$ expression on $CD4^+$ T-lymphocytes in patients with AIDS was reduced in comparison

with the patients without it. There were no differences detected in CXCR4$^+$ expression on CD4$^+$ T-lymphocytes in the compared groups.

Correlation of CXCR4$^+$ expression on blood lymphocytes with some other parameters were established (Tab.7).

Table 7.Correlations of CXCR4 expression on BL with immunological parameters in studied groups

Parameters	**Control group n=16**	**group 1 HIV/HCV n=51**	**group 2 HIV n=23**	**group 3 HCV n=10**
CD3$^-$CD16$^+$ CD56$^+$ (%)	R=-0.81 p<0.0002	NS	NS	NS
CD3$^-$CD16$^+$CD56$^+$ (cells/μL)	R=-0.50 p<0.0002	NS	NS	R=0.83 p=0.003
CD19+ (%)	R=0.59 p<0.02	R=0.32 p<0.02	NS	R=0.66 p<0.05
CD19+ (cells/μL)	NS	R=0.48 p<0.0003	NS	NS
CD3$^+$CD4$^+$(%)	R=0.69 p<0.003	R=0.38 p<0.005	R=0.53 p<0.01	NS
CD3$^+$CD4$^+$ (cells/μL)	NS	R=0.39 p=0.006	R=0.51 p=0.01	NS
CD3$^+$CD8$^+$ (%)	NS	R= –0.28 p<0.05	NS	NS
CD3$^+$CD4$^+$/ CD3$^+$CD8$^+$ (IRI*)	NS	R=0.35 p<0.01	NS	NS
CD4$^+$CD25$^+$ (cells/μL)	NS	R=0.45, p<0.03	NS	NS
CD4+CXCR4 (%)	R=0.65 p=0.06	R=0.81 p<0.0001	R=0.85 p<0.0001	R=0.82 p=0.004
CD4+CXCR4 (cells/μL)	R=0.59 p=0.02	R=0.78 p<0.0001	R=0.83 p<0.0001	R=0.62 p=0.03

Note: NS – not significant (Spearman Rank Order Correlations); *IRI - immunoregulatory index

Positive correlation of CXCR4$^+$ expression on blood lymphocytes with CD4$^+$CXCR4$^+$ T-cells was detected in all observation groups.

Positive correlation of CXCR4$^+$ with CD4$^+$CD25$^+$, CD3$^+$CD4$^+$, CD19$^+$ cells was detected as well as inverse correlation of CXCR4$^+$ with CD3$^+$CD8$^+$ count in HIV/HCV coinfected patients.

Correlation of CCR5$^+$ expression on blood lymphocytes with some other parameters is given in table 8.

Table 8. Correlations of CCR5 with immunological parameters in studied groups

Parameters	**Control group n=16**	**group 1 HIV/HCV n=51**	**group 3 HIV n=23**	**group 3 HCV n=10**
CD3$^-$CD16$^+$ CD56$^+$ (%)	R=0.56 p<0.02	NS	NS	NS
CD19+ (cells/μL)	NS	R= -0.55 p<0.0001	NS	NS
CD3$^+$CD4$^+$	R= -0.50 p<0.05	NS	R= -0.60 p<0.002	NS
CD3$^+$CD4$^+$ (cells/μL)	NS	R=-0.62 p<0.0001	R=-0.50 p=0.02	NS
CD3$^+$CD8$^+$ (%)	NS	R=0.47 p<0.001	R=0.87 p<0.0001	NS
CD3$^+$CD4$^+$/CD3$^+$CD8$^+$ (IRI*)	NS	R= -0.58 p<0.0001	R=-0.73 p<0.0001	NS
CD4$^+$CD25$^+$ (%)	NS	R= -0.39 p=0.005	NS	NS
CD4$^+$CD25$^+$ (cells/μL)	NS	R=-0.55 p<0.02	NS	NS
HLA-DR (%)	NS	R=0.62 p<0.0001	R=0.56 p=0.005	NS
CD3$^+$HLA-DR$^+$ (%)	R=0.51 p=0.04	R=0.66 p<0.0001	R=0.74 p<0.0001	R=0.66 p=0.04
CD3$^+$HLA-DR$^+$, cells/μL	NS	NS	R=0.51 p<0.01	
CD4$^+$HLA-DR$^+$ (%)	NS	NS	NS	R=0.64 p=0.04
CD4$^+$HLA-DR$^+$, cells/μL	NS	R=-0.28 p<0.05	NS	NS
CD8$^+$HLA-DR$^+$ (%)	NS	R=0.66 p<0.0001	R=0.72 p<0.0001	NS
CD8$^+$HLA-DR$^+$ cells/μL	NS	NS	R=0.45 p<0.03	NS
CD4+CCR5 (%)	R=0.73 P=0.001	NS	NS	NS
CD4+CCR5 (cells/μL)	NS	NS	NS	R=0.73 p=0.02

Note: NS – not significant (Spearman Rank Order Correlations); *IRI - immunoregulatory index

In group 1 positive correlations of $CCR5^+$ expression on blood lymphocytes were detected with number of T-lymphocytes, cytotoxic T-cells, activated T-lymphocytes and cytotoxic T-cells as well as inverse correlations were established with number of T helper cells, $CD4^+CD25^+$ cells.

DISCUSSION

Studies of $CCR5^+$ and $CRCX4^+$ expression on blood lymphocytes and $CD3^+CD4^+$ lymphocytes, as well as some immunity parameters enabled to detect some significant differences in the studied groups.

We estimated that in patients with HIV/HCV coinfection $CCR5^+$ expression on $CD4^+$ T-lymphocytes was decreased and on the other hand $CCR5^+$ expression on blood lymphocytes was increased. It may be associated with the changes of HIV tropism during course of HIV-infection. Opposite dynamics of $CCR5^+$ and $CXCR4^+$ expressions on blood lymphocytes were marked with the development of AIDS: $CXCR4^+$ expression was decreased and $CCR5^+$ expression was increased. Recently published data by Schwarze-Zander et al. demonstrated that favorable course of AIDS at coinfection GB-C/HIV was associated with down-regulation of $CCR5^+$ and $CXCR4^+$ expression on $CD4^+$ T-lymphocytes in patients without CCR5/Δ32 mutation [3]. In our study the presence of CCR5/Δ32 mutation in the patients was not detected. However, we revealed the decrease in an absolute parameter of $CCR5^+$ expression and a relative parameter of $CXCR4^+$ expression on $CD4^+$ T-lymphocytes in patients with AIDS in comparison with patients without it among HIV/HCV coinfected persons.

Positive correlation of $CXCR4^+$ expression on blood lymphocytes with number of $CD4^+CXCR4^+$ T-cells detected in all observation groups, it may be explained by predominant expression of CXCR4 molecule like HIV-coreceptor on CD4+T cells [1];

In contrast to $CXCR4^+$ positive correlations between $CCR5^+$ expression on blood lymphocytes with $CD4^+CCR5^+$ T-cells were not detected in HIV-infected patients (groups 1 and 2) due to expression of this HIV coreceptor on the blood lymphocytes outside T-cells system: macrophages, monocytes, NK, especially in HIV-infection [1, 2]. HIV coreceptors CCR5 and CXCR4 function as chemokine receptors and their expression on cells is associated with certain type of immune response [3].

Our results confirmed that $CCR5^+$ was correlated with activation of cellular immune response in HIV/HCV coinfected patients as well as in the remained studied groups while $CXCR4^+$ expression was associated with suppression of immune response. Positive correlations of $CCR5^+$ expression on blood lymphocytes were detected with expression of activation markers of immune response HLA-DR [1, 13] and negative correlations with inhibition markers of immune response ($CD4^+CD25^+$T-lymphocytes) [14-18], while $CXCR4^+$ expression positively correlated with $CD4^+CD25^+$ T-lymphocytes. $CCR5^+$ expression on blood lymphocytes positively correlated with the level of $CD8^+$T-lymphocytes, and negatively with the $CD19^+$ B-lymphocytes. While the expression of $CXCR4^+$ positively correlated with the level of $CD19^+$ B-lymphocytes, and negatively with the level of $CD8^+$-lymphocytes in HIV/HCV coinfected patients. Direct correlation $CCR5^+$ expression with the HIV viral load, and reverse correlation with the level of $CD4^+$-lymphocytes was also found out in patients with HIV/HCV coinfection, which was associated with increased immunity activation with the progression of HIV infection. According to received data development of AIDS leads to increase of Th1 type immune response activation in HIV/HCV coinfected patients.

The limitation of our study include the absence of detection of HIV tropism to CCR5 and CXCR4 coreceptors and correlations of HIV tropism with immunological and clinical data of HIV-infected patients involved in the study.

CONCLUSIONS

Based on the obtained results, analysis and scientific literature data we can conclude that in chronic HIV/HCV coinfected patients $CCR5^+$ expression on blood lymphocytes was increased and $CCR5^+$ expression on $CD4^+$ T-lymphocytes was decreased in comparison with healthy individuals and HCV-infected patients. With the development of AIDS $CXCR4^+$ and $CCR5^+$ expressions on $CD4^+$T-lymphocytes, $CXCR4^+$ expression on blood lymphocytes were decreased and $CCR5^+$ expression on blood lymphocytes was increased. Increased $CCR5^+$ expression on blood lymphocytes in HIV/HCV coinfected patients was associated with Th1 type immune response activation.

Conflicts of interest

We declare that we have no conflicts of interest.

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